The link between personality and sexual selection in a neotropical poison frog

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by
Peignier Mélissa
from France

Supervisor of the doctoral thesis:
Prof. Dr. Eva Ringler

Institute for Ecology and Evolution | Division of Behavioural Ecology

source: https://doi.org/10.48549/5721 | downloaded: 21.1.2025

This work is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License (https://creativecommons.org/licenses/by-sa/4.0/) except Appendix 1 Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/).

The link between personality and sexual selection in a neotropical poison frog

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by
Peignier Mélissa
from France

Supervisor of the doctoral thesis:
Prof. Dr. Eva Ringler

Institute for Ecology and Evolution | Division of Behavioural Ecology

Accepted by the Faculty of Science.

Bern, 7 December 2022

The Dean
Prof. Dr. Marco Herwegh

Author:
MSc Mélissa Peignier
Institute of Ecology and Evolution
University of Bern
Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland

Supervisors:
Prof. Dr. Eva Ringler
Head of Division, Behavioural Ecology
Institute of Ecology and Evolution
University of Bern
Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland

Dr. Virginie Canoine
Department of Behavioural Biology
University of Vienna
Djerassiplatz 1, 1030 Wien, Austria

External reviewer:
Prof. Dr. Thomas Bugnyar
Head of department, Behavioral and Cognitive Biology
Department of Behavioral and Cognitive Biology
University of Vienna
Djerassiplatz 1, 1030 Wien, Austria

Chair:
Prof. Dr. Claudia Bank
Head of Division, Theoretical Ecology and Evolution
Institute of Ecology and Evolution
University of Bern
Baltzerstrasse 6, CH-3012 Bern, Switzerland

Table of contents

Acknowledgments

Recently, many people have asked me if I was happy to finish my PhD. I always answered yes with a large smile, but in truth, I was always lying a little bit…As much as I am happy to finally be able to show the world all the work that I have been doing for the last four years, I am a bit sad that I won't be able to work with all of you everyday from now on, and that this story has to end. It is always difficult to go from one big part of your life to the next, but I am glad I had the chance to live this part.

I have many thanks to give today, but I want to start with you Eva. You have been an amazing supervisor, from the beginning to the end. You were always present for me, and helped me improved the quality of my science so much. You are one of these precious supervisors that the academic world needs to know about. You are a combination of intelligence and compassion, and I am honoured to have been your student. This PhD has exceeded all my expectations, and it will forever stay one of the biggest achievements of my life. This is all thanks to you. So, thank you Eva, for being who you are and for allowing me to take this journey at your sides.

Max, thank you for helping me so much throughout this PhD. Field work would not have been as amazing without you. You are probably one of the smartest persons I know, and I am really glad that you were there during this journey. Thank you for our endless conversations. I can't promise I remember everything, you know that my brain dies some times, but I really enjoyed talking with you and learning so much from you.

Yimen, thank you for all the help and numerous zoom meetings about my statistical analyses. You taught me a lot.

Prof. Bugnyar and Prof. Bank, thank you for agreeing to review my work. I hope you will enjoy it as much as I enjoyed working on it.

To the entire CNRS team in French Guiana. Thank you for all the support whether it be in work or in personal moments at the camp. You have made my experience in the field absolutely wonderful. Beyond that, you have all taught me a lot, I hope to see you again soon, I love you.

To the Hasli team, I want to thank all of you for your support, whether it be emotional or scientific. You are an amazing group of people, and I was incredibly lucky to work with you.

To all of you who were with me in the field: Lolo, Gaëlle, Momo, Kathi, Vincent, Christoph, Fabrice, thank you so much. You have made some of the best memories I will ever have. Thank you for all the hard work you have accomplished, this thesis would have never been possible without you. But most of all, thank you for all the fun you brought to the camp. More than fieldworkers, I gained friends in you.

To Lauriane…how can I even thank you? I still remember when you first came as a volunteer student and started helping me with my video analyses. Here we are, three years later. More than an incredible student, you have become one of my best friends. I love you so much, thank you for all the help, all the chocolate breaks, all the gossiping, the dancing, the singing. You know as well as I do that this thesis is also a bit yours.

To my next desk neighbour, Birgit. Thank you for the uncountable corrections and revisions, for the stats questions, for being there during the breakdowns and for putting me back on my feet when I needed. I have learned a lot at your sides, you are an incredible scientist and an inspiration. I do not think that I would have accomplished so much for this thesis without you.

Marina, you lovely girl. We have not met a long time ago, but from the very first minute I saw you, I knew we would be friends. Thank you so much for being there, for being you, and for being the best cheerleader. I love you.

À ma famille. Je veux vous remercier tous de m'avoir encouragé depuis le premier jour, même sans avoir la moindre idée de ce que je fais réellement. Vous êtes mon pilier. Un merci tout particulier à ma maman et ma sœur. C'est grâce à vous si j'en suis là aujourd'hui, merci de m'avoir toujours poussée, d'avoir toujours cru en moi, et de m'avoir toujours relevée quand je tombais. Je vous aime infiniment.

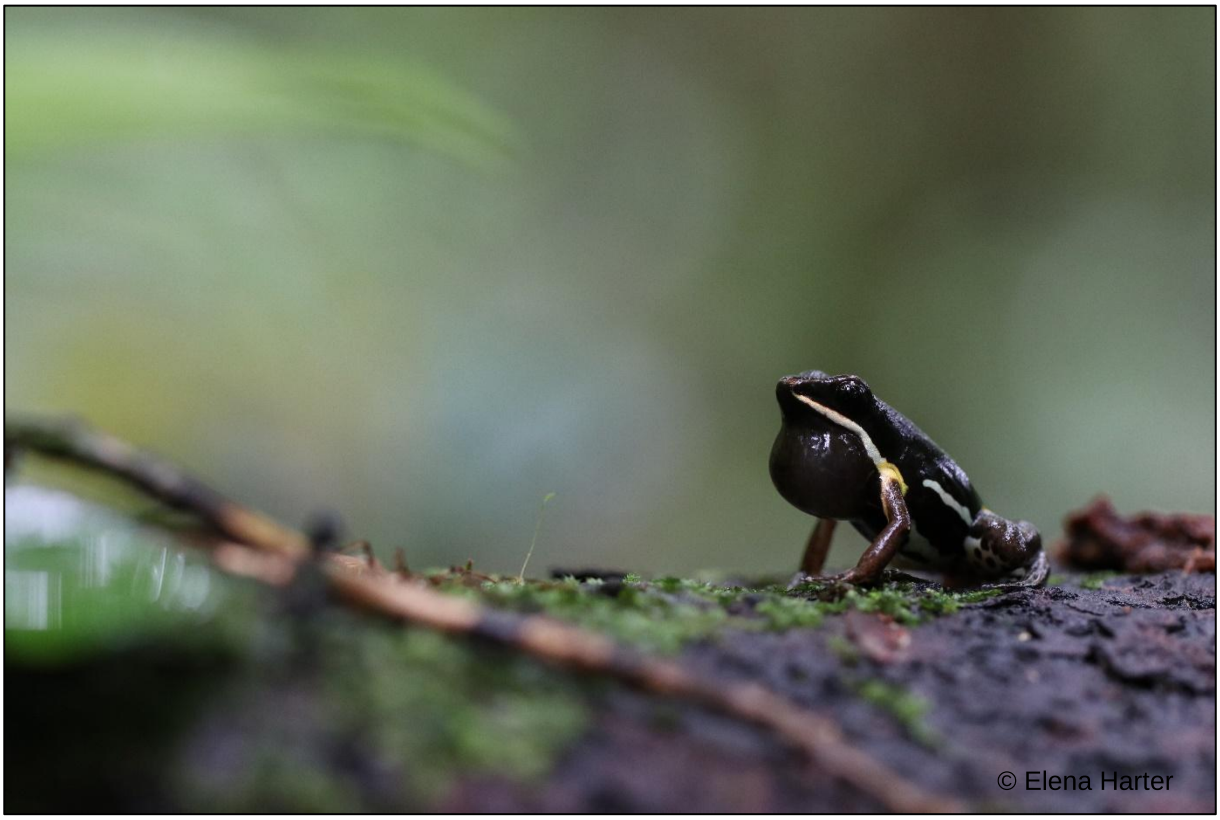
To you Fabrice. Without you, this entire journey would not have existed. Thank you for pushing me to apply in the first place. But above all thank you for supporting me all along, for reassuring me when I thought I was not good enough, and for forcing me to take breaks when I did not. Thank you for putting your life on hold to move with me to Austria and then Switzerland. Thank you for cheering me up in the worst moments when I thought I would never make it. You will never know how much it meant to me, to have you in my life during this PhD. I love you.

And finally, even if you will never read these lines, thank you Enjoy. You were there for my first diploma, and you are here for my last one. In your own way, you have supported me through every single day of this PhD. Thank you so much, my little angel. I think in a way, it was destiny, seeing that I used to call you "my little froggy" …

Summary

Over the past years, an increasing number of studies have provided empirical evidence of the existence of consistent between-individual differences in behaviour, also known as “animal personality”. However, we do not yet understand how these behavioural differences between individuals arise in the first place, and how they are maintained in animal populations. Throughout my thesis, I aimed to improve our understanding of the evolutionary mechanisms that maintain between-individual variation in behaviour. I investigated patterns in the distribution of behaviours across the natural and social environment, and I studied how personality traits relate to behaviours that are shaped by sexual selection. To do so, I used a combination of behavioural tests, molecular parentage analysis and fitness measures in a closed, wild, free-ranging experimental population of the Neotropical poison frog *Allobates femoralis*. Throughout the chapters that I present here, I show that male and female poison frogs display several personality traits that, combined with behavioural plasticity, helps them cope with variation in their natural and social environment. Further, I show that personality traits are related to several behaviours that are shaped by sexual selection, such as mate choice, reproductive success, and reproductive output. I also showed that personality traits can have different – even opposite – effects on the various components of reproductive success, which can lead to the evolution of different reproductive strategies. By studying variation in behaviour between and within individuals, I increased our understanding of how behavioural variation is maintained and found evidence that limited plasticity can arise because of a link with individual differences in life-history trade-offs.

General introduction



© Elena Harter

General introduction

The history of animal personality research

Behavioural variation between and within individuals is ubiquitous in nature. For instance, most zookeepers and pet owners would agree without any doubt that their animals differ in their behaviours, e.g., with one of them being bolder or more active than the other. Yet, between-individual variation in behaviour was not the focus of scientific research for a long time, often accused to apply a too anthropomorphic view on animal behaviour. Traditionally, focus was put on within-individual variation in behaviour; i.e., phenotypic plasticity, to infer how individuals cope with changing environmental conditions [1]. It is only recently that increasing attention has been given to between-individual variation in behaviour. In the last decades, empirical evidence has shown that individuals consistently differ in their behaviour and do not exhibit the full range of variation in their species' behavioural repertoire [2]. This concerns not only animals close to humans, like primates, but also animals phylogenetically very distant from human (e.g., hermit crab [3], anemones [4]). These between-individual behavioural differences that are consistent across time and contexts are commonly referred to as 'animal personality' [5].

The fact that animals differ in their behaviour has been acknowledged as early as the late nineteenth century [6]. However, at that time, the study of human personality was still at its very beginning. It was not until the end of the 20th century that researchers adopted the conceptual framework of the psychology of human personality to develop the field of animal personality. The study of animal personality within behavioural ecology was then popularized by the work of Réale and colleagues in 2007 [2]. The emergence of the field of animal personality has shifted classic thinking in behavioural ecology in two ways [7]. First, it shifted the focus from behavioural variation at the species/population/group-level to differences between individuals within the same species [7]. Second, a novel way of thinking was to look at how different behaviours are correlated across situations [7].

Terminology

Animal personality is characterized by two aspects: (1) within-individual consistency, or limited behavioural plasticity, and (2) between-individual variation in behaviour (Figure 1). Personality is commonly divided in five personality (or "behavioural") traits

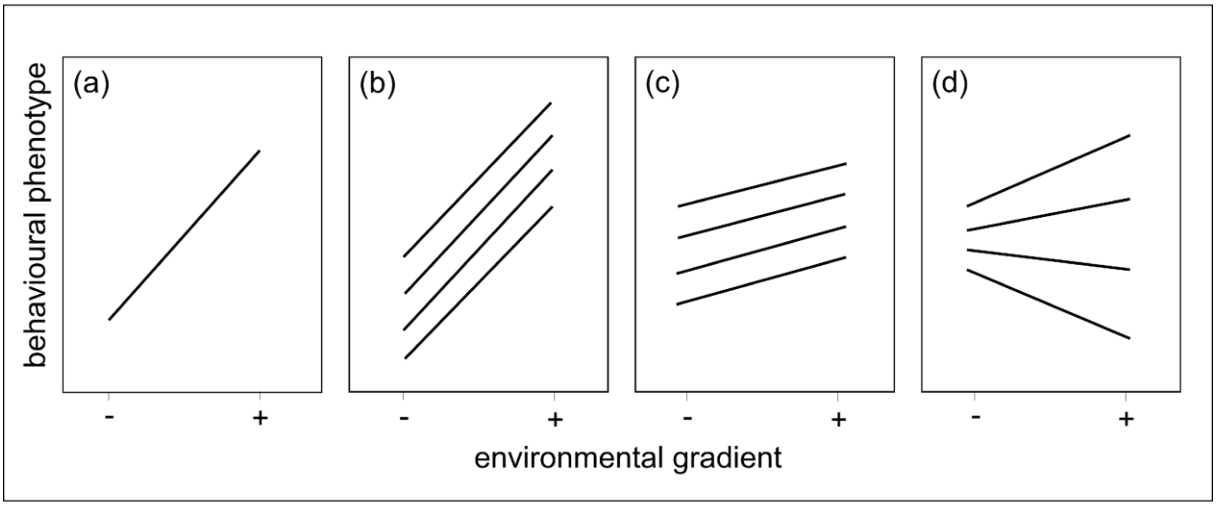


Figure 1. Behavioural responses along an environmental gradient. Lines represent behavioural responses of four hypothetical individuals to changes in time or context. For instance, levels of boldness in four mice as a function of temperature. In panel a, mice adjust to a change in temperature, but all individuals behave the same (there is behavioural plasticity, but no between-individual behavioural variation). In panel b, mice adjust to a change in temperature and behave differently (there is behavioural plasticity and between-individual behavioural variation). In panel c, mice do not adjust well to a change in the environment, and all behave differently (there is little behavioural plasticity and there is between-individual behavioural variation). This is when we find different personality types. Individuals can also differ in their levels of plasticity (see **manuscript 2**), for instance with shy individuals showing higher plasticity (panel d). Adapted from [74].

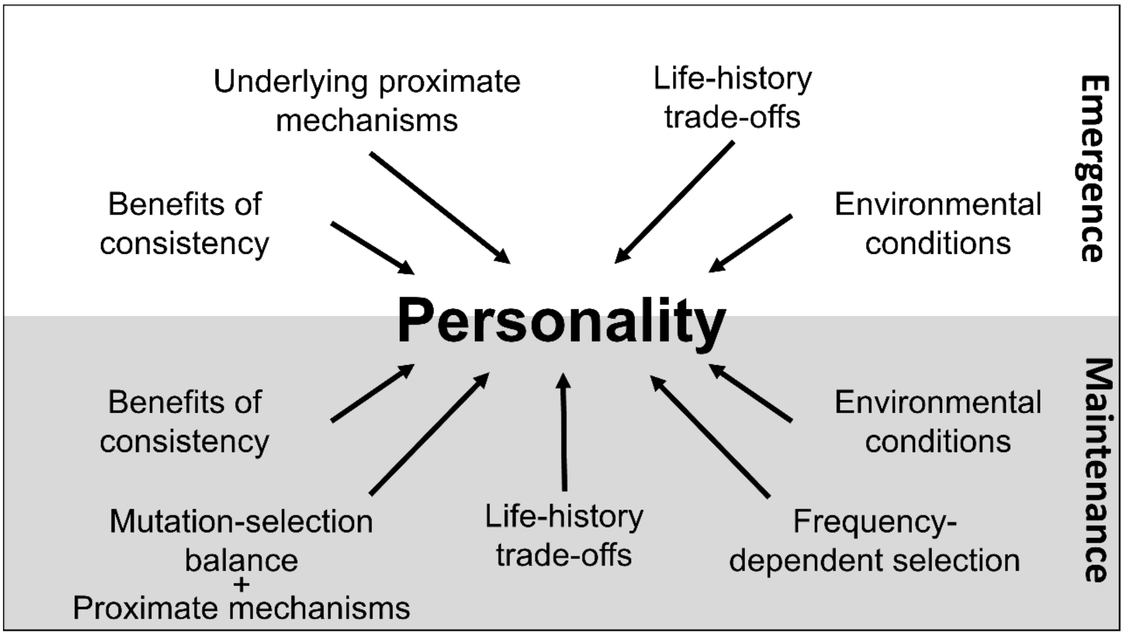


Figure 2. Summary of the hypothesized causes of the emergence and maintenance of consistent between-individual behavioural variation (i.e., “animal personality”).

(or “axes”): active/passive, aggressive/docile, bold/shy, exploratory/stationary, and sociable/non-sociable [2]. Individuals vary in their phenotypic expression and can take any value along these personality traits, thereby having a specific personality or behavioural type (e.g., more or less aggressive). Personality traits can be seen as latent variables that affect multiple quantifiable behaviours of an organism in certain contexts [8,9]. For instance, aggressiveness can be seen as an unobservable (i.e., latent) variable that affects several observable behaviours during agonistic encounters, which can be assessed and quantified in an experimental context (e.g., speed of territorial reaction, number of attacks). Additionally, personality traits often appear to be correlated into “behavioural syndromes”. For example, activity, exploration, boldness, and aggressiveness are correlated in many animal species [10]. This axis of (co)variation is commonly referred to as the reactive-proactive continuum, with proactive individuals being more active, aggressive, explorative, and bold than reactive individuals [11].

Between-individual behavioural differences have been shown to play a major role in reproduction and survival, e.g., by affecting the risk of being detected and caught by predators, the likelihood of dispersal, the foraging efficiency, and/or the attractiveness to mating partners [12–14]. While evidence for the influence of personality traits are numerous, it is still largely unknown how consistent between-individual differences in behaviour arise in the first place and why this variation is maintained.

How has animal personality emerged?

As mentioned above, personality traits are composed of several correlated behaviours (e.g., latency to approach a conspecific, number of attacks, etc. for aggressiveness) and are stable across time and contexts [2,5,8,9]. As a result, the existence of personality traits suggests a limited degree of plasticity, such that an individual's behaviour in one situation and at one point in time affects how it will behave in other situations and at other times. Such inflexibility can seem maladaptive at first. In theory, we would expect individuals to adjust their behavioural responses continuously and flexibly according to the current situation [1]; or that natural selection should remove all non-optimal behaviours from the population. Several non-necessarily mutually exclusive hypotheses have been developed to explain the evolution of limited plasticity and consistent between-individual differences in behaviours (summarized in Figure 2).

One hypothesis is that limited plasticity could persist, even when suboptimal behaviours should be selected against, because of underlying proximate mechanisms (e.g., hormones, genes, metabolism, and neuronal pathways). For instance, in the songbird (*Parus major*), steroid hormones can affect boldness related behaviours, with higher expression of mineralocorticoid and lower expression of glucocorticoid receptors being associated with shyer personalities [15]. Proximate mechanisms could also lead to between-individual differences in behaviour. Indeed, an individuals' state (morphology, physiology) is determined by proximate mechanisms, and influences the costs and benefits of the individual's actions, which in turn influences its behavioural responses [16]. In the context of intrasexual competition, smaller individuals are more likely to lose a fight and might be more at risk of dying from any injury, compared to bigger individuals. Thus, we expect smaller individuals to show less aggression compared to bigger ones, in order to avoid getting into costly fights. Furthermore, underlying proximate mechanisms could lead to the emergence of behavioural syndromes if they govern several behaviours [17,18]. Or, if behaviours are genetically correlated (e.g., boldness-related, and aggressiveness-related behaviours), then selection on one (e.g., increase in boldness level) could produce an indirect, correlated response in the other (e.g., increase in aggressiveness level) and prevent it from reaching its optimal value. In this case, the genetic correlations between traits act as evolutionary constraints [19].

Another hypothesis is that limited plasticity could have evolved because individuals benefit from being consistent in their behavioural responses [20–22]. Being consistent could be advantageous if it influences the behavioural responses of competitors in a way that will improve the focal individual's fitness [22]. For example, males would benefit from being constantly aggressive if other males adjust their behaviour accordingly, by not engaging in a competition. Or, females could benefit from choosing a male who is consistent in the level of care he provides to his offspring [23].

Between-individual differences in behaviour could also reflect behavioural strategies to cope with different environmental conditions [24,25]. For instance, while aggressive individuals might do poorly in a mating context, by attacking potential mates, they should do particularly well in situations with very high intra-sexual competition. Situations where personality traits are non-randomly distributed across the natural and social environment are typically referred to as "phenotype by environment correlations" [24,25]. Selective pressures induced by the heterogeneity of the environment could maintain, or even generate, individual differences in behaviour within a population [24,25]. For instance, in territorial species, only highly aggressive individuals may succeed in establishing a territory in high-density patches, where they have access to more mates but face elevated intra-sexual competition. Consistent differences in foraging tactics have been observed in the bluegill sunfish (*Lepomis macrochirus*) [26]. The most efficient foraging tactic differs between littoral and open water zones. Individuals that employ a certain tactic are usually found in the habitat that best fits their foraging tactic. In the open water, prey are more visible, and sunfish forage at a higher speed compared to individuals living in vegetated habitat where prey are more cryptic [26].

I aimed here at presenting several hypotheses explaining the evolution of limited plasticity and between-individual differences in behaviours. These hypotheses are not mutually exclusive. Understanding the evolution of animal personality will require to study these hypotheses together, alongside with the hypotheses explaining the maintenance of personality traits.

How is animal personality maintained?

Limited plasticity can seem maladaptive at first, yet we have evidence for the existence of limited plasticity and between-individual behavioural differences in a lot of taxa [27–31]. Which raises the question, why is selection not acting against suboptimal behaviours? Several hypotheses, non-mutually exclusive, have been developed to explain the maintenance of between-individual variation in behaviour (summarized in Figure 2). For instance, mutation-selection balance could favour the maintenance of genetic polymorphism [32]. Different personality types should co-exist due to a balance between the production by mutation of new alleles (which affect a personality trait) and the elimination (by natural selection) of those new alleles that do not bring the personality trait to its optimum [33].

Also, frequency-dependent selection could facilitate the coexistence of different behavioural types [34]. In the case of negative frequency dependent selection, different behavioural phenotypes could coexist when the fitness payoffs of a given personality type depends negatively on the frequency with which this personality type is spread in the population [34]. However, frequency-dependent selection fails to explain why animals are consistent in their behaviours [34]. Dall and colleagues [22] recently suggested that several personality types can coexist when either the cost of flexibility is high, or the benefit of consistency is high. Being consistent could be advantageous if it influences the behavioural responses of competitors in a way that will improve the focal individual's fitness [22]. For instance, males who are highly aggressive when competing for a resource could have an advantage in being predictable by signalling to other individuals their aggressive tendencies. As a result, potential competitors will be able to avoid costly fights with low prospects of winning [23].

Lastly, consistent between-individual differences in behaviour could be favoured and maintained by life-history trade-offs, with individuals differing in their investment into either current or future reproductive success [35,36]. For instance, we could expect proactive individuals to be better at attracting mates, finding food and repelling competitors but suffer from higher energy expenditure and an increased risk of predation, resulting in higher annual reproductive success but lower probability of surviving until the following reproductive season. Contrary, reactive individuals should have lower annual reproductive success but benefit from increased longevity. Thus, in the long run different behavioural phenotypes should contribute equally to the future gene pool to become evolutionarily stable [37]. Empirical studies testing this hypothesis have hinted towards the importance of personality as a component of life-history strategies. For example, in the grey mouse lemurs (*Microcebus murinus*), young males with low current but high expected future reproductive success are shyer than older males with high current but lower expected future fitness [38].

Some of the hypotheses presented here, and in the previous section, explain both the evolution and the maintenance of animal personality. More work is now needed to disentangle the mechanisms behind the evolution and maintenance of within-individual consistency and between-individual differences in behaviours.

Current state of knowledge

Despite the increasing number of studies showing the influence of personality traits on reproductive success and survival over the past years, we still do not understand how these behavioural differences between individuals arise in the first place, and how they are maintained in animal populations. For example, we need more empirical data to verify if personality types reflect behavioural strategies for coping with different environmental conditions, and then to disentangle the causes and consequences of non-random distribution of personality traits in the environment. We also need more data to test the hypothesis that different behavioural phenotypes face different life-history trade-offs, but that resulting long-term fitness is equal. Specifically, the relationship between personality traits and behaviours that are shaped by sexual selection (such as mate choice, intra-sexual competition, and parental care) is poorly understood. Long-term studies are required to understand the underlying mechanisms explaining the evolution and maintenance of animal personalities. Since captive individuals (compared to wild populations) are typically less repeatable in their behaviours [39], future studies should focus on collecting long-term data from entire wild populations.

Questions related to animal personality have mainly been studied in vertebrates, and particularly in fish, mammals, and birds [40–45] (but see [46] for a study on insects). However to date, amphibian personality remains largely unexplored [47,48]. Yet, because they undergo metamorphosis, amphibians are a particularly interesting and suitable group to study how between-individual behavioural differences arise and their link with physiology, morphology, or ecology [48]. In particular, when zooming into the amphibian tree of life, Neotropical poison frogs (Dendrobatidae *sensu* AmphibiaWeb [49]), feature behaviours with ideal prerequisites for within- and between-individual variation in behaviour to arise, such as territoriality, elaborate courtship behaviour, or complex parental care (e.g., [47,50–58]). Additionally, poison frogs display notably diverse and often complex reproductive behaviours, which makes them excellent systems to study the effects of personality on variation in reproductive behaviour across various social and ecological contexts [54].

Thesis aims

Throughout my thesis, I aimed to improve our understanding of the evolutionary mechanisms that shape and maintain between-individual variation in behaviour. Specifically, I used a free-living population of the Neotropical poison frog *Allobates femoralis* and investigated the distribution pattern of behaviour across the natural and social environment. I also studied how different personality traits relate to behaviours that are shaped by sexual selection, such as mate choice, intra-sexual competition, and parental care, and how they ultimately impact an individual’s reproductive success (summarized in Figure 3). To better understand the role of behavioural variation in the ecological and evolutionary dynamics of populations, I used a combination of behavioural tests and fitness measures on a closed free-living experimental population living on a river island.

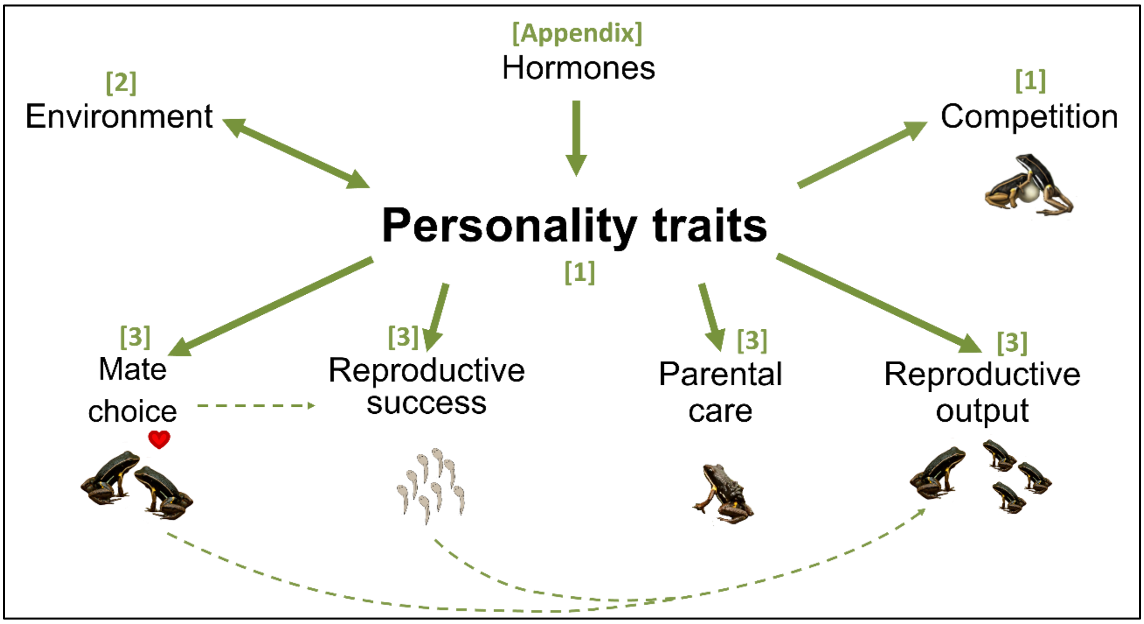


Figure 3. Summary of the aims of my thesis. The numbers refer to the chapters. In chapter 1, I investigated the existence of personality traits in *Allobates femoralis*, and the link between personality traits and plasticity in territory defence. In chapter 2, I studied the distribution pattern of personality traits across the natural and social environment. In chapter 3 I investigated how different personality traits relate to behaviours that are shaped by sexual selection, and how they ultimately impact an individual's reproductive success. Additionally, in **appendix 2**, I present a paper where a student (who I co-supervised) investigated whether individual androgen levels can explain between-individual variation in behaviours.

Figure 4. Picture of a male *Allobates femoralis* perching on a log.

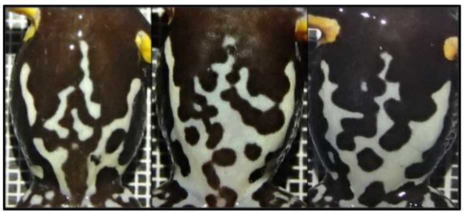


Figure 5. Belly pattern from three different *Allobates femoralis* adult individuals.

Study species

Throughout my thesis, I used the poison frog *Allobates femoralis* (Figure 4) as a model species. The brilliant-thighed poison frog *A. femoralis* has a pan-Amazonian distribution with isolated local populations. Both sexes are iteroparous and polygamous within prolonged but discrete reproductive periods that coincide with the rainy seasons [59]. During the reproductive season, males call from elevated structures on the forest floor to attract females and to announce their territory to male competitors, towards which they react aggressively when they enter the territory [60–62]. Females commute from their resting sites to males' territories, and pair formation, courtship and mating take place in the male's territory [63–67]. Externally fertilized terrestrial clutches of approximately 20 eggs are laid in the leaf litter. Tadpole transport takes place after 15–20 days of larval development (**appendix 1**) and is mainly performed by males. Males transport the tadpoles to water bodies located up to 200 m outside their territories and allocate tadpoles across several water bodies, probably as a bet-hedging strategy against total offspring loss [68–71]. Tadpoles require 40-50 days until metamorphosis and sexual maturity is reached after 8 (males) and 10 (females) months [63]. Annual survival of adult *A. femoralis* is low, with only about 20% surviving from one reproductive season to the next ([59], personal observations).

I expected to find consistent between-individual differences in behaviour in both males and females. Specifically, I expected to find evidence of the existence of the personality traits aggressiveness and boldness in males, because they display territorial aggression and face trade-offs between calling to secure a mating partner and exposure to predators [61]. Since females have never been observed in any kind of aggressive interaction with neither conspecific males nor females, I did not expect to find evidence for the existence of the personality trait aggressiveness and therefore did not test for it. Contrary, boldness might matter for females if it helps them deal with predators when they travel to males' territories or when they are foraging. Finally, I expected to find consistent between-individual differences in exploration in both sexes because males transport tadpoles to natural pools and females commute to male territories [65,68,70,72].

Male and female *A. femoralis* are distributed across the space in a non-random fashion (i.e., with patches of higher and lower density) [65], which makes them very good model to study distribution pattern of personality traits. In addition, each single adult individual is recognizable by a unique belly pattern (Figure 5), which facilitates the repetition of behavioural tests on the same individuals. Finally, *A. femoralis* is also suitable to study how different personality traits relate to behaviours that are shaped by sexual selection for several reasons. First, both males and females mate multiple time with multiple partners and, second, reproductive success can be accurately assessed by collecting tissue samples and using genetic parentage analysis to infer parent-offspring relationships [59]. Males also show paternal care, and we can elicit care (e.g., tadpole transport behaviour) by adding a tadpole on the back of a foster-parent [73]. It is also possible to manipulate tadpole deposition sites, as individual readily deposit in artificial pools that can be moved in space.

Chapters overview

Chapter 1: Animal personality and individual plasticity in the Neotropical poison frog *Allobates femoralis*

Manuscript 1: “Repeatable territorial aggression in a Neotropical poison frog”

In this study we investigated the existence of consistent between-individual variation in aggressiveness measured in a territorial defence context. We conducted repeated, standardized behavioural tests to assess levels of territorial aggression, using a playback call mimicking a conspecific intruding the focal male’s territory. We expected to find consistent between-individual differences in the latency to react to the playback (e.g., latency to orientate and to jump towards the intruder), and in the speed to approach the intruder. We found moderate repeatability in the behaviours measured in a territorial defence context, therefore hinting towards the existence of the personality trait “aggressiveness”.
This paper is published in *Frontiers in Ecology and Evolution*.

Manuscript 2: “Poison frogs show similar levels of plasticity during territory defence regardless of their personality”

In this study we investigated how the personality trait “aggressiveness” is linked to plasticity in a territory defence context. We conducted repeated standardized territorial intrusion experiments mimicking territory intruders of different body sizes via playback calls broadcasted at different peak frequencies. In general, we expected individuals to be more cautious towards larger intruders, but if a correlation between aggressiveness and plasticity exists, we expected less aggressive males to be more responsive, and adjust their response to intruders of different sizes, while more aggressive male frogs should be unresponsive and rely on routines. We found repeatability in the behaviours measured in a territorial defence context and showed that both aggressive and less aggressive males decreased their level of aggressiveness towards big intruders. However, we found no support for a correlation between personality and plasticity. We further discuss how the existence of such a link could vary in time or between contexts.
This paper will be summited to *Biology Letters*.

Chapter 2: Links between animal personality and environment

Manuscript 3: “Exploring links between personality traits and their social and non-social environments in wild poison frogs”

In this study we investigated the existence of consistent between-individual variation in “aggressiveness”, “exploration”, and “boldness” in *A. femoralis* male and female, and how these personality traits are linked to the frogs’ natural and social environment. We repeatedly measured several behaviours in a territorial defence context and in a Novel Environment Test. We expected that the three personality traits would encompass the behaviours measured in the tests. Additionally, we measured aspect of the natural and social environment of each individual male (e.g., complexity of the vegetation surrounding the territory, territory size, number of male and female neighbours). We found evidence for the existence of the three personality traits, but we did not find a relationship between individual behaviours and the natural environment. We did find a plastic response of males to changes in female density, probably reflecting how individuals cope with their socio-ecological environment.
This paper is published in *Behavioral Ecology and Sociobiology*.

Manuscript 4: “The link between animal personality and habitat selection in a neotropical poison frog”

In this study, we investigated whether the choice of a territory by males is influenced by their personality types. While the previous chapter did not find any link between males’ personality traits and properties of their natural environment, fine scale vegetation structures within the territories are likely to be important for the ecology of the species. In a laboratory population, we measured the repeatability of behaviours expressed in the home terrarium (i.e., “activity”) and in a Novel Environment Test (i.e., “exploration” and “boldness”). Additionally, we performed a two-choice test between habitats of varying complexity in their fine scale vegetation structures. We expected proactive individuals to occupy areas of low complexity where they are easier to spot for females, while reactive individuals were expected to occupy areas of higher complexity with more places to hide. We did not find an effect of personality types on habitat choice. However, we showed that personality traits measured in a highly standardized artificial setup indeed reflects behaviours expressed in a natural setup, which is particularly relevant as the use of artificial setups to measure personality has been highly criticized.

This paper is in revision in *Behavior*.

Chapter 3: The interplay between animal personality and sexual selection

Manuscript 5: “Mate choice in a promiscuous poison frog”

In this study we investigated the existence of mate choice by female *A. femoralis*. While it was previously thought that females do not make an active choice, the possibility of an active preference for novel mating partners and/or for males with specific call characteristics had not been tested. We performed a two-choice test in a laboratory population and investigated female preference towards a previous partner and a novel mate. We also recorded call characteristics of both males. We expected females to prefer novel mating partners to attenuate negative effects of mating with single low-quality mates, and males with specific call characteristics, as they likely reflect male quality. We found that females preferred previous mating partners and also males with shorter advertisement calls. Females likely recognized their previous mating partners based on individual call characteristics. Overall, our study provides first evidence for female mate choice in a poison frog with sequential polyandry and was a necessary step to better understand how personality traits affect mate choice (manuscript 6).

This paper is published in *Ethology*.

Manuscript 6: “Opposing effects of personality traits on mating and cross-generational reproductive success”

In this study we investigated the influence of the personality traits “boldness”, “aggressiveness”, and “exploration” on mating success, reproductive performance, and reproductive output in a free-ranging population using a combination of behavioural tests and genetic parentage analysis. On the one hand, we expected more aggressive and bolder males to obtain more mating partners and produce more clutches. On the other hand, males showing these characteristics might be more likely to accidentally attack females crossing their territory, resulting in a lower mating and reproductive success. These individuals likely have a greater probability to encounter predators while transporting tadpoles, resulting in a lower reproductive output

compared to less aggressive or shyer males. We further expected exploration to have a different impact on males and females. Males who spend more time exploring, instead of calling for females, should have less mating partners and reproductive success. On the contrary, females who are more explorative should have an advantage when looking for males territories, and thus have more mating partners and a higher reproductive success. We found that personality traits had opposing effects on mating and reproductive output of males and females, which could lead to the evolution of different reproductive strategies.
This paper will be submitted to *Ecology Letters*.

Manuscript 7: "Odour cues rather than personality affect tadpole deposition in a poison frog"

In this study, we investigated how external cues and personality traits influence the ability of individual males to find and use new rearing sites (i.e., paternal care). To do so, we used a combination of behavioural tests and genetic parentage analysis in a free-ranging population. We expected that proactive individuals should find new rearing sites quicker and use a higher number of different sites, or sites located farther away from their territory. We also expected odour cues from tadpoles to influence the likelihood of detection of a new rearing site. We did not find an effect of exploration or boldness on the discovery and use of new rearing sites. However, we found a positive influence of the presence of odour cues on the likelihood of deposition in a rearing site. We show that olfaction, together with a well-developed spatial memory, might play a key role when exploring for new rearing sites, and discuss the potential effect of activity on paternal care.
This paper will be submitted to *Molecular Ecology*.

Appendices

Appendix 1: "A comparative table for staging anuran embryos from terrestrial clutches based on the Brilliant-thighed Poison Frog, Allobates femoralis (Anura: Dendrobatidae)"

With this study we present a detailed description of the developmental stages of embryos and tadpoles of *A. femoralis* from oviposition to hatching, with comparison to their respective Gosner stages.
This paper is in press in *Herpetology notes.*

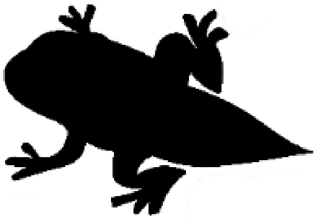
Appendix 2: “Personality is linked to differential testosterone modulation after behavioural challenges in male poison frogs”

In this study we investigated whether individual’s androgen level, and or their modulation, can explain between-individual behavioural differences in aggressiveness-, boldness- and exploration-related behaviours. We took repeated non-invasive water-borne hormonal samples of individual males before and after a series of behavioural tests assessing aggression, boldness, and exploratory tendencies. We found repeatability in testosterone levels and found a positive correlation between testosterone levels measured after behavioural testing and the frogs’ exploratory tendency. We also show that individuals with low baseline testosterone tended to have increased testosterone levels after behavioural testing, while it was the opposite for individuals with high baseline testosterone level. Our results hint towards a possible effect of individual’s androgen level on between-individual behavioural differences.

This paper will be submitted to *Hormones and Behaviour*.

All manuscripts are presented in the style of the journal they are published in/will be submitted to.

Chapter 1



Animal personality and individual plasticity in the Neotropical poison frog *Allobates femoralis*

Manuscript 1: “Repeatable territorial aggression in a Neotropical poison frog”

This manuscript is published in *Frontiers in Ecology and Evolution* (10:881387)
27 April 2022
doi: 10.3389/fevo.2022.881387

Sarah Chaloupka[1], Mélissa Peignier[1,2,3], Susanne Stückler[1], Yimen Araya-Ajoy[4],
Patrick Walsh[5], Max Ringler[1,3,6,7] and Eva Ringler[1,2,3*]

Affiliations :
[1] Department of Behavioral and Cognitive Biology, University of Vienna, Vienna, Austria
[2] Messerli Research Institute, University of Veterinary Medicine Vienna, Vienna, Austria,
[3] Division of Behavioral Ecology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland,
[4] Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway,
[5] School of Biological Sciences, Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom,
[6] Department of Evolutionary Biology, University of Vienna, Vienna, Austria,
[7] Institute of Electronic Music and Acoustics, University of Music and Performing Arts Graz, Graz, Austria

*Correspondence:
Eva Ringler
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
eva.ringler@unibe.ch

Abstract

Intra-specific aggressive interactions play a prominent role in the life of many animals. While studies have found evidence for repeatability in boldness, activity, and exploration in amphibians, we know relatively little about consistent among-individual variation in aggressiveness, despite its importance for male-male competition and territoriality. Amphibians, and Neotropical poison frogs (Dendrobatidae) in particular, are highly suitable for investigating among-individual variation in aggressiveness, as most species exhibit strong territoriality in at least one of the sexes. In the present study, we aimed to fill this gap in knowledge, by investigating within- and between-individual variation in territorial aggression in a semi-natural population of the Neotropical poison frog *Allobates femoralis* (Dendrobatidae) in French Guiana. We conducted repeated, standardized behavioral tests to assess if the level of territorial aggression is consistent within and different between individuals. Further, we tested a possible link between body size and level of territorial aggression. We found moderate repeatability in territorial aggressiveness, but no link to age and/or body size. In conclusion, our study represents the first documentation of repeatable aggressive behavior in a territorial context in amphibians.

Keywords

territoriality, aggression, animal personality, poison frogs, *Allobates femoralis*

Introduction

An increasing number of studies have investigated within-individual consistency and between individual variation of behavior over time and across contexts, termed animal personality (Réale et al., 2007; Dingemanse et al., 2010; Zidar et al., 2017; Goursot et al., 2019). Animal personality is typically characterized along five main axes, including activity, aggressiveness, boldness/shyness, exploration/avoidance, and sociability (Réale et al., 2007). These axes constitute behavioral traits that affect multiple behaviors of an organism in specific contexts. Theoretical models and empirical research have shown that behavioral differences, along any axis, can affect individual fitness through their effects on survival and mating success (Sih et al., 2004a,b; Smith and Blumstein, 2008; Amy et al., 2010; Réale and Dingemanse, 2010; Sih et al., 2012; Wolf and Weissing, 2012).

Several studies recently highlighted that frogs, toads, newts, and salamanders display repeatable behaviors along at least three personality axes: boldness, activity, and exploration (reviewed in Kelleher et al., 2018). Interestingly, no study to date has focused on the aggressiveness axis in amphibians, despite its importance for male-male competition and territoriality. This might be due to the focus so far being on aquatic species and/or species from temperate regions that commonly do not establish territories. However, aggressiveness is common in many amphibians where males defend and fight over resources or territories (Mathis et al., 1995; Wells, 2007; Dyson et al., 2013).This behavior is particularly well-known in Neotropical poison frogs (Dendrobatidae, *sensu* AmphibiaWeb, 2021) who offer ideal prerequisites to study within- and between-individual variation in aggressive behavior (e.g., Duellman, 1966; Summers and Amos, 1997; Summers, 2000; Gardner and Graves, 2005; Poelman and Dicke, 2008; Galeano and Harms, 2016; Gonzáles-Santoroet al., 2021; for a review see Pröhl, 2005).

Here, we use the Brilliant-Thighed Poison Frog *Allobates femoralis* (Aromobatinae) to investigate within- and between-individual variation in male aggressiveness in the context of territorial defense. This species occurs across the Amazon Basin and Guiana Shield in local disjunct populations. Males are highly territorial during the reproductive season and announce their territory to male competitors and female mating partners via a prominent advertisement call broadcasted from elevated structures like fallen branches or logs (Hödl et al., 2004).

Females are not territorial but display site fidelity to perches from where they travel to male territories for courtship and mating (Ringler et al., 2012; Fischer et al., 2020). Territorial males approach and aggressively repel calling conspecifics that intrude into their territory, as territory possession is a prerequisite for male mating success in *A. femoralis* (Narins et al., 2003; Montanarin et al., 2011; Ringler et al., 2011; Ursprung et al., 2011a; Stückler et al., 2019).

Although featuring individually distinct calls (Gasser et al., 2009), territorial *A. femoralis* have been shown to react aggressively to familiar neighbors and strangers in playback experiments, probably because during the reproductive season territory intrusions by either neighbors or strangers are equally threatening to the territory holder (Tumulty et al., 2018). A recent study further found that younger individuals were more likely to attack a non-threatening model (i.e., mimicking a female or a non-calling male) during acoustic playback than older, more experienced frogs, indicating the importance of experience and learning for identifying and distinguishing potential mating partners from rivaling individuals (Sonnleitner et al., 2020). Given that territorial advertisement and defense are costly in terms of energy expenditure and risks of predator exposure (Ryan et al., 1983; Taigen and Wells, 1985; Pough and Taigen, 1990; Wells, 2007; Dyson et al., 2013), we expect that the corresponding costs-benefits trade-off should lead to the emergence of the personality trait “aggressiveness” in males. Males featuring high levels of aggressiveness might be better at defending their territory, but might suffer from increased energy expenditure, risk of injury or predation. Males that show lower levels of aggression might in turn benefit from higher survival chances until the next season. Accordingly, we would expect to observe within-individual consistency and between-individual differences in aggressive responses to acoustic playbacks mimicking a territory intruder.

Methods

Ethical note

This study was approved by the scientific committee of the “Nouragues Ecological Research Station” and the ethics board of the University of Veterinary Medicine Vienna. Behavioral experiments were conducted in strict accordance with current French and EU law, following the Study of Animal Behaviour (ASAB) guidelines ASAB (2020). Permissions for working and sampling were provided by the CNRS Guyane

(“Centre National de la Recherche Scientifique Guyane”), and by the “Ministère de la Transition Écologique et Solidaire” (permit number: TREL2002508S/303).

Study Population and Area

The study was conducted in a free-living population of *A. femoralis*, located on a ~5 ha river island in the vicinity of the field camp “Saut Pararé” of the CNRS Nouragues Ecological Research Station (4°02’ N, 52°41’W; WGS84), within the nature reserve “Les Nouragues” in French Guiana (Bongers et al., 2001; Ringler et al., 2016). The population was established in 2012 by introducing 1,800 tadpoles to the island, where *A. femoralis* had not occurred previously (Ringler et al., 2014). Since then, a stable adult population of about 150 individuals has established, and detailed information about genetic relatedness, body size and age of all individuals is available from a long-term monitoring of the island population.

We conducted daily surveys of individuals in the study population to assess and monitor their distribution and movements. Every frog was photographed on scale paper from its dorsal- and ventral-side for later individual identification and assessment of body size. We recorded the exact location, sex, picture numbers, and current activity of each frog on a high-resolution background map of the island (Ringler et al., 2016), using tablet PCs (WinTab 9, Odys, Willich, Germany) and the mobile GIS software ArcPad 10.2 (ESRI, Redlands, CA, United States). Individuals were sexed based on the presence (males) or absence (females) of vocal sacs. For individual identification, we compared the pictures of the individually unique ventral patterns using the pattern matching software Wild-ID (Bolger et al., 2012). To assess individual body sizes, we measured snout-urostyle length with the aid of a scale paper using the software ImageJ (Rasband, 1997-2021).

Quantification of Aggressive Behavior

For the present study, we measured the level of aggressiveness as the agonistic response of an individual toward a simulated conspecific male entering its territory (Réale et al., 2007). During agonistic encounters, male *A. femoralis* typically orientate their head/body, jump toward the intruder and wrestle (Hödl, 1987; Narins et al., 2003). We simulated a territory intruder by broadcasting a standardized synthetic advertisement call from a loudspeaker with an integrated music player (MUVO 2c, Creative, Singapore) positioned 2 m from and facing the focal male (Figure 1). A twig from the forest floor was positioned 20 cm in front of the speaker as a perimeter marker for a successful approach to the speaker. After placing the speaker, we waited 2 min before starting the playback to take a suitable position to conduct the trial. The synthetic call featured the average spectral and temporal parameters of another free-ranging population of *A. femoralis* in French Guiana based on recordings by Gasser et al. (2009); for a detailed description see Ursprung et al. (2009) and Ringler et al. (2017). The playback contained 25 bouts of 10 calls each, with equally long interbout-intervals, totaling a duration of 6 min 42 s, and was presented from WAV-files (16-bit, 44.1 kHz). We calibrated the speaker once per week with a sound pressure meter (SL-100, Voltcraft, Hirschau, Germany) to produce the playback signal at a sound-pressure level (SPL) of at least 69 dB (re 20 mPa; A, fast) at a 2 m distance, which lies within the range of natural variation in this species and considered to be the minimum threshold for eliciting a positive phonotactic response in *A. femoralis* (Hödl, 1987).

During the playback, the experimenter stayed approximately 2 m behind the speaker and documented the movements of the focal male using a voice recorder (ICD-PX240, Sony, Tokyo). We recorded the following behaviors during the trial: first head-body orientation (“head”), first jump toward the speaker (“jump”), and when the frog crossed within 20 cm of the speaker (“finish”). Trials were scored as successful when either the frog came within 20 cm of the speaker or when the playback signal ended. We scored trials as unsuccessful and excluded from the analyses when the focal male began calling during the playback, as this can be interpreted as the intruder/speaker being outside of the defended area of the male’s territory (Ringler et al., 2011). Both behaviors, antiphonal calling as well as phonotactic approach, can be interpreted as “aggressive” territorial behaviors, but in our experiment we only focused on the phonotactic response as a measure of territorial aggression.

After the trials we captured the focal frog and took ventral and dorsal images for identification. To account for local variation in sound transmission, we then measured the SPL of the playback stimulus at the initial location of the focal frog, with the speaker in the same location as for the trial. We successfully tested 32 individual males, and replicated tests four times, with a minimum of 7 days between two consecutive trials to minimize habituation effects to the experimental setup.

From the audio recordings we then extracted the latencies from the start of the playback until the first head-body orientation, until the first jump, and until the arrival within 20 cm of the speaker using the software Audacity 2.2.1 (AudacityTeam, 1999-2017). We further determined the approach speed over 1.8 m from the time between the first jump and the arrival within 20 cm of the speaker (cf. Figure 1). Individuals who did not react to the speaker were not given a threshold value to avoid right or left censoring.

Statistical Analysis

All statistical analyses were conducted in R v3.6.0 (R CoreTeam, 2020) using the integrated development environment RStudio v1.3.1093 (R Studio Team, 2019). To investigate the prevalence of an aggressiveness personality trait in *A. femoralis*, we calculated the adjusted repeatability of the measured behaviors, as the proportion of phenotypic variation that can be attributed to between-subject variation (Nakagawa and Schielzeth, 2010).

First, we transformed the data that deviated from normality. We used the function “transformTukey” to apply a constant transformation on the latency until the first head-body orientation, until the first jump, until arrival to the speaker and on the speed to reach the speaker. We calculated repeatability from four linear mixed effect models with the function *lmer* in the *lme4* package (Bates et al., 2016), each with one of the transformed behaviors as the response variable. A behavior was considered as repeatable when the 95% confidence intervals did not overlap zero. For each model, we included the SPL, but also the trial number, and the individual size as fixed effects, to account for habituation and effect of body size on aggressive responses. Previous studies have shown that age influences the accuracy of aggressive responses in *A. femoralis*, and that body size is positively correlated to age (Ursprung et al., 2011b; Sonnleitner et al., 2020). In all models ID was included as random effect to account for repeated measurements. We inspected model residuals for normal distribution using diagnostic qqplots. Finally, we calculated the confidence interval using the function “confint.” The model featuring the latency until the first jump failed to converge with all possible optimizers, and therefore is excluded from our analysis.

Results

Males took on average 27.2 s (± 70.6 SD) to orientate their body toward the speaker and 43.8 s (± 85.8 SD) to first jump toward the speaker. They took 85.5 s (± 47.3 SD)

Table 1. Mixed effect model results. The estimates, standard-error and p-values are presented. Significant results (p-value < 0.05) are written in bold.

	Latency until head-body orientation		Latency to reach the speaker		Speed to reach the speaker	
Fixed effects (Estimate±SE | p-value)						
(Intercept)	-0.39±0.45	0.383	1.46±0.23	**<0.01**	-1.02±0.42	**0.015**
trial	0.01±0.01	0.403	-0.01±0.01	**0.028**	0.02±0.01	**0.016**
size	0.13±0.13	0.313	0.03±0.07	0.667	-0.03±0.13	0.809
dB	-0.00±0.00	0.459	-0.00±0.00	0.304	0.00±0.00	0.186
Random effects (Estimate±SD)						
ID	0.00±0.03		0.00±0.03		0.00±0.06	
residual	0.02±0.13		0.00±0.05		0.01±0.08	

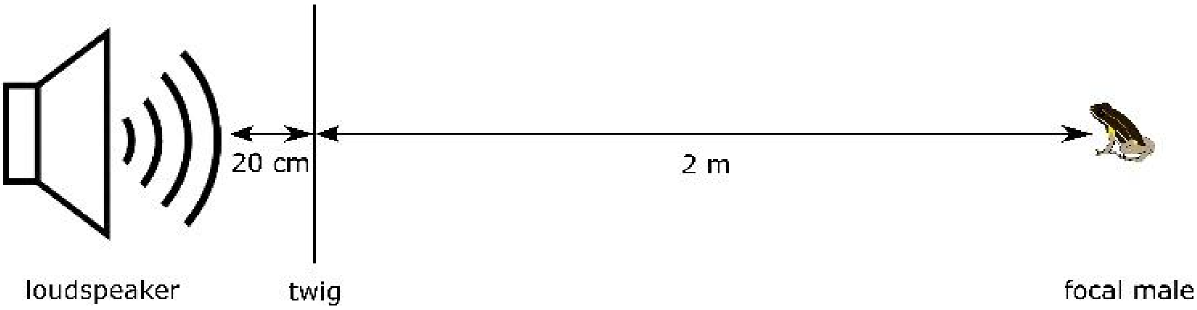


Figure 1. Experimental setup to assess individual aggressiveness of territorial *A. femoralis* males.

on average to reach the speaker, with a mean speed of 3.8 cm s^{-1} (± 2.4 SD).

We found that phonotactic approach speed of *A. femoralis* males was repeatable (R = 0.37, 95% CI = 0.22–0.45). When looking at the measurements, a highly repeatable behavior would show low within-individual variability but high between individual differences. In our case, individuals were indeed rather consistent in their speed, while between-individual variation was large (Figure 2). However, we did not find evidence for repeatability in the latency to perform a head-body orientation or the latency to arrive at the speaker (respectively, R = 0.07, 95%CI = 0–0.18 and R = 0.23, 95% CI = 0–0.33). Individuals were highly variable in the time they took to perform a head-body orientation, resulting in low among-individual variation and low within-individual consistency and therefore low repeatability (Figure 2). Conversely, individuals were more consistent in the time they took to reach the speaker, but between-individual variation was still low, resulting in a low repeatability (Figure 2). Finally, we observed that individuals reached the speaker quicker in the last compared to the first trial (Table 1, p = 0.028 and p = 0.016). Body size and SPL were not related to any behavioral measurement (Table 1).

Discussion

We designed a standardized in situ experiment to collect data on the agonistic response of an individual toward a simulated conspecific intruding its territory, to evaluate its applicability for population-wide studies on animal personality in terrestrial anurans. Despite the small sample size in this study, we found repeatable differences among individuals in the speed to reach the speaker, suggesting the existence of personality along the aggressiveness axis in *A. femoralis*. These values lie in the range of average repeatability scores previously reported in behavioral studies in amphibians (R = 0.24–0.39; Brodin et al., 2013; Carlson and Langkilde, 2013; Urszán et al., 2015; Kelleher et al., 2017), and our choice of not censoring the data might have resulted in rather conservative estimates of repeatability.

In *A. femoralis*, high levels of aggression could entice an individual to react fiercer toward a conspecific intruding its territory or help a male to take over another territory. This is particularly relevant because the possession of a territory by a male is a prerequisite for reproductive success (Ursprung et al., 2011a). Aggressive individuals will, however, probably be more likely to engage in energetically costly and potentially

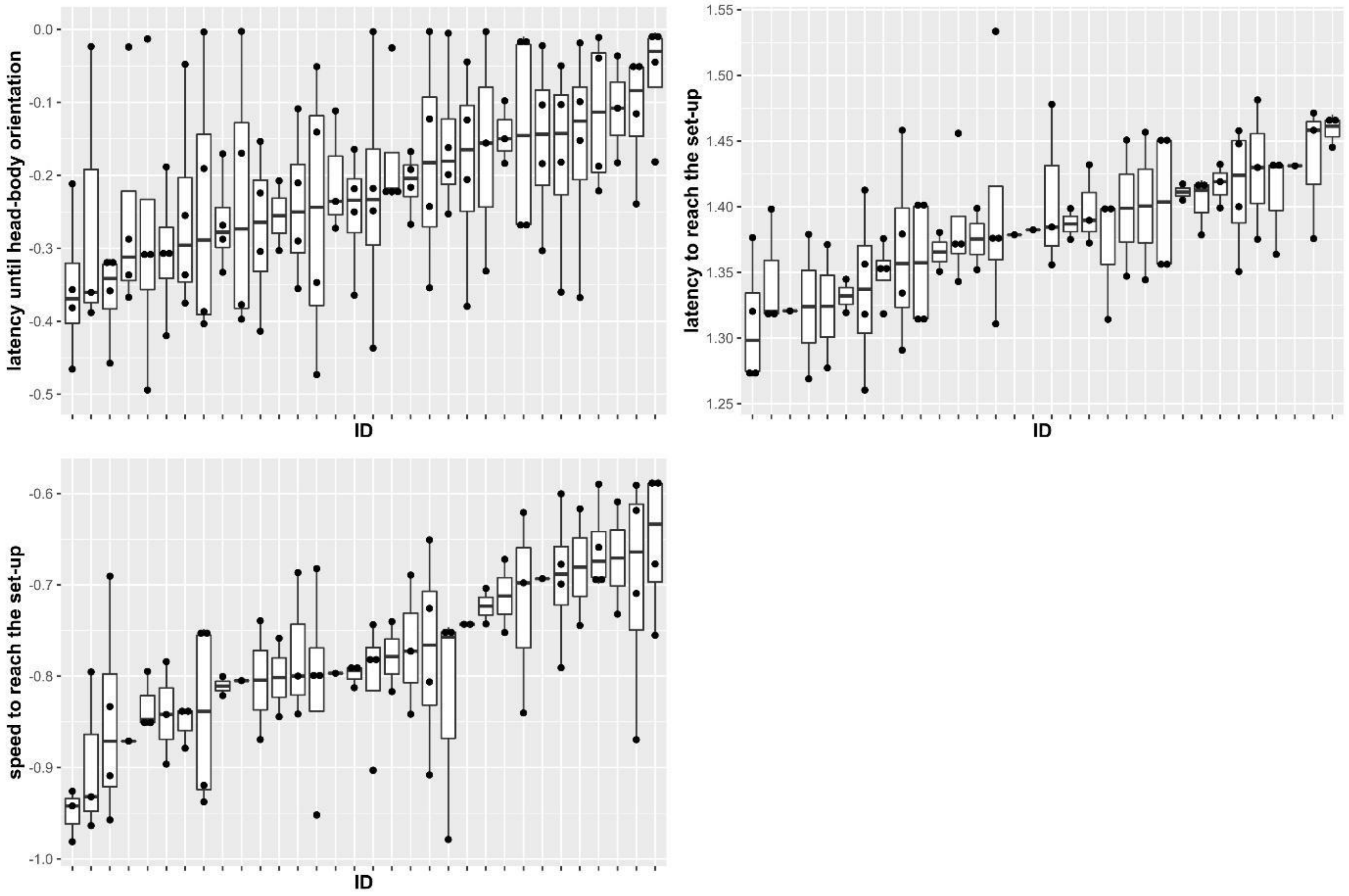


Figure 2. Latency to first head-body orientation, latency to arrive at the speaker and speed to arrive at the speaker for each of the 32 individual males. All variables have been transformed using a constant transformation. Males are ordered by median. For each individual, the four dots represent the results of each of the four trials in which the individual reacted to the speaker, while the horizontal bold line represents the median across these four trials. The upper and lower horizontal lines delimiting the boxes represent the first and third quartiles.

physically harmful fights. Future studies should investigate if levels of aggressiveness are ultimately linked to the chance of winning or losing a territorial conflict, and also how this is related to individual fitness.

We did not find that the initial latency to respond to a conspecific intruder was repeatable. We see two possible explanations for this result: First, the lack of repeatability could be due to local variation in habitat complexity (i.e., vegetation density, leaf litter, and perch height) at the specific location where each respective trial was performed. Indeed, spectral degradation and reverberation have been found as important cues for acoustic distance assessment in *A. femoralis* (Ringler et al., 2017). Secondly, the absence of repeatability for the latency to respond to a conspecific intruder could be the result of a cost-benefit trade-off of aggressive responses to intruders. The latency to respond to an intruder might contain two discrete behaviors: an evaluation of the circumstances (i.e., own breeding status, known neighbors, etc.) and the decision, based on evaluated risks and benefits, of whether to make an aggressive approach. Once the decision to approach is taken, the individual level of aggressiveness takes over and drives the speed to approach the conspecific intruder, therefore leading to repeatable results throughout trials. Future studies should investigate how the different behaviors emitted in a given context are structured into one or several functional units (i.e., personality traits) using structural equation modeling (Araya-Ajoy and Dingemanse, 2014).

Simulating a territory intruder by broadcasting a standardized call proved to be a powerful tool to repeatedly measure aggressive response of male *A. femoralis*. Phonotactic experiments with simulated advertisement calls of conspecifics are a very common method to study anuran behavior, for example to measure territory borders of territorial amphibians (Narins et al., 2003; Rojas et al., 2006; Ringler et al., 2011), female preferences for male call traits (Tárano and Herrera, 2003; Akre and Ryan, 2010), or acoustic properties for species discrimination (Schwartz, 1987; Bee et al., 2001). However, such playback experiments had never been applied to assess repeatability of aggressive responses in individual territory holders. In the present study we used the same playback for all trials to assess individual consistency of territorial aggression in the same context. Future studies should use a random set of advertisement calls featuring a range of different spectral and temporal acoustic parameters to better assess aggressive responses across contexts.

While animal personality has been broadly documented in mammals, birds, fish and even invertebrates (e.g., Bell, 2005; Dochtermann and Jenkins, 2007; Tremmel and Müller, 2013; Zidar et al., 2017; Goursot et al., 2019), unfortunately we only have limited knowledge about within and between-individual behavioral variation in amphibians (but see Kelleher et al., 2018).This is surprising, as amphibians might provide key insights on the evolution of animal personality and its link to physiology, morphology and ecology, as they face extreme shifts in their ecological niche when they undergo metamorphosis (Wilson and Krause, 2012). Also many amphibians are territorial and vigorously defend their territories against conspecific intruders (Wells, 2007), offering ample opportunities to investigate the link between male-male competition and individual fitness. The present study is the first to investigate the prevalence of repeatable among-individual differences in territorial aggressiveness in free ranging terrestrial neotropical anurans.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://osf.io/fdxqm/?view_only=9427c034f20f466f99d30fdd1397752e.

Authors contributions

SC, MR, and ER contributed to conception and design of the study. SC and SS collected the data. SC and MP organized the database. SC, YA-A, and MP performed the statistical analysis. MP did the data curation. SC wrote the first draft of the manuscript. MP, PW, MR, and ER reviewed, edited, and wrote sections of the manuscript. MR and ER supervised the study. All authors contributed to manuscript revision, and have read, and approved the submitted version.

Acknowledgments

We thank the Nouragues research field station (managed by CNRS), which benefits from “Investissement d’Avenir” grants managed by the Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25- 01). We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. This study was funded by the Austrian Science Fund (FWF) via the project P 31518 (PI: ER). MR was supported by an Erwin Schrödinger Grant (FWF J 3868) and a Standalone Grant (FWF P 33728) from the Austrian Science Fund (FWF).

References

Akre, K. L., and Ryan, M. J. (2010). Complexity Increases Working Memory for Mating Signals. Curr. Biol. 20, 502–505. doi: 10.1016/j.cub.2010.01.021

AmphibiaWeb. (2021). AmphibiaWeb: Information on Amphibian Biology and Conservation. Available online at: www.amphibiaweb.org (Accessed on Mar 032021)

Amy, M., Sprau, P., de Goede, P., and Naguib, M. (2010). Effects of Personality on Territory Defence in Communication Networks: A Playback Experiment with Radio-Tagged Great Tits. Proc. R. Soc. Lond. B. 277, 3685–3692. doi:10.1098/rspb.2010.0598

Araya-Ajoy, Y. G., and Dingemanse, N. J. (2014). Characterizing behavioural 'characters': an evolutionary framework. Proc. R. Soc. Lond. B. 281:20132645. doi: 10.1098/rspb.2013.2645

ASAB (2020). Guidelines for the Treatment of Animals in Behavioural Research and Teaching. Anim. Behav. 159, 1–11. doi : 10.1016/j.anbehav.2019.11.002

Audacity Team (1999-2017). Audacity. Available online at: http://audacityteam.org/ (Accessed on Apr 28 2017)

Bates, D., Mächler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2016). lme4: linear Mixed-Effects Models Using 'Eigen' and S4.Available online at: http://CRAN.R-project.org/package=lme4. (Accessed on Apr 14 2021)

Bee, M. A., Kozich, C. E., Blackwell, K. J., and Gerhardt, H. C. (2001). Individual Variation in Advertisement Calls of Territorial Male Green Frogs, Rana Clamitans: Implications for Individual Discrimination. Ethology 107, 65–84.doi: 10.1046/j.1439-0310.2001.00640.x

Bell, A. M. (2005). Behavioural Differences Between Individuals and Two Populations of Stickleback (Gasterosteus Aculeatus). J. Evol. Biol. 18, 464–473.doi: 10.1111/j.1420-9101.2004.00817.x

Bolger, D. T., Morrison, T. A., Vance, B., Lee, D., and Farid, H. (2012). A Computer-Assisted System for Photographic Mark–recapture Analysis. Methods Ecol. Evol.3, 813–822. doi: 10.1111/j.2041-210X.2012.00212.x

Bongers, F., Charles-Dominique, P., Forget, P., and Théry, M. (2001). Nouragues: Dynamics and Plant-Animal Interactions in a Neotropical Rainforest. Berlin: Springer Science.

Brodin, T., Lind, M. I., Wiberg, M. K., and Johansson, F. (2013). Personality Trait Differences Between Mainland and Island Populations in the Common Frog (Rana Temporaria). Behav. Ecol. Sociobiol. 67, 135–143. doi: 10.1007/s00265-012-1433-1

Carlson, B. E., and Langkilde, T. (2013). Personality Traits Are Expressed in Bullfrog Tadpoles During Open-Field Trials. J. Herpetol. 47, 378–383. doi: 10.1670/12-061

Dingemanse, N. J., Kazem, A. J., Réale, D., and Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol.Evol. 25, 81–89. doi: 10.1016/j.tree.2009.07.013

Dochtermann, N. A., and Jenkins, S. H. (2007). Behavioural Syndromes in Merriam's Kangaroo Rats (Dipodomys Merriami): A Test of Competing Hypotheses. Proc. R. Soc. Lond. B. 274, 2343–2349. doi: 10.1098/rspb.2007.0622

Duellman, W. E. (1966). Aggressive Behavior in Dendrobatid Frogs. Herpetologica. 22, 217–221.

Dyson, M. L., Reichert, M. S., and Halliday, T. M. (2013). "Contests in Amphibians," in Animal Contests, eds I. C. W. Hardy and M. Briffa (Cambridge,UK: Cambridge University Press), 228–257. doi: 10.1017/cbo9781139051248.013

Fischer, M., Ringler, M., Ringler, E., and Pašukonis, A. (2020). Reproductive Behavior Drives Female Space Use in a Sedentary Neotropical Frog. PeerJ.8:e8920. doi: 10.7717/peerj.8920

Galeano, S. P., and Harms, K. E. (2016). Coloration in the Polymorphic Frog Oophaga Pumilio Associates with Level of Aggressiveness in Intraspecific and Interspecific Behavioral Interactions. Behav. Ecol. Sociobiol. 70, 83–97. doi: 10.1007/s00265-015-2027-5

Gardner, E. A., and Graves, B. M. (2005). Responses of Resident Male Dendrobates Pumilio to Territory Intruders. J. Herpetol. 39, 248–253. doi: 10.1670/95-04A

Gasser, H., Amézquita, A. T., and Hödl, W. (2009). Who Is Calling? Intraspecific Call Variation in the Aromobatid Frog Allobates Femoralis. Ethology 115,596–607. doi: 10.1111/j.1439-0310.2009.01639.x

Gonzáles-Santoro, M. D., Hernández-Restrepo, J. P., and Palacios-Rodríguez,P. (2021). Aggressive Behaviour, Courtship and Mating Call Description of the Neotropical Poison Frog Phyllobates Aurotaenia (Anura: Dendrobatidae). Herpetol. Notes 14, 1145–1149.

Goursot, C., Düpjan, S., Kanitz, E., Tuchscherer, A., Puppe, B., and Leliveld,L. M. C. (2019). Assessing Animal Individuality: Links Between Personality and Laterality in Pigs. Curr Zool. 65, 541–551. doi: 10.1093/cz/zoy071

Hödl, W. (1987). "Dendrobates femoralis (Dendrobatidae): a handy fellow for frog bioacoustics," in Proceedings of the 4th Ordinary General meeting of the Societas Europaea Herpetologica, eds J. J. van Gelder, H. Strijbosch, and P. Bergers (Netherlands: Faculty of Sciences Nijmegen).

Hödl, W., Amézquita, A. T., and Narins, P. M. (2004). The Role of Call Frequency and the Auditory Papillae in Phonotactic Behavior in Male Dart-Poison Frogs Epipedobates Femoralis (Dendrobatidae). J. Comp. Physiol. A. 190, 823–829.doi: 10.1007/s00359-004-0536-1

Kelleher, S. R., Silla, A. J., and Byrne, P. G. (2018). Animal Personality and Behavioral Syndromes in Amphibians: A Review of the Evidence, Experimental Approaches, and Implications for Conservation. Behav. Ecol. Sociobiol.72:10539. doi: 10.1007/s00265-018-2493-7

Kelleher, S. R., Silla, A. J., Dingemanse, N. J., and Byrne, P. G. (2017). Body Size Predicts Between-Individual Differences in Exploration Behaviour in the Southern Corroboree Frog. Anim. Behav. 129, 161–170. doi: 10.1016/j.anbehav.2017.05.013

Mathis, A., Jaeger, R. G., Keen, W. H., Ducey, P. K., Walls, S. C., and Buchanan, B. W. (1995). "Aggression and Territoriality by Salamanders and a Comparison with the Territorial Behaviour of Frogs," in Amphibian Biology. Volume 2: Social Behaviour, eds B. K. Sullivan and H. Heatwole (Chipping Norton, UK: Surrey Beatty), 633–676. doi: 10.1016/0003-3472(95)90038-1

Montanarin, A., Kaefer, I. L., and Lima, A. P. (2011). Courtship and Mating Behaviour of the Brilliant-Thighed Frog Allobates Femoralis from Central Amazonia: Implications for the Study of a Species Complex. Ethol. Ecol. Evol.23, 141–150. doi: 10.1080/03949370.2011.554884

Nakagawa, S., and Schielzeth, H. (2010). Repeatability for Gaussian and Non-Gaussian Data: A Practical Guide for Biologists. Biol. Rev. Camb. Philos. Soc.85, 935–956. doi: 10.1111/j.1469-185X.2010.00141.x

Narins, P. M., Hödl, W., and Grabul, D. S. (2003). Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. Proc. Natl. Acad. Sci. U.S.A. 100, 577–580. doi: 10.1073/pnas.0237165100

Poelman, E. H., and Dicke, M. (2008). Space Use of Amazonian Poison Frogs: Testing the Reproductive Resource Defense Hypothesis. J. Herpetol. 42, 270–278. doi: 10.1670/07-1031.1

Pough, F. H., and Taigen, T. L. (1990). Metabolic Correlates of the Foraging and Social Behaviour of Dart-Poison Frogs. Anim. Behav. 39, 145–155. doi: 10.1016/S0003-3472(05)80734-1

Pröhl, H. (2005). Territorial Behavior in Dendrobatid Frogs. J. Herpetol. 39,354–365. doi: 10.1670/162-04A.1

R Core Team. (2020). R: a Language and Environment for Statistical Computing. Available online at :http://www.r-project.org (Accessed on Apr 14 2021)

R Studio Team. (2019). RStudio: Integrated Development for R. Boston, MA: RStudio, Inc.

Rasband, W. S. (1997-2021). ImageJ. Bethesda, MD: U. S. National Institutes of Health.

Réale, D., and Dingemanse, N. J. (2010). "Personality and Individual Social Specialisation," in Social Behaviour: Genes, Ecology and Evolution, eds T. Székely, A. J. Moore, and J. Komdeur (Cambridge, UK: Cambridge University Press), 417–441. doi: 10.1017/cbo9780511781360.033

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., and Dingemanse, N. J. (2007). Integrating

Animal Temperament Within Ecology and Evolution. Biol. Rev. 82,291–318. doi: 10.1111/j.1469-185X.2007.00010.x

Ringler, E., Mangione, R., and Ringler, M. (2014). Where Have All the Tadpoles Gone? Individual Genetic Tracking of Amphibian Larvae Until Adulthood. Mol. Ecol. Resour. 15, 737–746. doi: 10.1111/1755-0998.12345

Ringler, E., Ringler, M., Jehle, R., and Hödl, W. (2012). The Female Perspective of Mating in A. Femoralis, a Territorial Frog with Paternal Care – a Spatial and Genetic Analysis. PLoS One. 7:e40237. doi: 10.1371/journal.pone.0040237

Ringler, M., Mangione, R., Pašukonis, A., Rainer, G., Gyimesi, K., Felling-Wagner,J., et al. (2016). High-Resolution Forest Mapping for Behavioural Studies in the Nature Reserve 'Les Nouragues', French Guiana. J. Maps. 12, 26–32. doi:10.1080/17445647.2014.972995

Ringler, M., Ringler, E., Mendoza, D. M., and Hödl, W. (2011). Intrusion Experiments to Measure Territory Size: Development of the Method, Tests Through Simulations, and Application in the Frog Allobates Femoralis. PLoSOne. 6:e25844. doi: 10.1371/journal.pone.0025844

Ringler, M., Szipl, G., Hödl, W., Khil, L., Kofler, B., Lonauer, M., et al. (2017). Acoustic Ranging in Poison Frogs—It Is Not About Signal Amplitude Alone. Behav. Ecol. Sociobiol. 71:114. doi: 10.1007/s00265-017-2340-2

Rojas, B., Amézquita, A. T., and Delgadillo, A. (2006). Matching and Symmetry in the Frequency Recognition Curve of the Poison Frog Epipedobates Trivittatus. Ethology 112, 564–571. doi: 10.1111/j.1439-0310.2005.01190.x

Ryan, M. J., Bartholomew, G. A., and Rand, A. S. (1983). Energetics of Reproduction in a Neotropical Frog. Physalaemus Pustulosus. Ecology 64, 1456–1462. doi: 10.2307/1937500

Schwartz, J. (1987). The Function of Call Alternation in Anuran Amphibians: A Test of Three Hypotheses. Evolution 41, 461–471. doi: 10.1111/j.1558-5646.1987.tb05818.x

Sih, A., Bell, A., and Johnson, J. C. (2004a). Behavioral Syndromes: An Ecological and Evolutionary Overview. Trends Ecol. Evol. 19, 372–378. doi: 10.1016/j.tree.2004.04.009

Sih, A., Bell, A., Johnson, J. C., and Ziemba, R. E. (2004b). Behavioral Syndromes: An Integrative Overview. Q. Rev. Biol. 79, 241–277. doi: 10.1086/422893

Sih, A., Cote, J., Evans, M., Fogarty, S., and Pruitt, J. (2012). Ecological Implications of Behavioural Syndromes. Ecol. Lett. 15, 278–289. doi: 10.1111/j.1461-0248.2011.01731.x

Smith, B. R., and Blumstein, D. T. (2008). Fitness Consequences of Personality: A Meta-Analysis. Behav. Ecol. 19, 448–455. doi: 10.1093/beheco/arm144

Sonnleitner, R., Ringler, M., Loretto, M., and Ringler, E. (2020). Experience Shapes Accuracy in Territorial Decision-Making in a Poison Frog. Biol. Lett.16:20200094. doi: 10.1098/rsbl.2020.0094

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W., and Ringler,E. (2019). Spatio-Temporal Characteristics of the Prolonged Courtship in Brilliant-Thighed Poison Frogs. Allobates Femoralis. Herpetologica. 75, 268–279. doi: 10.1655/Herpetologica-D-19-00010.1

Summers, K. (2000). Mating and Aggressive Behaviour in Dendrobatid Frogs from Corcovado National Park, Costa Rica: A Comparative Study. Behaviour 137,7–24. doi: 10.1163/156853900501845

Summers, K., and Amos, W. (1997). Behavioral, Ecological, and Molecular Genetic Analyses of Reproductive Strategies in the Amazonian Dart-Poison Frog, Dendrobates Ventrimaculatus. Behav. Ecol. 8, 260–267. doi: 10.1093/beheco/8.3.260

Taigen, T. L., and Wells, K. D. (1985). Energetics of Vocalization by an Anuran Amphibian (Hyla Versicolor). J. Comp. Physiol. B. 155, 163–170. doi: 10.1007/BF00685209

Tárano, Z., and Herrera, E. A. (2003). Female Preferences for Call Traits and Male Mating Success in the Neotropical Frog Physalaemus Enesefae. Ethology 109,121–134. doi: 10.1046/j.1439-0310.2003.00848.x

Tremmel, M., and Müller, C. (2013). Insect Personality Depends on Environmental Conditions. Behav. Ecol. 24, 386–392. doi: 10.1093/beheco/ars175

Tumulty, J. P., Pašukonis, A., Ringler, M., Forester, J. D., Hödl, W., and Bee, M. A. (2018). Brilliant-Thighed Poison Frogs Do Not Use Acoustic Identity Information to Treat Territorial Neighbours as Dear Enemies. Anim. Behav.141, 203–220. doi: 10.1016/j.anbehav.2018.05.008

Ursprung, E., Ringler, M., and Hödl, W. (2009). Phonotactic Approach Pattern in the Neotropical Frog Allobates Femoralis: A Spatial and Temporal Analysis. Behaviour 146, 153–170. doi: 10.1163/156853909X410711

Ursprung, E., Ringler, M., Jehle, R., and Hödl, W. (2011a). Strong Male/male Competition Allows for Non choosy Females: High Levels of Polygynandry in a Territorial Frog with Paternal Care. Mol. Ecol. 20, 1759–1771. doi: 10.1111/j.1365-294X.2011.05056.x

Ursprung, E., Ringler, M., Jehle, R., and Hödl, W. (2011b). Toe regeneration in the neotropical frog Allobates femoralis. Herpetol. J. 21, 83–86.

Urszán, T. J., Török, J., Hettyey, A., Garamszegi, L. Z., and Herczeg, G. (2015). Behavioural Consistency and Life History of Rana Dalmatina Tadpoles. Oecologia 178, 129–140. doi: 10.1007/s00442-014-3207-0

Wells, K. D. (2007). The Ecology and Behavior of Amphibians. Chicago, IL: University of Chicago Press.

Wilson, A. D. M., and Krause, J. (2012). Personality and Metamorphosis: Is Behavioral Variation Consistent Across Ontogenetic Niche Shifts? Behav. Ecol.23, 1316–1323. doi: 10.1093/beheco/ars123

Wolf, M., and Weissing, F. J. (2012). Animal Personalities: Consequences for Ecology and Evolution. Trends Ecol. Evol. 27, 452–461. doi: 10.1016/j.tree.2012.05.001

Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O., and Løvlie, H. (2017). A Comparison of Animal Personality and Coping Styles in the Red Junglefowl. Anim. Behav. 130, 209–220. doi: 10.1016/j.anbehav.2017.06.024

Manuscript 2: “Poison frogs show similar levels of plasticity during territory defence regardless of their personality”

This manuscript will be submitted to *Biology Letters*

Mélissa Peignier[a*], Lauriane Bégué[a], Max Ringler[a,b], Birgit Szabo[a], & Eva Ringler[a]

Affiliations:
[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[b] Institute of Electronic Music and Acoustics, University of Music and Performing Arts Graz, Austria

*Correspondence:
Mélissa Peignier
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
melissa.peignier@unibe.ch

Abstract

Personality traits, or consistent between-individual differences in behaviour, can be related to an individual's degree of plasticity. In such cases, individuals displaying certain personality type will rely on routines (i.e., 'unresponsive' individuals), while others will be more responsive to environmental cues. Since personality traits and plasticity can affect male-male competition, integrating both can help better understand the complexity of aggressive contexts. Here, we used a free-ranging population of the Neotropical poison frog *Allobates femoralis* to investigate how territorial aggression is linked to plasticity. Males are highly territorial and display aggressive behaviours towards conspecifics intruding their territory. We conducted repeated standardized territorial intrusion experiments mimicking territory intruders of different body sizes via playback calls broadcasted at different peak frequencies. We found repeatability for the latency to reach and approach an intruder (the loudspeaker), and observed that both aggressive and less aggressive males decreased their level of aggression towards big intruders. However, more aggressive individuals were restricted in how much they could lower their level of aggression. While such a restriction might be associated with a link between personality and plasticity, we found no support for a correlation in the context of male territory defence during the breeding season.

Keywords

animal personality, anuran, male-male competition, playback, bioacoustics

Introduction

Between-individual differences in behaviour that are consistent across time and/or context -also termed ‘animal personality’- have been documented in a wide variety of animal taxa [1–5]. While animal personality typically implies limited plasticity, it does not entirely preclude the existence of plasticity [6,7]. Indeed, an individual’s average level of a behaviour can be correlated to its degree of plasticity in that behaviour [8–11]. In such cases, some individuals will function better under stable conditions and rely on routines (i.e., ‘unresponsive’ individuals), and others will be more responsive to environmental cues [10,11]. For instance, in mice (*Mus musculus domesticus*), less aggressive individuals adjust their level of aggression according to social context while highly aggressive individuals are more rigid [12].

Animal personality matters in terms of reproduction and survival by, for example, affecting male-male competition, dispersal, or foraging efficiency [13–15]. In particular, aggressiveness plays an important role in territorial species in which more aggressive individuals are often more successful in acquiring and/or defending a territory, but more at risk of engaging in potentially harmful fights [16]. Integrating both animal personality and plasticity could help understand more comprehensively the complexity of aggressive decisions during male-male competition.

Neotropical poison frogs (Dendrobatidae, sensu [17]) show particularly prominent territory defence behaviour which can be easily provoked in the wild. *Allobates femoralis* is a poison frog which acoustically establishes multi-purpose territories during the prolonged breeding season [18–21]. Individual males’ reproductive success is directly linked to their ability to hold a territory [21], and importantly, individuals show consistent differences in their level of territorial aggression (referring to the personality trait ‘aggressiveness’ [22]). Furthermore, call peak frequency is negatively correlated with body size in *A. femoralis*, so that bigger individuals produce calls with lower frequencies [23].

In this study, we conducted repeated standardized territorial intrusion experiments to investigate how territorial aggression is linked to plasticity in *A. femoralis* by mimicking territory intruders of different body sizes via playback calls broadcasted at different peak frequencies. In general, we expected individuals to be more cautious towards larger intruders. However, if personality is correlated to plasticity in *A. femoralis*, we expected less aggressive males to be more responsive, and adjust their response to intruders of different sizes, while more aggressive male frogs should be unresponsive and rely on routines, by showing similar levels of aggression regardless of intruder size.

Methods

Ethical note

This study was approved by the scientific committee of the "Nouragues Ecological Research Station". Sampling for this study was conducted in strict accordance with current French and EU law and followed the (ASAB 2020) guidelines on the treatment of study animals. Working and tissue sampling permissions were provided by the CNRS Guyane ("Centre National de la Recherche Scientifique Guyane"), by the "Ministere de la transition ecologique et solidaire" (permit number: TREL2002508S/303), and the Secretariat of the Convention on Biological Diversity (APA declaration: TREL1734890A/34).

Study site

We used an experimental population of *A. femoralis* on a small (~ 5 ha) river island (4°02′ N 52°41′ W; [24]) located within a lowland tropical rainforest in the Nouragues Nature Reserve, French Guiana. We carried out the study between April–May 2022 corresponding with the reproductive period of the species and performed all tests during the main peak calling activity, between 1400–1900 h.

Territorial defence test

In order to assess individual aggressiveness and plasticity, we used acoustic playbacks (5 min in length) of advertisement calls simulating an intruder to induce territorial defence behaviour in the territory holder (i.e. the focal male) [19,25]. Advertisement calls consist of four notes each sweeping up in frequency and range between 2.5–4.1 kHz (centre frequency 3.4 kHz) [26,27]. Territory holders typically respond to calling intruders by orientating their head/body towards the intruder, jumping towards the intruder, and sometimes wrestling with the intruder [19,25,26,28,29].

We used a music player with integrated loudspeaker (MUVO 2c, Creative, Singapore) for playbacks. We centred the loudspeaker on top of a black PVC disc (radius = 15 cm) that was positioned at a distance of 2 m (measured with a laser rangefinder, DLE 50, Bosch, Stuttgart, Germany) from a focal male facing it. The experimenter stood an additional meter behind the setup and recorded the behaviours performed by the focal male (head-body orientation, jump, touching the disc with at least one part of the body) using a digital voice recorder (ICD-PX333, Sony, Tokyo, Japan). Each focal male was given 30s acclimation time to resume normal behaviour after installation of the setup. We ended the trial when the focal male touched the disc or when the playback ended. If the focal male called during the experiment, we excluded the trial from our analysis because focal males only call in response to playbacks if the speaker is outside the defended area (cf. [19]).

To assess base levels of aggressiveness, we first repeatedly presented one of seven synthetic calls (chosen randomly) mimicking an ‘average size intruder’. The synthetic calls featured the spectral and temporal parameters of a nearby natural population (peak frequency 3.4 kHz ± 378 Hz SD), and varied in their inter-note and inter-call intervals [26,27]). We then assessed individual responses to a synthetic low and a high frequency call mimicking a ‘big’ and a ‘small’ intruder, respectively. These two calls had similar features as those used in the base aggressiveness test, but we changed the peak frequency by ±3 standard deviations (SD) (representing a change of 10.97 %). All playbacks were presented from the original WAV-files (16-bit, 44.1 kHz) with the same volume setting. Because sound can be reflected and/or distorted by the dense vegetation within the forest, we ensured that the received sound pressure level (‘SPL’) was above 56 dB in all trials by measuring SPL (in dB) in each trial using a sound pressure meter (SL-100, Voltcraft, Hirschau, Germany). Previous work demonstrated that a minimum of 56 dB are necessary to elicit a behavioural response in territorial males [30].

Data collection

Audio file names from the digital voice recordings were blinded. We extracted the latency until the first head-body orientation (in sec), until the first jump (in sec), and the speed to reach the speaker (in cm/s). A previous study performed on the same population has shown that the latency until the first head-body orientation, the latency until the first jump, and the speed to reach the intruder are repeatable, and that the personality trait aggressiveness can be derived from the covariance between these behaviours [22]. We assigned a censored value of 0 cm/s for the speed of males who did not reach the speaker. We also assigned a censored latency value of 300 sec (5 min = total duration of each trial) to males who did not perform a head-body orientation or a jump.

Statistical analyses

First, we confirmed that male *A. femoralis* showed consistent between-individual differences in the behaviours expressed during a territorial defence test. To this end, we estimated the repeatability of each behaviour (i.e., latency until head-body orientation and until first jump, speed to reach the speaker) using the ‘rptR’ package [31]. We log-transformed the response variables to fit a normal distribution and considered behaviours to be repeatable if the 95% confidence interval (CI) did not overlap zero.

To investigate if focal males adjusted their behavioural responses across contexts: encountering an average size, big and small intruder, we performed a random regression analysis [32] and fitted three models: for the (1) latency until head-body orientation, (2) latency until the first jump and (3) speed to reach the speaker (response variables). ‘Treatment’ (average, small or big intruder) was included as a fixed effect [32]. Again, response variables were log-transformed to fit a normal distribution. For each response variable, we compared a random intercept (individual identity) model to a random intercept and slope model (intercept: individual identity, slope: ‘treatment’). We used a Bayesian approach [33] and fitted the models using the package ‘brms’ [34]. We compared model fit using the widely applicable information criterion (WAIC [35]) and selected the best fitting model, with smaller values indicating higher parsimony [32,36]. For all models we used an uninformative prior and ran two Markov chains with 3,000 iterations each and a burn-in of 500. We selected every 2nd posterior parameter sample after the initial burn-in [32]. We present estimates and confidence intervals generated from the best fitting models. We made sure that our models converged by ensuring that Rhat parameters were close to 1 [32]. All statistical analyses were performed in R v3.6.0 [37].

Results

We repeatedly tested 64 males with a playback mimicking an average size intruder resulting in a total of 199 trials (mean ± SD = 3.11 ± 1.24 repetitions per individual). Of those 64 males, 34 were also tested with a playback simulating a small and a big intruder. On average, consecutive trials with the same individual were 5.36 ± 4.59 SD days apart, and the minimum amount of time between two trials was 24 h [22]. Both the latency until the first head-body orientation and the latency until the first jump had a repeatability of $R = 0.18$ (first head-body orientation: $p < 0.001$, 95%CI = 0.06 - 0.30; first jump: $p < 0.001$, 95%CI = 0.05 - 0.29). The speed to reach the speaker had a repeatability of $R = 0.30$ ($p < 0.001$, 95%CI = 0.15 - 0.43).

In all cases, the simpler model including only a random intercept fitted the data best (model (1) for the latency until head-body orientation: ΔWAIC = 1.79, model (2) for the latency until the first jump: ΔWAIC = 0.48, model (3) for the speed to reach the speaker ΔWAIC = 0.65), suggesting that plasticity was equal across the three treatments. We only found moderate evidence for an effect of treatment on the latency to orientate, as confidence intervals slightly overlapped zero. Overall, individuals were slower to turn towards a big intruder (95%CI = -0.06, 0.93). We also found significant evidence that treatment affected the latency to jump towards and the speed to approach an intruder, as individuals were slower to jump towards (95%CI = 0.10, 1.15) and approach (95%CI = -4.49, -0.02) a big intruder (Table 1, Figure 1). Individuals did not differ in their behavioural responses towards a small and an average sized intruder (Table 1, Figure 1).

Discussion

In this study, we used a wild population of the Neotropical poison frog *Allobates femoralis* to investigate whether personality is correlated to the degree of plasticity in male territorial defence behaviour. We found repeatability for the latency to approach and reach an average size intruder. We also observed that both aggressive and less aggressive males plastically adjusted their reaction to differently sized intruders, by decreasing their level of aggressiveness towards big intruders.

In *A. femoralis*, male reproductive success is directly linked to territory ownership [21]. Loss of a territory to a rival male would result in complete loss of reproduction with little chance to reproduce in the following season because only about 20% of all adults survive until the next reproductive season [21]. Thus, plasticity in the level of aggression should provide an advantage in maintaining territory ownership and avoiding costly fights. Yet, we observed a consistent difference in the responses

Table 1. Results of the Bayesian random regression models fitted with a random intercept (for the individual). Estimates and confidence intervals (l-95% and u-95%) are presented. The response variables are log-transformed. Significant confidence intervals (i.e., non-overlapping zero) are written in bold.

Response variable		**Estimate**	**Estimate error**	**l-95%**	**u-95%**
Latency to orientate	*Group-Level Effects (ID):*				
	sd(intercept)	0.73	0.15	**0.46**	**1.04**
	Population-Level Effects:				
	intercept	1.86	0.17	**1.54**	**2.19**
	high frequency call	0.04	0.25	-0.46	0.53
	low frequency call	0.43	0.25	-0.06	0.93
	Family specific parameters:				
	sigma	1.32	0.07	**1.19**	**1.48**
Latency to jump	*Group-Level Effects (ID):*				
	sd(intercept)	0.80	0.15	**0.43**	**1.04**
	Population-Level Effects:				
	intercept	2.64	0.18	**2.30**	**2.99**
	high frequency call	-0.01	0.28	-0.54	0.54
	low frequency call	0.62	0.27	**0.10**	**1.15**
	Family specific parameters:				
	sigma	1.41	0.08	**1.27**	**1.57**
Speed	*Group-Level Effects (ID):*				
	sd(intercept)	4.32	0.70	**3.08**	**5.87**
	Population-Level Effects:				
	intercept	-4.40	0.91	**-6.17**	**-2.59**
	high frequency call	-0.20	1.12	-2.33	1.97
	low frequency call	-2.20	1.13	**-4.49**	**-0.02**
	Family specific parameters:				
	sigma	5.91	0.32	**5.33**	**6.59**

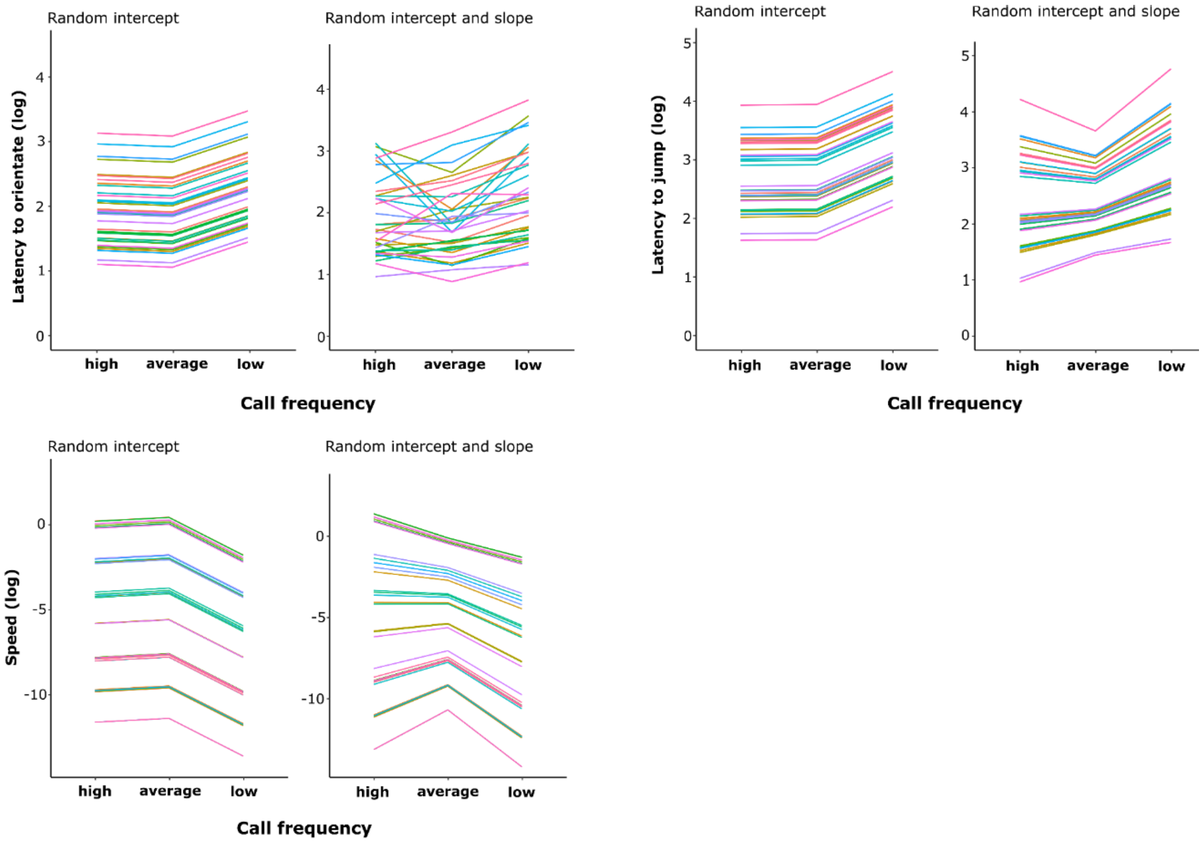


Figure 1. Individual latency to orientate, latency to jump and speed to approach an average size intruder (average frequency call), a small intruder (high frequency call) and a big intruder (low frequency call). For each variable the left panel presents the prediction lines assuming males adjust their behaviour equally between the three treatments (random intercept), and the right panel presents the prediction lines assuming males differ in the extent to which they adjust their behaviour between the three treatments (random intercept and slope). Each line represents a different individual. The three response variables are log transformed.

towards large intruders between more and less aggressive males. When faced with a bigger intruder, more aggressive males decreased their level of aggression but not to the point it would match the level of less aggressive individuals. While plasticity is advantageous in territorial defence in *A. femoralis*, more aggressive males are either not capable of fully adjusting their behaviour – for instance due to a correlation between personality and plasticity – or choose not to because the benefits of minimising the risk of a fight might not outweigh the costs linked to plasticity [38]. However, we found no support for a correlation between personality and plasticity in our data. Individuals showed differences in defence behaviour (i.e., personality) and plasticity, but did not adjust their reaction to differently sized intruders depending on their personality.

The existence of a link between personality and plasticity might be conditional on the costs and benefits such a link would bring in a given context. A correlation might be adaptive in one context but disadvantageous in another. For instance, male song sparrows (*Melospiza melodia*) respond more intensely to strangers than to neighbours when their mate is not fertile, but respond similarly to neighbours and strangers when their mates is fertile [39]. In *A. femoralis*, territories are only maintained during the breeding season [18], but in species maintaining year-round territories with specific mating seasons (e.g., the spotted antbird, *Hylophylax n. naevioides*), we could expect a correlation between personality and plasticity during the non-fertile period, when fitness expectations are lower. Less aggressive males should be more flexible (e.g., not responding to their neighbours and only responding lightly against strangers), while more aggressive males should be more rigid and be less efficient in differentiating between neighbours and strangers. In *A. femoralis*, future studies should investigate whether a correlation between personality and plasticity exists with other personality traits, and /or in periods when fitness expectations are not as high as in the middle of the breeding season.

Lastly, we found repeatability of aggressive behaviours in a range consistent with what has been shown in *A. femoralis* previously and in other personality studies on amphibians [22,40–43]. Our results show that some individuals reacted more quickly to conspecific intruding their territory, while other individuals responded more passively. While being constantly highly aggressive might provide an advantage in repelling competitors, it also means that individuals will likely engage more often in potentially harmful fights. Contrary, less aggressive individuals might be at risk of losing their territory to competitors but benefit in an increased survival.

Now that we have more sophisticated statistical analysis tools available we are able to disentangle personality and plasticity. We want to encourage more studies aiming to investigate this link in a range of species and contexts in the future, improving our understanding of when it pays off to be plastic and when it is better to be consistent. Species with distinct life phases, such as amphibians, are particularly good models to study the temporal variation and context specificity of the correlation between personality traits and plasticity. Individuals face different challenges and exploit different habitats across their life, and consequently, a correlation could emerge across certain life phases.

Data availability statement

The datasets generated during and/or analysed during the current study are available in the Open Science Framework repository: https://osf.io/ue29c/?view_only=14b4a9c4f3fb4724a191149b7fe2bc52

Authors contributions

Conceptualization: MP, LB, MR, ER; Methodology: MP, MR, ER; Formal Analysis: MP, LB, BS; Investigation: MP; Resources: MR, ER; Writing – original draft: MP, BS; Writing – review and editing: MP, LB, MR, BS, ER; Visualization: MP; Supervision: ER; Project administration: MP, ER; Funding acquisition: ER.

Acknowledgments

We thank Fabrice Petel, Vincent Premel, Gaëlle Raboisson and Moric Toszeghi for help collecting the data. This work was funded through a Standalone Grant (FWF P-31518) from the Austrian Science Fund (FWF). MR was supported by an Erwin Schrodinger Grant (FWF J-3868) and a Standalone Grant (FWF P-33728) from the Austrian Science Fund (FWF).

References

[1] Goursot, C., Düpjan, S., Kanitz, E., Tuchscherer, A., Puppe, B. & Leliveld, L. M. C. 2019 Assessing animal individuality: links between personality and laterality in pigs. Current zoology 65, 541–551.

[2] Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O. & Løvlie, H. 2017 A comparison of animal personality and coping styles in the red junglefowl. Anim Behav 130, 209–220.

[3] Tremmel, M. & Müller, C. 2013 Insect personality depends on environmental conditions. Behav Ecol 24, 386–392.

[4] Dochtermann, N. A. & Jenkins, S. H. 2007 Behavioural syndromes in Merriam's kangaroo rats (Dipodomys merriami): a test of competing hypotheses. Proc R Soc Lond B 274, 2343–2349.

[5] Bell, A. M. 2005 Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). Journal of Evolutionary Biology 18, 464–473.

[6] Sih, A., Bell, A. & Johnson, J. C. 2004 Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19, 372–378.

[7] Réale, D. & Dingemanse, N. J. 2010 Personality and individual social specialisation. In Social behaviour: Genes, ecology and evolution (ed. T. Székely, A. J. Moore & J. Komdeur), pp. 417–441. Cambridge, United Kingdom: Cambridge University Press.

[8] Dingemanse, N. J. & Dochtermann, N. A. 2013 Quantifying individual variation in behaviour. Mixed-effect modelling approaches. J Anim Ecol 82, 39–54.

[9] Dingemanse, N. J., Kazem, A. J. N., Reale, D. & Wright, J. 2010 Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol 25, 81–89.

[10] Wolf, M., van Doorn, G. S. & Weissing, F. J. 2008 Evolutionary emergence of responsive and unresponsive personalities. Proc Natl Acad Sci USA 105, 15825–15830.

[11] Ólafsdóttir, G. Á. & Magellan, K. 2016 Interactions between boldness, foraging performance and behavioural plasticity across social contexts. Behavioral ecology and sociobiology 70, 1879–1889.

[12] Benus, R. F., Daas, S. D., Koolhaas, J. M. & van OORTMERSSEN, G. A. 1990 Routine Formation and Flexibility in Social and Non-Social Behaviour of Aggressive and Non-Aggressive Male Mice. Behav 112, 176–193.

[13] Dingemanse, N. J., Both, C., Drent, P. J. & Tinbergen, J. M. 2004 Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc Lond B 271, 847.

[14] Smith, B. R. & Blumstein, D. T. 2008 Fitness consequences of personality: a meta-analysis. Behav Ecol 19, 448–455.

[15] Dingemanse, N. J. & Réale, D. 2005 Natural selection and animal personality. Behav 142, 1159–1184.

[16] Duque-Wilckens, N., Trainor, B. C. & Marler, C. A. 2019 Aggression and territoriality. In Encyclopedia of animal behavior (ed. J. C. Choe), Second edition, pp. 539–546. Amsterdam: Elsevier.

[17] AmphibiaWeb. 2022 AmphibiaWeb: Information on amphibian biology and conservation. www.amphibiaweb.org. Accessed 4 February 2022.

[18] Ringler, M., Ursprung, E. & Hödl, W. 2009 Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog Allobates femoralis (Aromobatidae). Behav Ecol Sociobiol 63, 1281–1293. (doi:10.1007/s00265-009-0793-7).

[19] Ringler, M., Ringler, E., Magaña Mendoza, D. & Hödl, W. 2011 Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. PLoS one 6, e25844. (doi:10.1371/journal.pone.0025844).

[20] Ringler, E., Ringler, M., Jehle, R. & Hödl, W. 2012 The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. PLoS one 7, e40237. (doi:10.1371/journal.pone.0040237).

[21] Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. 2011 Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Mol Ecol 20, 1759–1771. (doi:10.1111/j.1365-294X.2011.05056.x).

[22] Peignier, M., Araya-Ajoy, Y. G., Bégué, L., Chaloupka, S., Dellefont, K., Leeb, C., Walsh, P., Ringler, M. & Ringler, E. 2022 Exploring links between personality traits and their social and non-social environments in wild poison frogs. Behav Ecol Sociobiol 76.

[23] Amézquita Torres, A., Hödl, W., Pimentel Lima, A., Castellanos, L., Erdtmann, L. & Araújo, M. C. de. 2006 Masking interference and the evolution of the acoustic communication system in the Amazonian dendrobatid frog Allobates femoralis. Evolution 60, 1874–1887. (doi:10.1554/06-081.1).

[24] Ringler, M., Mangione, R., Pašukonis, A., Rainer, G., Gyimesi, K., Felling-Wagner, J., Kronaus, H., Réjou-Méchain, M., Chave, J. & Reiter, K. et al. 2016 High-resolution forest mapping for behavioural studies in the Nature Reserve 'Les Nouragues', French Guiana. J Maps 12, 26–32. (doi:10.1080/17445647.2014.972995).

[25] Ursprung, E., Ringler, M. & Hödl, W. 2009 Phonotactic approach pattern in the neotropical frog Allobates femoralis: a spatial and temporal analysis. Behaviour 146, 153–170. (doi:10.1163/156853909X410711).

[26] Narins, P. M., Hödl, W. & Grabul, D. S. 2003 Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. Proc Natl Acad Sci USA 100, 577–580. (doi:10.1073/pnas.0237165100).

[27] Gasser, H., Amézquita Torres, A. & Hödl, W. 2009 Who is calling? Intraspecific call variation in the aromobatid frog Allobates femoralis. Ethology 115, 596–607. (doi:10.1111/j.1439-0310.2009.01639.x).

[28] Hödl, W. 1987 Dendrobates femoralis (Dendrobatidae): a handy fellow for frog bioacoustics. In Proceedings of the 4th Ordinary General meeting of the Societas Europaea Herpetologica (ed. van Gelder J. J., H. Strijbosch & P. Bergers). Nijmegen, Netherlands.

[29] Sonnleitner, R., Ringler, M., Loretto, M.-C. & Ringler, E. 2020 Experience shapes accuracy in territorial decision-making in a poison frog. Biol Lett 16, 20200094.

[30] Hödl, W. 1983 Phyllobates femoralis (Dendrobatidae): Rufverhalten und akustische Orientierung der Männchen (Freilandaufnahmen). Begleitveröffentlichungen zu wissenschaftlichen Filmen. Vienna, Austria: Bundesstaatliche Hauptstelle für Wissenschaftliche Kinematographie.

[31] Stoffel, M. A., Nakagawa, S. & Schielzeth, H. 2017 rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8, 1639–1644.

[32] Hertel, A. G., Niemelä, P. T., Dingemanse, N. J. & Mueller, T. 2020 A guide for studying among-individual behavioral variation from movement data in the wild. Movement ecology 8, 30.

[33] Hadfield, J. D. 2010 MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmmR Package. J. Stat. Soft. 33, 1–22.

[34] Bürkner, P.-C. 2017 brms : An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Soft. 80.

[35] Gelman, A., Hwang, J. & Vehtari, A. 2014 Understanding predictive information criteria for Bayesian models. Stat Comput 24, 997–1016.

[36] Whalen, A. & Hoppitt, W. J. E. 2016 Bayesian Model Selection with Network Based Diffusion Analysis. Front Psychol 7, 409.

[37] R Core Team. 2020 R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

[38] DeWitt, T. J., Sih, A. & Wilson, D. S. 1998 Costs and limits of phenotypic plasticity. Trends Ecol Evol 13, 77–81.

[39] Moser-Purdy, C., MacDougall-Shackleton, E. A. & Mennill, D. J. 2017 Enemies are not always dear: male song sparrows adjust dear enemy effect expression in response to female fertility. Anim Behav 126, 17–22.

[40] Chaloupka, S., Peignier, M., Stückler, S., Araya-Ajoy, Y., Walsh, P., Ringler, M. & Ringler, E. 2022 Repeatable territorial aggression in a Neotropical poison frog. Front Ecol Evol 10.

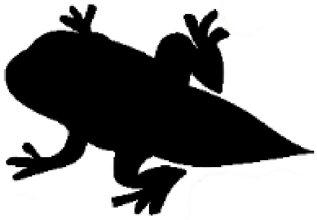
[41] Brodin, T., Lind, M. I., Wiberg, M. K. & Johansson, F. 2013 Personality trait differences between mainland and island populations in the common frog (Rana temporaria). Behav

Ecol Sociobiol 67, 135–143.

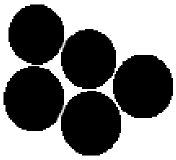
[42] Maes, J., van Damme, R. & Matthysen, E. 2013 Individual and among-population variation in dispersal-related traits in Natterjack toads. Behav Ecol 24, 521–531.

[43] González-Bernal, E., Brown, G. P. & Shine, R. 2014 Invasive cane toads: social facilitation depends upon an individual's personality. PLoS one 9, e102880.

Chapter 2



Links between animal personality and environment



Manuscript 3: "Exploring links between personality traits and their social and non-social environments in wild poison frogs"

This manuscript is published in *Behavioral Ecology and Sociobiology* (76:93)
21 June 2022
doi: 10.1007/s00265-022-03202-9

Mélissa Peignier[a,b,c,*], Yimen G. Araya-Ajoy[d], Lauriane Bégué[c,e], Sarah Chaloupka[f], Katharina Dellefont[c], Christoph Leeb[b,g,h], Patrick Walsh[i], Max Ringler[a,c,f,j] & Eva Ringler[a,b,c]

Affiliations:
[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[b] Messerli Research Institute, University of Veterinary Medicine Vienna, Austria
[c] Department of Behavioral and Cognitive Biology, University of Vienna, Austria
[d] Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[e] Department of Biology and Ecology, University of Montpellier, France
[f] Department of Evolutionary Biology, University of Vienna, Austria
[g] iES Landau, Institute for Environmental Sciences, University of Koblenz-Landau, Germany
[h] Central Research Laboratories, Natural History Museum Vienna, Austria
[i] Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh, United Kingdom
[j] University of Music and Performing Arts Graz, Austria

*Correspondence:
Mélissa Peignier
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
melissa.peignier@unibe.ch

Abstract

An animal’s behavioral phenotype comprises several traits, which are hierarchically structured in functional units. This is manifested in measured behaviors often being correlated, partly reflecting the need of a coordinated functional response. Unfortunately, we still have limited understanding whether consistent differences in animal behaviors are due to underlying physiological constraints or a result of plastic adaptation to their current environment. Therefore, characterizing the spatial distribution of behaviors can provide important insights into causes and consequences of behavioral variation. In the present study, we quantified behaviors in a wild, free ranging population of the Neotropical frog *Allobates femoralis*. We investigated how these behaviors were linked to the frogs’ natural and social environment and quantified the extent to which these behaviors consistently differed among individuals (i.e., animal personality). We assessed levels of aggressiveness, exploration, and boldness by measuring several underlying behaviors expressed in a set of experimental assays, and found evidence for consistent among-individual differences along these axes. Contrary to our expectation, there was no relationship between individual behaviors and their natural environment, but we found a plastic response of males to changes in female density, which might reflect how individuals cope with their socio-ecological environment.

Keywords

behavioral variation, animal personality, poison frogs, non-random distribution, environment

Introduction

Behavioral variation is ubiquitous in nature. Behaviors may vary considerably among species; and within species, we find variation both among and within individuals. Behavioral variation at all of these levels plays a major role in reproduction and survival, for example affecting the risk of being detected and caught by predators, likelihood of dispersal, foraging efficiency, and/or the attractiveness to mating partners (Pigliucci 2005; Réale and Dingemanse 2010; Sih et al. 2012). This variation can occur in the form of consistent among-individual differences in behavior, referred to as animal personality, which has been documented in many animal taxa (Bell 2005; Dochtermann and Jenkins 2007; Tremmel and Müller 2013; Zidar et al. 2017; Goursot et al. 2019). Animal personality, however, does not preclude the existence of individual plasticity (Dingemanse et al. 2010), and we need to take into consideration both sources of variation when thinking about adaptive significance of behavioral variation in animals.

Five main personality traits are generally characterized in animals along the following axes: active/passive, aggressive/docile, bold/shy, exploratory/stationary, and sociable/non-sociable (Réale et al. 2007). Personality traits can be seen as latent variables that affect multiple quantifiable behaviors of an organism in certain contexts (Pigliucci 2003; Araya-Ajoy and Dingemanse 2014). For instance, aggressiveness can be seen as an unobservable (i.e., latent) variable that affects several observable behaviors during agonistic encounters, which can be assessed and quantified in an experimental context (e.g., speed of territorial reaction, number of attacks). Situations where personality traits are non-randomly distributed across space are typically referred to as “phenotype by environment correlation”, where environment refers to both the natural and social surroundings of the individual (Conover and Schultz 1995; Dingemanse and Araya-Ajoy 2015). For example, anemones (*Condylactis gigantea*) living in areas with higher seagrass were found to be shyer than those living in more open areas (Hensley et al. 2012) and western bluebirds (*Sialia mexicana*) modify their aggression to match the level of aggressive behavior of their mate (Duckworth and Kruuk 2009).

Since personality traits can vary simultaneously within and among individuals, associations between behavior and environment can originate from multiple processes, such as a non-random distribution of behaviors and/or phenotypic plasticity

(Sprau and Dingemanse 2017). A non-random distribution of behaviors might be a direct effect of certain phenotypes (caused by genes and permanent environmental effects) showing a preference for certain environments. Alternatively, selective pressures induced by the heterogeneity of the environment can also maintain or generate individual differences in behavior within a population (Dingemanse et al. 2004). For instance, in territorial species, only highly aggressive individuals may succeed in establishing a territory in high-density patches, where they have access to more mates but face elevated intra-sexual competition. In turn, the link between aggressiveness and population density could also be caused by individual plasticity allowing individuals to match their aggressiveness to competition levels. Regardless of the mechanisms linking behavior and environment, identifying non-random spatial distribution of behaviors provides important insights into their function and their role in allowing organisms to cope with environmental variation. Long-term studies of the distribution of behaviors in the wild are thus a necessary first step towards understanding the mechanisms underlying the non-random distribution of behavioral traits (Archard and Braithwaite 2010).

Amphibians, and in particular Neotropical poison frogs (Dendrobatidae, *sensu* AmphibiaWeb 2022), are great models to study behavioral variation across their environment. Many species show territoriality or site fidelity that facilitates repeated measurements in wild individuals (Wells 2007; Kelleher et al. 2018), which can then be linked to local environmental parameters. Many species also exhibit elaborate courtship behavior, terrestrial oviposition, and obligatory tadpole transport of hatched larvae to aquatic sites (e.g. Crump 1972; Roithmair 1994; Pröhl 2005; Pašukonis et al. 2013; Rojas and Pašukonis 2019; Yang et al. 2019; Souza et al. 2021), offering ideal prerequisites for within- and between-individual variation in behavior to arise. In the present study we aim to quantify how male-male aggression, exploratory, and anti-predator behaviors are expressed as functional units that can be described as latent variables reflecting three personality axes (referred to by one dimension of the axis as “aggression”, “exploration”, and “boldness”). We also aim at identifying how behaviors relate to the individual’s natural and social environment using a free-ranging population of the Neotropical poison frog *Allobates femoralis* (Dendrobatidae).

Based on the natural history of *A. femoralis*, we make multiple predictions. In males, we expect to find consistent among-individual differences in boldness and aggression. During the reproductive season, males produce loud advertisement calls to warn intruders and to attract females (Ringler et al. 2011; Chaloupka et al. 2022). Differences in personality traits across males might be related to differences in the trade-off they face between calling to secure mating and exposure to predators. As females do not display territoriality but merely perch fidelity (Fischer et al. 2020) and are never observed in any other aggressive interaction, they are generally considered non-aggressive; we have no clear expectation if and how varying degrees of boldness could be maintained in females. However, we expect to find consistent individual differences in exploration in both sexes, because males transport tadpoles to natural pools located up to 200 m away from their territory and females commute to male territories within 20 m distance of their perch for mating (Ringler et al. 2012, 2013, 2018; Fischer et al. 2020). Finally, we expect males' personality traits to be non-randomly distributed across space because individual males face distinct challenges based on their social and/or natural environment. On the one hand, we expect more aggressive and bolder individuals to occupy areas of low complexity (e.g., with sparse vegetation and few ground structures) where they are easier to spot for females, while more passive or shyer individuals should occupy areas of higher complexity with more places to hide. On the other hand, we expect bolder and more aggressive individuals to occupy territories in areas with a higher population density than those occupied by more passive or shyer individuals.

Methods

Ethical note

This study was approved by the scientific committee of the "Nouragues Ecological Research Station" and the ethics and animal welfare committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice (GSP) guidelines and national legislation. Sampling for this study was conducted in strict accordance with current French and EU law and followed the (ASAB 2020) guidelines on the treatment of study animals. Working and tissue sampling permissions were provided by the CNRS Guyane ("Centre National de la Recherche Scientifique Guyane"), by the "Ministère de la transition écologique et solidaire" (permit number: TREL2002508S/303), and the Secretariat of the Convention on Biological Diversity (APA declaration: TREL1734890A/34).

Study population and area

We conducted our study in a population of *A. femoralis* on a lowland rainforest river island of approximately 5 ha. The island is situated in the “Les Nouragues” nature reserve in French Guiana (4°02´ N, 52°41´W; Bongers et al. 2001), near the “Saut Pararé” field camp of the CNRS Nouragues Ecological Research Station. The population was introduced in 2012 and has reached a stable size of approximately 150 adult individuals (see Ringler et al. 2014). The experiments took place from February to April of 2019, coinciding with the breeding season. We surveyed the population every day during its most active hours from 0900 to 1800 h, aiming to sample all adult males and females on the island in the course of the study period.

We caught frogs using a transparent plastic bag to minimize stress and direct contact, thereby limiting the potential influence of handling on behaviors. We identified all frogs via digital pictures of their distinct ventral patterns and with the help of the pattern matching software Wild-ID (Bolger et al. 2012), and sexed them by the presence (males) or absence (females) of vocal sacs. Ventral patterns clearly differ among individuals and are consistent across their adult lifespan, providing a reliable method of identification. Information on the age of individuals was available from a concurrent long-term monitoring project of the island’s population since its origins in 2012. We recorded the locations of all frogs on a detailed digital map (Ringler et al. 2016) using the mobile GIS software ArcPad 10 (ESRI, Redlands, CA, USA) on rugged Win10 tablets (CAT T20, Bullitt Group, Reading, UK), and further handled the data in ArcGIS 10.6 (ESRI). We determined body size of all adults (snout urostyle length; SUL) from dorsal photographs in front of a reference scale using the software Image J 1.52a (Rasband 1997-2021).

Quantification of aggressive behaviors

Aggression is often measured via “an individual’s agonistic reaction towards a conspecific” (Réale et al. 2007). To assess individual levels of aggression, we used acoustic playbacks to evoke territorial defense behavior in focal males (Fig. 1; Ursprung et al. 2009). During agonistic encounters, male *A. femoralis* display typical responses consisting of an orientation of their head/body, jumps towards the intruder and sometimes direct attack (i.e., wrestling) (Hödl 1987; Narins et al. 2003). We expect that in the context of territorial defense, the personality trait “aggression” affects the latency until the first head-body orientation, the latency to the first jump, the probability to jump during moments when the intruder does not call, and the speed to approach the intruder (Chaloupka et al. 2022). Aggressive territorial behavior was only assessed in males, as females do not defend territories.

We used a music player with integrated loudspeaker (MUVO 2c, Creative, Singapore), centered on top of a black PVC disc (radius = 15 cm), 2 m from and facing the calling focal male (Fig. 1). We established the 2 m distance between the focal frog and the loudspeaker with a laser rangefinder (DLE 50, Bosch, Stuttgart, Germany). The experimenter remained a further 1 m behind the loudspeaker. We gave a 30 s acclimation period to the frog, which was enough for the frog to return to normal behavior and calling, before randomly presenting one of seven synthetic calls. The calls varied in their inter-note and inter-call intervals to avoid habituation over the course of the experiment. Each synthetic call featured the spectral and temporal parameters of a nearby free-ranging population of *A. femoralis* (Narins et al. 2003; Gasser et al. 2009). Each of the playbacks lasted for 5 min and was presented from the original WAV-files (16-bit, 44.1 kHz) using the same volume settings across all trials.

Using a digital voice recorder (ICD-PX333, Sony, Tokyo, Japan), we commented on the behavior of the focal male, noting its first head-body orientation, its jumps, and its arrival at the speaker (i.e., touching the disc with at least one part of his body). The trial ended when the male entered the 15 cm perimeter or when the playback stopped after 5 min. Trials where a focal male began to call were stopped and excluded from further analysis, as this was most likely the result of the speaker being positioned outside the defended area of the focal male’s territory (cf. Ringler et al. 2011). Vegetation density may cause the sound to be reflected and/or distorted, so we measured the received sound pressure level (“SPL” in dB) of the signal after each trial using a sound pressure meter (SL-100, Voltcraft, Hirschau, Germany). In all cases, the received SPL was above the threshold of 56 dB to elicit a behavioral response (Hödl 1983). The minimum duration between two consecutive tests with the same individual was 24 h (on average tests were 12.11 ± 7.92 SD days apart). *Allobates femoralis* typically lives for 1.5 years (Ringler et al. 2009; Ursprung et al. 2011a, b) and males only display aggressive reactions to territory intruders during the reproductive season. In our study, we aimed to test individuals with similar among- and within-individual inter-test intervals to cover several weeks of the reproductive season.

Fig. 1 Picture of the territorial defense trial, presenting the speaker and a male on top of the perimeter.

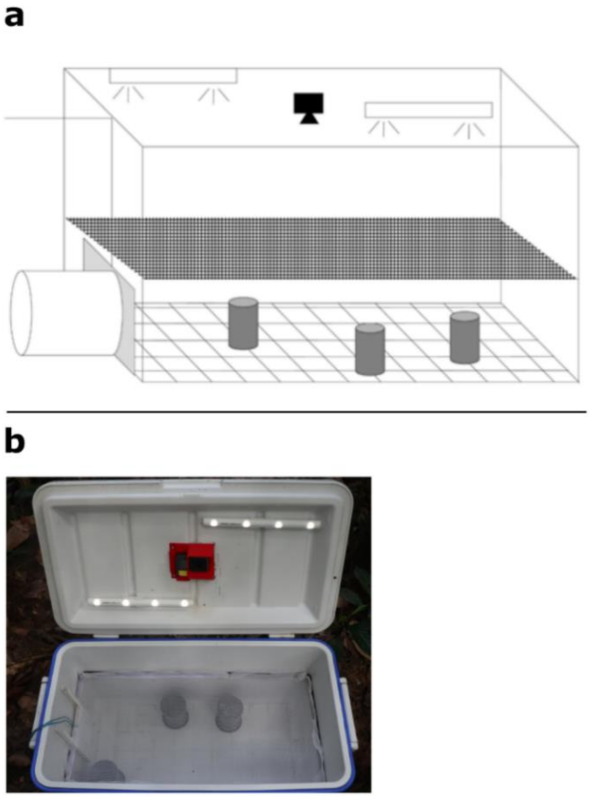


Fig. 2 Schematic (A) and picture (B) of the Novel Environment Test. The cooler box measured 50 x 25 x 29 cm, with a 10 cm PVC tube attached on one side of the box. A sliding door separated the shelter from the box. Two LED tubes and a Hero Black 5 Go pro camera were attached to the lid of the box. Three solid PVC tubes (10 cm height, 5 cm diameter) were placed inside the box as visual obstacles at randomized positions every day. A grid was drawn on the floor of the cooler box to help randomize the position of the obstacles. A mesh net was placed in the cooler box at 20 cm height to prevent the frog from jumping on a wall outside of the camera range.

Therefore, an average interval of 12 days between tests represents an effective compromise across the available time and life span of the species.

We blinded audio file names prior to analysis and coding to avoid observer bias. From the dictaphone recordings of the territorial defense trials, we extracted the latency until the first head-body orientation (in s), the latency until the first jump (in s), the speed to reach the speaker (in cm/s), and if the male jumped (1) or not (0) during inter-bout-intervals (hereafter “inter-bout jumps”, cf. Ursprung et al. 2009) using the software VLC (VideoLAN 2021). Males that did not reach the speaker were coded a censored speed of 0 cm/s. Following the same reasoning, males that did not perform a head-body orientation or a jump were given a censored latency of 300 s (corresponding to the total duration of the experiment). In total we conducted 163 valid territorial defense trials with 51 males (mean ± SD = 3.20 ± 1.31 repetitions per individual).

Quantification of exploratory tendency and boldness

To assess levels of exploratory tendency and boldness in male and female *A. femoralis* we used a Novel Environment Test (NET), an approach that has been commonly used in personality studies (Carter et al. 2013; Kelleher et al. 2018). “Boldness” (or the corollary “shyness”) is defined as “an individual’s reaction to any risky situation”, and “exploration” as “an individual’s reaction to a new situation” (Réale et al. 2007). Therefore, we expected the personality trait “boldness” to affect the latency to go out of a dark shelter into a bright (novel) environment and the probability to enter the novel environment, and the personality trait “exploration” to affect the distance travelled, the number of jumps performed, and the area covered in the novel environment. Those behaviors were chosen in accordance with previous amphibian personality studies (Kelleher et al. 2018). Although counterintuitive at first, the number of jumps and the area covered are both important aspects of a frog’s exploratory tendency. Indeed, two individuals performing an equal number of jumps can visit more or fewer distinct areas of the box depending on their respective average jump lengths. Individuals were tested at the same location they were found. Males were tested immediately after the territorial defense test, and females were caught at their encounter location and put straight inside the NET (Fig. 2). Although this method prevents us from comparing levels of exploratory tendency and boldness between sexes, it was chosen to keep handling as minimal as possible on males.

The NET setup consisted of a cooler box (50 x 25 x 29 cm, hereafter “Novel

Environment”), with a 10 cm PVC tube attached on one side of the box (hereafter “shelter”). An opaque sliding door separated the Novel Environment from the shelter. In the lid of the box, we installed a wide-angle video camera (Hero Black 5, GoPro, San Mateo, CA, USA) and two elongated, battery powered LED lights (LUMIstixx, Osram/Ledvance, Garching, Germany) for homogeneous illumination. We set the camera to “superview”-mode to ensure full visibility of the Novel Environment. We installed a mesh net in the Novel Environment at 20 cm height to prevent the frog from jumping on a wall outside of the camera range. We used three solid PVC tubes (10 cm height, 5 cm diameter) as visual obstacles, to motivate the frogs to explore the entire Novel Environment. The positions of the obstacles were changed daily and their positions were determined using a random number generator.

At the beginning of each trial, we placed all individuals in the shelter for 10 min to allow them to acclimatize. The shelter remained accessible throughout the trials to encourage natural behaviors within the Novel Environment (Carter et al. 2013; Kelleher et al. 2018), as the individual was free to remain inside or return to the shelter at any time. After this acclimation period, we switched on the lights and the camera, closed the lid of the Novel Environment, opened the sliding door between shelter and Novel Environment and filmed for 15 min. The minimum duration between two consecutive tests with the same individual was 24 h (on average tests were 11.36 ± 8.43 SD days apart).

We blinded video file names prior to analysis and coding to avoid observer bias. To analyze the video recordings obtained during the NET, we used the software TRACKER (Brown 2019) to correct for distances that were distorted by the camera wide-angle lens. Using the coding software BORIS (Friard et al. 2016), we assessed the latency from the opening of the sliding door until frogs left the shelter (in s) and the number of jumps performed inside the Novel Environment. For individuals who stayed inside the shelter during the entire experiment, the time spent in the shelter was censored to a value of 900 s (corresponding to the total duration of the experiment). We also coded the decision to enter the Novel Environment (1) or not (0) as a binomial response. Using the automated tracking software TOXTRAC (Rodriguez et al. 2018), we measured the distance travelled (in pixels) as well as the area visited inside the Novel Environment. For the latter variable, the software divided the floor of the Novel Environment into a 9 x 8 grid and automatically counted the number of distinct squares a frog visited during a trial. As individuals varied in the time they spent inside the Novel

Environment, we standardized the data collected by only considering movements performed during the first 2 min after leaving the shelter. On average, individuals spent 624.21 s inside the Novel Environment (± 303.13 SD). This timeframe allowed for meaningful levels of movement to occur but maximized the available data. Using this criterion, 21 out of 259 tests had to be excluded from the exploration analysis, leaving a total of 238 valid NET trials with 52 males and 35 females (mean ± SD = 2.74 ± 1.33 repetitions per individual). Only very few individuals did not leave the shelter in all repetitions.

Territory size, habitat complexity, and number of neighbors

To find out the distribution patterns of behaviors with respect to a male's natural and social environment, we determined the territory size and the local complexity of the habitat, as well as the number of female and male neighbors for each male. This was only studied in males, as in *A. femoralis* males acquire and defend non-overlapping territories, while female display only site fidelity (Ringler et al. 2009, 2011, 2012; Fischer et al. 2020). We used Dirichlet tessellation in ArcGIS to approximate male territories as Voronoi polygons (Voronoi 1908) on a day-to-day basis (for more information see Supplementary Materials). For the daily territory estimation, we applied a roving-window approach, using all capture points of the last 5 days a male was seen, including the focal day, but excluding all points where a male was likely found outside its territory (i.e., all capture points linked to tadpole transport). To ensure that the sizes of territories located at the population periphery were not over-estimated (Ringler et al. 2009), we included the vertices of the island outline into the Dirichlet tessellation procedure, to establish a buffer from the edge of the island until halfway to the outermost capture points of peripheral males. To ensure that estimates at the beginning and end of the season were not impacted by individuals not being captured we used the POPAN formula (Schwarz and Arnason 1996) in program MARK (White and Burnham 1999; White 2020) to correct our population estimates at these points in the breeding season (for more detailed information see Supplementary Materials).

After conducting the Dirichlet tessellation, we dissolved the resulting Voronoi polygons based on male identity to obtain "Voronoi territories" for each male and then calculated territory sizes in ArcGIS. We also counted the direct male neighbors for each focal male from the daily Voronoi territories. To obtain the number of female neighbors we calculated female centroid points (mean center) across the entire season. Based on the typical distances females commute for mating (Ringler et al. 2012; Fischer et al. 2020), we constructed 20 m buffer circles and counted the number of contained centroid points of male Voronoi territories.

To determine the complexity of the habitat for each individual male we took four photographs (camera in automatic (P) mode, focal length 50 mm, no flash, jpg images) from each of the cardinal directions (0°, 90°, 180°, and 270° towards magnetic north) of a 100 cm x 100 cm red fabric. The fabric was placed at 3.5 m from the centroid point of each male’s territory at forest floor level as this represents half the radius of the typical male territory (cf. Ringler et al. 2011). We positioned the camera 20 cm above the forest floor, corresponding to the perch location of a male during territorial advertisement calling. We calculated habitat complexity as the average percentage of red fabric that was covered by vegetation on the four pictures, with increasing coverage of the fabric representing more complex habitat. For that, we cropped each image in Paint.net v4.1.6 (Brewster 2019) to show only the lower half of the red fabric as this would represent the most visually relevant part of the habitat for the frogs. Finally, we counted the number of pixels of visible fabric to calculate the percentage of fabric covered by vegetation. To facilitate this process, we aimed at increasing the differences between fabric and vegetation by setting the image hue to 180 and decreasing the luminance and contrast to − 20. If males changed their territory location during the study period, analysis of habitat complexity was performed in the territory in which most experimental trials had been conducted.

Statistical analysis

We conducted all statistical analyses in R v3.6.0 (R Core Team 2020) using the integrated development environment RStudio v1.3.1093 (RStudio Team 2019).

Structure of behaviors: To determine how the measured behaviors are structured into functional units (i.e., aggression, exploration and boldness), we investigated the phenotypic covariance structure among different measurements during the behavioral tests. To infer a latent variable describing aggression from behaviors measured during the territorial defense test (e.g. speed to reach the speaker, latency until the first head-body orientation, latency until the first jump, inter-bout jumps), using the SEM package we applied structural equation modelling to the phenotypic covariance matrix derived from the means of each behavior for each individual, in order to avoid pseudoreplication (Fox et al. 2020). Models were compared, to determine the most parsimonious model (for details see Supplementary Fig. 1), based on differences in Akaike's information criterion (AIC) values, with small values indicating higher parsimony and a $\Delta AIC \geq 2$ indicating significant differences (Burnham and Anderson 2002). We expected the best model to have a latent variable explaining the covariance among the four measurements. We applied the same technique to determine whether two latent variables describing boldness and exploration can be inferred from the behaviors measured during the NET (i.e., distance travelled, number of jumps, number of areas, time spent in the shelter and probability to enter the box, for details see Supplementary Fig. 2). We expected the best model to have two correlated latent variables, one that would explain explorative behaviors (e.g., distance travelled, number of jumps, number of areas), and one that would explain boldness related behaviors (e.g., probability to enter the box, time spent in the shelter). In addition, Bayesian generalized linear mixed models were used to confirm that there were no impacts from the fact that SEM analysis combines within and between individual effects (for details see Supplementary Material).

Personality traits: To investigate if *A. femoralis* exhibits personality (i.e., between-individual variation in behaviors), we assessed the repeatability (R) of all measured behaviors using the "rpt" function in the rptR package (Stoffel et al. 2017). Latency until the first head-body orientation and until the first jump had to be log transformed to achieve normal distribution. We used the function "transformTukey" to apply a constant transformation on the speed to reach the speaker, the distance travelled, and the time spent in the shelter. Repeatability was estimated based on the models applied to the transformed data. We estimated repeatability from models fitted with a Gaussian error distribution for the latency until the first head-body orientation and until the first jump, the speed to reach the speaker, the time spent in the shelter and the distance travelled. We estimated repeatability from models fitted with a Poisson error distribution for the number of jumps and the number of areas, and from models fitted with a binary distribution for the inter-bout jumps and the probability to enter the box. For all models, ID was included as a random effect.

Distribution of behaviors across the natural and social environment: We used a bivariate approach to study how behaviors measured during the territorial defense trial and the NET correlate with variation in the habitat complexity, territory size, and number of male and female neighbors at the among- and within-individuals level using Bayesian generalized linear mixed models (Hadfield 2010). In the analyses, the latency until the first jump, distance travelled, and time spent in the shelter were chosen as they best represented the latent variables of aggression, exploration and boldness respectively (see the “Structure of behaviors” section and Fig. 3). Although the probability to go inside the box was slightly more correlated to boldness than “time spent in shelter”, we decided to use the latter variable to avoid using a binomial variable in the model. Models were built with the transformed data (see the “Personality traits” section).

To investigate the among-individual covariance between behaviors and environment variables, we divided each of the environmental variables by their mean value and added all of them as response variables (see Houslay and Wilson 2017). We included age (i.e., as a binomial trait: newly encountered adults vs. recaptures from previous years) as a trait-specific fixed effects to control for effects of age on all our response variables. To calculate the variance due to differences among individuals and the covariance between measured behaviors, we fitted an unstructured covariance matrix for the grouping variable ID. We then used the posterior distributions to estimate the among and within-individual correlations and covariances between each of the behavior measured and the environment. We assumed statistical significance if the 95% credible intervals did not overlap 0 and performed the same model verification as previously (see the “Structure of behaviors” section). We also fitted these relationships between behaviors and environment variables with univariate linear mixed models corrected for multiple comparisons, which led to the same biological conclusions.

Results

Personality traits

Among the measures taken from the territorial defense test, all were considered repeatable and ranged in repeatability from 0.17 to 0.37 (Table 1; Fig. 4a). The best SEM model supports a latent variable explaining the covariance of the four behavioral responses measured in a territorial defense test (Fig. 3a; Supplementary Fig. 1b). Together, these results suggest that *A. femoralis* exhibits a personality trait “aggressiveness” encompassing the latency until the first head-body orientation and

Table 1 Repeatability (R) and confidence intervals (CI) of the behaviors measured during the territorial defence test and the Novel Environment Test (NET).

Test	Variable	R	95% CI
Territorial defence test	Latency until head-body orientation	0.17	[0.02;0.34]
	Latency until first jump	0.24	[0.07;0.40]
	Speed to reach the perimeter	0.37	[0.19;0.53]
	Inter-bout jumps	0.20	[0.001;0.37]
NET	Probability to go in the box	0.44	[0.13;0.89]
	Time spent in the shelter	0.30	[0.15;0.44]
	Distance travelled in the box	0.36	[0.21;0.49]
	Number of jumps	0.45	[0.27;0.62]
	Number of areas visited	0.48	[0.30;0.62]

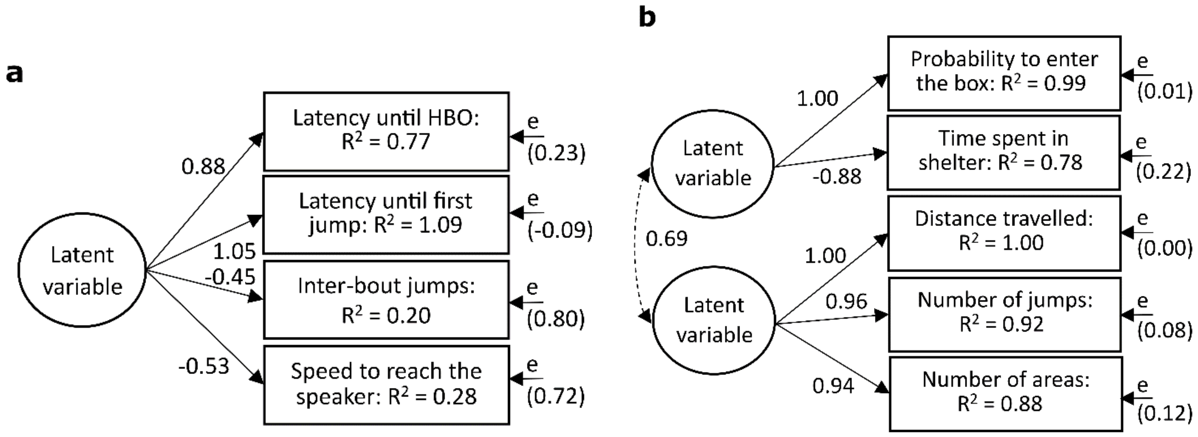


Fig. 3 Path diagrams of the best structure equation models (SEMs) (based on difference in Akaike's information criterion (AIC) values) explaining the covariance structure among four behaviors assessed during an aggressivity test (A), and five behaviors assessed during a Novel Environment Test (B). 'HBO' refers to the latency until the first head-body orientation. Squares represent the variances of the different behaviors explained by the SEM structure (R^2). Numbers associated with arrows are standardized factor loadings which represent how behavioral responses are predicted to change based on changes to the latent variable. Number in brackets represent variances of residuals or error variances (e) associated to each behavior. All simulated models can be found in the Supplementary material (Supplementary Fig. 1, 2).

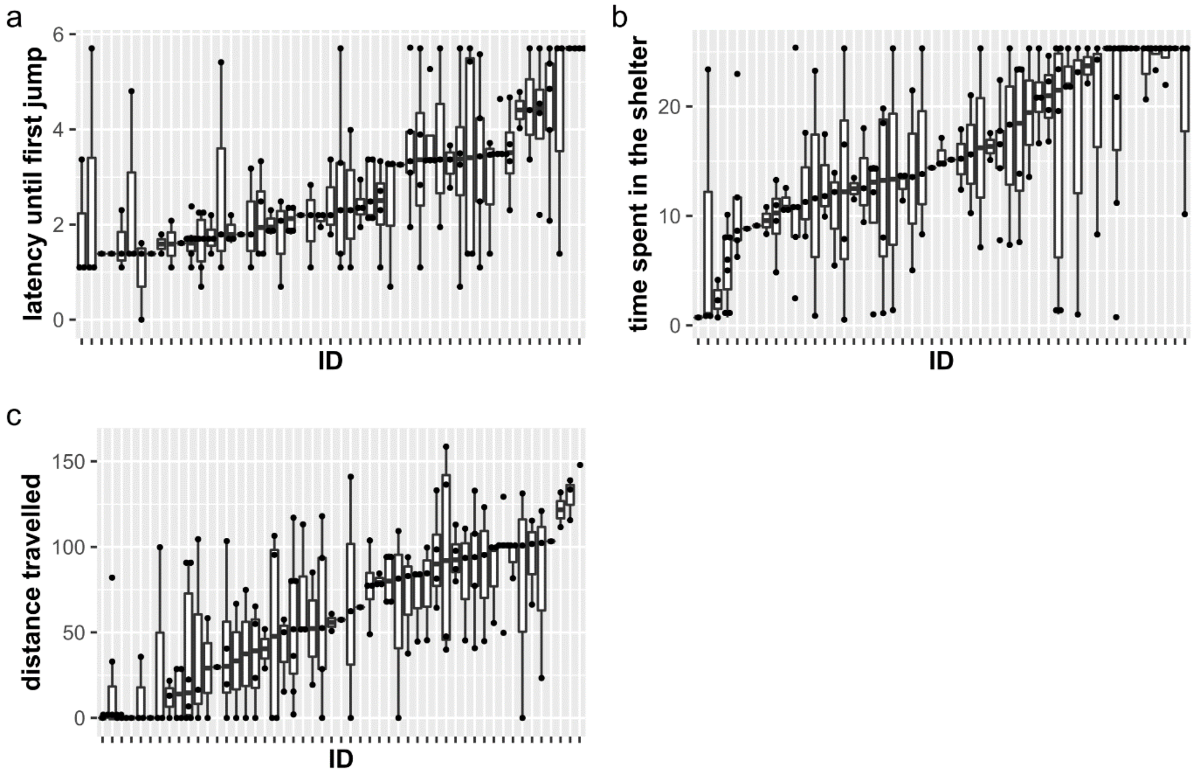


Fig. 4 Range of variation in the three behaviors that best represented the latent variables of aggression, exploration and boldness. The latency until the first jump (a), the time spent in the shelter (b), and the distance travelled in the Novel Environment Test (c) are presented for individual males. All variables have been transformed using a log (a) or constant (b and c) transformation. Boxes indicate the inter quartile range, with the central line depicting the median and the whiskers extending to 1.5*IQR. Dots represent the results of each trial. Males are ordered by their median (represented as a horizontal bold line).

until the first jump towards a calling intruder, the speed to reach the intruder and the probability to jump during inter-bout. However, the residual variances were high (Fig. 3a; Supplementary Fig. 1b) and another model encompassing two latent variables, had an AIC score close to the best model (Δ AIC = 1.7; Supplementary Fig. 1g).

Similarly, we measured individual behavioral responses in a NET, where we found significant repeatabilities in all behavioral measurements (Table 1; Fig. 4b, c). Based on the comparison of the SEM, the best model supported the existence of a latent variable explaining the covariance patterns of three behavioral measurements (distance travelled, number of jumps and number of areas). It also supports that another latent variable can be derived from the covariance of two behavioral measurements (time spent inside the shelter and probability to enter the box). The two latent variables are correlated (Fig. 3b; Supplementary Fig. 2h). Together, these results suggest that *A. femoralis* males and females exhibit a personality trait "exploration" encompassing the distance travelled, the number of jumps performed, and the area covered in a new environment. The results also suggest the existence of the personality trait "boldness", encompassing the latency to leave a safe place and enter a new environment and the probability to enter a new environment.

For both the behaviors measured in the territorial defense test and in the NET, the phenotypic covariances in the SEM were mostly driven by the within-individual variances. The results of the Bayesian models also show that the direction of the among and within individual covariances was similar (Supplementary Tables 2, 3). Therefore, doing the SEM using only the among- or within-individual covariance matrix would have resulted in a similar interpretation of the results.

Behavior and the characteristics of an individual and its environment

Next, we investigated the correlation between the behaviors measured during the territorial defense trial and habitat complexity, territory size, and the number of male and female neighbors. On average, habitats were relatively complex with 82.77% of the fabric covered by vegetation in the measure of habitat complexity (SD = 9.31). Males occupied territories of 669.31 m^2 on average (SD = 440.99). Males had on average 7 female and 5 male neighbors (mean of individual means) across the season (absolute range females: 1–15, absolute range males: 1–10). Throughout the season, the average variation in the number of both female and male neighbors was 2 for individual territory holders (range females: 0–7; range males: 0–6).

There was no relation between the aggressive responses of a male during a territorial defense test and its social or natural environment. However, there was a significant relation between the level of exploration and boldness of a male and the number of female neighbors at the within-individual level (Supplementary Table 4). This suggests that individuals respond plastically to their social environment, increasing their level of exploration and boldness when the number of females around increases. The spatial setup of the territories on the day with the most individuals present at the same time (07th of March 2019) is presented in Fig. 5. The average response of each individual in terms of latency until the first jump, time spent in the shelter and distance travelled in the box is also represented.

Discussion

In the present study, we investigated the structure of potential personality traits in *A. femoralis* and determined how behaviors related to aggressiveness, exploration, and boldness are structured into functional units (i.e., personality traits). We also investigated how individual behaviors relate to an individual's natural and social environment in a wild, free ranging, entire population of *A. femoralis*.

Personality traits

Our analysis showed that the repeatability of the variables measured in the territorial defense trial (0.17 to 0.37) was in the lower range of the repeatability found in most studies (with overlapping confidence intervals; Bell et al. 2009; mean = 0.37, 95% confidence limits = 0.35–0.38) but consistent with the average findings in other personality studies on amphibians (Brodin et al. 2013; Maes et al. 2013; Gifford et al. 2014; González-Bernal et al. 2014). Moreover, we found that a latent variable explains the covariance of the four behaviors measured during a territorial defense test (i.e., the latency until the first head-body orientation and until the first jump towards a calling intruder, the speed to reach the intruder and the probability to jump during the inter-bout interval). Together, these results suggest that *A. femoralis* males exhibit a personality trait "aggressiveness". However, a great portion of the speed and the inter-bout jumps measured during the territorial defense trial were explained by exogenous factors. Together with the existence of another model with similar support, these results suggest that the behavioral measures capture different aspects of aggressiveness. In this model (Supplementary Fig. 1g), a latent variable explains the covariance of the latencies until the first head-body orientation and until the first jump. Another latent variable explains the covariance between the speed to reach the speaker and the inter-bout jumps. We interpret these two latent variables as reactivity and offensiveness, respectively. Males of many amphibian species defend and fight over territories using acoustic and visual displays (Hödl and Amezquita 2001; Toledo et al. 2007). For instance, in the Bornean rock-skipper frog (*Staurois latopalmatus*), males perform foot-flagging and advertisement calls to defend their territories against conspecific intruders (Preininger et al. 2009). Our results show that more aggressive males react fiercer towards conspecific territory intruders. This is of particular ecological relevance, because territory possession is the most important prerequisite for male reproductive success in this species (Ursprung et al. 2011a). However, high levels of aggression might also come with a cost, if more aggressive individuals engage more often in energetically costly and potentially harmful fights.

We also found that the probability to enter a novel environment and the time it took to do so were highly repeatable, and a latent variable explained a relatively large part of the covariance among the two variables. Together, these results suggest the existence of the personality trait “boldness” in *A. femoralis* males and females. Boldness relates to the reaction of an individual to a predator, a novel object, or a conspecific and is relevant in many contexts such as predator avoidance, feeding, or mating (Réale et al. 2007). In *A. femoralis*, a generally high propensity to take risks might be reflected not only in the response to a predation threat, but also in how prominently the advertisement call is presented. Thus, higher risk taking could be reflected in males calling at higher rates, higher amplitude, over longer durations, from more exposed locations, or moving/turning more during calling. In *A. subfolionidificans*, a closely related species, calling activity has been found to be positively related to reproductive success (Souza et al. 2021). Being bold, however, can also be costly as it might lead to more frequent encounters with predators. Future studies are needed to identify the link between calling activity and individual reproductive success, in order to investigate possible trade-offs in *A. femoralis*.

Finally, we found that the distance travelled, the number of jumps performed, and the area covered in a new environment were highly repeatable. Moreover, we found that a latent variable explained some of the covariance among the three behavioral measurements. Together, these results suggest that *A. femoralis* males and females exhibit a personality trait “exploration”. Exploration behavior is especially relevant for dispersal and resource acquisition (Dingemanse et al. 2003; Gruber et al. 2017). In *A. femoralis*, males rely on spatial memory to find water bodies (Pašukonis et al. 2016) and distribute their tadpoles across multiple sites to decrease risks of losing entire clutches due to desiccation or predation (Erich et al. 2015; Ringler et al. 2018). Therefore, being more explorative might enable males to find more sites for tadpole deposition or a better territory to settle, while females put their explorative behaviors at use when looking for a mate and, more rarely, for tadpole transport (Ringler et al. 2015). A highly explorative individual will, however, be more conspicuous to predators or at risk of losing its territory during periods of absence.

Distribution of behaviors across the natural and social environment

Contrary to our expectation, we did not find a relationship between individual behaviors and their natural environment (i.e., habitat complexity and territory size). However, we cannot exclude that other habitat characteristics that we did not measure, such as quantity and quality of the leaf litter, tree species, or canopy cover, are linked to individual behavior. Likewise, we did not find any relationship between individual behavior and territory size, as more aggressive and bolder individuals settled in territories of varying sizes (Fig. 5a, b). This is in line with the previous finding, that in *A. femoralis* only the possession of a territory is important for reproductive success, but not territory size (Ursprung et al. 2011a), and as a consequence males cannot increase their reproductive success by extending territorial space. As already suggested in a previous study, variation in territory sizes is probably strongly dependent on characteristics of the natural environment (Ringler et al. 2017) rather than a consequence of varying levels of aggression in *A. femoralis* males.

More explorative individuals were not necessarily located near the artificial pools on the island (Fig. 5c), although water bodies are of critical importance for individual fitness, given that they are obligate for tadpole development after hatching. Previous studies have shown that males distribute their tadpoles across multiple water bodies located outside their territories (Ringler et al. 2013, 2018; Erich et al. 2015; Beck et al. 2017). This suggests that the ability of an individual to find water bodies once it has settled in a territory is critical, while the location of the territory is not. Finding water bodies in *A. femoralis* is strongly related to olfactory sensing (Serrano-Rojas and

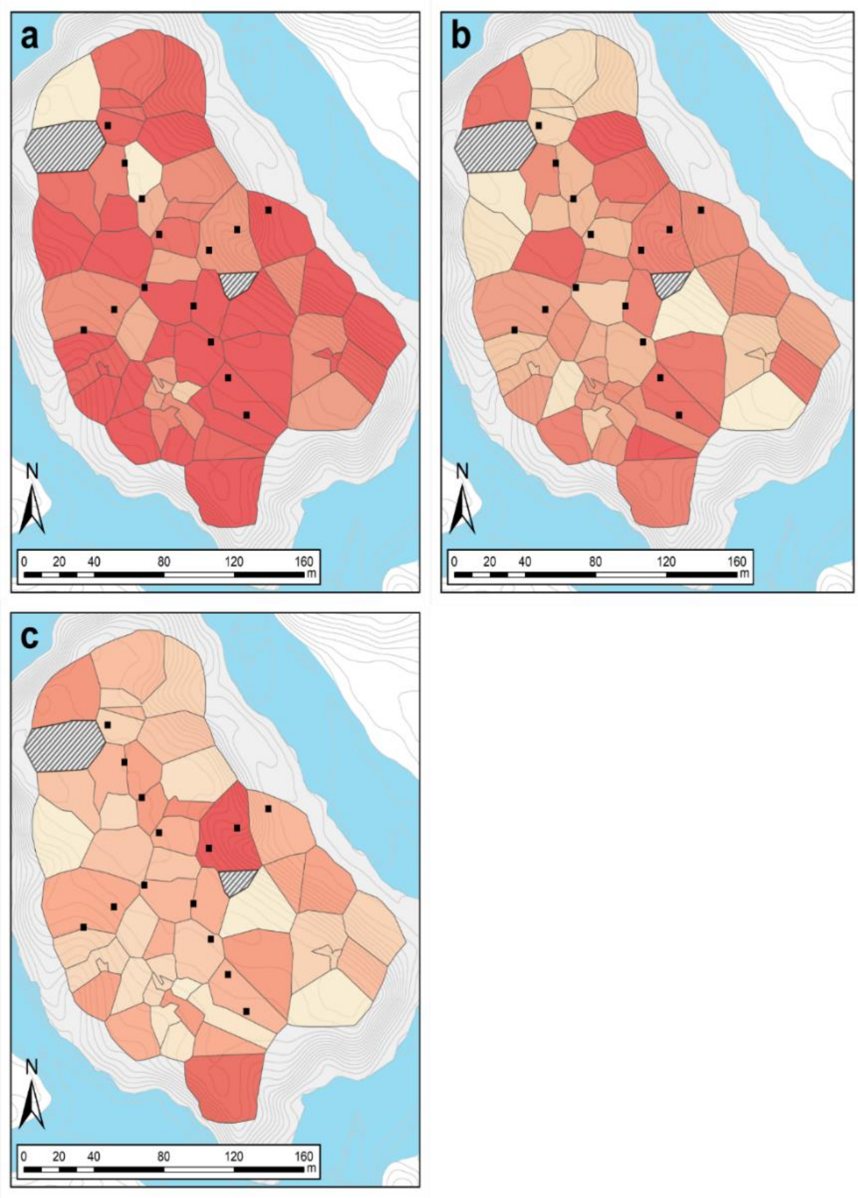


Fig. 5 Maps showing the spatial distribution of individual performance of male frogs on the island in the behavioral essays. The maps use the Voronoi territories of 07 March 2019 when the most individuals were present at the same time on the island. The maps show the mean value, calculated over all respective trials of (A) the individuals' latency to jump in the territorial defense trial, (B) the time spent in the shelter during the Novel Environment Test (NET), and (C) the distance travelled in the NET. All color ramps have 20 equal intervals across the full range of the respective value; darker colors represent shorter latency in (A), shorter time spent in the shelter in (B), and longer distances in (C). Black squares indicate the 14 artificial pools that were in place on the island since 2018; thin grey lines show 50 cm elevation isoclines; the blue area shows the river Arataye. The territories of two males that were not tested in the behavioral assays are shown with a hatched white pattern.

Pašukonis 2021) and spatial memory might help in revisiting such sites once they have been encountered (Pašukonis et al. 2016; Beck et al. 2017). Taken together, our results suggest that *A. femoralis* males establish their territories independent of large-scale resource distribution and the wider structure of the habitat. And likewise, the characteristics of their natural environment apparently are not associated with individual differences in behavior.

We also did not find support for a relationship between aggressiveness and the social environment. We initially expected more aggressive individuals to occupy territories in high density areas, where they would be more likely to find mating partners but also face elevated male-male competition. However, with this trade-off between female availability and intra-sexual competition, males might have equal reproductive outcomes regardless of their level of aggressiveness and independent of the overall density, as long as they manage to establish a territory at all. Future studies should investigate how the interplay between aggressiveness and population density affects reproductive success.

Our results show that exploration- and boldness-related behaviors were positively linked to the number of females in the vicinity to male territories. Since significant correlations were only found at the within-individual level, this suggests that the link between exploration or boldness and the social environment is mainly driven by individual plasticity. This indicates that males, who mostly have stable territories and move little, increase their overall levels of exploration and boldness when the number of females around their territory increases. There are currently no studies showing any direct mechanisms how males could assess the presence and number of nearby females. We suggest that future studies should investigate secondary or indirect cues, such as distribution or density of feeding sites, that might determine female density and that might allow males to identify areas with more females. However, because the among-individual effects have broad credible intervals that are not centered around zero, we cannot rule out the possibility of non-random settlement.

Our study does not claim or identify any causal relationship between behaviors or personality traits and their natural and/or social environment. Still, the identification of such relationship in a natural free-ranging population of animals provides a first step towards understanding the mechanisms underlying the distribution of behaviors across space (cf. Archard and Braithwaite 2010).

Conclusion

We studied the structure of personality traits and the distribution of behaviors across the environment in an entire free-ranging population of the poison frog *A. femoralis* by measuring several behaviors in situ in different contexts. We found that *A. femoralis* indeed exhibits animal personality along the aggressiveness, exploration and boldness axes. Furthermore, we found non-random distribution of behaviors across the animals' social environment, which may allow individuals to cope with their complex socio-ecological environment. While amphibians have been largely overlooked in animal personality research, this study is one of the first comprehensive study of animal personality in amphibian in the wild. By providing a detailed description of how behavioral measurements are structured in functional units, that allow individuals to cope with their socio-ecological environment, it broadens our understanding of the functional role of behavior in frogs and offers a first step towards understanding the mechanisms that play a role in the emergence and maintenance of behavioral variation.

Data availability statement

The datasets generated during and/or analyzed during the current study are available in the Open Science Framework repository: https://doi.org/10.17605/OSF.IO/AJV5X

Authors contributions

Conceptualization: MP, YA, ER; Methodology: MP, LB, SC, KD, CL, YA, PW, MR, ER; Formal Analysis: MP, LB, MR; Investigation: MP, SC, KD, CL, YA, MR, ER; Resources: ER; Data curation: MP, MR, CL; Writing – original draft: MP; Writing – review and editing: MP, YA, PW, MR, ER; Supervision: MP, MR, ER; Project administration: MR, ER; Funding acquisition: ER.

Acknowledgments

The authors thank B. Furdi for help in processing the data and R. Fouchier for providing Fig. 1. The authors also thank E. Haeler and C. Eliasch for their assistance during field work, and B. Szabo for comments on earlier versions of this manuscript. We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. We thank the Nouragues research field station (managed by CNRS) which benefits from 'Investissement d'Avenir' grants managed by Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). This work has been done on the Natural Reserve of Nouragues. We thank the editors and two anonymous reviewers for valuable comments on this and previous versions of the manuscript. Open access funding provided by University of Bern. This work was funded through a Standalone Grant (FWF P-31518) from the Austrian Science Fund (FWF). MR was supported by an Erwin Schrödinger Grant (FWF J-3868) and a Standalone Grant (FWF P-33728) from the Austrian Science Fund (FWF).

Supplementary material

Manuscript 3: “Exploring links between personality traits and their social and non-social environments in wild poison frogs”

Territory size approximation via Voronoi polygons

We used Dirichlet tessellation in ArcGIS to approximate male territories as Voronoi polygons (Voronoi 1908) on a day-to-day basis. With this temporal fine-scale approach we accounted for eventual territory shifts and dynamic fluctuations within the population. While Voronoi polygons tend to overestimate absolute territory size, as they partition the entire available space amongst all individuals, they however provide good relative estimates of the space to which an individual has exclusive access. Compared to other polygon methods, Voronoi polygons yield stable area estimates with very low numbers of capture points, and they are robust against differences in the number of capture points between individuals (Wray et al. 1992).

Across the field season, we are confident that we sampled most, if not all, adult males on the island in this period, but we did not manage to sample the entire population of males every day. This daily under-sampling would have resulted in an overestimation of territory sizes in the beginning and in the end by partitioning the entire available space only amongst the males actually sampled during this time. Prior to our personality assessment trials we performed five days of baseline capture-recapture sampling. Thus, for early trial days we did not need to compensate our territory estimations for incomplete sampling. To compensate for males that were not captured anymore during the last days of trials, and to not overestimate late territory sizes, we used the mark-recapture data from our baseline sampling to estimate the true population size at the end of the field season. We used daily individual capture histories of all individuals (males and females) with the POPAN formulation (Schwarz and Arnason 1996) in the program MARK (White and Burnham 1999; White 2020). We estimated groupwise (g; based on sex) day-to-day survival probability (φ), probability of detection (p), probability of entry into the population (pent), and cumulative population size (N), from where we derived daily estimates of male and female population size. We used the following link functions for our model parameters: φ: sin; p: sin; pent: MLogit; N: log. We built several group/sex (g) dependent models with constant (.) and time dependent (t) parameters and selected the best supported model for our data based on its Akaike's Information Criterion corrected for small sample sizes (AIC$_c$) (Supplementary Table 1).

We compared our population estimates with the number of males known to be alive (i.e. individuals sampled within the last 5 days in 2019 and those found to be still alive in 2020). We then went backwards in the capture histories and incorporated capture points of further males in the territory estimation, assuming that they still had been alive during the last days but had not been captured anymore. We did this until the number of males included in the territory estimation matched the number of males estimated from the best fitting POPAN model.

Supplementary Table 1 Models evaluated in our POPAN estimation using the parameters apparent survival (φ), recapture probability (p), probability of entry (pent), and cumulative population size (N). Indices denote constant (.), time-dependent (t), and group/sex-dependent (g) parameters.

Model	**AIC$_c$**	**Δ AIC$_c$**
φ(.,g), p(t,g), pent(t,g), N(g)	5079.44	
φ(.,g), p(.,g), pent(.,g), N(g)	5137.27	57.83
φ(t,g), p(.,g), pent(t,g), N(g)	5411.13	331.68
φ(.,g), p(.,g), pent(t,g), N(g)	5437.55	358.11
φ(t), p(t), pent(t), N(g)	5503.57	424.13
φ(t,g), p(t,g), pent(t,g), N(g)	5539.30	459.85
φ(t,g), p(.,g), pent(t,g), N(g)	16250.09	11170.64
φ(.,g), p(t,g), pent(.,g), N(g)	Never reached numerical convergence	
φ(t,g), p(t,g), pent(.,g), N(g)	Never reached numerical convergence	

Bayesian Generalized Linear Mixed Models

Since the covariance used in the SEM represents a combination of within and between individual effects, we also built two multivariate models using Bayesian Generalized Linear Mixed Models (Hadfield 2010) to study how the behaviors measured during the territorial defense trial and the NET vary within and among individuals. To investigate the among- and within- individual covariance between behaviors we scaled all the behaviors (i.e., centered to their mean value and standardized to units of 1 phenotypic standard deviation), with the exception of the binomial variables (i.e., inter-bout jumps and probability to enter the box). We included as response variables the four behaviors measured during the territory defense trial in one model, and the five behaviors measured during the NET in the second model. We used the function 'transformTukey' to apply a constant transformation on the speed to reach the speaker, the distance travelled, and the time spent in the shelter. We included age as a trait-specific fixed effects to control for effects of age on all our response variables. To calculate the variance due to differences among individuals and the covariance between measured behaviors, we fitted an unstructured covariance matrix for the grouping variable ID. To get the same information at the within-individual level, and to account for the repeated measurements, we fitted an unstructured covariance matrix for the residual (co)variation. We then used the posterior distributions to estimate the mean and credible intervals for the among and within-individual correlations and covariances between each of the behaviors measured (Supplementary Tables 2, 3). We verified the absence of autocorrelation (correlation between lags <0.1; Hadfield 2010), sufficient mixing (visual inspection of plots of Markov-Chain-Monte-Carlo chains), and performed a Heidelberg and Welch diagnostic test to assess that the Markov chain was run for long enough.

Supplementary Table 2 Covariance and 95% credible intervals between behaviors measured during a territorial defense trial. Estimates were calculated based on a MCMCglmm model investigating the correlations between the four behaviors measured ('latency until head-body orientation' and 'until the first jump', 'inter-bout jumps' and 'speed to reach the speaker').

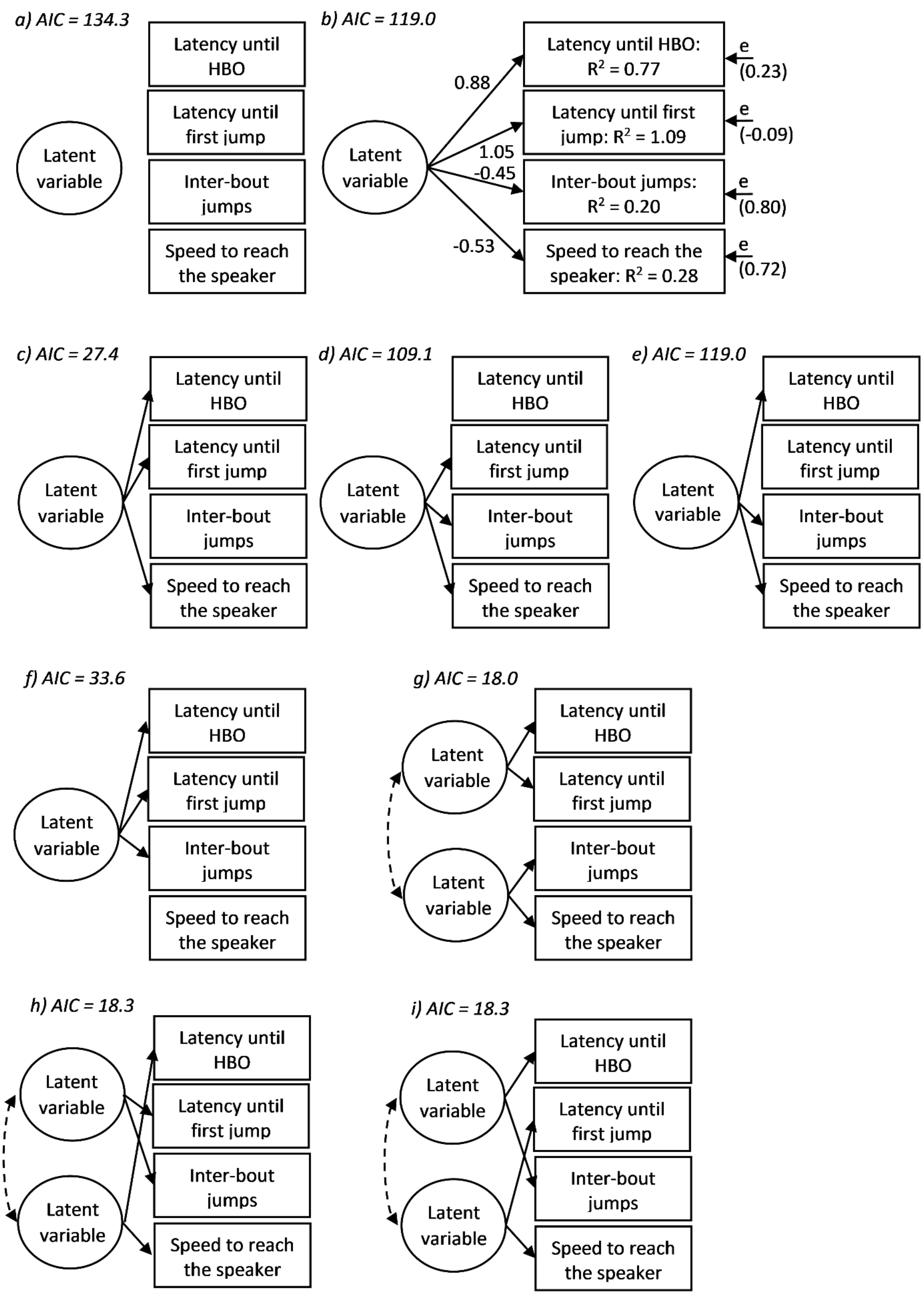
	among-individual	**within-individual**
head – head	0.24 (0.05; 0.53)	0.84 (0.64; 1.09)
jump – head	0.22 (0.05; 0.5)	0.73 (0.55; 0.95)
speed – head	-0.24 (-0.48; -0.06)	-0.25 (-0.41; -0.11)
inter-bout – head	-0.52 (-1.4; -0.01)	-0.77 (-0.98; -0.39)
head – jump	0.22 (0.05; 0.5)	0.73 (0.55; 0.95)
jump – jump	0.24 (0.07; 0.52)	0.84 (0.65; 1.07)
speed – jump	-0.26 (-0.51; -0.09)	-0.34 (-0.51; -0.2)
inter-bout – jump	-0.57 (-1.42; -0.06)	-0.85 (-1; -0.63)
head – speed	-0.24 (-0.48; -0.06)	-0.25 (-0.41; -0.11)
jump – speed	-0.26 (-0.51; -0.09)	-0.34 (-0.51; -0.2)
speed – speed	0.38 (0.16; 0.72)	0.67 (0.51; 0.88)
inter-bout – speed	0.52 (0.04; 1.21)	0.25 (-0.16; 0.62)
head – inter-bout	-0.52 (-1.4; -0.01)	-0.77 (-0.98; -0.39)
jump – inter-bout	-0.57 (-1.42; -0.06)	-0.85 (-1; -0.63)
speed – inter-bout	0.52 (0.04; 1.21)	0.25 (-0.16; 0.62)
inter-bout – inter-bout	1.85 (0.11; 6.03)	1 (1; 1)

Supplementary Table 3 Covariance and 95% credible intervals between behaviors measured during a Novel Environment Test (NET). Estimates were calculated based on a MCMCglmm model investigating the correlations between the five behaviors measured (‘time spent in the shelter’, ‘probability to enter the box’, ‘distance travelled’, ‘number of jumps’ and ‘number of areas visited’ in the NET).

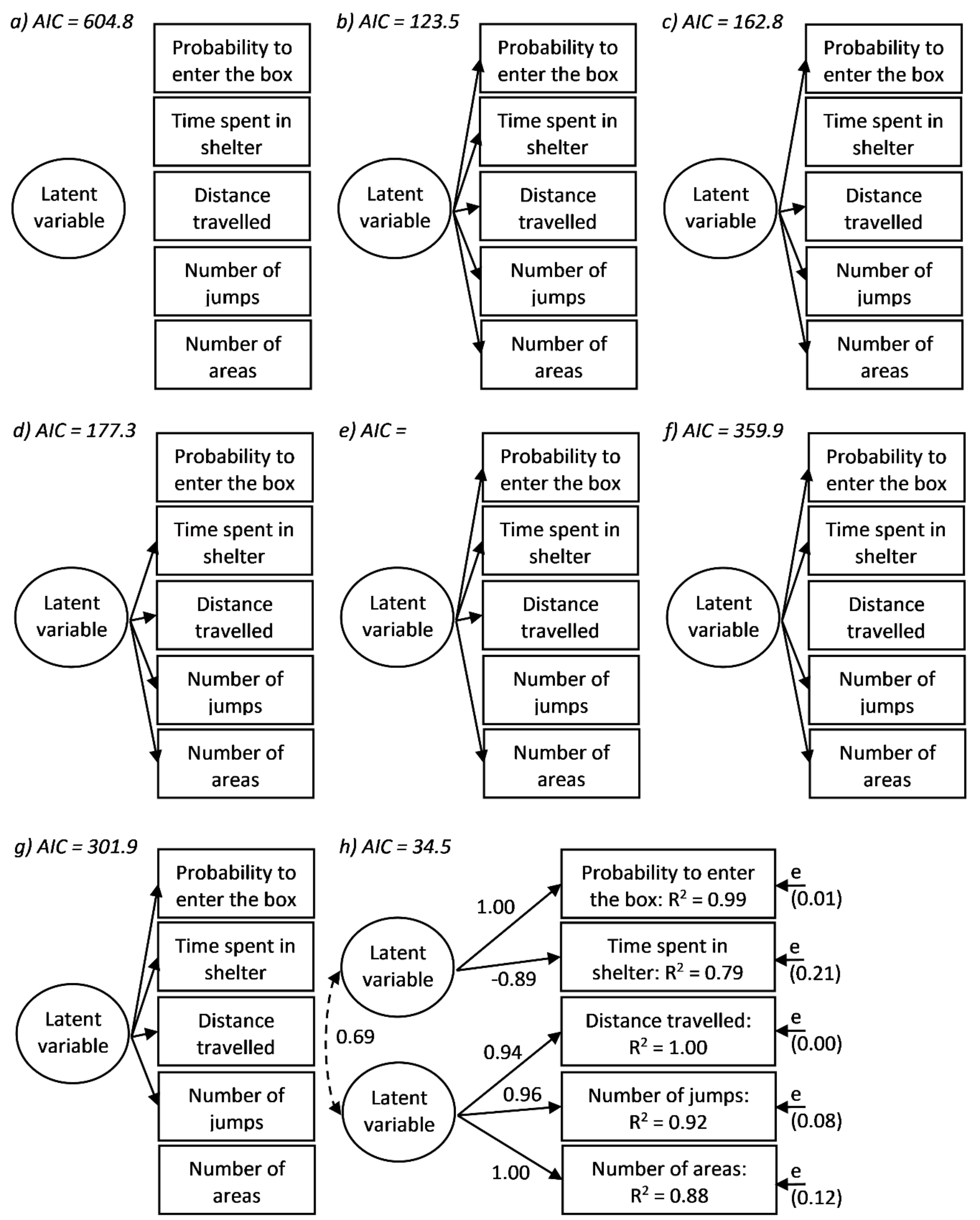
	among-individual	**within-individual**
time-time	0.39 (0.22, 0.63)	0.65 (0.53, 0.8)
jump-time	-0.33 (-0.52, -0.19)	-0.01 (-0.11, 0.08)
areas-time	-0.34 (-0.53, -0.2)	-0.01 (-0.11, 0.08)
distance-time	-0.39 (-0.59, -0.23)	-0.04 (-0.13, 0.04)
goin-time	-1.7 (-2.82, -0.93)	-0.51 (-0.69, -0.31)
time-jump	-0.33 (-0.52, -0.19)	-0.01 (-0.11, 0.08)
jump-jump	0.4 (0.22, 0.64)	0.66 (0.53, 0.81)
areas-jump	0.33 (0.17, 0.55)	0.58 (0.46, 0.73)
distance-jump	0.41 (0.23, 0.66)	0.54 (0.43, 0.67)
goin-jump	1.69 (0.9, 2.83)	0.43 (0.21, 0.64)
time-areas	-0.34 (-0.53, -0.2)	-0.01 (-0.11, 0.08)
jump-areas	0.33 (0.17, 0.55)	0.58 (0.46, 0.73)
areas-areas	0.34 (0.18, 0.56)	0.69 (0.56, 0.84)
distance-areas	0.39 (0.21, 0.62)	0.59 (0.48, 0.73)
goin-areas	1.62 (0.88, 2.7)	0.51 (0.29, 0.7)
time-distance	-0.39 (-0.59, -0.23)	-0.04 (-0.13, 0.04)
jump-distance	0.41 (0.23, 0.66)	0.54 (0.43, 0.67)
areas-distance	0.39 (0.21, 0.62)	0.59 (0.48, 0.73)
distance-distance	0.47 (0.28, 0.73)	0.58 (0.47, 0.7)
goin-distance	1.93 (1.07, 3.18)	0.56 (0.38, 0.71)
time-goin	-1.7 (-2.82, -0.93)	-0.51 (-0.69, -0.31)
jump-goin	1.69 (0.9, 2.83)	0.43 (0.21, 0.64)
areas-goin	1.62 (0.88, 2.7)	0.51 (0.29, 0.7)
distance-goin	1.93 (1.07, 3.18)	0.56 (0.38, 0.71)
goin-goin	8.59 (3.65, 17.58)	1 (1, 1)

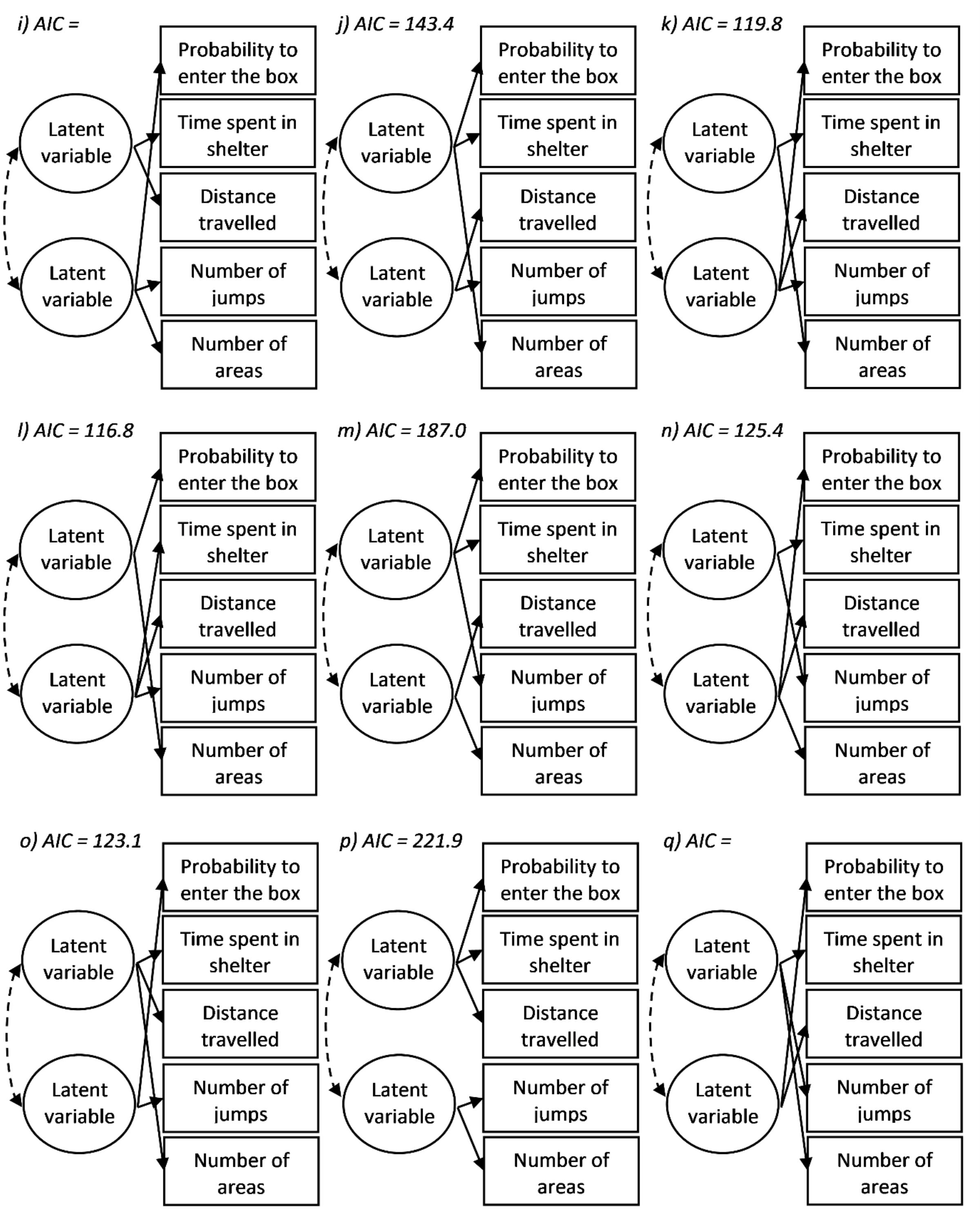
Supplementary Table 4 Between- and within-individual covariance and slope between three behaviors that best represents the personality traits aggressiveness (‘latency until first jump’), exploration (‘distance travelled’) and boldness (‘time in shelter’) and measurements of the natural and social environment. We present the mean and 95% credible intervals.

		covariance		**slope**	
		among-individual	**within-individual**	**among-individual**	**within-individual**
behavior	**environmental measure**	**mean (95% Credible Interval)**		**mean (95% Credible Interval)**	
latency to jump	habitat complexity	0 (-0.03; 0.03)	0 (0; 0)	0.08 (-2.26; 2.36)	-25.13 (-6045.11; 6005.22)
	territory size	0.02 (-0.16; 0.2)	-0.03 (-0.14; 0.08)	0.06 (-0.53; 0.66)	-0.16 (-0.82; 0.49)
	female neighbors	0 (-0.12; 0.12)	0.01 (-0.04; 0.07)	-0.01 (-0.83; 0.82)	0.3 (-1.05; 1.61)
	male neighbors	0.01 (-0.08; 0.1)	0.05 (-0.03; 0.13)	0.14 (-1.41; 1.69)	0.68 (-0.37; 1.71)
distance travelled	habitat complexity	-0.22 (-1.35; 0.80)	0 (0; 0)	-16.05 (-92.13; 56.86)	-1078.43 (-179728.2; 185096.0)
	territory size	-0.25 (-5.56; 4.93)	0.54 (-2.99; 4.19)	-0.88 (-18.65; 16.58)	3.13 (-17.74; 23.74)
	female neighbors	-0.86 (-4.70; 2.88)	1.80 (0.07; 3.83)	-6.30 (-32.41; 19.43)	40.63 (1.51; 80.78)
	male neighbors	-1.17 (-4.58; 1.57)	0.28 (-1.97; 2.52)	-18.11 (-67.59; 25.34)	3.87 (-26.85; 33.70)
time in shelter	habitat complexity	0.02 (-0.16; 0.20)	0 (0; 0)	1.66 (-11.45; 14.14)	-207.41 (-34767.74; 34900.15)
	territory size	0.39 (-0.49; 1.41)	-0.13 (-0.85; 0.54)	1.34 (-1.62; 4.71)	-0.76 (-4.60; 3.11)
	female neighbors	0.21 (-0.39; 0.91)	-0.36 (-0.74; -0.03)	1.53 (-2.62; 6.15)	-8.26 (-15.62; -0.85)
	male neighbors	-0.05 (-0.57; 0.45)	-0.07 (-0.50; 0.37)	-0.70 (-8.38; 7.19)	-0.96 (-6.79; 4.96)


a) AIC = 134.3
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
b) AIC = 119.0
Latent variable
0.88
1.05
-0.45
-0.53
Latency until HBO: $R^2 = 0.77$
Latency until first jump: $R^2 = 1.09$
Inter-bout jumps: $R^2 = 0.20$
Speed to reach the speaker: $R^2 = 0.28$
e (0.23)
e (-0.09)
e (0.80)
e (0.72)
c) AIC = 27.4
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
d) AIC = 109.1
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
e) AIC = 119.0
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
f) AIC = 33.6
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
g) AIC = 18.0
Latent variable
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
h) AIC = 18.3
Latent variable
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker
i) AIC = 18.3
Latent variable
Latent variable
Latency until HBO
Latency until first jump
Inter-bout jumps
Speed to reach the speaker

Supplementary Fig. 1 Path diagrams of nine structure equation models (SEMs) explaining the covariance structure among four behaviors assessed during an aggressivity test. ‘HBO’ refers to the latency until the first head-body orientation. Models propose different scenario in which a latent variable describing ‘aggressiveness’ is derived from all or part of the behaviors measured during an aggressivity test. Each model with two latent variables was tested without and with (double-sided dashed arrow) a correlation among the two latent variables. SEMs were evaluated based on difference in Akaike’s information criterion (AIC) values, with small values indicating a better parsimony. All models tested without a correlation was under identified and thus no AIC could be calculated. The more parsimonious model has numbers in the square which represents the variances of the different behaviors explained by the SEM structure (R^2). Numbers associated with arrows are standardized factor loadings which represent how behavioral responses are predicted to change based on changes to the latent variable. Number in brackets represent variances of residuals or error variances (e) associated to each behavior.


a) AIC = 604.8
b) AIC = 123.5
c) AIC = 162.8
d) AIC = 177.3
e) AIC =
f) AIC = 359.9
g) AIC = 301.9
h) AIC = 34.5
Latent variable
Probability to enter the box
Time spent in shelter
Distance travelled
Number of jumps
Number of areas
Probability to enter the box: R² = 0.99
Time spent in shelter: R² = 0.79
Distance travelled: R² = 1.00
Number of jumps: R² = 0.92
Number of areas: R² = 0.88
1.00
-0.89
0.69
0.94
0.96
1.00
e (0.01)
e (0.21)
e (0.00)
e (0.08)
e (0.12)


i) AIC =

j) AIC = 143.4

k) AIC = 119.8

l) AIC = 116.8

m) AIC = 187.0

n) AIC = 125.4

o) AIC = 123.1

p) AIC = 221.9

q) AIC =

Supplementary Fig. 2 Path diagrams of twenty-six structure equation models (SEMs) explaining the covariance structure among five behaviors assessed during a Novel Environment Test (NET). Models propose different scenario in which two latent variables describing ‘exploration’ and ‘boldness’ are derived from all or part of the behaviors measured during the NET. Each model with two latent variables was tested without and with (double-sided dashed arrow) a correlation among the two latent variables. SEMs were evaluated based on difference in Akaike’s information criterion (AIC) values, with small values indicating a better parsimony. All models tested without a correlation was under identified and thus no AIC could be calculated. Models (e) and (i) were also under identified and have no AIC. The more parsimonious model has numbers in the square which represents the variances of the different behaviors explained by the SEM structure (R^2). Numbers associated with arrows are standardized factor loadings which represent how behavioral responses are predicted to change based on changes to the latent variable. Number in brackets represent variances of residuals or error variances (e) associated to each behavior.

References

AmphibiaWeb (2022) AmphibiaWeb: Information on amphibian biology and conservation, www.amphibiaweb.org

Araya-Ajoy YG, Dingemanse NJ (2014) Characterizing behavioural ‘characters’: an evolutionary framework. Proc R Soc Lond B 281:20132645. https://doi.org/10.1098/rspb.2013.2645

Archard GA, Braithwaite VA (2010) The importance of wild populations in studies of animal temperament. J Zool 281:149–160. https://doi.org/10.1111/j.1469-7998.2010.00714.x

ASAB (2020) Guidelines for the treatment of animals in behavioural research and teaching. Anim Behav 159:I–XI. https://doi.org/10.1016/j.anbehav.2019.11.002

Beck KB, Loretto M-C, Ringler M, Hödl W, Pašukonis A (2017) Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. PeerJ 5:e3745. https://doi.org/10.7717/peerj.3745

Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). J Evol Biol 18:464–473. https://doi.org/10.1111/j.1420-9101.2004.00817.x

Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Anim Behav 77:771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Bolger DT, Morrison TA, Vance B, Lee D, Farid H (2012) A computer-assisted system for photographic mark–recapture analysis. Methods Ecol Evol 3:813–822. https://doi.org/10.1111/j.2041-210X.2012.00212.x

Bongers F, Charles-Dominique P, Forget P-M, Théry M (eds) (2001) Nouragues: Dynamics and plant-animal interactions in a Neotropical rainforest. Monographiae biologicae, v. 80. Kluwer Academic Publishers, Dordrecht, Netherlands

Brewster R (2019) paint.net, https://www.getpaint.net/

Brodin T, Lind MI, Wiberg MK, Johansson F (2013) Personality trait differences between mainland and island populations in the common frog (Rana temporaria). Behav Ecol Sociobiol 67:135–143. https://doi.org/10.1007/s00265-012-1433-1

Brown D (2019) Tracker: Video analysis and modeling tool for physics education. Open Source Physics, Davidson, NC, USA, https://physlets.org/tracker/

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical information-theoretic approach, 2nd edn. Springer, New York, NY, USA

Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R (2013) Animal personality: what are behavioural ecologists measuring? Biol Rev 88:465–475. https://doi.org/10.1111/brv.12007

Chaloupka S, Peignier M, Stückler S, Araya-Ajoy Y, Walsh P, Ringler M, Ringler E (2022) Repeatable territorial aggression in a Neotropical poison frog. Front Ecol Evol 10:881387. https://doi.org/10.3389/fevo.2022.881387

Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol Evol 10:248–252. https://doi.org/10.1016/S0169-5347(00)89081-3

Crump ML (1972) Territoriality and mating behavior in Dendrobates granuliferus (Anura: Dendrobatidae). Herpetologica 28:195–198

Dingemanse NJ, Araya-Ajoy YG (2015) Interacting personalities: Behavioural ecology meets quantitative genetics. Trends Ecol Evol 30:88–97

Dingemanse NJ, Both C, Drent PJ, Tinbergen JM (2004) Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc Lond B 271:847-852

Dingemanse NJ, Kazem AJN, Réale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol 25:81–89. https://doi.org/10.1016/j.tree.2009.07.013

Dingemanse NJ, Both C, van Noordwijk AJ, Rutten AL, Drent PJ (2003) Natal dispersal and personalities in great tits (Parus major). Proc R Soc Lond B 270:741–747. https://doi.org/10.1098/rspb.2002.2300

Dochtermann NA, Jenkins SH (2007) Behavioural syndromes in Merriam’s kangaroo rats (Dipodomys merriami): a test of competing hypotheses. Proc R Soc Lond B 274:2343–2349.

https://doi.org/10.1098/rspb.2007.0622

Duckworth RA, Kruuk LEB (2009) Evolution of genetic integration between dispersal and colonization ability in a bird. Evolution 63:968–977. https://doi.org/10.1111/j.1558-5646.2009.00625.x

Erich M, Ringler M, Hödl W, Ringler E (2015) Brood-partitioning behaviour in unpredictable environments: hedging the bets? Behav Ecol Sociobiol 69:1011–1017. https://doi.org/10.1007/s00265-015-1913-1

Fischer M-T, Ringler M, Ringler E, Pašukonis A (2020) Reproductive behavior drives female space use in a sedentary Neotropical frog. PeerJ 8:e8920. https://doi.org/10.7717/peerj.8920

Fox J, Nie Z, Byrnes J, Culbertson M, DebRoy S, Friendly M, Goodrich B, Jones RH, Kramer A, Monette G (2020) Package ‘sem’: Structural Equation Models, https://CRAN.R-project.org/package=sem

Friard O, Gamba M, Fitzjohn R (2016) BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol 7:1325–1330. https://doi.org/10.1111/2041-210X.12584

Gasser H, Amézquita Torres A, Hödl W (2009) Who is calling? Intraspecific call variation in the aromobatid frog Allobates femoralis. Ethology 115:596–607. https://doi.org/10.1111/j.1439-0310.2009.01639.x

Gifford ME, Clay TA, Careau V (2014) Individual (co)variation in standard metabolic rate, feeding rate, and exploratory behavior in wild-caught semiaquatic salamanders. Physiol Biochem Zool 87:384–396. https://doi.org/10.1086/675974

González-Bernal E, Brown GP, Shine R (2014) Invasive cane toads: social facilitation depends upon an individual’s personality. PLoS ONE 9:e102880. https://doi.org/10.1371/journal.pone.0102880

Goursot C, Düpjan S, Kanitz E, Tuchscherer A, Puppe B, Leliveld LMC (2019) Assessing animal individuality: links between personality and laterality in pigs. Curr Zool 65:541–551. https://doi.org/10.1093/cz/zoy071

Gruber J, Brown G, Whiting MJ, Shine R (2017) Is the behavioural divergence between range-core and range-edge populations of cane toads (Rhinella marina) due to evolutionary change or developmental plasticity? R Soc Open Sci 4:170789. https://doi.org/10.1098/rsos.170789

Hadfield JD (2010) MCMC Methods for multi-response generalized linear mixed models: the MCMCglmmR package. J Stat Soft 33:1–22. https://doi.org/10.18637/jss.v033.i02

Hensley NM, Cook TC, Lang M, Petelle MB, Blumstein DT (2012) Personality and habitat segregation in giant sea anemones (Condylactis gigantea). J Exp Mar Biol Ecol 426-427:1–4. https://doi.org/10.1016/j.jembe.2012.05.011

Hödl W (1983) Phyllobates femoralis (Dendrobatidae): Rufverhalten und akustische Orientierung der Männchen (Freilandaufnahmen). Wissenschaftlicher Film. Bundesstaatliche Hauptstelle für Wissenschaftliche Kinematographie, Wien

Hödl W (1987) Dendrobates femoralis (Dendrobatidae): a handy fellow for frog bioacoustics. In: van Gelder J. J., Strijbosch H, Bergers P (eds) Proceedings of the 4th Ordinary General meeting of the Societas Europaea Herpetologica, Nijmegen, Netherlands

Hödl W, Amezquita A (2001) Visual signaling in anuran amphibians. In: Ryan MJ (ed) Anuran communication. Smithsonian Institution Press, Washington, DC, USA, pp 121–141

Houslay TM, Wilson AJ (2017) Avoiding the misuse of BLUP in behavioural ecology. Behav Ecol 28:948–952. https://doi.org/10.1093/beheco/arx023

Kelleher SR, Silla AJ, Byrne PG (2018) Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. Behav Ecol Sociobiol 72:79. https://doi.org/10.1007/s00265-018-2493-7

Maes J, van Damme R, Matthysen E (2013) Individual and among-population variation in dispersal-related traits in Natterjack toads. Behav Ecol 24:521–531. https://doi.org/10.1093/beheco/ars193

Narins PM, Hödl W, Grabul DS (2003) Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. P Natl Acad Sci USA 100:577–580.

https://doi.org/10.1073/pnas.0237165100

Pašukonis A, Ringler M, Brandl HB, Mangione R, Ringler E, Hödl W (2013) The homing frog: high homing performance in a territorial dendrobatid frog Allobates femoralis (Dendrobatidae). Ethology 119:762–768. https://doi.org/10.1111/eth.12116

Pašukonis A, Trenkwalder K, Ringler M, Ringler E, Mangione R, Steininger J, Warrington I, Hödl W (2016) The significance of spatial memory for water finding in a tadpole-transporting frog. Anim Behav 116:89–98. https://doi.org/10.1016/j.anbehav.2016.02.023

Pigliucci M (2003) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol Lett 6:265–272. https://doi.org/10.1046/j.1461-0248.2003.00428.x

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 20:481–486. https://doi.org/10.1016/j.tree.2005.06.001

Preininger D, Böckle M, Hödl W (2009) Communication in noisy environments II: Visual signaling behavior of male foot-flagging frogs Staurois latopalmatus. Herpetologica 65:166–173. https://doi.org/10.1655/08-037R.1

Pröhl H (2005) Territorial behavior in dendrobatid frogs. J Herpetol 39:354–365. https://doi.org/10.1670/162-04A.1

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org

Rasband WS (1997-2021) ImageJ. U. S. National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/

Réale D, Dingemanse NJ (2010) Personality and individual social specialisation. In: Székely T, Moore AJ, Komdeur J (eds) Social behaviour: Genes, ecology and evolution. Cambridge University Press, Cambridge, UK, pp 417–441

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Ringler E, Mangione R, Ringler M (2014) Where have all the tadpoles gone? Individual genetic tracking of amphibian larvae until adulthood. Mol Ecol Resour 15:737–746. https://doi.org/10.1111/1755-0998.12345

Ringler E, Pašukonis A, Fitch WT, Huber L, Hödl W, Ringler M (2015) Flexible compensation of uniparental care: female poison frogs take over when males disappear. Behav Ecol 26:1219–1225. https://doi.org/10.1093/beheco/arv069

Ringler E, Pašukonis A, Hödl W, Ringler M (2013) Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. Front Zool 10:67. https://doi.org/10.1186/1742-9994-10-67

Ringler E, Ringler M, Jehle R, Hödl W (2012) The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. PLoS ONE 7:e40237. https://doi.org/10.1371/journal.pone.0040237

Ringler E, Szipl G, Harrigan RJ, Bartl-Binder P, Mangione R, Ringler M (2018) Hierarchical decision-making balances current and future reproductive success. Mol Ecol 27:2289–2301. https://doi.org/10.1111/mec.14583

Ringler M, Mangione R, Pašukonis A et al (2016) High-resolution forest mapping for behavioural studies in the Nature Reserve 'Les Nouragues', French Guiana. J Maps 12:26–32. https://doi.org/10.1080/17445647.2014.972995

Ringler M, Ringler E, Magaña Mendoza D, Hödl W (2011) Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. PLoS ONE 6:e25844. https://doi.org/10.1371/journal.pone.0025844

Ringler M, Szipl G, Hödl W, Khil L, Kofler B, Lonauer M, Provin C, Ringler E (2017) Acoustic ranging in poison frogs—it is not about signal amplitude alone. Behav Ecol Sociobiol 71:114. https://doi.org/10.1007/s00265-017-2340-2

Ringler M, Ursprung E, Hödl W (2009) Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog Allobates femoralis (Aromobatidae). Behav Ecol Sociobiol 63:1281–1293. https://doi.org/10.1007/s00265-009-0793-7

Rodriguez A, Zhang H, Klaminder J, Brodin T, Andersson PL, Andersson M (2018) ToxTrac: a fast and robust software for tracking organisms. Methods Ecol Evol 9:460–464. https://doi.org/10.1111/2041-210X.12874

Roithmair ME (1994) Male territoriality and female mate selection in the dart-poison frog Epipedobates trivattatus (Dendrobatidae, Anura). Copeia 1994:107–115. https://doi.org/10.2307/1446676

Rojas B, Pašukonis A (2019) From habitat use to social behavior: natural history of a voiceless poison frog, Dendrobates tinctorius. PeerJ 7:e7648. https://doi.org/10.7717/peerj.7648

RStudio Team (2019) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA, http://www.rstudio.com

Schwarz CJ, Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52:860–873. https://doi.org/10.2307/2533048

Serrano-Rojas SJ, Pašukonis A (2021) Tadpole-transporting frogs use stagnant water odor to find pools in the rainforest. J Exp Biol https://doi.org/10.1242/jeb.243122

Sih A, Cote J, Evans M, Fogarty S, Pruitt J (2012) Ecological implications of behavioural syndromes. Ecol Lett 15:278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x

Souza JRD, Kaefer IL, Lima AP (2021) Calling activity determines male mating success in a territorial frog with parental care. Ethology 127:359-365. https://doi.org/10.1111/eth.13135

Sprau P, Dingemanse NJ (2017) An approach to distinguish between plasticity and non-random distributions of behavioral types along urban gradients in a wild passerine bird. Front Ecol Evol 5:92. https://doi.org/10.3389/fevo.2017.00092

Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8:1639–1644. https://doi.org/10.1111/2041-210X.12797

Toledo LF, Araújo OGS, Guimarães LD, Lingnau R, Haddad CFB (2007) Visual and acoustic signaling in three species of Brazilian nocturnal tree frogs (Anura, Hylidae). Phyllomedusa 6:61–68. https://doi.org/10.11606/issn.2316-9079.v6i1p61-68

Tremmel M, Müller C (2013) Insect personality depends on environmental conditions. Behav Ecol 24:386–392. https://doi.org/10.1093/beheco/ars175

Ursprung E, Ringler M, Hödl W (2009) Phonotactic approach pattern in the neotropical frog Allobates femoralis: a spatial and temporal analysis. Behaviour 146:153–170. https://doi.org/10.1163/156853909X410711

Ursprung E, Ringler M, Jehle R, Hödl W (2011a) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Mol Ecol 20:1759–1771. https://doi.org/10.1111/j.1365-294X.2011.05056.x

Ursprung E, Ringler M, Jehle R, Hödl W (2011b) Toe regeneration in the neotropical frog Allobates femoralis. Herpetol J 21:83–86

VideoLAN (2021) VLC media player. VideoLAN, Paris, France, https://www.videolan.org/vlc/

Voronoi G (1908) Nouvelles applications des paramètres continus à la théorie des formes quadratiques. Premier mémoire. Sur quelques propriétés des formes quadratiques positives parfaites. J Reine Angew Math 1908:97-102. https://doi.org/10.1515/crll.1908.133.97

Wells KD (2007) The ecology and behavior of amphibians. University of Chicago Press, Chicago, IL, USA

White GC (2020) MARK. Colorado State University, Fort Collins, CO, USA, http://phidot.org/software/mark/index.html

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120–S139. https://doi.org/10.1080/00063659909477239

Wray S, Cresswell WJ, Rogers D (1992) Dirichlet tessellations: a new, non-parametric approach to home range analysis. In: Priede IG, Swift SM (eds) Wildlife telemetry: remote monitoring and tracking animals. Ellis Horwood, New York, pp 247–255.

Yang Y, Blomenkamp S, Dugas MB, Richards-Zawacki CL, Pröhl H (2019) Mate choice versus mate preference: inferences about color-assortative mating differ between field and lab assays of poison frog behavior. Am Nat 193:598–607. https://doi.org/10.1086/702249

Zidar J, Balogh A, Favati A, Jensen P, Leimar O, Løvlie H (2017) A comparison of animal personality and coping styles in the red junglefowl. Anim Behav 130:209–220. https://doi.org/10.1016/j.anbehav.2017.06.024

Manuscript 4: "The link between animal personality and habitat selection in a neotropical poison frog"

This manuscript is currently under review in *Behavior*
10 August 2022

Lauriane Bégué[1,2], Mélissa Peignier[1] & Eva Ringler[1]

Affiliations:
[1] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[2] Department of Biology and Ecology, University of Montpellier, France

*Correspondence:
Eva Ringler
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
eva.ringler@unibe.ch

Abstract

Environmental variation plays a key role in the evolution and maintenance of animal personality. Individuals with contrasting personalities might exhibit different habitat preferences. Alternatively, variation in individual behaviour across space could arise as a plastic adaptation to distinct habitats. Our study aims to investigate if habitat choice is influenced by an individual’s personality. To this end, we assessed individual levels of activity, boldness, and exploration in male poison frogs, and performed a habitat choice test under controlled laboratory conditions. Individuals were consistent in their behaviours, but all tested frogs chose the complex over the simple habitat. Individuals that were characterized as bold and very explorative also showed more movements between the two different habitats in the choice test. These results indicate that personality measured in a highly standardized artificial setup, such as a novel environment test, indeed can reflect boldness and exploration related behaviours measured in a more naturalistic setup.

Keywords

Habitat choice, Novel Environment Test, boldness, exploration, habitat complexity, captive population.

Introduction

Empirical evidence for the existence of consistent behavioural differences between individual animals across time and contexts, also termed ‘animal personality’, has been found in various taxa (reviewed in Réale et al., 2007; Gosling, 2008). Yet, we do not fully understand how animal personality arises and is maintained in animal populations (DiRienzo & Montiglio, 2015). The selective pressures induced by environmental heterogeneity have been proposed as potential mechanisms both generating and maintaining individual differences in behaviour within a population (Dingemanse et al., 2004). Situations where personality traits are non-randomly distributed across the natural and social environment are referred to as ‘phenotype by environment correlation’ (Conover & Schultz, 1995; Dingemanse & Araya-Ajoy, 2015). Unfortunately, to date very few studies have investigated whether habitat selection could be driven by animal personality, or whether behaviours are adjusted in response to the environmental conditions an individual is, or has been, exposed to (i.e., behavioural plasticity).

A study in dunnocks (*Prunella modularis*) has shown that bold individuals settled in areas with high human disturbance, suggesting that habitat choice was based on personality (Holtmann et al., 2017). Individuals also became bolder with age, but the effect of behavioural plasticity was weak compared to the effect of personality. While they cannot completely exclude an effect of developmental plasticity, their results provide first evidence that personality was the predominant factor determining the individuals’ distribution across the habitat. More empirical research is needed to better understand the processes that influence how animal personalities are distributed across the environment.

The existence of personality has been demonstrated in several species of amphibians (reviewed in Kelleher et al., 2018), and amphibians are typically distributed across heterogeneous environments making them excellent model species to investigate the distribution of personalities across the natural environment. A recent study in a wild population of the neotropical poison frog *Allobates femoralis* showed that males exhibit consistent within- and between-individual variation in territorial aggression, boldness and exploration (Peignier et al., 2022). There was no link between males’ personality traits and properties of their natural environment known to affect sound transmission and visibility to females and predators (i.e. overall vegetation complexity in the area surrounding the male’s territory and also territory size). But males seemed to adjust their level of exploration and boldness in response to changes in their social environment, especially to the density of females nearby. However, also fine scale vegetation structure within the territories are likely very important for the ecology of the species. During the breeding season, males need structures such as branches, logs and roots where they can perch during advertisement calling (Ursprung et al., 2011; Ringler et al., 2012) and suitable leaves for clutch deposition (Ringler et al., 2013; Ringler et al., 2018). Given that there is variation in fine scale vegetation structure among male territories, we asked if males with different behavioural phenotypes exhibit contrasting habitat preferences for territory establishment.

In the present study, we tested if habitat complexity drives habitat choice in *Allobates femoralis*. We further checked if habitat preferences are linked to specific personality traits. We focused on 36 males from a captive population of *A. femoralis* to quantify within- and between-individual consistency in activity, boldness and exploration. We investigated habitat selection by presenting males with a two-choice test between a non-complex (i.e., with fewer hiding places, perches, water bodies and leaf litter) and a complex habitat. When faced with a novel environment, we expected males to either all choose the same habitat - which would reflect an ideal habitat for the species - or select a specific habitat according to their personality. In the latter case, we expected bolder individuals to occupy areas of low complexity (i.e. sparse vegetation and few ground structures) where they are easier to spot for females, while shyer individuals are expected to occupy areas of higher complexity with more places to hide.

Methods

Ethical note

The frogs used in this experiment belonged to an ex-situ laboratory population at the animal care facility of the University of Bern and were retained to be used in future experiments. Original stock for this population, including all animals used for this study, was sampled in and exported from French Guiana in compliance with all legal requirements from the responsible French authorities (DIREN: Arrêté n°82 du 10.08.2012 and Arrêté n°4 du 14.01.2013). All testing was approved by the Suisse Federal Food Safety and Veterinary Office (National No. 33232, Cantonal No.

BE144/2020). Captive conditions were approved by the Suisse Federal Food Safety and Veterinary Office (Laboratory animal husbandry license: No. BE4/11). We followed the guidelines laid out by the ASAB for the treatment of animals in behavioural research and Teaching (Asab, 2020) and the ARRIVE guidelines (Percie du Sert et al., 2020).

Study species and experimental setup

Allobates femoralis is a small diurnal leaf litter frog common throughout the Amazon basin and the Guiana Shield (Amezquita et al., 2009). During the reproductive season, males emit prominent advertisement calls from elevated structures on the forest floor (e.g., branches, logs, etc.) to announce territory possession to conspecifics and attract female mating partners into their territory (Hödl et al., 2004). Males generally occupy territories ranging from 64.62 to 417.63 m² in size (Ringler et al., 2011). Females are not territorial and commute to males’ territories for courtship and mating (Ringler et al., 2012; Fischer et al., 2020). After tadpole hatching, males transport the larvae to water bodies located up to 200 m away from their territories (Ringler et al., 2013; Ringler et al., 2018).

We conducted our study in spring 2021 under controlled laboratory conditions in the animal care facilities at the Ethological station of Hasli from the University of Bern. The studied population consists of wild caught frogs from French Guiana and own captive bred frogs. Individuals are kept in breeding pairs in standard (60 × 40 × 40 cm) glass terraria furnished with a coconut shelter, a perch, a plant, a water bowl, and expanded clay pebbles covered with autoclaved oak leaves. The sides are covered with Xaxim (tree fern stems) mats in the lower and cork in the upper half to prevent visual contact between terraria. Light, temperature and humidity are automatically controlled to mimic natural conditions in French Guiana.

All behavioural tests were performed on 36 adult males during their reproductively active period (November-June). Individuals were unambiguously identified via their unique ventral coloration patterns (Ringler et al., 2014). We photographed all males over millimeter paper and measured their body size by determining their snout to urostyle length (SUL) using the software ImageJ (Rasband, 1997-2021).

We assessed individual levels of activity, boldness and exploration (see ‘Assessment of activity level’ and ‘Assessment of boldness and exploration levels’) for all focal males, by repeating each trial three times per individual (cf. Réale et al., 2007) resulting in a total of 108 tests per assessment. To prevent habituation and/or fatigue during the experiments, no individual was tested twice on the same day and we ran activity assessment and boldness/exploration assessment on alternated days (cf. Uher & Asendorpf, 2008; Roche et al., 2016).

Assessment of activity level

Similar to the behaviour of *A. femoralis* individuals in the wild, males in our laboratory population are usually more active during the afternoon and particularly after rainfall (Aichinger, 1991; Ringler et al., 2013). Therefore, we ran activity trials immediately after the daily ‘rainfall’ in the afternoon, from 1600 to 1900. To this end, we selected six breeding pairs per day, where we removed the respective females from the tanks and placed a wide-angle video camera (Hero Black 5 and 8, GoPro, San Mateo, CA, USA) on top of each terrarium. The first 45 minutes of recording were not considered in the analysis to allow individuals to resume normal behaviour after the experimenter had exited the room. Temperature in the room during those trials was constant (28,3 ± 0.4ºC).

We analyzed the subsequent 60 min of video recordings using the coding software BORIS (Friard et al., 2016). When males were visible and/or audible, we coded activity as: (i) number of jumps and (ii) call duration (in s). In instances where males were neither visible nor audible for the entirety of the recording (e.g., hiding under a shelter, n=27/108 observations), we coded them with a value of zero for both number of jumps and call duration.

Assessment of boldness and exploration levels

We collected data on individual levels of boldness and exploration using a Novel Environment Test (NET) (cf. Carter et al., 2013), and assessed both exploration and boldness within this setup. We chose similar variables as in Peignier et al. (2022), which has shown that both personality traits could be distinctly measured in the NET. The setup (Figure 1) consisted of a cooler box (50 x 25 x 29 cm), with a 10cm PVC tube attached on one side of the box (hereafter ‘release tube’). The floor of the box was visually divided into 40 squares. An opaque sliding door separated the box from the release tube, so that it provided a safe, dark environment where the frog could calm down after being caught. In the lid of the box, we installed a wide-angle video camera

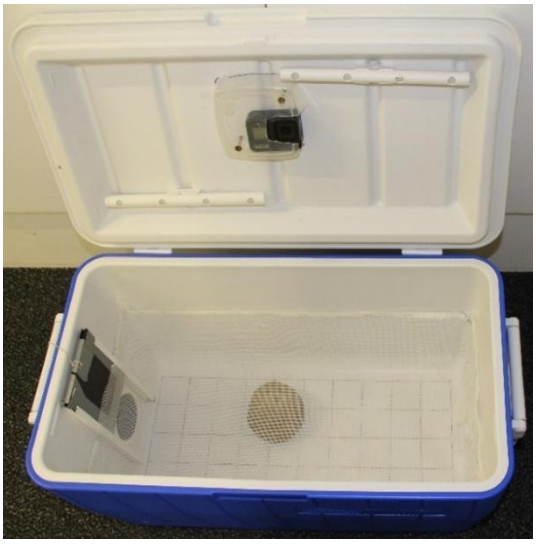


Figure 1. Picture of the Novel Environment Test setup with the release tube attached on the left side and a coconut shelter in the open area.

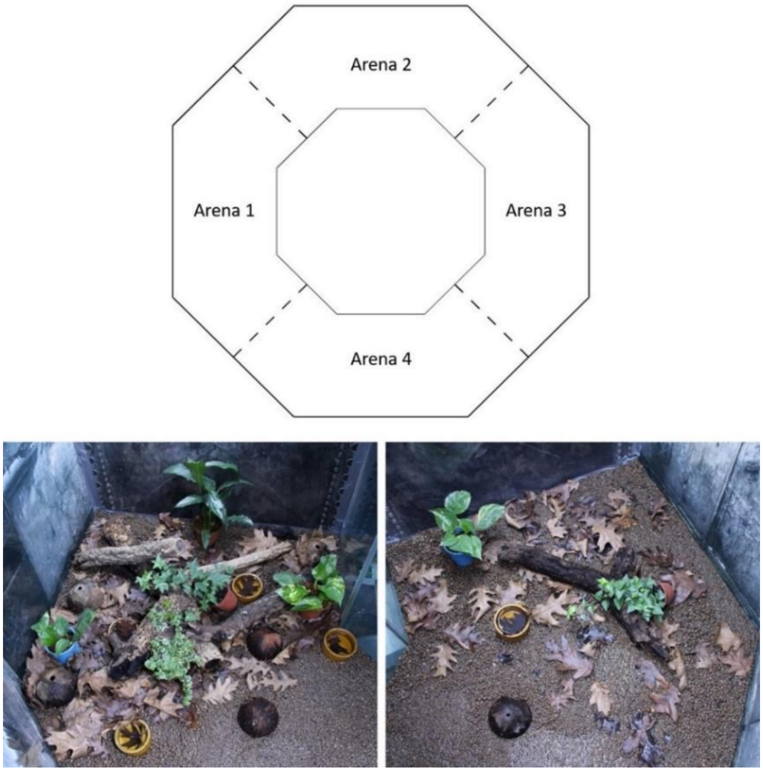


Figure 2. Scheme of the habitat choice test (top) and pictures of the complex and non complex habitats (bottom). The ring tank setup (top) was split in four arenas separated by black walls (dashed lines) to prevent any visual contact between males, and each arena was provided with a complex and a non-complex habitat separated by a small empty area. In the complex habitat (bottom), 80 to 90% of the clay pebbles were covered by a mix of leaves, plants, wood branches, water bowls and coconut shelters whereas in the non-complex habitat, 10 to 20% of the clay pebbles were covered by a mix of the same components.

(Hero Black 5, GoPro, San Mateo, CA, USA) and two elongated, battery powered LED lights (LUMIstixx, Osram/Ledvance, Garching, Germany). We also placed a coconut shelter in the box, similar to the one in the home terrarium, to hide part of the novel environment and motivate the frog to enter it. The position of the coconut shelter was constant within but varied between repetitions.

We ran experiments from 0900 to 1700. At the beginning of each trial, we caught a male in its home terrarium and placed it in the release tube for about 10 minutes to recover from capture. We then switched on the lights and camera, closed the lid of the box and opened the sliding door for 25 minutes. This allowed individuals to stay in the release tube or return to it at any moment after entering the box. As temperature varied during the day, we noted the exact ambient temperature (in °C) at the beginning of each trial.

We analyzed video recordings using the coding software BORIS (Friard et al., 2016). We coded (i) the probability for individuals to enter the box (1) or not (0), (ii) the latency to leave the release tube (i.e., time to enter into a bright novel environment, in s), (iii) the time spent in the box (i.e., when the individual was in the open area of the box and not hiding under the coconut shelter, in s) and (iv) the number of visited squares in the box. Males who did not leave the release tube (n = 44/108 observations), were given a censored value of 1500 s for the latency to leave the release tube (i.e., total duration of the experiment) and a value of 0 s for the time spent in the box.

Habitat choice test

To investigate if males prefer different levels of habitat complexity according to their personality, we used a two-choice test presenting each male two habitats with variable complexity. Experiments took place in an 8000 L ring tank. The soil of the ring tank was covered with clay pebbles and divided in four 2,4 m² arenas allowing us to test four individuals at the same time (Figure 2). The arenas were separated by black walls preventing any visual contact between males. In each arena, a ‘complex’ and a ‘non-complex’ habitat area was created with a small empty area separating them. In the complex habitat, 80 to 90 % of the clay pebbles were covered by a mix of oak leaves, plants, wood branches, water bowls and coconut shelters whereas in the non-complex habitat, only 10 to 20 % of the clay pebbles were covered by a mix of the same components (Figure 2).

Before the start of the trials, the raining system was switched on for 10 minutes to ensure similar humidity levels across all trials. We caught males in their home terraria and placed them in a release tube in the middle area without the possibility to see nor access the habitats. We switched on a speaker broadcasting a recording of the ambient background noise in the housing room (i.e., consisting of advertisement calls of several males). We left the speaker playing for the entire duration of the trial to mimic a natural environment and stimulate the focal males to settle in a territory in the habitat choice setup. After a break of 15 minutes to recover from the stress of the capture, we lifted the release tube and switched on the video cameras to record the focal frogs' behaviour during the next eight hours. At 1800, we caught the frogs and put them back in their home terraria. We switched on the raining system for five minutes to remove chemical cues from the setup. All experiments were conducted at a constant temperature of 29 ºC.

The first 30 minutes of the recordings were not considered in the analysis to allow individuals to acclimate to the new environment. We analyzed subsequent 7 h 30 min of video recordings using the software BORIS (Friard et al., 2016) to code (i) the time spent in each habitat type (in s) and (ii) the number of times individuals crossed over from one habitat type to the other.

Statistical analyses

We conducted all statistical analyses in R v.3.6.0 (R Core Team, 2020), using the integrated development environment RStudio v.1.2.1335 (RStudio Team, 2019). We used the function transformTukey to adjust variables which deviated from normality (i.e., calling duration during the activity trial, latency and time spent in the box during the NET).

First, we investigated whether origin (i.e., wild caught or captive bred), and body size influenced the behaviours measured in the activity trials (i.e., number of jumps and call duration) and in the NET (i.e., probability to enter the box, latency to leave the release tube, time spent in the box and number of visited squares). We additionally investigated the influence of temperature on the behaviours measured in the NET. For that, we fitted two generalized mixed effect model with a Poisson distribution and either the number of jumps or the number of visited squares in the NET as response variable. We also fitted a generalized mixed effect model with a binomial distribution, with the probability to enter the box as response variable. Finally, we fitted three linear mixed effect models with either the call duration, the latency to leave the release tube or the time spent in the box as response variables. In these six models we added origin, and body size as fixed effects and male ID as random effect. We also added temperature as fixed effect in the four models with the behaviours measured in the NET as response variable.

To assess the amount of behavioural variation in the population due to inter-individual variation, we estimated the repeatability of each behaviours measured during the activity trial and the NET with the ‘rptR’ package (Stoffel et al., 2017). We considered behaviours to be repeatable if the 95% confidence interval (CI) did not overlap zero.

To determine whether individuals chose a habitat over another based on its complexity, we first checked data for normal distribution with a Shapiro-Wilk test (statistic $= 0.49958$, $p < 0.001$) and decided to use a Wilcoxon one sample test comparing the time spend in the complex habitat to half of the total trial duration (13500 s). Then, we investigated if males’ personality influenced their movements between the two different habitats in the choice test. For this, we only used behaviours that were repeatable (i.e., number of jumps, as a proxy of activity, latency to leave the release tube as a proxy of boldness, time spend in the box and mean number of visited squares in the box as proxies of exploration). For each variable we calculated the average behaviour out of the data from the three trials. We fitted a generalized linear model with a Poisson distribution with the number of times individuals crossed over from one habitat type to the other as response variable and the four average variables as fixed effects.

Results

Personality

There was no influence of origin and body size on any of the behaviours measured during the activity trials and the NET. There was also no influence of temperature on the behaviours measured in the NET (Table 1). The number of jumps measured during the activity trials was repeatable ($R = 0.44$, $p < 0.001$, 95%CI = 0.01, 0.17), while the call duration measured during the activity trials was not ($R = 0.16$, $p = 0.064$, 95%CI = 0, 0.37). Among the measures taken during the NET, all but the probability to enter the box ($R = 0.22$, $p = 0.022$, 95%CI = 0, 0.45) turned out to be repeatable and ranged from 0.23 to 0.34. For the boldness-related behaviours, the latency to leave the release tube

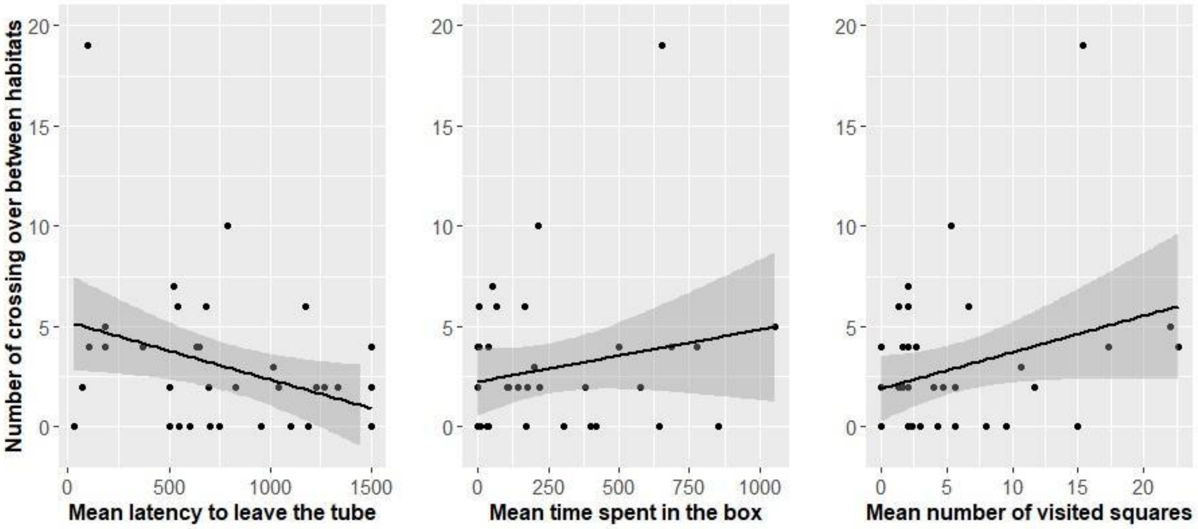


Figure 3. Number of times frogs crossed from one habitat to the other in the habitat selection test as a function of the mean latency to leave the tube (left), the mean time spent in the box (middle) and the mean number of squares visited (right) in the NET.

Table 1. Results of the (generalized) linear mixed effect models investigating the influence of body size and origin on behaviours measured during the activity trial and of body size, origin and temperature on behaviours measured during the NET. N = 108 observations on 36 individuals.

	Number of jumps		Call duration		Probably to enter the box		Latency to leave the release tube		Time spend in the box		Number of visited squares	
Fixed effects (Estimate±SE | p value)												
(Intercept)	-3.02±6.94	0.664	10.59±10.99	0.335	-4.58±8.03	0.569	13.1±18.17	0.471	-3.60±5.35	0.501	-5.10±5.34	0.339
Body size	1.51±2.41	0.532	-2.65±3.82	0.488	1.41±2.23	0.528	3.63±5.21	0.487	1.18±1.54	0.444	2.05±1.81	0.258
Origin	0.73±0.64	0.260	0.99±1.02	0.331	-0.78±0.62	0.205	0.80±1.40	0.570	-0.50±0.41	0.229	-0.22±0.49	0.647
Temperature					0.06±0.19	0.763	-0.49±0.40	0.212	0.08±0.12	0.489	0.01±0.04	0.718
Random effects (Estimate ± SD)												
ID	3.12±1.77		3.14±1.77		1.05±1.02		8.11±2.85		0.74±0.86		1.69±1.3	
Residual			15.59±3.95				22.27±4.72		1.85±1.36			

Table 2. Results of the generalized linear model investigating the influence of personality on crossing over in the habitat choice setup. Significant p values (< 0.05) are indicated with raised asterisks. N = 36 individuals.

	Number of crossing over from one habitat to the other	
Fixed effects (Estimate±SE | p value)		
(Intercept)	2.08±0.32	<0.001*
Mean number of jumps	-0.003±0.01	0.543
Mean latency to leave the release tube	-0.001±0.0003	<0.001*
Mean time spend in the box	-0.001±0.0005	0.014*
Mean number of visited squares	0.06±0.02	0.006*

had a repeatability of 0.23 ($p = 0.01$, 95%CI = 0.007, 0.43). For the exploration-related behaviours, the time spent in the box had a repeatability of 0.29 ($p = 0.004$, 95%CI = 0.06, 0.48), and the number of visited squares had a repeatability of 0.34 ($p = 0.004$, 95%CI = 0.02, 0.56).

Habitat choice test

During the habitat choice test, 23 out of 36 individuals explored both habitat types by crossing over from one habitat to the other. However, all individuals spent significantly more time in the complex habitat than in the non-complex one (Wilcoxon one sample test: $V = 665$, $p < 0.001$). The number of times individuals crossed over from one habitat type to the other was influenced by personality (Table 2). Individuals who were bolder (i.e. exited faster the release tube) and more explorative (i.e. stayed longer and visited more squares) in the NET, also crossed more often from one habitat type to the other in the choice test (Table 2; Figure 3).

Discussion

In the present study we investigated if habitat selection is driven by animal personality in the poison frog *Allobates femoralis*. By using a Novel Environment Test and assessing individual activity patterns, we confirmed the existence of personality along the active/passive, bold/shy, and exploration/avoidance axes in our laboratory frog population. We show that the repeatability of the variables measured (ranging from 0.23 to 0.44) was in the lower range of what has been found in most personality studies in other taxa so far (mean = 0.37, 95%CI = 0.35, 0.38) (Bell et al., 2009). However, our repeatability results are consistent with previous findings in amphibians (Brodin et al., 2013; Maes et al., 2013; Gifford et al., 2014; González-Bernal et al., 2014), and with what has been measured in wild *A. femoralis* (Peignier et al., 2022). The somewhat low repeatability in the present study is not surprising since ectotherms (compared to endotherms) and captive individuals (compared to wild populations) are typically less repeatable in their behaviours (Bell et al., 2009).

We also tested if habitat selection is driven by personality type using a two-choice test opposing two habitats with different level of complexity. When individuals enter a novel environment, they could either randomly disperse, all select the same environment (e.g., the environment that offers greatest resources) or select a habitat to match their personality (Holtmann et al., 2017). We expected shy individuals to select complex habitats (with more places to hide) and bold individuals to prefer non-complex habitats (where they are easier to spot for females). In our study, we observed that all individuals spent significantly more time in the complex compared to the non-complex habitat, regardless of their personality.

Vegetation composition plays a major role in the ecology of *A. femoralis*. During the reproductive season, males call from perches to both repel male competitors and attract females (Narins et al., 2003). Once the courtship initiated with the female, eggs are laid in the leaf litter (Ringler et al., 2012; Fischer et al., 2020). Therefore, choosing a complex habitat would be relevant regarding the species' ecology, as it offers more resources such as more plants and branches to perch and hide, more leaf litter to hide and lay clutches. We cannot rule out that the simple habitat we offered was eventually considered too scarcely vegetated to be considered attractive, or that frogs evaluate a territory based on the combination of its natural and social characteristics. Our study design was chosen to specifically test for habitat preferences in the absence of any other social cues, such as presence of conspecifics. We speculate that in the wild, the distribution of certain personalities across the habitat is the result of a combination of different factors, including individual preferences and social interactions. If certain personalities have a competitive advantage when choosing a territory, this could also lead to a habitat type by personality correlation. Future studies should look into the relative contribution of social interactions and individual preferences on habitat choice and territory settlement.

Finally, we found that bolder and more explorative individuals were more likely to cross between habitats in the choice test. While this was not initially the main focus of our study, this result suggests that the behaviours measured in the NET indeed reflect exploration and boldness related behaviours measured in a more naturalistic setup. Animal personality is currently an omnipresent topic in animal behaviour research, and as such has been highly contested (cf. Beekman & Jordan, 2017). One of the main criticisms is whether the use of artificial setups, such as a Novel Environment, really allows to measure behavioural patterns that reflect common behaviours of the species under natural conditions. Our results demonstrate that exploration behaviour measured in a highly artificial setup indeed reflect exploration behaviour in a more naturalistic setup. We encourage more studies to investigate personality traits across different experimental complexities and settings to assure the ecological relevance of the behaviours measured.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Authors contributions

Conceptualization: LB, MP, ER; Methodology: LB, MP, ER; Formal Analysis: LB, MP; Investigation: LB; Resources: ER; Data curation: LB; Writing – original draft: LB; Writing – review and editing: LB, MP, ER; Supervision: MP, ER; Project administration: ER; Funding acquisition: ER.

Acknowledgments

This study was funded by the Austrian Science Fund (FWF) [grant P 31518 to ER]. The authors thank M. Moser and D. Josi for help in setting up the video recording system and A. Bégué for help with figure 2.

References

Aichinger, M. (1991). Tadpole transport in relation to rainfall, fecundity, and body size in five species of poison-dart frogs from Amazonian Peru. — Amphib-reptil. 12: 49–55.

Amezquita, A., Lima, A.P., Jehle, R., Castellanos, L., Ramos, Ó., Crawford, A.J., Gasser, H. & Hödl, W. (2009). Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog Allobates femoralis. — Biol. J. Linn. Soc. 98: 826–838.

Beekman, M. & Jordan, L.A. (2017). Does the field of animal personality provide any new insights for behavioral ecology? — Behav. Ecol. 28: 617–623.

Bell, A.M., Hankison, S.J. & Laskowski, K.L. (2009). The repeatability of behaviour: a meta-analysis. — Anim. Behav. 77: 771–783.

Brodin, T., Lind, M.I., Wiberg, M.K. & Johansson, F. (2013). Personality trait differences between mainland and island populations in the common frog (Rana temporaria). — Behav. Ecol. Sociobiol. 67: 135–143.

Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G. & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? — Biol. Rev. 88: 465–475.

Conover, D.O. & Schultz, E.T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. — Trends Ecol. Evol. 10: 248–252.

Dingemanse, N.J. & Araya-Ajoy, Y.G. (2015). Interacting personalities: Behavioural ecology meets quantitative genetics. — Trends Ecol. Evol. 30: 88–97.

Dingemanse, N.J., Both, C., Drent, P.J. & Tinbergen, J.M. (2004). Fitness consequences of avian personalities in a fluctuating environment. — Proc. R Soc. Lond. B 271: 847.

DiRienzo, N. & Montiglio, P.-O. (2015). Four ways in which data-free papers on animal personality fail to be impactful. — Front. Ecol. Evol. 3: 23.

Fischer, M.-T., Ringler, M., Ringler, E. & Pašukonis, A. (2020). Reproductive behavior drives female space use in a sedentary Neotropical frog. — PeerJ 8: e8920.

Friard, O., Gamba, M. & Fitzjohn, R. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. — Methods Ecol. Evol. 7: 1325–1330.

Gifford, M.E., Clay, T.A. & Careau, V. (2014). Individual (co)variation in standard metabolic rate, feeding rate, and exploratory behavior in wild-caught semiaquatic salamanders. — Physiol. biochem. zool. 87: 384–396.

González-Bernal, E., Brown, G.P. & Shine, R. (2014). Invasive cane toads: social facilitation depends upon an individual's personality. — PLoS one 9: e102880.

Gosling, S.D. (2008). Personality in non-human animals. — Soc. Pers. Psychol. Compass 2: 985–1001.

Hödl, W., Amézquita Torres, A. & Narins, P.M. (2004). The role of call frequency and the auditory papillae in phonotactic behavior in male dart-poison frogs Epipedobates femoralis (Dendrobatidae). — J. Comp. Physiol. A 190: 823–829.

Holtmann, B., Santos, E.S.A., Lara, C.E. & Nakagawa, S. (2017). Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype-environment covariance. — Proc. Royal Soc. B 284: 20170943.

Kelleher, S.R., Silla, A.J. & Byrne, P.G. (2018). Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. — Behav. Ecol. Sociobiol. 72: 10539.

Maes, J., van Damme, R. & Matthysen, E. (2013). Individual and among-population variation in dispersal-related traits in Natterjack toads. — Behav. Ecol. 24: 521–531.

Narins, P.M., Hödl, W. & Grabul, D.S. (2003). Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. — Proc. Natl. Acad. Sci. USA 100: 577–580.

Peignier, M., Araya-Ajoy, Y.G., Bégué, L., Chaloupka, S., Dellefont, K., Leeb, C., Walsh, P., Ringler, M. & Ringler, E. (2022). Exploring links between personality traits and their social and non-social environments in wild poison frogs. — Behav. Ecol. Sociobiol. 76: 1–14.

R Core Team (2020). R: a language and environment for statistical computing. — R

Foundation for Statistical Computing, Vienna, Austria.

Rasband, W.S. (1997-2021). ImageJ. — U. S. National Institutes of Health, Bethesda, MD, USA.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. — Biol. Rev. 82: 291–318.

Ringler, E., Mangione, R. & Ringler, M. (2014). Where have all the tadpoles gone? Individual genetic tracking of amphibian larvae until adulthood. — Mol. Ecol. Resour. 15: 737–746.

Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. (2013). Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. — Front. Zool. 10: 67.

Ringler, E., Ringler, M., Jehle, R. & Hödl, W. (2012). The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. — PLoS one 7: e40237.

Ringler, E., Szipl, G., Harrigan, R.J., Bartl-Binder, P., Mangione, R. & Ringler, M. (2018). Hierarchical decision-making balances current and future reproductive success. — Mol. Ecol. 27: 2289–2301.

Ringler, M., Ringler, E., Magaña Mendoza, D. & Hödl, W. (2011). Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. — PLoS one 6: e25844.

Roche, D.G., Careau, V. & Binning, S.A. (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. — J. Exp. Biol. 219: 3832–3843.

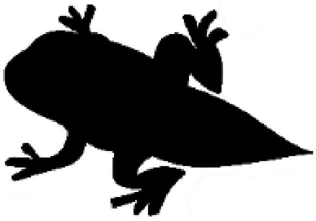
RStudio Team (2019). RStudio: Integrated Development for R. — RStudio, Inc., Boston, MA, USA.

Stoffel, M.A., Nakagawa, S. & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. — Methods Ecol. Evol. 8: 1639–1644.

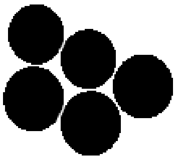
Uher, J. & Asendorpf, J.B. (2008). Personality assessment in the great apes: comparing ecologically valid behavior measures, behavior ratings, and adjective ratings. — J. Res. Pers. 42: 821–838.

Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. (2011). Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. — Mol. Ecol. 20: 1759–1771.

Chapter 3



The interplay between animal personality and sexual selection



Manuscript 5: “Mate choice in a promiscuous poison frog”

This manuscript is published in *Ethology* (1:11)
09 September 2022
doi: 10.1111/eth.13331

Mélissa Peignier[a,b,c,*], Lauriane Bégué[a,c,d], Alina Gieseke[b], Diana Petri[b], Max Ringler[a,c,e,f] & Eva Ringler[a,b,c]

Affiliations
[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[b] Messerli Research Institute, University of Veterinary Medicine Vienna, Austria
[c] Department of Behavioral and Cognitive Biology, University of Vienna, Austria
[d] Department of Biology and Ecology, University of Montpellier, France
[e] Department of Evolutionary Biology, University of Vienna, Austria
[f] Institute of Electronic Music and Acoustics, University of Music and Performing Arts Graz, Austria
Mélissa Peignier and Lauriane Bégué should be considered joint first authors.

*Correspondence :
Mélissa Peignier
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
melissa.peignier@unibe.ch

Abstract

In many animal species, members of one sex, most often females, exhibit a strong preference for mating partners with particular traits or resources. However, when females sequentially mate with multiple partners, strategies underlying female choice are not very well understood. Particularly, little is known if under such sequential polyandry females mate truly randomly, or if they actively try to spread mating events across multiple partners. In the present study, we used the highly promiscuous poison frog *Allobates femoralis* to investigate whether promiscuity could result from a preference for novel mates. Furthermore, we examined the importance of call characteristics for mate choice. We conducted mate choice experiments in a laboratory setup, by presenting females with recent mating partners or novel males. We recorded call characteristics of both males and the time females spent close to each male. In our trials, females preferred previous mating partners over novel males and also males with shorter advertisement calls. Results from previous studies on *A. femoralis* suggest that females in our trials recognized previous partners based on individual call characteristics. While mating decisions in the wild and in the laboratory might differ, our study provides first evidence for female mate choice in a poison frog with sequential polyandry.

Keywords

Anura, call characteristics, familiarity, female mate choice, polyandry, sequential mating

Introduction

Animals have evolved a wide variety of species-specific mating strategies to maximize their reproductive success (Emlen & Oring, 1977; Kokko et al., 2003; Krasnec et al., 2012). Mate choice and intra-sexual competition are key elements of many mating systems and together characterize sexual selection (Darwin, 1871; Rosenthal, 2017). In many cases, members of one sex, most often females (but see e.g., Werner & Lotem, 2003), exhibit a preference for mating partners with particular traits or resources. Females may benefit from being choosy by obtaining access to resources (e.g. nesting site, food), secure males that show high parental investment or benefit from high genetic quality or compatibility of the male that will enhance the viability in the offspring (Hamilton, 1990; Neff & Pitcher, 2005; Rosenthal, 2017; Tregenza & Wedell, 2000; Trivers, 1972). However, in populations living in highly dynamic environments, “desirable” or “optimal” traits of potential mating partners may change over time or might be difficult to assess (Bonsall & Klug, 2011). In general, females should become less choosy as costs of assessment or courtship increase. In this situation, the choosing sex may partition reproduction across multiple partners to ensure against total reproductive failure and thereby promiscuous mating might become adaptive (Carlisle, 1982; Fox & Rauter, 2003; Garcia-Gonzalez et al., 2015; Yasui, 2001; Yasui & Garcia-Gonzalez, 2016).

In species where females sequentially mate with multiple partners in subsequent reproductive events, strategies underlying female choice are not very well understood, and female multiple mating is often interpreted as a result of non-choosiness by the female (Krasnec et al., 2012; Yasui, 2001). However, such a mating pattern could actually be the result of various strategies. On one hand, females may indeed opportunistically mate with available males that by chance are spatially close or advertise availability, leading to a seemingly random mating pattern (Janetos, 1980; Meuche et al., 2013). On the other hand, such promiscuous mating could be caused by females that show a preference for males they have not previously mated with (Krasnec et al., 2012; Yasui, 2001). Sequential polyandry is known to attenuate negative effects of mating with single low-quality mates (Fox & Rauter, 2003; Yasui, 2001; Yasui & Garcia-Gonzalez, 2016). However, whether such a mating pattern is the result of true random mating, or the active preference for novel mates remains unknown for most species with promiscuous mating systems.

To study mate choice, amphibians are a particularly interesting taxon because of their high diversity and complexity of reproductive strategies and mating systems (Luz Nunes-de- Almeida et al., 2021; Sullivan et al., 1995; Wells, 2007). Females of many anuran species exhibit mate choice based on characteristics of male advertisement calls (e.g. Gerhardt, 1991; Giacoma et al., 1997; Klump & Gerhardt, 1987; Schwartz et al., 2001; Tárano & Fuenmayor, 2013; Welch et al., 1998). Temporal properties of a call such as call/note duration or number of calls within a bout can convey information on a male's endurance (Taigen & Wells, 1985), on its body size (Giacoma et al., 1997) or on the quality of paternal care (Pettitt et al., 2020). Spectral properties, such as the frequency or amplitude of the call, are often linked to age (Felton et al., 2006) and body size (Humfeld, 2013; McClelland et al., 1996). However, not all anurans show clear preferences for certain traits when it comes to mate choice. Several species have evolved high levels of promiscuity, suggesting non-selective mating by females (Roberts & Byrne, 2011).

In Neotropical poison frogs (Dendrobatidae *sensu* AmphibiaWeb, 2021), female mate choice is mostly based on visual and acoustic signals of potential mating partners. Visual cues predominantly play a role in the colourful, aposematic species, where dorsal brightness, spectral reflectance and colouration patters often enable assortative mating (e.g. in *Oophaga pumilio*, Dreher et al., 2017; Gade et al., 2016; Maan & Cummings, 2008, 2009, 2012; Reynolds & Fitzpatrick, 2007; Richards-Zawacki et al., 2012; Richards-Zawacki & Cummings, 2011; Summers et al., 1999). However, the visually mediated assortative mating in *O. pumilio* has recently been shown to become overridden by the outcome of direct male–male competition. In the wild, female *O. pumilio* prefer to mate with males of the local colour morph. A study showed that this preference is overridden by intra-sexual selection, as females tested in a laboratory setting preferred territory holders over non-territorial males, regardless of their colour morph (Yang & Richards-Zawacki, 2020). Acoustic signalling is present in the vast majority of poison frogs across all clades, with different acoustic characteristics being relevant for female mate choice (e.g. Dreher & Pröhl, 2014; Lüddecke, 2002; Meuche et al., 2013; Pettitt et al., 2020; Pröhl, 2003; Roithmair, 1994; Souza et al., 2021). Also territory size and breeding resources, which the female can assess during courtship within the male's territory, seems to play a role in female mating decisions (e.g. in *Allobates paleovarzensis* [Da Rocha et al., 2018], *Ameerega trivitatta* [Roithmair, 1994], *Oophaga pumilio* [Donnelly, 1989; Pröhl & Hödl, 1999]).

In the Brilliant-thighed Poison Frog *Allobates femoralis* (subfamily Aromobatinae sensu AmphibiaWeb, 2021), both sexes typically mate multiple times and with different partners, resulting in a highly promiscuous mating system (Montanarin et al., 2011; Stückler et al., 2019; Ursprung et al., 2011). Previous studies point towards the absence of active mate choice by females because of high levels of polyandry and low levels of reproductive skew among males (Ringler et al., 2012; Ursprung et al., 2011). Female preference for males with larges territories was described in an observational study by Roithmair (1992); however, these findings were not corroborated in a more recent study using molecular methods to measure reproductive success (Ursprung et al., 2011). So far, the possibility of an active preference for novel mating partners has not been studied and previous studies did also not incorporate male call characteristics into analyses of female choice. Given the substantial energetic costs and risks of predation that male frogs generally face when calling, calls could be expected to serve as a signal of male quality, directed at and used by females (Pough & Taigen, 1990; Ryan et al., 1982, 1983; Taigen & Wells, 1985; Zahavi, 1977). Furthermore, previous studies have shown that the calls of *A. femoralis* males are individually distinct which could help females identify and favour novel partners for mating (Gasser et al., 2009; Tumulty et al., 2018).

To investigate patterns of female mating in *A. femoralis*, we conducted a choice test where we presented a previous mating partner and a novel male to females in a two-arm maze. We made the following predictions:

1. if females prefer novel mating partners, they should spend more time close to a novel male compared with in the central section or close to their previous mating partner;
2. if females prefer males based on the amount and/or characteristics of the advertisement or courtship call, female choice should be correlated with the number, the duration, the frequency and/or the consistency of male calls.

Methods

Ethical note

This study was approved by the ethics and animal welfare committee of the University of Vienna (No. 2019-003) in accordance with Good Scientific Practice (GSP) guidelines and national Austrian legislation. The frogs used in this experiment belonged to an ex situ laboratory population at the animal care facility of the University of Vienna. Original stock for this population, including all animals used for this study, was sampled in and exported from French Guiana in compliance with all legal requirements from the responsible French authorities (DIREN: Arrêté n°82 du August 10, 2012 and Arrêté n°4 du January 14, 2013). We followed the guidelines laid out by the ASAB for the treatment of animals in behavioural research and Teaching (Asab, 2020) and the ARRIVE guidelines (Percie du Sert et al., 2020).

Study species

Allobates femoralis (Figure 1) is a Neotropical poison frog that occurs commonly throughout the Amazonian basin and Guiana shield (Amezquita et al., 2009). During the reproductive season, which coincides with local rainy seasons (Gottsberger & Gruber, 2004), males are highly territorial and advertise territory occupancy via prominent advertisement calls from elevated perches to repel male competitors and attract female mating partners (Hödl et al., 2004; Ringler et al., 2011). Advertisement calls consist of four notes which feature an upward frequency sweep, with calls being repeated at regular intervals to form bouts of up to 40 four-notes calls (Narins et al., 2003). Females do not establish territories but show site fidelity and commute to male territories within 20 m for courtship and mating (Fischer et al., 2020; Ringler et al., 2012). Males switch from advertisement calls to courtship calls once a female is in sight. The buzzing courtship call lasts for .5–1 s and features a broadband burst of pulses with a dominant frequency of 2500–2700 Hz (see Figure 1 in Stückler et al., 2019). Once the male switches to courtship calling, the pair begins an elaborate courtship sequence that can last up to several hours and almost always end up in successful oviposition (Montanarin et al., 2011; Stückler et al., 2019). The courtship march in *A. femoralis* is among the longest observed in poison frogs and likely serves the pair to identify a suitable oviposition site or collect fine-scale spatial information needed for subsequent parental care (Stückler et al., 2019). The female lays a clutch of approximately 20 eggs in the leaf litter, which is then externally fertilized by the male, and subsequently the female leaves the male's territory (Ringler et al., 2015). After 15–20 days of larval development, tadpoles hatch and are transported by the male to medium-sized natural pools located up to 200 m from their territory (median: 27.52 m ± 30.90 m iqr; Ringler et al., 2013; see also Beck et al., 2017; Ringler et al., 2018).

Males partition tadpoles of single clutches over several water bodies in an attempt to hedge their bets regarding parental care (Erich et al., 2015; Pašukonis et al., 2017). Occasionally females take over transport, but only when the father is absent (Ringler et al., 2015).

Housing conditions

We performed the behavioural experiments under controlled laboratory conditions from October to December 2019 in the animal care facilities at the University of Vienna using individuals from a captive *A. femoralis* population. Prior to the choice experiments, we kept the frogs in randomly assigned pairs in standard glass terraria of equal size (60 × 40 × 40 cm) with identical equipment and furnishings. The floor was made of expanded clay pebbles, back and side walls were covered with Xaxim (tree fern stems) mats in the lower and cork in the upper half to prevent visual contact between terraria. Half a coconut shell, a small plant, and a branch provided standardized shelter and elevated calling positions. We provided autoclaved oak leaves as substrate for oviposition, and a small glass bowl of 10 cm diameter filled with water for tadpole deposition. An automatic raining, heating and lighting system ensured standardized climatic conditions in all terraria, similar to natural conditions in French Guiana. The temperature ranged from 21°C at night to 28°C during the day. Lights were on from 7 a.m. to 7 p.m. and humidity in the terraria was constantly at 100%. Frogs were fed wingless fruit flies (*Drosophila*) three times a week but were never fed on the day of an experiment. Pairs used in our experiment had already been kept together over several months, and each pair had already produced clutches together. We also made sure that the novel male and the female used in a test had not been in contact (i.e., housed in the same terraria) before.

Mate choice test setup

We performed the mate choice test in a separate room using a two-arm maze. The middle area (120 × 35 × 70 cm) housed the female, while the two adjacent areas to the left and right (60 × 35 × 70 cm each) housed the previous mating partner from her home terrarium, and a novel male she had never had direct contact with, respectively (Figure 1). The side areas housing the males were separated from the middle part with sliding doors made of glass on the lower half and mesh on the upper half to allow the

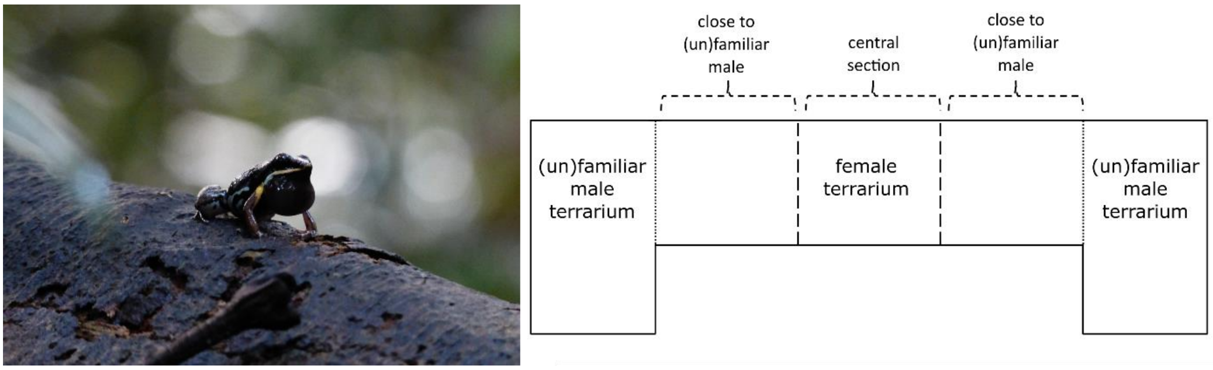


Figure 1. Picture of a male *Allobates femoralis* calling (left) and scheme of the two-arm maze (right). The set-up consisted of a middle terrarium (120 x 35 x 70 cm) housing the female and two adjacent terraria (60 x 35 x 70 cm) housing a previous mating partner and a novel male. The dashed lines represent the white markers used to divide the females' terrarium into three thirds for the analyses. The middle and adjacent terrarium were separated with sliding doors made of glass on the lower half and mesh on the upper half (dotted lines).

female to see, smell and hear both males. All area had expanded clay pebbles as soil material, which was covered with autoclaved oak leaves. We sprayed and rinsed each compartment with 2 L of demineralized and dechlorinated water before each trial to maintain an equal level of humidity for each trial and remove odour cues from the previous trial. The light and heat conditions were the same as the ones provided in the housing room. The males were provided with half a coconut shell for shelter and a cork branch for calling. In the female's compartment, we placed small white markers on the ground to divide the area into three thirds (corresponding to "close to previous partner," "central section," "close to novel male").

Mate choice test procedure

To ensure that females were physically ready to produce a new clutch and likely motivated to find a mate, we waited a minimum of 6 days after their last clutch to perform the test (cf. Weygoldt, 1980; in captivity on average 8 days between oviposition). We tested each female only once, while males could serve as the previous partner and novel male in one trial each. To control for a possible directional bias of females in the setup, we alternated the side where we presented previous partner/novel male. We also controlled for the effect of male body size by choosing a novel male of similar stature to the previous partner. All frogs had at least 60 min to acclimate to the testing terraria before the experiment started. Females could see, hear and smell both males during this acclimation period. We then recorded the behaviours of each female for 180 min between 3 p.m. and 6 p.m. with digital full-HD surveillance cameras (BX400 HD Minidome; IndigoVision) that were installed on top of the setup. Additionally, we recorded male calls with two Lavalier microphones (TY-109; renkforce) attached to pre-amplifiers (PS418S; Superlux) and a digital audio recorder (24-bit, 44.1 kHz; H4N, Zoom). Microphones were inserted into the male compartments to record calls during trials. After the trial, we photographed each individual on millimetre paper to measure snout-urostyle length (SUL) with ImageJ (Rasband, 1997–2021), before transferring them back to their home terraria. We conducted 23 trials in total, one trial per female.

Data collection

For coding the location of females in the video recordings of the trials we used the software BORIS (Friard et al., 2016). To determine whether females prefer a previous partner or rather a novel male, we analysed the relative duration a female spent close to each male without taking into account the time spent in the central section (hereafter “proportion of paired time”). The proportion of paired time was calculated by dividing the time spent near a particular male (previous partner or novel male) by the total time spent near either male within a trial. Additionally, we coded for each male whether the female chose him (1) or not (0) during the test. We coded a male as chosen if the female spent more than half of the proportion of paired time with him.

We used the bioacoustics software RAVEN PRO 1.6.1 (K. Lisa Yang Center for Conservation BioacousticsYang Center for Conservation Bioacoustics, 2011–2021) to determine the spectral and temporal characteristics of the advertisement and courtship calls. We counted the total number of advertisement (i.e., four-notes call) and courtship calls. Then we applied a band energy detector to find entire bouts of advertisement calls (detector settings: minimum frequency: 2000 Hz; maximum frequency: 5000 Hz; minimum duration: 1 s; maximum duration: 50 s; minimum separation: 2 s; minimum occupancy: 14%; SNR threshold: 29 dB; block size: 5 s; hop size: .1 s; percentile: 30). We further applied additional band energy detectors to find single *A. femoralis* advertisement and courtship calls (detector settings: minimum frequency: 2000 Hz; maximum frequency: 5000 Hz; minimum duration: .3 s; maximum duration: 1.7 s for advertisement calls and 3.0 s for courtship calls; minimum separation: .27 s for advertisement calls and .01 s for courtship calls; minimum occupancy: 40% for advertisement calls and 75% for courtship calls; SNR threshold: 29 dB for advertisement calls and 13 dB for courtship calls; block size: 5 s; hop size: .1 s; percentile: 30). Finally, to find the four separate notes constituting the advertisement calls, we applied another band energy detector (detector settings: minimum frequency: 2000 Hz; maximum frequency: 10000 Hz; minimum duration: .03 s; maximum duration: .2 s; minimum separation: .01 s; minimum occupancy: 10%; SNR threshold: 29 dB; block size: 5 s; hop size: .1 s; percentile: 30) on the cut-out files of the detected bouts. From the automatic detections, we used the mean duration (s) and mean peak frequency (Hz) of the four-note advertisement calls and of the courtship calls. Then, we calculated the advertisement call rate as the number of calls in a bout per min. We also calculated the coefficients of variation within males of the time interval between the first and second, second and third, and third and fourth notes (i.e., standard deviation divided by the mean). Likewise, we calculated the coefficient of variation of the inter-call interval (i.e., time between the last note of a call and the first note of another call within a bout). Finally, we calculated the coefficient of variation of the frequency range and of the mid-frequency of each note. We coded males that did not call with a call number, call rate and a mean call duration of 0, and “NA” for the other measurements.

Statistical analyses

We conducted all statistical analyses in R v3.6.0 (R Core Team, 2020), using the integrated development environment Rstudio v1.3.1093 (Rstudio Team, 2019). First, we verified that we used males of similar size by comparing the size of the previous partner and novel male using an independent two samples t-test. We checked data for normal distribution of each group with a Shapiro–Wilk test (size of the previous partner: statistic = .944, *p*-value = .223; size of the novel male: statistic = .948, *p*-value = .266) and for homogeneity of variance with Levene’s test (df1 = 1, df2 = 44, statistic = .609, *p*-value = .439). We also checked for a potential directional bias in females. We checked for data normality (Shapiro–Wilk test: statistic = .822, *p*-value <.001) and used a Wilcoxon one sample test comparing the proportion of paired time (whether it was with the previous or novel male) spent on the left side to .5. As both tests did not reveal significant differences in body size or any side bias across tests, we did not include these variables in any further analyses.

Next, we investigated whether females were responsive to the presence of males. We built three Wilcoxon one sample tests comparing the proportion of time spent in each section to .3333, which represents a third of the total trial time. In other words, we investigated whether females spent more or less than 1/3 of the time in each section. Here, we used non-parametric tests because the time spent in each section did not follow a normal distribution (Shapiro–Wilk test: proportion of time close to the novel male: statistic = .839, *p*-value = .002; proportion of time close to the previous partner: statistic = .862, *p*-value = .004; proportion of time in the central section: statistic = .740, *p*-value < .001). Then, we analysed the female’s preference for a male based on familiarity by using a Wilcoxon one sample test to compare the paired proportion of time spent close to the novel male to .5. Again, we used a non-parametric test because the response variable did not follow a normal distribution (Shapiro–Wilk test: statistic = .811, *p*-value = .001).

We investigated the influence of different characteristics of the advertisement and courtship call on female choice behaviour. We built three generalized linear mixed effect models (GLMMs) following a binomial distribution with the function “glmer” in the lme4 package (Bates et al., 2016). We used the choice of the female (if the female spent most of the time with the male −1− or not −0−) as a response variable in all three models. In the first model, we added the coefficients of variation of the inter-notes intervals, inter-call interval, frequency ranges of each note, mid frequencies of each note and the mean peak frequency of calls as fixed effects. All fixed effects were scaled using the “scale” function in R (i.e., centred to their mean value and standardized to units of 1 phenotypic standard deviation). In the second model, we added the number and duration of advertisement and courtship calls, and the rate of advertisement calls as fixed effects. All fixed effects were scaled. In the last model, we added the mean frequency of courtship calls as a fixed effect. In all three models, we included trial as a random effect. We built several separate models instead of one full model with all parameters to make use of the maximum amount of data in each model. For instance, since not all individuals performed courtship calls, including the mean peak frequency of courtship calls in the model with the number and duration of calls would have led to a loss of data.

Both the familiarity and the call duration seemed to influence the time a female spent close to a male (i.e., females spent more time close to their previous partner and close to males whose advertisement call was shorter; see “Results” for more details). Therefore, we further investigated whether this could be the result of males producing shorter calls for females they already know and have mated with. To test whether there is a link between call duration and familiarity, we fitted a linear model with the call duration as response variable and the status of the male (previous partner *vs.* novel male) as a fixed effect. We added trial and male ID as random effects. We applied a constant transformation on the call duration using the function transformTukey and inspected model residuals for normal distribution using diagnostic qq-plots. We acknowledge that introducing an interaction between familiarity status and call characteristics would have been particularly interesting to deepen our understanding of female choice behaviour, but unfortunately our sample size does not allow for such analysis.

Results

We first confirmed that we used novel males and previous partners of similar size (independent two samples t-test, t-value = −1.031, p-value = .308). We also verified the absence of a potential side bias on female choice (Wilcoxon one sample test, V = 162, p-value = .472).

When testing for a preference based on familiarity, we first checked that females were responsive to the presence of male. In average females spent 50% of their time close to the previous partner, 35% close to the novel male and 15% in the central section. We found very strong evidence (*sensu* Muff et al., 2021) that females spent significantly less than 1/3 of the time in the central section ($z = -3.35$, $V = 28$, pseudo median = .09, p-value <.001), while we found weak evidence that they spent more than 1/3 of the total trial time close to the previous partner ($z = 1.83$, $V = 198$, pseudo median = .51, p-value = .07; Figure 2). Finally, females spent around a 1/3 of the time close to the novel male ($z = .34$, $V = 149$, pseudo median = .34, p-value = .749; Figure 2). We also found that females did not spend more or less than half of the total paired time close to the novel male ($z = -1.19$, $V = 99$, pseudo median = .44, p-value = .238).

When investigating the effect of call characteristics on female choice, our model results showed moderate evidence that females spent more time close to the males whose advertisement calls were on average shorter (Figure 3; Table 1) and we found weak evidence that females spent more time close to the males who called at a higher rate (Table 1). The amount and average frequency of calls had no effect on female's choice (Table 1). The consistency of the time interval between two notes of the frequency range and mid frequency of the notes did not influence female's choice (Table 1). None of the measured courtship call characteristics had an effect on female choice (Table 1). Based on our results, we wondered if males possibly produced shorter calls for the females they have already mated with (i.e., previous partners). However, we did not find a link between call duration and male familiarity status (Table 1). Call duration of previous partner and novel males did not differ, which confirms that call duration in itself is an important factor in female mate choice, no matter if the female already mated with the calling male or not. To sum up, in our trials females preferred previous mating partners over novel males. They also preferred males with shorter advertisement call, but previous partners and short-calling males were not necessarily the same individuals.

Table 1. Results of the generalized linear mixed effect models looking at how call characteristics of male advertisement and courtship calls affect female choice behaviour. Sample size (N) are presented for each model. Results indicating at least weak evidence (*sensu* Muff et al., 2021) are written in bold. The fixed effects in the first two models have been scaled (i.e., centred to their mean value and standardized to units of 1 phenotypic standard deviation).

Response variable	**Fixed effects**	**N**	**Estimate**	**Standard-error**	***p*-value**
Female's choice	intercept	24	-1.05	0.98	0.285
	time interval note 1 – note 2		-2.06	1.77	0.246
	time interval note 2 – note 3		0.47	1.56	0.763
	time interval note 3 – note 4		1.48	1.55	0.340
	time interval between calls		-2.83	1.81	0.119
	frequency range note 1		1.35	2.38	0.570
	frequency range note 2		1.34	3.52	0.703
	frequency range note 3		-2.34	3.45	0.498
	frequency range note 4		-0.34	1.55	0.829
	mid frequency note 1		0.91	2.14	0.670
	mid frequency note 2		-1.76	2.56	0.491
	mid frequency note 3		2.72	3.84	0.480
	mid frequency note 4		-0.63	2.97	0.833
	advertisement call frequency		1.91	2.79	0.494
	Random effects				
	trial		0	0	
Female's choice	intercept	34	0.01	0.44	0.984
	number of advertisement calls		0.30	0.54	0.577
	advertisement calls duration		-4.75	2.10	**0.024**
	advertisement calls rate		3.52	1.97	**0.074**
	number of courtships calls		0.70	0.57	0.220
	courtships calls duration		-0.53	0.58	0.360
	Random effects				
	trial		0	0	
Female's choice	intercept	20	5.58	4.14	0.178
	courtship call frequency		-0.00	0.00	0.194
	Random effects				
	trial		0	0	
Advertisement call duration	intercept	38	0.02	0.00	<0.001
	status (previous partner *vs* novel)		0.00	0.00	0.436
	Random effects				
	male ID		$1.89*10^{-4}$	0.01	
	trial		0.00	0.00	
	residual		$5.58*10^{-5}$	0.01	

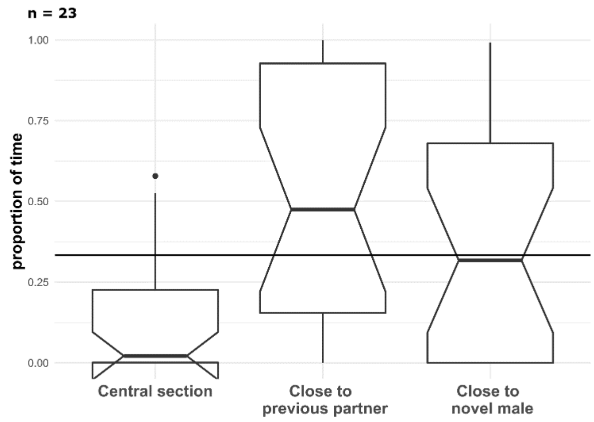


Figure 2. Boxplot of the proportion of time spent by females in each section. The horizontal line crossing the entire plot represents a third of the total time. The median is represented by thick dark lines, the interquartile range is represented by the upper and lower edges of each box, the qualitative difference in median is represented by the notches, and the upper and lower quantiles (1.5*IQR) are represented by the whiskers.

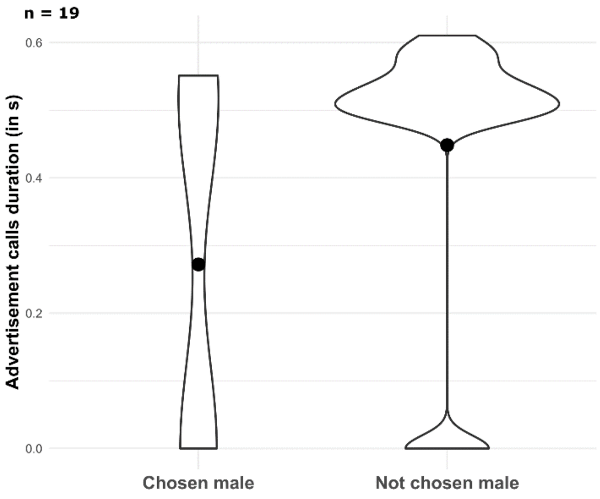


Figure 3. Violin plot of the call duration (in seconds) of the chosen and non-chosen males (i.e., males with whom the females spent respectively more and less than half of the paired proportion of time). The dot represents the mean.

Discussion

In the present study, we investigated mechanisms underlying female mate choice in the Neotropical poison frog *Allobates femoralis*. Previous studies in this species had revealed a highly promiscuous mating system, suggesting low opportunity for sexual selection and a seemingly random mating strategy in females (Montanarin et al., 2011; Ringler et al., 2012; Stückler et al., 2019; Ursprung et al., 2011). Yet, a preference for novel mating partners by females, or a choice based on the characteristics of male advertisement or courtship calls had not been investigated and therefore could not be ruled out so far. Here, we show that females tested in the laboratory in a two-choice test seem to have a preference for their previous mating partner over novel males and also for males (previous partners or not) with shorter 4-note advertisement calls.

We did not find a significant difference in the time females spent with the previous versus the novel partner, yet we found a trend towards a preference for their previous partner. Females spent about half of the time close to their previous partner against 35% of the time close to the novel male. Our initial expectation that females would prefer novel males was motivated by potential benefits associated with multiple mating partners (Evans & Magurran, 2000; Hosken & Blanckenhorn, 1999; Jennions & Petrie, 2000). In our captive colony, females had been cohabitated with the respective previous mating partners in a restricted space (i.e., terrarium) for a relatively long time prior to the experiment. We cannot rule out that these particular housing conditions might have induced habituation and/or caused pair bonding and thereby influenced the decision to stay with these males in our choice experiment. However, although pair bonding and even monogamy have been observed in other poison frogs (Brown et al., 2010; Caldwell, 1997), this behaviour is unknown from the field in *A. femoralis*. The long cohabitation prior to the experiment also means that all females had previously witnessed successful reproduction with, and beneficial paternal care provided by the respective male. Therefore, choosing the previous partner might have been the females' most promising way to ensure future reproduction based on the knowledge gained from previous experience.

As another possibility, in direct contests female *A. femoralis* might prefer known territory holders over males with unknown territorial status, similar to observations in the related poison frog *Oophaga pumilio*, where direct male–male contest overrules a strong tendency for assortative mating by colour morph (Yang & Richards-Zawacki, 2020). In our experimental setup, females only had information about the territorial status of the familiar male, whereas the status of the new male was unknown to them. We also cannot rule out that females had made their choice already prior to being in the experimental setup. All females needed to be ready to mate at the time we transferred them into the setup, which we decided based on the time since their last clutch. Therefore, they could have already decided to mate with the only available male at the time before the experiment, which was the previous partner, before we gave them the option of another, novel male during the experiment. However, in our opinion it is unlikely that this had been the case for the majority of females tested in our experiment.

In the wild, females *A. femoralis* do not stay in close proximity to males but rather commute from their resting perches to male territories for mating (Fischer et al., 2020; Ringler et al., 2012). After oviposition, they do not stay long enough to directly witness and assess the parental abilities of the male (Fischer et al., 2020; Stückler et al., 2019). Therefore, under natural conditions females are unable to predict benefits that might arise from actively choosing to mate again with a previous partner. Preliminary data and long-term monitoring of a wild population show that, in low-density areas or isolated situations, females mate repeatedly with the same male (E. Ringler, unpublished data). In these areas, females probably trade-off the potential benefits of multiple mating partner against the costs to search for and access them and decide for an apparently monogamous mating strategy. We see the possibility that, in our experiment, where we presented only two males to the female, this condition resembled such a low-density situation.

Although we cannot rule out that visual recognition also played a role in our experiment (e.g., via the ventral colour pattern or the shape of the lateral bright line), we assume that females recognized previous partners based on their call characteristics. Previous research has shown that the calls of *A. femoralis* males are individually distinct and would allow for individual discrimination (Gasser et al., 2009; Tumulty et al., 2018). In our captive breeding colony, all *A. femoralis* were housed together as pairs in separate tanks but in the same room. In this setting, males from the other tanks were strongly attenuated and audible only as a conflated background chorus. Each female was therefore more subjected to the calls of the male she was housed with, making it more likely for her to learn and subsequently discriminate distinctive features of his call. In the wild, females commute from their resting site to males' territories within 20 m and typically make the decision on whom to mate with before approaching a male (Stückler et al., 2019). Therefore, it is unlikely that females would choose a mating partner based on familiar morphological features or chemical stimuli. Recognition based on call acoustic cues is much more plausible.

In the present study, we also investigated whether female choice is based on certain characteristics of the advertisement or courtship call. Given the costs associated with advertising in terms of energy expenditure and elevated risk of predation (Ryan et al., 1982; Wells, 2001; Zahavi, 1977), call characteristics in anurans may convey important information about overall male quality. We found that the acoustic characteristics of courtship calls did not affect female behaviour in our experiment. As male *A. femoralis* only switch to courtship calls when the female is visible and in the immediate vicinity, this result is in line with previous observations in the field, that females make their mating decisions before approaching males and engaging in close distance courtship (Stückler et al., 2019).

Our results also show that females prefer the male whose advertisement calls were shorter on average, whether they were previous mating partners or not. Advertisement calls became shorter or longer by a change in the note and inter-note interval duration. We also found weak evidence that females prefer males with higher advertisement call rates. In numerous anuran species, spectral and temporal call properties are used by females to assess the quality of potential mates (Gerhardt, 1991; Giacoma et al., 1997; Klump & Gerhardt, 1987; Schwartz et al., 2001; Tárano & Fuenmayor, 2013; Welch et al., 1998). For example, call duration is often correlated with male body size (e.g., in the green toad *Bufotes viridis*: Giacoma et al., 1997). However, since we controlled for equal body size of the two presented males in our experiment, we see no evidence that the choice for shorter calls could be related to a preference for smaller males. Producing short calls requires a quick adjustment of the muscles involved in the production of calls and therefore could be a reliable indicator of high motivation, better cognitive performance and/or motor control in calling males (Prestwich, 1994). Likewise, to call at a higher rate requires more power and therefore could lead to a higher energy expenditure, potentially representing an honest signal of male quality. A recent study also found a preference of females for males with a higher call rate in the Cope's gray treefrog (*Dryophytes chrysoscelis*). However, this preference decreased if the timing of individual calls became increasingly inconsistent (Tanner & Bee, 2020). In our experiment, the consistency of the males' calls in the temporal and spectral domain did not influence female choice. Future studies should look at the energy expenditure in the production of different components in the advertisement call.

Advertisement calls have also been shown to signal the quality of paternal care in another dendrobatid frog, the Golden Rocket Frog (*Anomaloglossus beebei*). Males who produced longer calls also provided higher quality care and were preferred by females (Pettitt et al., 2020). So far, we do not have any indication that advertisement calls in *A. femoralis* convey information about male parental state or that parental males are actually preferred by females. But to investigate the relationship between male care, call characteristics and female preferences might be an interesting topic for future studies.

While mating decisions in the wild and in the laboratory might differ, our study provides first evidence for active female mate choice in a poison frog with sequential polyandry. One aspect that a two-choice test fails to address is the availability of further males, as it is the regular situation in the wild, and which could lead the females to make less than optimal choices as a result of a decoy effect. Lea and Ryan (2015) found that Tungara Frogs (*Physalaemus pustulosus*) subjected to two males choose the one with the most appealing call (i.e., with low dominant frequency, longer durations and faster call rates in their study) but reverse their choice when a third male with the least appealing call is introduced. Although we found evidence for choice being based on certain characteristics of the advertisement call produced by males, further studies are needed to investigate the link between characteristics of advertisement calls, mate choice and reproductive success in *A. femoralis*. In other dendrobatid species, a link between female mate choice and overall calling activity of individual males has been found (Pröhl, 2003; Roithmair, 1994; Souza et al., 2021). Future studies in the wild should investigate whether female choice in *A. femoralis* is based on immediate call characteristics or on the accumulated information on long-term calling effort and acoustic interactions between advertising males. Our study thereby provides a basis for further studies into female mate choice in *A. femoralis*.

Data availability statement

The datasets generated during and/or analyzed during the current study are available in the Open Science Framework repository : https://doi.org/10.17605/ OSF.IO/U82YH.

Authors contributions

Mélissa Peignier: Conceptualization; methodology; formal analysis; investigation; writing –original draft; writing –review and editing; visualization; project administration. Lauriane Bégué: Methodology; formal analysis; investigation; writing –original draft; writing –review and editing. Alina Gieseke: Methodology; formal analysis; investigation; writing –original draft. Diana Petri: Methodology; investigation; formal analysis; writing –original draft. Max Ringler: Methodology; resources; writing –review and editing. Eva Ringler: Conceptualization; methodology; resources; writing –review and editing; supervision; project administration; funding acquisition.

Acknowledgments

This study was funded by the Austrian Science Fund (FWF) via the project P 31518 (PI : Eva Ringler). MR was supported by an Erwin Schrödinger Grant (FWF J 3868) and a Standalone Grant (FWF P 33728) from the Austrian Science Fund (FWF). We are grateful to Josef Ursprung for building the experimental terraria for the choice experiments together with MR. We thank Birgit Szabo for comments on the manuscript. Open access funding provided by Universitat Bern.

References

Amezquita, A., Lima, A. P., Jehle, R., Castellanos, L., Ramos, Ó., Crawford, A. J., Gasser, H., & Hödl, W. (2009). Calls, colours, shape, and genes : A multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biological Journal of the Linnean Society*, *98*, 826–838. https://doi.org/10.1111/j.1095-8312.2009.01324.x

AmphibiaWeb. (2021). AmphibiaWeb : Information on amphibian biology and conservation. www.amphibiaweb.org Asab. (2020). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, *159*, I–XI. https://doi.org/10.1016/j.anbeh av.2019.11.002

Bates, D., Mächler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai B., Scheipl F., Grothendieck G., Green P., & Fox J., Bauer A. (2016). Lme4 : Linear mixed-effects models using 'Eigen' and S4. http://CRAN.R-proje ct.org/packa ge=lme4

Beck, K. B., Loretto, M.-C., Ringler, M., Hödl, W., & Pašukonis, A. (2017). Relying on known or exploring for new ? Movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ*, *5*, e3745. https://doi.org/10.7717/peerj.3745

Bonsall, M. B., & Klug, H. (2011). The evolution of parental care in stochastic environments. *Journal of Evolutionary Biology*, *24*, 645–655. https://doi.org/10.1111/j.1420-9101.2010.02203.x

Brown, J. L., Morales, V. R., & Summers, K. (2010). A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *The American Naturalist*, *175*, 436–446. https://doi.org/10.1086/650727

Caldwell, J. P. (1997). Pair bonding in spotted poison frogs. *Nature*, *385*, 211. https://doi.org/10.1038/385211a0

Carlisle, T. R. (1982). Brood success in variable environments : Implications for parental care allocation. *Animal Behaviour*, *30*, 824–836. https :// doi.org/10.1016/S0003-3472(82)80156-5

da Rocha, S. M. C., Pimentel Lima, A., & Kaefer, I. L. (2018). Territory size as a main driver of male-mating success in an Amazonian nurse frog (*Allobates paleovarzensis*, Dendrobatoidea). *Acta Ethologica*, *21*(1), 51–57. https://doi.org/10.1007/s10211-017-0280-5

Darwin, C. (1871). *Principles of sexual selection in the descent of man, and selection in relation to sex*. John Murray. https://doi.org/10.1037/12293-008

Donnelly, M. A. (1989). Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. *Oecologia*, *81*(2), 212–218. https://doi.org/10.1007/BF003 79808

Dreher, C. E., & Pröhl, H. (2014). Multiple sexual signals : Calls over colors for mate attraction in an aposematic, color-diverse poison frog. *Frontiers in Ecology and Evolution*, *2*(29), 3224. https://doi.org/10.3389/fevo.2014.00022

Dreher, C. E., Rodríguez, A., Cummings, M. E., & Pröhl, H. (2017). Mating status correlates with dorsal brightness in some but not all poison frog populations. *Ecology and Evolution*, *7*(24), 10503–10512. https://doi.org/10.1002/ece3.3531

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science*, *197*, 215–223. https://doi.org/10.1126/scien ce.327542

Erich, M., Ringler, M., Hödl, W., & Ringler, E. (2015). Brood-partitioning behaviour in unpredictable environments : Hedging the bets ? *Behavioral Ecology and Sociobiology*, *69*, 1011–1017. https://doi.org/10.1007/s00265-015-1913-1

Evans, J. P., & Magurran, A. E. (2000). Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 10074–10076. https://doi.org/10.1073/pnas.18020 7297

Felton, A., Alford, R. A., Felton, A. M., & Schwarzkopf, L. (2006). Multiple mate choice criteria and the importance of age for male mating success in the microhylid frog, Cophixalus ornatus. *Behavioral Ecology and Sociobiology*, *59*, 786–795. https://doi.org/10.1007/s00265-005-0124-6

Fischer, M.-T., Ringler, M., Ringler, E., & Pašukonis, A. (2020). Reproductive behavior drives female space use in a sedentary neotropical frog. *PeerJ*, *8*, e8920. https://doi.org/10.7717/peerj.8920

Fox, C. W., & Rauter, C. M. (2003). Bet-hedging and the evolution of multiple mating. *Evolutionary Ecology Research*, *5*, 273–286.

Friard, O., Gamba, M., & Fitzjohn, R. (2016). BORIS : A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*, 1325–1330. https://doi.org/10.1111/2041-210X.12584

Gade, M. R., Hill, M., Saporito, R. A., & Foster, S. (2016). Color assortative mating in a mainland population of the poison frog *Oophaga pumilio*. *Ethology*, *122*(11), 851–858. https://doi.org/10.1111/ eth.12533

Garcia-Gonzalez, F., Yasui, Y., & Evans, J. P. (2015). Mating portfolios : Bet-hedging, sexual selection and female multiple mating. *Proceedings of the Royal Society London – Biological Sciences*, *282*, 20141525. https://doi.org/10.1098/rspb.2014.1525

Gasser, H., Amézquita Torres, A., & Hödl, W. (2009). Who is calling ? Intraspecific call variation in the aromobatid frog *Allobates femoralis*. *Ethology*, *115*, 596–607. https://doi.org/10.1111/j.1439-0310.2009.01639.x

Gerhardt, H. C. (1991). Female mate choice in treefrogs : Static and dynamic acoustic criteria. *Animal Behaviour*, *42*, 615–635. https://doi.org/10.1016/S0003-3472(05)80245-3

Giacoma, C., Zugolaro, C., & Beani, L. (1997). The advertisement calls of the green toad (*Bufo viridis*) : Variability and role in mate choice. *Herpetologica*, *53*, 454–464.

Gottsberger, B., & Gruber, E. (2004). Temporal partitioning of reproductive activity in a neotropical anuran community. *Journal of Tropical Ecology*, *20*, 271–280. https://doi.org/10.1017/S0266 46740 3001172

Hamilton, W. D. (1990). Mate choice near or far. *Integrative and Comparative Biology*, *30*, 341–352. https://doi.org/10.1093/ icb/30.2.341

Hödl, W., Amézquita Torres, A., & Narins, P. M. (2004). The role of call frequency and the auditory papillae in phonotactic behavior in male dart-poison frogs *Epipedobates femoralis* (Dendrobatidae). *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *190*, 823–829. https://doi.org/10.1007/ s00359-004-0536-1

Hosken, D. J., & Blanckenhorn, W. U. (1999). Female multiple mating, inbreeding avoidance, and fitness : It is not only the magnitude of costs and benefits that counts. *Behavioral Ecology*, *10*, 462–464. https://doi.org/10.1093/behec o/10.4.462

Humfeld, S. C. (2013). Condition-dependent signaling and adoption of mating tactics in an amphibian with energetic displays. *Behavioral Ecology*, *24*, 859–870. https://doi.org/10.1093/behec o/art024

Janetos, A. C. (1980). Strategies of female mate choice : A theoretical analysis. *Behavioral Ecology and Sociobiology*, *7*, 107–112.

Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply ? A review of the genetic benefits. *Biological Reviews*, *75*, 21–64. https://doi.org/10.1111/j.1469-185X.1999.tb000 40.x

K. Lisa Yang Center for Conservation Bioacoustics. (2011–2021). *Raven Pro (Version 1.6.1) [Computer software]*. The Cornell Lab of Ornithology. https://ravensoundsoftw are.com/

Klump, G. M., & Gerhardt, H. C. (1987). Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature*, *326*, 286–288. https://doi.org/10.1038/326286a0

Kokko, H., Brooks, R. C., Jennions, M. D., & Morley, J. (2003). The evolution of mate choice and mating biases. *Proceedings of the Royal Society London –Biological Sciences*, *270*, 653–664. https://doi.org/10.1098/rspb.2002.2235

Krasnec, M. O., Cook, C. N., & Breed, M. D. (2012). Mating systems in sexual animals. *Nature Education Knowledge*, *10*, 72.

Lea, A. M., & Ryan, M. J. (2015). Irrationality in mate choice revealed by túngara frogs. *Science*, *349*, 964–966. https://doi.org/10.1126/ scien ce.aab2012

Lüddecke, H. (2002). Male and female responses to call playback in the Andean frog *Colostethus subpunctatus*. *Amphibia-Reptilia*, *23*(2), 141–150. https://doi.org/10.1163/15685 38027 60061787

Luz Nunes-de- Almeida, C. H., Batista Haddad, C. F., & Toledo, L. F. (2021). A revised classification of the amphibian reproductive modes. *Salamandra*, *57*, 413–427.

Maan, M. E., & Cummings, M. E. (2008). Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution*, *62*(9), 2334–2345. https://doi.org/10.1111/j.1558-5646.2008.00454.x

Maan, M. E., & Cummings, M. E. (2009). Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(45), 19072–19077. https://doi.org/10.1073/pnas.09033 27106

Maan, M. E., & Cummings, M. E. (2012). Poison frog colors are honest signals of toxicity, particularly for bird predators. *The American Naturalist*, *179*(1), E1–E14. https://doi.org/10.1086/663197

McClelland, B. E., Wilczynski, W., & Ryan, M. J. (1996). Correlations between call characteristics and morphology in male cricket frogs (Acris crepitans). *The Journal of Experimental Biology*, *199*, 1907–1919.

Meuche, I., Brusa, O., Linsenmair, K., Keller, A., & Pröhl, H. (2013). Only distance matters – non- choosy females in a poison frog population. *Frontiers in Zoology*, *10*, 29. https://doi.org/10.1186/1742-9994-10-29

Montanarin, A., Kaefer, I. L., & Pimentel Lima, A. (2011). Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia : Implications for the study of a species complex. *Ethology Ecology and Evolution*, *23*, 141–150. https://doi.org/10.1080/03949 370.2011.554884

Muff, S., Nilsen, E. B., O'Hara, R. B., & Nater, C. R. (2021). Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution*, *37*(3), 203–210. https://doi.org/10.1016/j.tree.2021.10.009

Narins, P. M., Hödl, W., & Grabul, D. S. (2003). Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 577–580. https://doi.org/10.1073/pnas.02371 65100

Neff, B. D., & Pitcher, T. E. (2005). Genetic quality and sexual selection : An integrated framework for good genes and compatible genes. *Molecular Ecology*, *14*, 19–38. https://doi.org/10.1111/j.1365-294X.2004.02395.x

Pašukonis, A., Beck, K. B., Fischer, M.-T., Weinlein, S., Stückler, S., & Ringler, E. (2017). Induced parental care in a poison frog : A tadpole cross-fostering experiment. *Journal of Experimental Biology*, *220*, 3949–3954. https://doi.org/10.1242/jeb.165126

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0 : Updated guidelines for reporting animal research. *PloS Biology*, *18*(7), e3000410. https://doi.org/10.1371/ journ al.pbio.3000410

Pettitt, B. A., Bourne, G. R., & Bee, M. A. (2020). Females prefer the calls of better fathers in a neotropical frog with biparental care. *Behavioral Ecology*, *31*, 152–163. https://doi.org/10.1093/behec o/ arz172

Pough, F. H., & Taigen, T. L. (1990). Metabolic correlates of the foraging and social behaviour of dart-poison frogs. *Animal Behaviour*, *39*, 145–155. https://doi.org/10.1016/S0003-3472(05)80734-1

Prestwich, K. N. (1994). The energetics of acoustic signaling in anurans and insects. *American Zoologist*, *34*, 625–643.

Pröhl, H. (2003). Variation in male calling behaviour and relation to male mating success in the strawberry poison frog (*Dendrobates pumilio*). *Ethology*, *109*(4), 273–290. https://doi.org/10.1046/j.1439-0310.2003.00863.x

Pröhl, H., & Hödl, W. (1999). Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behavioral Ecology and Sociobiology*, *46*, 215–220. https://doi.org/10.1007/s0026 50050612

R Core Team. (2020). *R : A language and environment for statistical computing (version 4.0.2) [Computer software]*. R Foundation for Statistical Computing. http://www.r-project.org

Rasband, W. S. (1997–2021). *ImageJ (version 1.50i) [computer software]*. U. S. National Institutes of Health. http://imagej.nih.gov/ij/

Reynolds, R. G., & Fitzpatrick, B. M. (2007). Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution*, *61*(9), 2253–2259. https://doi.org/10.1111/j.1558-5646.2007.00174.x

Richards-Zawacki, C. L., & Cummings, M. E. (2011). Intraspecific reproductive character displacement in a polymorphic poison dart frog, *Dendrobates pumilio*. *Evolution*, *65*(1), 259–267. https://doi.org/10.1111/j.1558-5646.2010.01124.x

Richards-Zawacki, C. L., Wang, I. J., & Summers, K. (2012). Mate choice and the genetic basis for colour variation in a polymorphic dart frog : Inferences from a wild pedigree. *Molecular Ecology*, *21*(15), 3879–3892. https://doi.org/10.1111/j.1365-294X.2012.05644.x

Ringler, E., Pašukonis, A., Fitch, W. T., Huber, L., Hödl, W., & Ringler, M. (2015). Flexible compensation of uniparental care : Female poison frogs take over when males disappear. *Behavioral Ecology*, *26*, 1219–1225. https://doi.org/10.1093/behec o/arv069

Ringler, E., Pašukonis, A., Hödl, W., & Ringler, M. (2013). Tadpole transport logistics in a neotropical poison frog : Indications for strategic planning and adaptive plasticity in anuran parental care. *Frontiers in Zoology*, *10*, 67. https://doi.org/10.1186/1742-9994-10-67

Ringler, E., Ringler, M., Jehle, R., & Hödl, W. (2012). The female perspective of mating in *A. femoralis*, a territorial frog with paternal care –A spatial and genetic analysis. *PloS One*, *7*, e40237. https://doi.org/10.1371/journ al.pone.0040237

Ringler, E., Szipl, G., Harrigan, R. J., Bartl-Binder, P., Mangione, R., & Ringler, M. (2018). Hierarchical decision-making balances current and future reproductive success. *Molecular Ecology*, *27*, 2289–2301. https://doi.org/10.1111/mec.14583

Ringler, M., Ringler, E., Magaña Mendoza, D., & Hödl, W. (2011). Intrusion experiments to measure territory size : Development of the method, tests through simulations, and application in the frog *Allobates femoralis*. *PloS One*, *6*, e25844. https://doi.org/10.1371/journ al.pone.0025844

Roberts, J. D., & Byrne, P. G. (2011). Polyandry, sperm competition, and the evolution of anuran amphibians. In T. J. Roper, M. Naguib, & J. C. Mitani (Eds.), *Advances in the study of behavior* (Vol. *43*, pp. 1–53). Academic Press.

Roithmair, M. E. (1992). Territoriality and male mating success in the dart-poison frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology*, *92*(4), 331–343. https://doi.org/10.1111/j.1439-0310.1992.tb009 70.x

Roithmair, M. E. (1994). Male territoriality and female mate selection in the dart-poison frog *Epipedobates trivattatus* (Dendrobatidae, Anura). *Copeia*, *1994*(1), 107–115. https://doi.org/10.2307/1446676

Rosenthal, G. G. (2017). *Mate choice. The evolution of sexual decision making from microbes to humans*. Princeton University Press.

Rstudio Team. (2019). *Rstudio : Integrated development for R (version 1.3.1093) [Computer software]*. Rstudio, Inc. http://www.rstudio.com

Ryan, M. J., Bartholomew, G. A., & Rand, A. S. (1983). Energetics of reproduction in a neotropical frog, *Physalaemus pustulosus*. *Ecology*, *64*, 1456–1462. https://doi.org/10.2307/1937500

Ryan, M. J., Tuttle, M. D., & Rand, S. A. (1982). Bat predation and sexual advertisement in a neotropical anuran. *The American Naturalist*, *119*, 136–139. https://doi.org/10.1086/283899

Schwartz, J. J., Buchanan, B. W., & Gerhardt, H. C. (2001). Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behavioral Ecology and Sociobiology*, *49*, 443–455. https://doi.org/10.1007/s0026 50100317

Souza, J. R. D., Kaefer, I. L., & Pimentel Lima, A. (2021). Calling activity determines male mating success in a territorial frog with parental care. *Ethology*, *127*, 359–365. https://doi.org/10.1111/eth.13135

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W., & Ringler, E. (2019). Spatio-temporal characteristics of the prolonged courtship in brilliant-thighed poison frogs, *Allobates femoralis*. *Herpetologica*, *75*, 268–279. https://doi.org/10.1655/Herpetologica-D-

19-00010.1

Sullivan, B. K., Ryan, M. J., & Verrell, P. A. (1995). Female choice and mating system structure. In B. K. Sullivan & H. Heatwole (Eds.), *Amphibian biology. Volume 2 : Social behaviour* (pp. 469–517). Surrey Beatty.

Summers, K., Symula, R., Clough, M. E., & Cronin, T. W. (1999). Visual mate choice in poison frogs. *Proceedings of the Royal Society London –Biological Sciences*, *266*, 2141–2145. https://doi.org/10.1098/ rspb.1999.0900

Taigen, T. L., & Wells, K. D. (1985). Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *Journal of Comparative Physiology B*, *155*, 163–170. https://doi.org/10.1007/BF006 85209

Tanner, J. C., & Bee, M. A. (2020). Inconsistent sexual signaling degrades optimal mating decisions in animals. *Science Advances*, *6*(20), eaax3957. https://doi.org/10.1126/sciadv.aax3957

Tárano, Z., & Fuenmayor, E. (2013). Mate choice based on acoustic features in Johnstone's whistling frog *Eleutherodactylus johnstonei* : An experimental approach. *South American Journal of Herpetology*, *8*, 52–59. https://doi.org/10.2994/SAJH-D-12-00016.1

Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage : Invited review. *Molecular Ecology*, *9*, 1013–1027. https://doi.org/10.1046/j.1365-294x.2000.00964.x

Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man : 1871–1971* (2nd ed., pp. 136–179). Aldine.

Tumulty, J. P., Pašukonis, A., Ringler, M., Forester, J. D., Hödl, W., & Bee, M. A. (2018). Brilliant-thighed poison frogs do not use acoustic identity information to treat territorial neighbours as dear enemies. *Animal Behaviour*, *141*, 203–220. https://doi.org/10.1016/j.anbeh av.2018.05.008

Ursprung, E., Ringler, M., Jehle, R., & Hödl, W. (2011). Strong male/male competition allows for nonchoosy females : High levels of polygynandry in a territorial frog with paternal care. *Molecular Ecology*, *20*, 1759–1771. https://doi.org/10.1111/j.1365-294X.2011.05056.x

Welch, A. M., Semlitsch, R. D., & Gerhardt, H. C. (1998). Call duration as an indicator of genetic quality in male gray tree frogs. *Science*, *280*, 1928–1930. https://doi.org/10.1126/science.280.5371.1928

Wells, K. D. (2001). The energetics of calling in frogs. In M. J. Ryan (Ed.), *Anuran communication* (pp. 45–60). Smithsonian Institution Press.

Wells, K. D. (2007). *The ecology and behavior of amphibians*. University of Chicago Press.

Werner, N. Y., & Lotem, A. (2003). Choosy males in a haplochromine cichlid : First experimental evidence for male mate choice in a lekking species. *Animal Behaviour*, *66*, 293–298. https://doi.org/10.1006/ anbe.2003.2208

Weygoldt, P. (1980). Zur Fortpflanzungsbiologie von *Phyllobates femoralis* (Boulenger) im Terrarium. *Salamandra*, *16*, 215–226.

Yang, Y., & Richards-Zawacki, C. L. (2020). Male–male contest limits the expression of assortative mate preferences in a polymorphic poison frog. *Behavioral Ecology*, *32*(1), 151–158. https://doi.org/10.1093/ behec o/araa114

Yasui, Y. (2001). Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. *Ecological Research*, *16*, 605–616. https://doi.org/10.1046/j.1440-1703.2001.00423.x

Yasui, Y., & Garcia-Gonzalez, F. (2016). Bet-hedging as a mechanism for the evolution of polyandry, revisited. *Evolution*, *70*, 385–397. https://doi.org/10.1111/evo.12847

Zahavi, A. (1977). The cost of honesty. *Journal of Theoretical Biology*, *67*, 603–605. https://doi.org/10.1016/0022-5193(77)90061-3

Manuscript 6: "Opposing effects of personality traits on mating and cross-generational reproductive success".

This manuscript will be submitted to *Ecology Letters*

Mélissa Peignier[a,b,*], Yimen G. Araya-Ajoy[c], Max Ringler[a,d,e,f], & Eva Ringler[a,b]

[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[b] Messerli Research Institute, University of Veterinary Medicine Vienna, Austria
[c] Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[d] Department of Behavioral and Cognitive Biology, University of Vienna, Austria
[e] Department of Evolutionary Biology, University of Vienna, Austria
[f] Institute of Electronic Music and Acoustics, University of Music and Performing Arts Graz, Austria

*Correspondence :
Mélissa Peignier
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
melissa.peignier@unibe.ch

Abstract

Reproductive success is shaped by multiple factors, such as the ability to acquire mating partners, offspring production and offspring survival until adulthood. Personality traits – such as boldness or aggressiveness – can impact single components of reproductive success, but we have limited information about how these traits influence the various components of reproductive success. Yet, personality traits which are positively linked to one component of reproductive success might not necessarily be equally beneficial for all components, and correlational selection may favour different combinations of traits. We used the Neotropical poison frog *Allobates femoralis* to investigate the influence of boldness, aggressiveness, and exploration on mating success, reproductive performance, and reproductive output. We found that personality traits had different – even opposing – effects on mating and reproductive output of males and females, which could lead to the evolution of different reproductive strategies.

Keywords

animal personality, reproductive strategies, poison frog, mating success, aggressiveness, exploration, boldness

Introduction

Individual reproductive success is shaped by different processes, such as the acquisition of mating partners, offspring production, and offspring survival until adulthood. Animal personality, defined as behavioural differences between individuals that are consistent across time and context, has also been shown to impact individual reproductive success (Sánchez-Tójar et al., 2022; Smith & Blumstein, 2008). Multiple studies have investigated the influence of personality on single processes such as the number of offspring produced or surviving until adulthood, or parental performances (Janczak et al., 2003; Mutzel et al., 2013; Réale et al., 2009; Schuett et al., 2012; Smith & Blumstein, 2008). For instance, fast-exploring Blue Tit females (*Cyanistes caeruleus*) have a higher reproductive success than slow-exploring ones because they feed their offspring at higher rates (Mutzel et al., 2013). Yet, we are still missing detailed insight into how personality differently affects the various processes shaping reproductive success.

Multiple hypotheses have been proposed to explain the emergence and maintenance of individual behavioural differences (Réale & Dingemanse, 2012). The hypothesis that has received the strongest support to date stipulates that between-individual differences in behaviour, and behavioural consistency, have evolved and are maintained because of their link with individual differences in life-history trade-offs (Biro & Stamps, 2008; Wolf et al., 2007). On the one hand, more aggressive males might benefit from increased reproductive success, while less aggressive males might benefit from increased survival. On the other hand, individuals with high future expectations in terms of reproduction should be more risk-averse (e.g., less aggressive, less bold, less explorative) than individuals with low expectations.

So far, studies that tried to provide empirical support for this hypothesis have only looked at the link between one personality trait and one measure of reproductive success / survival (e.g., Ariyomo & Watt, 2013; Bonnot et al., 2018; Collins et al., 2019; Monceau et al., 2017; Réale et al., 2009). No study has looked at the potentially differential effects that personality traits can have on the various processes shaping reproductive success (e.g., acquisition of mating partners, number of offspring produced and offspring survival). Yet, personality traits which are positively linked to one component of reproductive success might not necessarily be equally beneficial for all components. For instance, more aggressive individuals might benefit in gaining access to mates, but be less performant in parental care, consequentially affecting their offspring' survival. Furthermore, correlational selection may favour different combinations of personality traits (Montiglio et al., 2017). Typically, correlational selection occurs when the influence of an individual's personality trait on a component of reproductive success depends on that individual's score for another personality trait (Svensson et al., 2021). Selection then acts to maintain phenotypic correlations among traits. For instance, it could be beneficial (in terms of reproductive success) to be aggressive only if individuals are also bold. Importantly, the effect of particular personality traits on reproductive success might not be the same in males and females, as both sexes often face differential selective pressures on their reproductive behaviours (Darwin, 1871; Rosenthal & Ryan, 2022).

In this study, we monitored an entire, free-ranging population of the Brilliant-thighed Poison Frog (*Allobates femoralis*) during their reproductive season to examine the influence of personality on different processes shaping reproductive success. Amphibians have diverse and often complex reproductive behaviours (Luz Nunes-de-Almeida et al., 2021; Schulte et al., 2020; Wells, 2007) and, Neotropical poison frogs (Dendrobatidae *sensu* AmphibiaWeb 2022) possess behaviours with ideal prerequisites for within- and between-individual behavioural variation, including territoriality, elaborate courtship behaviour, and complex parental care (Crump, 1972; Lötters et al., 2007; Pašukonis et al., 2013; Pröhl, 2005; Roithmair, 1994; Rojas & Pašukonis, 2019; Souza et al., 2021; Stynoski et al., 2015; Wells, 2007).

We studied the influence of boldness, aggression, and exploration levels on the number of mating partners, clutches produced (hereafter 'reproductive performance'), and offspring that survived until adulthood (hereafter 'reproductive output') in males and females *A. femoralis*. To this end, we conducted repeated behavioural experiments and collected tissue samples for genetic analysis to infer parent-offspring relationships. We expected boldness, exploration and aggression levels to impact the three processes shaping reproductive success (i.e., mating success, reproductive performance, and reproductive output) differently. On the one hand, we expected more aggressive and bolder males, that advertise and defend their territory more vigorously (Hödl, 1983; Ringler et al., 2011), to obtain more mating partners, and consequently, produce more clutches. On the other hand, males showing these characteristics might be more likely to accidentally attack females crossing their territory, resulting in a lower mating success compared to less aggressive or bold males (Sonnleitner et al., 2020).

Highly aggressive and bold males might also have a greater probability to be detected by predators while transporting their tadpoles by being less careful, resulting in a lower reproductive output. We further expected exploration to have a different impact on both sexes. Males who spend more time exploring, and therefore less time advertising for females, should have less mating partners and reproductive performance. However, females who must commute from their resting site to male territories for mating should have more mating partners and a higher reproductive success if they spend more time exploring (Fischer et al., 2020; Ringler et al., 2012). Overall, we found support for our predictions. Personality traits differentially impacted the three components of reproductive success, which we argue could lead to the evolution of different reproductive strategies.

Material and methods

Ethical note

This study was approved by the ethics and animal welfare committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice (GSP) guidelines and national legislation. It was also approved by the scientific committee of the ‘Nouragues Ecological Research Station’. We conducted sampling in strict accordance with current French and EU law and we followed the ASAB guidelines (2021) on the treatment of study animals. The CNRS Guyane (‘Centre National de la Recherche Scientifique Guyane’), the ‘Ministère de la transition écologique et solidaire’ (permit number: TREL2002508S/303), and the Secretariat of the Convention on Biological Diversity (APA declaration: TREL1734890A/34) provided working and tissue sampling permissions.

Study system

Allobates femoralis is a diurnal frog with a highly promiscuous mating system (Montanarin et al., 2011; Stückler et al., 2019; Ursprung et al., 2011a). Males and females vary in exploration and boldness levels, and males vary in their aggression level (Chaloupka et al., 2022; Peignier et al., 2022a). Males are territorial, and the possession of a territory is directly linked to their mating success (Ursprung et al., 2011a). Females commute from their perching site to male territories located within 20 m radius to mate, and the mating decision is typically made prior to approaching a male

(Fischer et al., 2020; Ringler et al., 2012; Stückler et al., 2019). Females lay clutches in the leaf litter and males provide care by transporting the newly hatched tadpoles to water bodies located outside of their territory (Beck et al., 2017; Ringler et al., 2013b, 2018). Spreading reproductive events across multiple partners, rather than mating multiple times with the same individual, could help reduces risks of failed reproduction (e.g., genetic incompatibility) or poor paternal care (e.g., failure from the male to transport the tadpoles). For this reason, we used the number of different mating partners, rather than the number of mating events, as the measure of mating success in our study. Also, we used the number of clutches, rather than the number of tadpoles produced, to avoid underestimating an individual's reproductive performance. Previous work has shown that body size is not related to mating or reproductive success in *A. femoralis* (Ursprung et al., 2011a).

Study site and population survey

We conducted our study on an experimental population of *Allobates femoralis* located on a river island of approximately 5 ha in a lowland rainforest. The island is situated in the proximity of the field camp 'Saut Pararé' of the CNRS Nouragues Ecological Research Station in the nature reserve 'Les Nouragues' in French Guiana (4°02´ N, 52°41´W). The population was introduced in 2012 and has been stable since then, with approximately 150 adult individuals (for detailed information see Ringler et al., 2015). The frogs in the study area rely primarily on an array of artificial pools for tadpole deposition. In 2018, 14 pools were positioned across the island in a cross-shaped array (volume: ~ 15 l). We sampled all tadpoles from these pools before removing them at the beginning of the 2019 field season. We then opened 16 new pools (volume: ~5 l) positioned in a cross-shaped array, turned by 45° in relation to the previous locations for the purpose of another study (Peignier et al., in prep). We placed the 14 old pools back at their original location at the end of the field season.

We monitored the population on the island every day from 0900 to 1800 h between February and April 2019 and caught every adult we encountered. We identified all adult frogs via visual identification of their distinct ventral patterns using the pattern matching software Wild-ID (Bolger et al., 2012), and collected tissue samples of all newly encountered individuals for genetic analysis. We sexed frogs by the presence (males) or absence (females) of vocal sacs. For each capture, we recorded the exact spatial locations of the frog on a digital map using rugged Win10 tablets (CAT T20, Bullitt Group, Reading, United Kingdom) with the mobile GIS software ArcPad 10 (ESRI, Redlands, CA, USA), and further handled the data in ArcGIS 10.6 (ESRI). To measure mating success, reproductive performance, and reproductive output, we sampled tadpoles from the new pools regularly between February and May 2019. Furthermore, we returned in 2020 (from February to mid-March) to collect tissue samples of the newly encountered adults (representing metamorphosed tadpoles from 2019 who survived until they reached adulthood) (see below ‘Parentage analysis’).

Behavioral tests

In the course of another study, adult individuals encountered in 2019 were tested for within- and between-individual variation in aggression, boldness, and exploration (Peignier et al., 2022a). In this study, levels of territorial aggression were assessed using acoustic playbacks to evoke territorial defence behaviour in focal males (but not in females, who do not exhibit aggressive territorial behaviours). Individual levels of exploration and boldness were assessed in both males and females using a Novel Environment Test. In total 163 territorial defence tests were conducted with 51 males (mean ± SD = 3.20 ± 1.31 repetitions per individual) and 238 Novel Environment tests with 52 males and 35 females (mean ± SD = 2.74 ± 1.33 repetitions per individual). Results from this previous study showed that the measured behaviours from both tests were repeatable and found evidence for the prevalence of the personality traits aggressiveness (only in males), exploration and boldness. The latency to jump towards an intruder in the territorial defence test, the time spent in the shelter and the distance travelled in the Novel Environment Test best represented the personality traits aggressiveness, boldness and exploration, respectively (for more details see Peignier et al., 2022a). In the present study, we thus used these three behaviours as proxies for aggressiveness, boldness and exploration for each individual.

Parentage analysis

We used a molecular parentage analysis and performed a pedigree estimation to determine the matin success, reproductive performance and reproductive output of the adult males of 2019. We sampled DNA from the adults by removing the third toe of both hind limbs, and immediately preserved it in 96% ethanol (Ringler, 2018; Ursprung et al., 2011b). Additionally, we had access to DNA of older individuals (survivors from previous years, encountered again in 2019) from a long-term monitoring on the island population. To extract DNA from tadpoles, we clipped the tip of their tail and preserved it in 96% ethanol. We always released tadpoles in artificial pools after clipping to allow them to complete their development. We sampled a total of 121 adults (64 males and 57 females) (hereafter ‘adults from 2019’) in 2019, and 1142 tadpoles (hereafter ‘tadpoles from 2019’). Additionally, we sampled 71 adults (27 males and 44 females) in 2020, among which 55 were new encounters (i.e., descendants of the 2019 cohort; hereafter ‘adult descendants from 2019’).

To isolate genomic DNA of the toe samples, we performed a Proteinase K digestion immediately followed by an extraction using a DNeasy kit (QIAGEN, Valencia, CA) to isolate genomic DNA from the tadpoles’ tails samples. We then used fluorescent-labelled primers and PCR protocols to amplify microsatellites at twelve highly variable loci (Afem03, Afem04, Afem05, Afem09, Afem12, Afem13, Afem16, Afem20, Afem22, Afem24, Afem25, Afem27) (Jehle et al., 2008; Ringler et al., 2013a). We ran the amplified products, diluted with water and mixed with internal size standard LIZ, on a capillary sequencer (ABI 3730, Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA). We identified all loci visually and determined the allele sizes in PeakScanner 1.0 (Applied Biosystems). We used the binning software Tandem 1.01 (Matschiner & Salzburger, 2009) to determine their final sizes. We removed individuals from further analyses when four or more loci could not be scored, resulting in a total of 57 males and 53 females adults from 2019, 1109 tadpoles from 2019, and 55 adult descendants from 2019 that could be used for pedigree reconstruction.

We conducted the parentage analysis in the software COLONY 2.0.6.7 (Wang, 2009), by building a medium precision full likelihood model allowing for polygamous mating in both sexes, without setting a sibship prior. We measured mating success and reproductive performance, by treating individual adults from 2019 as potential ‘fathers’ and ‘mothers’, whereas all tadpoles from 2019 were treated as potential ‘offspring’. To further investigate the influence of personality on the reproductive output of the adult males from 2019, we treated all adults from 2019 as potential parents, whereas all adult descendants from 2019 were treated as potential ‘offspring’. We used the ‘Best (ML) Configuration’ for the analyses. The software simulated parental genotype when one or both parents were not found for a tadpole. COLONY assigned 1006 tadpoles from 2019 out of 1109 (90.7%) to at least one known parent, and 695 of these 1006 individuals were assigned to both parents (69.1 %). From 2019, 52 adult males out of 57 (91.2%) and 47 females from 2019 out of 53 (88.7%) produced at least one tadpole, and 47 males (85.5%) and 48 females (87.3%) produced at least one tadpole that reached adulthood in 2020. All the 55 descendants of the adults from the 2019 cohort were assigned to at least one known parent.

To determine mating success and reproductive performance, we counted how many different mates each adult had in 2019, and how many clutches they produced. It has been shown that males distribute their clutches across several pools (Erich et al., 2015), and that captive *A. femoralis* female can lay a clutch on average every 8 days (Weygoldt, 1980). Therefore, we assumed that tadpoles from an identified pair that were deposited in one or several pools less than 6 days apart belonged to the same clutch, and represented one mating event. We also considered that tadpoles with only one parent assigned belonged to the same clutch than other tadpoles deposited less than 6 days apart with both parents assigned. In the case where all tadpoles deposited on a given day had only one parent assigned, we considered that they originated from the same clutch produced by the assigned parent and a new mate. We considered tadpoles from the same mating pair to originate from two separate clutches if they were deposited more than 6 days apart. In cases when only one parent was assigned for two clutches deposited more than 6 days apart, we assumed that the clutches originated from two different mating pairs. Finally, we counted the number of adult descendants in 2020 that each adult from 2019 produced. To this end, we only used parent–offspring triads for which at least one parent was identified. Lastly, we also investigated the prevalence of (dis)assortative mating in *A. femoralis*. Methods and results for the prevalence of (dis)assortative mating are presented in the supplementary material.

Statistical analysis

All statistical analyses were done in R v3.6.0 (R Core Team 2019). We had one value of mating success, reproductive performance, and reproductive output per individual. We re-scaled the behavioural data to facilitate interpretation (i.e., we divided the exploration score by 1000 to turn into megapixels, and the aggressiveness and boldness scores by 60 to turn them into minutes). To avoid artificially increasing sample size due to shared clutches, and because we had different expectations for both sexes, we assessed the influence of personality traits on reproductive success separately for males and females.

To investigate the influence of personality traits on males and females' reproductive success, we built three generalized linear model (GLM) per sex using the 'lme4' package (Bates et al., 2016), with mating success, reproductive performance, or reproductive output as response variables. We added an interaction between the best linear unbiased predictors (scaled BLUPs, extracted from random intercept models) of boldness and exploration scores as fixed effects in the female models, and an interaction between the BLUPs of aggressiveness, boldness, and exploration scores in the male models. We also included the number of different mates as a fixed effect in the model with the reproductive performance as the response variable, and the number of different mates and the number of clutches produced as fixed effects in the model with the reproductive output as response variable. Adding these variables as fixed effects in the models is essentially similar to a path analysis, and enabled us to study the direct and indirect effects of behaviours on the different reproductive success components. All models followed a Poisson distribution, making the estimates of the relationship between phenotype and reproductive success a very good approximation of selection gradients (Morrissey & Goudie, 2022).

We further confirmed our results using error-in-variable models, by estimating the joint likelihood of the path model parameters via Bayesian inference using the 'rstan' package (Carpenter et al., 2017). These models allow acquiring unbiased estimates of selection gradients and their uncertainty, while controlling for both measurement error and phenotypic plasticity (Dingemanse et al. 2021; Ponzi et al., 2018). We built two models (one per sex) which assumed that mating success, reproductive performance, and reproductive output could be modelled with a Poisson distribution. We added an interaction between boldness and exploration scores as fixed effects in the female model, and an interaction between aggressiveness, boldness, and exploration scores as fixed effects in the male model. Additionally, we included the number of different mates as a fixed effect in the model with the reproductive performance as the response variable, and the number of different mates and the number of clutches produced as fixed effects in the model with the reproductive output as response variable. For both models we ran 101,000 iterations with a burn-in of 1,000. We selected every 100th posterior parameter sample after the initial burn-in. Because of the high complexity of the models, we used strong priors for the relationships that we knew (from the GLMs) should be positive (e.g., effect of some reproductive parameters on others). We also used informative priors for the means of the different reproductive success components, but we used diffuse priors for the effects of behaviours on reproductive success measures. Details of model parametrization, point estimates and confidence intervals of the posterior distributions can be found in the supplementary material.

Results

On average, males had 2 different mates (± 1.15 SD), produced 3 clutches (± 1.91 SD), and had 1 offspring who survived until adulthood (± 2.02 SD), while females had 1 mate (± 1.15 SD), produced 2 clutches (± 1.98 SD), and had 1 offspring who survived until adulthood (± 1.67).

Both the generalized linear models and the error-in-variable models gave comparable results in terms of value and direction of the estimates (Supplementary tables 1, 2). As we expected, the number of different mates positively influenced reproductive performance in both males and females (Table 1, Figure 1). In females, number of mates influenced reproductive output, while in males, reproductive output was influenced by the number of clutches (Table 1, Figure 1).

We found moderate evidence (*sensu* Muff et al., 2021) for an effect of the interaction between the level of aggression and exploration on the number of mates obtained in males (Table 1). Less aggressive males obtained more mating partners if their level of exploration was low, while more aggressive males obtained more mating partners if their level of exploration was high (Figure 2). We also found strong evidence that exploratory behaviour affected mating success in females, and that this effect was stronger when females were also bolder (Table 1). More explorative females obtained more mating partners if their level of boldness was high, while females with low levels of exploration had a high number of mating partners regardless of their boldness level (Figure 2).

We did not find any effect of personality on reproductive performance (Table 1). We also showed that personality did not influence the reproductive output of females. However, we found strong evidence for an effect of the interaction between the level of aggression, exploration and boldness on reproductive output in males (Table 1). Males who were shy, not aggressive and highly explorative had the highest reproductive output (Figure 2). Males who were bold, aggressive and explorative also had a high reproductive

Table 1. Results of the generalized linear models investigating the link between personality traits and different processes shaping reproductive success for males and females. Models estimates, standard-error, and p-values are presented. Based on Muff et al. (2021), evidence of effects is reported with stars (i.e., 0.1 < p-value < 0.05: weak evidence *; 0.05 < p-value < 0.01: moderate evidence **; 0.01 < p-value: strong evidence ***). Error-in-variable models are reported in Supplementary table 1.

	Number of different mates			**Number of clutches produced**			**Number of offspring surviving to adulthood**		
Results for males (N = 51)	estimate	SE	p-value	estimate	SE	p-value	estimate	SE	p-value
(Intercept)	0.59	0.13	< 0.001***	0.17	0.21	0.399	-2.42	0.68	< 0.001***
number of mates				0.38	0.07	< 0.001***	0.06	0.22	0.795
number of clutches							0.34	0.14	0.014**
aggressiveness	-0.14	0.16	0.366	-0.20	0.14	0.138	0.34	0.19	0.080*
boldness	-0.10	0.16	0.541	-0.04	0.15	0.807	-0.89	0.47	0.060*
exploration	-0.02	0.14	0.907	-0.07	0.11	0.530	0.25	0.28	0.366
aggressiveness * boldness	-0.19	0.23	0.396	-0.01	0.21	0.956	0.72	0.36	0.047**
aggressiveness * exploration	-0.28	0.14	0.041**	-0.07	0.12	0.547	1.16	0.38	0.002***
boldness * exploration	0.04	0.14	0.792	-0.02	0.12	0.858	0.33	0.45	0.467
aggressiveness * boldness * exploration	-0.19	0.19	0.301	0.06	0.16	0.697	1.96	0.52	< 0.001***
Results for females (N = 36)	estimate	SE	p-value	estimate	SE	p-value	estimate	SE	p-value
(Intercept)	-0.17	0.26	0.505	-0.20	0.22	0.369	-1.14	0.39	0.004***
number of mates				0.53	0.10	< 0.001***	0.99	0.41	0.016**
number of clutches							-0.30	0.26	0.247
boldness	-0.40	0.24	0.095*	-0.24	0.17	0.162	-0.17	0.30	0.573
exploration	-0.88	0.36	0.015**	-0.07	0.26	0.799	-0.24	0.46	0.599
boldness * exploration	-0.53	0.20	0.010***	-0.00	0.16	0.988	0.70	0.46	0.131

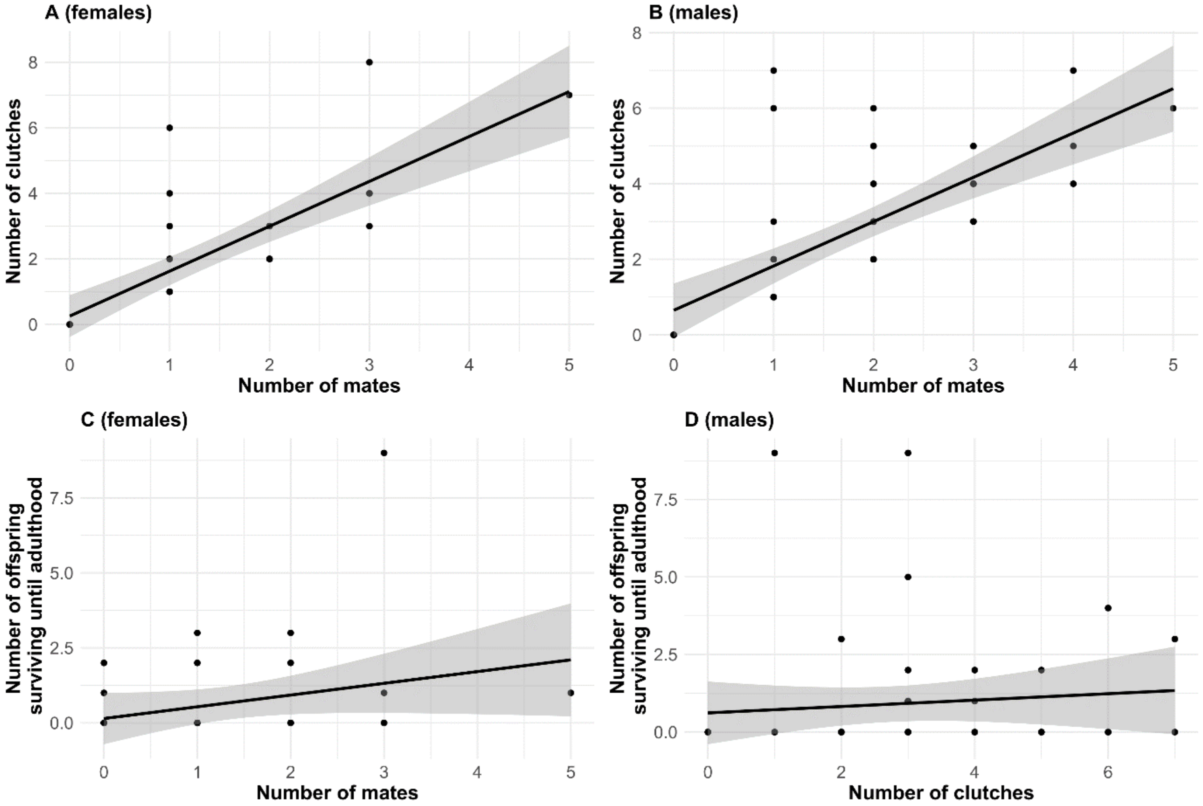


Figure 1. Influence of components of reproductive success on others. The grey area around the regression line (in black) represents the 95% confidence interval.

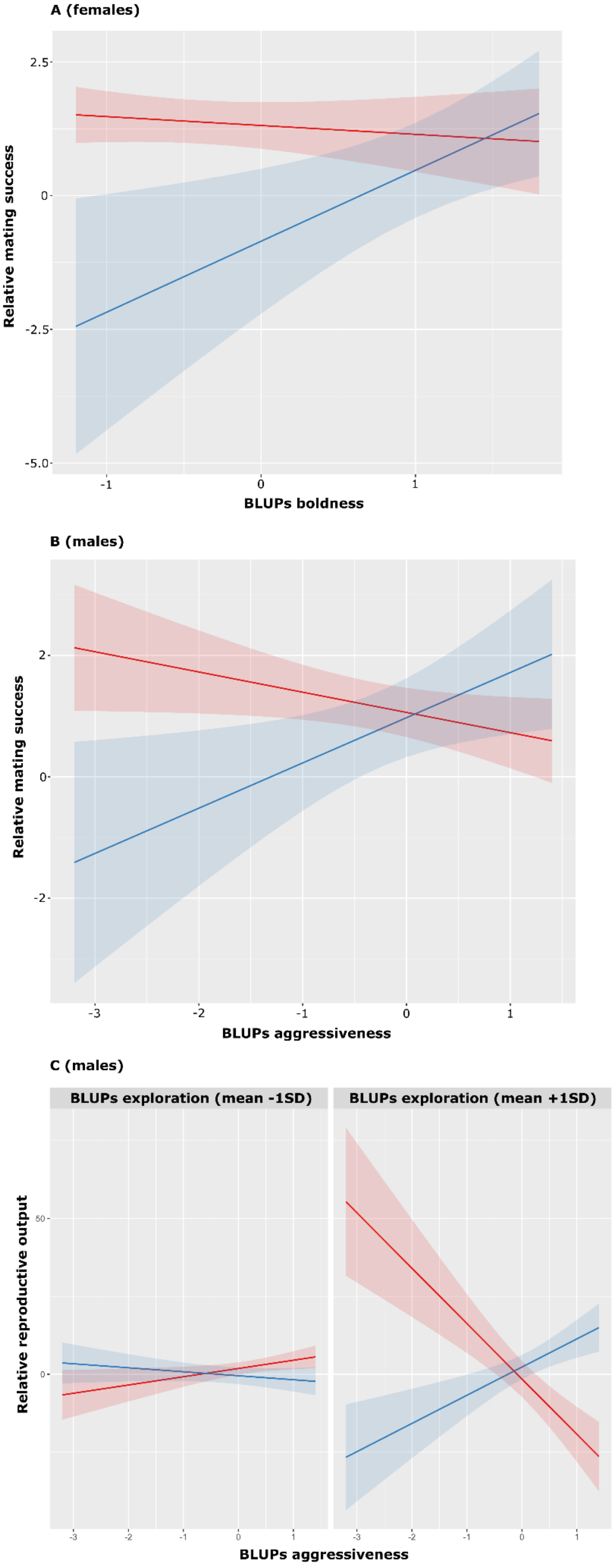


Figure 2. Influence of personality traits on components of reproductive success. The values for the phenotypes are BLUPs extracted from random regression models, and the BLUPs of aggressiveness and boldness have been multiplied by -1 so that higher values represent higher levels of aggression and boldness. Reproductive success measures are relative measures, calculated by dividing each value by the mean population value, to show only the between-individual covariance between phenotype and reproductive success (Houslay & Wilson 2017). In plot A and B, red lines represent exploration scores inferior to mean – 1 SD, and blue lines represent exploration scores superior to mean + 1 SD. In plot C, red lines represent boldness scores inferior to mean – 1 SD, and blue lines represent boldness scores superior to mean + 1 SD.

output (Figure 2). The reproductive output of individuals with a low level of exploration was impacted less by their level of aggression or boldness (Figure 2).

Discussion

We used a wild, free ranging population of *A. femoralis* to study how personality traits affect the various processes shaping reproductive success. We found that specific personality traits can impact components of reproductive success in different ways, and that this differs between the sexes (Figure 3). We also found evidence that the effects of a personality trait on a component of reproductive success can depend on the level of other personality traits (Figure 3).

In females, we found that level of exploration and boldness influenced their number of mating partners. More explorative and bolder females mated with a higher number of males. Likewise, females with low levels of exploration had a high number of mating partners regardless of their boldness level. In *A. femoralis*, females move from their resting sites to males' territories located on average 20 m away for mating (Fischer et al., 2020; Ringler et al., 2012), thus more explorative and bolder females probably benefit by finding more males. At the same time, exploring is costly (e.g., increased predation risk, time and energy loss) and females who are not explorative might benefit from going back to the same males they have mated with in the past (Peignier et al., 2022b).

In males, we found that the level of aggression and boldness influences the number of mates obtained within a reproductive season. Less aggressive males obtained more mating partners when their level of exploration was low, while more aggressive males obtained more mating partners when their level of exploration was high. In *A. femoralis* male, the level of aggression influences the reactions towards conspecific males intruding the territory (Chaloupka et al. 2022; Peignier et al., 2022a), and possession of a territory is a prerequisite for reproductive success (Ursprung et al., 2011a). Therefore, less aggressive males who might struggle to repel competitors could enhance the number of mates they obtain by being present in their territory most of the time, thus increasing their availability to females and ensuring they do not lose their territory to a competitor. More aggressive individuals who defend their territory fiercer might also be less accurate and attack any intruding conspecific, even females (Sonnleitner et al., 2020). Consequently, being more explorative might enhance the

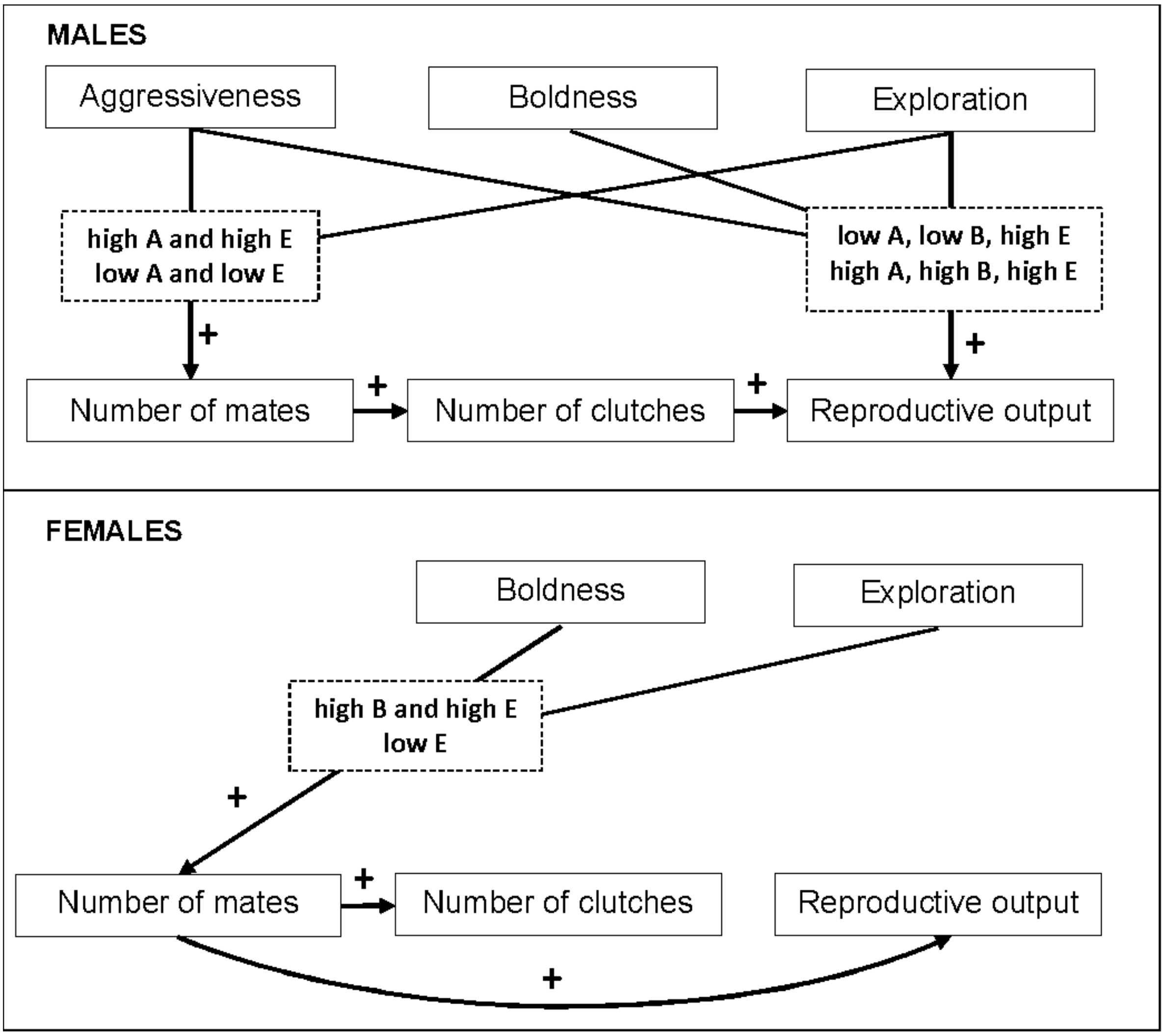


Figure 3. Representation of the influence of personality traits on components of reproductive success in males and females. In dashed squares are the personality types who have the highest value of the focal component of reproductive success, with A the level of aggression, B the level of boldness and E the level of exploration.

number of mates a male can obtain if it increases their chances of settling in locations with more females around.

Our results also showed that the effect of aggressiveness and boldness on a male’s reproductive output was dependent on the male's level of exploration. More aggressive and bolder males, or less aggressive and shyer males, had a higher reproductive output when their level of exploration was high. While the reproductive output of individuals with a low level of exploration did not change with their level of aggressiveness or boldness. A high level of exploration might result in a higher reproductive output because more explorative individuals could benefit by finding more or better water bodies for tadpole deposition. However, we initially expected more aggressive and bolder males, who might be more likely to encounter predators while transporting their tadpoles, to have a lower reproductive output. Probably, bolder males who encounter predators more often are better at recognising them and escaping, while shyer males benefit from being more cautious and avoiding predators.

Another possible explanation is that the effect of male personality on reproductive output could depend on the behaviour of its offspring. Animal personality variation is, at least partially, genetically determined (Dochtermann et al., 2015; Réale et al., 2007) and personality has been shown to have a heritable component in several species (Drent et al., 2003; van Oers et al., 2004a, 2004b). For instance, a study on great tits (*Parus major*) reported a realized heritability of 54 % (± 5%) for early exploratory behaviour (Drent et al., 2003). Flight initiation distance, a common measure of boldness, is also highly heritable in burrowing owls (*Athene cunicularia*; Carrete et al., 2016). If *A. femoralis* tadpoles inherit their personality traits from their parents, we expect that aggressiveness and boldness levels will be beneficial for tadpoles to survive until adulthood. On the one hand, highly aggressive offspring might get better access to food during their development or have more chances to find a suitable territory to settle once they reach sexual maturity. On the other hand, very shy offspring might benefit from increased survival by successfully hiding from predators during their development. Therefore, future studies should investigate the heritability of personality traits in *Allobates femoralis*, and their consequences on offspring performance.

Most studies to date have estimated personality effects on single processes. Yet, behaviours that are positively linked to one process (e.g., mating success), might not necessarily be equally beneficial to another process (e.g., offspring survival). This is precisely what we observed here, as less aggressive males benefited both if they were also explorative (in terms of reproductive output) or if they were not explorative (in terms of mating success), while it was only beneficial for *A. femoralis* male to be aggressive if they were also really explorative (in terms of mating success). We also observed that females and males' phenotype affected their reproductive success in different ways. Where boldness did not affect males mating success, females obtained more mating partners when their boldness and exploration levels were high. Given that we used models fitted for a Poisson distribution, the estimates we obtained were very good approximation of selection gradients and of how phenotype affects relative reproductive success (Morrissey & Goudie, 2022). Consequently, we can infer that in males, the behavioural phenotype affects reproductive output more strongly than mating success. In females, the behavioural phenotype considerably affected mating success, which was almost twice in magnitude compared to males.

Overall, our results provide support for the hypothesis that behavioural consistency arises because of a link with individual differences in life-history trade-offs and suggest a potential effect of correlational selection (Montiglio et al., 2017). This link between personality traits and individual differences in life-history trade-offs could further lead to the evolution of different reproductive strategies, and we would expect males that are not aggressive and not explorative to invest more in finding mates than in parental care, to improve their overall fitness. Future studies should investigate how personality traits are related to multiple components of both reproductive success and survival to assess and compare effects of animal personality on lifetime fitness.

Data availability statement

The datasets generated during and/or analysed during the current study are available in the Open Science Framework repository: https://osf.io/tcz3d/?view_only=6d82a9bb166a457ea8f969722ddd5129

Authors contributions

Conceptualization: MP, ER; Methodology: MP, YA, MR, ER; Formal Analysis: MP, YA, ER; Investigation: MP, ER; Resources: MR, ER; Writing – original draft: MP; Writing – review and editing: MP, YA, MR, ER; Visualization: MP; Supervision: ER; Project administration: MP, ER; Funding acquisition: ER.

Acknowledgments

We want to thank S. Chaloupka, K. Dellefont and C. Leeb for their help in acquiring the data, as well as C. Baranyi, I. Aebi-Huber and N. Besuchet Schmutz for their help processing the genetic data. We also thank L. Bégué and B. Furdi for help in processing the behavioural data, and D. Blumstein and B. Szabo for comments on an earlier version of this manuscript. We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. We thank the Nouragues research field station (managed by CNRS) which benefits from ‘Investissement d’Avenir’ grants managed by Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). This work has been done on the Natural Reserve of Nouragues. This work was funded through a Standalone Grant (FWF P-31518) from the Austrian Science Fund (FWF). MR was supported by an Erwin Schrödinger Grant (FWF J-3868) and a Standalone Grant (FWF P-33728) from the Austrian Science Fund (FWF).

Supplementary material

Manuscript 6: “Opposing effects of personality traits on mating and cross-generational reproductive success”

Assortative mating

An individual’s personality has been shown to influence mating decisions, through behavioural phenotype matching (Collins et al., 2019; Schuett et al., 2011). This can happen in two opposite ways: assortative or disassortative mating, which describe an individuals' tendency to mate with those who are similar or different to their (behavioural) phenotype at a higher rate than expected from random (Jiang et al., 2013). In this study, we monitored an entire, free-ranging population of the Brilliant-thighed Poison Frog (*Allobates femoralis*) during their reproductive season and examined the prevalence of non-random mating by personality type. As in *A. femoralis* females make a mating decision before approaching a male (Stückler et al., 2019), (dis)assortative mating could only happen if they know the male’s personality before commuting towards him. Therefore, the more parsimonious expectation here is an absence of (dis)assortative mating. To investigate the existence of assortative mating by personality type, we performed Kendall correlations for the average exploration score and for the average boldness score of males’ and females’ mating partners, respectively. We did not find evidence for (dis)assortative mating by personality type in *A. femoralis* (male exploration – female exploration: $z = -0.59$, $p = 0.554$; male boldness – female boldness: $z = -0.16$, $p = 0.869$), supporting our initial expectation.

Supplementary table 1. Results of the error-in-variable models investigating the link between personality traits and three components of reproductive success for females. The posterior mean, 95% Credible Intervals, standard-deviation (SD) and residual variance (RV) are presented. Significant results (i.e., 95% CI not overlapping 0) are written in bold.

response variable	*fixed effect*	mean	low-95%CI	high-95%CI
number of mates	intercept	-0.135	-0.319	0.046
	boldness	-0.244	-0.687	0.241
	exploration	-0.551	**-1.054**	**-0.017**
	boldness*exploration	-0.397	-0.867	0.013
number of clutches	intercept	-0.206	**-0.385**	**-0.021**
	number of mates	0.493	**0.351**	**0.634**
	boldness	-0.245	-0.58	0.088
	exploration	-0.085	-0.522	0.329
	boldness*exploration	-0.075	-0.476	0.307
number of offspring surviving to adulthood	intercept	-1.153	**-1.341**	**-0.96**
	number of mates	0.512	**0.305**	**0.71**
	number of clutches	-0.272	-0.74	0.121
	boldness	-0.425	-1.567	0.732
	exploration	-0.949	-2.295	0.392
	boldness*exploration	0.896	-0.98	2.38
	random effects			
	SD boldness	4.252	**3.541**	**5.089**
	SD exploration	0.681	**0.555**	**0.85**
	RV boldness	2.961	**1.679**	**4.107**
	RV exploration	0.56	**0.331**	**0.81**

Supplementary table 2. Results of the error-in-variable models investigating the link between personality traits and three components of reproductive success for males. The posterior mean, 95% Credible Intervals, standard-deviation (SD) and residual variance (RV) are presented. Significant results (i.e., 95% CI not overlapping 0) are written in bold.

response variable	*fixed effect*	mean	low-95%CI	high-95%CI
number of mates	intercept	0.552	**0.4**	**0.705**
	aggressiveness	0.038	-0.349	0.429
	boldness	-0.133	-0.473	0.204
	exploration	-0.079	-0.438	0.289
	aggressiveness*boldness	-0.024	-0.53	0.481
	boldness*exploration	0.038	-0.395	0.546
	aggressiveness*exploration	-0.139	-0.649	0.386
	aggressiveness*boldness*exploration	-0.037	-0.673	0.606
number of clutches	intercept	-0.037	-0.215	0.136
	number of mates	0.463	**0.365**	**0.563**
	aggressiveness	-0.161	-0.547	0.249
	boldness	-0.031	-0.345	0.282
	exploration	-0.056	-0.397	0.276
	aggressiveness*boldness	-0.043	-0.538	0.48
	boldness*exploration	-0.029	-0.481	0.421
	aggressiveness*exploration	-0.025	-0.511	0.473
	aggressiveness*boldness*exploration	-0.048	-0.743	0.671
number of offspring surviving to adulthood	intercept	-2.216	**-2.413**	**-2.02**
	number of mates	-0.456	-1	0.02
	number of clutches	0.491	**0.301**	**0.675**
	aggressiveness	0.157	-1.413	1.761
	boldness	-0.851	-2.066	0.411
	exploration	-0.241	-1.57	1.107
	aggressiveness*boldness	-0.05	-1.821	1.897
	boldness*exploration	-0.577	-1.994	1.235
	aggressiveness*exploration	0.76	-1.617	2.476
	aggressiveness*boldness*exploration	0.174	-1.851	2.161
	random effects			
	SD aggressiveness	1.456	**1.276**	**1.658**
	SD boldness	4.597	**4.053**	**5.209**
	SD exploration	1.013	**0.879**	**1.174**
	RV aggressiveness	0.497	**0.038**	**0.964**
	RV boldness	2.489	**1.392**	**3.481**
	RV exploration	0.558	**0.191**	**0.851**

References

AmphibiaWeb (2022). AmphibiaWeb: Information on amphibian biology and conservation. [WWW document]. URL https://amphibiaweb.org.

Ariyomo, T.O. & Watt, P.J. (2013). Disassortative mating for boldness decreases reproductive success in the guppy. *Behavioral Ecolology*, 24, 1320–1326.

Asab (2021). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour*, 171, I–XI.

Bates, D., Mächler, M., Bolker, B., Walker, S., Christensen, R.H.B. & Singmann, H. et al. (2016). lme4: linear mixed-effects models using 'Eigen' and S4.

Beck, K.B., Loretto, M.-C., Ringler, M., Hödl, W. & Pašukonis, A. (2017). Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ*, 5, e3745.

Biro, P.A. & Stamps, J.A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecolology and Evolution*, 23, 361–368.

Bolger, D.T., Morrison, T.A., Vance, B., Lee, D. & Farid, H. (2012). A computer-assisted system for photographic mark–recapture analysis. *Methods in Ecolology and Evolution*, 3, 813–822.

Bonnot, N.C., Goulard, M., Hewison, A.M., Cargnelutti, B., Lourtet, B. & Chaval, Y. et al. (2018). Boldness-mediated habitat use tactics and reproductive success in a wild large herbivore. *Animal Behaviour*, 145, 107–115.

Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B. & Betancourt, M. et al. (2017). Stan : A Probabilistic Programming Language. Columbia Univ., New York, NY (United States); Harvard Univ., Cambridge, MA (United States), 76.

Carrete, M., Martínez-Padilla, J., Rodríguez-Martínez, S., Rebolo-Ifrán, N., Palma, A. & Tella, J.L. (2016). Heritability of fear of humans in urban and rural populations of a bird species. *Scientific Reports*, 6, 31060.

Chaloupka, S., Peignier, M., Stückler, S., Araya-Ajoy, Y., Walsh, P. & Ringler, M. et al. (2022). Repeatable territorial aggression in a Neotropical poison frog. *Frontiers in Ecolology and Evolution*, 10.

Collins, S.M., Hatch, S.A., Elliott, K.H. & Jacobs, S.R. (2019). Boldness, mate choice and reproductive success in Rissa tridactyla. *Animal Behaviour*, 154, 67–74.

Crump, M.L. (1972). Territoriality and mating behavior in Dendrobates granuliferus (Anura: Dendrobatidae). *Herpetologica*, 28, 195–198.

Darwin, C. (1871). Principles of sexual selection in The descent of man, and Selection in relation to sex. John Murray, London, United Kingdom.

Dingemanse, N.J., Araya-Ajoy, Y.G. & Westneat, D.F. (2021). Most published selection gradients are underestimated: Why this is and how to fix it. *Evolution*, 75, 806–818.

Dochtermann, N.A., Schwab, T. & Sih, A. (2015). The contribution of additive genetic variation to personality variation: heritability of personality. *Proceedings of the Royal Society London B*, 282, 20142201.

Drent, P.J., van Oers, K. & van Noordwijk, A.J. (2003). Realized heritability of personalities in the great tit (Parus major). *Proceedings of the Royal Society London B*, 270, 45–51.

Erich, M., Ringler, M., Hödl, W. & Ringler, E. (2015). Brood-partitioning behaviour in unpredictable environments: hedging the bets? *Behavioral Ecolology and Sociobiology*, 69, 1011–1017.

Fischer, M.-T., Ringler, M., Ringler, E. & Pašukonis, A. (2020). Reproductive behavior drives female space use in a sedentary Neotropical frog. *PeerJ,* 8, e8920.

Hödl, W. (1983). Phyllobates femoralis (Dendrobatidae): Rufverhalten und akustische Orientierung der Männchen (Freilandaufnahmen). Bundesstaatliche Hauptstelle für Wissenschaftliche Kinematographie, Wien.

Houslay, T.M. & Wilson, A.J. (2017). Avoiding the misuse of BLUP in behavioural ecology. *Behavioral Ecolology*, 28, 948–952.

Janczak, A.M., Pedersen, L.J., Rydhmer, L. & Bakken, M. (2003). Relation between early fear- and anxiety-related behaviour and maternal ability in sows. *Applied Animal Behaviour*

Science, 82, 121–135.

Jehle, R., Gasser, H., Pfunders, M., Amézquita Torres, A., Pimentel Lima, A. & Hödl, W. (2008). Ten polymorphic microsatellite loci for Allobates femoralis, an Amazonian dendrobatoid frog. *Molecular Ecology Resources*, 8, 1326–1328.

Jiang, Y., Bolnick, D.I. & Kirkpatrick, M. (2013). Assortative mating in animals. *The American Naturalist*, 181, E125-38.

Lötters, S., Jungfer, K.-H., Henkel, F.W. & Schmidt, W. (2007). Poison frogs. Biology, species & captive husbandry. Edition Chimaira, Frankfurt am Main, Germany.

Luz Nunes-de-Almeida, C.H., Batista Haddad, C.F. & Toledo, L.F. (2021). A revised classification of the amphibian reproductive modes. *Salamandra*, 57, 413–427.

Matschiner, M. & Salzburger, W. (2009). TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics*, 25, 1982–1983.

Monceau, K., Dechaume-Moncharmont, F.-X., Moreau, J., Lucas, C., Capoduro, R. & Motreuil, S. et al. (2017). Personality, immune response and reproductive success: an appraisal of the pace-of-life syndrome hypothesis. An appraisal of the pace-of-life syndrome hypothesis. *Journal of Animal Ecolology*, 86, 932–942.

Montanarin, A., Kaefer, I.L. & Pimentel Lima, A. (2011). Courtship and mating behaviour of the brilliant-thighed frog Allobates femoralis from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution*, 23, 141–150.

Montiglio, P.-O., Wey, T.W., Chang, A.T., Fogarty, S. & Sih, A. (2017). Correlational selection on personality and social plasticity: morphology and social context determine behavioural effects on mating success. *Journal of Animal Ecology*, 86, 213–226.

Morrissey, M.B. & Goudie, I.B.J. (2022). Analytical results for directional and quadratic selection gradients for log-linear models of fitness functions. *Evolution*, 76, 1378–1390.

Muff, S., Nilsen, E.B., O'Hara, R.B. & Nater, C.R. (2021). Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution*.

Mutzel, A., Dingemanse, N.J., Araya-Ajoy, Y.G. & Kempenaers, B. (2013). Parental provisioning behaviour plays a key role in linking personality with reproductive success. *Proceedings of the Royal Society London B*, 280, 20131019.

Pašukonis, A., Ringler, M., Brandl, H.B., Mangione, R., Ringler, E. & Hödl, W. (2013). The homing frog: high homing performance in a territorial dendrobatid frog Allobates femoralis (Dendrobatidae). *Ethology*, 119, 762–768.

Peignier, M., Araya-Ajoy, Y.G., Bégué, L., Chaloupka, S., Dellefont, K. & Leeb, C. et al. (2022a). Exploring links between personality traits and their social and non-social environments in wild poison frogs. *Behavioral Ecolology and Sociobiology*, 76.

Peignier, M., Bégué, L., Gieseke, A., Petri, D., Ringler, M. & Ringler, E. (2022b). Mate choice in a promiscuous poison frog. *Ethology*, 128, 693–703.

Ponzi, E., Keller, L.F., Bonnet, T. & Muff, S. (2018). Heritability, selection, and the response to selection in the presence of phenotypic measurement error: Effects, cures, and the role of repeated measurements. *Evolution*, 72, 1992–2004.

Pröhl, H. (2005). Territorial behavior in dendrobatid frogs. *Journal of Herpetology*, 39, 354–365.

R Core Team (2019). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Réale, D. & Dingemanse, N.J. (2012). Animal Personality. John Wiley & Sons, Ltd.

Réale, D., Martin, J., Coltman, D.W., Poissant, J. & Festa-Bianchet, M. (2009). Male personality, life-history strategies and reproductive success in a promiscuous mammal. *Journal of Evolutionary Biolology*, 22, 1599–1607.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291–318.

Ringler, E. (2018). Testing skin swabbing for DNA sampling in dendrobatid frogs. *Amphibia Reptilia*, 98, 245–251.

Ringler, E., Mangione, R. & Ringler, M. (2015). Where have all the tadpoles gone? Individual genetic tracking of amphibian larvae until adulthood. *Molecular Ecology Resources*, 15, 737–746.

Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. (2013a). Characterization of seven new polymorphic microsatellite loci in the brilliant-thighed poison frog Allobates femoralis (Dendrobatidae), and their cross-species utility in three other dendrobatid species. *The Herpetological Journal*, 23, 175–178.

Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. (2013b). Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. *Frontiers in Zoology*, 10, 67.

Ringler, E., Ringler, M., Jehle, R. & Hödl, W. (2012). The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. *PLoS one*, 7, e40237.

Ringler, E., Szipl, G., Harrigan, R.J., Bartl-Binder, P., Mangione, R. & Ringler, M. (2018). Hierarchical decision-making balances current and future reproductive success. *Molecular Ecology*, 27, 2289–2301.

Ringler, M., Ringler, E., Magaña Mendoza, D. & Hödl, W. (2011). Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. *PLoS one*, 6, e25844.

Roithmair, M.E. (1994). Male territoriality and female mate selection in the dart-poison frog Epipedobates trivattatus (Dendrobatidae, Anura). *Copeia*, 1994, 107–115.

Rojas, B. & Pašukonis, A. (2019). From habitat use to social behavior: natural history of a voiceless poison frog, Dendrobates tinctorius. *PeerJ*, 7, e7648.

Rosenthal, G.G. & Ryan, M.J. (2022). Sexual selection and the ascent of women: Mate choice research since Darwin. *Science*, 375, eabi6308.

Sánchez-Tójar, A., Moiron, M. & Niemelä, P.T. (2022). Terminology use in animal personality research: a self-report questionnaire and a systematic review. *Proceedings of the Royal Society London B*, 289, 20212259.

Schuett, W., Dall, S.R.X. & Royle, N.J. (2011). Pairs of zebra finches with similar ‘personalities’ make better parents. *Animal Behaviour*, 81, 609–618.

Schuett, W., Laaksonen, J. & Laaksonen, T. (2012). Prospecting at conspecific nests and exploration in a novel environment are associated with reproductive success in the jackdaw. *Behavioral Ecology and Sociobiology*, 66, 1341–1350.

Schulte, L.M., Ringler, E., Rojas, B. & Stynoski, J.L. (2020). Developments in amphibian parental care research: history, present advances and future perspectives. *Herpetological Monographs*, 34, 71-97.

Smith, B.R. & Blumstein, D.T. (2008). Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, 19, 448–455.

Sonnleitner, R., Ringler, M., Loretto, M.-C. & Ringler, E. (2020). Experience shapes accuracy in territorial decision-making in a poison frog. *Biology Letters*, 16, 20200094.

Souza, J.R.D., Kaefer, I.L. & Pimentel Lima, A. (2021). Calling activity determines male mating success in a territorial frog with parental care. *Ethology*.

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W. & Ringler, E. (2019). Spatio-temporal characteristics of the prolonged courtship in Brilliant-Thighed Poison Frogs, Allobates femoralis. *Herpetologica*, 75, 268–279.

Stynoski, J.L., Schulte, L.M. & Rojas, B. (2015). Poison frogs. *Current Biology*, 25, R1026-R1028.

Svensson, E.I., Arnold, S.J., Bürger, R., Csilléry, K., Draghi, J. & Henshaw, J.M. et al. (2021). Correlational selection in the age of genomics. *Nature Ecology & Evolution*, 5, 562–573.

Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. (2011a). Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. *Molecular Ecology*, 20, 1759–1771.

Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. (2011b). Toe regeneration in the neotropical frog Allobates femoralis. *The Herpetological Journal*, 21, 83–86.

van Oers, K., Drent, P.J., Goede, P. de & van Noordwijk, A.J. (2004a). Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proceedings of the Royal Society London B*, 271, 65–73.

van Oers, K., Drent, P.J., Jong, G. de & van Noordwijk, A.J. (2004b). Additive and nonadditive genetic variation in avian personality traits. *Heredity*, 93, 496–503.

Wang, J. (2009). A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, 18, 2148–2164.

Wells, K.D. (2007). The ecology and behavior of amphibians. University of Chicago Press, Chicago, IL, USA.

Weygoldt, P. (1980). Zur Fortpflanzungsbiologie von Phyllobates femoralis (Boulenger) im Terrarium. *Salamandra*, 16, 215–226.

Wolf, M., van Doorn, G.S., Leimar, O. & Weissing, F.J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, 447, 581–584.

Manuscript 7: “Odour cues rather than personality affect tadpole deposition in a poison frog”

This manuscript will be submitted to *Molecular Ecology*

Mélissa Peignier[a,b,*], Max Ringler[a,c,d,e], & Eva Ringler[a,b]

[a] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland
[b] Messerli Research Institute, University of Veterinary Medicine Vienna, Austria
[c] Department of Behavioral and Cognitive Biology, University of Vienna, Austria
[d] Department of Evolutionary Biology, University of Vienna, Austria
[e] Institute of Electronic Music and Acoustics, University of Music and Performing Arts Graz, Austria

*Correspondence:
Mélissa Peignier
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
melissa.peignier@unibe.ch

Abstract

Appropriate decision-making is a key aspect of everyday life for animals. It is particularly relevant in contexts such as mate choice, intra-sexual competition and parental care which can have a strong impact on fitness. Several factors can influence decision-making, such as external cues (e.g., from the natural and social environment) and the animal’s own internal state (e.g., physiology, motivation, behavioural phenotype). Making adaptive decisions requires the integration of all available information. Thus, it is necessary to study both external cues and internal state together to fully understand how animals make decisions. Yet, we only have limited knowledge how different factors act together on decision-making, and whether one source is more important the other. Here, we used an entire, free-ranging, population of the Neotropical poison frog *Allobates femoralis* to investigate how two factors (olfactory cues and personality traits) influence the ability of individual males to find and use new rearing sites. We experimentally manipulated the location of tadpole deposition sites and the olfactory cues they emitted, we repeatedly measured exploration and boldness in adult males, and we inferred parent-offspring relationships via pedigree reconstruction using molecular parentage analysis. We found that the discovery and use of new rearing sites was influenced by olfactory cues, but we did not find an effect of exploration or boldness or their interplay with external cues on parental care. In highly dynamic environments such as the neotropics, individuals likely benefit more from relying on reliable external cues, rather than personality traits, to discover and make use of rearing resources.

Keywords

animal personality, parental care, decision-making, tadpole transport, poison frog, Anura

Introduction

On a daily basis, animals need to decide which risks to take (e.g., predation, injury from fights with conspecifics) to maximise their fitness (McFarland, 1977). Several factors play a role in decision making, such as external cues (e.g., from the natural and social environment) and internal state (e.g., physiology, motivation) (Budaev et al., 2019; McFarland, 1977). Consistent between-individual differences in behaviour, also called ‘animal personality’, can also influence decision-making (Carter et al., 2013). Boldness, for instance, can influence the likelihood of migration or the choice of a foraging patch (Chapman et al., 2011; Mella et al., 2015). The key to making adaptive choices is the integration of all available information, both external and internal (Budaev et al., 2019). Studying both together is, therefore, necessary to fully understand the processes by which animals make decisions. Currently, we only have limited knowledge about the interplay between external cues and personality traits on decision-making.

Decision-making is particularly relevant in the contexts of mate choice, intra-sexual competition and parental care, due to the expected large impact of the behaviours on fitness (Clutton-Brock, 1991). Parental care is defined as any parental behaviour that increases offspring survival, while parents often bear some cost (i.e., reduction in survival or future reproductive success; Smiseth, 2019). Therefore, appropriate decision-making is essential to optimize the costs and benefits of care to ensure offspring survival. Studies have shown that personality traits can impact decision making in the context of parental care (Mutzel et al., 2013; Thys et al., 2021; Zhao et al., 2016) such as in male blue tits (*Cyanistes caeruleus*), in which more aggressive males feed their offspring at lower rates compared to less aggressive males (Mutzel et al., 2013). However, more studies are needed to understand if cue assessment differs between individuals with different personalities, and whether external cues and personality traits act equally on decision-making during parental care or whether one factor is more important than the other.

Behaviours related to parental care can range from protection against predators to the exploitation of certain resources for the benefit of the offspring (Royle et al., 2012). There might be variation in how different individuals cope with these challenges, for example in how innovative individuals are to discover new resources. Many neotropical poison frog species (Dendrobatidae, AmphibiaWeb, 2022) lay their eggs on land and transport the newly hatched tadpoles to patchy water resources (Wells, 2007). The ability of adults to find suitable water bodies is vital for their fitness, but tadpole transport is expensive in terms of time and energy, and is highly risky (e.g., increased predation pressure) to the transporting parent. Furthermore, tropical rainforests are highly dynamic environments and the availability and quality of suitable rearing sites might change unpredictably (Fouilloux et al., 2021; Rudolf & Rödel, 2005; Schulte & Lötters, 2013). Therefore, individuals should rely on all available information to find, assess and choose the appropriate rearing sites that maximize offspring survival (McKeon & Summers, 2013; Poelman et al., 2013; Ringler et al., 2013b; Ringler et al., 2018). Previous studies have highlighted the importance of olfactory cues (external cues e.g., Rojas, 2014; Schulte et al., 2011; Schulte & Lötters, 2013, 2014), and natal site imprinting or spatial memory (internal states e.g., Beck et al., 2017; Erich et al., 2015; Pašukonis et al., 2016, 2019; Ringler et al., 2013b) for tadpole transport. How personality (e.g., exploration or boldness), another internal state, affects decisions in the context of tadpole transport has not been explored as of yet.

Here, we used a free-ranging population of the Neotropical poison frog *Allobates femoralis* (Dendrobatidae, AmphibiaWeb, 2022) to investigate how external cues and personality traits influence the ability of individual males to find and use new rearing sites. We experimentally manipulated the position of tadpole deposition sites and the olfactory cues they emitted. We repeatedly measured exploration and boldness in adult, territorial males. We also collected tissue samples of adult frogs and tadpoles and inferred parent-offspring relationships via pedigree reconstruction using molecular parentage analysis. We did so in order to reconstruct the location and timing of tadpole depositions of all males in the population. Male *Allobates femoralis* which exhibit either explorative, shy and aggressive, or explorative, bold and not aggressive personalities, have a higher number of offspring surviving until adulthood (Peignier et al. in prep). Thus, we expected that more explorative and bolder males would find new rearing sites quicker and use a higher number of different rearing sites, or rearing sites located farther away from their territory than less explorative or shyer males. Previous studies have hinted towards the importance of olfactory cues associated with stagnant water and/or tadpole odour for the initial discovery of new rearing sites (Pašukonis et al., 2016; Serrano-Rojas & Pašukonis, 2021). Hence, we expected pools with odour cues to be detected earlier than pools without any odour cues.

Methods

Ethical note

The ethics and animal welfare committee of the University of Veterinary Medicine Vienna, in accordance with Good Scientific Practice (GSP) guidelines and national legislation, approved this study. The scientific committee of the ‘Nouragues Ecological Research Station’ also approved this study. It was conducted in strict accordance with current French and EU law, and we followed the ASAB guidelines (2020) on the treatment of study animals. Working and tissue sampling permissions were provided by the CNRS Guyane (‘Centre National de la Recherche Scientifique Guyane’), the ‘Ministère de la transition écologique et solidaire’ (permit number: TREL2002508S/303), and the Secretariat of the Convention on Biological Diversity (APA declaration: TREL1734890A/34).

Study system

Allobates femoralis is a diurnal Neotropical poison frog with a highly promiscuous mating system (Montanarin et al., 2011; Stückler et al., 2019; Ursprung et al., 2011a). During the reproductive season, males are highly territorial and perform advertisement calls from elevated perches to both repel other males and attract females (Hödl et al., 2004; Ringler et al., 2011). Females commute from their perching site to males within a 20 m radius to mate, and lay clutches on the leaf litter (Fischer et al., 2020; Ringler et al., 2012). After 15–21 days of clutch development, males provide care by transporting the newly hatched tadpoles to water bodies located up to 200 m outside of their territory where tadpoles will complete their development to metamorphosis within 40–50 days (Ringler et al., 2013b; Ringler et al., 2018). Males actively spread larvae from single and successive clutches across several water bodies (Erich et al., 2015). Previous studies have hinted towards the importance of olfactory cues associated with stagnant water and/or tadpole odour for the initial discovery of new rearing sites (Pašukonis et al., 2016; Serrano-Rojas & Pašukonis, 2021). Additionally, previous studies have shown that some males prefer returning to their natal pools despite the presence of predators, and even when closer pools are available (Ringler et al., 2018). Males also display personality along the aggressive/docile, bold/shy, and exploratory/stationary axes (Chaloupka et al., 2022; Peignier et al., 2022). Importantly, parental personality is associated with offspring survival. More explorative males who

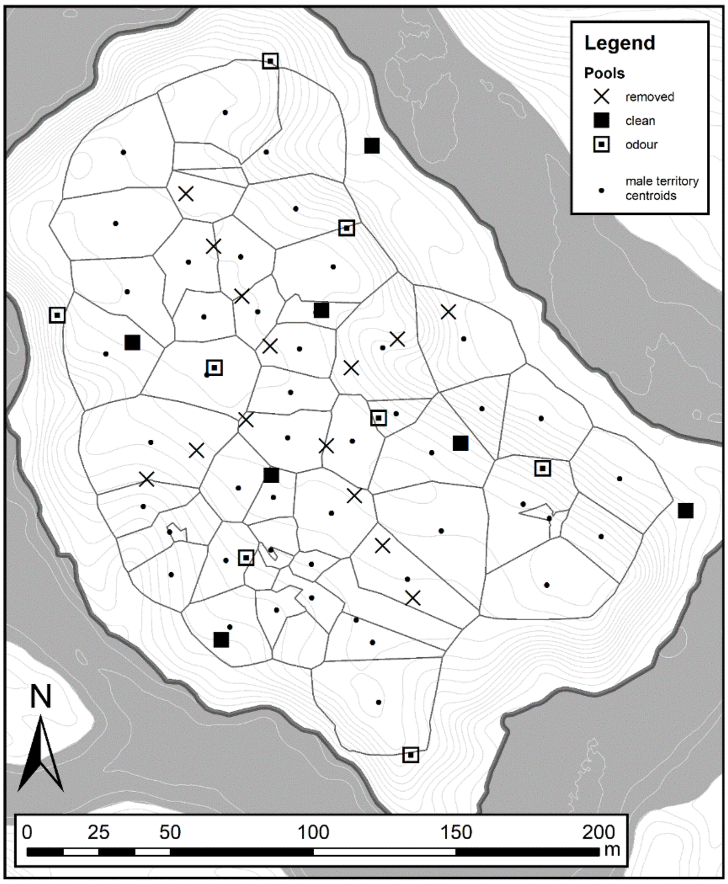


Figure 1. Maps showing the spatial distribution of male territories (delimited by black lines) and artificial rearing sites. The crosses represent the artificial rearing sites that were removed at the beginning of the field season, while the squares represent the new artificial rearing sites with (black squares) or without (white and black squares) odour cues. The centroids of male territories are represented by a black dot. The map uses the Voronoi territories of the day when the most individuals were present at the same time on the island (7 March 2019). The thin grey lines represent the 50 cm elevation isoclines, and the dark grey area represents the river Arataye.

are also aggressive and bold or docile and shy have more offspring who survive until adulthood (Peignier et al. in prep).

Rearing pools set-up

We conducted our study using a wild, experimental population of *Allobates femoralis* located on a ~5 ha river island in a lowland rainforest, in the vicinity of the field camp ‘Saut Pararé’ of the CNRS Nouragues Ecological Research Station (nature reserve ‘Les Nouragues’ in French Guiana; 4°02´ N, 52°41´W). We introduced the population in 2012 and it has been stable since then, with ~ 150 adult individuals (Ringler et al., 2015). In 2018, we positioned 14 artificial pools across the island in a cross-shaped array (volume: ~ 15 L), which the frogs used for tadpole deposition. We sampled all tadpoles from these pools and removed the pools at the beginning of February 2019 (hereafter referred to as ‘old pools’). We then positioned 16 new pools (volume: ~5 L) in a cross-shaped array, turned by 45° in relation to the old pools (Figure 1; hereafter referred to as ‘new pools’). At the end of the study, we put the 14 old pools back at their original location.

We filled all new pools with rainwater and leaves. Additionally, at every other new pool we positioned a 5 L bucket 2 m above ground filled with river water and leaves. We allocated tadpoles that had been sampled from the old pools in a semi-random order so that in the end each bucket received equal number of tadpoles (Figure 1). The buckets were positioned 1 m to the side of the new pool to prevent water and tadpoles to drop into the new pool underneath. The pools with nearby overhanging buckets served as ‘pools with odour cues’, while the other pools were taken as control.

To identify factors influencing tadpole deposition and pool discovery, we sampled tadpoles from the new pools every day from the 15th of February to the 02nd of April 2019, and from the 06th to the 22nd of April 2019. We also did one additional sampling in May 2019. We collected tissue samples (i.e., fin clip) of all these tadpoles for further genetic analysis. Afterwards, each tadpole was randomly assigned and deposited into one of the odour cue buckets. We removed all predators from the new pools and exchanged the water on a regular basis.

Frog population survey

We surveyed the frog population on the island every day from 0900 to 1800 h between

February–April 2019, and caught all encountered adults, aiming for total sampling of adult males and females on the island in this period. We collected tissue samples of all newly encountered adults for genetic analysis and identified all adult frogs via digital pictures of their distinct ventral patterns and with the help of the pattern matching software Wild-ID (Bolger et al., 2012). We sexed them by the presence (males) or absence (females) of vocal sacs. We recorded the exact spatial locations of all frogs on a digital map using rugged Win10 tablets (CAT T20, Bullitt Group, Reading, United Kingdom) with the mobile GIS software ArcPad 10 (ESRI, Redlands, CA, USA), and further handled the data in ArcGIS 10.6 (ESRI).

Parentage analysis

We identified deposition patterns via pedigree reconstruction using molecular parentage analysis. For the adults, we sampled DNA by removing the third toe of both hind limbs which we immediately preserved in 96% ethanol (Ringler, 2018; Ursprung et al., 2011b). We also had access to genetic data of older individuals (survivors from previous years, found again in 2019) from the long-term monitoring on the island population. For the tadpoles, we clipped the tail tips of all individuals and likewise preserved them in 96% ethanol. Our sample comprised a total of 121 adults (64 males and 57 females) and 1142 tadpoles.

We isolated genomic DNA of all samples using a Proteinase K digestion followed by an extraction using a DNeasy kit (QIAGEN, Valencia, CA). We then amplified all samples at twelve highly variable microsatellite loci (Afem03, Afem04, Afem05, Afem09, Afem12, Afem13, Afem16, Afem20, Afem22, Afem24, Afem25, Afem27) using fluorescent-labelled primers and PCR protocols described in Jehle et al. (2008) and Ringler et al. (2013a). Finally, we diluted the amplified products with water, mixed them with internal size standard LIZ, and ran them on a capillary sequencer (ABI 3730, Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA). We visually identified all loci and determined the allele sizes using PeakScanner 1.0 (Applied Biosystems). We determined the final allele sizes using the binning software Tandem 1.01 (Matschiner & Salzburger, 2009). We removed individuals for which four or more loci could not be scored. In total we performed the pedigree reconstruction on 57 males, 53 females, and 1109 tadpoles.

We conducted parentage assignments using the software COLONY 2.0.6.7 (Jones & Wang, 2010). We built a full likelihood model allowing for polygamous mating in both sexes with a medium precision and without setting a sibship prior. To determine deposition patterns, we treated individual adults as potential ‘fathers’ and ‘mothers’, whereas all tadpoles were treated as potential ‘offspring’. We used the ‘Best (ML) Configuration’ for further analyses. Of the 1109 tadpoles, COLONY assigned 1006 individuals (90.7%) to at least one known parent. When one or both parents were not found within our sampled adult genotype dataset, the parental genotype was simulated by the software. For 695 of these 1006 individuals, both parents were assigned (69.1 %).

Males typically distribute their clutches across several pools, possibly to improve offspring survival (Erich et al., 2015). Therefore, to quantify parental performances, we determined how many different old and new pools each individual used. We also identified how quickly each male discovered a new pool by determining if males deposited a clutch in a new pool within 45 days of installation (1) or not (0). Although, this value depends on whether (or not) a male had a clutch to deposit within the first 45 days, the study was performed in the peak of the rearing season and almost all males had already produced clutche(s) at that time. Hence, we do not expect this to considerably bias our results. To understand which factors affect the choice to deposit in a pool, we determined for each clutch in which pool(s) it was deposited, if the pool contained odour cues or not, the distance between the pool and the male’s territory and the distance between the pool and the closest old pool that has been removed (see ‘Spatial analysis’). To be able to quantify paternal performances for each male, we only used tadpoles for which at least the father was known. Since captive *A. femoralis* female can lay a clutch every 8 days on average (Weygoldt, 1980), we assumed that tadpoles from the same parent pair that were deposited in one or several pools within 6 days belonged to the same clutch. We also considered that tadpoles deposited on a specific day with only one parent assigned belonged to the same clutch than tadpoles deposited on the same day with the two parents assigned. Finally, we considered that tadpoles produced by the same parent pair that were deposited more than 6 days apart were from two separate clutches.

Spatial analysis

To identify the factors influencing the discovery of new rearing sites, we determined the distance between male territories (centroids) and each one of the new pools at the date when a deposition occurred. We also determined the distances between the new pool used and the closest old pool (irrespective whether it was actually used by the male). These parameters were used to verify if males discovered new pools by searching close to their territory or close to old pools. Additionally, we measured the average distance between the male territories (centroids) and each one of the old pools to verify if the disturbance we created by removing rearing sites in the peak of the mating season changed the dynamic of tadpole deposition (e.g., if there was a link between personality traits and paternal performance before but not after pool removal). To determine daily territory centroids with the corresponding function in ArcGIS 10.6 (ESRI) we used the daily male territories that had been estimated in a previous study using a roving-window approach and Dirichlet tessellation (for details see Peignier et al., 2022).

Assessment of the level of exploration and boldness

In the course of another study, adult individuals found on the island were tested for within- and between-individual variation in exploration and boldness, from February to April 2019 (Peignier et al., 2022). In this study, individual levels of exploration and boldness were assessed using a Novel Environment Test (NET). The latency to emerge from a dark shelter into a bright (novel) environment and the probability to enter this novel environment, as well as the number of jumps performed in the novel environment were measured using the coding software BORIS (Friard et al., 2016). Furthermore, the distance travelled (in pixels) and the area covered in the novel environment were measured using the automated tracking software TOXTRAC (Rodriguez et al., 2018; Peignier et al., 2022). Individuals who did not emerge from the shelter were given a censored value of 900 s (total duration of the experiment). In total 156 NET trials with 50 males (mean ± SD = 3.31 ± 1.50 repetitions per individual) were conducted. Results from this previous study showed that the behaviours measured in the NET are repeatable and found evidence for the prevalence of the personality traits exploration and boldness in males (Peignier et al., 2022). Further, the time spent in the shelter and the distance travelled best represented the latent variables of boldness and exploration, respectively. In the present study, we thus used these two variables as proxies for exploration and boldness for each individual (hereafter named ‘boldness’ and ‘exploration’ scores).

Statistical analysis

We conducted all statistical analyses in R v3.6.0 (R Core Team, 2019) using the integrated development environment RStudio v1.3.1093 (RStudio Team, 2019).

Influence of personality traits on paternal care: We used a bivariate approach to study how behaviours measured in the NET correlate with variation in paternal performances at the between- and within-individuals level using Bayesian Generalized Linear Mixed Models (package ‘MCMCglmm’, Hadfield, 2010). We constructed five bivariate models to quantify the relationship between exploration (distance travelled in the NET) and (1) the number of old and (2) new pools used, (3) the likelihood to discover a new pool within 45 days of installation, and (4) the average distance between the male’s territory and the old and (5) new pool he deposited in (for a total of 10 bivariate models). We also constructed an additional five bivariate models to look at the relationship between boldness (time spent in the shelter in the NET) and the five paternal performance variables listed above.

In order to investigate between-individual covariance between exploration/boldness and the paternal performance variables, we divided each of the paternal performance variables by their mean value before adding them as the second response variable (see Houslay & Wilson, 2017). We used the function ‘transformTukey’ to apply a constant transformation on the distance and the time spent in the shelter to better approach a normal distribution. Models with the likelihood to discover a new pool within 45 days were fitted as binomial. We further used the posterior distributions to estimate the between and within-individual correlations and covariances between personality and paternal performance. We fitted an unstructured covariance matrix for the grouping variable ID to be able to calculate the variance caused by differences between individuals and the covariance between exploration/boldness.

We ran the models using a parameter expanded prior (described in the supplementary material), with 1,000,000 iterations, a burn-in of 10,000, and selected every 500th posterior parameter sample (thinning interval). We confirmed the absence of autocorrelation (correlation between lags < 0.1), sufficient mixing (visual inspection of plots of MCMC chains) and that we ran the Markov chain for long enough (Heidelberg and Welch diagnostic tests; Hadfield, 2010). We present estimates and credible intervals generated from our models, and we estimated that statistical significance was reached if the 95% credible intervals did not overlap 0.

Influence of external cues on paternal care: Next, we identified the parameters that best predict patterns of tadpole deposition in a given pool. To this end, we structured the data in a way that included all possible deposition options (i.e., all available 16 new artificial pools) of a given tadpole transport event (i.e., the deposition of tadpoles from one single clutch across single or multiple pools) and noted whether the assigned father deposited tadpoles at a given pool or not (‘deposition’: yes = 1/ no = 0). We further included the ‘distance between the pool and the male’s territory’, the ‘distance between the pool and the previously closest (used or not) old pool’, the male's and clutch identity, the male’s exploration and boldness scores, and the treatment of the pool (with or without odour cues) in the data set.

We fitted a generalized linear mixed effect model (GLMM) using the package lme4 (Bates et al., 2016), with ‘deposition’ as the response variable. As fixed effects we included ‘treatment’ (odour of a pool with or without tadpoles), ‘distance between the pool and the male’s territory’, and ‘distance between the pool and the closest old pool’. The parameters ‘clutchID’ nested within ‘maleID’ were used as random factors. We conducted a likelihood ratio test based on the maximum likelihood fits of the full and the null model using the ‘fixedLRT’ function to obtain p-value estimates for the overall fit of the model. Additionally, we performed a Conditional Inference Tree using the package ‘partykit’ to better understand the ranking based on relative importance of the fixed effects (Hothorn & Zeileis, 2015). We used the same response and partitioning variables as for the GLMM (fixed effects) to grow the conditional inference tree (Hothorn et al., 2006).

Influence of the interplay between personality traits and external cues on paternal care: Lastly, we looked at the interplay between olfactory cues and personality traits on paternal care. We fitted two GLMMs with a Poisson distribution using the number of new pools as the response variable, and two further GLMMs with a binomial distribution using the likelihood to discover a new pool within 45 days as the response variable. For each model we included an interaction between the exploration and boldness scores and either ‘treatment’ or ‘distance between the pool and the male’s territory’ as fixed effects, and male identity as the random effect. In these models, we used an average per individual of the distance travelled in the NET and of the time spent in the shelter as exploration and boldness scores, respectively.

Results

Within the 57 males and 53 females, 52 males (91.2%) and 47 females (88.7%) produced at least one tadpole in 2019. In total 37/52 males deposited in an old pool and 41/52 deposited in a new pool. We could collect behavioural data on all but 7 of these 52 individuals. Of the 16 new pools installed, 12 were used in total. Furthermore, we observed that all depositions that occurred within the first 3 weeks after the opening of the new pools were in pools with tadpole odour cues (Figure 2). Only after three weeks did males start to discover and use the control pools. Only 10 males out of 41 discovered and used new pools within the first 45 days of installation. On average, males moved 98 m to deposit their tadpoles (± 49 m SD), and pools were located between 19 and 100 m (average = 48 m, SD = 21 m) from an old pool.

Influence of personality traits on paternal care

There was no correlation between levels of exploration or boldness and paternal performance at the between- and within-individuals level (Supplementary Table 1). Males with varying level of exploration or boldness did not differ in the number of pools they used, how far from their territory they went to deposit or how quickly they found new pools.

Influence of external cues on paternal care

The likelihood ratio test revealed that the full model explained the variation in the likelihood to deposit tadpoles better than the null model ($v2 = 193.09$, $df = 4$, $p < 0.01$). The full model showed that all three fixed effects significantly affected the probability of deposition inside a given pool. The deposition likelihood significantly increased with decreasing distance between the pool and the male’s territory or the closest old pool, and with the presence of odour cues (Figure 2, Supplementary Table 2). The conditional inference tree analysis confirmed the relative importance ranking of the fixed effects and showed that the distance from the new pool to the male’s territory ($p < 0.001$; Figure 3), followed by the treatment ($p < 0.001$; Figure 3), and the distance between the pool and the closest old pool ($p = 0.01$; Figure 3) were significant predictors for deposition probability. Pools that were close to the male’s territory (closer

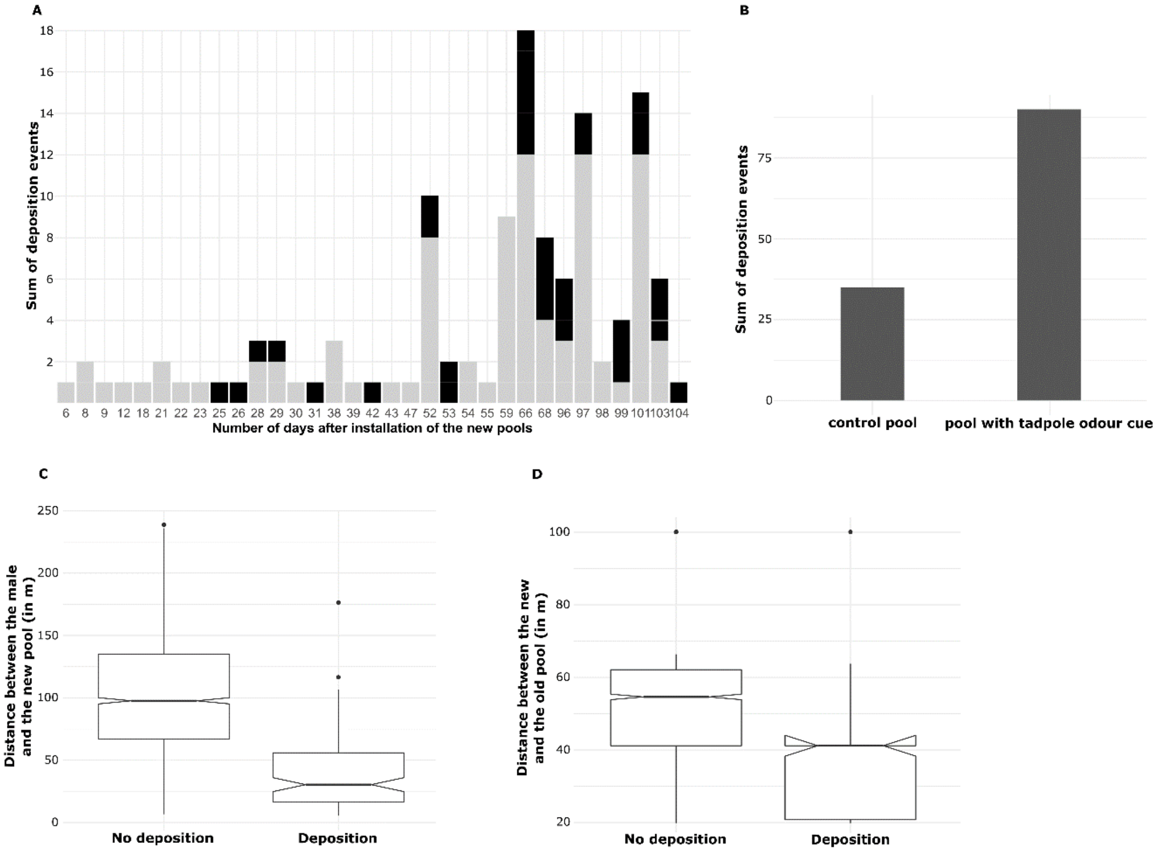


Figure 2. Patterns of discovery and use of new rearing resources for tadpole deposition. Panel A presents how many days after installation of the new pools depositions happened in pools with (light grey) and without (black) odour cues. Pools were sampled daily from the 1st to the 46th day after installation, then from the 51st to the 68th day and from the 95th to the 103rd day post-installation. Panel B presents the total number of depositions in pools with or without odour cues. Panel C presents the likelihood of deposition in pools depending on their distance to the centroid of the male's territory. Panel D presents the likelihood of deposition in pools depending on their distance to the closest old pool.

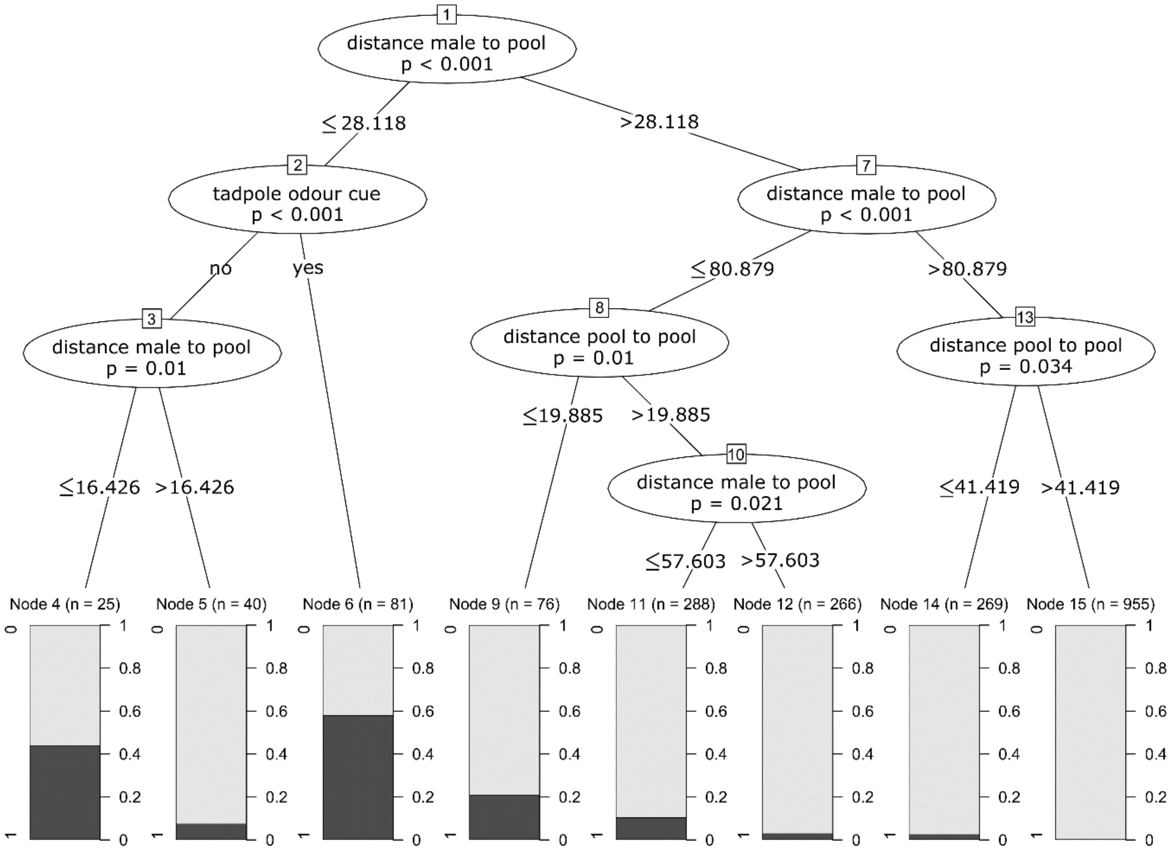


Figure 3. Conditional inference tree examining where male poison frogs choose to deposit their tadpoles. Pools that did receive a deposition (1 in the barplot) versus those that did not (0 in the barplot) were best classified according to three categories. The highest deposition frequency (58 %) was observed for pools that were in close spatial proximity to a male's territory and that had odour cues (node 6). ‘distance male to pool’ represents the distance between the new pool used for deposition and the male’s territory. ‘tadpole odour cue’ represents the treatment of the pool (with or without tadpole odour cue). ‘distance pool to pool’ represents the distance between the new pool and the closest old pool (used or not by the frog before).

than 28 m; Figure 3) and that had tadpole odour cues were the most likely to receive tadpoles (58.0% of the pools that fell into this category were used for deposition; Figure 3, nodes 6). On the other end of the spectrum, pools that were far from a male's territory (farther than 81 m; Figure 3) and far from an old pool (farther than 41 m; Figure 3) were least likely to receive tadpoles (Figure 3, node 15).

Influence of the interplay between personality traits and external cues on paternal care

We found no influence of the interaction between personality and treatment or distance between the pool to the male's territory on paternal care. Precisely, the number of new pools used or the likelihood to discover a new pool within 45 days were not affected by an interaction between exploration or boldness and treatment or distance pool-male's territory (Supplementary Table 3).

Discussion

In the present study, we used an entire free-ranging population of the Neotropical poison frog *Allobates femoralis* to investigate the role of the interplay between external cues (specifically olfactory cues) and personality traits (specifically exploration and boldness) on the ability of individual males to find and use new rearing sites.

Influence of personality traits on paternal care

While we have empirical evidence of the interplay between personality and reproductive success in several taxa (for a review see Smith & Blumstein, 2008), we still have limited knowledge on how a personality trait is mechanistically translated into fitness (Dingemanse & Réale, 2013). One hypothesis is that personality traits influence parental care which in turn influences fitness (Mutzel et al., 2013). Males of the common goby (*Pomatoschistus microps*) with a more active personality type are less efficient at parental care by cannibalizing more eggs, thereby reducing their reproductive success, compared to less active males (Vallon et al., 2016). Since we know that in *A. femoralis*, males' personality influences their reproductive output (i.e., the number of their tadpoles who survive until adulthood; Peignier et al. in prep), we expected that males with higher levels of exploration and boldness increase their reproductive output by using a higher number of rearing sites or being more efficient at discovering new rearing sites. However, we found no correlation between levels of exploration or boldness and variation in paternal performances at the between- and within-individual levels.

The field of animal personality has received a lot of criticism, mainly because there is no consensus about whether behaviours measured in artificial setups (such as the NET) could reflect reliable natural behaviours of the species (cf. Beekman & Jordan, 2017). The absence of correlation between exploration or boldness levels and paternal performances could suggest that the behaviours measured in the NET do not represent natural explorative or boldness-related behaviours of *A. femoralis* males. However, a previous study performed in a lab population of *A. femoralis* showed that exploration behaviours measured in the NET indeed reflect natural exploration-related behaviours expressed in the context of territory settlement (Bégué et al. in prep). Another context in which exploration could be beneficial for *A. femoralis* males is during their courtship march, which is a necessary step of the mating process (Stückler et al., 2019). Further studies are needed to clearly understand in which natural contexts individuals explore. However, the lack of correlation between personality traits and paternal performances we observed was unlikely due to NET-measured behaviours not reflecting natural explorative or boldness-related behaviours.

Previous findings have shown that male *A. femoralis* use spatial memory to navigate in their environment (Pašukonis et al., 2013, 2014, 2016). Males rely on experience and are able to quickly find their way back to their territory when translocated (Pašukonis et al., 2013, 2014). Moreover, males also know the location of several different deposition sites in the area (Pašukonis et al., 2016). These findings suggest that males have an accurate representation of their familiar surroundings. It is possible that exploration and boldness do not matter for tadpole deposition in a familiar environment compared to the level of activity. Activity, as a personality trait, is defined as ‘the general level of activity of an individual’ in a non-novel environment (Réale et al., 2007). Removing familiar deposition sites effectively changed the known environment but did not create a novel environment. In addition, our results show that individuals tend to deposit more frequently in new pools that are located closer to their territory. The area surrounding their territory is likely an area that individuals know well, and deposition within this area might be therefore influenced by activity, rather than exploration or boldness.

It is also possible that the link between male’s personality and reproductive output (Peignier et al. in prep) is not due to male’s deposition behaviour, but rather to the offspring' ability to survive until adulthood. Many personality traits have been shown to have a heritable component (Ariyomo et al., 2013; Dochtermann et al., 2015; Drent et al., 2003). If tadpoles inherit their personality traits from their parents, we expect that more aggressive and bolder individuals might get better access to food, while less aggressive and shy tadpoles might benefit in successfully hiding from predators. Individuals with higher levels of exploration might benefit in being more likely to find a suitable territory to settle. Future studies should take into account the offspring's personality to better understand the mechanistic pathways by which parents' personality is linked to their reproductive output.

Influence of external cues on paternal care

It is possible that the effect of sensory cues on decision-making during parental care is much stronger than the effect of personality, which would explain the absence of a link between exploration or boldness and paternal performances. Several studies have highlighted the importance of olfactory cues for tadpole transport (Rojas, 2014; Schulte et al., 2011; Schulte & Lötters, 2013, 2014). Here, we also investigated which external cues drive the discovery of new rearing sites. We found an effect of the location of old and new rearing sites on deposition behaviour and showed that frogs use olfactory cues to find pools in which to deposit their tadpoles. Males deposited more in pools closer to their territory, with conspecific tadpole odour, or closer to old rearing sites.

Previous work already hinted towards the importance of tadpole and stagnant water odour cues for *A. femoralis* male when finding new rearing sites (Pašukonis et al., 2016; Serrano-Rojas & Pašukonis, 2021). Moreover, previous studies have shown that *A. femoralis* male prefer to return to their natal pools to deposit their tadpoles, even when closer pools are available (Ringler et al., 2018), and that males rely on spatial memory to relocate known deposition sites (Pašukonis et al., 2016). We observed that, while males used few new rearing sites (1.54 ± 0.71 SD, range = 1 to 4, against 2.20 ± 1.56 SD, range = 1 to 7, for the old rearing sites) they continuously went back to the same new pools to deposit their clutches, supporting the findings of previous studies that males make use of spatial memory in the context of paternal care (Pašukonis et al., 2016; Ringler et al., 2018; Serrano-Rojas & Pašukonis, 2021).

Tadpole transport is costly for the transporting parent, as it increases risks of predation and the loss of the territory or mating opportunities. Consequently, males should minimize the time and effort spent in transportation (Beck et al., 2017; Ringler et al., 2013b). Our findings, combined with previous studies, indicate that male *A. femoralis* have developed a highly efficient system, combining spatial learning and memory, and the use of external cues to find new rearing sites when deciding where to deposit their offspring.

Influence of the interplay between personality traits and external cues on paternal care

We did not find a role of the interaction between external cues and exploration or boldness on paternal care. Overall, our results suggest that external cues are the main source of information used by individuals to decide where to transport their tadpoles. Neotropical amphibians, who live in highly unpredictable environment, benefit from finding novel rearing resources quickly. We performed our study in the peak of the rearing season, when most males had clutches that needed to be transported. With the sudden removal of all rearing sites, males were under high pressure to find new rearing sites quickly. In this context, relying on strong external cues ensured the discovery of a deposition site, and prevented total clutch loss. This result suggests that, in *A. femoralis*, sensory cues might override personality in the context of parental care.

Animals constantly need to make decisions that will influence their fitness (McFarland, 1977), and the key to making adaptive decisions is to integrate all information available as best as possible (Budaev et al., 2019). Personality traits have been shown to influence decision-making, particularly in the context of parental care where appropriate decision-making is essential to ensure offspring survival (Mutzel et al., 2013; Thys et al., 2021; Zhao et al., 2016). However, in highly dynamic environments, the availability of resources and the environmental pressures change constantly. Relying on predictable external cues likely ensures individuals to discover and use rearing resources, and thereby improves offspring survival. Male *A. femoralis* have developed highly efficient techniques (e.g., olfaction, well-developed spatial memory), which are optimized in terms of time and energy expenditure, to rapidly find new rearing sites in an unpredictable environment (Beck et al., 2017; Pašukonis et al., 2016). Animals need to make decisions in a range of different contexts (e.g., foraging, mate choice, intra-sexual competition) to maximize their fitness. Future studies should investigate how external cues and personality traits affect decision-making in various contexts and the ultimate fitness consequences of the decisions.

Data availability statement

The datasets generated during and/or analysed during the current study are available in the Open Science Framework repository: https://osf.io/pk2mf/?view_only=d1b58c1e16ca4b54900d0a0ff52a2e89

Authors contributions

Conceptualization: MP, ER; Methodology: MP, MR, ER; Formal Analysis: MP, ER; Investigation: MP; Resources: MR, ER; Writing – original draft: MP; Writing – review and editing: MP, MR, ER; Visualization: MP; Supervision: ER; Project administration: ER; Funding acquisition: MR, ER.

Acknowledgments

The authors thank S. Chaloupka, K. Dellefont and C. Leeb for their help in acquiring the data, and C. Baranyi, I. Aebi-Huber and N. Besuchet Schmutz for their help processing the genetic data. We also thank L. Bégué and B. Furdi for help in processing the behavioural data. We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. We thank the Nouragues research field station (managed by CNRS) which benefits from 'Investissement d'Avenir' grants managed by Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). This work has been done on the Natural Reserve of Nouragues. This work was funded through a Standalone Grant (FWF P-31518) from the Austrian Science Fund (FWF). MR was supported by an Erwin Schrödinger Grant (FWF J-3868) and a Standalone Grant (FWF P-33728) from the Austrian Science Fund (FWF).

Supplementary material

Manuscript 7: “Odour cues rather than personality affect tadpole deposition in a poison frog”

Prior used in the bivariate models

```
prior1 □ list(R = list(V = diag(c(1, 0.0001),2,2), nu = 0.002, fix = 2),
              G = list(G1 = list(V = diag(2), nu = 2,
                        alpha.mu = rep(0,2),
                        alpha.V = diag(25^2,2,2))))
```

Supplementary table 1. Between- and within-individual covariance and slope between the behaviours that best represents the personality traits exploration and boldness and measurements of parental performance. We present the mean and 95% credible intervals.

		covariance		**slope**	
		among-individual	**within-individual**	**among-individual**	**within-individual**
behaviour	**measures of parental performance**	**mean (95% Credible Interval)**		**mean (95% Credible Interval)**	
exploration score	number of new pools used	-0.82 (-6.83, 4.24)	-0.02 (-0.87, 0.91)	-4.36 (-34.58, 20.83)	-162.47 (-8705.88, 9107.92)
	number of old pools used	-1.29 (-20, 16.23)	-0.01 (-0.82, 0.83)	-0.66 (-10.29, 8.42)	-71.95 (-8243.93, 8278.78)
	discovered within 45 days (1/0)	-7.94 (-32.53, 10.89)	-0.11 (-0.68, 0.67)	-5.79 (-20.96, 11.09)	-1070.24 (-6791.46, 6677.24)
	average distance to new pools used	-5.44 (-17.11, 2.86)	-0.01 (-0.87, 0.86)	-11.64 (-33.12, 6.04)	-96.11 (-8674.11, 8636.45)
	average distance to old pools used	-4.56 (-15.99, 3.49)	-0.01 (-0.83, 0.84)	-12.31 (-42.1, 9.17)	-85.02 (-8347.83, 8399.67)
boldness score	number of new pools used	0.2 (-0.38, 0.91)	0.00 (-0.08, 0.08)	1.03 (-2.14, 4.54)	6.45 (-751.21, 755.99)
	number of old pools used	-0.79 (-2.99, 0.94)	-0.00 (-0.06, 0.07)	-0.4 (-1.43, 0.47)	-2.4 (-647.84, 667.34)
	discovered within 45 days (1/0)	0.35 (-1.06, 1.8)	0.01 (-0.07, 0.07)	0.46 (-1.06, 2.31)	134.03 (-675.55, 686.12)
	average distance to new pools used	0.84 (-0.11, 2.15)	-0.00 (-0.07, 0.07)	1.76 (-0.25, 3.98)	-8.01 (-664.13, 693.83)
	average distance to old pools used	0.37 (-0.58, 1.49)	-0.00 (-0.07, 0.07)	1.02 (-1.58, 3.79)	-0.26 (-687.49, 710.46)

Supplementary table 2. Results of the generalized linear mixed effect models investigating the influence of external cues on tadpole deposition likelihood. We present estimates, standard-error (SE), standard-deviation (SD), p-values and 95% confidence intervals (95%CI). Significant results (p-value < 0.05) are written in bold. N = 2000 observation on 41 males and 90 clutches.

	Deposition likelihood			
Fixed effects	*Estimate*	*SE*	*p-value*	*95%CI*
(intercept)	0.08	0.33	0.813	-0.57, 0.72
treatment	1.11	0.22	**< 0.001**	0.68, 1.56
distance between pool and male’s territory	-0.05	0.00	**< 0.001**	-0.05, -0.04
distance between pool and closest old pool	-0.01	0.01	**0.026**	-0.03, -0.002
Random effects	*Estimate*	*SD*		
clutch ID:male ID	0	0		
maleID	0	0		

Supplementary table 3. Results of the generalized linear mixed effect models investigating the role of the interplay between external cues and personality traits on parental care. We present estimates, standard-error (SE), standard-deviation (SD), p-values and 95% confidence intervals (95%CI). The fixed effect ‘dist.male-pool’ presents the distance between the pool and the male’s territory. Significant results (p-value < 0.05) are written in bold.

	Number of new pools used			**Number of new pools used**			**Likelihood to discover a pool within 45 days**			**Likelihood to discover a pool within 45 days**		
Fixed effects	*Estimate*	*SE*	*p-value*	*Estimate*	*SE*	*p-value*	*Estimate*	*SE*	*p-value*	*Estimate*	*SE*	*p-value*
(intercept)	0.63	0.11	**<0.001**	0.58	0.1	**<0.001**	-5.12	5.28	0.332	-3.06	1.35	**0.024**
boldness score	0.17	0.11	0.135	0.16	0.11	0.16	-0.45	1.74	0.797	0.44	0.86	0.606
exploration score	0.15	0.12	0.218	0.13	0.11	0.262	-1.47	1.53	0.337	-0.92	0.87	0.294
treatment	0.03	0.09	0.773				0.78	1.16	0.499			
dist.male-pool				0.03	0.09	0.773				-0.17	0.59	0.778
boldness score:exploration score	0.19	0.12	0.103	0.14	0.1	0.191	-0.01	1.45	0.994	0.42	0.8	0.602
boldness score:treatment	0.07	0.11	0.499				2.63	2.34	0.259			
exploration score:treatment	0.14	0.12	0.237				2.29	1.86	0.218			
boldness score:exploration score:treatment	0.06	0.09	0.489				1.18	1.37	0.39			
boldness score: dist.male-pool				-0.03	0.1	0.759				-0.75	0.83	0.365
exploration score: dist.male-pool				0	0.11	0.989				0.29	0.79	0.715
boldness score:exploration score:dist.male-pool				-0.03	0.11	0.782				-1.17	0.76	0.123
Random effects	*Estimate*	*SD*		*Estimate*	*SD*		*Estimate*	*SD*		*Estimate*	*SD*	
maleID	0.03	0.18		0.04	0.21		18.43	4.29		5.54	2.35	

References

AmphibiaWeb (2022) AmphibiaWeb: Information on amphibian biology and conservation. Available from: https://amphibiaweb.org [Accessed 7 October 2022].

Ariyomo, T. O., Carter, M. & Watt, P. J. (2013) Heritability of boldness and aggressiveness in the zebrafish. Behavior Genetics, 43(2), 161–167. https://doi.org/10.1007/s10519-013-9585-y.

Bates, D., Mächler, M., Bolker, B., Walker, S., Christensen, R. H. B. & Singmann, H. et al. (2016) lme4: linear mixed-effects models using 'Eigen' and S4 (Version 1.1-27.1). Available from: http://CRAN.R-project.org/package=lme4 [Accessed 14 April 2021].

Beck, K. B., Loretto, M.-C., Ringler, M., Hödl, W. & Pašukonis, A. (2017) Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. PeerJ, 5, e3745. https://doi.org/10.7717/peerj.3745.

Beekman, M. & Jordan, L. A. (2017) Does the field of animal personality provide any new insights for behavioral ecology? Behavioral Ecology, 28(3), 617–623.

Bolger, D. T., Morrison, T. A., Vance, B., Lee, D. & Farid, H. (2012) A computer-assisted system for photographic mark–recapture analysis. Methods in Ecology and Evolution, 3(5), 813–822. https://doi.org/10.1111/j.2041-210X.2012.00212.x.

Budaev, S., Jørgensen, C., Mangel, M., Eliassen, S. & Giske, J. (2019) Decision-making from the animal perspective: bridging ecology and subjective cognition. Frontiers in Ecology and Evolution, 7, 164. https://doi.org/10.3389/fevo.2019.00164.

Carter, A. J., Marshall, H. H., Heinsohn, R. & Cowlishaw, G. (2013) Personality predicts decision making only when information is unreliable. Animal Behaviour, 86(3), 633–639. https://doi.org/10.1016/j.anbehav.2013.07.009.

Chaloupka, S., Peignier, M., Stückler, S., Araya-Ajoy, Y., Walsh, P. & Ringler, M. et al. (2022) Repeatable territorial aggression in a Neotropical poison frog. Frontiers in Ecology and Evolution, 10. https://doi.org/10.3389/fevo.2022.881387.

Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L.-A., Nilsson, J.-Å. & Brodersen, J. et al. (2011) To boldly go: individual differences in boldness influence migratory tendency. Ecology Letters, 14(9), 871–876. https://doi.org/10.1111/j.1461-0248.2011.01648.x.

Clutton-Brock, T. H. (1991) The evolution of parental care. Princeton University Press: Princeton, NJ, USA.

Dingemanse, N. J. & Réale, D. (2013) What is the evidence that natural selection maintains variation in animal personalities? In: Carere, C. & Maestripieri, D. (Eds.) Animal personalities: Behavior, physiology, and evolution. University of Chicago Press: Chicago, IL, USA, pp. 201–220.

Dochtermann, N. A., Schwab, T. & Sih, A. (2015) The contribution of additive genetic variation to personality variation: heritability of personality. Proceedings of the Royal Society London - Biological Sciences, 282(1798), 20142201. https://doi.org/10.1098/rspb.2014.2201.

Drent, P. J., van Oers, K. & van Noordwijk, A. J. (2003) Realized heritability of personalities in the great tit (Parus major). Proceedings of the Royal Society London - Biological Sciences, 270(1510), 45–51.

Erich, M., Ringler, M., Hödl, W. & Ringler, E. (2015) Brood-partitioning behaviour in unpredictable environments: hedging the bets? Behavioral Ecology and Sociobiology, 69(6), 1011–1017. https://doi.org/10.1007/s00265-015-1913-1.

Fischer, M.-T., Ringler, M., Ringler, E. & Pašukonis, A. (2020) Reproductive behavior drives female space use in a sedentary Neotropical frog. PeerJ, 8, e8920. https://doi.org/10.7717/peerj.8920.

Fouilloux, C. A., Serrano Rojas, S. J., Carvajal-Castro, J. D., Valkonen, J. K., Gaucher, P. & Fischer, M.-T. et al. (2021) Pool choice in a vertical landscape: Tadpole-rearing site flexibility in phytotelm-breeding frogs. Ecology and Evolution, 11(13), 9021–9038. https://doi.org/10.1002/ece3.7741.

Friard, O., Gamba, M. & Fitzjohn, R. (2016) BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and

Evolution, 7(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584.

Hadfield, J. D. (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmmR Package. Journal of Statistical Software, 33(2), 1–22. https://doi.org/10.18637/jss.v033.i02.

Hödl, W., Amézquita Torres, A. & Narins, P. M. (2004) The role of call frequency and the auditory papillae in phonotactic behavior in male dart-poison frogs Epipedobates femoralis (Dendrobatidae). Journal of Comparative Physiology a - Neuroethology, Sensory, Neural, and Behavioral Physiology, 190(10), 823–829. https://doi.org/10.1007/s00359-004-0536-1.

Hothorn, T., Hornik, K. & Zeileis, A. (2006) Unbiased recursive partitioning: a conditional inference framework. Journal of Computational and Graphical Statistics, 15(3), 651–674. https://doi.org/10.1198/106186006X133933.

Hothorn, T. & Zeileis, A. (2015) partykit: A modular toolkit for recursive partytioning in R. The Journal of Machine Learning Research, 16(1), 3905–3909. https://doi.org/10.5555/2789272.2912120.

Houslay, T. M. & Wilson, A. J. (2017) Avoiding the misuse of BLUP in behavioural ecology. Behavioral Ecology, 28(4), 948–952. https://doi.org/10.1093/beheco/arx023.

Jehle, R., Gasser, H., Pfunders, M., Amézquita Torres, A., Pimentel Lima, A. & Hödl, W. (2008) Ten polymorphic microsatellite loci for Allobates femoralis, an Amazonian dendrobatoid frog. Molecular Ecology Resources, 8(6), 1326–1328. https://doi.org/10.1111/j.1755-0998.2008.02304.x.

Jones, O. R. & Wang, J. (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10(3), 551–555. https://doi.org/10.1111/j.1755-0998.2009.02787.x.

Matschiner, M. & Salzburger, W. (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics, 25(15), 1982–1983. https://doi.org/10.1093/bioinformatics/btp303.

McFarland, D. J. (1977) Decision making in animals. Nature, 269(5623), 15–21. https://doi.org/10.1038/269015a0.

McKeon, C. S. & Summers, K. (2013) Predator driven reproductive behavior in a tropical frog. Evolutionary Ecology, 27(4), 725–737. https://doi.org/10.1007/s10682-013-9641-3.

Mella, V. S. A., Ward, A. J. W., Banks, P. B. & McArthur, C. (2015) Personality affects the foraging response of a mammalian herbivore to the dual costs of food and fear. Oecologia, 177(1), 293–303. https://doi.org/10.1007/s00442-014-3110-8.

Montanarin, A., Kaefer, I. L. & Pimentel Lima, A. (2011) Courtship and mating behaviour of the brilliant-thighed frog Allobates femoralis from Central Amazonia: implications for the study of a species complex. Ethology, Ecology & Evolution, 23(2), 141–150. https://doi.org/10.1080/03949370.2011.554884.

Mutzel, A., Dingemanse, N. J., Araya-Ajoy, Y. G. & Kempenaers, B. (2013) Parental provisioning behaviour plays a key role in linking personality with reproductive success. Proceedings of the Royal Society London - Biological Sciences, 280(1764), 20131019. https://doi.org/10.1098/rspb.2013.1019.

Pašukonis, A., Loretto, M.-C. & Rojas, B. (2019) How far do tadpoles travel in the rainforest? Parent-assisted dispersal in poison frogs. Evolutionary Ecology, 44, 473. https://doi.org/10.1007/s10682-019-09994-z.

Pašukonis, A., Trenkwalder, K., Ringler, M., Ringler, E., Mangione, R. & Steininger, J. et al. (2016) The significance of spatial memory for water finding in a tadpole-transporting frog. Animal Behaviour, 116, 89–98. https://doi.org/10.1016/j.anbehav.2016.02.023.

Peignier, M., Araya-Ajoy, Y. G., Bégué, L., Chaloupka, S., Dellefont, K. & Leeb, C. et al. (2022) Exploring links between personality traits and their social and non-social environments in wild poison frogs. Behavioral Ecology and Sociobiology, 76(7). https://doi.org/10.1007/s00265-022-03202-9.

Poelman, E. H., Wijngaarden, R. A. P. & Raaijmakers, C. E. (2013) Amazon poison frogs (Ranitomeya amazonica) use different phytotelm characteristics to determine their suitability for egg and tadpole deposition. Evolutionary Ecology, 27(4), 661–674.

https://doi.org/10.1007/s10682-013-9633-3.

R Core Team (2019) R: a language and environment for statistical computing (Version 3.6.0), Vienna, Austria. R Foundation for Statistical Computing. Available from: http://www.r-project.org [Accessed 14 April 2021].

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007) Integrating animal temperament within ecology and evolution. Biological Reviews, 82(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x.

Ringler, E. (2018) Testing skin swabbing for DNA sampling in dendrobatid frogs. Amphibia-Reptilia, 98, 245–251. https://doi.org/10.1163/15685381-17000206.

Ringler, E., Mangione, R. & Ringler, M. (2015) Where have all the tadpoles gone? Individual genetic tracking of amphibian larvae until adulthood. Molecular Ecology Resources, 15(4), 737–746. https://doi.org/10.1111/1755-0998.12345.

Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. (2013a) Characterization of seven new polymorphic microsatellite loci in the brilliant-thighed poison frog Allobates femoralis (Dendrobatidae), and their cross-species utility in three other dendrobatid species. Herpetological Journal, 23(3), 175–178. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126724/pdf/emss-59832.pdf.

Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. (2013b) Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. Frontiers in Zoology, 10(1), 67. https://doi.org/10.1186/1742-9994-10-67.

Ringler, E., Ringler, M., Jehle, R. & Hödl, W. (2012) The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. PloS one, 7(6), e40237. https://doi.org/10.1371/journal.pone.0040237.

Ringler, E., Szipl, G., Harrigan, R. J., Bartl-Binder, P., Mangione, R. & Ringler, M. (2018) Hierarchical decision-making balances current and future reproductive success. Molecular Ecology, 27(9), 2289–2301. https://doi.org/10.1111/mec.14583.

Ringler, M., Ringler, E., Magaña Mendoza, D. & Hödl, W. (2011) Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. PloS one, 6(10), e25844. https://doi.org/10.1371/journal.pone.0025844.

Rodriguez, A., Zhang, H., Klaminder, J., Brodin, T., Andersson, P. L. & Andersson, M. (2018) ToxTrac: a fast and robust software for tracking organisms. Methods in Ecology and Evolution, 9(3), 460–464. https://doi.org/10.1111/2041-210X.12874.

Rojas, B. (2014) Strange parental decisions: fathers of the dyeing poison frog deposit their tadpoles in pools occupied by large cannibals. Behavioral Ecology and Sociobiology, 68(4), 551–559. https://doi.org/10.1007/s00265-013-1670-y.

Royle, N. J., Smiseth, P. T. & Kölliker, M. (Eds.) (2012) The evolution of parental care. Oxford University Press: Oxford, United Kingdom.

RStudio Team (2019) RStudio: Integrated Development for R (Version 1.3.1093), Boston, MA, USA. RStudio, Inc. Available from: http://www.rstudio.com [Accessed 28 March 2021].

Rudolf, V. H. W. & Rödel, M.-O. (2005) Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecific cues. Oecologia, 142(2), 316–325. https://doi.org/10.1007/s00442-004-1668-2.

Schulte, L. M. & Lötters, S. (2013) The power of the seasons: rainfall triggers parental care in poison frogs. Evolutionary Ecology, 27(4), 711–723. https://doi.org/10.1007/s10682-013-9637-z.

Schulte, L. M. & Lötters, S. (2014) A danger foreseen is a danger avoided: how chemical cues of different tadpoles influence parental decisions of a Neotropical poison frog. Animal Cognition, 17(2), 267–275. https://doi.org/10.1007/s10071-013-0659-2.

Schulte, L. M., Yeager, J., Schulte, R., Veith, M., Werner, P. & Beck, L. A. et al. (2011) The smell of success: choice of larval rearing sites by means of chemical cues in a Peruvian poison frog. Animal Behaviour, 81(6), 1147–1154. https://doi.org/10.1016/j.anbehav.2011.02.019.

Serrano-Rojas, S. J. & Pašukonis, A. (2021) Tadpole-transporting frogs use stagnant water odor to find pools in the rainforest. Journal of Experimental Biology, 224(21), jeb243122.

https://doi.org/10.1242/jeb.243122.

Smiseth, P. T. (2019) Parental care. In: Fath, B.D. & Jørgensen, S.E. (Eds.) Encyclopedia of ecology. Elsevier: Amsterdam, pp. 301–309.

Smith, B. R. & Blumstein, D. T. (2008) Fitness consequences of personality: a meta-analysis. Behavioral Ecology, 19(2), 448–455. https://doi.org/10.1093/beheco/arm144.

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W. & Ringler, E. (2019) Spatio-temporal characteristics of the prolonged courtship in Brilliant-Thighed Poison Frogs, Allobates femoralis. Herpetologica, 75(4), 268–279. https://doi.org/10.1655/Herpetologica-D-19-00010.1.

Thys, B., Eens, M., Pinxten, R. & Iserbyt, A. (2021) Pathways linking female personality with reproductive success are trait- and year-specific. Behavioral Ecology, 32(1), 114–123. https://doi.org/10.1093/beheco/araa110.

Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. (2011a) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Molecular Ecology, 20, 1759–1771. https://doi.org/10.1111/j.1365-294X.2011.05056.x.

Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. (2011b) Toe regeneration in the neotropical frog Allobates femoralis. Herpetological Journal, 21(1), 83–86. Available from: http://www.ingentaconnect.com/content/bhs/thj/2011/00000021/00000001/art00011.

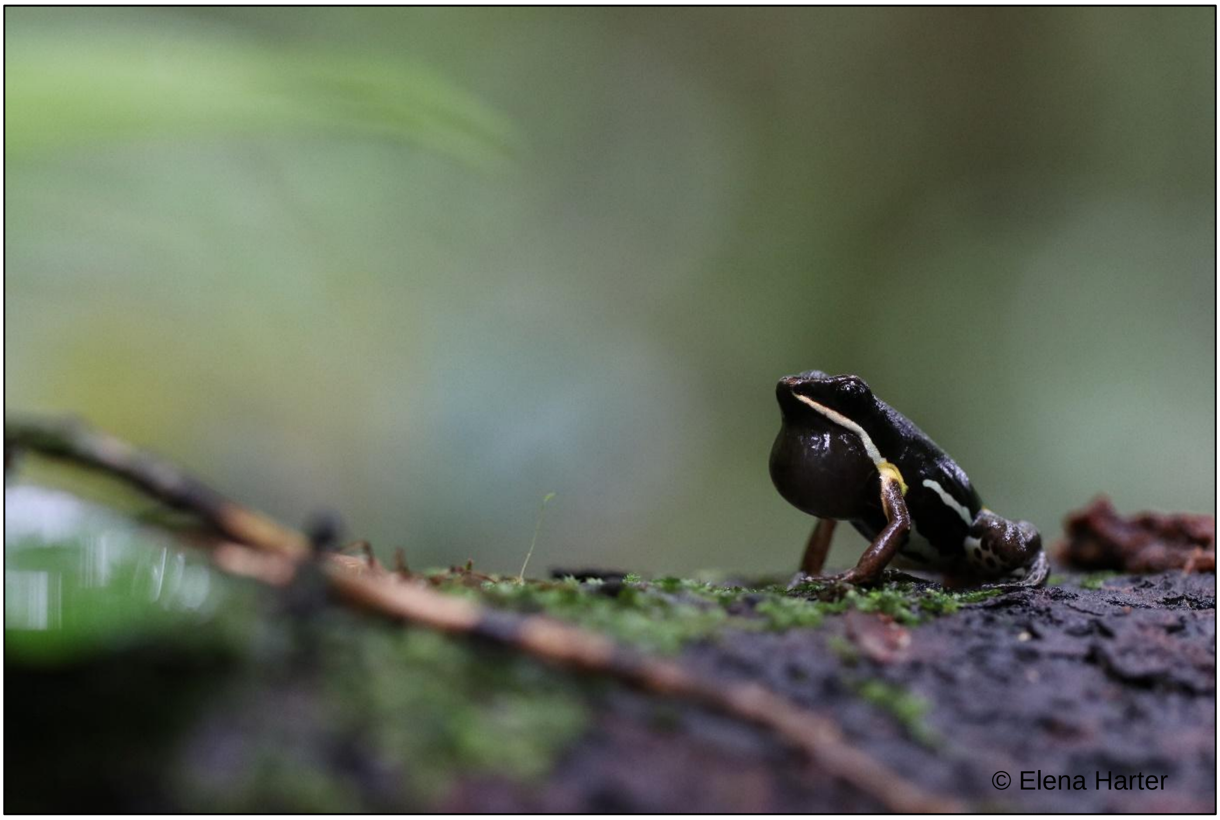
Vallon, M., Grom, C., Kalb, N., Sprenger, D., Anthes, N. & Lindström, K. et al. (2016) You eat what you are: personality-dependent filial cannibalism in a fish with paternal care. Ecology and Evolution, 6(5), 1340-1352. https://doi.org/10.1002/ece3.1966.

Wells, K. D. (2007) The ecology and behavior of amphibians. University of Chicago Press: Chicago, IL, USA.

Weygoldt, P. (1980) Zur Fortpflanzungsbiologie von Phyllobates femoralis (Boulenger) im Terrarium. Salamandra, 16(4), 215–226. Available from: https://www.salamandra-journal.com/index.php/home/contents/1980-vol-16/1279-weygoldt-p-4/file.

Zhao, Q.-S., Hu, Y.-B., Liu, P.-F., Chen, L.-J. & Sun, Y.-H. (2016) Nest site choice: a potential pathway linking personality and reproductive success. Animal Behaviour, 118, 97–103. https://doi.org/10.1016/j.anbehav.2016.05.017.

General discussion


© Elena Harter

General discussion

Animal personality is a current hot topic in behavioural research. Over the years, an increasing amount of empirical evidence has been gathered about the influence of animal personality on different traits related to reproduction and survival [40,45,75,76]. Yet, we still have limited insight in how personality arises and why it is maintained in animal populations [16–18,20,22,32,77,78]. In my thesis, I aimed to increase our understanding of the maintenance of personality traits, using an entire, free-ranging, population of the Neotropical poison frog *Allobates femoralis* (Figure 4).

Characterization of personality traits

In my first chapter, I started by showing evidence of the existence of consistent between-individual behavioural differences in both male and female *A. femoralis*. I found repeatable behavioural differences along the personality traits aggressiveness, exploration and boldness for males, and exploration and boldness for females (**manuscripts 1 and 3**). I used structural equation modelling to determine how distinct behaviours covary and are structured into functional units. This way, I was able to characterise personality traits as latent variables, understand what behaviours each trait encompassed and which of these behaviours best represented each personality trait. A proper characterization of the behaviours underlying personality traits is crucial to understand how personality affects behaviours in different contexts, and ultimately, reproduction and survival. Using a single behaviour as a proxy for a personality trait, without checking how well the behaviour represents the personality trait, hampers our understanding of the influence of personality traits on different outcomes (e.g., mating success, reproductive success). For instance, in our study the latency to jump towards an intruder characterizes the personality trait aggressiveness slightly better than the speed to reach the intruder (manuscript). Therefore, I advise all future studies on animal personality to use structural equation modelling and aim at finding the different behaviours that cluster together to form a personality trait.

Personality and plasticity

Several hypotheses have been developed to explain the existence and maintenance of personality traits. While we could expect individuals to adjust their behavioural responses according to the current situation [1], limited plasticity could be adaptive because individuals might benefit from being predictable, especially in a social context [22]. For example, in an intra-sexual competition context, males would benefit from being constantly aggressive if other males adjust their behaviour accordingly [22].

Using a behavioural reaction norm approach, I investigated if an individual's average level of territorial defence is correlated to plasticity [79]. This idea has been studied in both the animal personality and animal cognition field (under the term 'coping style' [80]), and theory predicts that some individuals should rely on routines and be inflexible or 'unresponsive' to environmental cues, while other individuals should be more plastic [81]. In the case of *A. femoralis*, I found no support for a correlation between personality and plasticity. Both aggressive and less aggressive males plastically adjusted their reaction to differently sized intruders (**manuscript 2**). These findings suggest that in situations in which the costs of poor decisions are high, individuals respond flexibly to environmental cues no matter their personality type.

Personality and environment

Another hypothesis that has been developed to explain the existence and maintenance of animal personality stipulates that consistent between-individual differences in behaviour could reflect behavioural strategies to cope with different environmental conditions [77]. Selective pressures exerted by the environment could generate and/or maintain individual differences in behaviour within a population [82]. In such cases, behaviours should appear non-randomly distributed across the natural and social environment [24,25]. I investigated the patterns of distribution of aggressiveness, boldness and exploration-related behaviours across the environment. I found that males adjusted their level of exploration and boldness to the density of females around their territory (**manuscript 3**). However, I did not find evidence for a non-random settlement of different personality types in space (**manuscripts 3** **and 4**). Of course, we cannot exclude that other habitat characteristics that we did not measure, such as quantity and quality of the leaf litter, tree species, or canopy cover, are linked to individual behaviour. These results, overall, suggest that the environment is unlikely to drive the evolution of consistent between-individual behavioural differences in *Allobates femoralis.*

Phenotype by environment correlation exists in other species (e.g., [4,83]), but understanding whether the environment influences personality or personality influences the choice of an environment to settle in will require future studies. Such questions are best answered in the wild, where individuals face complex environmental challenges. However, controlling the different aspects of the experiment and accurately measuring the environmental pressures (e.g., density of males and females surrounding an individual, habitat complexity, resources availability) can be difficult. While a laboratory setup would solve these issues, measuring personality traits in the lab typically gives less repeatable results than in the wild [39]. Consequently, future studies should aim at integrating both field and lab-based experiments to understand the causes and consequences of non-random settlement of behaviours.

Personality and sexual selection

Several hypotheses have been developed under the framework of sexual selection to explain the evolution and maintenance of animal personality. In their review, Schuett and colleagues [23] draw attention to the role of sexual selection on the emergence of both between-individual behavioural differences and limited behavioural plasticity. They argue that sexual selection should influence the maintenance of between-individual differences in behaviour because the personality traits of both the choosy and the chosen sex can impact mate choice [23]. Behavioural phenotype matching (or “assortative mating”) exists in several species [43,45,84]. In *A. femoralis*, females typically make the decision with whom to mate before commuting to the male’s territory [67]. Assortative mating could only happen if females know the male’s personality before commuting towards them, which is possible only if call characteristics are reliable signals of a male’s personality. The most parsimonious prediction, therefore, is that assortative mating is unlikely to be a driver of between-individual differences in behaviour in *A. femoralis*. This prediction was supported in **manuscript 6**, where I found no support for assortative mating. This result suggests that there is no influence of behavioural phenotype matching in the maintenance of between-individual behavioural differences in *A. femoralis*. Future studies should now investigate whether call characteristics are reliable signals of a male’s personality, and whether females use this information.

Personality and paternal care

Numerous studies show that consistent between-individual differences in behavioural traits covary with proxies for fitness, such as reproductive success or survival [20,75], thus implying that behavioural phenotypes are subject to natural selection. Yet, we still have limited information how a personality trait is translated into fitness. Parental care could be one mechanistic path [76,85], if for instance, a personality trait influences the choice of parents for high-quality breeding resources [86] or if it influences parents responsiveness towards offspring solicitations [43], which in turn increases offspring survival.

In **manuscript 7**, I did not find evidence that individuals' level of exploration and boldness influence their ability to discover and use new rearing resources. Males relied primarily on external cues (e.g., olfactory cues) to perform paternal care (**manuscript 7**). The lack of correlation between personality and paternal care seems surprising at first, since I found in **manuscript 6** that males with certain personality types have a greater reproductive output (i.e., number of tadpoles surviving until adulthood). While it is possible that I did not consider the right measures of paternal care, two hypotheses seem more likely to explain the lack of correlation. First, we know from previous studies that male *A. femoralis* have an accurate representation of their familiar surroundings [55,87,88]. I did not create a novel environment by removing rearing sites that males were familiar with, and opening new ones in the vicinity, but simply changed the known environment. Therefore, activity, rather than exploration or boldness, could covary with paternal care. Second, it is also possible that tadpoles inherited their personality traits from their parents, which would explain the link between male's personality and reproductive output [89–91]. More aggressive and bolder tadpoles could increase their survival if they get better access to food, while less aggressive and shy tadpoles could benefit in successfully hiding from predators.

In the context of parental care, appropriate decision-making is crucial to ensure offspring survival [92]. Relying on sensory cues likely ensures the best possible reproductive success by avoiding the costs of variation due to personality differences. This result goes in the same direction as **manuscript 2**, showing that when the costs of poor decisions are high, individuals respond to environmental cues no matter their personality type.

Personality and life-history trade-offs

Another hypothesis explaining the emergence and maintenance of animal personality, which has received the strongest support to date, stipulates that between-individual differences in behaviour should be favoured and maintained because of their link with

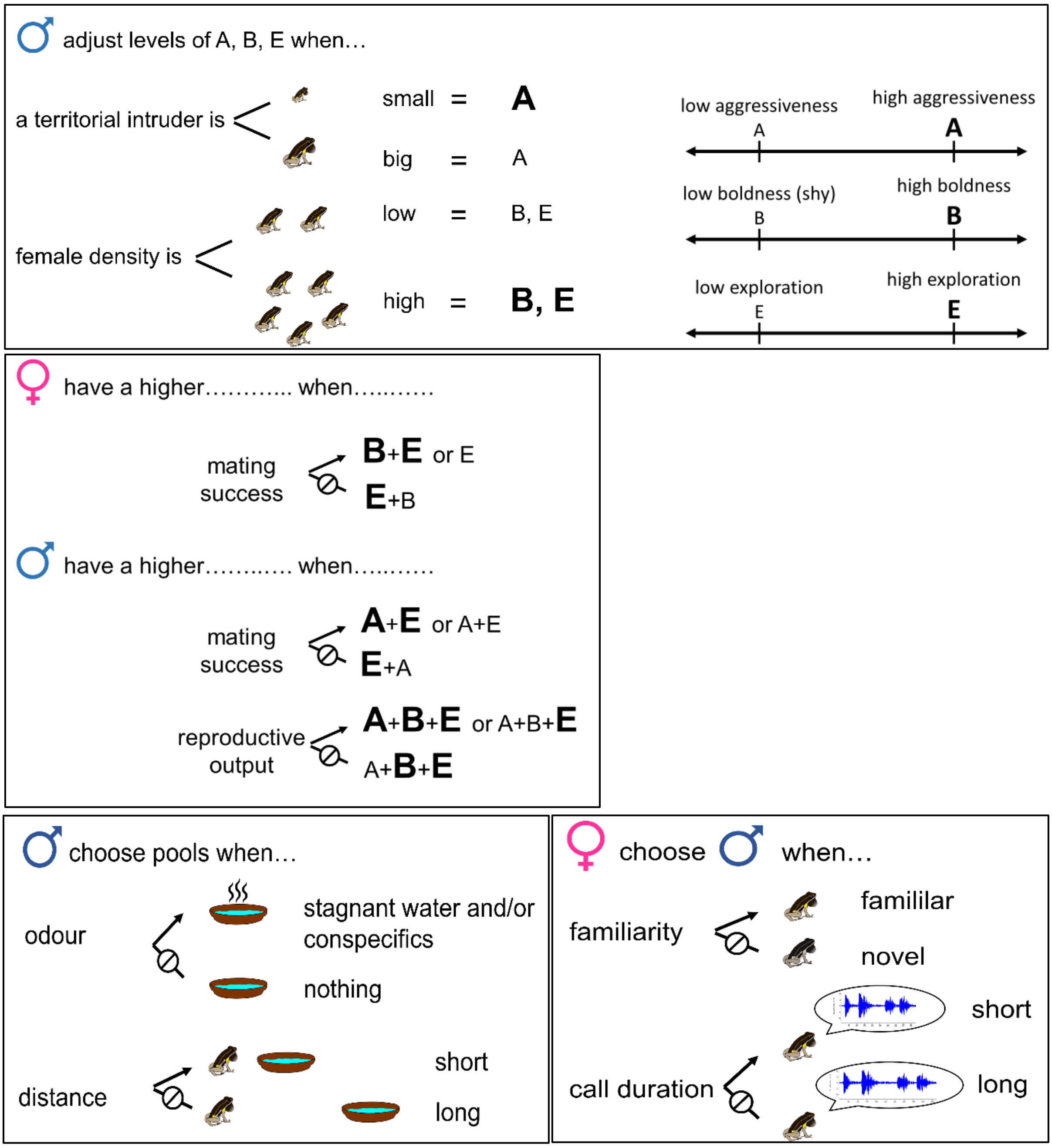


Figure 6. Summary of results gathered from the different manuscripts presented in the thesis. Crossed out lines represent the less favourable option. Levels of aggressiveness, boldness and exploration are represented by the letters A, B and E respectively, with bigger letters for higher levels of the trait and smaller letters for lower levels of the trait.

life-history trade-offs [35,36]. For instance, young individuals with high future expectations in terms of reproduction should be more risk-averse (e.g., less aggressive, less bold, less explorative) than older individuals with low expectations. Or more aggressive males might benefit from increased reproductive success, while less aggressive males might benefit from increased survival.

So far, many studies have tried to provide empirical support for this hypothesis, but most have only looked at the link between one personality trait and one measure of reproductive success or survival [36,75]. No study has looked at the potentially differential effects that personality traits can have on the various processes shaping reproductive success. In **manuscript 6**, I showed that aggressiveness, exploration and boldness differentially impacted three components of reproductive success (mating success, reproductive performance and reproductive output) in males and females. For instance, males with low levels of aggressiveness obtained more mates if they were not explorative, but had a higher reproductive output if they were explorative. While males with high levels of aggression only benefited in terms of mating success if they were also really explorative. So, we expect males that are not aggressive and not explorative to invest more in finding mates than in parental care, to improve their fitness.

Overall, these results suggest a potential effect of correlational selection [93] and provide support for the hypothesis that animal personality arises because of a link with individual differences in life-history trade-offs [36]. This in turn could lead to the evolution of different reproductive strategies.

Future directions

With my thesis, I was able to show the influence of personality traits on individual decision-making and reproductive success. I did not find evidence for an effect of environmental conditions on the emergence and maintenance of animal personality. However, results suggest a possible effect of life-history trade-offs on the emergence and maintenance of consistent between-individual differences in behaviours. I also showed that consistency does not equal absence of plasticity, and individuals are still capable of adjusting their behaviours within the limits of their personality, or use more reliable cues when the costs of poor decisions are high.

Animal personality is an integral part of behavioural ecology, and I want to highlight the importance of studying personality traits together with plasticity and environmental cues to understand the factors influencing reproduction and survival, and the drivers of decisions-making in animals. It will still require more work to better understand the evolution and maintenance of animal personality, but the results obtained throughout this thesis bring us one step closer. It is now primordial to approach these questions by integrating proximate mechanisms with long-term studies of the development of personality across ontogeny. Only by doing so will we improve our understanding of consistency, plasticity, between-individual variation in behaviour, and clarify how selection acts on animal personality.

References (of the general introduction and discussion)

[1] Krebs, J. R. & Davies, N. B. 2009 Behavioural Ecology. An Evolutionary Approach, 4th ed. Oxford: Wiley.

[2] Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007 Integrating animal temperament within ecology and evolution. *Biol Rev* 82, 291–318.

[3] Bridger, D., Bonner, S. J. & Briffa, M. 2015 Individual quality and personality: bolder males are less fecund in the hermit crab Pagurus bernhardus. *Proceedings. Biological sciences* 282, 20142492.

[4] Hensley, N. M., Cook, T. C., Lang, M., Petelle, M. B. & Blumstein, D. T. 2012 Personality and habitat segregation in giant sea anemones (*Condylactis gigantea*). *J Exp Mar Biol Ecol* 426-427, 1–4.

[5] Sánchez-Tójar, A., Moiron, M. & Niemelä, P. T. 2022 Terminology use in animal personality research: a self-report questionnaire and a systematic review. *Proc R Soc Lond B* 289, 20212259.

[6] Vonk, J., Weiss, A. & Kuczaj, S. A. 2017 *Personality in nonhuman animals.* Springer eBook Collection Behavioral Science and Psychology. Cham, Switzerland: Springer.

[7] Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. 2004 Behavioral syndromes: an integrative overview. *Q Rev Biol* 79, 241–277.

[8] Pigliucci, M. 2003 Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecol Lett* 6, 265–272.

[9] Araya-Ajoy, Y. G. & Dingemanse, N. J. 2014 Characterizing behavioural ‘characters’: an evolutionary framework. *Proc R Soc Lond B* 281, 20132645.

[10] Sih, A. & Bell, A. M. 2008 Insights for behavioral ecology from behavioral syndromes. *Adv Stud Behav* 38, 227–281.

[11] Koolhaas, J. M., Korte, S. M., Boer, S. F. de, van der Vegt, B. J., van Reenen, C. G., Hopster, H., Jong, I. C. de, Ruis, M. A. & Blokhuis, H. J. 1999 Coping styles in animals. Current status in behavior and stress-physiology. *Neurosci Biobehav Rev* 23, 925–935.

[12] Pigliucci, M. 2005 Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol* 20, 481–486. (doi:10.1016/j.tree.2005.06.001).

[13] Réale, D. & Dingemanse, N. J. 2010 Personality and individual social specialisation. In *Social behaviour: Genes, ecology and evolution* (ed. T. Székely, A. J. Moore & J. Komdeur), pp. 417–441. Cambridge, United Kingdom: Cambridge University Press.

[14] Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. 2012 Ecological implications of behavioural syndromes. *Ecol Lett* 15, 278–289.

[15] Baugh, A. T., Senft, R. A., Firke, M., Lauder, A., Schroeder, J., Meddle, S. L., van Oers, K. & Hau, M. 2017 Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: A multilevel experiment in Parus major. *Horm Behav* 93, 99–108.

[16] Carere, C. & Maestripieri, D. 2013 *Animal personalities*. *Behavior, physiology, and evolution.* Chicago, IL, USA: University of Chicago Press.

[17] Mell, H., Josserand, R., Decencière, B., Artacho, P., Meylan, S. & Le Galliard, J.-F. 2016 Do personalities co-vary with metabolic expenditure and glucocorticoid stress response in adult lizards? *Behav Ecol Sociobiol* 70, 951–961.

[18] Careau, V., Thomas, D., Humphries, M. M. & Réale, D. 2008 Energy metabolism and animal personality. *Oikos* 117, 641–653.

[19] Lynch, M. & Walsh, B. 1998 *Genetics and analysis of quantitative traits.* Sunderland, MA, USA: Sinauer.

[20] Sih, A., Bell, A. & Johnson, J. C. 2004 Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* 19, 372–378.

[21] Sih, A. 1992 Prey Uncertainty and the Balancing of Antipredator and Feeding Needs. *Am Nat* 139, 1052–1069.

[22] Dall, S. R. X., Houston, A. I. & McNamara, J. M. 2004 The behavioural ecology of personality. Consistent individual differences from an adaptive perspective. *Ecol Lett* 7,

734–739.
[23] Schuett, W., Tregenza, T. & Dall, S. R. X. 2010 Sexual selection and animal personality. *Biol Rev* 85, 217–246.
[24] Conover, D. O. & Schultz, E. T. 1995 Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol Evol* 10, 248–252.
[25] Dingemanse, N. J. & Araya-Ajoy, Y. G. 2015 Interacting personalities. Behavioural ecology meets quantitative genetics. *Trends Ecol Evol* 30, 88–97.
[26] Ehlinger, T. J. & Wilson, D. S. 1988 Complex foraging polymorphism in bluegill sunfish. *Proc Natl Acad Sci USA* 85, 1878–1882.
[27] Bell, A. M. 2005 Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). *Journal of Evolutionary Biology* 18, 464–473.
[28] Dochtermann, N. A. & Jenkins, S. H. 2007 Behavioural syndromes in Merriam's kangaroo rats (Dipodomys merriami): a test of competing hypotheses. *Proc R Soc Lond B* 274, 2343–2349.
[29] Tremmel, M. & Müller, C. 2013 Insect personality depends on environmental conditions. *Behav Ecol* 24, 386–392.
[30] Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O. & Løvlie, H. 2017 A comparison of animal personality and coping styles in the red junglefowl. *Anim Behav* 130, 209–220.
[31] Goursot, C., Düpjan, S., Kanitz, E., Tuchscherer, A., Puppe, B. & Leliveld, L. M. C. 2019 Assessing animal individuality: links between personality and laterality in pigs. *Current zoology* 65, 541–551.
[32] Jong, G. de & Gavrilets, S. 2000 Maintenance of genetic variation in phenotypic plasticity: the role of environmental variation. *Genetical research* 76, 295–304.
[33] Verweij, K. J. H., Yang, J., Lahti, J., Veijola, J., Hintsanen, M., Pulkki-Råback, L., Heinonen, K., Pouta, A., Pesonen, A.-K. & Widen, E. *et al.* 2012 Maintenance of genetic variation in human personality: testing evolutionary models by estimating heritability due to common causal variants and investigating the effect of distant inbreeding. *Evolution; international journal of organic evolution* 66, 3238–3251.
[34] Wolf, M. & McNamara, J. M. 2012 On the evolution of personalities via frequency-dependent selection. *Am Nat* 179, 679–692.
[35] Wolf, M., van Doorn, G. S., Leimar, O. & Weissing, F. J. 2007 Life-history trade-offs favour the evolution of animal personalities. *Nature* 447, 581–584.
[36] Biro, P. A. & Stamps, J. A. 2008 Are animal personality traits linked to life-history productivity? *Trends Ecol Evol* 23, 361–368.
[37] Wilson, D. S. 1998 Adaptive individual differences within single populations. *Phil Trans R Soc Lond B* 353, 199–205.
[38] Dammhahn, M. 2012 Are personality differences in a small iteroparous mammal maintained by a life-history trade-off? *Proc R Soc Lond B*, rspb20120212.
[39] Bell, A. M., Hankison, S. J. & Laskowski, K. L. 2009 The repeatability of behaviour: a meta-analysis. *Anim Behav* 77, 771–783.
[40] Vargas, R., Mackenzie, S. & Rey, S. 2018 'Love at first sight': The effect of personality and colouration patterns in the reproductive success of zebrafish (*Danio rerio*). *PLoS one* 13, e0203320.
[41] Bonnot, N. C., Goulard, M., Hewison, A. M., Cargnelutti, B., Lourtet, B., Chaval, Y. & Morellet, N. 2018 Boldness-mediated habitat use tactics and reproductive success in a wild large herbivore. *Anim Behav* 145, 107–115.
[42] Collins, S. M., Hatch, S. A., Elliott, K. H. & Jacobs, S. R. 2019 Boldness, mate choice and reproductive success in *Rissa tridactyla*. *Anim Behav* 154, 67–74.
[43] Both, C., Dingemanse, N. J., Drent, P. J. & Tinbergen, J. M. 2005 Pairs of extreme avian personalities have highest reproductive success. *Journal of Animal Ecology* 74, 667–674.
[44] Réale, D., Martin, J., Coltman, D. W., Poissant, J. & Festa-Bianchet, M. 2009 Male personality, life-history strategies and reproductive success in a promiscuous mammal. *J Evol Biol* 22, 1599–1607.
[45] Ariyomo, T. O. & Watt, P. J. 2013 Disassortative mating for boldness decreases reproductive success in the guppy. *Behav Ecol* 24, 1320–1326.

[46] Monceau, K., Dechaume-Moncharmont, F.-X., Moreau, J., Lucas, C., Capoduro, R., Motreuil, S. & Moret, Y. 2017 Personality, immune response and reproductive success: an appraisal of the pace-of-life syndrome hypothesis. An appraisal of the pace-of-life syndrome hypothesis. *J Anim Ecol* 86, 932–942.

[47] Kelleher, S. R., Silla, A. J. & Byrne, P. G. 2018 Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. *Behav Ecol Sociobiol* 72, 10539.

[48] Wilson, A. D. M. & Krause, J. 2012 Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? *Behav Ecol* 23, 1316–1323.

[49] AmphibiaWeb. 2022 *AmphibiaWeb: Information on amphibian biology and conservation*. https://amphibiaweb.org. Accessed 7 October 2022.

[50] Crump, M. L. 1972 Territoriality and mating behavior in *Dendrobates granuliferus* (Anura: Dendrobatidae). *Herpetologica* 28, 195–198.

[51] Roithmair, M. E. 1994 Male territoriality and female mate selection in the dart-poison frog *Epipedobates trivattatus* (Dendrobatidae, Anura). *Copeia* 1994, 107–115. (doi:10.2307/1446676).

[52] Pröhl, H. 2005 Territorial behavior in dendrobatid frogs. *J Herpetol* 39, 354–365. (doi:10.1670/162-04A.1).

[53] Lötters, S., Jungfer, K.-H., Henkel, F. W. & Schmidt, W. 2007 *Poison frogs*. *Biology, species & captive husbandry.* Frankfurt am Main, Germany: Edition Chimaira.

[54] Wells, K. D. 2007 *The ecology and behavior of amphibians.* Chicago, IL, USA: University of Chicago Press.

[55] Pašukonis, A., Ringler, M., Brandl, H. B., Mangione, R., Ringler, E. & Hödl, W. 2013 The homing frog: high homing performance in a territorial dendrobatid frog *Allobates femoralis* (Dendrobatidae). *Ethology* 119, 762–768.

[56] Stynoski, J. L., Schulte, L. M. & Rojas, B. 2015 Poison frogs. *Curr Biol* 25, R1026-R1028.

[57] Rojas, B. & Pašukonis, A. 2019 From habitat use to social behavior: natural history of a voiceless poison frog, *Dendrobates tinctorius*. *PeerJ* 7, e7648.

[58] Souza, J. R. D., Kaefer, I. L. & Pimentel Lima, A. 2021 Calling activity determines male mating success in a territorial frog with parental care. *Ethology*.

[59] Ursprung, E., Ringler, M., Jehle, R. & Hödl, W. 2011 Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. *Mol Ecol* 20, 1759–1771. (doi:10.1111/j.1365-294X.2011.05056.x).

[60] Hödl, W. 1987 *Dendrobates femoralis* (Dendrobatidae): a handy fellow for frog bioacoustics. In *Proceedings of the 4th Ordinary General meeting of the Societas Europaea Herpetologica* (ed. van Gelder J. J., H. Strijbosch & P. Bergers). Nijmegen, Netherlands.

[61] Ringler, M., Ringler, E., Magaña Mendoza, D. & Hödl, W. 2011 Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog *Allobates femoralis*. *PLoS one* 6, e25844. (doi:10.1371/journal.pone.0025844).

[62] Narins, P. M., Hödl, W. & Grabul, D. S. 2003 Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. *Proc Natl Acad Sci USA* 100, 577–580. (doi:10.1073/pnas.0237165100).

[63] Weygoldt, P. 1980 Zur Fortpflanzungsbiologie von *Phyllobates femoralis* (Boulenger) im Terrarium. *Salamandra* 16, 215–226.

[64] Montanarin, A., Kaefer, I. L. & Pimentel Lima, A. 2011 Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethol Ecol Evol* 23, 141–150. (doi:10.1080/03949370.2011.554884).

[65] Ringler, E., Ringler, M., Jehle, R. & Hödl, W. 2012 The female perspective of mating in *A. femoralis*, a territorial frog with paternal care – a spatial and genetic analysis. *PLoS one* 7, e40237. (doi:10.1371/journal.pone.0040237).

[66] Roithmair, M. E. 1992 Territoriality and male mating success in the dart-poison frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology* 92, 331–343.

(doi:10.1111/j.1439-0310.1992.tb00970.x).

[67] Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W. & Ringler, E. 2019 Spatio-temporal characteristics of the prolonged courtship in Brilliant-Thighed Poison Frogs, *Allobates femoralis*. *Herpetologica* 75, 268–279.

[68] Ringler, E., Pašukonis, A., Hödl, W. & Ringler, M. 2013 Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. *Front Zool* 10, 67. (doi:10.1186/1742-9994-10-67).

[69] Beck, K. B., Loretto, M.-C., Ringler, M., Hödl, W. & Pašukonis, A. 2017 Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ* 5, e3745.

[70] Ringler, E., Szipl, G., Harrigan, R. J., Bartl-Binder, P., Mangione, R. & Ringler, M. 2018 Hierarchical decision-making balances current and future reproductive success. *Mol Ecol* 27, 2289–2301.

[71] Erich, M., Ringler, M., Hödl, W. & Ringler, E. 2015 Brood-partitioning behaviour in unpredictable environments: hedging the bets? *Behav Ecol Sociobiol* 69, 1011–1017.

[72] Fischer, M.-T., Ringler, M., Ringler, E. & Pašukonis, A. 2020 Reproductive behavior drives female space use in a sedentary Neotropical frog. *PeerJ* 8, e8920.

[73] Pašukonis, A., Beck, K. B., Fischer, M.-T., Weinlein, S., Stückler, S. & Ringler, E. 2017 Induced parental care in a poison frog: a tadpole cross-fostering experiment. *J Exp Biol* 220, 3949–3954.

[74] Dingemanse, N. J., Kazem, A. J. N., Reale, D. & Wright, J. 2010 Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol Evol* 25, 81–89.

[75] Smith, B. R. & Blumstein, D. T. 2008 Fitness consequences of personality: a meta-analysis. *Behav Ecol* 19, 448–455.

[76] Mutzel, A., Dingemanse, N. J., Araya-Ajoy, Y. G. & Kempenaers, B. 2013 Parental provisioning behaviour plays a key role in linking personality with reproductive success. *Proc R Soc Lond B* 280, 20131019.

[77] Dall, S. R. X., Bell, A. M., Bolnick, D. I. & Ratnieks, F. L. W. 2012 An evolutionary ecology of individual differences. *Ecol Lett* 15, 1189–1198.

[78] Maynard Smith, J. 1986 *Evolution and the theory of games*, Reprint. Cambridge: Cambridge Univ. Pr.

[79] Mathot, K. J., Wright, J., Kempenaers, B. & Dingemanse, N. J. 2012 Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. *Oikos* 121, 1009–1020.

[80] Coppens, C. M., Boer, S. F. de & Koolhaas, J. M. 2010 Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365, 4021–4028.

[81] Wolf, M., van Doorn, G. S. & Weissing, F. J. 2008 Evolutionary emergence of responsive and unresponsive personalities. *Proc Natl Acad Sci USA* 105, 15825–15830.

[82] Dingemanse, N. J., Both, C., Drent, P. J. & Tinbergen, J. M. 2004 Fitness consequences of avian personalities in a fluctuating environment. *Proc R Soc Lond B* 271, 847.

[83] Duckworth, R. A. & Kruuk, L. E. B. 2009 Evolution of genetic integration between dispersal and colonization ability in a bird. *Evolution* 63, 968–977.

[84] Laubu, C., Dechaume-Moncharmont, F.-X., Motreuil, S. & Schweitzer, C. 2016 Mismatched partners that achieve postpairing behavioral similarity improve their reproductive success. *Sci Adv* 2, e1501013.

[85] Dingemanse, N. J. & Réale, D. 2013 What is the evidence that natural selection maintains variation in animal personalities? In *Animal personalities. Behavior, physiology, and evolution* (ed. C. Carere & D. Maestripieri), pp. 201–220. Chicago, IL, USA: University of Chicago Press.

[86] Zhao, Q.-S., Hu, Y.-B., Liu, P.-F., Chen, L.-J. & Sun, Y.-H. 2016 Nest site choice: a potential pathway linking personality and reproductive success. *Anim Behav* 118, 97–103.

[87] Pašukonis, A., Loretto, M.-C., Landler, L., Ringler, M. & Hödl, W. 2014 Homing trajectories and initial orientation in a Neotropical territorial frog, *Allobates femoralis* (Dendrobatidae). *Front Zool* 11, 29. (doi:10.1186/1742-9994-11-29).

[88] Pašukonis, A., Trenkwalder, K., Ringler, M., Ringler, E., Mangione, R., Steininger, J., Warrington, I. & Hödl, W. 2016 The significance of spatial memory for water finding in a tadpole-transporting frog. *Anim Behav* 116, 89–98.

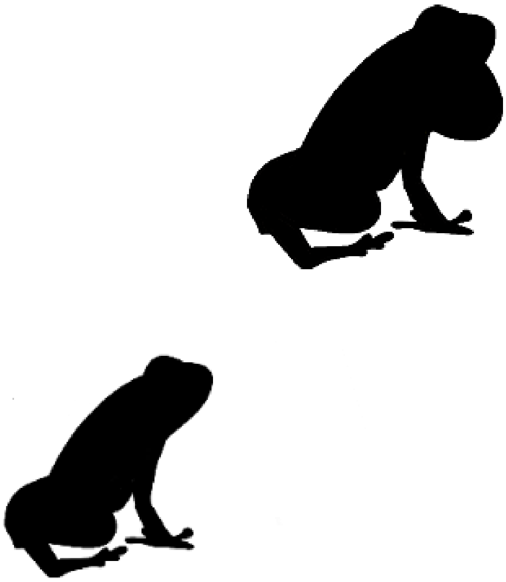
[89] Drent, P. J., van Oers, K. & van Noordwijk, A. J. 2003 Realized heritability of personalities in the great tit (*Parus major*). *Proc R Soc Lond B* 270, 45–51.

[90] Dochtermann, N. A., Schwab, T. & Sih, A. 2015 The contribution of additive genetic variation to personality variation: heritability of personality. *Proc R Soc Lond B* 282, 20142201.

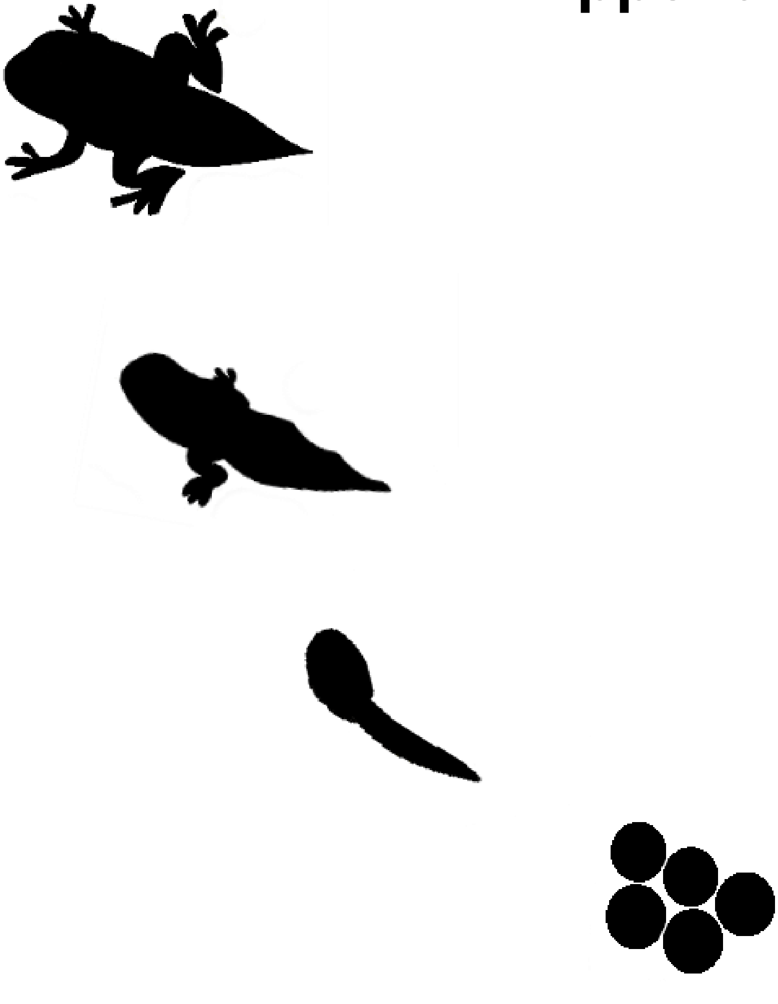
[91] Ariyomo, T. O., Carter, M. & Watt, P. J. 2013 Heritability of boldness and aggressiveness in the zebrafish. *Behav Genet* 43, 161–167.

[92] Clutton-Brock, T. H. 1991 *The evolution of parental care.* Monographs in behavior and ecology. Princeton, NJ, USA: Princeton University Press.

[93] Montiglio, P.-O., Wey, T. W., Chang, A. T., Fogarty, S. & Sih, A. 2017 Correlational selection on personality and social plasticity: morphology and social context determine behavioural effects on mating success. *Journal of Animal Ecology* 86, 213–226.



Appendices



Appendix 1: “A comparative table for staging anuran embryos from terrestrial clutches based on the Brilliant-thighed Poison Frog, *Allobates femoralis* (Anura: Dendrobatidae)”

This manuscript is in press in *Herpetology Notes*
13 July 2022

Mélissa Peignier[1,2,†], Barbara Furdi[3,†], Lauriane Bégué[1], and Eva Ringler[1,2,*]

[1] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Bern, Switzerland.
[2] Messerli Research Institute, University of Veterinary Medicine Vienna, 1210 Vienna, Austria.
[3] Department of Behavioral and Cognitive Biology, University of Vienna, 1210 Vienna, Austria.
[†] These authors contributed equally to this paper.

*Correspondence:
Eva Ringler
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50A
CH-3032 Hinterkappelen
eva.ringler@unibe.ch

Developmental stages in tadpoles are usually classified according to the scheme created by Gosner (Gosner, 1960). This staging table shows a generalized system that can be used to describe embryonal and larval development in anurans. This reference

Developmental stages in tadpoles are usually classified according to the scheme created by Gosner (Gosner, 1960). This staging table shows a generalised system that can be used to describe embryonal and larval development in anurans. This reference table, however, was initially developed for temperate zone anurans with aquatic oviposition, so the development of species that differ from this breeding mode might deviate from this description. Terrestrial oviposition has evolved independently in several anuran species (Vági et al., 2019; Furness et al., 2022), such as within the genus Eleutherodactylus (Townsend and Steward, 1994; Bourne, 1997), *Adenomera hylaedactyla* or *Amazophrynella minuta* (Lima and Magnusson, 2006), as well as poison frogs (Dendrobatidae sensu AmphibiaWeb, 2022).

In poison frogs, eggs are deposited on land, often directly in the leaf litter. After hatching, the terrestrial eggs turn into aquatic tadpoles, and therefore, in most dendrobatid species, one of the parents transports the tadpoles on its back to suitable water bodies (Ringler et al., 2013; Killius and Dugas, 2014; Frazão Luiz et al., 2015; Schulte and Mayer, 2017). It is still unknown which are the morphological changes that the newly hatched tadpoles go through before they can climb onto their parent’s back to be transported.

The aim of this study was to provide a detailed description of the developmental stages of embryos and tadpoles of the Neotropical poison frog *Allobates femoralis* (Boulenger, 1884) from oviposition to hatching, with comparison to their respective Gosner stages. On one hand, this staging table will provide us with a reference table to assess the time elapsed since oviposition and the time until tadpole transport will occur for clutches under both field and lab conditions. On the other hand, it shall give insight into possible developmental differences between aquatic and terrestrial developing embryos, and thereby improve our knowledge of the embryonic morphological changes.

Allobates femoralis is a small diurnal leaf litter frog common throughout the Amazon basin and the Guiana Shield (Amezquita et al., 2009). During the reproductive season, males occupy territories of 64–417 m2 (Ringler et al., 2011) from which they call to repel male competitors and attract females, while perched on elevated surfaces (Narins et al., 2003; Hödl et al., 2004). Males aggressively defend their territories against calling intruders (Narins et al., 2003; Hödl et al., 2004). Females display site fidelity and commute to male territories for courtship and mating (Fischer et al., 2020). The possession of a territory is of great importance for male reproductive success, as it is where pair formation, courtship, mating, and egg deposition occur (Montanarin et al., 2011; Ursprung et al., 2011; Stückler et al., 2019). Both sexes can mate multiple times with multiple partners (Ursprung et al., 2011). The courtship march in *A. femoralis* is one of the longest among poison frogs (Stückler et al., 2019). After the courtship march, eggs are laid in the leaf litter, where they develop for 15 to 20 days before hatching. Once hatched, tadpoles are transported by the males to medium sized natural pools located up to 200 m (on average 27.52 ± 30.90 m) away from their territory (Ringler et al., 2013, 2018). Males typically distribute the tadpoles across several water bodies (Erich et al., 2015). Occasionally, females take over tadpole transport, but only when males disappear (Ringler et al., 2015).

Herein, we provide a detailed description of the developmental changes that *A. femoralis* embryos and larvae undergo before tadpole transport. We recorded the morphological changes in tadpoles during development and related these stages to the ones proposed by Gosner (1960). Observations were conducted under controlled conditions in an ex-situ breeding population of A. femoralis at the University of Bern. We kept pairs of frogs in glass terraria (60 × 40 × 40 cm) with expanded clay pebbles on the floor. The back and side walls were covered with xaxim (tree fern stems) mats in the lower and cork in the upper half. We provided half a coconut shell, a small plant, and a branch in each tank. We provided autoclaved oak leaves as substrate for oviposition, and a small glass bowl of 10 cm diameter filled with water for tadpole deposition. We used an automatic rain, heating, and lighting system to ensure standardised climatic conditions in all terraria, similar to natural conditions in French Guiana. The temperature ranged from 21 °C at night to 28 °C during the day. Lights were on from 07:00 to 19:00 h and humidity in the terraria was constantly at 100%.

To record morphological changes during the development, we checked the tanks for new clutches (Fig. 1) every day during the peak of the reproductive season and took daily pictures of the clutches from egg-laying to tadpole transport. We took daily photos of nine clutches (for a total of 120 eggs, mean = 13.33 ± 3.24 SD per clutch), using a Canon EOS 77D camera (Canon © Deutschland GMBH, Krefeld, Germany), equipped with a macro lens (Canon EF 100mm 1:2.8 USM). The clutches were placed in an open Petri dish, on white background, under an external artificial light source, to ensure that the light was homogeneously distributed and the image had a high contrast. All the clutches placed in Petri dishes were left in the parents’ tank between daily inspections to prevent desiccation, as they were subjected to the automatic rain system, and to enable the father to transport the clutch to the water bowl once the development completed. We show the pictures with reference to corresponding Gosner stages in Figure 2.

All 25 pre-feeding stages that Gosner (1960) described were also observed during A. femoralis embryonic development. Even if no stage is strictly related to day-to-day development, the three-week development period enabled us to successfully differentiate stages. As expected, we recorded small differences in the development of individual embryos even within the same clutch. Variability increased with time, most prominently with the start of muscular responses (stage 18; Fig. 2O). The first eight stages (fertilisation and cleavage processes) were finished within a day. First a lightening appeared on one hemisphere (Fig. 2A), then cell division began (Fig. 2B–G). Then, the gastrulation started and the blastopore became conspicuous (stages 11–12; Fig. 2H–J). Following this, neural folding usually began five days after oviposition (stages 13–15; Fig. 2K–L). The neural tube was formed quickly after, on the same or on the following day, and the embryo began developing a recognisable head (stage 16; Fig. 2M). The tail bud appeared between days five and six (stage 17; Fig. 2N), and noticeably elongated from then on, while the gill plate became less and less visible. Together with the development of the tail bud, the adhesive organ started developing (Fig. 2N). The organ is large, with soft edges, supposedly to enable the tadpoles to fix themselves on their father’s back. Contrarily to most North American pelobatids, bufonids, hylids, and ranids, whose adhesive organs are initially united as a ridge before they become bifid (Gosner, 1960), in *A. femoralis* the mouth part developed as an invagination which later developed into the mouth part (stages 23–25). About a week post oviposition, we observed an initiation of spasmodic muscular responses, and the division of the gill plate into ridges (stage 18; Fig. 2O).

From this stage, the variability in developmental time increased. While a heartbeat was visible in most temperate zone anurans with aquatic oviposition at stage 19, we were not able to observe it in *A. femoralis*. The external gill filaments fully developed on stage 21, usually between 9 and 12 days post-oviposition (Fig. 2R). At this stage, the larvae transitioned to free-swimming tadpoles, the cornea became transparent and the eyes were clearly discernible. The fins became more transparent around day 12 (stage 22; Fig. 2S). After the first two weeks post-oviposition, the external gills disappeared, the oral disc and labial tooth developed and we observed a modification of the pigmentary patterns (stages 23–25; Fig. 2T). Only one clutch was successfully transported by the father. In that case, tadpole transport occurred on day 21 post-oviposition. While this is still within the range of what has been observed in the wild previously (Ringler et al., 2013), we noticed that males sat for a few days close to the Petri dish. Probably setting-up the clutch in a Petri dish disturbed the father and prevented him from reaching the tadpoles, or artificially increased the delay before transport.

Our results suggest that *A. femoralis* tadpoles develop a recognisable tadpole-like shape (i.e., with a discernible fin and head) between days 8 to 11. Most developmental modifications were similar to the ones described by Gosner (1960) in temperate zone anurans with aquatic oviposition, with exception of the development of the adhesive organ. This difference could be due to the physical requirements of holding on to the parent during tadpole transport, which is obligatory in all dendrobatid species. Future studies should investigate the differences in the development of adhesive organs between species with and without tadpole transport. Additionally, comparative studies between species with lentic and lotic tadpoles might serve to identify functional similarities and differences in specific morphological traits (cf. Baldo et al., 2014; Candioti et al., 2016, 2020). Further studies are also required to understand when newly hatched tadpoles are physically able to climb onto their father’s back to be transported, and how fathers know when each clutch needs to be transported. For instance, visual cues of the developmental stage of the clutch (e.g., the loss of external gills and the changes in pigmentation) might be a signal for parents to initiate tadpole pick up and transport. With this study, we provide a detailed staging table, related to the one proposed by Gosner (1960), describing embryonal and larval development in terrestrial breeding anurans. Discussed characteristics were easily traceable due to a long developmental time. The staging table we developed can serve for further laboratory experimentation, for example as a tool to reconstruct the day of oviposition in *A. femoralis*.

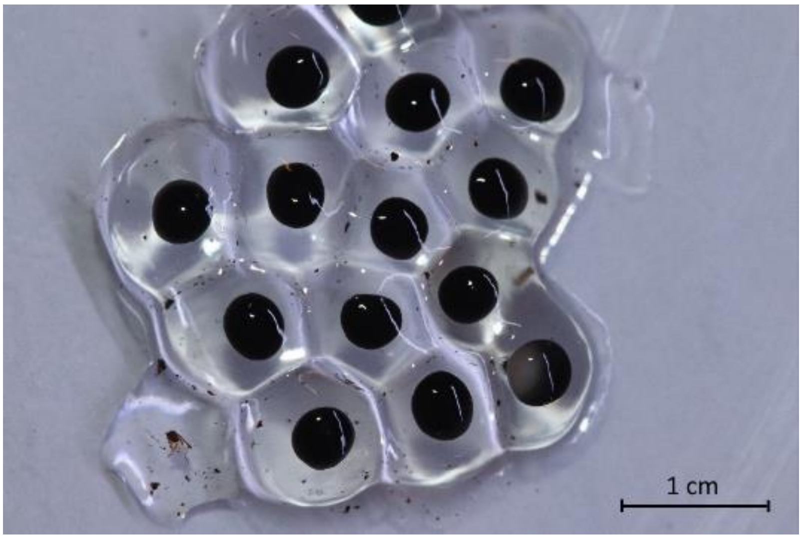


Figure 1. Picture of a clutch oviposited and fertilised during the preceding night. Photo by Mélissa Peignier.

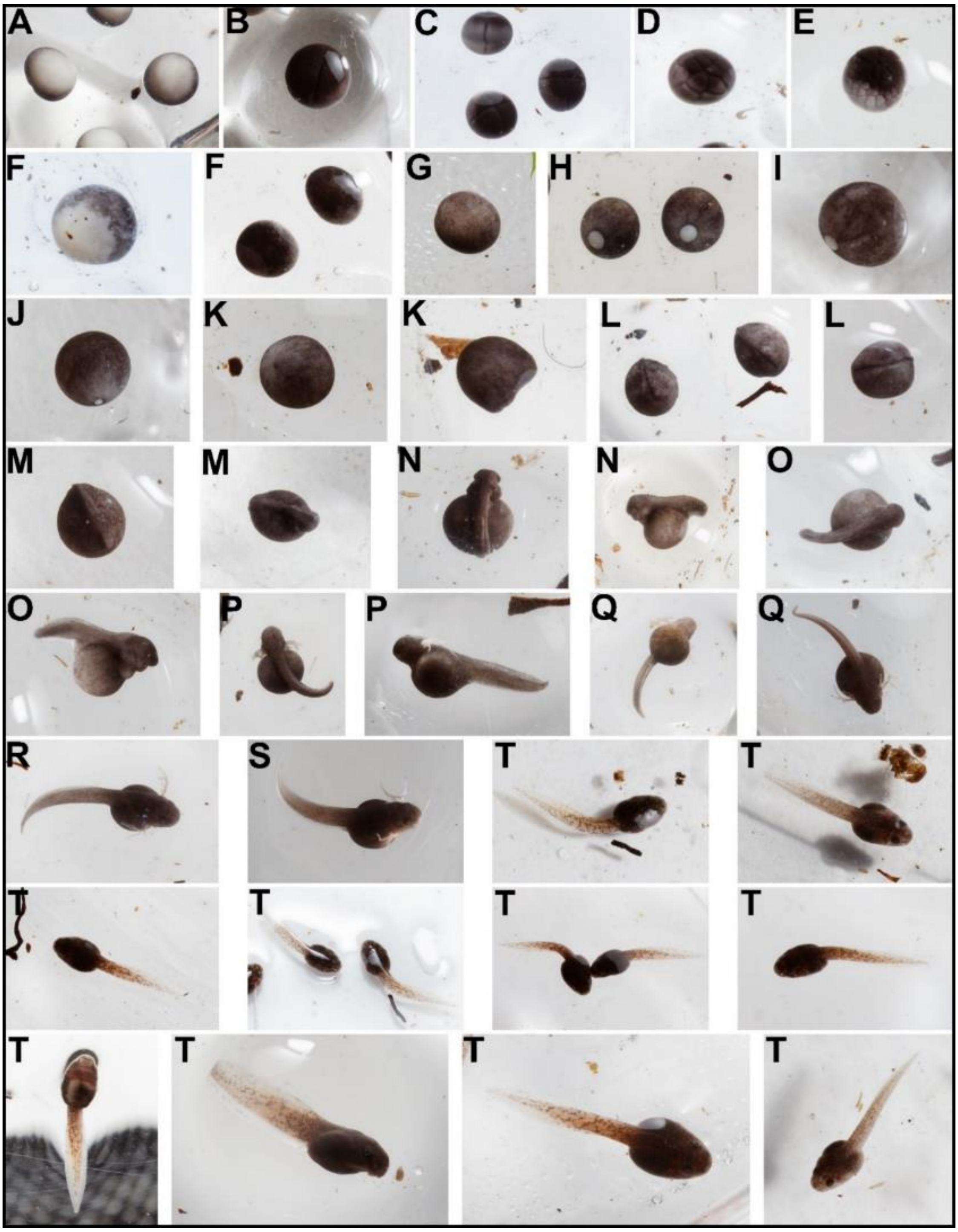


Figure 2. Pictures of the embryonic development of *Allobates femoralis*. For each picture, the day of development, the principal embryonic changes and the embryonic stages equivalent to Gosner (1960) are given. A–G: days 1–2. fertilisation and cleavage (stages 1–9); H-I: days 2–3. mid-gastrula (stages 10–11); J: days 2–3. late gastrula (stage 12); K: days 3–5. neural plate (stage 13); L: days 4–5. neural folds (stage 14); M: days 4–5. neural tube (stage 16); N: days 5-–6. tail bud and mouth (stage 17); O: days 6–8. muscular response and ridges (stage 18); P–Q: days 7–13. external gills (stages 19–20); R-S: days 9–14. cornea and fins become transparent, full development of external gills (stages 21-22); T: days 14–21. changes in pigmentation, disappearance of external gills, oral disc and labial tooth development (stages 23–25). Photos by Lauriane Bégué and Mélissa Peignier.

Acknowledgments

We acknowledge the financial support of the Swiss National Science Fund (SNSF) [grant 197921 to ER] and the Austrian Science Fund (FWF) [grant P 31518 to ER]. We thank J. Culebras and F. Angiolani-Larrea for their help with preparing the photo set-up. We also thank B. Szabo for suggestions on an earlier version of this manuscript and Pedro Henrique dos Santos Dias for pre-reviewing the manuscript and providing helpful comments.

References

Amezquita, A., Lima, A.P., Jehle, R., Castellanos, L., Ramos, Ó., Crawford, A.J., Gasser, H., Hödl, W. (2009): Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. Biological Journal of the Linnean Society **98**: 826–838.

AmphibiaWeb (2022): AmphibiaWeb: Information on amphibian biology and conservation. Berkeley, CA, USA, University of California. Available at https://amphibiaweb.org. Accessed on 4 February 2022.

Baldo, D., Candioti, F.V., Haad, B., Kolenc, F., Borteiro, C., Pereyra, M.O., et al. (2014): Comparative morphology of pond, stream and phytotelm-dwelling tadpoles of the South American Redbelly Toads (Anura: Bufonidae: *Melanophryniscus*). Biological Journal of the Linnean Society **112**: 417–441.

Bourne, G.R. (1997): Reproductive behavior of terrestrial breeding frogs *Eleutherodactylus johnstonei* in Guyana. Journal of Herpetology **31**: 221–229.

Candioti, F.V., Grosso, J., Haad, B., Pereyra, M.O., Bornschein, M.R., Borteiro, C., et al. (2016): Structural and heterochronic variations during the early ontogeny in toads (Anura: Bufonidae). Herpetological Monographs **30**: 79–118.

Candioti, F.V., Grosso, J., Pereyra, M.O., Haad, M.B., Lescano, J., Siu-Ting, K., Aguilar, C., Baldo, D. (2020): Larval anatomy of Andean toads of the *Rhinella spinulosa* group (Anura: Bufonidae). Herpetological Monographs **34**: 116–130.

Erich, M., Ringler, M., Hödl, W., Ringler, E. (2015): Brood-partitioning behaviour in unpredictable environments: hedging the bets? Behavioral Ecology and Sociobiology **69**: 1011–1017.

Fischer, M.-T., Ringler, M., Ringler, E., Pašukonis, A. (2020): Reproductive behavior drives female space use in a sedentary Neotropical frog. PeerJ **8**: e8920.

Frazão Luiz, L., Contrera, F.A.L., Neckel-Oliveira, S. (2015): Diet and tadpole transportation in the poison dart frog *Ameerega trivittata* (Anura, Dendrobatidae). The Herpetological Journal **25**: 187–190.

Furness, A.I., Venditti, C., Capellini, I. (2022): Terrestrial reproduction and parental care drive rapid evolution in the trade-off between offspring size and number across amphibians. PLOS Biology **20**: e3001495.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 183–190.

Hödl, W., Amézquita, A., Narins, P.M. (2004): The role of call frequency and the auditory papillae in phonotactic behavior in male Dart-poison frogs *Epipedobates femoralis* (Dendrobatidae). Journal of Comparative Physiology A **190**: 823–829.

Killius, A.M., Dugas, M.B. (2014): Tadpole transport by male *Oophaga pumilio* (Anura: Dendrobatidae): An observation and brief review. Herpetology Notes **7**: 747–749.

Lima, A.P., Magnusson, W.E., Menin, M., Erdtmann, L.K., Rodrigues, D.J., Keller, C., Hödl, W. (2006): Guide to the frogs of Reserva Adolpho Ducke, Central Amazonia: Guia de sapos da Reserva Adolpho Ducke, Amazônia central.Manaus, Brazil, Attema Design Editorial.

Montanarin, A., Kaefer, I.L., Lima, A.P. (2011): Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. Ethology Ecology & Evolution **23**: 141–150.

Narins, P.M., Hödl, W., Grabul, D.S. (2003): Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. Proceedings of the National Academy of Sciences**100**: 577–580.

Ringler, E., Pašukonis, A., Fitch, W.T., Huber, L., Hödl, W., Ringler, M. (2015): Flexible compensation of uniparental care: female poison frogs take over when males disappear. Behavioral Ecology **26**: 1219–1225.

Ringler, E., Pašukonis, A., Hödl, W., Ringler, M. (2013): Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. Frontiers in Zoology **10**: 67.

Ringler, M., Ringler, E., Magaña Mendoza, D., Hödl, W. (2011): Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog *Allobates femoralis*. PLoS ONE **6**: e25844.

Ringler, E., Szipl, G., Harrigan, R.J., Bartl-Binder, P., Mangione, R., Ringler, M. (2018): Hierarchical decision-making balances current and future reproductive success. Molecular Ecology **27**: 2289–2301.

Schulte, L.M., Mayer, M. (2017): Poison frog tadpoles seek parental transportation to escape their cannibalistic siblings. Journal of Zoology **303**: 83–89.

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W., Ringler, E. (2019): Spatio-temporal characteristics of the prolonged courtship in Brilliant-Thighed Poison Frogs, *Allobates femoralis*. Herpetologica **75**: 268–279.

Townsend, D.S., Stewart, M.M. (1994): Reproductive ecology of the Puerto Rican frog *Eleutherodactylus coqui*. Journal of Herpetology **28**: 34–40.

Ursprung, E., Ringler, M., Jehle, R., Hödl, W. (2011): Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Molecular Ecology **20**: 1759–1771.

Vági, B., Végvári, Z., Liker, A., Freckleton, R.P., Székely, T. (2019): Parental care and the evolution of terrestriality in frogs. Proceedings of the Royal Society B: Biological Sciences **286**: 20182737.

Appendix 2: “Personality is linked to differential testosterone modulation after behavioural challenges in male poison frogs”

This manuscript will be submitted to *Hormones and behaviour*

Eva Ringler[1,2,3,*], Katharina Dellefont[1,*], Mélissa Peignier[1,2,3], Virginie Canoine[1]

[1] Department of Behavioural and Cognitive Biology, University of Vienna, Vienna, Austria.
[2] Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, University of Vienna, Vienna, Austria.
[3] Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland.
[*] shared first-authorship

Correspondence:
Eva Ringler
Institute of Ecology and Evolution
Division of Behavioural Ecology
University of Bern
Wohlenstrasse 50a
3032 Hinterkapellen
eva.ringler@unibe.ch

Abstract

Hormones play a fundamental role in mediating social behaviours of animals. However, it is less well understood to what extent among-individual behavioural variation can be attributed to variation in underlying hormonal profiles. The goal of the present study was to infer if differences in individual androgen levels, and/or the modulation thereof, can explain among-individual variation in aggressiveness, boldness and exploration. We used as a model the dart-poison frog *Allobates femoralis* and took repeated non-invasive water-borne hormonal samples of individual males before and after a series of behavioural tests for assessing aggression, boldness, and exploratory tendency. Our results show that intra- and inter-individual water-borne testosterone ($_{wb}$T) levels are repeatable. Time of day, age of the frog, and trial order did not show any significant impact on measured $_{wb}$T levels. We did not find a significant relationship between baseline $_{wb}$T and the assessed behavioural traits, however we found a significant positive correlation between $_{wb}$T levels after behavioural testing and the frogs’ exploratory tendency. In general, $_{wb}$T levels decreased after behavioural tests compared to the respective baseline levels. However, we identified two different patterns with regard to androgen modulation in response to behavioural testing: individuals with low baseline $_{wb}$T tended to have increased hormonal levels, while individuals with comparatively high baseline $_{wb}$T concentrations rather showed a decrease in hormonal levels after the behavioural testing. Our results show that differences in hormonal profiles and/or responses to social challenges can indeed explain among-individual differences in behavioural traits.

Keywords

testosterone, animal personality, behavioural challenge

Introduction

Hormones play a fundamental role in the expression of morphological and behavioural traits (Adkins-Regan, 2005). The causal relationship between hormones and behaviour is bidirectional, as hormones regulate the expression of social behaviours, but at the same time being exposed to behaviour of con- or heterospecifics can also induce a hormonal response in the focal individual (Adkins-Regan, 2005; Vitousek et al., 2014; Wingfield et al., 1990). However, the precise interplay between hormones and behaviour may differ among individuals of a population, as it depends on various factors, such as experience during early development, an animal's physiological condition, environment and adaptation to specific life-history stages in order to optimise fitness.

In recent years, several studies have shown that there is high among-individual variation and within-individual consistency of behaviour across time and contexts in several animal taxa (i.e. termed animal personality; Araya-Ajoy and Dingemanse, 2014; Réale et al., 2007), and that these differences in behaviour ultimately impact an animal's prospects of survival, competitive ability, mating success and other fitness relevant traits (Dingemanse et al., 2004; Sih and Bell, 2008). One key question in the study of animal personality is to what extent differences in behavioural phenotypes can be attributed to differences in hormonal profiles, and/or if different behavioural phenotypes arise from differences in their physiological response to challenges in their social/natural environment (cf. Biro and Stamps, 2010; Réale et al., 2010; Sih et al., 2015).

Animal personality is typically measured along five main axes, namely aggressive-docile, exploration affine-averse, sociable-solitary, bold-shy and active-passive (Réale et al., 2007). These five axes are known to be modulated by sex steroids, in particular androgens, especially during the reproductive season (see: Adkins-Regan, 2005; Hau, 2007; Nelson, 2005). In male vertebrates, androgens play a key role in the development and maintenance of primary and secondary sexual traits but also regarding the modulation of different behaviours related to reproduction, such as territorial aggression, courtship and mating (Fusani, 2008; Hirschenhauser et al., 2003; Hunt et al., 2019; Rosvall et al., 2020; Rosvall et al., 2012). It is thus not surprising that androgens, e.g., testosterone (T), undergo seasonal fluctuations, with the highest concentration during the breeding season and lowest during the nonreproductive period (Goymann et al., 2019; Hau, 2007; Husak et al., 2021; Wingfield et al., 1990).

However, persistent high levels of T are expected to bear considerable costs (Wingfield et al., 2001), such as reduced immune function (Dufty, 1989; McGlothlin and Ketterson, 2008), increased risk-taking and resulting elevated predation risk (Marler and Moore, 1988; Raynaud and Schradin, 2014), interference with parental behaviour (Hegner and Wingfield, 1987; McGlothlin and Ketterson, 2008), and metabolic expenses (Buchanan et al., 2001; Tobler et al., 2007). Therefore, fine-tuned temporal fluctuations in T modulating adequate behavioural and physiological response to sudden environmental challenges may help to optimize the cost-benefit trade-off associated with high and low levels of circulating T in males. High inter-and intra-species differences in the levels of circulating T have been shown for many vertebrate taxa, and these differences have been linked to respective social and environmental factors, such as breeding season length, type of mating system, and latitude (cf. Husak et al., 2021). Identifying the ecological and physiological factors that shape behaviour at the species, population, but also individual level will advance our understanding about the mechanisms that give rise to behavioural variation across different levels of biological organization.

The aim of the present study was to infer if differences in individual baseline androgen levels, and/or the modulation thereof after behavioural challenges, can explain among-individual variation in the personality traits aggressiveness, boldness and exploration. We used as a model the Neotropical poison frog *Allobates femoralis*, a highly territorial species that shows highly aggressive behavioural response towards acoustic playbacks, simulating calling intruders (Hödl, 1983a; Narins et al., 2003; Ringler et al., 2011; Rodríguez et al., 2022). To this end, we took repeated samples of individual baseline water borne testosterone ($_{wb}$T) levels (i.e. without any manipulation prior hormonal sampling), and also directly after behavioural tests which assessed territorial aggression, boldness and explorative behaviour of individual males. For the hormonal sampling, we used a non-invasive water bath method (Baugh et al., 2018; Rodríguez et al., 2022), which enabled us to take repeated samples of the same individuals in the field. We assessed inter- and intra-individual consistency of $_{wb}$T to gain information about the consistency of individual hormonal profiles over the course of several weeks during the breeding season. Additionally, we identified if and how preceding behavioural testing will alter obtained hormonal measurements. The combination of repeated hormonal sampling and behavioural testing allowed us to link the endocrine profile of each individual to its personality.

We expected $_{\mathrm{wb}}$T levels to be highly repeatable, especially when sampled without any preceding behavioural manipulation, but slightly higher in the afternoon, when the males are actively advertising territory ownership to male and female conspecifics (cf. Rodríguez et al., 2022). We also expected $_{\mathrm{wb}}$T to be positively linked to levels of territorial aggression and/or exploration in the behavioural tests, given that previous studies in several species, including *A. femoralis*, suggested a link between androgen modulation and spatial behaviour in the context of territory defence or homing (cf. Herman and Wallen, 2007; Hodgson et al., 2008; Pašukonis et al., 2022; Rodríguez et al., 2022).

Material and methods

Ethical note

This study was approved by the scientific committee of the “Nouragues Ecological Research Station” and the ethics board of the University of Veterinary Medicine Vienna, as well as the University of Vienna. The hormonal such as the behavioural sampling was conducted in strict accordance with current French and EU law, according to the Study of Animal Behaviour (ASAB) guidelines.

Study site and study species

This study was conducted in a free-ranging population of *A. femoralis* on a river island of approx. 5ha, close to the field camp ‘Saut Pararé’ (4°02′ N, 52°41′ W) in the nature reserve ‘Les Nouragues’, in French Guiana (Bongers et al., 2001; Ringler et al., 2016). The island population of *A. femoralis* was established in 2012 by introducing tadpoles from the nearby mainland population and has been stable ever since with approximately 150 individuals (Ringler et al., 2015). We conducted fieldwork during the rainy season, from the beginning of February 2019 until the end of April 2019, which coincides with the reproductive season of the focal species (Gottsberger and Gruber, 2004).

Allobates femoralis (Boulenger 1883) is a small, diurnal Neotropical poison frog (Dendrobatidae sensu AmphibiaWeb), which is distributed throughout the Amazon Basin and Guiana Shield. During the reproductive season, males are highly territorial and advertise territory occupancy to male competitors and potential female mating partners by producing loud advertisement calls from exposed, elevated positions (Hödl, 1983b; Ringler et al., 2011; Rodríguez López and Hödl, 2020; Roithmair, 1992). Males virguously defend their territory against conspecific intruders (Narins et al., 2003, 2005). Females exhibit site fidelity but are typically not aggressive to either sex (Ringler et al., 2012; Ringler et al., 2009), and actively approach neighbouring calling males for courtship and mating (Montanarin et al., 2011; Ringler et al., 2012; Stückler et al., 2019). Egg deposition takes place in the male's territory and both sexes mate multiple times with multiple partners (Ringler et al., 2012; Ursprung et al., 2011). After hatching, tadpoles are typically transported by the male to medium sized water bodies located up to 200m outside the territory (Beck et al., 2017; Ringler et al., 2013).

Population monitoring

We surveyed the entire population every day from 0900 to 1800 h. We identified all frogs on site via digital pictures of their unique ventral patterns and later verified their identity with the pattern matching software Wild-ID (Bolger et al., 2012). Frogs were sexed by the presence (males) or absence (females) of a vocal sac. We recorded the precise location of the frogs on a digital map, using a tablet PC (WinTab 9, Odys, Willich, Germany) equipped with the mobile GIS software ArcPad 10.2 (ESRI, Redlands, CA, U.S.A.). We determined body size (snout urostyle length) from dorsal photographs taken on top of a measurement grid using the software Image J 1.52a (Rasband, 1997-2021). Information on the age of individuals was available form a concurrent long-term monitoring on the island population since its origin in 2012.

Experimental design

To gain information about the inter- and intra-individual variation of individual ${}_{wb}T$ levels and further investigate the effect of preceding behavioural tests on their T response, we repeatedly sampled ${}_{wb}T$ under two following conditions: First we collected 'baseline' levels by capturing a focal frog without the use of any acoustic stimuli (e.g. playback) and immediately transferred it to the water bath (for details see 'Hormonal sampling'). Second, we also measured ${}_{wb}T$ concentrations immediately after the focal individual had completed a suite of behavioural tests to assess personality traits ('experimental'; for details see 'Behavioural experiments'). In every trial we noted the date and time of day (am or pm) when the measurement was taken, as well as individual parameters such as body size (in mm) and age, measured as a binomial variable (first reproducer vs. recapture from previous years). We aimed for obtaining three replicates in each condition per individual, summing up to a total of six measurements per frog. Half of the tested individuals started with ‘baseline’, while the other half started with the ‘experimental’ sampling. Consecutive samples were always taken more than 24 hours apart. After every second trial we added a break of at least 3 days to minimize any confounding effects of the procedure on the measurements.

Behavioural tests

All individuals underwent a set of behavioural tests to quantify the following behavioural traits: territorial aggression, boldness and explorative tendency. The procedure of these combined tests lasted for a total of about 30 min and to facilitate reading, we will from now on define both tests with ‘behavioural test’ unless we a specifically refer to one of these tests only.

Territorial aggression: We assessed within- and between-individual variation in the levels of territorial aggression in individual males by simulating a calling intruder inside a male’s territory. To do so, we used a simulated territorial intrusion test to induced territorial defence behaviour of the territorial male by broadcasting /presenting synthetic conspecific call by a loudspeaker (for details see Peignier et al., 2022). These conspecific male calls elicit instantly an aggressive response of a territorial male (Rodríguez et al., 2022; Sonnleitner et al., 2020; Ursprung et al., 2009) which can be categorised/quantified in following behavioural parameters: a) latency until the first head-body orientation and b) until the first jump, the likelihood to jump in moments when the speaker was silent (i.e., between bouts of calls), and c) the speed to approach the speaker (cf. Chaloupka et al., 2022; Peignier et al., 2022).

Boldness and Exploration: Immediately after the previous test, we caught the frog and assessed exploration- and boldness-related behaviours using a Novel Environment Setup (cf. Peignier et al., 2022). For this, we first put the frog in a dark shelter for five minutes, to allow the individual to recover after handling. Afterwards we opened the shelter and allowed the focal frog to explore the Novel Environment for 15 minutes. We measured the a) latency and probability to leave the shelter as well as the distance travelled, b) the number of jumps performed, and c) the area covered in the novel environment (for more details see Peignier et al., 2022).

To assess within- and between-individual variation in behaviour we repeated those tests several times: We conducted 163 territorial defence tests with 51 males (mean ± SD = 3.20 ± 1.31 repetitions per individual) and 156 Novel Environment Tests with 50 males (mean ± SD = 3.31 ± 1.50 repetitions per individual). In a previous study, using the same behavioural dataset, it was shown that the behaviours measured during both tests are repeatable and that the latency until the first jump, the distance travelled, and the time spent in the shelter best represented aggression, exploration, and boldness, respectively (Peignier et al. 2022). In the present study, we use the average value per individual for these three behaviours as personality scores to investigate the link between personality and $_{\text{wb}}$T levels.

Hormonal sampling and analysis

We used a non-invasive water-bath method (Baugh et al., 2018; Baugh and Gray-Gaillard, 2021; Gabor et al., 2013) following the protocol described in (Rodríguez et al., 2022) to collect repeated $_{\text{wb}}$T measurements of male *A. femoralis*. In brief, after capture, we put the frogs in a small glass box (14cmx9cmx5cm), filled with 40 mL of distilled water and left them in this box for one hour. An opaque cover was placed over the box to minimize any disturbances from outside and to minimize stress of the focal individual. After one hour the frog was gently released at the original capture location. Non-polar hormones were extracted by processing each water sample through 20 mL sterile syringes coupled to an individual C18 cartridge (SPE, Sep-Pak C18 Plus, 360 mg Sorbent, 55–105 µm particle size, #WAT020515, Waters corp., Milford, MA) with a flow rate of ca. 10 mL/min. Afterwards, cartridges were eluted with 4 mL of 96% EtOH into 8 mL borosilicate vials and stored at 4 °C until further processing in the endocrinological lab at the University of Vienna.

In order to quantify the $_{\text{wb}}$T concentrations of the samples, we used a commercially available ELISA kit (Enzo Life science #ADI-900 065). Beforehand, 1ml out of the 4ml of 96% EtOH eluded samples were pipetted into a glass tube and dried down under a N2-stream and then re-suspended in 250ul Assay buffer provided by the manufacturer. Previous tests have shown that 1 ml of 96% EtOH, was sufficient to quantify reliably testosterone concentration. Because the antibody has a very low cross-reaction with other androgens (19-hydroxytestosterone <15%, androstendione <7.2%, Estradiol < 0.4, all others < 0,001%) we dare to assume to have mostly measured testosterone. Final concentration of the samples was corrected for dilution factor. The detection limit for the assay was 5.67 pg/ml. The CV% of all duplicates was below 5.3%.

Statistical analysis

The statistical analyses were conducted in RStudio (RStudio Team, 2019). We log transformed the ${}_{wb}$T concentration as it strongly deviated from normality. To investigate factors that affect the overall androgen concentration, we fitted a linear mixed model using the function ‘lmer’, in the package ‘lme4’, with condition (‘baseline’/‘experimental’), time of day (am/pm), age (new encounters/survivors from a previous reproductive season), whether the frog was calling or not before the hormonal measurement, and trial order as fixed effects. We included ID as random effect, and ${}_{wb}$T concentration (log transformed) as response variable. We assured that model assumptions of residual normality were met by visually inspecting qqplots. The condition was the only factor influencing overall ${}_{wb}$T concentration. We further investigated the consistency of ${}_{wb}$T levels within and between individuals, using both reduced (‘baseline’ only or ‘experimental’ only) datasets, with the ‘rpt’ function in the rptR package (Stoffel et al., 2017). To identify if ${}_{wb}$T concentration at ‘baseline’ itself had an influence on androgen modulation during/after the behavioural tests, we calculated $\Delta\,{}_{wb}$T by subtracting individual average ‘baseline’ levels from the respective average ‘experimental’ levels from all individual males. We then tested for a possible correlation between ‘baseline’ ${}_{wb}$T concentration and $\Delta\,{}_{wb}$T using a Pearson correlation test.

We also looked for an influence of aggression, exploration and boldness related behaviours on ${}_{wb}$T concentration. We built two linear mixed effect models with the log transformed ${}_{wb}$T concentration as a response variable, the three personality scores as fixed effects and ID as random factor. One model ran on the baseline concentration (‘baseline’) and the other model ran on the ${}_{wb}$T concentration measured after the behavioural experiments (‘experimental’). We assured that model assumptions of residual normality were met by visually inspecting qqplots.

We observed that not all individuals responded to the behavioural manipulations in the same way, with individuals increasing or decreasing hormone levels after the behavioural test (Figure 1). Therefore, we further investigated if the variation in ${}_{wb}$T level between conditions was related to personality. We calculated the average ${}_{wb}$T level in the ‘baseline’ and the ‘experimental’ condition for all individuals and built a generalized linear model following a binomial distribution with whether an individual increased or decreased its average level of testosterone between conditions as response variable, and added the three personality traits as fixed effects.

Results

In total we collected 252 hormonal samples from 40 individual males. We obtained samples for baseline ${}_{\text{wb}}\text{T}$ measurements from 37 males (‘baseline’: 1–6 samples per male, mean ± SD = 3.51 ± 1.19 samples per male), and samples after the behavioural manipulations from 39 males (‘experimental’: 1–5 samples per male; mean ± SD = 3.13 ± 1.08 samples per male).

Hormonal concentrations were not influenced by the time of day when samples were collected, the age of the individual, the activity (i.e., calling or not) of the male, or the trial order (all $p > 0.05$; Table 1). However, ${}_{\text{wb}}\text{T}$ concentrations were significantly lower ($\beta = -0.3$, $p < 0.001$, Table 1) when measured after behavioural experiments (mean average ${}_{\text{wb}}\text{T}$ ± SD = 306.15 ± 107.5 pg/mL) compared to the respective baseline samples (mean average ${}_{\text{wb}}\text{T}$ ± SD = 387.43 ± 171.1 pg/mL). Repeatability was quite high for the baseline samples (‘baseline’: R = 0.45; 95%CI = [0.24;0.61]), but much lower when hormones were collected after behavioural manipulations (‘experimental’: R = 0.24; 95%CI = [0.03;0.43]).

Not all individuals responded to the behavioural manipulations with a reduction in ${}_{\text{wb}}\text{T}$. Interestingly, we found a significant negative correlation between average baseline ${}_{\text{wb}}\text{T}$ concentration and $\Delta\,{}_{\text{wb}}\text{T}$ in males (Pearson correlation test: $r = -0.764$, $t = -6.81$, $df = 33$, $p < 0.001$, Figure 1a). This means, that individuals with a relatively low baseline ${}_{\text{wb}}\text{T}$ tended to increase hormone levels after the behavioural tests, while individuals with a comparatively high baseline level showed a decrease in their androgen levels after the behavioural manipulation (Figure 1b).

We found very weak evidence (*sensu* Muff et al., 2021) for an effect of exploration on baseline ${}_{\text{wb}}\text{T}$ levels, and moderate evidence that the ${}_{\text{wb}}\text{T}$ concentration measured after behavioural experiments was positively correlated with individual exploration levels (Table 1). The difference in average ${}_{\text{wb}}\text{T}$ level in the baseline condition and after behavioural experiments was not linked to personality (Table 1).

Table 1. Results of the (generalized) linear mixed effect models looking at how personality and abiotic factors influence ${}_{\mathrm{wb}}$T concentration. Sample size (N) are presented for each model. Results indicating at least weak evidence (*sensu* Muff et al., 2021) are written in bold. The personality scores in the three models have been scaled (i.e., centred to their mean value and standardized to units of 1 phenotypic standard deviation).

	Estimate β	**Standard-Error**	**p-value**
Factors influencing overall ${}_{\mathrm{wb}}$T concentration (N = 244 for 39 individuals)			
Intercept	5.80	0.12	< 0.001
Time of the day (am/pm)	0.06	0.10	0.535
Trial order	-0.00	0.01	0.999
Condition	-0.30	0.08	**< 0.001**
Age (0/1)	-0.11	0.12	0.377
Calling (yes/no)	0.10	0.11	0.358
Random effects	*Variance*	*Standard-Deviation*	
ID	0.11	0.33	
Residual	0.20	0.45	
Link between personality and baseline ${}_{\mathrm{wb}}$T concentration (N = 128 for 35 individuals)			
Intercept	5.79	0.08	< 0.001
Aggressiveness score	-0.03	0.09	0.715
Exploration score	0.18	0.11	**0.097**
Boldness score	-0.08	0.11	0.432
Random effects	*Variance*	*Standard-Deviation*	
ID	0.18	0.43	
Residual	0.22	0.47	
Link between personality and ${}_{\mathrm{wb}}$T concentration measured after behavioural testing (N = 121 for 38 individuals)			
Intercept	5.60	0.06	< 0.001
Aggressiveness score	-0.08	0.06	0.195
Exploration score	0.16	0.07	**0.023**
Boldness score	-0.06	0.07	0.424
Random effects	*Variance*	*Standard-Deviation*	
ID	0.05	0.22	
Residual	0.20	0.45	
Link between personality and androgen modulation after behavioural testing (N = 35)			
Intercept	-1.38	0.53	< 0.001
Aggressiveness score	-0.94	0.70	0.178
Exploration score	-0.81	0.70	0.251
Boldness score	0.50	0.58	0.392

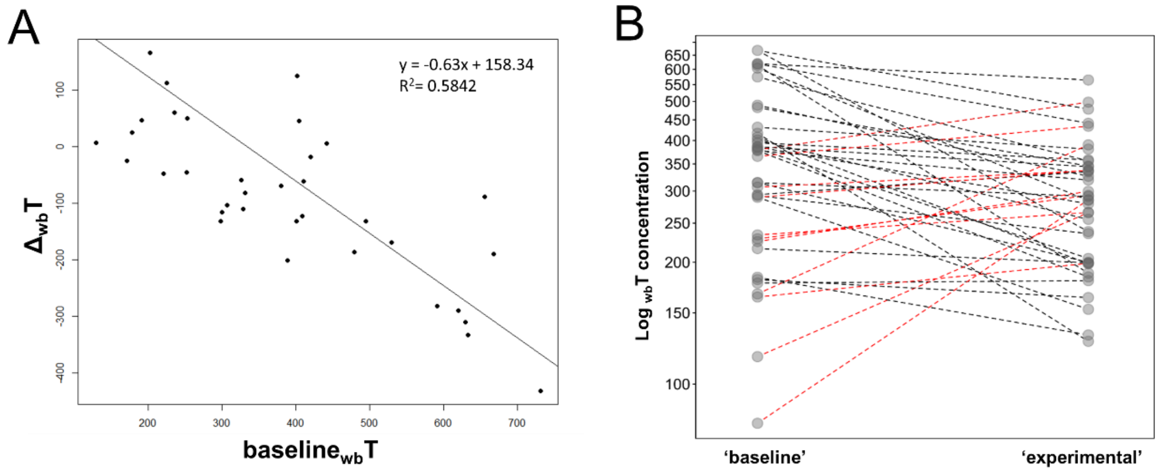


Figure 1. Comparison of average individual $_{wb}T$ concentration from baseline samples and samples taken immediately after behavioural experiments. A) Correlation between average baseline $_{wb}T$ and the relative change $\Delta_{wb}T$ after behavioural testing; B) Pairwise comparisons of individual males. Red lines indicate individuals for which average $_{wb}T$ measured in the 'experimental' condition is increased compared to 'baseline'. Black lines indicate individuals for which average $_{wb}T$ level is decreased in the 'experimental' condition compared to 'baseline'.

Discussion

Variation in androgen levels

Our results show that male *A. femoralis* have relatively consistent $_{wb}T$ levels throughout the breeding season. The repeatability of hormonal concentrations that were obtained from non-invasive water bath samples was quite high, especially for the 'baseline' samples (R = 0.45). This indicates that T levels are relatively constant over the course of several weeks within the reproductive season and there are consistent differences between individuals in their average androgen level. As a consequence, even a low number of repeated measurements allowed reliable estimations of individual baseline hormonal profiles in male poison frogs.

We did not find a significant difference between hormonal samples collected in the morning and in the afternoon. This was contrary to what we expected, as in a previous study androgen levels were found to clearly increase over the course of the day in a nearby *A. femoralis* population (Rodríguez et al., 2022). We cannot rule out that situations which happened immediately before collecting the sample, for example a recently ended courtship, a fight or other stressful situations (cf Rodríguez et al., 2022), might have influenced the hormonal state of the male. However, we don't expect such instances to have considerably affected our dataset, since 'baseline' samples were always collected when males were perching (and advertising) in their respective territory, with no other conspecific nearby. In vertebrates, steroid concentrations commonly undergo a circadian rhythm; they increase during early morning hours and drop in the afternoon (Nelson, 2005). Several studies have documented the existence of diurnal cycles of circulating testosterone (fish: Lorenzi et al., 2008, monkeys: Schlatt et al., 2008, humans: Diver et al., 2003, but see also Licht et al., 1985 for green sea turtles). In several animal taxa, T concentrations are positively related to latitude and negatively to the length of the breeding season (Eikenaar et al., 2012; Husak et al., 2021), which suggests that tropical animals usually exhibit lower T levels with very low seasonal fluctuation during the reproductive season compared to temperate-zone species (see also Canoine et al., 2007; Hau et al., 2008). This could be a possible reason why we did not find any significant difference in $_{wb}T$ concentration between morning and afternoon in *A. femoralis* males.

The factor "age" did not show a significant relationship with androgen concentration. There is evidence for an age-related change of T levels in various animal taxa (Schlatt et al., 2008; Těšický et al., 2022), however those taxa typically show a greater lifespan than our studied species. In *A. femoralis* the majority of the population only survives one reproductive season (cf. Ringler et al., 2015), and therefore age likely is not a relevant factor for the variation in androgen levels in this short-lived species.

Influence of behavioural tests on testosterone levels

An increase in T has been observed in many species following social challenges (Goymann et al., 2019; Wingfield et al., 2020 but see also Assis et al., 2012; Moore et al., 2020). In our study, ${}_{wb}$T concentration generally dropped after the behavioural tests. This is in contrast with a recent study which found a positive androgenic response to simulated territorial intrusions in *A. femoralis* males, providing support for the Challenge Hypothesis (Rodríguez et al., 2022). In this previous study, water-borne androgen concentration was elevated after presenting a conspecific playback compared to baseline conditions, but only in males that actually approached the loudspeaker, but not in males that did not react to the playback. As in the present study we collected the hormonal samples not immediately after the territorial aggression test, but after a suite of behavioural tests, the resulting androgen concentrations actually represent a combined/integrated hormonal response to the entire test sequence.

Most interestingly, not all individuals responded to the behavioural tests in the same way. We observed two different patterns when comparing 'baseline' and 'experimental' ${}_{wb}$T levels: individuals with low baseline ${}_{wb}$T tended to show an increase in hormonal levels, while individuals with comparatively high baseline ${}_{wb}$T rather showed a decrease in hormonal concentration following the behavioural manipulation (Figure 1). This phenomenon could potentially be caused by differential personality types being linked to differential physiological (i.e. hormonal) responses to stress and/or social challenges. This finding is in line with other studies showing that animal personality is linked to differential physiological response to social challenges (Baugh et al., 2017; Baugh et al., 2012; Cockrem, 2013). Alternatively, it could indicate that the physiological maximum of certain individuals was already reached before the start of the behavioural tests, so that no further increase in androgen levels was possible (Goymann et al., 2007; Wingfield et al., 1990).

Link between animal personality and androgen concentration

Contrary to our prediction, we found that neither 'baseline' nor 'experimental' androgen levels were associated with territorial aggression. This is in line with a previous study showing that the intensity of the phonotactic approach towards a playback was not related to androgen responsiveness in *A. femoralis* males (Rodríguez et al., 2022). However, ${}_{\mathrm{wb}}$T concentration measured after behavioural experiments were positively linked to the personality trait 'exploration'. With other words, individuals with a higher exploratory tendency in the novel environment test had higher ${}_{\mathrm{wb}}$T levels after the behavioural test. These findings are in line with a recent study that found androgens to be associated with navigation-associated behaviours in three species of poison frogs (Pašukonis et al., 2022). Higher baseline androgen levels were found in individuals that also showed more exploration after translocation in *Dendrobates tinctorius*. The amount of exploration during the navigation task was associated with an increase in androgen levels in *A. femoralis*, while successful homing was found to result in a significant decrease in androgen compared to baseline levels (Pašukonis et al., 2022). These results together with the findings of our present study highlight a prominent role of androgens for among-individual variation in exploration-related spatial behaviours in males. However, our experimental design does not allow to disentangle cause and consequence of this relationship. Future studies using hormonal manipulation experiments in combination with behavioural experiments are needed to precisely disentangle the causal relationship between testosterone modulation and exploration behaviour.

Methodological implications

One of the key questions in animal personality research is why there are consistent individual differences in behaviour. For example, heritable traits leading to genetically determined physiological differences among individuals could give rise to consistent behavioural differences (Baugh et al., 2012; Drent et al., 2003; Stamps and Groothuis, 2010). When trying to link hormonal with behavioural profiles, it is crucial at what timepoint the hormonal sample is collected. To minimize handling time and experimental effort, it would be ideal to measure hormonal levels directly before and/or after behavioural tests, however this procedure could affect the hormonal and behavioural experiments, respectively. Whether hormonal measurements should be taken independently from behavioural tests or immediately thereafter, depends on the research question. To gain information about long-term differences in hormonal profiles, measurements should be taken without any preceding behavioural experiments. However, when determining differences in physiological responses after behavioural challenges, it is important to carry out hormonal sampling completely independently from other experimental manipulations as well as directly after behavioural testing.

Conclusions

Our findings show that ${}_{\mathrm{wb}}\mathrm{T}$ levels in *A. femoralis* are quite stable across the reproductive season. Time of day, age and calling activity had no influence on hormone levels. Preceding behavioural experiments had a strong negative effect on resulting ${}_{\mathrm{wb}}\mathrm{T}$ concentrations, but the direction of the hormonal response appears to be linked to animal personality. Individuals with low baseline levels showed an increase in ${}_{\mathrm{wb}}\mathrm{T}$ levels, while individuals with high baseline levels showed a reduction of ${}_{\mathrm{wb}}\mathrm{T}$ concentrations after the behavioural tests. Taken together, these results show that differences in hormonal profiles and/or responses to social challenges can explain among-individual differences in behavioural traits.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Authors contributions

VC and ER designed the study. KD collected the data. MP performed the statistical analysis and did the data curation. KD wrote the first draft of the manuscript. MP, VC and ER reviewed, edited and wrote sections of the manuscript. VC and ER supervised the study. All authors contributed to manuscript revision, and have read, and approved the submitted version.

Acknowledgments

We thank the Nouragues research field station (managed by CNRS), which benefits from ‘Investissement d'Avenir’ grants managed by the Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. Many thanks to Camilo Rodriguez Lopez for providing help and advice regarding the hormonal sampling in the field. We also thank Sarah Chaloupka and Christoph Leeb for their help in acquiring the data. This study was funded by the Austrian Science Fund (FWF) via the project P 31518 (PI: Eva Ringler).

References

Adkins-Regan, E., 2005. Hormones and animal social behavior. Princeton University Press.

Araya-Ajoy, Y.G., Dingemanse, N.J., 2014. Characterizing behavioural ‘characters’: an evolutionary framework. Proc R Soc Lond B 281, 20132645. https://doi.org/10.1098/rspb.2013.2645.

Assis, V.R. de, Navas, C.A., Mendonça, M.T., Gomes, F.R., 2012. Vocal and territorial behavior in the Smith frog (Hypsiboas faber): relationships with plasma levels of corticosterone and testosterone. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 163, 265–271. https://doi.org/10.1016/j.cbpa.2012.08.002.

Baugh, A.T., Bastien, B., Still, M.B., Stowell, N., 2018. Validation of water-borne steroid hormones in a tropical frog (Physalaemus pustulosus). Gen Comp Endocrin 261, 67–80.

Baugh, A.T., Gray-Gaillard, S.L., 2021. Excreted testosterone and male sexual proceptivity: A hormone validation and proof-of-concept experiment in túngara frogs. General and comparative endocrinology 300, 113638. https://doi.org/10.1016/j.ygcen.2020.113638.

Baugh, A.T., Schaper, S.V., Hau, M., Cockrem, J.F., Goede, P. de, van Oers, K., 2012. Corticosterone responses differ between lines of great tits (Parus major) selected for divergent personalities. General and comparative endocrinology 175, 488–494. https://doi.org/10.1016/j.ygcen.2011.12.012.

Baugh, A.T., Senft, R.A., Firke, M., Lauder, A., Schroeder, J., Meddle, S.L., van Oers, K., Hau, M., 2017. Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: A multilevel experiment in Parus major. Horm Behav 93, 99–108. https://doi.org/10.1016/j.yhbeh.2017.05.011.

Beck, K.B., Loretto, M.-C., Ringler, M., Hödl, W., Pašukonis, A., 2017. Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. PeerJ 5, e3745. https://doi.org/10.7717/peerj.3745.

Biro, P.A., Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology & Evolution 25, 653–659. https://doi.org/10.1016/j.tree.2010.08.003.

Bolger, D.T., Morrison, T.A., Vance, B., Lee, D., Farid, H., 2012. A computer-assisted system for photographic mark–recapture analysis. Methods Ecol Evol 3, 813–822. https://doi.org/10.1111/j.2041-210X.2012.00212.x.

Bongers, F., Charles-Dominique, P., Forget, P.-M., Théry, M. (Eds.), 2001. Nouragues: Dynamics and plant-animal interactions in a Neotropical rainforest. Kluwer Academic Publishers, Dordrecht, Netherlands, 421 pp.

Buchanan, K.L., Evans, M.R., Goldsmith, A.R., Bryant, D.M., Rowe, L.V., 2001. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? Proc R Soc Lond B 268, 1337–1344. https://doi.org/10.1098/rspb.2001.1669.

Canoine, V., Fusani, L., Schlinger, B., Hau, M., 2007. Low sex steroids, high steroid receptors: Increasing the sensitivity of the nonreproductive brain. Developmental Neurobiology 67, 57–67. https://doi.org/10.1002/dneu.20296.

Chaloupka, S., Peignier, M., Stückler, S., Araya-Ajoy, Y., Walsh, P., Ringler, M., Ringler, E., 2022. Repeatable territorial aggression in a Neotropical poison frog. Front Ecol Evol 10, 881387. https://doi.org/10.3389/fevo.2022.881387.

Cockrem, J.F., 2013. Individual variation in glucocorticoid stress responses in animals. General and comparative endocrinology 181, 45–58. https://doi.org/10.1016/j.ygcen.2012.11.025.

Dingemanse, N.J., Both, C., Drent, P.J., Tinbergen, J.M., 2004. Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc Lond B 271, 847.

Diver, M.J., Imtiaz, K.E., Ahmad, A.M., Vora, J.P., Fraser, W.D., 2003. Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. Clinical endocrinology 58, 710–717. https://doi.org/10.1046/j.1365-2265.2003.01772.x.

Drent, P.J., van Oers, K., van Noordwijk, A.J., 2003. Realized heritability of personalities in the great tit (Parus major). Proc R Soc Lond B 270, 45–51. https://doi.org/10.1098/rspb.2002.2168.

Dufty, A.M., 1989. Testosterone and survival: A cost of aggressiveness? Horm Behav 23, 185–193. https://doi.org/10.1016/0018-506x(89)90059-7.

Eikenaar, C., Husak, J., Escallón, C., Moore, I.T., 2012. Variation in testosterone and corticosterone in amphibians and reptiles: relationships with latitude, elevation, and breeding season length. Am Nat 180, 642–654. https://doi.org/10.1086/667891.

Fusani, L., 2008. Testosterone control of male courtship in birds. Horm Behav 54, 227–233. https://doi.org/10.1016/j.yhbeh.2008.04.004.

Gabor, C.R., Bosch, J., Fries, J.N., Davis, D.R., 2013. A non-invasive water-borne hormone assay for amphibians. Amphib Reptil 34, 151–162. https://doi.org/10.1163/15685381-00002877.

Gottsberger, B., Gruber, E., 2004. Temporal partitioning of reproductive activity in a neotropical anuran community. J Trop Ecology 20, 271–280. https://doi.org/10.1017/S0266467403001172.

Goymann, W., Landys, M.M., Wingfield, J.C., 2007. Distinguishing seasonal androgen responses from male–male androgen responsiveness—Revisiting the Challenge Hypothesis. Horm Behav 51, 463–476. https://doi.org/10.1016/j.yhbeh.2007.01.007.

Goymann, W., Moore, I.T., Oliveira, R.F., 2019. Challenge Hypothesis 2.0: A Fresh Look at an Established Idea. BioScience 69, 432–442. https://doi.org/10.1093/biosci/biz041.

Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29, 133–144. https://doi.org/10.1002/bies.20524.

Hau, M., Gill, S.A., Goymann, W., 2008. Tropical field endocrinology: ecology and evolution of testosterone concentrations in male birds. General and comparative endocrinology 157, 241–248. https://doi.org/10.1016/j.ygcen.2008.05.008.

Hegner, R.E., Wingfield, J.C., 1987. Effects of Experimental Manipulation of Testosterone Levels on Parental Investment and Breeding Success in Male House Sparrows. The Auk 104, 462–469. https://doi.org/10.2307/4087545.

Herman, R.A., Wallen, K., 2007. Cognitive performance in rhesus monkeys varies by sex and prenatal androgen exposure. Horm Behav 51, 496–507. https://doi.org/10.1016/j.yhbeh.2007.01.005.

Hirschenhauser, K., Winkler, H., Oliveira, R.F., 2003. Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation. Horm Behav 43, 508–519. https://doi.org/10.1016/s0018-506x(03)00027-8.

Hodgson, Z.G., Meddle, S.L., Christians, J.K., Sperry, T.S., Healy, S.D., 2008. Influence of sex steroid hormones on spatial memory in a songbird. J Comp Physiol A 194, 963–969. https://doi.org/10.1007/s00359-008-0369-4.

Hödl, W., 1983a. Phyllobates femoralis (Dendrobatidae): Rufverhalten und akustische Orientierung der Männchen (Freilandaufnahmen). Begleitveröffentlichungen zu wissenschaftlichen Filmen 30:12-19. Bundesstaatliche Hauptstelle für Wissenschaftliche Kinematographie, Vienna, Austria.

Hödl, W., 1983b. Rufverhalten und Phonotaxis bei Männchen von Phyllobates femoralis (Dendrobatidae). Verh Dtsch Zool Ges 172.

Hunt, K.E., Hahn, T.P., Buck, C.L., Wingfield, J.C., 2019. Effect of testosterone blockers on male aggression, song and parental care in an arctic passerine, the Lapland longspur (Calcarius lapponicus). Horm Behav 110, 10–18. https://doi.org/10.1016/j.yhbeh.2019.02.001.

Husak, J.F., Fuxjager, M.J., Johnson, M.A., Vitousek, M.N., Donald, J.W., Francis, C.D., Goymann, W., Hau, M., Kircher, B.K., Knapp, R., Martin, L.B., Miller, E.T., Schoenle, L.A., Williams, T.D., 2021. Life history and environment predict variation in testosterone across vertebrates. Evolution; international journal of organic evolution 75, 1003–1010. https://doi.org/10.1111/evo.14216.

Licht, P., Wood, J.F., Wood, F.E., 1985. Annual and diurnal cycles in plasma testosterone and thyroxine in the male green sea turtle Chelonia mydas. Gen Comp Endocrin 57, 335–344. https://doi.org/10.1016/0016-6480(85)90212-6.

Lorenzi, V., Earley, R.L., Rodgers, E.W., Pepper, D.R., Grober, M.S., 2008. Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone, and 17beta-estradiol in the bluebanded goby (Lythrypnus dalli). Gen Comp Endocrin 155, 438–446. https://doi.org/10.1016/j.ygcen.2007.07.010.

Marler, C.A., Moore, M.C., 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. Behav Ecol Sociobiol 23, 21–26. https://doi.org/10.1007/BF00303053.

McGlothlin, J.W., Ketterson, E.D., 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Phil Trans R Soc Lond B 363, 1611–1620. https://doi.org/10.1098/rstb.2007.0002.

Montanarin, A., Kaefer, I.L., Pimentel Lima, A., 2011. Courtship and mating behaviour of the brilliant-thighed frog Allobates femoralis from Central Amazonia: implications for the study of a species complex. Ethol Ecol Evol 23, 141–150. https://doi.org/10.1080/03949370.2011.554884.

Moore, I.T., Hernandez, J., Goymann, W., 2020. Who rises to the challenge? Testing the Challenge Hypothesis in fish, amphibians, reptiles, and mammals. Horm Behav 123, 104537. https://doi.org/10.1016/j.yhbeh.2019.06.001.

Narins, P.M., Grabul, D.S., Soma, K.K., Gaucher, P., Hödl, W., 2005. Cross-modal integration in a dart-poison frog. Proc Natl Acad Sci USA 102, 2425–2429. https://doi.org/10.1073/pnas.0406407102.

Narins, P.M., Hödl, W., Grabul, D.S., 2003. Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. Proc Natl Acad Sci USA 100, 577–580. https://doi.org/10.1073/pnas.0237165100.

Nelson, R.J., 2005. An introduction to behavioral endocrinology.

Pašukonis, A., Serrano-Rojas, S.J., Fischer, M.-T., Loretto, M.-C., Shaykevich, D.A., Rojas, B., Ringler, M., Roland, A.-B., Marcillo-Lara, A., Ringler, E., Rodríguez, C., Coloma, L.A., O’Connell, L.A., 2022. Contrasting parental roles shape sex differences in poison frog space use but not navigational performance. bioRxiv, 2022.05.21.492915. https://doi.org/10.1101/2022.05.21.492915.

Peignier, M., Araya-Ajoy, Y.G., Bégué, L., Chaloupka, S., Dellefont, K., Leeb, C., Walsh, P., Ringler, M., Ringler, E., 2022. Exploring links between personality traits and their social and non-social environments in wild poison frogs. Behav Ecol Sociobiol 76. https://doi.org/10.1007/s00265-022-03202-9.

Rasband, W.S., 1997-2021. ImageJ. U. S. National Institutes of Health, Bethesda, MD, USA, Bethesda, MD, USA.

Raynaud, J., Schradin, C., 2014. Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers. Physiol Behav 129, 57–63. https://doi.org/10.1016/j.physbeh.2014.02.005.

Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., Montiglio, P.-O., 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 365, 4051–4063. https://doi.org/10.1098/rstb.2010.0208.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82, 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x.

Ringler, E., Mangione, R., Ringler, M., 2015. Where have all the tadpoles gone? Individual genetic tracking of amphibian larvae until adulthood. Mol Ecol Resour 15, 737–746. https://doi.org/10.1111/1755-0998.12345.

Ringler, E., Pašukonis, A., Hödl, W., Ringler, M., 2013. Tadpole transport logistics in a Neotropical poison frog: indications for strategic planning and adaptive plasticity in anuran parental care. Front Zool 10, 67. https://doi.org/10.1186/1742-9994-10-67.

Ringler, E., Ringler, M., Jehle, R., Hödl, W., 2012. The female perspective of mating in A. femoralis, a territorial frog with paternal care – a spatial and genetic analysis. PLoS one 7, e40237. https://doi.org/10.1371/journal.pone.0040237.

Ringler, M., Mangione, R., Pašukonis, A., Rainer, G., Gyimesi, K., Felling-Wagner, J., Kronaus, H., Réjou-Méchain, M., Chave, J., Reiter, K., Ringler, E., 2016. High-resolution forest mapping for behavioural studies in the Nature Reserve ‘Les Nouragues’, French Guiana. J Maps 12, 26–32. https://doi.org/10.1080/17445647.2014.972995.

Ringler, M., Ringler, E., Magaña Mendoza, D., Hödl, W., 2011. Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. PLoS one 6, e25844. https://doi.org/10.1371/journal.pone.0025844.

Ringler, M., Ursprung, E., Hödl, W., 2009. Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog Allobates femoralis (Aromobatidae). Behav Ecol Sociobiol 63, 1281–1293. https://doi.org/10.1007/s00265-009-0793-7.

Rodríguez, C., Fusani, L., Raboisson, G., Hödl, W., Ringler, E., Canoine, V., 2022. Androgen responsiveness to simulated territorial intrusions in Allobates femoralis males: evidence supporting the challenge hypothesis in a territorial frog. General and comparative endocrinology 326, 114046, 114046. https://doi.org/10.1016/j.ygcen.2022.114046.

Rodríguez López, C., Hödl, W., 2020. Sound radiation pattern of the advertisement call of the highly territorial poison frog Allobates femoralis. Behav Process 170, 103996. https://doi.org/10.1016/j.beproc.2019.103996.

Roithmair, M.E., 1992. Territoriality and male mating success in the dart-poison frog, Epipedobates femoralis (Dendrobatidae, Anura). Ethology 92, 331–343. https://doi.org/10.1111/j.1439-0310.1992.tb00970.x.

Rosvall, K.A., Bentz, A.B., George, E.M., 2020. How research on female vertebrates contributes to an expanded challenge hypothesis. Horm Behav 123, 104565. https://doi.org/10.1016/j.yhbeh.2019.104565.

Rosvall, K.A., Bergeon Burns, C.M., Barske, J., Goodson, J.L., Schlinger, B.A., Sengelaub, D.R., Ketterson, E.D., 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. Proceedings. Biological sciences 279, 3547–3555. https://doi.org/10.1098/rspb.2012.0442.

RStudio Team, 2019. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA, Boston, MA, USA.

Schlatt, S., Pohl, C.R., Ehmcke, J., Ramaswamy, S., 2008. Age-related changes in diurnal rhythms and levels of gonadotropins, testosterone, and inhibin B in male rhesus monkeys (Macaca mulatta). Biology of reproduction 79, 93–99. https://doi.org/10.1095/biolreprod.107.066126.

Sih, A., Bell, A.M., 2008. Insights for behavioral ecology from behavioral syndromes. Adv Stud Behav 38, 227–281. https://doi.org/10.1016/S0065-3454(08)00005-3.

Sih, A., Mathot, K.J., Moirón, M., Montiglio, P.-O., Wolf, M., Dingemanse, N.J., 2015. Animal personality and state–behaviour feedbacks: A review and guide for empiricists. Trends Ecol Evol 30, 50–60.

Sonnleitner, R., Ringler, M., Loretto, M.-C., Ringler, E., 2020. Experience shapes accuracy in territorial decision-making in a poison frog. Biol Lett 16, 20200094. https://doi.org/10.1098/rsbl.2020.0094.

Stamps, J., Groothuis, T.G.G., 2010. The development of animal personality: relevance, concepts and perspectives. Biol Rev 85, 301–325. https://doi.org/10.1111/j.1469-185X.2009.00103.x.

Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8, 1639–1644. https://doi.org/10.1111/2041-210X.12797.

Stückler, S., Ringler, M., Pašukonis, A., Weinlein, S., Hödl, W., Ringler, E., 2019. Spatio-temporal characteristics of the prolonged courtship in Brilliant-Thighed Poison Frogs, Allobates femoralis. Herpetologica 75, 268–279. https://doi.org/10.1655/Herpetologica-D-19-00010.1.

Těšický, M., Krajzingrová, T., Eliáš, J., Velová, H., Svobodová, J., Bauerová, P., Albrecht, T., Vinkler, M., 2022. Inter-annual repeatability and age-dependent changes in plasma testosterone levels in a longitudinally monitored free-living passerine bird. Oecologia 198, 53–66. https://doi.org/10.1007/s00442-021-05077-5.

Tobler, M., Nilsson, J.-K., Nilsson, J.F., 2007. Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. Biol Lett 3, 408–410. https://doi.org/10.1098/rsbl.2007.0127.

Ursprung, E., Ringler, M., Hödl, W., 2009. Phonotactic approach pattern in the neotropical frog Allobates femoralis: a spatial and temporal analysis. Behaviour 146, 153–170. https://doi.org/10.1163/156853909X410711.

Ursprung, E., Ringler, M., Jehle, R., Hödl, W., 2011. Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Mol Ecol 20, 1759–1771. https://doi.org/10.1111/j.1365-294X.2011.05056.x.

Vitousek, M.N., Zonana, D.M., Safran, R.J., 2014. An integrative view of the signaling phenotype: Dynamic links between signals, physiology, behavior and social context. Current zoology 60, 739–754. https://doi.org/10.1093/czoolo/60.6.739.

Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136, 829–846. https://doi.org/10.1086/285134.

Wingfield, J.C., Lynn, S., Soma, K.K., 2001. Avoiding the ‘Costs’ of Testosterone: Ecological Bases of Hormone-Behavior Interactions. Brain Behav Evol 57, 239–251. https://doi.org/10.1159/000047243.

Wingfield, J.C., Ramenofsky, M., Hegner, R.E., Ball, G.F., 2020. Whither the challenge hypothesis? Horm Behav 123, 104588. https://doi.org/10.1016/j.yhbeh.2019.104588.

Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: Peignier Mélissa

Registration Number: 20-113-544

Study program: PhD Ecology and Evolution

Bachelor ☐ Master ☐ Dissertation ☑

Title of the thesis: The link between personality and sexual selection in a neotropical poison frog

Supervisor: Prof. Dr. Eva Ringler

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of September 5th, 1996 and Article 69 of the University Statute of June 7th, 2011 is authorized to revoke the doctoral degree awarded on the basis of this thesis.

For the purposes of evaluation and verification of compliance with the declaration of originality and the regulations governing plagiarism, I hereby grant the University of Bern the right to process my personal data and to perform the acts of use this requires, in particular, to reproduce the written thesis and to store it permanently in a database, and to use said database, or to make said database available, to enable comparison with theses submitted by others.

Bern / 14.10.2022

Place/Date

Signature