

MECHANISMS THAT SHAPE THE EVOLUTION OF COOPERATIVE BREEDING

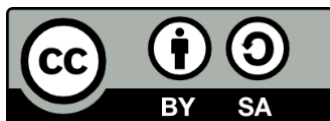
Inaugural dissertation
of the Faculty of Science,
University of Bern

Presented by
Irene García Ruiz
from Spain

Supervisor of the doctoral thesis:
Prof. Dr. Michael Taborsky

Institute of Ecology and Evolution | Department of Behavioral Ecology

Original document saved on the web server of the University Library of Bern.



This work is licensed under the Creative Commons Attribution—ShareAlike 4.0 International License. To view a copy of this license, visit <https://creativecommons.org/licenses/by-sa/4.0/>



MECHANISMS THAT SHAPE THE EVOLUTION OF COOPERATIVE BREEDING

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by
Irene García Ruiz
from Spain

Supervisor of the doctoral thesis:
Prof. Dr. Michael Taborsky

Institute of Ecology and Evolution
Behavioural Ecology Division

Accepted by the Faculty of Science.

Bern, 4 May 2022

The Dean
Prof. Dr. Zoltan Balogh

Written in Bern between December 2017 and May 2022 Printed in May 2022
Defence: 4. May 2022

Author

Irene García Ruiz
igaru.13@gmail.com
<https://irenegarcia.ruiz.com/>

Supervisor

Prof. Dr. Michael Taborsky
University of Bern
Institute of Ecology and Evolution, Behavioural Ecology Division
Wohlenstrasse 50a, CH-3032 Hinterkappelen
michael.taborsky@iee.unibe.ch

External Expert

Prof. Dr. Michael Cant
University of Exeter
Centre for Ecology and Conservation
Daphne du Maurier Building, Penryn Campus Penryn, Cornwall, TR10 9FE, UK
M.A.Cant@exeter.ac.uk

Chair at the Defence

Prof. Dr. Claudia Bank
University of Bern
Institute of Ecology and Evolution
Baltzerstrasse 6, 3012 Bern
claudia.bank@iee.unibe.ch

Manuscripts

Chapter 1: The evolution of cooperative breeding by direct and indirect fitness effects. In press in Science Advances.

Chapter 2: Group augmentation on trial: helpers in small groups enhance antipredator defence of eggs. Submitted to Biology Letters.

Chapter 3: Kin selection mediates coercion in asymmetric negotiations in a cooperatively breeding cichlid fish. In preparation for submission.

Appendix 1: Egg predator defence depends on both rank and size in a cooperatively breeding cichlid fish. Preliminary results.

Appendix 2: Influence of help prevention time on punishment and alloparental care in a cooperative breeder that pays to stay. Preliminary results.

Index

SUMMARY	7
GENERAL INTRODUCTION	9
REFERENCES	15
CHAPTER 1	21
THE EVOLUTION OF COOPERATIVE BREEDING BY DIRECT AND INDIRECT FITNESS EFFECTS	21
ABSTRACT	22
INTRODUCTION.....	23
RESULTS.....	24
DISCUSSION.....	34
MATERIALS AND METHODS	37
REFERENCES	42
SUPPLEMENTARY MATERIALS.....	48
CHAPTER 2	59
GROUP AUGMENTATION ON TRIAL: HELPERS IN SMALL GROUPS ENHANCE ANTIPREDATOR DEFENCE OF EGGS	59
ABSTRACT	60
INTRODUCTION.....	61
METHODS	61
RESULTS.....	65
DISCUSSION.....	66
REFERENCES	68
CHAPTER 3	73
KIN SELECTION MEDIATES COERCION IN ASYMMETRIC NEGOTIATIONS IN A COOPERATIVELY BREEDING CICHLID FISH	73
ABSTRACT	74
INTRODUCTION.....	75
METHODS	76
RESULTS.....	82
DISCUSSION.....	85
REFERENCES	88
SUPPLEMENTARY MATERIALS.....	93
APPENDIX 1	97
EGG PREDATOR DEFENCE DEPENDS ON BOTH RANK AND SIZE IN A COOPERATIVELY BREEDING CICHLID FISH	97
SUMMARY.....	98
METHODS	98
PRELIMINARY RESULTS	101
BRIEF DISCUSSION	102
REFERENCES.....	103
SUPPLEMENTARY MATERIAL	105
APPENDIX 2	107

INFLUENCE OF HELP PREVENTION TIME ON PUNISHMENT AND ALLOPARENTAL CARE IN A COOPERATIVE BREEDER THAT PAYS TO STAY.....	107
SUMMARY	108
METHODS	108
PRELIMINARY RESULTS	112
BRIEF DISCUSSION	114
REFERENCES	115
SUPPLEMENTARY MATERIAL	118
GENERAL DISCUSSION	120
REFERENCES	124
ACKNOWLEDGMENTS	127
DECLARATION OF CONSENT.....	128
CURRICULUM VITAE	129

Summary

The evolution of cooperation is an area of intensive theoretical and empirical research not only in biology but also in anthropology, political sciences and economics. Cooperative breeding, a form of cooperation in which helpers provide alloparental care to increasing the productivity of dominant individuals in the group has received considerable interest among behavioural ecologists. This behaviour poses a striking problem because helpers forego their own reproduction well after reaching sexual maturity, while investing in costly forms of help. Evolutionary mechanisms selecting for this form of cooperation are to this day not well understood.

Cooperative breeding is spread among a wide range of different taxa (i.e. mammals, birds, fish and social insects), across different ecological environments ranging from benign and stable to harsh and unpredictable, and groups range from family groups to groups with very low relatedness. Therefore, finding general evolutionary forces selecting for alloparental care has proven challenging. Kin selection can be a strong selective force in some instances, particularly when groups are formed by offspring that delay dispersal due to challenges in independent breeding. But neither all family groups provide help, nor all cooperative breeders have high within-group relatedness. Alternatively, direct fitness benefits could select for cooperative breeding, but evidence on the relevance of distinct mechanisms is limited in nature. In addition, research on the interplay of direct and indirect fitness benefits is scarce, but mixed groups of related and unrelated helpers are not uncommon. This thesis explores from a theoretical and empirical approach different evolutionary mechanisms based on direct fitness benefits and their interplay with kin selection.

In **Chapter 1** we investigated whether direct fitness benefits derived from living in large groups are an important selective force for cooperative breeding to evolve. We modelled the coevolution of delayed dispersal and alloparental care across different ecological scenarios. We assumed that helping increased the fecundity of the breeder at a survival cost to the helper. Individuals could adjust philopatry and helping levels to their resource holding potential or show fixed evolutionary strategies. We allowed relatedness to arise from population dynamics, and blocked relatedness to build-up to contrast your results. We found that direct fitness benefits from grouping are the main driver for the evolution of philopatry while kin selection is mainly responsible for the emergence of alloparental care. Direct benefits of increasing group size also selected for alloparental care to evolve in harsh environments. Although philopatry is a prerequisite for alloparental care to evolve, both philopatry and help were subjected to positive feedback. Moreover, behavioural plasticity in their decision to disperse allowed individuals to maximise group living benefits while reducing competition with kin for the breeding position.

In **Chapter 2**, we further explore in an empirical study the evolution of alloparental care driven by direct fitness benefits of living in larger groups (as predicted by the group augmentation hypothesis). For this we use the cooperative breeding cichlid *Neolamprologus pulcher* as a model species. After controlling for alternative mechanisms like kin selection, coercion and load-lightening, we found that subordinates provided more help in the form of defence against an egg predator when in small compared to large groups. This difference was

only evident in large helpers due to size-specific task specialization. These results confirm predictions for the group augmentation hypothesis, as in small groups there is a greater need to increase group size.

In **Chapter 3** we studied the negotiation process between dominant and subordinate group members, in which helpers may trade alloparental brood care against safety and resource access in the dominants' territory (as predicted by the pay-to-stay hypothesis). We investigated how unequal partners in bargaining power solve the conflict of fitness interests, and whether indirect fitness benefits alleviate this conflict in *N. pulcher*. To test this, in a full factorial design we experimentally disturbed the equilibrium in the negotiation process between dominant breeders and related or unrelated subordinates by simulating transgression from the helpers while allowing or preventing breeders the possibility to promote helping by coercion. Our results show that coercion by breeders is crucial for the performance of alloparental egg care by subordinate helpers, but that kinship reduces the importance of coercion as predicted by theoretical models.

General introduction

The evolution of cooperation ranks among the most fundamental questions in biology. But despite decades of study, the 125th anniversary issue of the journal *Science* still identified the question of “how did cooperative behaviour evolve?” as one for the fundamental unanswered question in biology (Pennisi 2005). The reason why our knowledge of the evolution of cooperation is still unsatisfactory is the lack of understanding of the different interacting evolutionary pressures involved. In this thesis, I aim to investigate different evolutionary forces driving the evolution of cooperative breeding, a particularly striking example of cooperation in which individuals forgo their own reproduction and assist in the rearing of offspring other than their own (Brown 1987; Cockburn 1998), as well as their interaction.

Kin selection hypothesis

Cooperative breeding has received much attention because it defies the prediction of natural selection theory that individuals should invest on their own reproduction (Darwin 1859; Fisher 1930). Kin selection emerges as the most prominent explanation for this seemingly altruistic behaviour by proposing that subordinates can gain indirect inclusive fitness benefits if they increase the production of related individuals (Hamilton 1964; Maynard Smith 1964; Wilson 1971; Bourke 2011). Broad amount of evidence is found across different taxa supporting this hypothesis (Emlen and Wrege 1988; Komdeur 1994; Queller and Strassmann 1998; Peters et al. 1999; Russell and Hatchwell 2001; Baglione et al. 2003; Covas et al. 2006; Nam et al. 2010; Wright et al. 2010; Briga et al. 2012). However, most of the evidence for the importance of kin selection for the evolution of cooperative behaviour relies on correlational studies that do not control for alternative mechanisms (West et al. 2002; Griffin and West 2003; Bourke 2014), and other studies do not find this correlation (Strassmann et al. 1997; Cockburn 1998; Queller and Strassmann 1998; Clutton-Brock et al. 2000). High relatedness between the interacting parts can be a result of limited dispersal and does not constitute in itself sufficient evidence that kin selection is responsible for promoting the evolution of help (Emlen 1995; Clutton-Brock 2002). In addition, in many cooperative breeding species not all subordinates are related to the offspring they care for, and unrelated helpers often invest as heavily as close relatives (e.g., Dunn et al. 1995; Magrath and Whittingham 1997; Clutton-Brock et al. 2000; Legge 2000; Dickinson 2004; Canestrari et al. 2005; Wright et al. 2010). In addition, competition between relatives can counterbalance the benefits of kin selection, and indirect fitness benefits might often not be enough to compensate for delayed reproduction (West et al. 2001, 2002; Dickinson and Hatchwell 2004). Therefore, kin selection alone cannot explain the evolution of cooperative breeding, and additional or alternative mechanisms must be invoked (Griffin and West 2002).

Cooperative breeding by direct fitness benefits

Direct benefits are postulated in cases of species in which unrelated helpers are commonly found (West et al. 2007; Clutton-Brock 2009; Kingma et al. 2011; Riehl 2013; Komdeur et al. 2017). For instance, subordinates that provide alloparental care can obtain increased chances to reproduce by acquisition of parental experience (Komdeur 1996), by securing parentage (Magrath and Whittingham 1997; Dickinson 2004), or by an increase in social prestige if costly help functions as a signal of quality (Zahavi 1974, 1995). In cases when subordinates obtain survival benefits from group membership, for instance due to access to limited resources or enhanced protection from predators, subordinates may help to increase group size (group augmentation hypothesis; (Kokko et al. 2001; Kingma et al. 2014). If the dominants incur costs from the presence of subordinates, for example through competition for resources or for reproduction, they may enforce help to compensate for these costs. Therefore, helping can also be a form of rent paid in order to be tolerated within the group and to stay on the territory (pay-to-stay hypothesis; (Gaston 1978; Kokko et al. 2002). However, predictions of the different mechanisms for the establishment of evolutionary stable levels of cooperation have hardly been experimentally tested, or have not successfully ruled out alternative evolutionary pressures selecting of cooperation. Throughout this thesis, we explore the validity and scenarios in which group augmentation, pay-to-stay and kin selection fitness benefits may select for the evolution of alloparental care, and their interrelation.

Group augmentation hypothesis

The group augmentation hypothesis states that if subordinates in cooperatively breeding animals raise the reproductive output of the breeders, the benefits of living in a resulting larger group due to improved survival or future reproductive success, favour the evolution of seemingly altruistic helping behaviour (Kingma et al. 2014). These fitness benefits can be divided in short- and long-term (Kingma et al. 2014).

Short-term group augmentation benefits refer to fitness benefits obtained during the subordinate's phase of the helper in the group, derived from survival fitness benefits by the increased group size due to reducing predation (e.g. safety-in-numbers through risk dilution, contributing to vigilance and predator repellence (Hamilton 1971; Mares et al. 2012)) or by increasing their body condition, for instance by grooming (Stacey and Ligon 1987; Creel and Creel 1995; Madden and Clutton-Brock 2009). However, most studies only provide circumstantial support for short-term group augmentation fitness benefits in the form of a positive correlation between group size and individual survival and/or group reproductive outcome (Wright 1998; Clutton-Brock et al. 1999; Covas et al. 2008; Cant et al. 2016; Ridley 2016). Under short-term benefits, helping effort is expected to be higher in small groups than in large groups, as the need to increase group size is larger. In large groups, the positive effects of help on the breeders' production are reduced, and an increase in group size may even show diminishing returns due to an increase in competition and risk of parasite or

disease transmission (Kingma et al. 2014). However, this hypothesis has not been explicitly tested, and studies on the relationship between help and group size had failed to control for load-lightening effects or ceiling effects (Bergmüller and Taborsky 2005; Brouwer et al. 2005). In **Chapter 2**, we address this shortcoming and investigate whether individuals adjust helping to group size while controlling for kin selection, coercion and load lightening.

Besides short-term fitness benefits derived from living in a large group, individuals may also obtain long-term benefits if the helper inherits a breeding position and offspring they helped produce, in turn, increase their survival or fecundity. Alternatively, group augmentation benefits may also be acquired if the new recruits disperse together with the former helper (Heg et al. 2008; Rodrigues and Taylor 2018). Therefore, if individuals benefit from long-term group augmentation fitness benefits, higher levels of help are expected when there is a higher chance for the subordinate to inherit the territory (Nowak and Sigmund 1998; Kingma et al. 2014). The largest support for alloparental care evolving under long-term group augmentation fitness benefits comes from purple crowned fairy wrens, in which subordinates adjusted nestling feeding rates to the probability of inheriting the breeding position (Kingma et al. 2011). In Lake Tanganyika cichlids, larger helpers that are more likely to inherit the territory also invest more in alloparental care behaviours, although alternative hypothesis like or pay-to-stay were not excluded, as breeders may enforce higher cooperation to larger subordinates due to inflicting higher reproductive competition (Taborsky and Limberger 1981; Wong and Balshine 2011). In **Appendix 1** we investigate whether help investment depends on the rank of the individuals while controlling for helper size and coercion in a cichlid fish.

It is also unclear whether group augmentation is, in itself, a sufficient evolutionary force to originate and maintain cooperative breeding behaviour. Helping to acquire group augmentation benefits may not be resistant to cheaters as helping incurs individual fitness cost but conveys communal shared benefits and, therefore, may suffer from the classic ‘tragedy of the commons’ problem (Hardin 1968; Killingback et al. 2006). A model on group augmentation found that help can evolve and be maintained stable even in the absence of kin selection benefits (Kokko et al. 2001). However, they did not include the coevolution of philopatry, a prerequisite for the evolution of cooperative breeding to evolve. Philopatric propensity influences the population kinship structure (Le Galliard et al. 2005; Hochberg et al. 2008; Mullon et al. 2018). While genetic relatedness among group members can facilitate the efficacy of kin selection (Hamilton 1964; West et al. 2007), grouping with kin may also generate competition between relatives for resources and the breeding position (Hamilton and May 1977; Taylor 1992; West et al. 2002; Platt and Bever 2009). In addition, in cases in which group size increases group member’s fitness, limited dispersal opportunities can promote as a by-product direct fitness benefits of group augmentation (Clutton-Brock et al. 1999; Kokko et al. 2001; Taborsky et al. 2005; Kingma et al. 2014). However, is it unknown whether philopatric tendencies can also evolve due to the direct fitness advantages of living in large groups or are the result of movement constraints imposed by the environment or as a result of kin selection (Hatchwell and Komdeur 2000). In **Chapter 1** we model the

coevolution of philopatry and alloparental care under the assumption that large groups entail survival benefits for group members. We let relatedness build up from group dynamics and contrast the results with a benchmark model in which we prevent relatedness from evolving. Because selection is subjected to the ecological, social and life history contexts (Clobert et al. 2009; Jacob et al. 2016), we let individuals show plastic behavioural reaction norms in their levels of philopatric and help tendencies according to their resource holding potential, and study the evolved mechanisms in a different range of ecological scenarios.

Pay-to-stay hypothesis

The pay-to-stay hypothesis proposes that help provided by helpers can be viewed as a form negotiation over the provision of different commodities between the dominant breeders and subordinate helpers, in which subordinates pay ‘rent’ to dominants in return for being allowed to stay in their territory (Gaston 1978). Because subordinates impose cost in the form of breeding competition, if helpers do not provide sufficient help, the dominants should punish helpers by imposing costs, or terminate the relationship by evicting them from their territory (Clutton-Brock and Parker 1995; Cant 2011; Riehl and Frederickson 2016). Even if there is a strong asymmetry in bargaining power between dominants and subordinates, subordinates may also seek outside options if the fitness of remaining in the group altogether with the costs of providing alloparental care and delaying reproduction are lower than the benefits of attempting independent breeding or migrating to another group (Clutton-Brock and Parker 1995; Cant 2011; Riehl and Frederickson 2016). Because there is a strong selection to avoid triggering threats in the negotiation process, these threats are often “hidden”, which may contribute to the reason why there is scarce evidence of the pay-to-stay evolutionary mechanism in nature (Reeve 1992; Mulder and Langmore 1993). However, some evidence has begun to accumulate in the cooperatively breeding cichlid *Neolamprologus pulcher* (Balshine-Earn et al. 1998; Bergmüller and Taborsky 2005; Bergmüller et al. 2005; Stiver et al. 2005; Zöttl et al. 2013; Naef and Taborsky 2020a). For the threats to be noticeable the status quo should be experimentally disturbed, for instance by simulating transgression (i.e. preventing the subordinate from providing help). In agreement with the pay-to-stay hypothesis, breeders in this species increase aggression for experimentally reduced help (Fischer et al. 2014; Naef and Taborsky 2020b), and aggression seems to lead to increased levels of help (Fischer et al. 2014). Apparent punishment for not helping scaled positively with the need for help (Heg and Taborsky 2010), and related helpers provided less rent payment than unrelated ones to the breeding pair (Zöttl et al. 2013). However, these studies did not control for the possibility of the breeders to enforce help after transgression. If subordinates provide help under pay-to-stay, we would only expect an increase in helping behaviour after transgression when the breeders can interact with them. In **Chapter 3** we experimentally manipulated the access to provide alloparental care for the subordinates, and the possibility for breeders to enforce help after transgression. We measured whether breeders increased punishment to the helpers when prevented to help, and whether helpers increased alloparental care only when breeders could coerce them after being prevented to do

so. Another unexplored prediction of pay-to-stay is that if aggression represent a form of punishment, a longer help prevention time should also lead to an increase in attack frequency and/or the likelihood of eviction. We test this hypothesis in **Appendix 2**.

Coexistence of different evolutionary mechanisms

It is important to note that the three mentioned mechanisms: kin-selection, group augmentation and pay-to-stay are not mutually exclusive and may operate in a social system in conjunction (Lehmann and Keller 2006; Kingma et al. 2011; Nonacs 2011). It is, however, largely unexplored how behaviour in cooperative societies is shaped by the interplay of these mechanisms. The coexistence of both related and unrelated individuals within breeding groups is widespread among cooperative breeders (Clutton-Brock 2009; Riehl 2013) and, therefore, kin selection may act in synergy with other evolutionary mechanisms. We explore the interrelation of direct group benefits with kin selection in different ecological scenarios in **Chapter 1**. In **Chapter 3**, we explore how kin selection may mediate in alloparental care driven by coercion by weakening selection for effective punishment against defectors (Marshall and Rowe 2003; Quiñones et al. 2016), while kin may instead provide voluntary help.

Model species

We used the cooperatively breeding cichlid *Neolamprologus pulcher* as a model species. This species offers a unique opportunity to study the evolution of different evolutionary mechanisms that drive the evolution of alloparental care. Short generational time and body size makes it easy to rear in the lab and obtain transgenerational data. Additionally, relatedness, group composition, environmental conditions and the behaviour of group members can be easily manipulated both in aquaria and in the wild (Taborsky 2016).

All necessary preconditions for the evolution of alloparental care by group augmentation have been found in *N. pulcher*. Helpers in this species benefit from increased survival in large groups due to reduced predation risk (Balshine et al. 2001; Heg et al. 2004; Taborsky et al. 2005), which is a prerequisite for group augmentation to evolve. In addition, helpers prefer to join a group instead of breeding independently (Taborsky 1985), and they prefer to join larger vs smaller groups despite thereby reducing their chance of inheriting the territory (Jordan et al. 2010; Reddon et al. 2011), which indicates that there is a strong selective pressure for helpers to belong in large groups. Furthermore, helpers increase the reproductive output of breeders (Taborsky 1984; Brouwer et al. 2005), which translates in an increase in group size since most offspring remain philopatric for long (Taborsky and Limberger 1981; Dierkes et al. 2005; Heg et al. 2008). Although alloparental care has not been definitely linked to group size, a field study showed that helpers visited breeding shelters more often when a big proportion of helpers were experimentally removed (Brouwer et al. 2005).

In addition, the greatest amount of evidence for help evolving under the pay-to-stay mechanism comes from this species (Balshine-Earn et al. 1998; Bergmüller and Taborsky 2005; Bergmüller et al. 2005; Hamilton and Taborsky 2005; Brintjes and Taborsky 2008; Zöttl et al. 2013; Fischer et al. 2014; Naef and Taborsky 2020*b*, 2020*a*; for more detail see above in section “Pay to stay”). Lastly, the presence of related and unrelated helpers allows to test predictions of kin selection interacting with direct fitness benefits driving the evolution of cooperative breeding.

Aims of the dissertation

In this thesis we aim to increase our knowledge on the different evolutionary pressures that select for cooperative breeding. We use a combination of theoretical and empirical approaches to investigate different direct and indirect fitness benefits selecting for alloparental care, and assess the relative importance of the distinct mechanisms in an ecological context.

References

- Baglione, V., D. Canestrari, J. M. Marcos, and J. Ekman. 2003. Kin selection in cooperative alliances of carrion crows. *Science* 300:1947–1949.
- Balshine, S., B. Leach, F. Neat, H. Reid, M. Taborsky, and N. Werner. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* 50:134–140.
- Balshine-Earn, S., F. C. Neat, H. Reid, and M. Taborsky. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology* 9:432–438.
- Bergmüller, R., D. Heg, and M. Taborsky. 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences* 272:325–331.
- Bergmüller, R., and M. Taborsky. 2005. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- Bourke, A. F. G. 2011. *Principles of social evolution*. Oxford University Press.
- . 2014. Hamilton’s rule and the causes of social evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369:20130362.
- Briga, M., I. Pen, and J. Wright. 2012. Care for kin: within-group relatedness and allomaternal care are positively correlated and conserved throughout the mammalian phylogeny. *Biology Letters* 8:533–536.
- Brouwer, L., D. Heg, and M. Taborsky. 2005. Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behavioral Ecology* 16:667–673.
- Brown, J. L. 1987. Helping and Communal Breeding in Birds. *Journal of Evolutionary Biology* 2:62–65.
- Bruintjes, R., and M. Taborsky. 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour* 75:1843–1850.
- Canestrari, D., J. M. Marcos, and V. Baglione. 2005. Effect of parentage and relatedness on the individual contribution to cooperative chick care in carrion crows *Corvus corone corone*. *Behavioral Ecology and Sociobiology* 57:422–428.
- Cant, M. A. 2011. The role of threats in animal cooperation. *Proceedings of the Royal Society B: Biological Sciences* 278:170–178.
- Cant, M. A., H. J. Nichols, F. J. Thompson, and E. Vitikainen. 2016. Banded mongooses: Demography, life history, and social behavior. Pages 318–337 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Clobert, J., J. le Galliard, J. Cote, S. Meylan, and M. Massot. 2009. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters* 12:197–209.
- Clutton-Brock, T. H. 2002. Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72.
- . 2009. Cooperation between non-kin in animal societies. *Nature* 462:51–57.

- Clutton-Brock, T. H., P. N. M. Brotherton, M. J. O’Riain, A. S. Griffin, D. Gaynor, L. Sharpe, R. Kansky, et al. 2000. Individual contributions to babysitting in a cooperative mongoose, *Suricata suricatta*. *Proceedings of the Royal Society B: Biological Sciences* 267:301–305.
- Clutton-Brock, T. H., D. Gaynor, G. M. McIlrath, A. D. C. Maccoll, R. Kansky, P. Chadwick, M. Manser, et al. 1999. Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *Journal of Animal Ecology* 68:672–683.
- Clutton-Brock, T. H., and G. A. Parker. 1995. Punishment in animal societies. *Nature* 373:209–216.
- Cockburn, A. 1998. Evolution of Helping Behavior in Cooperatively Breeding Birds. *Annual Review of Ecology and Systematics* 29:141–177.
- Covas, R., A. Dalecky, A. Caizergues, and C. Doutrelant. 2006. Kin associations and direct vs indirect fitness benefits in colonial cooperatively breeding sociable weavers *Philetairus socius*. *Behavioral Ecology and Sociobiology* 60:323–331.
- Covas, R., M. A. du Plessis, and C. Doutrelant. 2008. Helpers in colonial cooperatively breeding sociable weavers *Philetairus socius* contribute to buffer the effects of adverse breeding conditions. *Behavioral Ecology and Sociobiology* 63:103–112.
- Creel, S., and N. M. Creel. 1995. Communal hunting and pack size in African wild dogs, *Lycaon pictus*. *Animal Behaviour* 50:1325–1339.
- Darwin, C. 1859. *The origin of species by means of natural selection*. Pub One Info.
- Dickinson, J. L. 2004. A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behavioral Ecology* 15:233–238.
- Dickinson, J. L., and B. J. Hatchwell. 2004. Fitness consequences of helping. Pages 48–66 *in* *Ecology and evolution of cooperative breeding in birds*. Cambridge: Cambridge University Press.
- Dierkes, P., D. Heg, M. Taborsky, E. Skubic, and R. Achmann. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* 8:968–975.
- Dunn, P. O., A. Cockburn, and R. A. Mulder. 1995. Fairy-wren helpers often care for young to which they are unrelated. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 259:339–343.
- Emlen, S. T. 1995. An evolutionary theory of the family. *Proceedings of the National Academy of Sciences* 92:8092–8099.
- Emlen, S. T., and P. H. Wrege. 1988. The role of kinship in helping decisions among white-fronted bee-eaters. *Behavioral Ecology and Sociobiology* 23:305–315.
- Fischer, S., M. Zöttl, F. Groenewoud, and B. Taborsky. 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 281:20140184.
- Fisher, R. A. 1930. Genetics, Mathematics, and Natural Selection. *Nature* 126:805–806.
- Gaston, A. J. 1978. *The Evolution of Group Territorial Behavior and Cooperative Breeding*. *The American Naturalist* 112:1091–1100.
- Griffin, A. S., and S. A. West. 2003. Kin Discrimination and the Benefit of Helping in Cooperatively Breeding Vertebrates. *Science* 302:634–636.

- Griffin, A., and S. A. West. 2002. Kin selection: fact and fiction. *Trends in Ecology and Evolution* 17:15–21.
- Hamilton, I. M., and M. Taborsky. 2005. Unrelated helpers will not fully compensate for costs imposed on breeders when they pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 272:445–454.
- Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology* 7:17–52.
- . 1971. Geometry for the selfish herd. *Journal of theoretical Biology* 31:295–311.
- Hamilton, W. D., and R. M. May. 1977. Dispersal in stable habitats. *Nature* 269:578–581.
- Hardin, G. 1968. The tragedy of the commons: the population problem has no technical solution; it requires a fundamental extension in morality. *science* 162:1243–1248.
- Hatchwell, B. J., and J. Komdeur. 2000. Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour* 59:1079–1086.
- Heg, D., Z. Bachar, L. Brouwer, and M. Taborsky. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences* 271:2367–2374.
- Heg, D., L. Brouwer, Z. Bachar, and M. Taborsky. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg, D., Z. Heg-Bachar, L. Brouwer, and M. Taborsky. 2008. Experimentally induced helper dispersal in colonially breeding cooperative cichlids. *Environmental Biology of Fishes* 83:191–206.
- Heg, D., and M. Taborsky. 2010. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- Hochberg, M. E., D. J. Rankin, and M. Taborsky. 2008. The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. *BMC Evolutionary Biology* 8:238.
- Jacob, S., P. Wehi, J. Clobert, D. Legrand, N. Schtickzelle, M. Huet, and A. Chaine. 2016. Cooperation-mediated plasticity in dispersal and colonization. *Evolution: International Journal of Organic Evolution* 70:2336–2345.
- Jordan, L. A., M. Y. L. Wong, and S. S. Balshine. 2010. The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biology Letters* 6:301–303.
- Killingback, T., J. Bieri, and T. Flatt. 2006. Evolution in group-structured populations can resolve the tragedy of the commons. *Proceedings of the Royal Society B: Biological Sciences* 273:1477–1481.
- Kingma, S. A., M. L. Hall, and A. Peters. 2011. Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist* 177:486–495.
- Kingma, S. A., P. Santema, M. Taborsky, and J. Komdeur. 2014. Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution* 29:476–484.
- Kokko, H., R. A. Johnstone, and T. H. Clutton-Brock. 2001. The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B: Biological Sciences* 268:187–196.
- Kokko, H., R. A. Johnstone, and J. Wright. 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behavioral Ecology* 13:291–300.

- Komdeur, J. 1994. The effect of kinship on helping in the cooperative breeding seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society B: Biological Sciences* 256:47–52.
- . 1996. Influence of age on reproductive performance in the Seychelles warbler. *Behavioral Ecology* 7:417–425.
- Komdeur, J., D. S. Richardson, M. Hammers, C. Eikenaar, L. Brouwer, and S. A. Kingma. 2017. The evolution of cooperative breeding in vertebrates. *Els* 1–11.
- Le Galliard, J. F., R. Ferrière, and U. Dieckmann. 2005. Adaptive evolution of social traits: Origin, trajectories, and correlations of altruism and mobility. *American Naturalist* 165:206–224.
- Legge, S. 2000. Helper contributions in the cooperatively breeding laughing kookaburra: feeding young is no laughing matter. *Animal Behaviour* 59:1009–1018.
- Lehmann, L., and L. Keller. 2006. The evolution of cooperation and altruism - A general framework and a classification of models. *Journal of Evolutionary Biology* 19:1365–1376.
- Madden, J. R., and T. H. Clutton-Brock. 2009. Manipulating grooming by decreasing ectoparasite load causes unpredicted changes in antagonism. *Proceedings of the Royal Society B: Biological Sciences* 276:1263–1268.
- Magrath, R. D., and L. A. Whittingham. 1997. Subordinate males are more likely to help if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. *Behavioral Ecology and Sociobiology* 41:185–192.
- Mares, R., A. J. Young, and T. H. Clutton-Brock. 2012. Individual contributions to territory defence in a cooperative breeder: weighing up the benefits and costs. *Proceedings of the Royal Society B: Biological Sciences* 279:3989–3995.
- Marshall, J. A. R., and J. E. Rowe. 2003. Kin selection may inhibit the evolution of reciprocation. *Journal of Theoretical Biology* 222:331–335.
- Maynard Smith, J. 1964. Group selection and kin selection. *Nature* 201:1145–1147.
- Mulder, R. A., and N. E. Langmore. 1993. Dominant males punish helpers for temporary defection in Superb Fairy-wrens. *Animal Behaviour*.
- Mullon, C., L. Keller, and L. Lehmann. 2018. Social polymorphism is favoured by the co-evolution of dispersal with social behaviour. *Nature Ecology and Evolution* 2:132–140.
- Naef, J., and M. Taborsky. 2020a. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* 168:137–147.
- . 2020b. Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science* 7:191808.
- Nam, K.-B., M. Simeoni, S. P. Sharp, and B. J. Hatchwell. 2010. Kinship affects investment by helpers in a cooperatively breeding bird. *Proceedings of the Royal Society B: Biological Sciences* 277:3299–3306.
- Nonacs, P. 2011. Kinship, greenbeards, and runaway social selection in the evolution of social insect cooperation. *Proceedings of the National Academy of Sciences* 108:10808–10815.
- Nowak, M. A., and K. Sigmund. 1998. The dynamics of indirect reciprocity. *Journal of theoretical Biology* 194:561–574.
- Pennisi, E. 2005. How did cooperative behavior evolve? *Science* 309:93.

- Peters, J. M., D. C. Queller, V. L. Imperatriz-Fonseca, D. W. Roubik, and J. E. Strassmann. 1999. Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 266:379–384.
- Platt, T. G., and J. D. Bever. 2009. Kin competition and the evolution of cooperation. *Trends in Ecology and Evolution* 24:370–377.
- Queller, D. C., and J. E. Strassmann. 1998. Kin selection and social insects. *Bioscience* 48:165–175.
- Quiñones, A. E., G. S. van Doorn, I. Pen, F. J. Weissing, and M. Taborsky. 2016. Negotiation and appeasement can be more effective drivers of sociality than kin selection. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150089.
- Reddon, A. R., D. Balk, and S. Balshine. 2011. Sex differences in group-joining decisions in social fish. *Animal Behaviour* 82:229–234.
- Reeve, H. K. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature* 358:147–149.
- Ridley, A. R. 2016. Southern pied babblers: The dynamics of conflict and cooperation in a group-living society. Pages 115–132 *in* W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Riehl, C. 2013. Evolutionary routes to non-kin cooperative breeding in birds. *Proceedings of the Royal Society B: Biological Sciences* 280.
- Riehl, C., and M. E. Frederickson. 2016. Cheating and punishment in cooperative animal societies. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371.
- Rodrigues, A. M. M., and T. B. Taylor. 2018. Ecological and demographic correlates of cooperation from individual to budding dispersal. *Journal of Evolutionary Biology* 31:1058–1070.
- Russell, A. F., and B. J. Hatchwell. 2001. Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268:2169–2174.
- Stacey, P. B., and J. D. Ligon. 1987. Territory quality and dispersal options in the acorn woodpecker and a challenge to the habitat-saturation model of cooperative breeding. *American Naturalist* 130:654–676.
- Stiver, K. A., P. Dierkes, M. Taborsky, H. Lisle Gibbs, and S. Balshine. 2005. Relatedness and helping in fish: examining the theoretical predictions. *Proceedings of the Royal Society B: Biological Sciences* 272:1593–1599.
- Strassmann, J. E., C. J. Klingler, E. Arevalo, F. Zacchi, A. Husain, J. Williams, P. Seppä, et al. 1997. Absence of within-colony kin discrimination in behavioural interactions of swarm-founding wasps. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 264:1565–1570.
- Taborsky, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour* 32:1236–1252.
- . 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* 95:45–75.
- . 2016. Cichlid fishes: A model for the integrative study of social behavior. Pages 272–293 *in* W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.

- Taborsky, M., and D. Limberger. 1981. Helpers in fish. *Behavioral Ecology and Sociobiology* 8:143–145.
- Taylor, P. D. 1992. Altruism in viscous populations - an inclusive fitness model. *Evolutionary Ecology* 6:352–356.
- West, S. A., A. S. Griffin, and A. Gardner. 2007. Evolutionary Explanations for Cooperation. *Current Biology* 17:661–672.
- West, S. A., M. G. Murray, C. A. Machado, A. S. Griffin, and E. A. Herre. 2001. Testing Hamilton's rule with competition between relatives. *Nature* 409:510–513.
- West, S. A., I. Pen, and A. S. Griffin. 2002. Cooperation and competition between relatives. *Science* 296:72–75.
- Wilson, E. O. 1971. *The insect societies*. The insect societies.
- Wong, M., and S. Balshine. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biological Reviews* 86:511–530.
- Wright, J. 1998. Helping-at-the-Nest and Group Size in the Arabian Babbler *Turdoides squamiceps*. *Journal of Avian Biology* 29:105.
- Wright, J., P. G. McDonald, L. te Marvelde, A. J. N. Kazem, and C. M. Bishop. 2010. Helping effort increases with relatedness in bell miners, but 'unrelated' helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B: Biological Sciences* 277:437–445.
- Zahavi, A. 1974. Communal nesting by the Arabian babbler: a case of individual selection. *Ibis* 116:84–87.
- . 1995. Altruism as a handicap: the limitations of kin selection and reciprocity. *Journal of Avian Biology* 26:1–3.
- Zöttl, M., D. Heg, N. Chervet, and M. Taborsky. 2013. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* 4:1341.



CHAPTER 1

The evolution of cooperative breeding by direct and indirect fitness effects

Irene García-Ruiz^{1*}, Andrés Quiñones^{2,3†} and Michael Taborsky^{1†}

¹ Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Switzerland.

² Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia

³ Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

*Corresponding author.

†These authors contributed equally to this work

In press in Science Advances

Publish version available: García-Ruiz I, Quiñones A, Taborsky M (2022). The evolution of cooperative breeding by direct and indirect fitness effects. Science Advances, 8(21), eab17853.

[DOI: 10.1126/sciadv.abl7853](https://doi.org/10.1126/sciadv.abl7853)

Abstract

The evolution of cooperative breeding has been traditionally attributed to the effect of kin selection. While there is increasing empirical evidence that direct fitness benefits are relevant, the relative importance of alternative selection mechanisms is largely obscure. Here, we model the coevolution of the cornerstones of cooperative breeding, delayed dispersal and alloparental care, across different ecological scenarios and allowing individuals to adjust philopatry and helping levels. Our results suggest that (1) direct fitness benefits from grouping are the main driver for the evolution of philopatry; (2) kin selection is mainly responsible for the emergence of alloparental care, but group augmentation can be a sufficient promoter in harsh environments; (3) the coevolution of philopatry and alloparental care is subject to positive feedback; (4) age-dependent dispersal is triggered by both group benefits and relatedness. Model predictions are supported by empirical data and provide novel opportunities for comparative analyses and experimental tests of causality.

Teaser

Alternative evolutionary pathways for the emergence of cooperative breeding depend on the harshness of the environment.

Introduction

Cooperative breeding involves apparent altruism, as subordinate helpers forgo their own reproduction by delaying dispersal and investing in the care of offspring that are not their own. This intriguing social and life-history trait is distributed widely throughout animals (1–3). Kin selection can explain alloparental care because of fitness benefits accrued to related individuals (4, 5), and genetic relatedness among group members is indeed a good predictor of evolutionary transitions to cooperative breeding (6–8). However, in many cases the group members are not related to each other (9–12). Notably, group living as a result of limited dispersal may bear inevitable direct fitness benefits that can also select for philopatry and helping (13–16). It remains unclear, however, whether fitness benefits from grouping suffice to select for cooperative breeding. Furthermore, it is unknown how crucial selective forces such as kin selection and group benefits may interact (17, 18). Eventually, the relationship between delayed dispersal and alloparental care should be scrutinised in a co-evolutionary framework that includes both types of fitness benefits.

Dispersal obviously influences the population's kinship structure (19–21). Limited dispersal is central to the evolution of cooperative societies, as it builds up genetic relatedness among group members and thereby facilitates the efficacy of kin selection (22, 23). Nevertheless, while grouping with kin may generate indirect fitness benefits, it also increases competition between relatives (24–27). Hence, the effect of dispersal on competition and cooperation within groups needs to be clarified if aiming to understand the evolution of cooperative breeding.

Selection acts on significant life-history decisions subject to the respective ecological, social and life history contexts (28, 29). Importantly, the dynamics of dispersal and helping decisions influence how localised competition plays out (26). For instance, individuals expressing alloparental care early in life and dispersing to breed later may enhance indirect fitness benefits when young, while at the same time reducing reproductive competition with kin through leaving the group before starting to reproduce. Thus, dispersal decisions that vary across life stages or ecological contexts might resolve the cooperation-competition trade-off resulting from philopatry and promote the evolution of cooperation (29, 30).

Here, we model the coevolution of philopatry and cooperation driven by both kin selection and group benefits. Ultimately, we aim at identifying the conditions that favour the evolution of delayed dispersal and alloparental care. We construct a series of models in which individuals either adopt fixed behavioural strategies of dispersal and help throughout life or show age-dependent plasticity. To disentangle the role of kin selection and the effects of grouping, we compare the outcome of these models with a benchmark model in which the influence of genetic relatedness is controlled for. Our results suggest that direct survival benefits of group living are the main driver for philopatry and can be sufficient for the evolution of alloparental care under certain ecological conditions. Conversely, indirect fitness benefits can be the main driver for helping behaviour after group formation allows for kin structured populations. The relevance of direct vs indirect fitness benefits for the evolution of helping, however, changes depending on the harshness of the environment. Furthermore, behavioural plasticity allows for a developmental response reducing reproductive competition while at the same time enhancing help provided to relatives.

Results

Rationale

The structure and parameters of our model are informed by the basic components characterising cooperative breeding systems at large. Systematic variation of ecological conditions is meant to provide a diverse scope enabling subsequent empirical tests of the model predictions. We consider a class-structured population with overlapping generations living in a habitat with a limited number of breeding territories, which are monopolised by groups consisting of one breeder and an undefined number of subordinates. Individuals belong to one of three classes, (i) breeders that monopolise reproduction, (ii) subordinates in their natal or in a different group, and (iii) dispersers that are not part of a group. Group size in our model is an emergent property of dispersal decisions, mortality and help-dependent breeders' fertility. We track the age of individuals in the population and use age as a proxy for their resource holding potential (RHP(31)) when competing for breeding positions. Dispersers as well as local subordinates compete for the breeding position when joining a group and win with a probability proportional to their age, which is a realistic assumption in many species(32–34). The turnover for the breeding position in a territory occurs when the breeder dies.

If subordinates help to care for the dominant breeders' offspring, this raises the fertility of the breeder at a cost to the survival of helpers, as shown in cooperatively breeding mammals(35), birds(36), fishes(37), insects(38), and spiders(39). We assume complete reproductive skew, i.e. helpers will not raise their own offspring but only those of the dominant breeders. Increased productivity of breeders raises the group size, which enhances survival prospects for all group members. We let survival scale positively with group size, according to parameter x_n . Thus, if $x_n = 0$, then individuals in larger groups do not enjoy higher survival than individuals in smaller groups. As we increase x_n , the relationship between group size and survival is steeper. Similarly, survival depends on the cost of alloparental care; parameter x_h (Table 1) reflects how much the subordinate's survival decreases with increasing investment in care (see Methods for details).

In order to assess how different habitats affect life-history decisions, we systematically varied the quality of the habitat across simulations. Low baseline mortality (i.e., $m_0=1$) denotes high-quality environments (Table 1). Increasing values of m_0 reflect a reduced likelihood of individuals surviving another life cycle and therefore raises the turnover of breeding positions. This opens more breeding spots for dispersers and floaters. At the same time, we account for dispersal costs by increasing mortality for individuals outside a group. Values of $m_d > 1$ (Table 1) indicate higher mortality for dispersing individuals than expected only due to their solitary status. Hence, $m_d > 1$ reflects a survival benefit of group membership independent of group size. Finally, we also varied the likelihood of dispersers to find a new group to start breeding (parameter f , Table 1). This captures a variety of factors like the difficulty to reach or find a new group and the 'permeability' of groups to accept new members. Higher values of f translate into a higher likelihood for floaters to find a breeding position. This implies a lower probability of helpers inheriting the breeding position in their territory. Simultaneous variation of these three parameters, m_0 , m_d and f , simulates a range of

habitats that differ in their quality and the likelihood of dispersers to survive and to breed, allowing different strategies to evolve depending on the ecological parameters. The life cycle of the model is shown in Fig. 1.

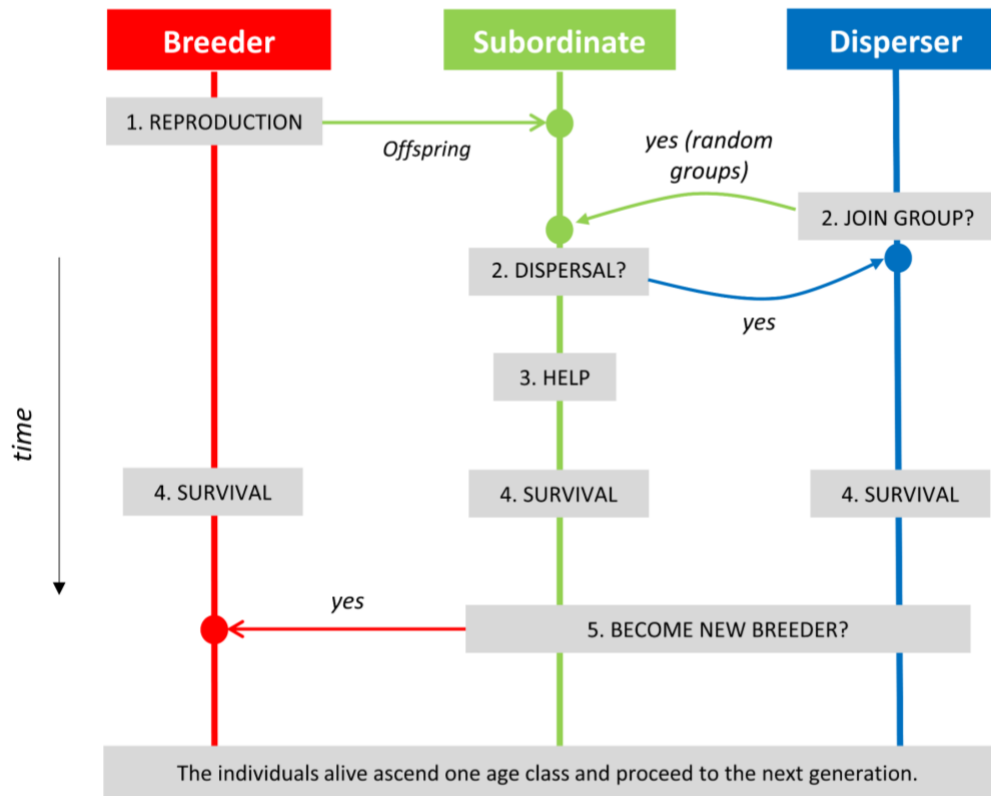


Fig. 1. Process overview and scheduling in the model. Arrows indicate a change of an individual to a different class. The Y-axis represents the timeline of the life cycle. (1) A breeder reproduces. Its productivity depends on the cumulative level of brood care provided in the group in the previous life cycle. (2) Subordinates may disperse to become floaters, or they may stay in the group and help. Dispersers/floaters may join a random group to become subordinates therein. (3) Subordinates in the group display help. (4) Individuals survive contingent on group living benefits and dispersal costs. (5) If a breeder dies, helpers in the group and a sample of floaters compete for the breeding position and win with a probability proportional to their age (as a proxy of RHP). Individuals still alive ascend one age class, and the cycle starts all over (i.e., next generation).

Direct vs indirect fitness benefits

To investigate the relative importance of direct fitness benefits of group living and indirect fitness benefits from relatedness for the evolution of cooperative breeding, we let relatedness emerge from the species' population dynamics. This allows drawing meaningful predictions for the role of kin selection without a priori assuming a particular evolutionary pathway. To distinguish direct fitness benefits derived from group size from those of kin selection, we created two parallel models for comparison, in one of which relatedness was prevented from building up through the dispersal decisions of individuals (benchmark model; see Methods

section for details). In the other model, the effect of indirect fitness benefits was separated from direct fitness benefits by removing the survival advantage arising from group size (i.e., $x_n=0$).

Our results show that cooperative breeding can evolve solely due to direct or indirect benefits. However, the conditions under which cooperation is selected are strict when considering each of these mechanisms in isolation. Cooperative breeding can evolve solely by kin selection in cases in which alloparental care has a low cost for the survival of helpers (low x_h) while at the same time greatly increasing the productivity of the breeders (high k_h ; Fig. S1). In this scenario, the degree of philopatry remains low. More costly forms of help are likely to evolve in harsh environments that greatly decrease the survival of individuals (Fig. S1; $m_0=0.3$).

Direct group benefits can also be exclusively responsible for the evolution of cooperative breeding, as suggested by the group augmentation hypothesis. The group augmentation mechanism denotes a scenario where helpers gain fitness benefits by enhancing group size if the recruits that are produced as a result of helping behaviour, in turn, increase the survival and/or reproduction of helpers (13, 14). Our results show that when group size benefits on survival take effect, the conditions under which philopatry can evolve are less restrictive with respect to the cost/benefit ratio in Hamilton's rule (Fig. 2: when $x_n>0$) than when only relatedness effects are considered. Direct group benefits, therefore, seem to be more likely to drive group formation. In addition, alloparental care can also evolve by group augmentation benefits alone (Fig. 2B&C: when $x_n \geq 3$ and relatedness is blocked). This applies in harsh environments where group living confers a substantial survival benefit, which is often the case in natural systems (16, 40–42). In contrast, in benign environments where habitat saturation is reached and mainly direct fitness benefits select for group formation, help can only evolve in combination of direct and indirect fitness benefits (Fig. 2A). This holds when benefits of helping can be inherited once a former helper attains the breeding position (e.g., by nest building, territory maintenance, long-term benefits of group augmentation (13, 14)), implying that during their first breeding attempt the new breeder's fertility is influenced by the help it had previously provided. If no benefits of helping are passed on to the breeder status, the probability that alloparental care evolves solely by group augmentation, i.e. in the absence of relatedness effects, is significantly reduced (Fig. S2). Under these conditions helping is unlikely to evolve by group augmentation benefits alone due to the resulting production of direct competitors for the breeding position (Fig. S3).

While direct group benefits seem to be the primary evolutionary driver of group formation, indirect fitness benefits are likely to select for alloparental care once groups have formed (Fig. 2). Nevertheless, the build-up of relatedness also selects for dispersal due to the effects of kin competition limiting the benefits of philopatry (Fig. 2; for more details see the "Age-dependent reaction norms" section below).

These results are in line with previous theoretical work suggesting a joint effect of group augmentation and kin selection (13). However, our model reveals that under the coevolution of philopatry and help, in harsh environments direct benefits on survival are the main driver of philopatry, which enables the evolution of helping. These results suggest a novel pathway to the evolution of cooperative breeding, where philopatry is originally selected by direct

fitness benefits. The ensuing relatedness within the groups in combination with the direct benefits by group augmentation select for the emergence of alloparental care. This evolutionary pathway is consistent with both the well-known correlation between cooperative breeding and relatedness (43, 44) and the demonstrated group-size dependent survival effects in cooperative breeders (16, 41, 42). Our findings are also in accordance with empirical results revealing that advantages of philopatry often do not offset the costs of delayed reproduction when group-size benefits are absent or weak (45).

Our results demonstrate that different habitat qualities may induce cooperative breeding by divergent selection mechanisms (Fig. 2). Group benefits are more important in harsh environments in which safety in numbers can significantly increase survival prospects, where joint effort may also facilitate the expulsion of predators and defence of resources against competitors. In benign environments, by contrast, helping evolves in connection with indirect fitness benefits obtained by the enhanced production of relatives, even if group survival benefits are the selective driver of philopatry (Fig. 2A). Benign environments bring about habitat saturation, as low mortality results in low breeder replacement rates. Therefore, the main evolutionary driver of philopatry is to queue to inherit the breeding territory. In species that do not follow a gerontocratic succession but instead a scramble contest for the breeding position, this result also holds true (Fig. S4). When survival is high due to philopatry, group size increases and alloparental care raises the number of competitors for the breeding position for which they are queuing. Consequently, in benign environments helping is more likely to evolve where indirect fitness benefits accrue, even if a certain level of dispersal serves to reduce competition for the breeding position among relatives. Previous models similarly predicted that philopatric tendencies leading to group formation vary in accordance with habitat quality (46). In stable and predictable environments, high population density and resulting habitat saturation can lead to a severe shortage of territory openings, while in variable and unpredictable environments the cost of successfully reproducing can be magnified to prohibitive levels, which may select for individuals to remaining as nonbreeders within their natal groups (46). Another model studying ecological pressures in benign and harsh environments, predicted that resource-defense benefits select for cooperative breeding in saturated habitats, while collective action benefits matter in harsh environments in which group size aids to cope with environmental challenges (47, 48). The results presented here hence support a growing consensus that cooperative breeding evolves by alternative mechanisms depending on environmental harshness.

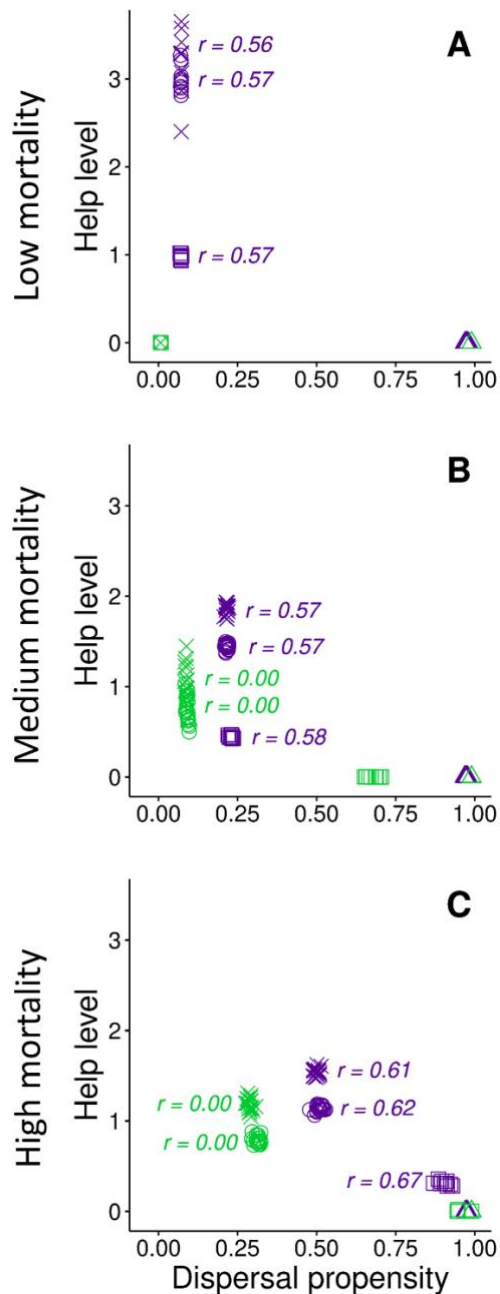


Fig. 2. Effect of survival benefits of group size on dispersal propensity and alloparental care in the presence and absence of relatedness effects, under three different habitat qualities. The evolutionary equilibria for levels of helping and dispersal are shown when group membership benefits through reduced mortality risk are absent (Δ , $x_n = 0$), low (\square , $x_n = 1$), high (\circ , $x_n = 3$), or very high (\times , $x_n = 4$). Relatedness was either an emergent property of the model dynamics (purple), or it was blocked by random shuffling (green; see Methods). Numbers in the figure indicate the levels of relatedness at equilibrium when helping evolved. Different habitat qualities are reflected by three divergent levels of baseline mortality. The results show that survival benefits of group size are the main driver for the evolution of philopatry ($x_n > 0$), while helping evolves mainly due to kin selection or group augmentation depending on environmental conditions. **(A)** In benign environments ($m_0=0.1$) helping can only evolve under kin selection, but some reduction of mortality risk by group membership is still required. **(B and C)** In harsher environments ($m_0=0.2$ or 0.3), helping can evolve due to both kin selection ($x_n = 1 - 4$) and group augmentation ($x_n = 3 - 4$), while mortality risk primarily affects differences in dispersal propensity. Results are shown across 20 replicas to assess

repeatability. Symbols denoting the absence of relatedness when $x_n = 0$ were slightly jittered horizontally to improve visual discrimination. Other input parameters are: $m_d=1$, $f=2$, $x_h=4$, $x_0 = 1.5$, $k_0=1$, $k_h=1$.

The coevolution of helping and philopatry

Group living is a precondition for the emergence of alloparental care. Once group living evolves due to direct survival benefits as shown in the previous section, kin structure builds up, which affects the level of cooperation and competition between group members. Hence it is worth clarifying how helping and philopatry coevolve against the backdrop of relatedness structure. To this end, we first scrutinised the evolution of dispersal rate until reaching its equilibrium, which was satisfied after 25'000 generations for all 20 runs. Concerning the coevolution of helping and dispersal, we found that philopatry is required for the evolution of alloparental care, whereas helping, in turn, selects for higher levels of philopatry in a positive feedback loop (see the "*Age-dependent reaction norms*" section below). This corroborates previous modelling results (19, 20, 49). Our simulation demonstrates that this positive feedback is also maintained by direct group benefits even in the absence of kin selection (Fig. S5). The results were similar both when the evolution of age-dependent reaction norms was considered or not.

Age-dependent reaction norms

The likelihood of subordinates obtaining a breeding position within a group typically varies with their rank or resource holding potential, which may affect their dispersal and helping strategies (10, 50, 51). To investigate this relationship, we analysed a state-dependent model in which individuals show age-dependent plasticity based on reaction norms for their dispersal propensity and alloparental investment. Age is typically linked to competitive ability, hence individuals may adjust their dispersal and helping strategies with time according to changes in their likelihood to obtain breeder status. Evolved reaction norms of dispersal and helping decisions according to different levels of habitat saturation and relatedness are summarised in Figs. 3 & 4.

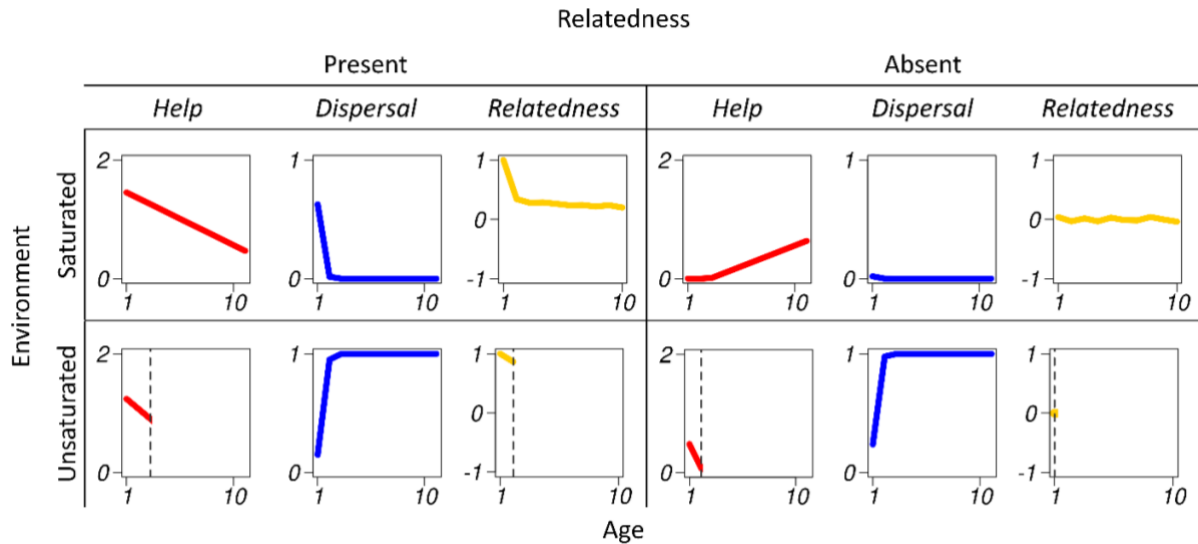


Fig. 3. Evolution of age-dependent reaction norms of dispersal propensity and help levels, at different habitat saturation levels. Age (abscissa) is plotted against helping levels (red), dispersal propensity (blue), and relatedness (yellow) at the equilibrium (mean values from 20 replicas each). Dotted lines in the ‘help’ graphs denote the age from which help is no longer expressed due to the lack of helpers from that age-group onwards. Relatedness per age group is only shown when a sufficient sample was available to calculate relatedness reliably, i.e. until the dotted lines. In saturated habitats (which often coincides with benign environments), subordinates show philopatric tendencies and they may either reduce help with age when kin selection is enabled, or increase help with age when indirect fitness benefits are absent, thereby obtaining long-term group augmentation benefits. In unsaturated habitats (often concurring with harsh environments), subordinates stay philopatric at young age benefitting from a safe haven until they are competitive enough to disperse and breed independently. When relatedness is present, young subordinates obtain indirect fitness benefits by providing help to close kin. Input parameters for saturated habitats are: $f=1$, $m_0=0.2$, $m_d=1$, $x_n=3$, $x_h=4$, $x_0=1.5$, $k_0=1$, $k_h=1$. Input parameters for unsaturated habitats are the same except for: $f=2$, $m_0=0.3$. Input parameters for presence/absence of relatedness are the same for the corresponding condition. Top left panel summarises results from Fig. 5, top right panel from Fig. S5, bottom left panel from Fig. S6 and bottom right panel from Fig. S7.

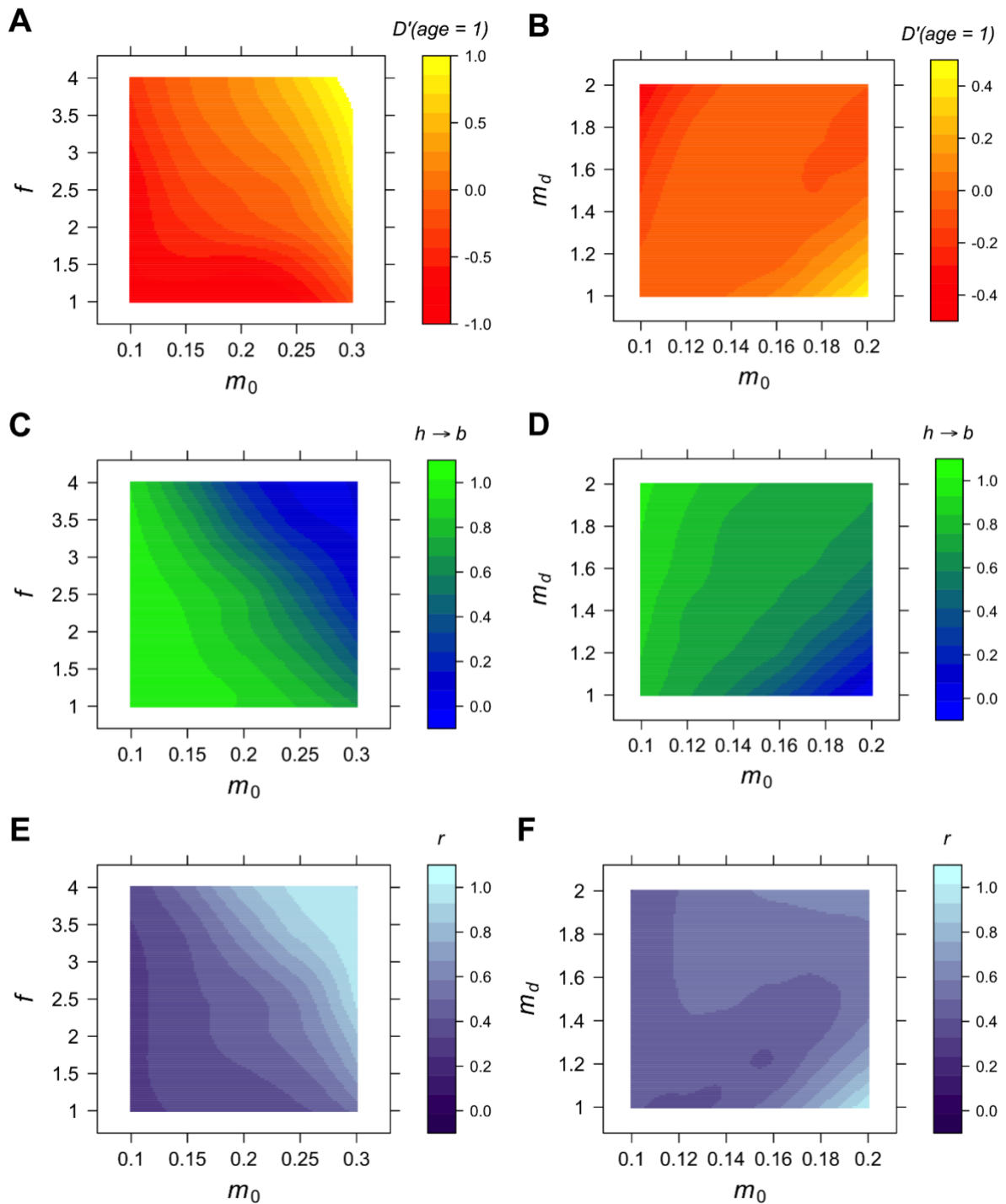


Fig. 4. Evolution of age-dependent reaction norms for dispersal across different ecological scenarios, and the proportion of helpers becoming breeders when relatedness is present. Different levels of habitat saturation were modelled by varying the overall mortality (m_0), the likelihood that floaters find a group to breed (f), and the mortality linked to dispersal (m_d). **A, B:** The derivative of the reaction norm (evaluated at age 1, D'), at the evolutionary equilibrium, determining dispersal propensity was plotted for scenarios when relatedness is present. Positive values of D' denote a positive slope and an increase in dispersal with age, while negative values of D' indicate a decrease in dispersal propensity with age. Both overall mortality (m_0) and group permeability (f) select for positive D' , that is an increase in the level of dispersal with age (**A**). In contrast, mortality associated with dispersal (m_d) has the opposite effect (**B**). In (**C**) and (**D**) we show the proportion of

helpers that become breeders (inheritance of breeding position as opposed to takeover by floaters) at the equilibrium. In (E) and (F) we show relatedness levels between breeders and subordinates in the group at the population level.

High dispersal costs and high environmental quality (reflected by high m_d and low values of m_o and f) result in reduced chances of dispersers to breed outside of their natal group early in life, hence individuals are selected to queue for the breeding position either in their natal or a foreign group (Fig. 3 top-left panel, and Fig. 4 C,D). With the resulting rise in habitat saturation, philopatric tendencies increase with age, i.e. when subordinate group members are more competitive to become breeders (Fig. 4 A,B). High dispersal rates at early life stages instead of staying in the natal territory allow individuals to avoid competing with relatives for the breeding position (Fig. 3 & 4). This result is further supported by the comparison between the two scenarios with and without the build-up of relatedness within groups, since after the removal of relatedness all individuals choose to stay in their home territory to queue for the breeding position (Fig. 3 top panels & Fig. S8). Under these environmental conditions, groups are formed by a mixture of related and unrelated subordinates (Fig. 4 E,F).

Our results indicate that individuals staying in their natal territory should decrease help over time as the degree of relatedness between them and the young declines with their own age due to time-dependent breeder replacement and dispersal dynamics (9) (Fig. 3 top-left panel). Indeed, reduced helping levels with low relatedness have been observed in several cooperative breeders (52). Nevertheless, the opposite tendency may also emerge when other selective forces are at play, such as a pay-to-stay negotiation process (18, 53, 54). Our model further shows that when the build-up of genetic relatedness is prevented, individuals increase help as their likelihood of becoming a breeder increases, thereby obtaining delayed reciprocity benefits if they inherit the territory, a phenomenon referred to as long-term group augmentation benefits (13, 14, 17) (Fig. 3 top-right panel & Fig. S5 B).

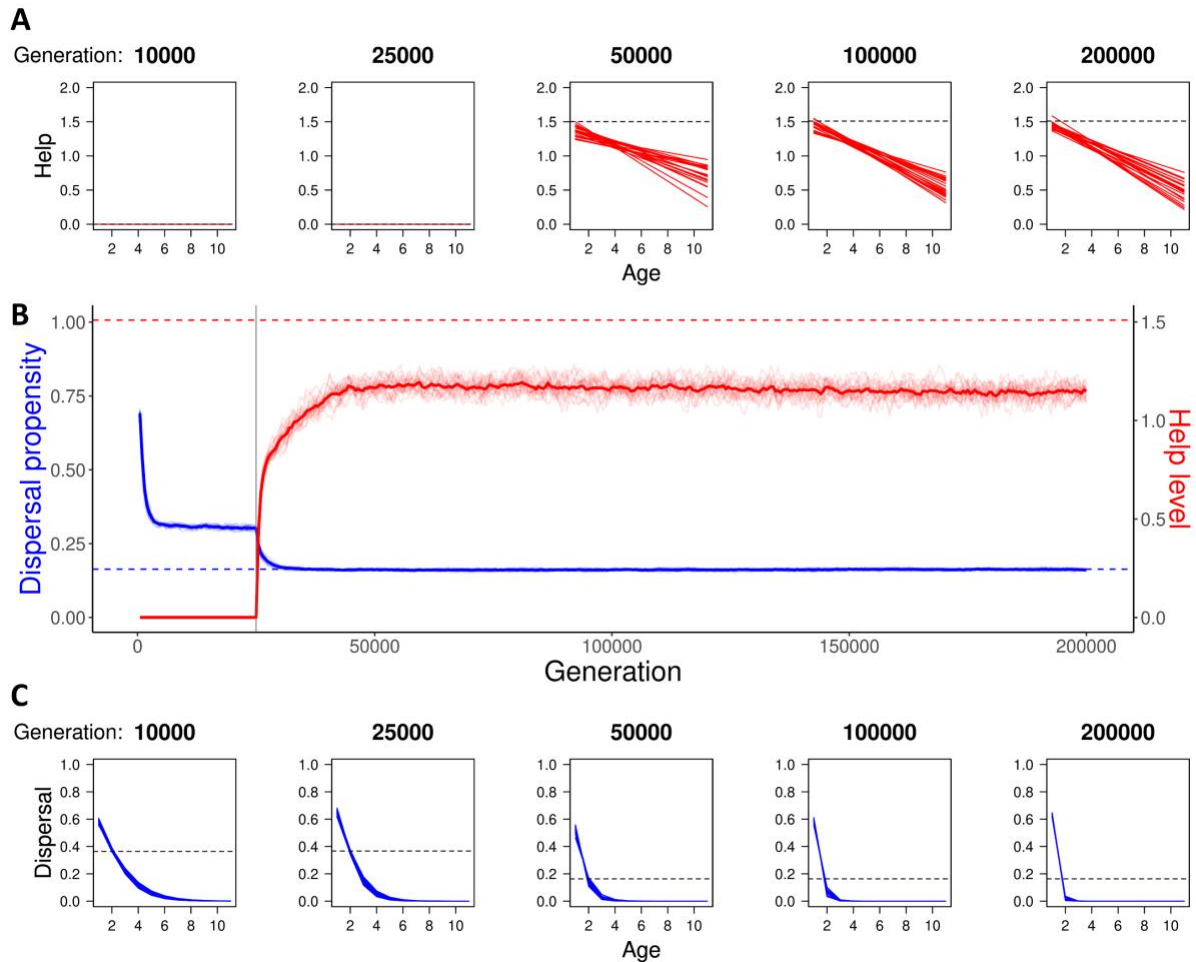


Fig. 5. Evolution of age-dependent reaction norms of philopatry and help levels in a highly saturated habitat when relatedness takes effect and subordinates queue to inherit the breeding position. B: Evolutionary dynamics of helping levels (red lines, right y-axis) and dispersal (blue lines, left y-axis). Bold lines represent the total mean values across replicas, while shades show the mean values for each of the 20 replicas of the stochastic model. Help was allowed to evolve from generation 25000 onwards (grey vertical line). (A and C) Reaction norms of help (A) and dispersal (C) at five different points in time. Dotted lines in all three figures represent the equilibrium values for levels of help and dispersal in the absence of the evolution of reaction norms. The input parameter values are: $f=1$, $m_0=0.2$, $m_d=1$, $x_n=3$, $x_h=4$, $x_0=1.5$, $k_0=1$, $k_h=1$.

When habitat saturation is low (reflecting high values of m_0 and f , and low m_d), individuals disperse from the natal group at some point to become breeders elsewhere. Initially they stay in their natal territory for protection, which serves as a "safe haven", and to help raising related young (Fig. 3 bottom-left & Fig. 4 A,B). At an older age, dispersal propensity increases (Fig. 3 bottom-left & Fig. 4 A,B). Under these environmental conditions, groups are mainly formed by helpers related to the breeding pair (Fig 4 E,F). These results are in line with data from a range of species (2), where delayed dispersal of young yields survival benefits and philopatry prevails until local vacancies become available. In these cases, subordinates may obtain additional indirect fitness benefits by enhancing the production and survival of kin.

Our results show that the evolution of reaction norms for the levels of help and dispersal propensity allow for a well-adjusted response to individual, social and ecological circumstances determining the likelihood of inheriting the territory and the level of relatedness among group members. Importantly, optimal dispersal strategies may vary during an individual's lifetime and across different ecological and social scenarios. When behavioural plasticity is not evolving and environmental properties select for individuals to remain in a group and queue for the breeding position, evolved helping levels are likely suboptimal for a particular age of a subordinate, since behavioural strategies cannot adjust to the dynamics of relatedness among group members (Fig. 5B). Likewise, non-plastic subordinates will show an intermediate tendency for dispersal, reflecting a compromise between the optimal strategies for young and old individuals. In highly saturated habitats, inflexible subordinates exhibit a degree of philopatry that reflects a compromise between queueing for the breeding position and the reduction of kin competition (Fig. 5C). In contrast, in unsaturated environments, non-plastic individuals respond with a low dispersal propensity corresponding to a compromise between remaining in the territory for safe resource use and helping kin, and dispersing for independent breeding (Fig. S6 C).

Our model effectively merges the ecological constraints and benefits of philopatry hypotheses that are often viewed as alternative explanations for the evolution of philopatry. Habitat saturation is a result of environmental variables that affect mortality and dispersal rates rather than an externally determined constraint that favours philopatry (55, 56). Low baseline mortality and constraints to disperse or to find a breeding position lead to both philopatry and saturated habitats as a result, which may generate a positive feedback loop. Therefore, both hypotheses are part of a continuum in which individuals are selected to weigh the odds between achieving independent breeding by dispersing against successfully queueing to inherit the breeding position at home. Additional indirect fitness benefits can then be obtained when individuals delay dispersal from their natal territory and are still related to their current owners.

Discussion

Our results suggest that for the evolution of cooperative breeding, direct benefits of group living are often the main driver of group formation, which is a requirement for the consequent emergence of helping. At the same time, delayed dispersal builds up genetic relatedness within groups, which facilitates the evolution of alloparental care by kin selection in addition to direct fitness benefits derived from enhanced group size. Even though kin selection and group augmentation can both select for the evolution of alloparental care in isolation, the conditions are restrictive and a combination of both mechanisms selecting for cooperative breeding is the most plausible scenario under natural conditions. In contrast to the common view, our model suggests that indirect fitness benefits alone are unlikely to select for the evolution of cooperative breeding without direct survival benefits of group living, seemingly because the prevalence of kin competition prevents the evolution of the relatedness structure that generates sufficient indirect fitness benefits. Apparently, the selection pressures on philopatry are in general not enough to allow high levels of relatedness in Hamilton's rule to favour helping (25, 26). Previous theoretical work has shown that the prevalence of kin competition can prevent the coevolution of philopatry and help (19, 49). In these models, the

hurdle created by kin competition can be overcome by more extreme costs to dispersal, or by kin discrimination (49). Here we show that direct fitness benefits based on grouping can overcome the negative effect of kin competition, which leads to the evolution of philopatry and promotes a positive feedback between philopatry and help, the two pillars of cooperative breeding. At the same time, kin selection facilitates the evolution of alloparental care by relaxing the competition between new recruits and the subordinates queuing for the breeding position in the territory. This points towards the importance of identifying eco-evolutionary feedbacks, in particular when the change in a trait value affects population structure, which in turn modifies the selective pressures on that trait (57, 58). To unravel the evolutionary pathway to cooperative breeding based on the joint action of direct fitness benefits and kin selection, future studies might disentangle the order of appearance of traits along evolutionary time using modern phylogenetically controlled comparative methods, which can unveil the pathway and causal direction relating those traits, while taking into account the ecological context (59).

Our findings highlight that kin selection is likely not the initial evolutionary force behind cooperative breeding despite high relatedness between subordinates and breeders. For instance, in the cooperatively breeding purple-crowned fairy wrens, subordinates feed siblings more than unrelated nestlings, at first glance suggesting that kin selection explains cooperation. However, the effect of relatedness on feeding effort and defence from nest predators varies depending on the probability of inheriting a breeding position, suggesting that long-term group augmentation might in fact explain alloparental investment by subordinate group members (17, 60). In the Florida scrub jay, group size increases territory size, which raises the chances of males splitting off part of the territory for independent breeding (56). Therefore, helping in this species not only increases the production of kin, but also the chances to inherit part of the territory due to the ensuing rise in group size. This points toward the importance of considering multiple hypotheses when investigating the evolution of cooperative breeding (4, 17). The emergence of helping after group formation can result from both group augmentation benefits and kin selection. Direct and mutual fitness benefits from increased group size as a driver of cooperation can explain puzzling phenomena like the ‘kidnapping’ of members from other groups observed in several species (40, 61), or the presence of unrelated helpers within groups (9, 12).

Life history decisions typically vary during an animal’s lifetime (62–64), yet previous studies have generally modelled static behavioural rules. In contrast, our approach allows individuals to evolve age-dependent plasticity in their decisions to disperse and help according to the ecological context. This yields more realistic predictions on fundamental life-history decisions based on the level of habitat saturation, a crucial parameter for the evolution of group living and cooperation. Our results show that cooperative breeding is driven by both grouping and kinship effects, but the prevalence of each mechanism is predicted to vary depending on the quality of the habitat. A comparative study on birds found that while cooperatively breeding starling species tend to occur in harsh and unpredictable environments in which helpers greatly increase the reproductive success of the breeders, cooperatively breeding hornbills tend to live in benign and stable environments where overcoming competition is likely to be the ecological driver for the evolution of cooperation (48).

In highly saturated habitats or where group membership is strongly linked to survival, our model predicts early dispersal to reduce kin competition for the breeding position. Empirical data in a range of taxa are in accordance with the prediction that early dispersal is triggered by breeding competition with siblings. For example, in the hover wasp (*Liostenogaster flavolineata*) and the cooperatively breeding stripe-backed wren (*Campylorhynchus nuchalis*), lower-ranked helpers are the most likely individuals to disperse from their natal nests (65, 66). In fact, in most social mammals, female dispersal from their natal groups seems to be linked to the avoidance of local competition with kin for resources and breeding opportunities (67). Local competition among kin may also explain why in some species subordinates expel siblings from their natal territory (68). Our model further predicts that alloparental care can evolve in low-quality habitats with high mortality rates, where the natal territory serves as a safe haven. Offspring that delay dispersal to benefit from group protection may help to raise kin, thereby gaining indirect fitness benefits while waiting for a safe opportunity to leave for independent reproduction elsewhere. Empirical results from a wide range of animal taxa conform with this prediction, including birds (69), mammals (70) and insects (71). This composite conditionality helps to solve the paradox of environmental quality and sociality, as both benign and harsh environments seem to promote the evolution of sociality, and supports previous predictions on the duality of different selective benefits depending on the harshness of the environment (46–48). A formal test of the predictions from our model would require analyses considering effects of habitat saturation and mortality levels either by experimental manipulation at a species level, or phylogenetically controlled interspecific comparisons.

We would like to point out potential deviations from our model assumptions that might yield different predictions. These deviations can be broadly classified along four lines. First, patterns of genetic relatedness; we assumed asexual reproduction, which affects relatedness. Consequently, in sexual systems, we expect two opposing factors that might balance each other out: avoiding competition with kin may be weaker, whereas avoiding inbreeding may be more important. Second, social determination of the behavioural phenotype; in our model dispersal and help are determined individually, but this is not necessarily the case. For example, rules of dispersal may be different where individuals leaving their home territory form coalitions or join certain groups preferably (72, 73). Third, enforcement and eviction by dominants; we have not included this possibility in our model, which is why it does not account for pay-to-stay negotiation processes (53, 54). Finally, environmental variation; we considered an island population model with random dispersal, overlapping generations and a constant environment in space and time. These assumptions imply that habitat saturation and relatedness levels are maintained roughly constant throughout the life of individuals. In contrast, drastic changes in habitat saturation can occur in predictably seasonal environments. An example of this is the bivoltine model of the evolution of eusociality (57), where the formation of breeding territories takes place at a specific time of the year (spring). Social insects that follow this type of life-history show variation in the tendency to disperse and help, not along an individual's lifetime but among the different generations that complete the life-cycle. Despite the potential effects of these deviations, which might highlight different routes in social evolution, we believe that our model captures a great portion of the variance existing in different cooperatively breeding species under various ecological settings, thereby helping to explain the interplay between dispersal and cooperation that is driven by direct and indirect fitness benefits.

In conclusion, our model suggests that fitness benefits of group living, and not kin selection, are the main driver of the evolution of philopatry, a prerequisite for the emergence of alloparental care. Furthermore, the model indicates that a combination of direct and indirect fitness benefits are the most likely selective pressures behind the evolution of cooperative breeding. We found that a positive feedback relationship between philopatry and alloparental care is maintained by direct group benefits even in the absence of kin selection, while relatedness can be an important promoter of cooperation. Additionally, age-dependent plasticity allows individuals to adjust their dispersal and helping strategies to social and environmental conditions to maximise their own reproductive outcome, while at the same time increasing cooperation and reducing competition among kin. Predictions from the model are met by empirical results from a wide range of taxa, and they can be scrutinized experimentally or by phylogenetically controlled comparisons across different biological systems.

Materials and Methods

We aim to determine circumstances under which subordinate group members gain direct fitness benefits by staying and helping dominants to raise offspring in their group due to survival benefits related to group size. We further examine the interplay of group benefits with the level of relatedness emerging from dispersal dynamics. To this end, we develop an individual-based model in which helping behaviour and dispersal coevolve. We start from an ancestral state featuring the absence of alloparental care and dispersal of all individuals, and assume net benefits of survival from living in larger groups.

Life cycle

We consider an asexual, haploid population consisting of 5000 breeding territories. Each breeding territory consists of a dominant breeder monopolising reproduction and an indefinite number of subordinates, which may aid the breeder's reproduction. In the simulations, groups are initialised with one breeder and three helpers. In the subsequent generations, the number of helpers is determined by the reproduction of the breeders and the dispersal decisions of the offspring. The breeder's fecundity depends on the cumulative level of help provided by the subordinates within the group. The offspring inherit the dispersal and helping tendency from the breeders (see following sections, Fig. 1, step 1). We keep track of the age of individuals in terms of the number of breeding cycles they keep up.

After breeders reproduce, the recently created offspring and subordinates in the group may disperse, and dispersers may settle in another randomly chosen group or remain floaters (Fig. 1, step 2). For simplification, we consider only individual dispersal, not budding dispersal (72, 73). Subordinates choosing to disperse stay in a vagrancy state for at least one generation, until they may bid for a breeding position or decide to join another group as subordinate.

Individuals that stay within a group henceforth express some level of help (Fig. 1, step 3). Since we are interested in the evolution of alloparental care, breeders are exempted from helping. The level of help performed has a survival cost. Note that the decision to help occurs after the decision to disperse, and individuals can potentially evolve a help level of 0. This

reflects the fact that in some species delayed dispersal occurs without the offspring helping their parents (74, 75), suggesting that direct fitness benefits can be sufficient to select for delayed dispersal (46, 76). It also allows for the evolution of solitary life if offspring never remain as subordinates in the group.

Individual-specific survival depends on (i) the environmental conditions, (ii) group membership, (iii) group size, and (iv) the level of help provided to the breeder (*Figure 1, step 4*). Note that the fitness benefits of increasing the breeder's productivity by helping only occur after mortality takes place, ensuring selection acts on the cost of helping before benefits are perceived.

If the breeder in a group dies, all helpers inside the group and a sample of floaters compete for the breeding position (*Fig. 1, step 5*). We let the number of floaters bidding for a breeding position be proportional to the relative abundance of floaters with respect to the number of breeding positions. Specifically, the number of floaters in each bid is given by $f * N_f / N_b$, where N_f and N_b are the numbers of floaters and territories, respectively; and f is a parameter that measures the access floaters have to breeding positions. This implementation reflects the likelihood of floaters to join a new group to breed, for instance, conditioned by spatial viscosity since it is unlikely that floaters can prospect all open breeding spots, or by the acceptance probability of new members. It also accounts for the fact that a high number of floaters in the population results in a higher probability that floaters will visit groups for reproductive purposes. If there are fewer floaters available than the calculated floater bidding sample, all floaters are taken as bidders.

The likelihood of filling an empty breeding position is implemented as a lottery weighted by the age of the candidates, with older individuals having a higher probability, regardless of relatedness levels. This contrasts with previous models assuming that dispersers join the queue for inheritance at the end (13, 77, 78) and conforms with empirical observations (79, 80). Age was chosen as a proxy for RHP since it enables individuals to assess their competitiveness, a simplification allowing for the evolution of a simple rule to adjust helping and dispersal strategies to the likelihood of becoming a breeder. In fact, age has been shown to be a good proxy of rank in a range of species (17, 33, 81), even if it is not universal (82). Nonetheless, other life-history traits related to resource holding potential and rank are likely to produce similar reaction norms based on these alternative traits (e.g. size). If a territory has no breeder or helpers remaining, it is open for takeover from floaters in the same way. Therefore, subordinates may inherit the dominant position within their group (9, 50, 56, 81, 83), but they may also gain a breeding position elsewhere after dispersing to another group (54, 83–85). While floaters may be at a disadvantage compared to helpers due to higher mortality, they enjoy the advantage of being able to sample a larger number of territories in which a breeding vacancy may have opened up (80).

Strategies

In order to assess the rules that govern the evolution of philopatry and the consequent emergence of help, as well as their interaction, we first outline a basic model in which individuals show a fixed strategy for the likelihood to disperse and for the level of help

provided throughout their life. We then incorporate age-dependent plasticity by letting dispersal and help to be determined by reaction norms. The coevolution of the reaction norms, determining philopatry and help, will be driven by the likelihood of obtaining a breeding position.

Fixed strategies of help and dispersal

We first model the coevolution of philopatry and help in which individuals express a fixed strategy throughout life. Help levels and dispersal propensity are quantitative phenotypic traits in the basic model, the values of which are solely determined by different alleles of a gene locus. In this basic model, the phenotypic dispersal propensity equals the allelic value of the gene β applying boundaries between 0 and 1. Likewise, the phenotypic value of help equals the allelic value of the gene α . If α takes negative values, help = 0. Additionally, to assess the influence of the evolution of help on philopatry, the mutation rate μ is initially set to 0 for α during the first 25000 generations until the population reaches an equilibrium for the dispersal propensity, and then μ is reset to allow for the evolution of help (Table 1). Initial values of $\alpha = 0$ and $\beta = 1$.

Reaction norms of help and philopatry

In addition, we construct a model in which the coevolution of help and philopatry is governed by behavioural reaction norms that allow individuals to express different levels of help and dispersal depending on their age. The dispersal likelihood D takes a logistic function with boundaries between 0 and 1 as given in Eq. 1.

$$D = \frac{1}{1 + \exp(-\beta_t t - \beta_0)} \quad (1)$$

The dispersal propensity is, therefore, conditioned by the age t of the individual, the gene β_t that modifies the strength and direction of the effect of age on the likelihood to disperse, and the gene β_0 that acts as the intercept (Table 1). The level of help H is conditioned by the age of the individual, the gene α_t that modifies the strength and direction of the effect of age on helping levels, and the gene α_0 that is the baseline in the linear function for the levels of help provided as given in Eq. 2.

$$H = \alpha_0 + \alpha_t t \quad (2)$$

If the level of help resulting from the function is negative, the phenotypic level of help = 0. As in the model of the fixed strategies for dispersal and help previously described, the mutation rate μ is initially set to 0 for α_0 and α_t during the first 25000 generations until the population reaches an equilibrium for the dispersal propensity, and then μ is reset to allow for the evolution of help. Initial values are $\alpha_0 = \alpha_t = \beta_t = 0$ and $\beta_0 = 1$.

Survival and reproduction

In each cycle some individuals die, according to baseline mortality (m_0), group membership and help provided to dominants. Higher values of m_0 indicate higher overall mortality for all

individuals in the population, where $1 - m_0$ is the maximum survival likelihood of the individuals in the population. In addition, the survival probability of breeders (S_B) and subordinates (S_H) depends on group size N , while the survival probability of a helper also depends on the cost of the amount of alloparental care H provided. Dispersers survive with probability S_F that depends on m_0 and an additional mortality attained to dispersal m_d , since several studies demonstrated higher mortality for dispersers by several orders of magnitude (76, 86). These survival probabilities are given by the logistic Eq. 3.

$$S_B = \frac{1-m_0}{1+\exp(x_0-x_n N)} \quad (3.1)$$

$$S_H = \frac{1-m_0}{1+\exp(x_0+x_h H-x_n N)} \quad (3.2)$$

$$S_F = \frac{1-m_0 m_d}{1+\exp(x_0)} \quad (3.3)$$

where x_n is a scaling parameter that quantifies the effect size of the benefit of group size in survival, x_h the effect size of the cost of helping, and x_0 an intercept.

Reproduction in a territory is monopolised by the breeder, and it is asexual. We assume that the breeders' fecundity is a random value drawn from a Poisson distribution with mean K . K depends on the baseline fecundity (k_0) and the cumulative level of help provided by the helpers in the group assuming diminishing fecundity returns as given in the Eq. 4.

$$K = k_0 + \frac{k_h \sum_{i=1}^{i=n} H_i}{1 + \sum_{i=1}^{i=n} H_i} \quad (4)$$

where k_h is a scaling parameter that quantifies the effect size of the cumulative help of subordinates on the fecundity of the breeder.

Offspring inherit the alleles from their parent unless mutations occur. Mutations occur independently at each of the loci, at a low rate ($\mu = 0.05$) per locus and reproduction event. Mutations change slightly the value of an allele inherited from the parent by adding a value drawn from a normal distribution with mean 0 and a standard deviation of $\sigma_\mu = 0.04$ (Table 1); hence the allelic value in the offspring is similar to the parental allele.

Relatedness

To calculate the coefficient of relatedness between the breeder and subordinates in a group, we track the evolution of another locus that changes exclusively by genetic drift. As in the phenotypic loci, alleles in this neutral locus represent different numerical values that are inherited from parent to offspring and modified by mutational processes. Relatedness between breeders and helpers in a group is calculated as the coefficient of a linear regression between the allelic values of the breeders and helpers for the neutral gene (87).

To distinguish the effect of kin selection from group augmentation, we created a parallel model for comparison in which individuals just born (i.e. age = 1) that decide to stay in the natal group as subordinates (Fig. 1, step 2) are shuffled to another group of the same size without passing through a dispersal phase, thereby removing relatedness from the model without interfering with dispersal patterns or group sizes. Although this implementation removes the effect of relatedness on the evolution of alloparental care, there might be a selective force to be philopatric at age 1 that involves indirect fitness benefits, as the decision to refrain from dispersing will impact the effective group size of the related breeder positively. To assess this potential effect we implemented a model in which the shuffling of the newborn helpers was done to random groups regardless whether they also produced offspring, while blocking the evolution of helping. Effects of the potential cryptic kin selection influence on selection for philopatry as outlined above were mainly restricted to environments with medium mortality rates at low group size benefits and, therefore, do not greatly impact the conclusions of our model (see Fig. S9).

Table 1. Overview of notation.

Symbol	Meaning
f	Mean number of groups a floater samples for becoming a breeder
N	Group size
N_f	Total number of floaters
N_b	Number of breeding territories
t	Age as a proxy of RHP
β	Genetic propensity to disperse, without reaction norm to age
β_0	Intercept in the dispersal reaction norm
β_t	Effect size of age on dispersal when reaction norms evolve
D	Dispersal propensity
α	Genetic predisposition to help without reaction norm to age
α_0	Level of help independent of age when reaction norms evolve
α_t	Linear effect size of age on help when reaction norms evolve
H	Level of help provided to the breeder
m_0	Baseline mortality
m_d	Multiplier of the baseline mortality for dispersers
x_0	Intercept in the survival function
x_h	Effect size of the cost of help in terms of survival
x_n	Effect size of the benefit of group size in terms of survival
S	Survival rate
k_0	Fecundity of the breeder when no help is provided
k_h	Effect size of the cumulative help of subordinates on the fecundity of the breeder
K	Fecundity of the breeder
μ	Mutation rate
σ_μ	Mutation step size

References

1. J. C. Choe, B. J. Crespi, *The evolution of mating systems in insects and arachnids* (Cambridge University Press, 1997).
2. W. D. Koenig, J. L. Dickinson, *Cooperative Breeding in Vertebrates* (Cambridge University Press, Cambridge, 2016).
3. M. Taborsky, Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Advances in the Study of Behavior*. **23**, e100 (1994).
4. T. H. Clutton-Brock, Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* (1979). **296**, 69–72 (2002).
5. A. Griffin, S. A. West, Kin selection: fact and fiction. *Trends in Ecology and Evolution*. **17**, 15–21 (2002).
6. W. O. H. Hughes, B. P. Oldroyd, M. Beekman, F. L. W. Ratnieks, Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* (1979). **320**, 1213–1216 (2008).
7. C. K. Cornwallis, S. A. West, K. E. Davis, A. S. Griffin, Promiscuity and the evolutionary transition to complex societies. *Nature*. **466**, 969–972 (2010).
8. D. Lukas, T. H. Clutton-Brock, Cooperative breeding and monogamy in mammalian societies. *Proceedings of the Royal Society B: Biological Sciences*. **279**, 2151–2156 (2012).
9. P. Dierkes, D. Heg, M. Taborsky, E. Skubic, R. Achmann, Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters*. **8**, 968–975 (2005).
10. J. Field, E. Leadbeater, Cooperation between non-relatives in a primitively eusocial paper wasp, *Polistes dominula*. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **371**, 20150093 (2016).
11. G. S. Wilkinson, G. G. Carter, K. M. Bohn, D. M. Adams, Non-kin cooperation in bats. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **371**, 20150095 (2016).
12. C. Riehl, Evolutionary routes to non-kin cooperative breeding in birds. *Proceedings of the Royal Society B: Biological Sciences*. **280** (2013), doi:10.1098/rspb.2013.2245.
13. H. Kokko, R. A. Johnstone, T. H. Clutton-Brock, The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B: Biological Sciences*. **268**, 187–196 (2001).
14. S. A. Kingma, P. Santema, M. Taborsky, J. Komdeur, Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution*. **29**, 476–484 (2014).
15. T. H. Clutton-Brock, D. Gaynor, G. M. McIlrath, A. D. C. Maccoll, R. Kansky, P. Chadwick, M. Manser, J. D. Skinner, P. N. M. Brotherton, Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *Journal of Animal Ecology*. **68**, 672–683 (1999).
16. D. Heg, L. Brouwer, Z. Bachar, M. Taborsky, Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour*. **142**, 1615–1641 (2005).

17. S. A. Kingma, M. L. Hall, A. Peters, Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist*. **177**, 486–495 (2011).
18. M. Taborsky, M. A. Cant, J. Komdeur, *The Evolution of Social Behaviour* (Cambridge University Press, 2021).
19. J. F. Le Galliard, R. Ferrière, U. Dieckmann, Adaptive evolution of social traits: Origin, trajectories, and correlations of altruism and mobility. *American Naturalist*. **165**, 206–224 (2005).
20. C. Mullon, L. Keller, L. Lehmann, Social polymorphism is favoured by the co-evolution of dispersal with social behaviour. *Nature Ecology and Evolution*. **2**, 132–140 (2018).
21. M. E. Hochberg, D. J. Rankin, M. Taborsky, The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. *BMC Evolutionary Biology*. **8**, 238 (2008).
22. W. D. Hamilton, The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*. **7**, 17–52 (1964).
23. S. A. West, A. S. Griffin, A. Gardner, Evolutionary Explanations for Cooperation. *Current Biology*. **17**, 661–672 (2007).
24. W. D. Hamilton, R. M. May, Dispersal in stable habitats. *Nature*. **269**, 578–581 (1977).
25. P. D. Taylor, Altruism in viscous populations - an inclusive fitness model. *Evolutionary Ecology*. **6**, 352–356 (1992).
26. S. A. West, I. Pen, A. S. Griffin, Cooperation and competition between relatives. *Science* (1979). **296**, 72–75 (2002).
27. T. G. Platt, J. D. Bever, Kin competition and the evolution of cooperation. *Trends in Ecology and Evolution*. **24**, 370–377 (2009).
28. J. Clobert, J. le Galliard, J. Cote, S. Meylan, M. Massot, Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*. **12**, 197–209 (2009).
29. S. Jacob, P. Wehi, J. Clobert, D. Legrand, N. Schtickzelle, M. Huet, A. Chaine, Cooperation-mediated plasticity in dispersal and colonization. *Evolution: International Journal of Organic Evolution*. **70**, 2336–2345 (2016).
30. J. W. Pepper, B. B. Smuts, A mechanism for the evolution of altruism among nonkin: Positive assortment through environmental feedback. *American Naturalist*. **160**, 205–213 (2002).
31. G. A. Parker, Assessment strategy and the evolution of fighting behaviour. *Journal of Theoretical Biology*. **47**, 223–243 (1974).
32. J. L. Brown, E. R. Brown, J. Sedransk, Dominance, Age, and Reproductive Success in a Complex Society: A Long-Term Study of the Mexican Jay. *Auk*. **114**, 279–286 (1997).
33. M. Taborsky, in *Cooperative Breeding in Vertebrates*, W. D. Koenig, J. L. Dickinson, Eds. (Cambridge University Press, Cambridge, 2016), pp. 272–293.
34. A. Bang, R. Gadagkar, Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*. *Proceedings of the National Academy of Sciences*. **109**, 14494–14499 (2012).

35. J. P. Rood, Group size, survival, reproduction, and routes to breeding in dwarf mongooses. *Animal Behaviour*. **39**, 566–572 (1990).
36. S. A. Kingma, M. L. Hall, E. Arriero, A. Peters, Multiple benefits of cooperative breeding in purple-crowned fairy-wrens: a consequence of fidelity? *Journal of Animal Ecology*. **79**, 757–768 (2010).
37. L. Brouwer, D. Heg, M. Taborsky, Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behavioral Ecology*. **16**, 667–673 (2005).
38. P. H. W. Biedermann, M. Taborsky, Larval helpers and age polyethism in ambrosia beetles. *Proceedings of the National Academy of Sciences*. **108**, 17064–17069 (2011).
39. M. Salomon, Y. Lubin, Cooperative breeding increases reproductive success in the social spider *Stegodyphus dumicola* (Araneae, Eresidae). *Behavioral Ecology and Sociobiology*. **61**, 1743–1750 (2007).
40. R. G. Heinsohn, Kidnapping and reciprocity in cooperatively breeding white-winged choughs. *Animal Behaviour*. **41**, 1097–1100 (1991).
41. M. Z. Khan, J. R. Walters, Effects of helpers on breeder survival in the red-cockaded woodpecker (*Picoides borealis*). *Behavioral Ecology and Sociobiology*. **51**, 336–344 (2002).
42. F. Courchamp, B. Grenfell, T. H. Clutton-Brock, Population dynamics of obligate cooperators. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. **266**, 557–563 (1999).
43. M. Briga, I. Pen, J. Wright, Care for kin: within-group relatedness and allomaternal care are positively correlated and conserved throughout the mammalian phylogeny. *Biology Letters*. **8**, 533–536 (2012).
44. A. S. Griffin, S. A. West, Kin Discrimination and the Benefit of Helping in Cooperatively Breeding Vertebrates. *Science* (1979). **302**, 634–636 (2003).
45. P. B. Stacey, J. D. Ligon, The benefits-of-philopatry hypothesis for the evolution of cooperative breeding: variation in territory quality and group size effects. *American Naturalist*. **137**, 831–846 (1991).
46. S. T. Emlen, The evolution of helping. I. An ecological constraints model. *The American Naturalist*. **119**, 29–39 (1982).
47. S. F. Shen, S. T. Emlen, W. D. Koenig, D. R. Rubenstein, The ecology of cooperative breeding behaviour. *Ecology Letters*. **20**, 708–720 (2017).
48. Y.-H. Lin, S.-F. Chan, D. R. Rubenstein, M. Liu, S.-F. Shen, Resolving the paradox of environmental quality and sociality: the ecological causes and consequences of cooperative breeding in two lineages of birds. *The American Naturalist*. **194**, 207–216 (2019).
49. N. Perrin, L. Lehmann, Is sociality driven by the costs of dispersal or the benefits of philopatry? A role for kin-discrimination mechanisms. *American Naturalist*. **158**, 471–483 (2001).
50. S. Balshine-Earn, F. C. Neat, H. Reid, M. Taborsky, Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology*. **9**, 432–438 (1998).

51. M. A. Cant, Patterns of helping effort in co-operatively breeding banded mongooses (*Mungos mungo*). *Journal of Zoology*. **259**, 115–121 (2003).
52. J. Komdeur, The effect of kinship on helping in the cooperative breeding seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society B: Biological Sciences*. **256**, 47–52 (1994).
53. A. E. Quiñones, G. S. van Doorn, I. Pen, F. J. Weissing, M. Taborsky, Negotiation and appeasement can be more effective drivers of sociality than kin selection. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **371**, 20150089 (2016).
54. R. Bergmüller, D. Heg, M. Taborsky, Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences*. **272**, 325–331 (2005).
55. H. Kokko, P. Lundberg, Dispersal, migration, and offspring retention in saturated habitats. *American Naturalist*. **157**, 188–202 (2001).
56. G. E. Woolfenden, J. W. Fitzpatrick, The Inheritance of Territory in Group-Breeding Birds. *BioScience*. **28**, 104–108 (1978).
57. A. E. Quiñones, I. Pen, A unified model of Hymenopteran preadaptations that trigger the evolutionary transition to eusociality. *Nature Communications*. **8**, 1–13 (2017).
58. T. W. Fawcett, B. Kuijper, F. J. Weissing, I. Pen, Sex-ratio control erodes sexual selection, revealing evolutionary feedback from adaptive plasticity. *Proceedings of the National Academy of Sciences*. **108**, 15925–15930 (2011).
59. A. Gonzalez-Voyer, A. Von Hardenberg, in *Modern phylogenetic comparative methods and their application in evolutionary biology* (Springer, 2014), pp. 201–229.
60. N. Teunissen, S. A. Kingma, M. Fan, M. J. Roast, A. Peters, Context-dependent social benefits drive cooperative predator defense in a bird. *Current Biology*. **31**, 4120–4126 (2021).
61. J. B. Silk, Kidnapping and female competition among captive bonnet macaques. *Primates*. **21**, 100–110 (1980).
62. B. Fischer, G. S. van Doorn, U. Dieckmann, B. Taborsky, The evolution of age-dependent plasticity. *American Naturalist*. **183**, 108–125 (2014).
63. M. A. Cant, J. Field, Helping effort and future fitness in cooperative animal societies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. **268**, 1959–1964 (2001).
64. A. M. M. Rodrigues, Demography, life history, and the evolution of age-dependent social behaviour. *Journal of Evolutionary Biology*. **31**, 1340–1353 (2018).
65. J. Field, G. Shreeves, S. Sumner, Group size, queuing and helping decisions in facultatively eusocial hover wasps. *Behavioral Ecology and Sociobiology*. **45**, 378–385 (1999).
66. S. Zack, K. N. Rabenold, Assessment, age and proximity in dispersal contests among cooperative wrens: field experiments. *Animal Behaviour*. **38**, 235–247 (1989).
67. T. H. Clutton-Brock, D. Lukas, The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology*. **21**, 472–492 (2012).
68. D. Strickland, Juvenile dispersal in gray jays: dominant brood member expels siblings from natal territory. *Canadian Journal of Zoology*. **69**, 2935–2945 (1991).

69. L. A. Hunter, Acquisition of territories by floaters in cooperatively breeding purple gallinules. *Anim Behav.* **35**, 402–410 (1987).
70. R. Mares, A. J. Young, T. H. Clutton-Brock, Individual contributions to territory defence in a cooperative breeder: weighing up the benefits and costs. *Proceedings of the Royal Society B: Biological Sciences.* **279**, 3989–3995 (2012).
71. J. Korb, S. Schmidinger, Help or disperse? Cooperation in termites influenced by food conditions. *Behavioral Ecology and Sociobiology.* **56**, 89–95 (2004).
72. S. P. Sharp, M. Simeoni, B. J. Hatchwell, Dispersal of sibling coalitions promotes helping among immigrants in a cooperatively breeding bird. *Proceedings of the Royal Society B: Biological Sciences.* **275**, 2125–2130 (2008).
73. A. M. M. Rodrigues, T. B. Taylor, Ecological and demographic correlates of cooperation from individual to budding dispersal. *Journal of Evolutionary Biology.* **31**, 1058–1070 (2018).
74. J. Ekman, B. Sklepkovych, H. Tegelstrom, Offspring retention in the Siberian jay (*Perisoreus infaustus*): the prolonged brood care hypothesis. *Behavioral Ecology.* **5**, 245–253 (1994).
75. D. J. Green, A. Cockburn, Post-fledging care, philopatry and recruitment in brown thornbills. *Journal of Animal Ecology.* **70**, 505–514 (2001).
76. D. Heg, Z. Bachar, L. Brouwer, M. Taborsky, Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences.* **271**, 2367–2374 (2004).
77. G. Wild, J. Korb, Evolution of delayed dispersal and subsequent emergence of helping, with implications for cooperative breeding. *Journal of Theoretical Biology.* **427**, 53–64 (2017).
78. H. Kokko, R. A. Johnstone, Social queuing in animal societies: A dynamic model of reproductive skew. *Proceedings of the Royal Society B: Biological Sciences.* **266**, 571–578 (1999).
79. S. P. Doolan, D. W. Macdonald, Dispersal and extra-territorial prospecting by slender-tailed meerkats (*Suricata suricatta*) in the south-western Kalahari. *Journal of Zoology.* **240**, 59–73 (1996).
80. A. Jungwirth, J. Walker, M. Taborsky, Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Animal Behaviour.* **106**, 107–114 (2015).
81. C. Bridge, J. Field, Queuing for dominance: Gerontocracy and queue-jumping in the hover wasp *Liostenogaster flavolineata*. *Behavioral Ecology and Sociobiology.* **61**, 1253–1259 (2007).
82. S. Sumner, H. Kelstrup, D. Fanelli, Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proceedings of the Royal Society B: Biological Sciences.* **277**, 1721–1728 (2010).
83. E. M. Russell, I. Rowley, Philopatry or dispersal: competition for territory vacancies in the splendid fairy-wren, *Malurus splendens*. *Animal Behaviour.* **45**, 519–539 (1993).
84. J. R. Walters, P. D. Doerr, J. H. Carter III, The cooperative breeding system of the red-cockaded woodpecker. *Ethology.* **78**, 275–305 (1988).
85. M. Zöttl, L. Chapuis, M. Freiburghaus, M. Taborsky, Strategic reduction of help before dispersal in a cooperative breeder. *Biology Letters.* **9**, 20120878 (2013).

86. J. R. Lucas, P. M. Waser, S. R. Creel, Death and disappearance: estimating mortality risks associated with philopatry and dispersal. *Behavioral Ecology*. **5**, 135–141 (1994).
87. A. Gardner, S. A. West, G. Wild, The genetical theory of kin selection. *Journal of Evolutionary Biology*. **24**, 1020–1043 (2011).

Acknowledgements: We thank Tobias Zobrist for help with the coding implementation, and the editor and two anonymous referees for very helpful comments on earlier versions of this manuscript. We acknowledge the use of the University of Bern HPC cluster UBELIX (<http://www.id.unibe.ch/hpc>) in carrying out the simulations.

Funding: Funding was provided by SNSF-grant 31003A 176174 to Michael Taborsky.

Author contributions: I.G-R., A.Q. and M.T. designed the study and wrote the manuscript. The model simulations were set up and run by I.G-R.

Competing interests: The authors declare no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Custom code used to generate the simulations is available at <https://zenodo.org/record/5862148>.

Supplementary materials

The supplement materials include:

Fig. S1. Cooperative breeding arising solely by kin selection ($x_n = 0$), in different ecological scenarios.

Fig. S2. Evolution of helping and dispersal by group augmentation when effects of alloparental care cannot be inherited by the new breeder (i.e., the former helper).

Fig. S3. Effects of delayed sexual maturity on the competition between helpers and new recruits in harsh environments.

Fig. S4. Evolution of helping and dispersal when modelling a scramble context to inherit the breeding position.

Fig. S5. Evolution of age-dependent reaction norms of philopatry and help levels in highly saturated habitats when relatedness effects are excluded and subordinates queue to inherit the breeding position.

Fig. S6. Evolution of age-dependent reaction norms of philopatry and help in unsaturated habitats when relatedness takes effect and subordinates delay dispersal due to safe haven effects.

Fig. S7. Evolution of age-dependent reaction norms of philopatry and help in unsaturated habitats when relatedness is excluded and subordinates delay dispersal due to safe haven effects.

Fig. S8. Evolution of age-dependent reaction norms for dispersal across different ecological scenarios, and the proportion of helpers becoming breeders when relatedness is excluded.

Fig. S9. Evolution of dispersal for different implementations of the shuffling algorithm when removing relatedness in the benchmark model.

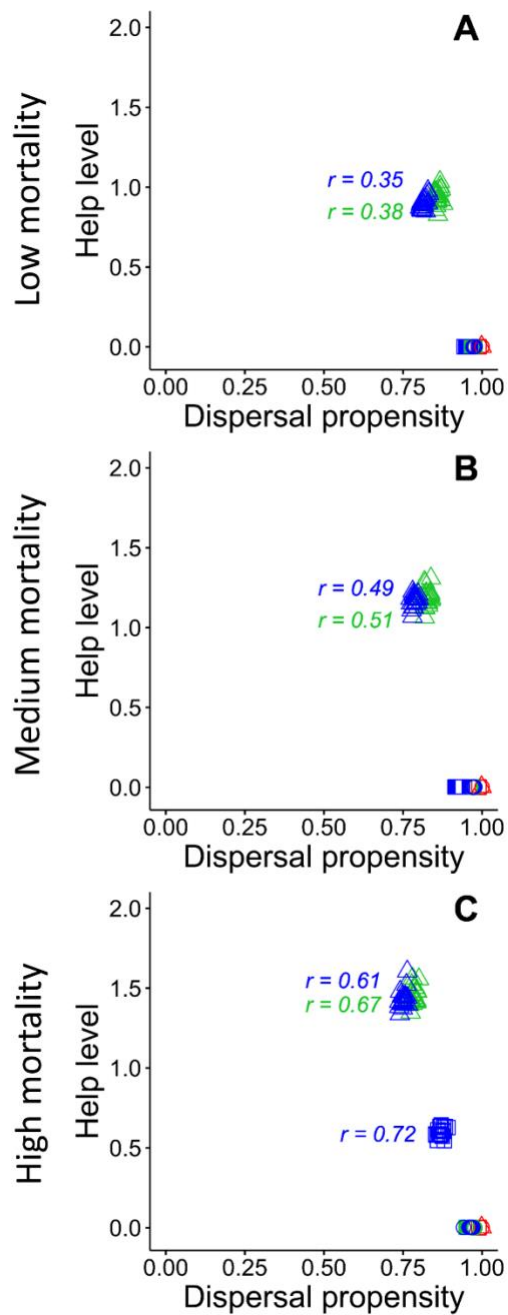


Fig. S1. Cooperative breeding arising solely by kin selection ($x_n = 0$), in different ecological scenarios. The evolutionary equilibria for levels of helping and dispersal are shown for different levels of cost of help: low (Δ , $x_h = 0.5$), medium (\square , $x_h = 2$), and high (\circ , $x_h = 4$), and for different effects of help for the fecundity of the breeder: low (red, $k_h = 1$), medium (green, $k_h = 2$), and high (blue, $k_h = 3$). Numbers in the figure indicate the levels of relatedness for evolutionary equilibria of helping. Different habitat qualities are represented by variations in baseline mortality: (A) benign environment ($m_0=0.1$), (B) medium environment ($m_0=0.2$), and harsh environment ($m_0=0.3$). Results show that low costs of help together with large effects on the productivity of the breeder are needed for cooperative breeding to evolve solely through kin selection, and that high levels of philopatry cannot evolve without involvement of concurrent alternative mechanisms (such as group size benefits). Results are shown across 20 replicas to assess repeatability. Other input parameters are: $m_d=1$, $f=2$, $x_0=1.5$, $k_0=1$.

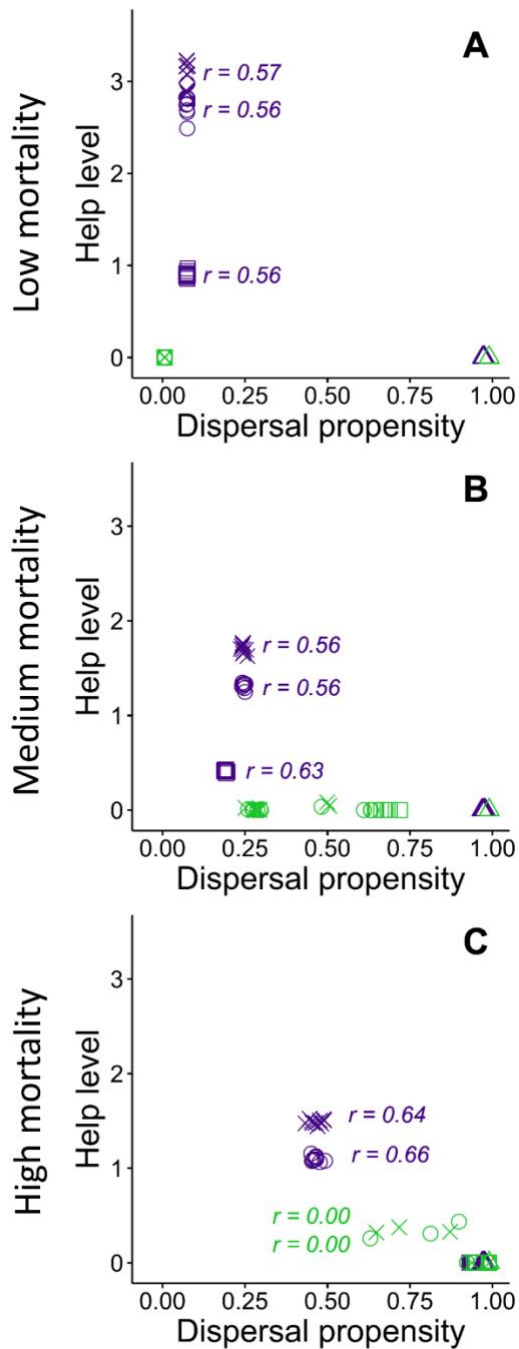


Fig. S2. Evolution of helping and dispersal by group augmentation when effects of alloparental care cannot be inherited by the new breeder (i.e., the former helper). The evolutionary equilibria for levels of direct brood care and dispersal are shown when group benefits through reduced mortality risk are absent (Δ , $x_n = 0$), low (\square , $x_n = 1$), medium (\circ , $x_n = 3$), or high (\times , $x_n = 4$). Relatedness was either an emergent property of the model dynamics (purple) or it was blocked (green). Numbers in the figure indicate the levels of relatedness for evolutionary equilibria of helping. Different habitat qualities are represented by variations in baseline mortality: (A) benign environment ($m_0=0.1$), (B) medium environment ($m_0=0.2$), and (C) harsh environment ($m_0=0.3$). Results reveal that group augmentation alone selects for helping sometimes in very harsh environments, but it seems unstable though time. Results are shown across 20 replicas to assess repeatability. Symbols denoting absence of relatedness when $x_n = 0$ were slightly jittered horizontally to improve visual discrimination. Same parameter inputs as in Fig. 2.

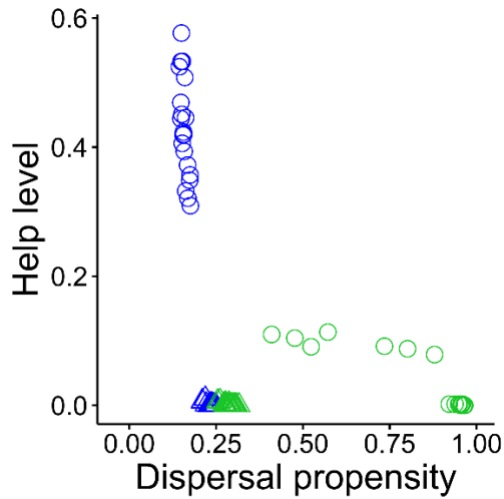


Fig. S3. Effects of delayed sexual maturity on the competition between helpers and new recruits in harsh environments. The evolutionary equilibria for levels of direct brood care and dispersal are shown for a delay in the age of sexual maturity (blue, minimum age to become breeder = 2), or no delay (green, minimum age to become breeder = 1). Values are shown for medium (Δ , $m_0=0.2$), and high (\circ , $m_0=0.3$) levels of environmental harshness. Delaying the age of reproduction decouples the effects from enhanced productivity caused by the help provided and from the concomitant increment of new competitors for the breeding position, thereby promoting the evolution of alloparental care by direct fitness benefits (i.e. group augmentation). Results are shown across 20 replicas to assess repeatability. Other input parameters are: $m_d=1$, $f=2$, $x_h=4$, $x_n=4$, $x_0=1.5$, $k_0=1$, $k_h=3$ and no relatedness was allowed to build up.

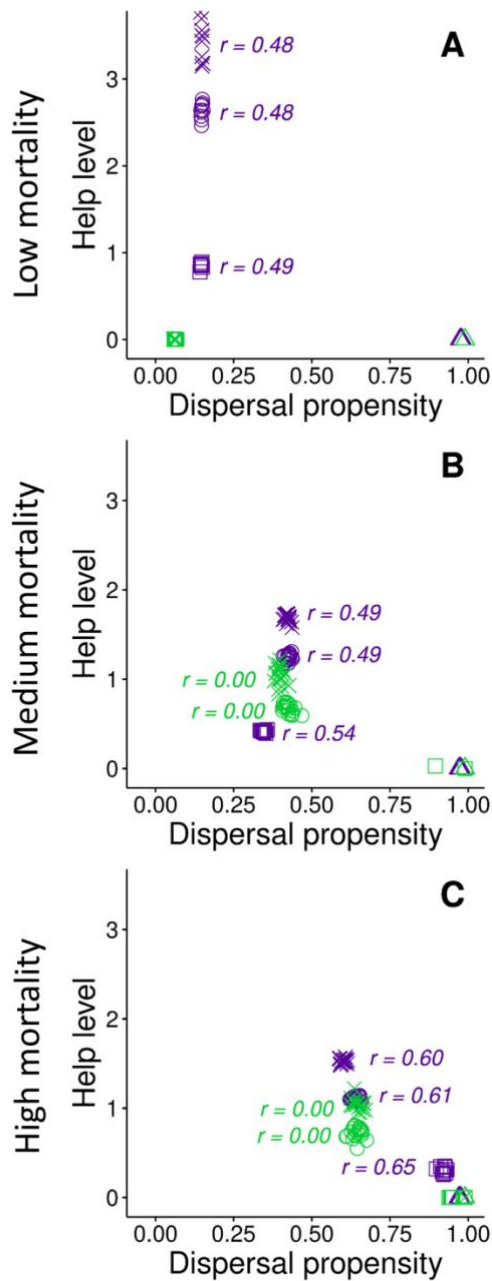


Fig. S4. Evolution of helping and dispersal when modelling a scramble context to inherit the breeding position. The evolutionary equilibria for levels of direct brood care and dispersal are shown when group benefits through reduced mortality risk are absent (Δ , $x_n = 0$), low (\square , $x_n = 1$), medium (\circ , $x_n = 3$), or high (\times , $x_n = 4$). Relatedness was either an emergent property of the model dynamics (purple) or it was blocked (green). Numbers in the figure indicate the levels of relatedness for evolutionary equilibria of helping. Different habitat qualities are represented by variations in baseline mortality: (A) benign environment ($m_0=0.1$), (B) medium environment ($m_0=0.2$), and (C) harsh environment ($m_0=0.3$). Results reveal that a scramble context for the breeding position instead of a gerontocratic context (Fig.2), does not change qualitatively the conclusions of the model. Results are shown across 20 replicas to assess repeatability. Symbols denoting absence of relatedness when $x_n = 0$ were slightly jittered horizontally to improve visual discrimination. Same parameter inputs as in Fig. 2.

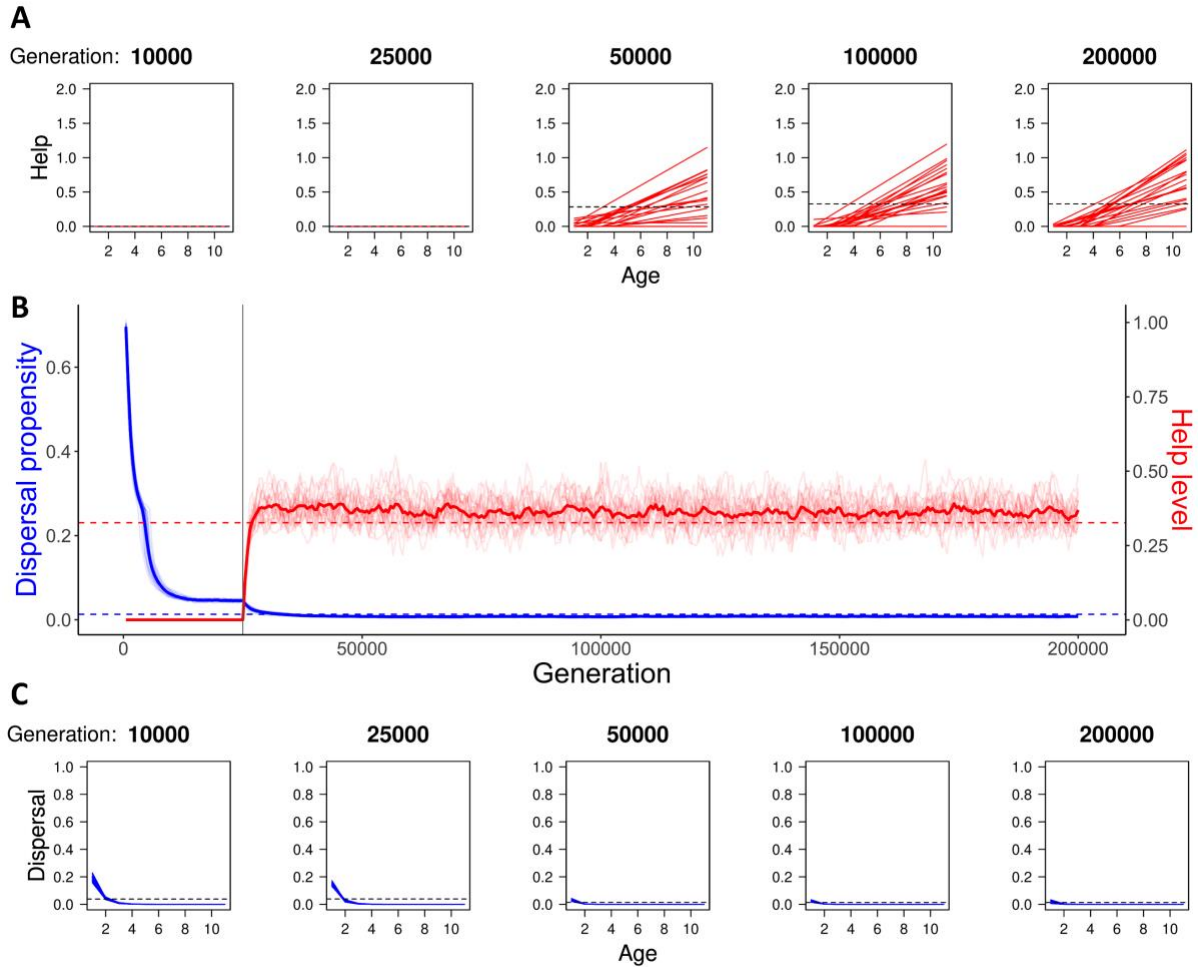


Fig. S5. Evolution of age-dependent reaction norms of philopatry and help levels in highly saturated habitats when relatedness effects are excluded and subordinates queue to inherit the breeding position. (B) Evolutionary dynamics of helping levels (red lines, right y-axis) and dispersal (blue lines, left y-axis). Bold colour lines represent the total mean values across replicas, while shades show the mean values for each of the 20 replicas of the stochastic model. Help was allowed to evolve from generation 25000 (grey vertical line). (A and C) Reaction norms of help (A) and dispersal (C) at five different points in time. Dotted lines in all three figures represent the equilibrium values for levels of help and dispersal in the absence of the evolution of reaction norms. The input parameter values are the same as in Fig. 5.

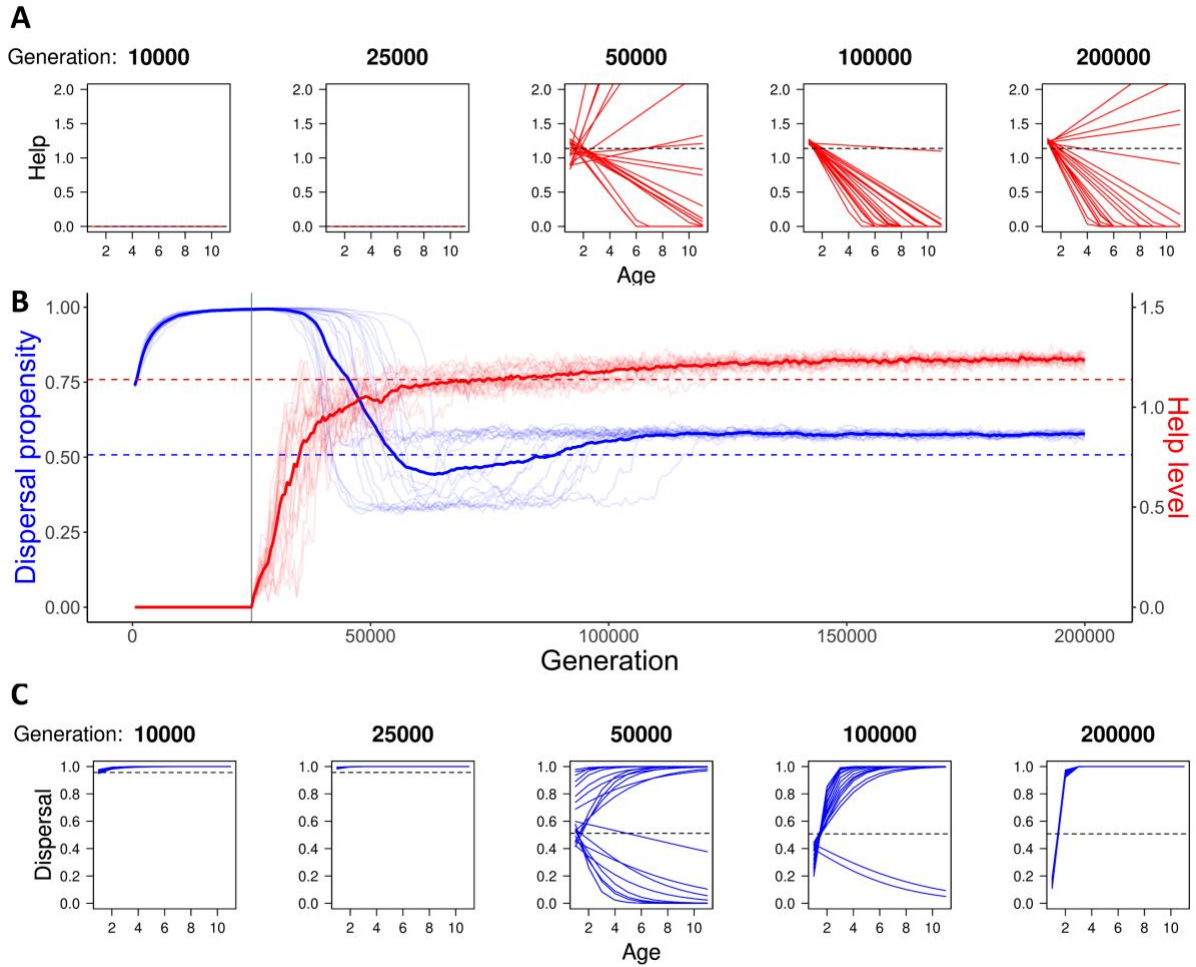


Fig. S6. Evolution of age-dependent reaction norms of philopatry and help in unsaturated habitats when relatedness takes effect and subordinates delay dispersal due to safe haven effects. (B) Evolutionary dynamics of help level (red lines, right y-axis) and dispersal (blue lines, left y-axis). Bold colour lines represent the total mean values across replicas, while shades show the mean values for each of the 20 replicas of the stochastic model. Help was allowed to evolve from generation 25000 (grey vertical line). **(A and C)** Reaction norms of help and dispersal, respectively, at five different points in time. Dotted lines in all three figures represent the equilibrium for levels of help and dispersal in the absence of the evolution of reaction norms. The input parameter values are: $f=2$, $m_0=0.3$, $m_d=1$, $x_n=3$, $x_h=4$, $x_0=1.5$, $k_0=1$, $k_h=1$.

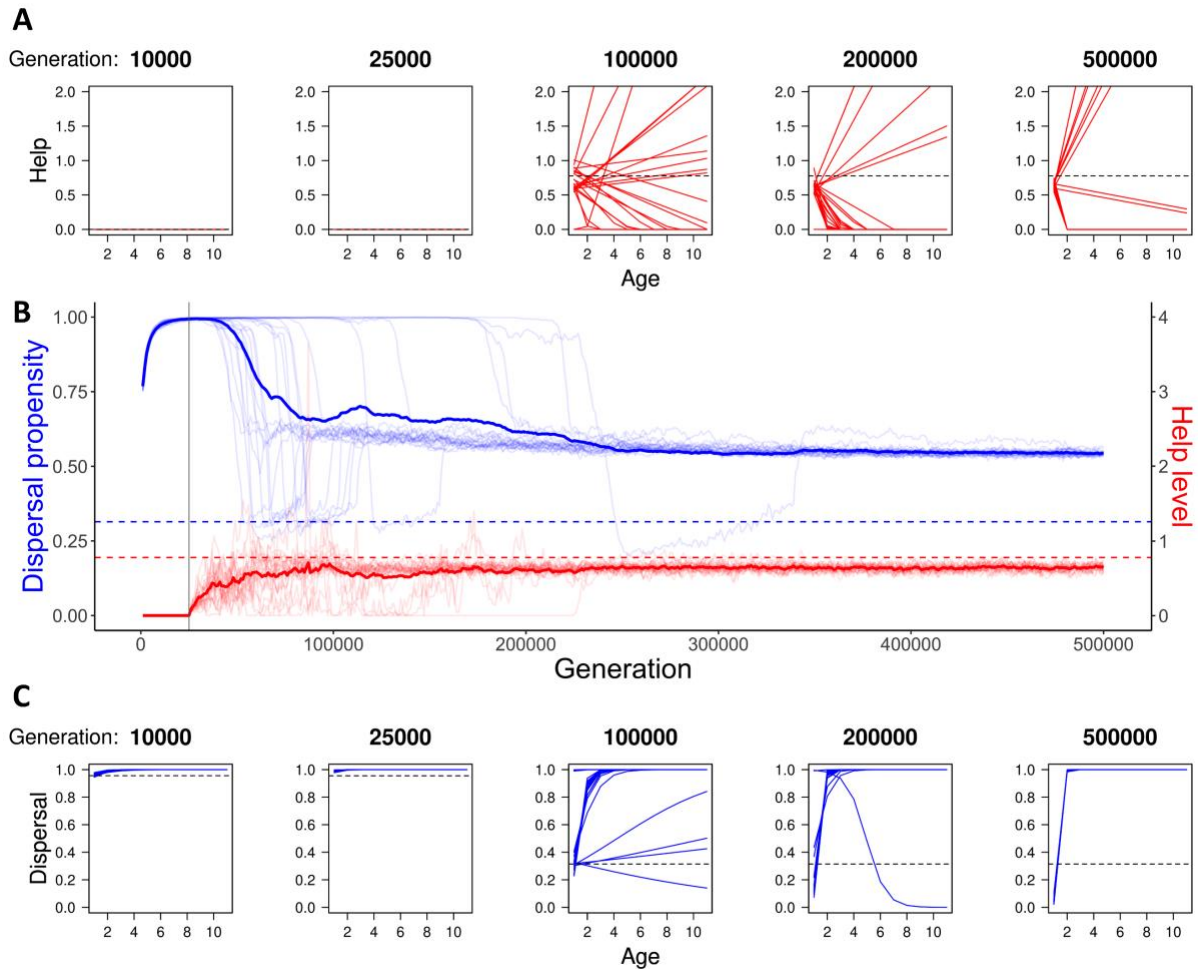


Fig. S7. Evolution of age-dependent reaction norms of philopatry and help in unsaturated habitats when relatedness is excluded and subordinates delay dispersal due to safe haven effects. (B) Evolutionary dynamics of help level (red lines, right y-axis) and dispersal (blue lines, left y-axis). Bold colour lines represent the total mean values across replicas, while shades show the mean values for each of the 20 replicas of the stochastic model. Help was allowed to evolve from generation 25000 (grey vertical line). (A and C) Reaction norms of help and dispersal, respectively, at five different points in time. Dotted lines in all three figures represent the equilibrium for levels of help and dispersal in the absence of the evolution of reaction norms. The input parameter values are the same as in Fig. S6.

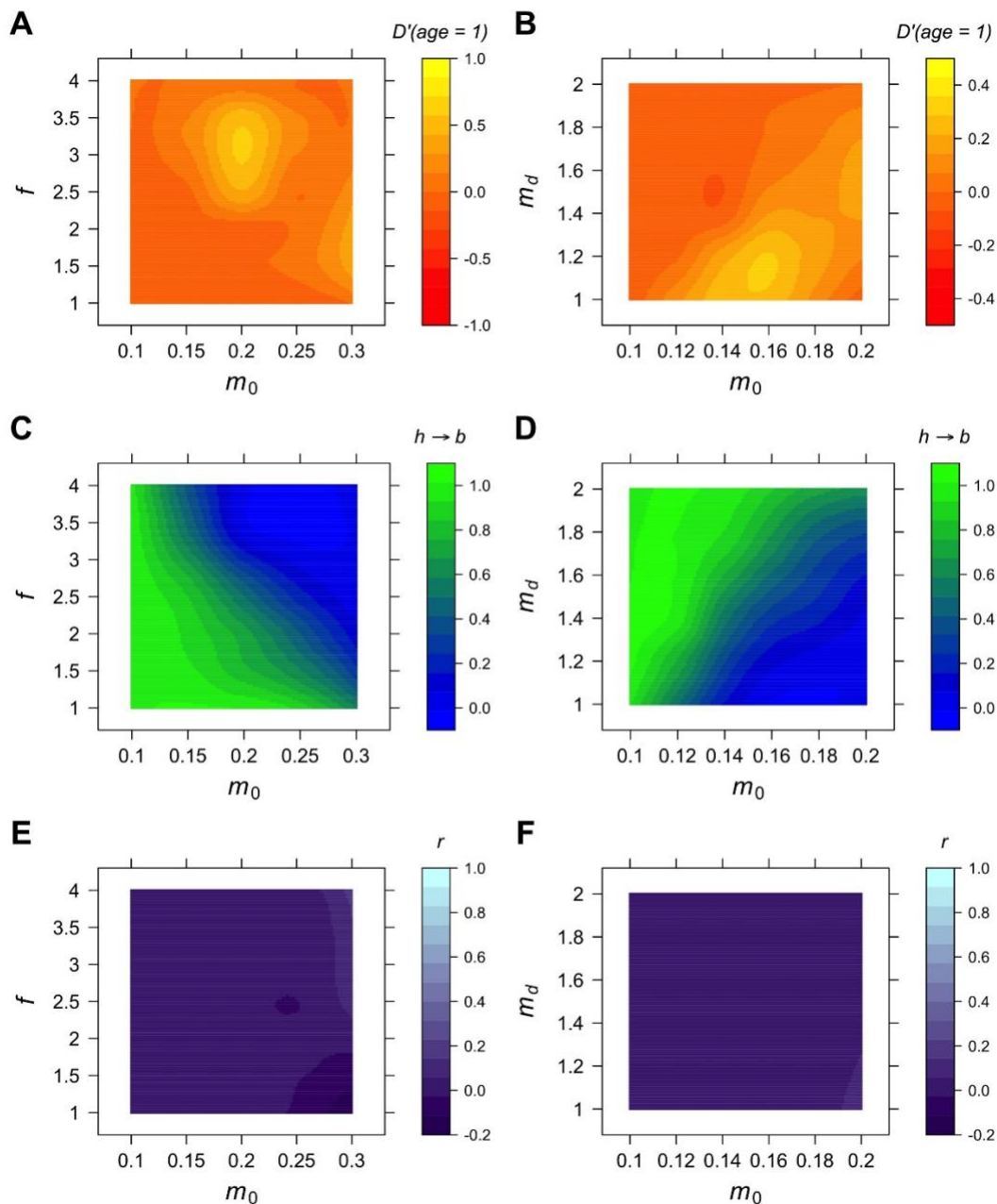


Fig. S8. Evolution of age-dependent reaction norms for dispersal across different ecological scenarios, and the proportion of helpers becoming breeders when relatedness is excluded. Different levels of habitat saturation were modelled by varying the overall mortality (m_0), the likelihood that floaters find a group to breed (f), and the mortality linked to dispersal (m_d). **A, B:** The derivative of the reaction norm (evaluated at age 1), at the evolutionary equilibrium, determining dispersal propensity was plotted for scenarios when relatedness is absent. Positive values of D' denote a positive slope and an increase in dispersal with age, while negative values of D' indicate a decrease in dispersal propensity with age. Both overall mortality (m_0) and group permeability (f) select for positive D' , that is an increase in the level of dispersal with age (**A**). In contrast, mortality associated with dispersal (m_d) has the opposite effect (**B**). This effect correlates with the reproductive perspectives of floaters. In (**C** and **D**) we show the proportion of helpers that become breeders (inheritance of breeding position as opposed to takeover by floaters) at the equilibrium. In (**E**) and (**F**) we show relatedness levels between breeders and subordinates in the group at the population level.

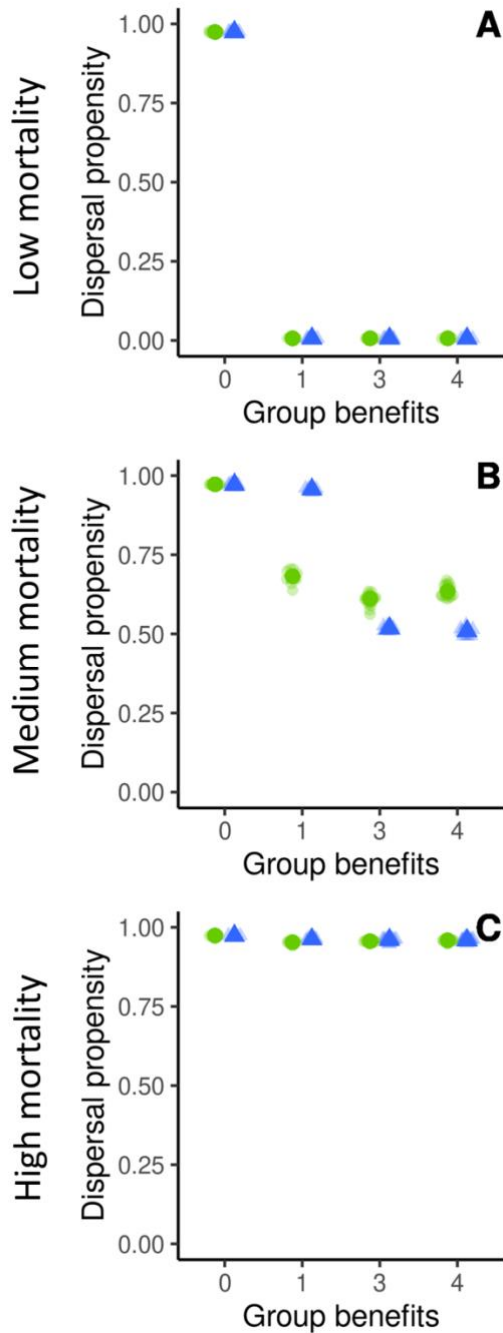


Fig. S9. Evolution of dispersal for different implementations of the shuffling algorithm when removing relatedness in the benchmark model. Equilibria for philopatry levels are shown for the original implementation in which group structure is maintained when removing relatedness (green circles), and for a benchmark model in which kin effects selecting for philopatry at age = 1 in the absence of relatedness are removed (blue triangles). Help was prevented from evolving for comparative purposes. The evolutionary equilibria for levels of dispersal are shown for different values of group benefits (x_n) and environmental harshness: (A) benign environment ($m_0=0.1$), (B) medium environment ($m_0=0.2$), and (C) harsh environment ($m_0=0.3$). Results reveal that potential cryptic kin effects may influence our results only for intermediate environmental mortality, especially when group benefits are low. Results are shown across 20 replicas to assess repeatability. Same parameter inputs as in Fig. 2.



CHAPTER 2

Group augmentation on trial: helpers in small groups enhance antipredator defence of eggs

Irene García-Ruiz^{1*} and Michael Taborsky¹

¹ Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Switzerland.

*Corresponding author.

Submitted to Biology Letters

Publish version available: García-Ruiz I, Taborsky M (2022). Group augmentation on trial: helpers in small groups enhance antipredator defence of eggs. Biology Letters, 18(10), 20220170. DOI: [10.1098/rsbl.2022.0170](https://doi.org/10.1098/rsbl.2022.0170)

Abstract

Mechanisms selecting for the evolution of cooperative breeding are hotly debated. While kin selection theory has been the central paradigm to explain the seemingly altruistic behaviour of non-reproducing helpers, it is increasingly recognized that direct fitness benefits may be highly relevant. The group augmentation hypothesis proposes that alloparental care may evolve to enhance group size when larger groups yield increased survival and/or reproductive success. However, there is a lack of empirical tests. Here we use a cooperatively breeding cichlid fish, in which group size predicts survival and group stability, to test this hypothesis experimentally by prompting two cooperative tasks: defence against an egg predator and digging out sand from the breeding shelter. We controlled for alternative mechanisms such as kin selection, load-lightening and coercion. As predicted by the group augmentation hypothesis, helpers increased defense against an egg predator in small compared to large groups. This difference was only evident in large helpers due to size-specific task specialization. Furthermore, helpers showed more digging effort in the breeding chamber compared to alternative personal shelters, indicating that digging was an altruistic service to the dominant breeders.

Introduction

The most common evolutionary explanations of alloparental care include kin selection, where closely related helpers benefit from indirect fitness benefits, and group augmentation, where direct fitness benefits accrue to helpers from enhanced group size [1,2]. The group augmentation hypothesis posits that helping is favoured by natural selection if it increases the productivity of breeders and the additionally produced young in turn improve the helper's survival and/or future reproductive success [3,4]. Immediate benefits of increased group size include, for instance, enhanced safety from dilution effects and cooperative territory and anti-predator defence [5–7]. Nevertheless, in contrast to substantial research efforts on the effect of kin selection, studies on the operation of group augmentation are scant [1,8,9].

We aim to remedy this shortcoming by an experimental study using manipulation of group composition and behaviour in a cooperatively breeding fish. In the cichlid *Neolamprologus pulcher*, helpers living in large groups benefit from increased survival due to reduced predation risk [10–12], which is a prerequisite for group augmentation to evolve. In addition, helpers prefer to join a group instead of breeding independently [13], and they prefer to join larger vs smaller groups despite thereby reducing their chance of inheriting the territory [14,15]. Furthermore, helpers increase the reproductive output of breeders [16,17], which yields larger groups since most offspring remain philopatric for long [18–20]. All this suggests that the preconditions for the evolution of alloparental care by group augmentation are met in this species.

If group augmentation selects for helping, alloparental care is predicted to be higher in small than in large groups due to the greater need for group size enhancement [3]. We tested this prediction by comparing the helping effort between small and large experimental groups during two different tasks, defence against an egg predator and digging out sand from the breeding shelter. At the same time we controlled for alternative selection mechanisms like kin selection, load lightening and pay-to-stay.

Methods

Study species

Neolamprologus pulcher is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa [21]. Breeding groups consist of a dominant breeding pair that largely monopolizes reproduction, and 0-20 related and unrelated helpers [11,12,18,22,23]. Groups typically use self-dug burrows under rocks and small holes and crevices as shelters and for breeding [12,19]. The dominance hierarchy is strictly size based [19,24,25]. Helpers actively engage in brood care (cleaning and fanning eggs and mouthing larvae and free-swimming fry), territory maintenance (removing sand and particles from shelters), and territory defence (against predators, space competitors and conspecifics) [16,19,26,27].

To induce predation threat on eggs we used *Telmatochromis vittatus*, a cichlid fish that preys on eggs and fry of *N. pulcher* [20,26]. Both dominant and subordinate group members defend the territory against *T. vittatus*, even though it is no direct threat to subordinates [26,28–30].

Experimental design

Test fish used in this experiment were laboratory-reared descendants from *N. pulcher* individuals caught in the wild near Kasakalawe point in Lake Tanganyika (Zambia). Experimental groups were housed in 200L compartments of 400L aquaria that were subdivided by a transparent partition. Helpers in the group all differed in body size to match natural conditions, which also attenuates aggression related to establishing dominance hierarchy. Focal helpers comprised two size classes referred to as small (at start of experiment: 19-26mm) and large (29-33mm), which were size-matched between small and large groups. Non-focal helpers included also larger individuals (up to 43mm), which were not used as focal test subjects due to the inherent risk of egg cannibalism [31,32]. Helpers were unrelated to the breeders in their group. Another experimental group of matched size was housed behind the transparent partition, as visual contact to neighbours helps stabilising groups by inducing territory defence [33]. Each experimental compartment was subdivided into four chambers: (a) the experimental chamber, (b) a housing compartment for a *T. vittatus*, (c) an isolation chamber for subordinates during the experiment, and (d) a chamber to isolate the breeders (Fig. 1).

Groups were checked for clutches every day, and experiments took place the day after spawning. We obtained a total sample size of 25 groups ($n = 12$ small and 13 large groups). The experiments were conducted from March to July 2020.

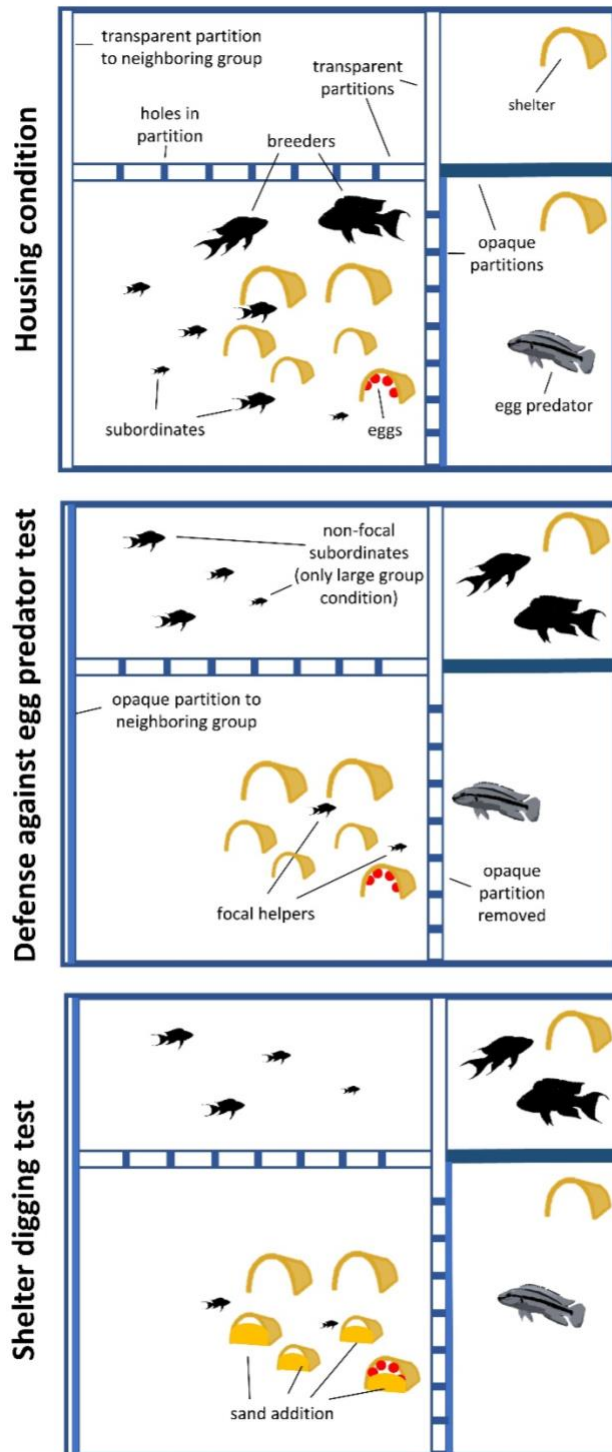


Figure 1. Experimental design. Before the experiment began, we isolated the breeders as well as the non-focal helpers (non-focal helpers were present only in the large group size condition), and added an opaque partition to the neighbouring group compartment. In the defence test against the egg predator, we removed the opaque partition hiding the egg predator and recorded the behaviour of the focal helpers for 10 min. Consecutively, in the digging test, we restored the opaque partition toward the egg predator and added sand to all shelters except for two. Subsequently we recorded digging events for 20 min.

Experimental procedure

We established groups of different sizes to compare the effect of group size on helping behaviours in *N. pulcher*. Small groups were composed of a breeding pair and two helpers, while large groups consisted of the breeding pair and six helpers.

On the day a clutch was found, we moved the breeding shelter with the eggs close to the partition separating the main compartment from the egg predator compartment, and added an opaque partition towards the neighbouring group so that their behaviour would not influence the experimental fish (Fig. 1). The experiment started the day after the clutch was produced. To control for potential load lightening effects [34], in the large groups we isolated four of the six helpers behind a transparent partition to prevent them from helping during the recording period. Therefore, in both group size conditions, only two focal helpers of matched size were effectively able to provide alloparental care. In this species breeders may coerce subordinates to provide help [29,30,32,35–37]. Since our aim is to measure voluntary alloparental care provisioning, we isolated the breeders before the behavioural recording started. The breeders' compartment had an opaque partition that prevented them from seeing the egg predator and whether the helpers were providing care, which precluded the breeders from any sort of enforcement while they were still visually present for the helpers to maintain the dominance hierarchy within the group. We waited 5 min for the fish to acclimatise after this manipulation before starting the experiment.

To measure helping behaviour we constructed two distinct consecutive tests. First an “egg predator defence test” and then a “removing sand from shelters test”. For the egg predator test we first started video recording, and subsequently removed the opaque partition that separated the egg predator compartment from the focal helpers' compartment (Fig.1). We scored the focal helpers' response for 10 min starting with the first behavioural response performed by any of the two helpers. We analysed frontal approach, operculum spread, S-bend and head down displays as aggressive behaviours. We also included biting, bow swimming and tail beat when these behaviours were performed against the transparent partition and the egg predator was in close proximity to it. Concurrently, we scored direct brood care behaviours including egg cleaning and fanning. For a description of these behaviours see [16].

After 20 min of *T. vittatus* presentation, we placed back the opaque partition and filled the shelters with sand, including the breeding chamber containing the eggs (Fig. 1). In both group size conditions, two shelters were not filled with sand. We scored behaviours for 20 min starting from the first digging event. We differentiated digging from a shelter from digging in the breeding chamber.

Data analysis

We used zero altered Poisson generalized linear mixed models (GLMM-ZAP) to analyse the data. For the presence / absence of the dependent variables we used a GLM with binomial error distribution, and for the count data we assumed a zero-truncated Poisson error distribution using the R package glmmADMB [38]. We used group size and the categorical helpers' size as fixed factors. The interactions between fixed factors were initially included in all models and dropped if they were not significant ($p > 0.05$). We used group ID as random

effects. To check for model fit and overdispersion, we used the function `simulateResiduals` from the R package DHARMA. No significant deviations in the predicted vs expected residuals were detected. We used the Wilcoxon matched-pairs signed-ranks test to check whether there are different amounts of digging performed in the breeding chamber compared to all other shelters. All simulations and statistical tests were performed in R 3.6.3. (R Core Team 2020).

Results

Defence against the egg predator

Large helpers in the small group size condition defended more against the egg predator than large helpers in large groups (non-zero count data: $z = 5.84$, $p < 0.001$; Fig. 2A). Furthermore, large helpers defended in general on more occasions than small helpers (binary data: $z = 2.01$, $p = 0.04$). During this experimental phase, helpers also provided brood care in the form of egg cleaning and fanning, with small helpers tending to provide egg care on more occasions than large helpers (binary data: $z = 1.69$, $p = 0.09$).

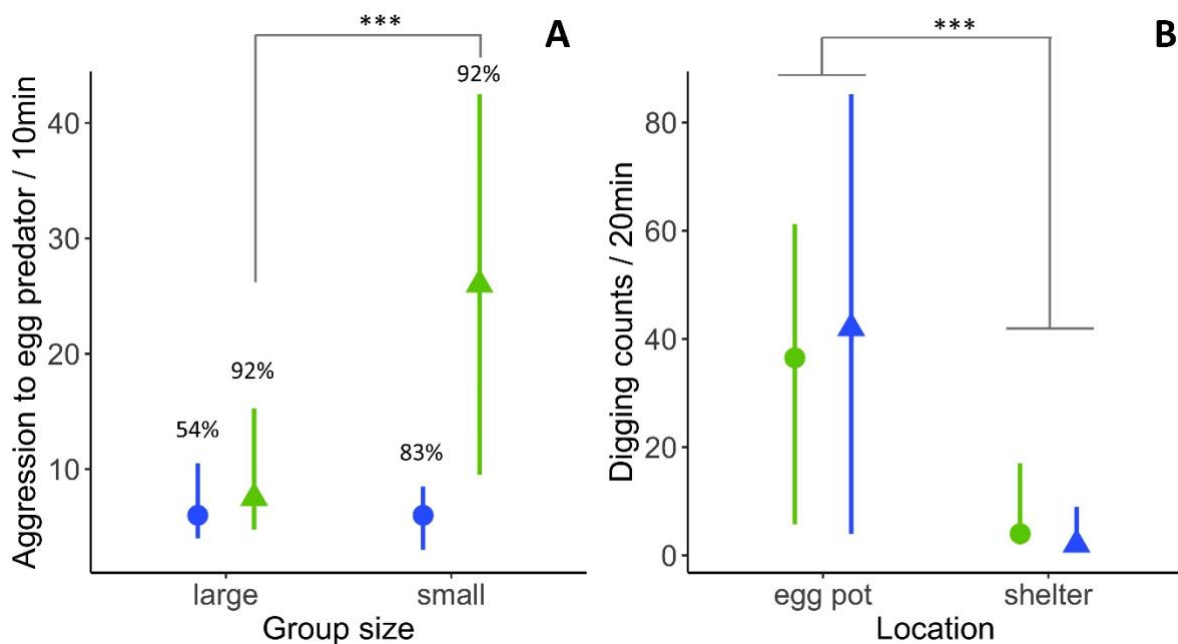


Figure 2. A: Numbers of aggressive displays towards the egg predator during 10min after the first reaction, separated by group size and helper size (green triangles: large helpers, blue circles: small helpers). Depicted are medians \pm interquartile ranges of behaviours when they occurred (i.e., zero occurrences are omitted). Numbers indicate the percentage of cases in which aggressive displays were shown at all against the egg predator. **B:** Digging counts in the breeding chamber and other shelters. Results are separated by large (green circles) and small (blue triangles) groups. Asterisks denote significance ($p < 0.001$).

Shelter digging test

Helpers in both small and large groups dug much more often in the breeding chamber compared to the other shelters combined ($V = 544$, $p < 0.001$; Fig. 2B). The amount of

digging in the breeding chamber did not differ between small and large groups (non-zero count data: $z = 0.70$, $p = 0.48$), nor between large and small helpers (non-zero count data: $z = 0.40$, $p = 0.69$). Correspondingly, the occurrence of digging in the breeding chamber neither differed between different group sizes (binary part: $z = 0.38$, $p = 0.71$) nor between helper sizes (binary part: $z = 0.00$, $p = 1.00$), and this was true also for the occurrence of digging in other shelters (group size: non-zero count data, $z = -0.62$, $p = 0.54$; binary data, $z = -0.41$, $p = 0.69$; helper size: non-zero count data, $z = -1.72$, $p = 0.09$; binary data: $z = -0.62$, $p = 0.54$).

Discussion

As predicted by the group augmentation hypothesis, helpers in small groups defended more against an egg predator than helpers in large groups. Importantly, this differentiation emerged when experimentally controlling for potential alternative effects such as load-lightening, coercion and relatedness. The fact that this difference was only apparent in large helpers corresponds to the size-specific task differentiation of helpers shown in nature [19,26]. The sand challenge revealed that both large and small helpers engaged more in digging out the breeding shelter than in digging out alternative personal shelters, which reflects a costly altruistic service to the brood and breeders; shelter digging was shown to be the most energetically demanding behaviour exhibited in this species, increasing routine metabolic rates more than six-fold [39,40]. No differences were found for digging behaviour between large and small groups, however. A previous experiment has shown that shelter digging is enforced by dominants [29]. Hence digging behaviour in *N. pulcher* seems to be controlled by coercion, corresponding to the pay-to-stay mechanism regulating altruistic help in this species [23], and not by selection for group augmentation. Future experiments might clarify whether breeders in small groups demand more digging effort from their helpers than breeders in large groups, which would serve the greater needs for group size enhancement in smaller groups.

Our results are consistent with previous studies suggesting group size effects on helping in *N. pulcher*. In the field, helpers visited breeding shelters more often when almost half of the helpers were experimentally removed [17], which is a proxy for direct brood care [41]; own unpubl. data). Helpers were also shown to compensate for an experimental reduction of help in other group members by increasing their own defence effort against conspecific intruders [42]. In addition, group members benefit also from the vicinity of other groups, as joint effort in antipredator defence allows them to reduce their own expense, revealing a significant benefit of coloniality in addition to group living [27].

In some cooperatively breeding birds and mammals, helpers were also found to show more alloparental care when living in small than in large groups [43,44], and in a few cases group size is increased even by kidnapping members of other groups [45–47], further highlighting the importance of group size. Investment in costly border patrols is also group size dependent in chimpanzees, for example [48]. Together with data showing that large group size enhances survival prospects and other fitness benefits for group members [5,7,46,49] we hypothesize that group augmentation is an important and negligently underrated driver of cooperation in social animals [1,3].

The size-specific task differentiation of helpers in *N. pulcher* may reflect different fitness benefits from group size attained at different age. An experimental field study showed that

large and medium-sized helpers had higher survival chances in large than in small groups through protection from predators, but this effect did not emerge in small helpers [10]. This suggests that group augmentation effects may provide substantial direct fitness benefits primarily to relatively large group members, apart from offspring [12,17], which may explain why only large helpers in this experiment showed enhanced levels of antipredator defence in small groups. In addition, higher ranked individuals queuing for the breeding position may help more because potential benefits of territory inheritance due to increased recruitment of future helpers are restricted to those that eventually acquire breeding status, a situation that might resemble cooperatively breeding wasps [50,51] and birds [9,52]. The increased egg predator defence of helpers in small groups may yield both short- and long-term group augmentation benefits [3].

In conclusion, our study suggests that group augmentation may be an important factor in the evolution of helping behaviour in *N. pulcher* and other cooperative breeders. It is likely to take effect in combination with other mechanisms selecting for alloparental care, such as kin selection and pay-to-stay [8,32].

Ethics. Experiments were approved by the Veterinary Office of the Kanton Bern (licence no. 74/15)

Funding. This work was supported by the Swiss National Science Foundation (grant no. 31003A-176174)

Acknowledgements. We thank Markus Wymann for helping in the construction of the tank partitions and Evi Zwygart for help in taking care of the fish.

References

1. Clutton-Brock TH. 2002 Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science (1979)* **296**, 69–72. (doi:10.1126/science.296.5565.69)
2. Taborsky M, Cant MA, Komdeur J. 2021 *The Evolution of Social Behaviour*. Cambridge University Press. (doi:10.1017/9780511894794)
3. Kingma SA, Santema P, Taborsky M, Komdeur J. 2014 Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution* **29**, 476–484. (doi:10.1016/j.tree.2014.05.013)
4. Kokko H, Johnstone RA, Clutton-Brock TH. 2001 The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B: Biological Sciences* **268**, 187–196. (doi:10.1098/rspb.2000.1349)
5. Clutton-Brock TH, Gaynor D, McIlrath GM, Maccoll ADC, Kansky R, Chadwick P, Manser M, Skinner JD, Brotherton PNM. 1999 Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *Journal of Animal Ecology* **68**, 672–683. (doi:10.1046/j.1365-2656.1999.00317.x)
6. Heg D, Taborsky M. 2010 Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* **5**. (doi:10.1371/journal.pone.0010784)
7. Cant MA, Nichols HJ, Thompson FJ, Vitikainen E. 2016 Banded mongooses: Demography, life history, and social behavior. In *Cooperative Breeding in Vertebrates* (eds WD Koenig, JL Dickinson), pp. 318–337. Cambridge: Cambridge University Press. (doi:10.1017/CBO9781107338357.019)
8. Kingma SA, Hall ML, Peters A. 2011 Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist* **177**, 486–495. (doi:10.1086/658989)
9. Kingma SA. 2017 Direct benefits explain interspecific variation in helping behaviour among cooperatively breeding birds. *Nature Communications* **8**, 1–7. (doi:10.1038/s41467-017-01299-5)
10. Heg D, Bachar Z, Brouwer L, Taborsky M. 2004 Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences* **271**, 2367–2374. (doi:10.1098/rspb.2004.2855)
11. Heg D, Brouwer L, Bachar Z, Taborsky M. 2005 Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* **142**, 1615–1641. (doi:10.1163/156853905774831891)
12. Balshine S, Leach B, Neat F, Reid H, Taborsky M, Werner N. 2001 Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* **50**, 134–140. (doi:10.1007/s002650100343)
13. Taborsky M. 1985 Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* **95**, 45–75. (doi:10.1163/156853985X00046)
14. Jordan LA, Wong MYL, Balshine SS. 2010 The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biology Letters* **6**, 301–303. (doi:10.1098/rsbl.2009.0732)
15. Reddon AR, Balk D, Balshine S. 2011 Sex differences in group-joining decisions in social fish. *Animal Behaviour* **82**, 229–234. (doi:10.1016/j.anbehav.2011.04.017)
16. Taborsky M. 1984 Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour* **32**, 1236–1252. (doi:10.1016/S0003-3472(84)80241-9)

17. Brouwer L, Heg D, Taborsky M. 2005 Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behavioral Ecology* **16**, 667–673. (doi:10.1093/beheco/ari042)
18. Dierkes P, Heg D, Taborsky M, Skubic E, Achmann R. 2005 Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* **8**, 968–975. (doi:10.1111/j.1461-0248.2005.00801.x)
19. Taborsky M, Limberger D. 1981 Helpers in fish. *Behavioral Ecology and Sociobiology* **8**, 143–145. (doi:10.1007/BF00300826)
20. Heg D, Heg-Bachar Z, Brouwer L, Taborsky M. 2008 Experimentally induced helper dispersal in colonially breeding cooperative cichlids. *Environmental Biology of Fishes* **83**, 191–206. (doi:10.1007/s10641-007-9317-3)
21. Duftner N, Sefc KM, Koblmüller S, Salzburger W, Taborsky M, Sturmbauer C. 2007 Parallel evolution of facial stripe patterns in the *Neolamprologus brichardi/pulcher* species complex endemic to Lake Tanganyika. *Molecular Phylogenetics and Evolution* **45**, 706–715. (doi:DOI: 10.1016/j.ympev.2007.08.001)
22. Stiver KA, Fitzpatrick J, Desjardins JK, Balshine S. 2006 Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Animal Behaviour* **71**, 449–456. (doi:10.1016/j.anbehav.2005.06.011)
23. Taborsky M. 2016 Cichlid fishes: A model for the integrative study of social behavior. In *Cooperative Breeding in Vertebrates* (eds WD Koenig, JL Dickinson), pp. 272–293. Cambridge: Cambridge University Press. (doi:10.1017/CBO9781107338357.017)
24. Riebli T, Taborsky M, Chervet N, Apolloni N, Zürcher Y, Heg D. 2012 Behavioural type, status and social context affect behaviour and resource allocation in cooperatively breeding cichlids. *Animal Behaviour* **84**, 925–936. (doi:10.1016/j.anbehav.2012.07.017)
25. Bender N, Hamilton IM, Heg D. 2005 Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. *Behaviour* **142**, 1591–1613. (doi:10.1163/156853905774831846)
26. Bruintjes R, Taborsky M. 2011 Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* **81**, 387–394. (doi:10.1016/j.anbehav.2010.10.004)
27. Jungwirth A, Josi D, Walker J, Taborsky M. 2015 Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Functional Ecology* **29**, 1218–1224. (doi:10.1111/1365-2435.12430)
28. Kasper C, Colombo M, Aubin-Horth N, Taborsky B. 2018 Brain activation patterns following a cooperation opportunity in a highly social cichlid fish. *Physiology & Behavior* **195**, 37–47. (doi:10.1016/j.physbeh.2018.07.025)
29. Naef J, Taborsky M. 2020 Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science* **7**, 191808. (doi:10.1098/rsos.191808)
30. Naef J, Taborsky M. 2020 Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* **168**, 137–147. (doi:10.1016/j.anbehav.2020.08.006)

31. Siemens M. 1990 Broodcare or Egg Cannibalism by Parents and Helpers in *Neolamprologus brichardi* (Poll 1986) (Pisces: Cichlidae): a Study on Behavioural Mechanisms. *Ethology* **84**, 60–80. (doi:10.1111/j.1439-0310.1990.tb00785.x)
32. Zöttl M, Heg D, Chervet N, Taborsky M. 2013 Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* **4**, 1341. (doi:10.1038/ncomms2344)
33. Brintjes R, Taborsky M. 2008 Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour* **75**, 1843–1850. (doi:10.1016/j.anbehav.2007.12.004)
34. Zöttl M, Fischer S, Taborsky M. 2013 Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour* **85**, 1471–1478. (doi:10.1016/j.anbehav.2013.03.045)
35. Bergmüller R, Taborsky M. 2005 Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* **69**, 19–28. (doi:10.1016/j.anbehav.2004.05.009)
36. Heg D, Taborsky M. 2010 Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* **5**. (doi:10.1371/journal.pone.0010784)
37. Fischer S, Zöttl M, Groenewoud F, Taborsky B. 2014 Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20140184–20140184. (doi:10.1098/rspb.2014.0184)
38. Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, Nielsen A, Sibert J. 2012 AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* **27**, 233–249. (doi:10.1080/10556788.2011.597854)
39. Grantner A, Taborsky M. 1998 The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **168**, 427–433. (doi:10.1007/s003600050162)
40. Taborsky M, Grantner A. 1998 Behavioural time–energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Animal Behaviour* **56**, 1375–1382. (doi:10.1006/anbe.1998.0918)
41. Balshine-Earn S, Neat FC, Reid H, Taborsky M. 1998 Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology* **9**, 432–438. (doi:10.1093/beheco/9.5.432)
42. Bergmüller R, Taborsky M. 2005 Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* **69**, 19–28. (doi:10.1016/j.anbehav.2004.05.009)
43. Clutton-Brock TH, Russell AF, Sharpe LL. 2004 Behavioural tactics of breeders in cooperative meerkats. *Animal Behaviour* **68**, 1029–1040. (doi:10.1016/j.anbehav.2003.10.024)
44. Pike KN, Ashton BJ, Morgan K v., Ridley AR. 2019 Social and Individual Factors Influence Variation in Offspring Care in the Cooperatively Breeding Western Australian Magpie. *Frontiers in Ecology and Evolution* **7**, 92. (doi:10.3389/fevo.2019.00092)

45. Heinsohn RG. 1991 Kidnapping and reciprocity in cooperatively breeding white-winged choughs. *Animal Behaviour* **41**, 1097–1100. (doi:10.1016/S0003-3472(05)80652-9)
46. Ridley AR. 2016 Southern pied babblers: The dynamics of conflict and cooperation in a group-living society. In *Cooperative Breeding in Vertebrates* (eds WD Koenig, JL Dickinson), pp. 115–132. Cambridge: Cambridge University Press. (doi:10.1017/CBO9781107338357.008)
47. Müller CA, Bell MBV. 2009 Kidnapping and infanticide between groups of banded mongooses. *Mammalian Biology* **74**, 315–318. (doi:10.1016/j.mambio.2008.08.003)
48. Langergraber KE, Watts DP, Vigilant L, Mitani JC. 2017 Group augmentation, collective action, and territorial boundary patrols by male chimpanzees. *Proceedings of the National Academy of Sciences* **114**, 7337–7342. (doi:10.1073/pnas.1701582114)
49. Covas R, du Plessis MA, Doutrelant C. 2008 Helpers in colonial cooperatively breeding sociable weavers *Philetairus socius* contribute to buffer the effects of adverse breeding conditions. *Behavioral Ecology and Sociobiology* **63**, 103–112. (doi:10.1007/s00265-008-0640-2)
50. Field J, Cronin A, Bridge C. 2006 Future fitness and helping in social queues. *Nature* **441**, 214–217. (doi:10.1038/nature04560)
51. Field J, Leadbeater E. 2016 Cooperation between non-relatives in a primitively eusocial paper wasp, *Polistes dominula*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**, 20150093. (doi:10.1098/rstb.2015.0093)
52. Downing PA, Griffin AS, Cornwallis CK. 2018 Sex differences in helping effort reveal the effect of future reproduction on cooperative behaviour in birds. *Proceedings of the Royal Society B: Biological Sciences* **285**. (doi:10.1098/rspb.2018.1164)



CHAPTER 3

Kin selection mediates coercion in asymmetric negotiations in a cooperatively breeding cichlid fish

Irene García-Ruiz^{1*} and Michael Taborsky¹

¹ Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Switzerland.

*Corresponding author.

In preparation for submission

Publish version available: García-Ruiz I, Taborsky M (2024). Nepotism mediates enforced cooperation in asymmetric negotiations. iScience, 27(7),110334. DOI: [10.1016/j.isci.2024.110334](https://doi.org/10.1016/j.isci.2024.110334)

Abstract

Cooperative breeding is the source of the most complex social organisations known among animals. In cooperative breeders, dominant and subordinate group members typically exchange different commodities among each other, which involves an incessant negotiation process. For example, helpers may trade alloparental brood care against safety and resource access in the dominants' territory. The crucial question is how in this continual bargaining process the conflict of fitness interests is resolved between the unequal partners, so that maintaining the cooperative interaction is optimal for all parties involved. Relatedness between breeders and helpers can alleviate the conflict of fitness interests between them, but evidence is accumulating that direct fitness benefits are pivotal for the evolution of such social systems. To evaluate the relative importance of direct and indirect fitness effects, here we experimentally disturb the negotiation process between dominant breeders and related or unrelated subordinates in a cooperative cichlid by simulating transgression from the helpers while allowing or preventing the ability of breeders to respond to this dereliction of duty in a full factorial design. Our results show that coercion by breeders is crucial for the performance of alloparental egg care by subordinate helpers, but that kinship reduces the importance of coercion as predicted by theoretical models. By experimentally manipulating both the behaviour of all involved parties and their responses to each other, we unravelled the interaction between the most fundamental selection mechanisms responsible for the evolution of complex social systems.

Introduction

Cooperative interactions in which individuals exchange same or different commodities are common in nature. However, for cooperative associations to form and remain stable, participating individuals must find an equilibrium affording that each party obtains a net fitness benefit (Buston and Zink 2009; Cant and Johnstone 2009). The conflict over maximizing one's own payoff can lead to negotiations in which individuals adjust their behaviour in response to the behaviour of the other party (Cant and Shen 2006; Binmore 2010; Quiñones et al. 2016). Negotiations occur often between unequal social partners due to asymmetries in resource holding potential or the possession of inalienable commodities (Taborsky 1994; Solomon and French 1997; Cant 2011; Koenig and Dickinson 2016; Taborsky et al. 2021). These asymmetries in bargaining power are often found in dominance hierarchies and provide scope for coercion to evolve (Kokko et al. 2002; Naef and Taborsky 2020a). However, negotiations involving unequal partners have been hitherto largely neglected.

A suitable model to study negotiation among unequal social partners is cooperative breeding (Zöttl et al. 2013c; Donaldson et al. 2014). In cooperatively breeding species, subordinate individuals help to rear the offspring produced by dominant breeders, while delaying their own reproduction (Brown 1987; Cockburn 1998). In these systems, breeders benefit from alloparental care received from the subordinates, while the subordinates benefit from the safety of the territory the dominants defend (Taborsky 1984). While dominant individuals normally have a higher bargaining power and may enforce help by attacking subordinates, subordinates may leave if the net benefits are no longer positive by staying (Taborsky et al. 2021). Dominant individuals may also evict the subordinates when the cost of reproductive competition is higher than the benefits they obtain from the presence/help of subordinates (Dierkes et al. 1999). The threat of eviction plays a central role in 'pay-to-stay' models of helping behaviour, in which subordinates provide help as a pay of rent to be tolerated in the territory (Gaston 1978; Kokko et al. 2002; Hamilton and Taborsky 2005). Outside options as well as market effects define the level of tolerance on whether to maintain the cooperative association or not. However, these forms of threats in the negotiation processes are rarely observed in nature due to the immediate decrease in inclusive fitness for the participants (Clutton-Brock and Parker 1995; Cant 2011).

To test these hidden threats, we must experimentally disturb the interaction, for instance by simulating transgression (i.e. preventing the subordinate from providing help). Help prevention should result in an increased level of coercion by the breeders, while in turn eliciting a compensatory response from the subordinate, either by increasing submission or the levels of help. In addition, to test whether punishment leads to an increase in cooperation, we must experimentally prevent punishment by the dominant breeders and check for a difference in cooperation when punishment is allowed or not. Therefore, experimental manipulation of all involved negotiation partners is required to unravel the bargaining process. In addition, joint interests (e.g. by shared genes) should reduce the importance of coercion in negotiations, as related subordinates have the incentive to help because of inclusive fitness benefits, whereas it weakens selection for breeders to effectively punish defectors (Marshall and Rowe 2003; Zöttl et al. 2013c; Quiñones et al. 2016; Schweinfurth and Taborsky 2018).

A study system showing asymmetric negotiation involving coercion and different degrees of relatedness is the cooperatively breeding cichlid fish *Neolamprologus pulcher*. Previous experiments have shown that simulated idle helpers increased their submission and received more aggression (Fischer et al. 2014; Naef and Taborsky 2020a, 2020b), as expected under pay-to-stay models. In addition, helpers increased helping behaviour after being prevented from helping, which has been interpreted as pre-emptive appeasement (Bergmüller and Taborsky 2005a; Naef and Taborsky 2020a). Larger helpers which impose a higher risk of reproductive competition to the dominant pair received more punishment and helped more than smaller helpers (Bruitjes and Taborsky 2008; Heg and Taborsky 2010). When subordinates were temporarily removed and hence could not participate in brood care duties, dominants were more likely to evict returning subordinates (Balshine-Earn et al. 1998; Fischer et al. 2014), but evicted helpers were allowed to return when help was required (Taborsky 1985), which corresponds with predictions from biological markets (Noë et al. 1991; Hammerstein and Noë 2016). In the same line, helpers reduced help levels when they were provided with outside options like the opportunity to breed independently (Bergmüller et al. 2005). In addition, unrelated subordinates provide more alloparental care than related ones, suggesting that kin selection may hamper the evolution of enhanced cooperative investment, as expected under cooperation driven by enforcement (Zöttl et al. 2013c; Quiñones et al. 2016). However, it is yet unclear whether help in related individuals is primarily driven by relatedness (via kin selection) or enforcement.

Here our aim is to experimentally test the control of alloparental care in *N. pulcher* by enforcement and relatedness in a full factorial design. We manipulated the ability of helpers to provide alloparental care, and the possibility of breeders to punish idle helpers. At the same time, we manipulated relatedness between helpers and breeders. Our results show that related and unrelated subordinates provide alloparental care driven by coercion from the breeders, but enforcement is apparently mediated by kin selection. This experiment provides the first evidence of alloparental care being controlled by an interaction between enforcement and kin selection, a result obtained by experimentally manipulating all parties involved in the negotiation process.

Methods

Study species

Neolamprologus pulcher is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa. Groups consist of a dominant pair and typically 1 to 25 subordinates of different size classes (Taborsky and Limberger 1981; Balshine et al. 2001; Taborsky et al. 2005; Groenewoud et al. 2016). There is a high reproductive skew, with subordinates of both sexes producing between 5 and 15 per cent of offspring in the group (Dierkes et al. 1999; Heg and Hamilton 2008; Bruitjes et al. 2011; Hellmann et al. 2015; Taborsky 2016). Overall relatedness between subordinates and dominants is low and decreases with helper age mainly due to the replacement of the breeders (Dierkes et al. 2005; Stiver et al. 2005). Both related and unrelated subordinates participate in brood care of the dominants' broods by cleaning and fanning the eggs (Taborsky 1984; Zöttl et al. 2013c), in territory maintenance by digging out sand from shelters and the breeding chamber (Taborsky and Limberger 1981; Bruitjes and

Taborsky 2011), and in territory defence against predators, space competitors and conspecific intruders (Taborsky and Limberger 1981; Taborsky 1984, 1985; Balshine et al. 2001; Bergmüller and Taborsky 2005*b*; Naef and Taborsky 2020*b*). Subordinates show a size-dependent polyethism in task specialization (Bruitjes and Taborsky 2011). Individual and kin recognition have been demonstrated in this species (Hert 1985; Le Vin et al. 2010). Subordinates remain in their natal group queuing for dominance, or they disperse into other groups either as subordinates or by taking over a vacant breeding position, usually long after sexual maturity (Bergmüller et al. 2005; Stiver et al. 2006; Jungwirth et al. 2015). Predation risk is the major environmental factor constraining dispersal (Taborsky 1984; Heg et al. 2004); see (Taborsky 2016) for review).

Experimental set-up

Test fish used in this experiment were captive descendants from *N. pulcher* individuals caught in the wild near Kasakalawe point at the southern end of Lake Tanganyika (Zambia). Fish were kept in non-reproductive aggregations in separate tanks.

Experimental groups were established of a pair and a subordinate of unknown sex; reliable sexing of immature subordinates by external anatomical features is impossible in this species, but there are no apparent sex differences in helping behaviours (Taborsky 1985). Groups were established following the natural group structure, where dominant males are the largest animals in the groups (mean: 64.4mm, range: 54-74mm of body length (BL)) followed by the dominant female (mean: 54.5mm, range: 45-61mm) with a minimum size difference of at least 5mm (Taborsky 1984) and an average of 10mm. Unrelated (mean: 26.4mm, range: 19-32mm) and related subordinates (mean: 25.0mm, range: 19-33mm) did not differ in size ($t_{36.156} = -1.083$, $p = 0.286$). The degree of relatedness between the breeders and the subordinate was experimentally varied, with half of the groups containing related subordinates (offspring: $r = 0.5$) and the other half containing unrelated subordinates ($r = 0.0$).

The groups were assembled following a standardized protocol: For related helpers, breeders were allowed to produce offspring in the tank. When the offspring reached around 20mm BL, all offspring but one was removed from the tank. The subordinate chosen was never the biggest, to avoid rank effects. For unrelated helpers, first the subordinate was released and allowed to acclimatize to the new territory for 24 h. Then both pair members were released. If the subordinate was evicted from the group, the aggressor was isolated for 24h. If the subordinate was still not accepted, it was replaced by another individual following the same procedure. To control for potential effects of familiarity differences between groups with related and unrelated helpers, the experiment started only after the second clutch produced by the pair with the helper's presence. Therefore, the helpers already had experience in egg care before the experiment started. Related and unrelated helpers were size-matched to avoid helper's size effects.

Groups were housed in a 100L compartment of a 200L aquarium subdivided by a glued opaque partition. Each experimental compartment contained three clay flowerpot-halves serving as shelters and as breeding substrate, a semi-transparent tube and an opaque tube suspended below the water surface to serve as refuge, and a filter. The bottom of the

aquarium was covered with a mixture of fine gravel and sand about 3cm deep. An experimental cage made of coarse-mesh was constructed to prevent the breeders from accessing a shelter over which the cage was placed (see below for a description of the experimental procedure), while the subordinates could freely pass through the mesh. After group formation and before the experiment started, this cage was put over one of the clay shelters in the tank to habituate group members to the object. The water temperature was held constant at 26-28°C and the light regime was set at a 13h-light and 11h-dark cycle including dawn and dusk, simulating natural conditions in Lake Tanganyika. The fish were fed with dry food five times a week and with de-frozen food on one day. An additional provision of krill was provided once a week to promote egg-laying.

Groups were checked for clutches every day. After the second clutch following group formation, experiments took place during the egg phase on the first and second day after spawning. We obtained a total sample size of 42 groups (n= 22 non-kin and 20 kin treatment groups, 3 non-kin groups had missing data).

The experiments were conducted from February 2019 to June 2020 at the Ethological station Hasli at the University of Bern, Switzerland under the ethical approval license of Veterinaermt Bern 74/15.

Experimental design and procedure

The experiment involved three phases, a baseline phase, an experience phase, and a test phase (Figure 1). In the experience phase, we simulate idle helpers by preventing them from helping and measure the response by the breeders. In the test phase, we assess whether helpers compensate for the lack of help in the previous phase by increasing alloparental care, and whether compensation is driven by the breeders' interaction with the helpers. Previous studies have shown that helpers increase their brood care investment after time periods where they were unable to conduct alloparental care (Zöttl et al. 2013a), but whether this increase was voluntary or due to enforcement was unclear. Helpers used in this experiment were either related or unrelated to the dominant pair to assess the different mechanisms that explain the evolution of alloparental care when kin selection might or not be an explanatory variable. We also recorded a pre-experimental phase before any manipulations happened in which the breeders could access the breeding shelter to assess the impact of restraining the breeders from accessing the breeding shelter.

We started the experiment when the group was stable and after they produced their second clutch. We used alloparental egg care as the target behaviour (egg cleaning and fanning), because it reflects truly altruistic behaviour (Zöttl et al. 2013c) that causes considerable opportunity and energy costs without immediate direct fitness benefits to the actor (Grantner and Taborsky 1998; Taborsky and Grantner 1998; Heg and Taborsky 2010; Wong and Balshine 2011). We also scored other cooperative behaviours such as digging out sand from the breeding shelter, as well as submission and agonistic interactions between the group members. Before the start of the experiment, the fish were habituated to the experimental equipment (the mesh-cage) and the subordinates learned that they could pass freely through the mesh net. The coarse-meshed cage consisted of a transparent plastic front for visualization purposes, and a mesh covering the other sides and the top with a mesh width of

(10x10mm). In all instances, subordinates were observed accessing the covered shelter through the mesh without difficulties. The experimental cage used for help prevention during the experience phase consisted of a transparent isolation chamber inverted which allowed water flow but prevented fish from accessing the covered shelter.

During all experimental phases, breeders were excluded from brood care by the experimental cage described above. During the “baseline phase” (Figure 1), the meshed cage was placed over the breeding shelter, allowing only the subordinate to access the eggs. After 5min acclimatisation, we scored the fish behaviours for 15min. In the “experience phase” (Figure 1) groups were divided into treatment groups (*T*), in which the whole group was prevented from brood care by a transparent cage for 30min, and control groups (*C*), in which the meshed cage was placed over the breeding shelter like in the baseline condition, allowing the subordinate to care for the eggs. The last 15min of the 30min experimental period in both treatment and control conditions were scored. Subsequently, during the “test phase” (Figure 1), breeders were either allowed to interact physically with the helper (+*I*) or prevented from physical contact (-*I*). Preventing social interaction was achieved by restricting the breeders through a half transparent-half opaque plexiglass cylinder put over the individuals (Figure 1). The opaque side of the cylinder was facing the breeding chamber to prevent breeders from visually perceiving whether the subordinates were providing brood care. Hence, they remained visually present but were unable to exert coercion according to the current level of help performed by the subordinate. This allowed keeping the social context and dominance hierarchy unaltered while experimentally modifying the opportunity for help enforcement. In the control (+ *I*) the cylinders were inserted in a similar fashion, but the breeders remained outside and unconstrained in their ability to punish the subordinate. During this phase, the mesh-cage was placed over the breeding chamber like in the baseline. We scored the helper behaviour for 15min after a 5min acclimatisation period.

After each phase, we manipulated the mesh-cage to control for manipulation between control and treatment. We performed a full factorial experimental design with randomised order of the four trials. After finishing each trial, a gap of 2h was kept before starting the next trial.

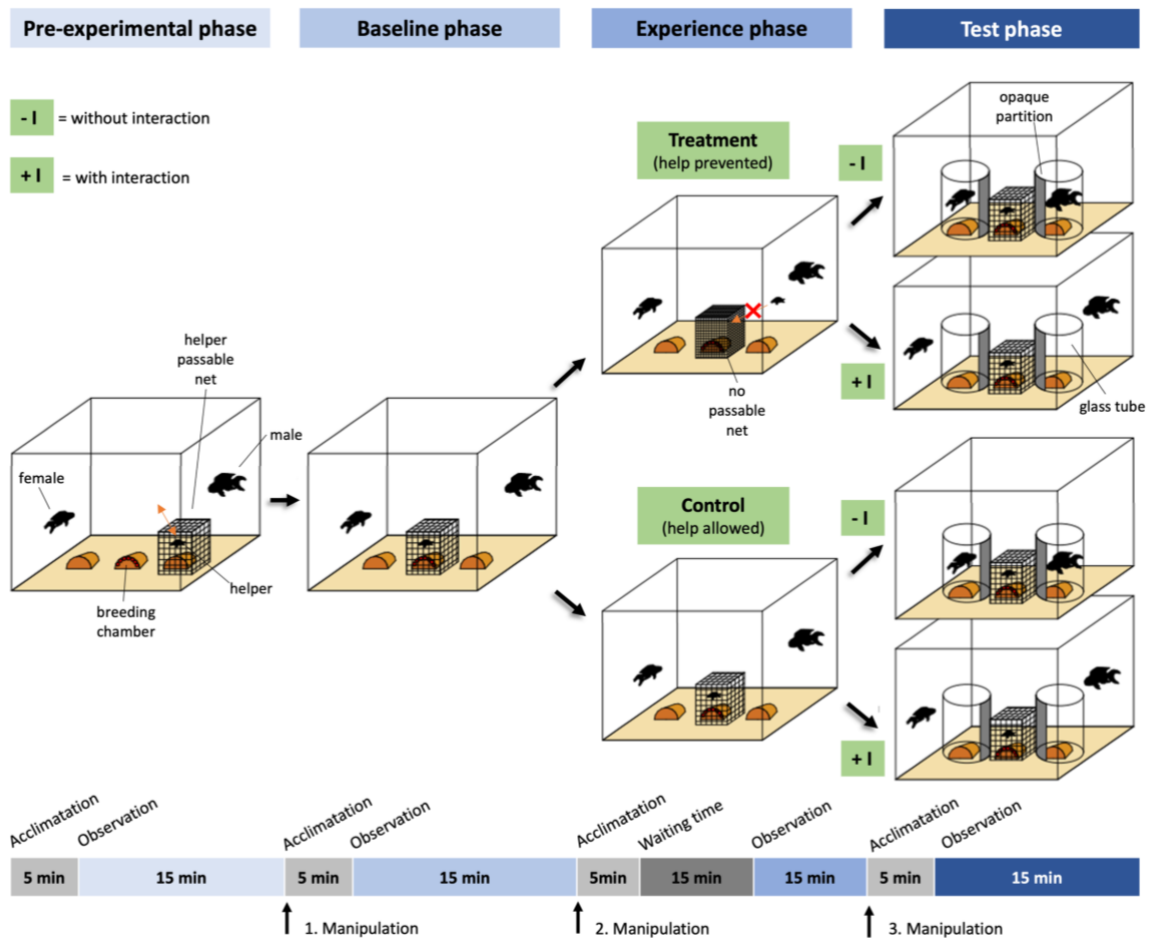


Figure 1. Schematic of the experimental set-up. The experiment started the day after the dominant female spawned. Pre-experimental phase: the recording device was placed in front of the aquarium, both breeders and helper were able to access the breeding chamber. Baseline phase: the mesh-cage only passable by the helper was placed over the breeding chamber, only the helper was able to access the eggs. Experience phase: either a non-passable net was placed over the breeding chamber preventing the helper from alloparental care during 30 min (*treatment*), or the passable net was reallocated on top of the breeding chamber (*control*). Test phase: either the breeders were prevented to interact with the helper by a half transparent-half opaque cylinder (*-I*) or they were allowed to interact with the helper (*+I*). All groups underwent all four possible combinations in a randomised full factorial experimental design. A minimum of 2h passed between trials.

Behavioural observations

We scored two aspects of direct brood care behaviour: the frequency of micro nipping of the eggs, which serves a hygienic function, referred to as “egg cleaning”, and the frequency of generating water flow to the clutch, which increases the oxygen supply, referred to as “egg fanning” (Grantner and Taborsky 1998; Taborsky and Grantner 1998). Additionally, we measured sand digging inside the breeding chamber and in shelters separately. All social interactions between breeders and helpers were scored, including submission, affiliation (bumping), overt and restrained aggression, and other agonistic interactions like fleeing or

avoiding (for brief descriptions of behaviours see (Taborsky 1984). The time spent in the breeding chamber or in shelters was also scored. The experimental phases were video recorded and encoded for blind scoring purposes. The recordings were manually scored using the Boris 7.9 software (Friard and Gamba 2016).

Data analysis

During the “test phase” we assessed the hypothesis that under pay-to-stay, helpers would increase alloparental care after being previously prevented to help during the “experience phase” only if the breeders could interact with the helper (T/+I), while under kin selection we expect no influence of the presence of breeders (+I = -I), (Figure 1; Table 1). Under kin selection, voluntary compensatory help may also be present. We used brood care (egg cleaning and fanning), and sand digging in the breeding chamber as response variables. The fixed effects variables of the model were prevention to access the eggs during the previous phase (T vs C), interaction with the breeders (+I vs -I), relatedness degree between helper and breeders (related vs unrelated), helper size, and the number of eggs. We also included the interaction of egg care prevention, interaction for the breeders with the helper and relatedness status when analysing brood care as the response variable, but not for digging behaviour due to the low frequency of digging events. Group ID was added as a random effect in all analyses to account for repeated use of the same groups for the different treatments.

We ran zero-altered generalised linear mixed-effects models using the R package glmmADMB (Zuur et al. 2009; Fournier et al. 2012). Huddle models allows us to test the significance of the presence or absence of a behaviour together with the quantitative significance. The probability of occurrence can be a more reliable factor as the amount of a given behaviour can be affected by many uncontrolled factors, such as the costs that the behaviour imposes in a given context (Emlen and Wrege 1988; Heinsohn and Legge 1999). For the presence/absence of the behaviour we fitted a binomial distribution, and a Poisson distribution for the count data (ZAP-GLMMs). In cases when the model did not fit a Poisson distribution, we fitted a negative binomial distribution instead (ZANB-GLMMs). To check for model fit and overdispersion, we used the function simulateResiduals from the R package DHARMA. The interactions between fixed factors were initially included in all models and dropped if they were not significant.

During the “experience phase,” we tested whether breeders would enforce helping when subordinates were prevented from helping. In this scenario, we expect that enforcement only occurs under pay-to-stay but not under kin selection. We run a ZAP-GLMMs as described above using aggression (restrained and overt) performed by the breeders as the response variable. The explanatory variables were prevention to access the eggs (T vs C), relatedness degree between helper and breeders and sex of the breeder, as well as the interactions of the fixed effects. In addition, we tested whether there was a difference in the type of aggression used against the helper depending on the treatment, relatedness degree or the sex of the breeder. For that, we run a weighted GLMM of the binomial family using the proportion of overt aggression vs total as the response variable. We also tested the response of the subordinate by analysing submissive and avoidance behaviours (avoid and flee) towards the breeders during this phase. An increase in submission and avoidance behaviours of the

subordinate would be expected for the prevention treatment only when enforcement occurs. We run ZAP-GLMMs as described above for both response variables. The fixed effects variables for both analyses were prevention to access the eggs and relatedness degree between helper and breeders, as well as the interactions between both. Non-significant interactions were removed from the analysis.

During the “baseline phase”, we examined whether relatedness influenced alloparental care and the aggression levels of the breeders. We run ZAP-GLMMs for levels of alloparental care and breeding chamber digging behaviours, using relatedness as a fixed factor and helper size and clutch size as covariates. For aggression levels, we run a ZAP-GLMM using relatedness and sex of the breeder as fixed effects. To control whether helpers used the breeding chamber to avoid direct aggression by the breeders, we analysed the impact of aggression by the breeders to the time the helper spent in the breeding chamber, controlling for the effect of help and relatedness. A negative relationship would mean that we cannot interpret differences in aggression between treatment and control in the “experience phase”.

All simulations and statistical tests were performed in R 3.6.3. (R Core Team 2020).

Table 1. Predictions evaluated in this study for the related subordinate. T: help prevention, C: control, +I: interaction possible between helper and breeder, -I: interaction prevented between helper and breeder, NK: non-kin, K: kin.

		Pay-to-stay	Kin selection	PS + KS	Results
Experience phase	<i>Aggression by breeders</i>	Yes, T > C	No	NK > K	T > C, NK > K
	<i>Submission by subordinates</i>	Yes, T > C	No	NK > K	T > C, NK > K
Test phase	<i>Alloparental care by subordinates</i>	T > C (when +I)	T ≥ C / +I = -I	NK ≥ K	T > C (when +I), NK = K

Results

Aggression by the breeders

Confirming predictions from the pay-to-stay hypothesis, when helpers were prevented from helping during the *experience phase*, both kin and non-kin subordinates were exposed to less aggression by the breeders (ZAP binomial: 1.17 ± 0.34 , $Z = 3.47$, $p < 0.001$; count: 1.04 ± 0.41 , $Z = 2.54$, $p = 0.01$; Figure 2a). In addition, breeders also used overt aggressive behaviours more often than restrained aggression when charging the helpers (1.70 ± 0.81 , $Z = 2.11$, $p = 0.03$; Figure 2b).

Relatedness alleviated dominant aggression, as related helpers were attacked less in the *experience phase* than unrelated helpers (ZAP binomial: -1.26 ± 0.47 , $Z = -2.67$, $p = 0.007$; count: -1.44 ± 0.49 , $Z = -2.92$, $p = 0.003$; Figure 2a), whereas the ratio between overt and restrained aggression did not differ between related and unrelated helpers (-0.77 ± 0.75 , $Z = -$

1.04, $p = 0.30$). Lower levels of aggression by breeders towards kin than towards non-kin were shown also in the *baseline condition* (ZAP count: -1.46 ± 0.61 , $Z = -2.39$, $p = 0.02$). These results provide evidence that both kin and non-kin are enforced to help, but relatedness modifies coercion.

When comparing aggressive responses towards helpers between male and female breeders, we found that male breeders were aggressive on less often than female breeders (ZAP binomial: -0.88 ± 0.32 , $Z = -2.72$, $p = 0.006$), but their aggression levels were higher when they did charge helpers (ZAP count: 0.62 ± 0.17 , $Z = 3.67$, $p = 0.0002$). Females aggressed more frequently the helpers also in the baseline condition (ZAP binomial: -6.15 ± 1.38 , $Z = -4.46$, $p < 0.001$).

Due to the experimental design, breeder aggression might have been influenced by the fact that helpers could retreat to the inaccessible breeding chamber when being attacked. However, in the *baseline condition* the time spent in the breeding chamber was not negatively influenced by the amount of aggression shown by the breeders (LMM, $t = 0.42$, $p = 0.68$). Instead, the time spent in the breeding chamber was influenced by relatedness (LMM, $t = -4.24$, $p = 0.0001$) and the amount of alloparental care (brood care and digging) performed (LMM, $t = 9.83$, $p < 0.0001$).

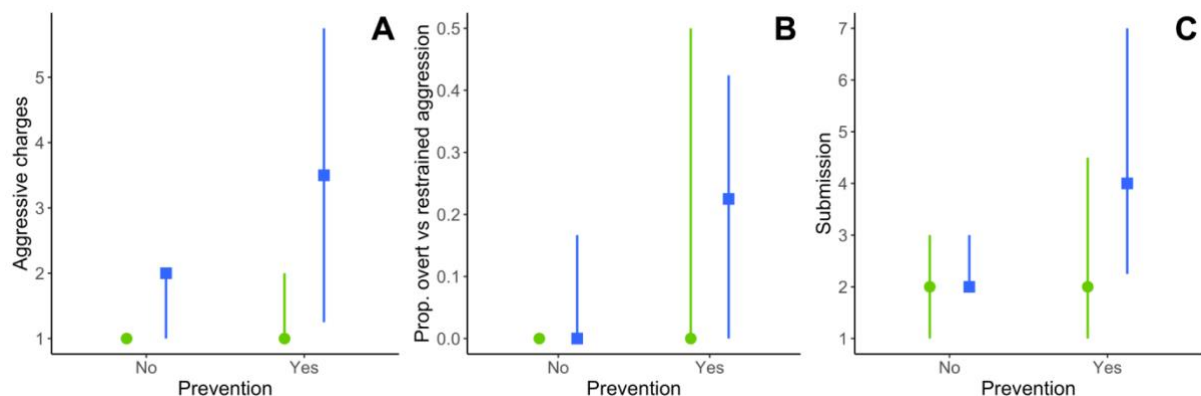


Figure 2. Overt aggression and restrained aggressive displays by breeders and submission by helpers are shown during the *experience phase*. (a) The frequency of the breeders' overt and restrain aggressive behaviours was higher in the help prevention treatment compared to the control, and unrelated helpers (blue squares) received more aggression than related helpers (green circles). Detailed statistics given in Table S1. (b) The breeders tended to use more overt aggressive displays than restrained aggression when helping was prevented than in the control condition. Detailed statistics given in Table S2. (c) Both related and unrelated helpers increased submissive displays when they were prevented from helping. Detailed statistics given in Table S3. Depicted are medians \pm interquartile ranges of behaviours when they occurred (i.e., zero occurrences are omitted).

Coinciding with the increased breeder aggression in the prevention treatment, helpers increased submissive displays when prevented from helping (ZAP binomial: 2.05 ± 0.46 , $Z = 4.48$, $p < 0.001$; count: 0.91 ± 0.17 , $Z = 5.52$, $p < 0.001$; Figure 2c). Likewise, helpers also showed more avoidance behaviours against breeders (ZAP binomial: 2.69 ± 0.91 , $Z = 2.94$, $p = 0.003$; count: 1.01 ± 0.17 , $Z = 6.07$, $p < 0.001$) when prevented from helping than in the control condition.

Related helpers showed less submission compared to unrelated helpers (ZAP binomial: -1.36 ± 0.53 , $Z = -2.56$, $p = 0.01$; count: -0.43 ± 0.20 , $Z = -2.17$, $p = 0.03$; Figure 2c), which coincides with the lower aggression levels they received from breeders (spearman correlation related: $R = 0.46$, $p < 0.001$; unrelated: $R = 0.42$, $p < 0.001$). Related helpers showed less avoidance behaviours towards breeders when prevented from helping than unrelated helpers (ZAP binomial: -2.18 ± 1.16 , $Z = -1.88$, $p = 0.06$; count: -0.58 ± 0.22 , $Z = -2.62$, $p = 0.009$).

Alloparental care

As predicted by the pay-to-stay hypothesis, in the *test phase* helpers provided higher levels of egg care when previously prevented from helping, but only when breeders were enabled to interact with them and thereby able to enforce help (ZAP count Prevention x Interaction: 0.50 ± 0.11 , $Z = 4.38$, $p < 0.001$; Figure 3a).

Kin provided brood care less frequently than non-kin when they had been prevented from helping (ZAP binomial Prevention x Relatedness: -2.52 ± 1.21 , $Z = -2.08$, $p = 0.04$), which suggest that related helpers have to pay a lower price than unrelated ones. However, they did provide in general alloparental care more frequently than unrelated helpers (ZAP binomial: 3.25 ± 1.43 , $Z = 2.28$, $p = 0.02$). This difference was not perceptible in the baseline (ZAP binomial: 0.31 ± 0.89 , $Z = 0.35$, $p = 0.72$; count: -0.13 ± 0.15 , $Z = -0.87$, $p = 0.38$).

Helpers provide alloparental care also in the form of digging out sand from the breeding shelter. This behaviour was shown in only 26% of the trials compared to 80% for direct egg care, as we did not add additional sand to any of the shelters during the experiment. Like with alloparental egg care, helpers also dug more often in the *test phase* after they had been prevented from helping before (ZAP count: 0.56 ± 0.19 , $Z = 2.94$, $p = 0.003$; Figure 3b), and when breeders were able to interact with them (ZAP count: 0.58 ± 0.23 , $Z = 2.47$, $p = 0.01$; Figure 3b). In addition, related helpers overall dug less than unrelated helpers (ZAP count: -0.80 ± 0.31 , $Z = -2.56$, $p = 0.01$).

Alloparental care also increased with the need of help, as more direct brood care was observed for larger clutches during the *baseline* (ZANB count: 0.01 ± 0.002 , $Z = 2.49$, $p = 0.01$). However, digging in the brood chamber showed the opposite tendency (ZANB count: -0.02 ± 0.01 , $Z = -2.21$, $p = 0.03$). This result might stem from the concurrent increase in direct egg care, possibly sifting the helpers' behaviour from one form of alloparental care to another. No egg cannibalism was observed for any of the experimental subordinates.

Larger, although immature, helpers provided more alloparental care in the form of direct brood care (ZANB count: 0.41 ± 0.19 , $Z = 2.23$, $p = 0.03$) and digging in the breeding shelter (ZANB count: 1.21 ± 0.47 , $Z = 2.56$, $p = 0.01$).

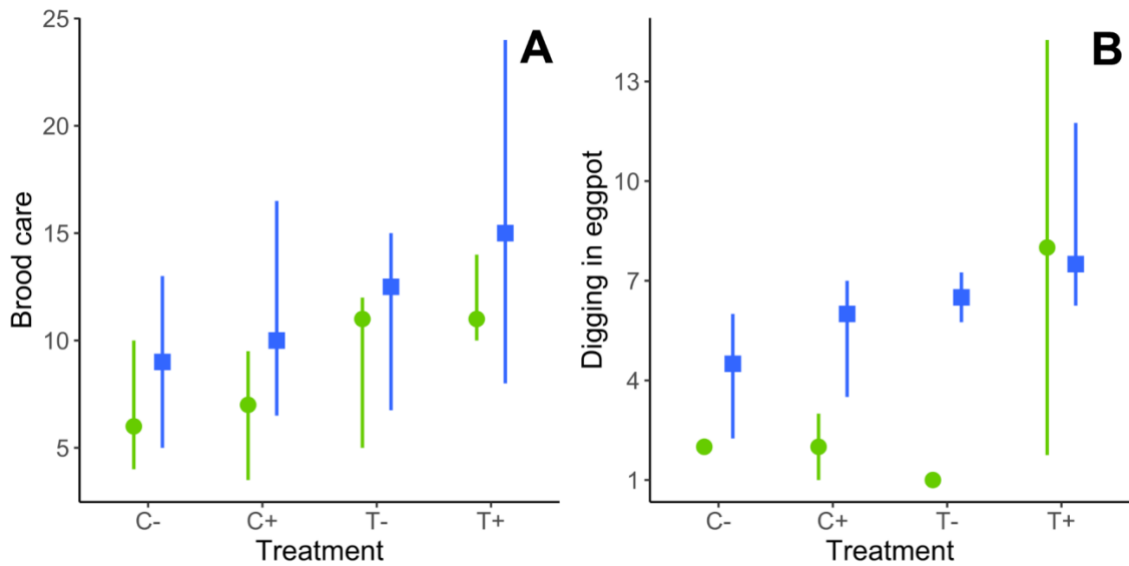


Figure 3. Alloprenatal care during the *test phase*. (a) Both kin (green circles) and non-kin (blue squares) helpers provided more brood care when previously prevented from helping (T: treatment vs C: control), but only if the breeders could interact with them (“+”: breeders could interact with helpers, vs “-”: breeders were prevented to interact with helpers). (b) Helpers dug in the breeding shelter more often when previously prevented to help only if the breeders could interact with them, with more digging performed by unrelated helpers. Depicted are medians \pm interquartile ranges of behaviours when they occurred (i.e., zero occurrences are omitted). Detailed statistics given in Table S4.

Discussion

The threat of punishment has been invoked as a key factor promoting the evolution of cooperation among non-relatives (Taborsky 1985; Clutton-Brock and Parker 1995; Cant and Johnstone 2006; Cant 2011; Cant and Young 2013; Quiñones et al. 2016; Ågren et al. 2019; Engelhardt and Taborsky 2020), but few studies have demonstrated the link between punishment and cooperation in animal societies (Bshary and Grutter 2005; Wong et al. 2007; Naef and Taborsky 2020a, 2020b). Here, we find support for the evolution of alloprenatal care as a result of a negotiation processes involving unequal partners in which each group member tries to maximise their own pay-off.

Consistent with predictions from pay-to-stay models (Gaston 1978; Kokko et al. 2002; Hamilton and Taborsky 2005), we found that help-prevention increased the dominants’ aggression (both frequency and intensity). Crucially, punishment increased cooperation (direct brood care and digging in the breeding chamber), and the increase in cooperation was mediated by the ability of breeders to enforce help. As a result of the increase in punishment, subordinates raised submissive displays, corroborating previous findings in the field (Heg and Taborsky 2010). Helpers may increase submission as a response to increased levels of aggression by the breeders, as often submissive displays were following an aggressive display by the breeders, but they may also serve as a pre-emptive appeasement to reduce aggression and increase tolerance in the territory (Taborsky 1985; Bergmüller and Taborsky 2005a).

Enforcement was also involved if helpers were related to breeders, but relatedness clearly reduced coercion in the negotiations between helper and breeders as predicted (Quiñones et al. 2016). Dominant breeders attacked related helpers less than unrelated helpers during experimental help prevention. In response to reduced aggression levels by the breeders, related helpers also displayed less submission than unrelated helpers. Submissive behaviour inflicts substantial energy costs for helpers (Grantner and Taborsky 1998; Taborsky and Grantner 1998). Therefore, related helpers apparently also pay rent like unrelated subordinates, but a lower demand of breeders from related helpers to pay rent is reflected by lower levels of the exchange of aggression and submission. Additionally, related helpers compensated help prevention by providing egg care less often than unrelated helpers, which confirms previous experimental results (Zöttl et al. 2013c). Overall, alloparental egg care levels were similar between related and unrelated helpers, which is not a very costly behaviour (Taborsky and Grantner 1998). However, unrelated helpers showed more shelter digging than related helpers, which is the most energy-demanding form of help causing a six-fold increase of routine metabolic rate (Grantner and Taborsky 1998). This is in accordance with a model predicting higher helping efforts resulting from enforcement than from kin selection (Quiñones et al. 2016). Contrary to the prediction of a previous theoretical model, however, helping by related subordinates also seems to be partly mediated by the threat of aggression by the dominant breeders, instead of a completely voluntary commodity (Quiñones et al. 2016).

In instances for which subordinates impose a higher cost to the breeders, negotiation theory predicts that these individuals should pay a higher price to stay in the territory. In general, the reproductive competition differs between the sexes, being stronger between males (Oliveira et al. 2008). Subordinate males may steal fertilizations from the dominant male, while subordinate females may just spawn in a different shelter from the dominants' territory, which may yield resource competition and involve a potential risk of egg cannibalism (Dierkes et al. 1999; Heg and Hamilton 2008; Heg et al. 2009; Mitchell et al. 2009). We found differences between the aggression levels of dominant males and females, with males being marginally more aggressive when they attacked a helper, but females charging helpers on more occasions. This result might depend on the reproductive stage. Since the fertilization of the eggs had already happened in our experimental period, the main threat during this study was egg cannibalism (Zöttl et al. 2013c), which might explain why the females were more prone to show aggression toward subordinates. This corroborates results from a previous study, where females also showed more aggression against helpers than pair males did during the early egg stages (Taborsky and Grantner 1998).

Previous findings in *N. pulcher* supported predictions that helpers pay-to-stay (Taborsky 2016): (1) punishment for not helping scaled positively with the need for help (Heg and Taborsky 2010), (2) punishment led to increased levels of help (Fischer et al. 2014; Naef and Taborsky 2020a, 2020b), (3) there was a higher acceptance of helpers when breeders were in need (Taborsky 1985; Zöttl et al. 2013b), (4) and less help was given by closer related helpers (Zöttl et al. 2013c). Our experiment now adds crucial evidence: (5) breeders punish helpers for reduced levels of help and (6) this punishment of breeders in turn raises helping levels. Furthermore, our experiment suggests that also related helpers pay-to-stay, albeit to a lower degree.

Additional mechanisms may be involved in the evolution of alloparental care in this species. A likely possibility is group augmentation, which proposes that helping is favoured if it enhances group size, which in turn increases helper survival and/or future reproductive output (Woolfenden 1975). Dispersing subordinates of *N. pulcher* prefer to join larger groups, and group size is associated with long-term group stability and survival (Taborsky et al. 2005; Reddon et al. 2011), which is consistent with predictions of group augmentation. Since helping is costly, altruistic behaviour could also have evolved as a prestige signal that serves as advertisement of quality to increase dominance rank and ultimately gain breeding status (Zahavi 1995). However, rank in *N. pulcher* is highly correlated with body size (Hamilton et al. 2005), which changes permanently in species exhibiting lifelong growth, and helpers that tend to help more also have a higher tendency to disperse to other groups instead of inheriting the territory (Antunes and Taborsky 2020). Therefore, this mechanism is an unlikely explanation for the evolution of help in this species.

A limitation of our methodology is that the mesh cage used to prevent the breeders from accessing the breeding chamber could have also served as a protected shelter for the subordinates during the control conditions. However, we did not find a correlation between the time spent in the breeding chamber and the agonistic behaviours performed by the dominants during the baseline condition. Additionally, in the few instances in which a helper was strongly charged, it used the floating shelters above the territory as a safe place instead of the mesh cage. Likewise, breeders could have increased agonistic behaviour due to the prevention of access to the breeding chamber. This effect is also likely higher for the female than for the male breeder, which contributes typically much more direct brood care. In fact, breeders did show aggressive displays when the helper was inside the breeding chamber, but the same behaviour was shown when the helper was in a covered shelter instead the breeding chamber with the mesh cage during the pre-experimental phase, so this behaviour did not seem to be related to the access of the helpers to the eggs. Regardless, this effect is the same for all treatment conditions, since the breeders were not allowed to access the breeding chamber ever during the duration of the experiment.

To our knowledge, this is the first experimental study testing explicitly for a negotiation process controlling for all interactions involved in a cooperatively breeding species. We conclusively show that alloparental care is at least partly driven by enforcement in *N. pulcher*, and kin selection seems to mediate the need for payment. Our study highlights the importance of using an integrative approach to study alloparental care behaviour, where several evolutionary mechanisms are considered simultaneously in order to disentangle interactions between different selection forces. Previous observational studies point in the same direction (Sumner et al. 2010; Wright et al. 2010; Kingma et al. 2011). Importantly, kin selection should not be automatically assumed to be the sole force selecting for cooperation even in species in which helpers are related. Additionally, direct fitness benefits might be the original force selecting for group formation, which may enable kin selection to evolve (Garcia-Ruiz et al., 2022). Therefore, this approach will help to resolve the ongoing debate about the importance of direct and indirect fitness benefits driving the evolution of cooperative breeding (e.g., (Clutton-Brock 2002, 2009; Dickinson and Hatchwell 2004; Heinsohn 2004; Lehmann and Keller 2006; West et al. 2006, 2007; Taborsky et al. 2021).

References

- Ågren, J. A., N. G. Davies, and K. R. Foster. 2019. Enforcement is central to the evolution of cooperation. *Nature Ecology and Evolution* 3.
- Antunes, D. F., and B. Taborsky. 2020. Early social and ecological experience triggers divergent reproductive investment strategies in a cooperative breeder. *Scientific Reports* 10:1–8.
- Balshine, S., B. Leach, F. Neat, H. Reid, M. Taborsky, and N. Werner. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* 50:134–140.
- Balshine-Earn, S., F. C. Neat, H. Reid, and M. Taborsky. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology* 9:432–438.
- Bergmüller, R., D. Heg, and M. Taborsky. 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences* 272:325–331.
- Bergmüller, R., and M. Taborsky. 2005a. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- . 2005b. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- Binmore, K. 2010. Bargaining in biology? *Journal of evolutionary biology* 23:1351–1363.
- Brown, J. L. 1987. Helping and Communal Breeding in Birds. *Journal of Evolutionary Biology* 2:62–65.
- Bruintjes, R., D. Bonfils, D. Heg, and M. Taborsky. 2011. Paternity of subordinates raises cooperative effort in cichlids. *PLoS One* 6:e25673.
- Bruintjes, R., and M. Taborsky. 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour* 75:1843–1850.
- . 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* 81:387–394.
- Bshary, R., and A. S. Grutter. 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters* 1:396–399.
- Buston, P. M., and A. G. Zink. 2009. Reproductive skew and the evolution of conflict resolution: a synthesis of transactional and tug-of-war models. *Behavioral Ecology* 20:672–684.
- Cant, M. A. 2011. The role of threats in animal cooperation. *Proceedings of the Royal Society B: Biological Sciences* 278:170–178.
- Cant, M. A., and R. A. Johnstone. 2006. Self-serving punishment and the evolution of cooperation. *Journal of Evolutionary Biology* 19:1383–1385.
- Cant, M. A., and R. A. Johnstone. 2009. How threats influence the evolutionary resolution of within-group conflict. *The American Naturalist* 173:759–771.
- Cant, M. A., and S.-F. Shen. 2006. Endogenous timing in competitive interactions among relatives. *Proceedings of the Royal Society B: Biological Sciences* 273:171–178.

- Cant, M. A., and A. J. Young. 2013. Resolving social conflict among females without overt aggression. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368:20130076.
- Clutton-Brock, T. H. 2002. Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72.
- . 2009. Cooperation between non-kin in animal societies. *Nature* 462:51–57.
- Clutton-Brock, T. H., and G. A. Parker. 1995. Punishment in animal societies. *Nature* 373:209–216.
- Cockburn, A. 1998. Evolution of Helping Behavior in Cooperatively Breeding Birds. *Annual Review of Ecology and Systematics* 29:141–177.
- Dickinson, J. L., and B. J. Hatchwell. 2004. Fitness consequences of helping. Pages 48–66 *in* *Ecology and evolution of cooperative breeding in birds*. Cambridge: Cambridge University Press.
- Dierkes, P., D. Heg, M. Taborsky, E. Skubic, and R. Achmann. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* 8:968–975.
- Dierkes, P., M. Taborsky, and U. Kohler. 1999. Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behavioral Ecology* 10:510–515.
- Donaldson, L., F. J. Thompson, J. Field, and M. A. Cant. 2014. Do paper wasps negotiate over helping effort? *Behavioral Ecology* 25:88–94.
- Emlen, S. T., and P. H. Wrege. 1988. The role of kinship in helping decisions among white-fronted bee-eaters. *Behavioral Ecology and Sociobiology* 23:305–315.
- Engelhardt, S. C., and M. Taborsky. 2020. Broad definitions of enforcement are unhelpful for understanding evolutionary mechanisms of cooperation. *Nature Ecology & Evolution* 4:322.
- Fischer, S., M. Zöttl, F. Groenewoud, and B. Taborsky. 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 281:20140184.
- Fournier, D. A., H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. N. Maunder, A. Nielsen, et al. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* 27:233–249.
- Friard, O., and M. Gamba. 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.
- Gaston, A. J. 1978. The Evolution of Group Territorial Behavior and Cooperative Breeding. *The American Naturalist* 112:1091–1100.
- Grantner, A., and M. Taborsky. 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 168:427–433.
- Groenewoud, F., J. G. Frommen, D. Josi, H. Tanaka, A. Jungwirth, and M. Taborsky. 2016. Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences* 113:4104–4109.
- Hamilton, I. M., D. Heg, and N. Bender. 2005. Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. *Behaviour* 142:1591–1613.

- Hamilton, I. M., and M. Taborsky. 2005. Unrelated helpers will not fully compensate for costs imposed on breeders when they pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 272:445–454.
- Hammerstein, P., and R. Noë. 2016. Biological trade and markets. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150101.
- Heg, D., Z. Bachar, L. Brouwer, and M. Taborsky. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences* 271:2367–2374.
- Heg, D., L. Brouwer, Z. Bachar, and M. Taborsky. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg, D., and I. M. Hamilton. 2008. Tug-of-war over reproduction in a cooperatively breeding cichlid. *Behavioral Ecology and Sociobiology* 62:1249–1257.
- Heg, D., E. Jutzeler, J. S. Mitchell, and I. M. Hamilton. 2009. Helpful female subordinate cichlids are more likely to reproduce. *PLoS One* 4:e5458.
- Heg, D., and M. Taborsky. 2010. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- Heinsohn, R. G. 2004. Parental care, load-lightening, and costs. Pages 67–80 in W. D. Koenig and J. L. Dickinson, eds. *Ecology and evolution of cooperative breeding in birds*. Cambridge University Press Cambridge, UK.
- Heinsohn, R., and S. Legge. 1999. The cost of helping. *Trends in Ecology and Evolution* 14:53–57.
- Hellmann, J. K., I. Y. Ligocki, C. M. O'Connor, A. R. Reddon, K. A. Garvy, S. E. Marsh-Rollo, H. L. Gibbs, et al. 2015. Reproductive sharing in relation to group and colony-level attributes in a cooperative breeding fish. *Proceedings of the Royal Society B: Biological Sciences* 282:20150954.
- Hert, E. 1985. Individual recognition of helpers by the breeders in the cichlid fish *Lamprologus brichardi* (Poll, 1974). *Zeitschrift für Tierpsychologie* 68:313–325.
- Jungwirth, A., J. Walker, and M. Taborsky. 2015. Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Animal Behaviour* 106:107–114.
- Kingma, S. A., M. L. Hall, and A. Peters. 2011. Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist* 177:486–495.
- Koenig, W. D., and J. L. Dickinson. 2016. *Cooperative Breeding in Vertebrates*. (W. D. Koenig & J. L. Dickinson, eds.). Cambridge University Press, Cambridge.
- Kokko, H., R. A. Johnstone, and J. Wright. 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behavioral Ecology* 13:291–300.
- Le Vin, A. L., B. K. Mable, and K. E. Arnold. 2010. Kin recognition via phenotype matching in a cooperatively breeding cichlid, *Neolamprologus pulcher*. *Animal Behaviour* 79:1109–1114.
- Lehmann, L., and L. Keller. 2006. The evolution of cooperation and altruism - A general framework and a classification of models. *Journal of Evolutionary Biology* 19:1365–1376.
- Marshall, J. A. R., and J. E. Rowe. 2003. Kin selection may inhibit the evolution of reciprocation. *Journal of Theoretical Biology* 222:331–335.

- Mitchell, J. S., E. Jutzeler, D. Heg, and M. Taborsky. 2009. Dominant members of cooperatively-breeding groups adjust their behaviour in response to the sexes of their subordinates. *Behaviour* 1665–1686.
- Naef, J., and M. Taborsky. 2020a. Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science* 7:191808.
- . 2020b. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* 168:137–147.
- Noë, R., C. P. van Schaik, and J. van Hooff. 1991. The market effect: an explanation for pay-off asymmetries among collaborating animals. *Ethology*. Berlin, Hamburg 87:97–118.
- Oliveira, R. F., M. Taborsky, and H. J. Brockmann. 2008. *Alternative reproductive tactics: an integrative approach*. Cambridge University Press.
- Quiñones, A. E., G. S. van Doorn, I. Pen, F. J. Weissing, and M. Taborsky. 2016. Negotiation and appeasement can be more effective drivers of sociality than kin selection. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150089.
- Reddon, A. R., D. Balk, and S. Balshine. 2011. Sex differences in group-joining decisions in social fish. *Animal Behaviour* 82:229–234.
- Schweinfurth, M. K., and M. Taborsky. 2018. Relatedness decreases and reciprocity increases cooperation in Norway rats. *Proceedings of the Royal Society B: Biological Sciences* 285:20180035.
- Solomon, N. G., and J. A. French. 1997. *Cooperative breeding in mammals*. Cambridge University Press.
- Stiver, K. A., P. Dierkes, M. Taborsky, H. Lisle Gibbs, and S. Balshine. 2005. Relatedness and helping in fish: examining the theoretical predictions. *Proceedings of the Royal Society B: Biological Sciences* 272:1593–1599.
- Stiver, K. A., J. Fitzpatrick, J. K. Desjardins, and S. Balshine. 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Animal Behaviour* 71:449–456.
- Sumner, S., H. Kelstrup, and D. Fanelli. 2010. Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proceedings of the Royal Society B: Biological Sciences* 277:1721–1728.
- Taborsky, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour* 32:1236–1252.
- . 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* 95:45–75.
- . 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Advances in the Study of Behavior* 23:e100.
- . 2016. Cichlid fishes: A model for the integrative study of social behavior. Pages 272–293 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Taborsky, M., M. A. Cant, and J. Komdeur. 2021. *The Evolution of Social Behaviour*. Cambridge University Press.

- Taborsky, M., and A. Grantner. 1998. Behavioural time–energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Animal Behaviour* 56:1375–1382.
- Taborsky, M., and D. Limberger. 1981. Helpers in fish. *Behavioral Ecology and Sociobiology* 8:143–145.
- West, S. A., A. Gardner, and A. S. Griffin. 2006. Altruism. *Current Biology* 16:R482–R483.
- West, S. A., A. S. Griffin, and A. Gardner. 2007. Evolutionary Explanations for Cooperation. *Current Biology* 17:661–672.
- Wong, M., and S. Balshine. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biological Reviews* 86:511–530.
- Wong, M. Y. L., P. M. Buston, P. L. Munday, and G. P. Jones. 2007. The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. *Proceedings of the Royal Society B: Biological Sciences* 274:1093–1099.
- Woolfenden, G. E. 1975. Florida scrub jay helpers at the nest. *The Auk* 92:1–15.
- Wright, J., P. G. McDonald, L. te Marvelde, A. J. N. Kazem, and C. M. Bishop. 2010. Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B: Biological Sciences* 277:437–445.
- Zahavi, A. 1995. Altruism as a handicap: the limitations of kin selection and reciprocity. *Journal of Avian Biology* 26:1–3.
- Zöttl, M., S. Fischer, and M. Taborsky. 2013a. Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour* 85:1471–1478.
- Zöttl, M., J. G. Frommen, and M. Taborsky. 2013b. Group size adjustment to ecological demand in a cooperative breeder. *Proceedings of the Royal Society B: Biological Sciences* 280:20122772.
- Zöttl, M., D. Heg, N. Chervet, and M. Taborsky. 2013c. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* 4:1341.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Zero-truncated and zero-inflated models for count data. Pages 261–293 *in* Mixed effects models and extensions in ecology with R. Springer.

Supplementary materials

Table S1. Effect of help prevention and relatedness of the helper on the levels of overt and restrained aggression by the breeders, during the experience phase and the baseline. The analysis scrutinised the presence/absence of aggression and the amount of aggression performed. Reference categories are no prevention (for prevention), unrelated (for relatedness) and female (for breeder's sex). Bold numbers despite significant main effect.

	<i>Experience phase</i>							
	<i>Estimate</i>	Count			<i>p</i>	Presence / absence		
<i>SE</i>		<i>z</i>		<i>Estimate</i>		<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	-0.35	0.40	-0.88	0.38	-1.34	0.39	-3.44	0.0005
<i>Prevention</i>	1.04	0.41	2.54	0.01	1.17	0.34	3.47	0.0005
<i>Relatedness</i>	-1.44	0.49	-2.92	0.003	-1.26	0.47	-2.67	0.007
<i>Sex</i>	0.62	0.17	3.67	0.0002	-0.88	0.32	-2.72	0.006

	<i>Baseline phase</i>							
	<i>Estimate</i>	Count			<i>p</i>	Presence / absence		
<i>SE</i>		<i>z</i>		<i>Estimate</i>		<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	0.37	0.27	1.38	0.16	-8.38	1.22	-6.87	<0.001
<i>Relatedness</i>	-1.46	0.61	-2.39	0.02	-0.77	1.38	-0.56	0.57
<i>Sex</i>	-0.63	0.48	-1.32	0.19	-6.15	1.38	-4.46	<0.001

Table S2. Effect of help prevention and relatedness of the helper on the levels of overt vs restrained aggression by the breeders, during the experience phase and the baseline. The analysis scrutinised the proportion of overt aggression vs the total of aggression performed. Reference categories are no prevention (for prevention), unrelated (for relatedness) and female (for breeder's sex). Bold numbers despite significant main effect.

	<i>Experience phase</i>			
	Estimate	SE	Z	p-value
<i>Intercept</i>	-2.56	0.76	-3.37	0.0007
<i>Prevention</i>	1.70	0.81	2.11	0.03
<i>Relatedness</i>	-0.77	0.75	-1.04	0.30
<i>Sex</i>	0.45	0.46	0.96	0.34

	<i>Baseline phase</i>			
	Estimate	SE	Z	p-value
<i>Intercept</i>	-1.19	0.34	-3.44	0.0006
<i>Relatedness</i>	-0.34	0.34	0.00	1.00
<i>Sex</i>	0.27	0.68	0.39	0.69

Table S3. Effect of help prevention and relatedness of the helper on the levels of submission and breeders' avoidance by the helper, during the experience phase and the baseline. The analysis scrutinised the presence/absence of submission and avoidance and the amount performed of these behaviours. Reference categories are no prevention (for prevention), unrelated (for relatedness) and female (for breeder's sex). Bold numbers despite significant main effect.

Submission by the helper

		<i>Experience phase</i>							
		Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
<i>Intercept</i>	0.55	0.19	2.93	0.003	0.33	0.39	0.85	0.40	
<i>Prevention</i>	0.91	0.17	5.52	<0.001	2.05	0.46	4.48	<0.001	
<i>Relatedness</i>	-0.43	0.20	-2.17	0.03	-1.36	0.53	-2.56	0.01	

		<i>Baseline phase</i>							
		Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
<i>Intercept</i>	0.81	0.12	6.52	<0.001	-0.29	0.25	-1.15	0.25	
<i>Relatedness</i>	-0.53	0.24	-2.21	0.03	-0.54	0.36	-1.48	0.14	

Avoidance by the helper

		<i>Experience phase</i>							
		Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
<i>Intercept</i>	0.74	0.16	4.54	<0.001	0.94	0.51	1.84	0.07	
<i>Prevention</i>	1.01	0.17	6.07	<0.001	2.69	0.91	2.94	0.003	
<i>Relatedness</i>	0.15	0.21	0.72	0.47	1.22	0.74	1.65	0.10	
<i>Prevention x Relatedness</i>	-0.58	0.22	-2.62	0.009	-2.18	1.16	-1.88	0.06	

		<i>Baseline phase</i>							
		Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
<i>Intercept</i>	0.68	0.14	4.93	<0.001	0.74	0.27	2.70	0.007	
<i>Relatedness</i>	0.12	0.18	0.70	0.49	0.41	0.39	1.06	0.29	

Table S4. Effect of help prevention, breeder interaction with helpers and relatedness on the levels of brood care (egg cleaning and fanning) and digging in the brood chamber, during the test phase and the baseline. The analysis scrutinised the presence/absence and the amount of alloparental care performed. Reference categories are no prevention, no interaction, and unrelated. Bold numbers despite significant results. Due to low digging counts, only main effects were run for digging behaviour.

Brood care: egg cleaning and fanning

	<i>Test phase</i>							
	Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	0.94	0.50	1.86	0.06	2.02	3.47	0.58	0.56
<i>Prevention</i>	0.11	0.10	1.05	0.29	1.03	0.85	1.22	0.22
<i>Interaction</i>	0.01	0.11	0.11	0.92	-0.30	0.81	-0.37	0.71
<i>Relatedness</i>	-0.25	0.17	-1.46	0.15	3.25	1.43	2.28	0.02
<i>Helper size</i>	0.46	0.18	2.49	0.01	-0.21	1.22	-0.17	0.87
<i>Clutch size</i>	0.001	0.002	0.60	0.55	-0.005	0.02	-0.30	0.77
<i>Prevention x Interaction</i>	0.50	0.11	4.38	<0.001	0.40	1.07	0.38	0.71
<i>Prevention x Relatedness</i>	-0.001	0.11	-0.01	0.99	-2.52	1.21	-2.08	0.04
<i>Interaction x Relatedness</i>	-0.04	0.11	-0.33	0.74	0.39	1.13	0.35	0.73

	<i>Baseline phase</i>							
	Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	0.97	0.54	1.79	0.07	0.18	3.03	0.06	0.95
<i>Relatedness</i>	-0.13	0.15	-0.87	0.38	0.31	0.89	0.35	0.72
<i>Helper size</i>	0.41	0.19	2.23	0.03	0.71	1.14	0.62	0.54
<i>Clutch size</i>	0.01	0.002	2.49	0.01	0.01	0.01	0.75	0.45

Sand digging in the brood chamber

	<i>Test phase</i>							
	Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	-0.42	1.11	-0.38	0.71	-3.27	1.74	-1.88	0.06
<i>Prevention</i>	0.56	0.19	2.94	0.003	-0.57	0.41	-1.39	0.16
<i>Interaction</i>	0.58	0.23	2.47	0.01	0.51	0.41	1.26	0.21
<i>Relatedness</i>	-0.80	0.31	-2.56	0.01	0.67	0.50	1.33	0.18
<i>Helper size</i>	0.72	0.43	1.69	0.09	1.21	0.65	1.86	0.06
<i>Clutch size</i>	-0.01	0.01	-1.11	0.26	0.02	0.01	-2.00	0.046

	<i>Baseline phase</i>							
	Count				Presence / absence			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
<i>Intercept</i>	-0.16	1.18	-0.13	0.89	-2.04	2.07	-0.99	0.32
<i>Relatedness</i>	-0.01	0.33	-0.04	0.97	-0.10	0.61	-0.16	0.87
<i>Helper size</i>	1.21	0.47	2.56	0.01	0.82	0.77	1.06	0.29
<i>Clutch size</i>	-0.02	0.01	-2.21	0.03	-0.01	0.01	-1.33	0.19

A decorative graphic consisting of a vertical dark blue bar on the left side of the page. From the top of this bar, a trail of white fish silhouettes of various sizes and orientations extends diagonally across the page towards the top right. From the bottom of the bar, another trail of dark blue fish silhouettes extends diagonally across the page towards the bottom right. The silhouettes vary in size, with larger ones appearing closer to the bar and smaller ones further away.

APPENDIX 1

Egg predator defence depends on both rank and size in a cooperatively breeding cichlid fish

Irene García-Ruiz^{1*}, Alexis Perret¹ and Michael Taborsky¹

¹ Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Switzerland.

*Corresponding author.

Preliminary results

Summary

In cooperatively breeding species, helping might evolve through long-term group augmentation benefits (Kokko et al. 2001; Kingma et al. 2014). These can be obtained, for instance, if the helper inherits a breeding position and offspring they helped to produce, in turn, increase their survival or fecundity. Under this hypothesis, higher helping levels are expected when there is an enhanced chance for the subordinate to inherit the territory (Kingma et al. 2014). In accordance with this prediction, in the Lake Tanganyika cichlid *Neolamprologus pulcher* larger helpers that are more likely to inherit the territory also invest more in alloparental care behaviours (Taborsky and Limberger 1981; Heg and Taborsky 2010a). Nevertheless, this is consistent also with the alternative hypothesis that breeders may enforce higher cooperation levels from larger subordinates due to imminent reproductive competition (pay-to-stay; (Taborsky 1985, 2016; Balshine-Earn et al. 1998). Here we study whether cooperative investment depends on the rank of subordinate individuals or whether there is rank-independent, size-specific task specialization, while controlling for alternative hypotheses like kin selection and coercion. We found that high rank individuals seem to defend more against an egg predator independently of size, meeting predictions of the long-term group augmentation hypothesis. However, the size of the focal also influenced significantly, with larger helpers providing less defence. No effect of rank was detected for territory maintenance.

Methods

Study species

Neolamprologus pulcher is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa (Duftner et al. 2007). Breeding groups consist of a dominant breeding pair that largely monopolizes reproduction, and 0-20 related and unrelated helpers (Balshine et al. 2001; Dierkes et al. 2005; Heg et al. 2005; Stiver et al. 2006; Taborsky 2016). Groups typically use self-dug burrows under rocks and small holes and crevices as shelters and for breeding (Taborsky and Limberger 1981; Balshine et al. 2001). The dominance hierarchy is strictly size based (Taborsky and Limberger 1981; Hamilton et al. 2005; Riebli et al. 2012). Helpers actively engage in brood care (cleaning and fanning eggs and mouthing larvae and free-swimming fry), territory maintenance (removing sand and particles from shelters), and territory defence (against predators, space competitors and conspecifics) (Taborsky and Limberger 1981; Taborsky 1984; Bruintjes and Taborsky 2011; Jungwirth et al. 2015).

To induce predation threat on eggs we used *Telmatochromis vittatus*, a cichlid fish that preys on eggs and fry of *N. pulcher* (Heg et al. 2008; Bruintjes and Taborsky 2011). Both dominant and subordinate group members defend the territory against *T. vittatus*, even though it is no direct threat to subordinates (Bruintjes and Taborsky 2011; Kasper et al. 2018; Naef and Taborsky 2020a, 2020b).

Experimental design

Test fish used in this experiment were laboratory-reared descendants from *N. pulcher* individuals caught in the wild near Kasakalawe point in Lake Tanganyika (Zambia). Experimental groups were housed in 200L aquaria.

Groups consisted of two breeders, a focal helper and a non-focal stimulus helper. To study the effect of rank in helping behaviours, we size matched focal helpers, and added a non-focal helper of larger or smaller size. Focal helpers were exposed to both rank treatment conditions at a random order. Even though focal helpers were chosen to be the same size, delays in the experiment due to time needed for group establishment produced small size differences (focal helpers at start of experiment: 29-34mm standard length, SL). In the high rank treatment (HR), non-focal helpers were between 3-8mm SL smaller than the focal helper. In the low rank (LR), non-focal helpers were between 2-9mm SL larger than the focal. All helpers were unrelated to the breeders in their group. We hosted two additional breeders behind a transparent partition to stimulate group stability by inducing territory defence (Bruintjes and Taborsky 2008).

If breeders spawned, we removed the eggs and waited three days to start the experiment. We obtained a total sample size of 19 groups.

Experimental procedure

To measure helping behaviour we constructed two distinct tests: an “egg predator defence test” and a “shelters digging test”. In this species breeders may coerce subordinates to provide help (Bergmüller and Taborsky 2005; Heg and Taborsky 2010b; Zöttl et al. 2013b; Fischer et al. 2014; Naef and Taborsky 2020a, 2020b). Since our aim is to measure voluntary alloparental care provisioning, we isolated the breeders before each test started behind an opaque partition (Fig. 1). In addition, to control for help provided by the non-focal helper, we isolated it behind a transparent partition with holes. Therefore, the non-focal remained present but unable to provide alloparental care. We waited 5 to 10 min for the fish to acclimatise after this manipulation before starting the experiment.

Before we started the egg predator test, we removed the shelter in the egg predator compartment so that it had a higher incentive to be close to the helper’s territory and to remain visually present. At the start of the test, we removed the opaque partition that separated the egg predator compartment from the focal helpers’ compartment (Fig.1). We video recorded and scored the focal helpers’ response for 15 min starting with the first behavioural response. We analysed frontal approach, operculum spread, and head down displays as aggressive behaviours. For a description of these behaviours see (Taborsky 1984). The percentage of time the egg predator was active was also scored to control for predation stimulus.

Two days after the egg predator test, we did the digging test. Before the start of the experiment, helpers were presented with the sand digging challenges to stimulate the behaviour. For the test, we removed all shelter but one and filled it up with sand. We scored sand digging for 45 min.

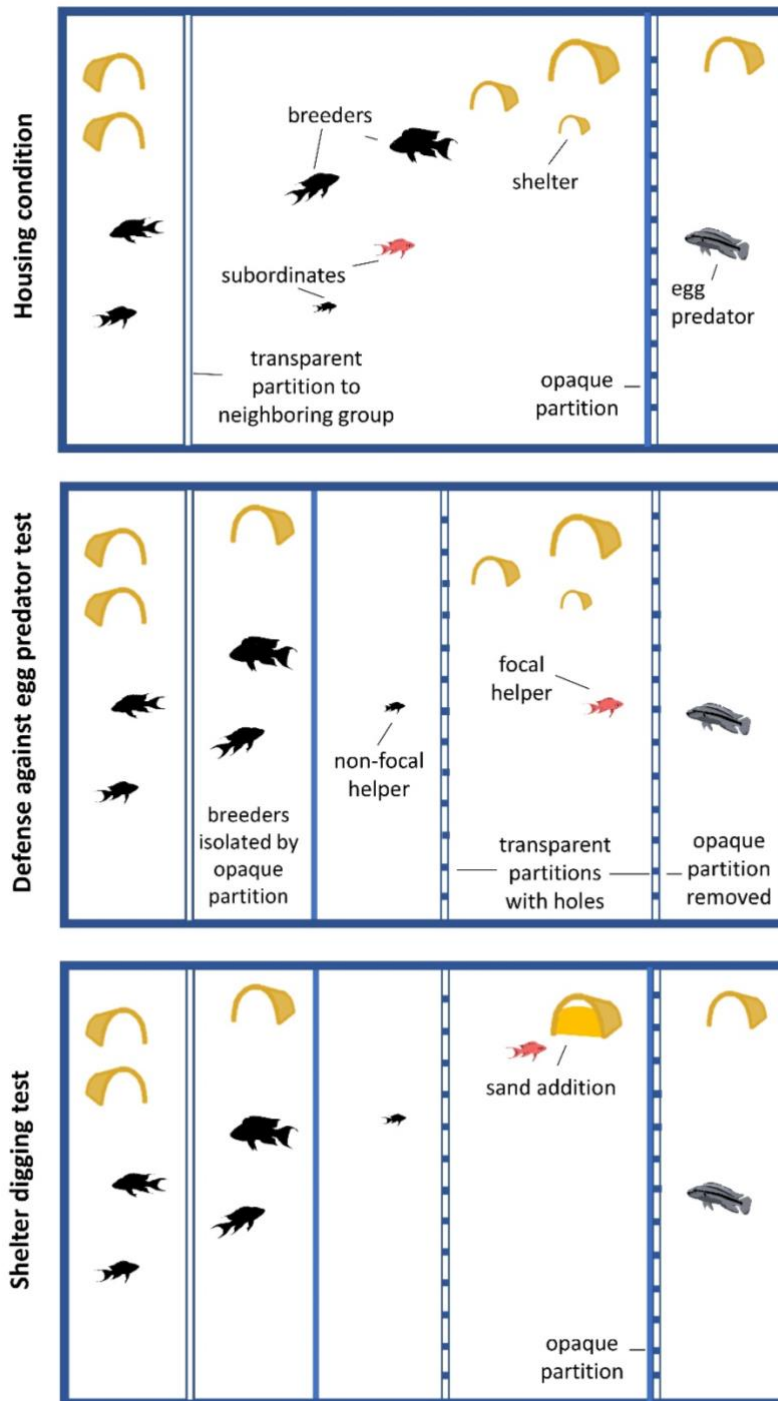


Figure 1. Experimental design. Before the test began, we isolated the breeders behind an opaque partition to prevent possible help enforcement. We also isolated the non-focal subordinate (larger than the focal in the low rank condition [LR], and smaller in the high rank condition [HR]) behind a transparent partition so that it remained present but without the possibility to provide help. In the defence test against the egg predator, we removed the opaque partition hiding the egg predator and recorded the behaviour of the focal helper for 15 min. In the digging test, we removed all shelter but one and filled it with sand. Subsequently we recorded digging events for 45 min.

Data analysis

We used defence against egg predator and digging events as dependent variables, treatment (HR or LR) as a factor, and controlled for the size of the helper and the activity of the egg predator (the last expressed as a percentage of time in the egg predator defence test). Helper ID was used as a random factor to control for repeated measures. We used generalized linear mix model to analyse the defence against the egg predator data with a Poisson error distribution. Because of the large number of absences of digging behaviour, we did a zero inflated negative binomial model to study the frequency of digging events. We used the R packages `lm4` and `glmmTMB` respectively. The interactions between independent variables were initially included in all models and dropped if they were not significant ($p > 0.05$). To check for model fit and overdispersion, we used the function `simulateResiduals` from the R package `DHARMA`. No significant deviations in the predicted vs expected residuals were detected. All simulations and statistical tests were performed in R 3.6.3. (R Core Team 2020).

Preliminary results

Defence against the egg predator

High rank individuals seem to defend more against an egg predator independently of size ($z = 3.10$, $p = 0.002$; Fig. 2). This result meets predictions of the long-term group augmentation hypothesis. However, the size of the focal also influenced significantly, with larger helpers providing less defence ($z = -2.49$, $p = 0.01$). Additionally, we found that high rank helpers seem to defend less when larger in size ($z = -3.06$, $p = 0.002$; Table 1).

Shelter digging test

No significant differences were found neither for rank ($z = 0.76$, $p = 0.45$) nor size ($z = -0.77$, $p = 0.441$) for digging behaviour (Fig. 2, Table 1).

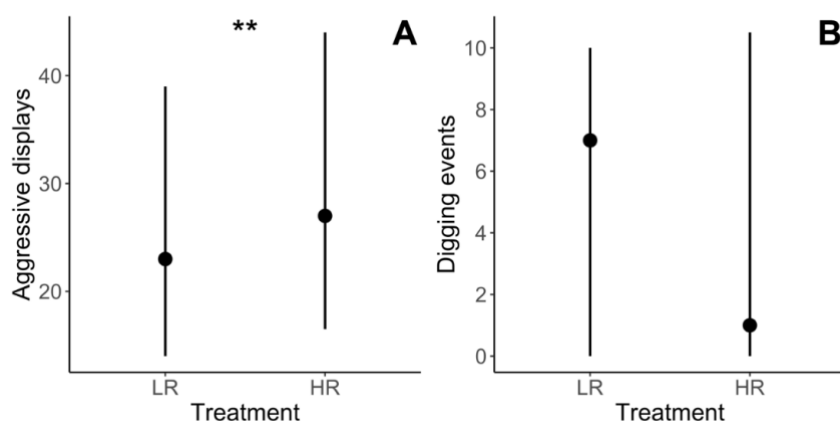


Figure 2. **A:** Numbers of aggressive displays towards the egg predator during 15min after the first reaction according to treatment (low rank LR or high rank HR focal). **B:** Digging counts in the shelters. Depicted are medians \pm interquartile ranges of behaviours. Asterisks denote significance ($p < 0.01$).

Brief discussion

Here, we show that helpers in the cooperatively cichlid fish *N. pulcher* adjust their levels of defence against an egg predator to their rank, with higher rank helpers defending more. This result is in line with predictions from the long-term group augmentation hypothesis, a form of transgenerational reciprocity, under which subordinates may help to increase group production, if the new recruits will in turn assist them if they obtain the breeding position (Kokko et al. 2001; Kingma et al. 2014). Because high rank individuals are higher in the queue to inherit the territory, higher rank individuals are expected to help more. A previous study in purple crowned fairy wrens also found that subordinates adjusted nestling feeding rates to the probability of inheriting the breeding position (Kingma et al. 2011). However, our results show helper size also influenced the defence frequency. Although the aim in this study was to control for helper size, a decrease in defence with size was also detected in a previous study in the field (Bruitjes and Taborsky 2011).

The absence of rank effects on digging behaviour may indicate that digging out shelters did not necessarily attain long-term fitness benefits. In this experiment, there were not eggs present and, therefore, shelter digging may correspond to a more immediate by product mutualistic benefit in the form of creating sheltering cavities from fish predators. In addition, territory maintenance in the breeding chamber has been linked to processes like pay-to-stay (Chapter 3, Appendix 2). Whether digging in the breeding chamber is also linked to long-term benefits is, however, still unknown.

References

- Balshine, S., B. Leach, F. Neat, H. Reid, M. Taborsky, and N. Werner. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* 50:134–140.
- Balshine-Earn, S., F. C. Neat, H. Reid, and M. Taborsky. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology* 9:432–438.
- Bergmüller, R., and M. Taborsky. 2005. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- Bruintjes, R., and M. Taborsky. 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour* 75:1843–1850.
- . 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* 81:387–394.
- Dierkes, P., D. Heg, M. Taborsky, E. Skubic, and R. Achmann. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* 8:968–975.
- Duftner, N., K. M. Sefc, S. Koblmüller, W. Salzburger, M. Taborsky, and C. Sturmbauer. 2007. Parallel evolution of facial stripe patterns in the *Neolamprologus brichardi/pulcher* species complex endemic to Lake Tanganyika. *Molecular Phylogenetics and Evolution* 45:706–715.
- Fischer, S., M. Zöttl, F. Groenewoud, and B. Taborsky. 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 281:20140184.
- Hamilton, I. M., D. Heg, and N. Bender. 2005. Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. *Behaviour* 142:1591–1613.
- Heg, D., L. Brouwer, Z. Bachar, and M. Taborsky. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg, D., Z. Heg-Bachar, L. Brouwer, and M. Taborsky. 2008. Experimentally induced helper dispersal in colonially breeding cooperative cichlids. *Environmental Biology of Fishes* 83:191–206.
- Heg, D., and M. Taborsky. 2010a. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- . 2010b. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- Jungwirth, A., D. Josi, J. Walker, and M. Taborsky. 2015. Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Functional Ecology* 29:1218–1224.
- Kasper, C., M. Colombo, N. Aubin-Horth, and B. Taborsky. 2018. Brain activation patterns following a cooperation opportunity in a highly social cichlid fish. *Physiology & Behavior* 195:37–47.
- Kingma, S. A., M. L. Hall, and A. Peters. 2011. Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist* 177:486–495.
- Kingma, S. A., P. Santema, M. Taborsky, and J. Komdeur. 2014. Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution* 29:476–484.

Kokko, H., R. A. Johnstone, and T. H. Clutton-Brock. 2001. The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B: Biological Sciences* 268:187–196.

Naef, J., and M. Taborsky. 2020a. Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science* 7:191808.

———. 2020b. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* 168:137–147.

Riebli, T., M. Taborsky, N. Chervet, N. Apolloni, Y. Zürcher, and D. Heg. 2012. Behavioural type, status and social context affect behaviour and resource allocation in cooperatively breeding cichlids. *Animal Behaviour* 84:925–936.

Stiver, K. A., J. Fitzpatrick, J. K. Desjardins, and S. Balshine. 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Animal Behaviour* 71:449–456.

Taborsky, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour* 32:1236–1252.

———. 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* 95:45–75.

———. 2016. Cichlid fishes: A model for the integrative study of social behavior. Pages 272–293 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.

Taborsky, M., and D. Limberger. 1981. Helpers in fish. *Behavioral Ecology and Sociobiology* 8:143–145.

Zöttl, M., D. Heg, N. Chervet, and M. Taborsky. 2013. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* 4:1341.

Supplementary material

Table 1. Effect of rank and helper size on different helping behaviours (egg predator defence and digging sand from shelters). Reference categories are low rank (LR) for treatment, and phase 1. Bold numbers denote significance.

	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
<i>Egg predator defence</i>				
Intercept	16.64	3.35	4.97	< 0.001
Treatment	7.89	2.55	3.10	0.002
Size focal helper	-1.75	0.70	-2.49	0.01
Phase	0.05	0.11	0.47	0.64
Egg predator activity	-0.25	0.56	-0.45	0.66
Treatment * Size focal	-2.50	0.82	-3.06	0.002
<i>Sand digging</i>				
Intercept	13.23	14.66	0.90	0.367
Treatment	0.54	0.71	0.76	0.448
Size focal helper	-3.74	4.85	-0.77	0.441
Phase	0.81	1.14	0.71	0.479

A decorative graphic consisting of a vertical dark blue bar on the left side of the page. From the top of this bar, a trail of white fish silhouettes extends diagonally across the page towards the bottom right. The silhouettes vary in size and orientation, creating a sense of movement. The background of the page is white, and the text is in a dark blue color.

APPENDIX 2

Influence of help prevention time on punishment and alloparental care in a cooperative breeder that pays to stay

Irene García-Ruiz^{1*}, Lorenzo Arduini¹ and Michael Taborsky¹

¹ Division of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032 Hinterkappelen, Switzerland.

*Corresponding author.

Preliminary results

Summary

In cooperative breeders, dominant and subordinate group members typically exchange different commodities among each other, which involves an incessant negotiation process. For example, helpers may trade alloparental brood care as a form of rent against safety and resource access in the dominants' territory. Here, we aim to test the prediction derived from the pay-to-stay hypothesis that breeders increase punishment as the time of transgression of helpers increases. We also test whether helpers increase help provisions as a response to increased aggression, as expected under pay-to-stay negotiation processes. We found that breeders increased aggression as time of transgression increased, and that they tended to use more aggressive forms of punishment with time. We also found that digging in the breeding chamber increased with punishment, but brood care only correlated positively with the need for help.

Methods

Study species

Neolamprologus pulcher is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa. Groups consist of a dominant pair and typically 1 to 25 subordinates of different size classes (Taborsky and Limberger 1981; Balshine et al. 2001; Heg et al. 2005; Groenewoud et al. 2016). There is a high reproductive skew, with subordinates of both sexes producing between 5 and 15 per cent of offspring in the group (Dierkes et al. 1999; Heg and Hamilton 2008; Bruintjes et al. 2011; Hellmann et al. 2015; Taborsky 2016). Overall relatedness between subordinates and dominants is low and decreases with helper age mainly due to the replacement of the breeders (Dierkes et al. 2005; Stiver et al. 2005). Both related and unrelated subordinates participate in brood care of the dominants' broods by cleaning and fanning the eggs (Taborsky 1984; Zöttl et al. 2013b), in territory maintenance by digging out sand from shelters and the breeding chamber (Taborsky and Limberger 1981; Bruintjes and Taborsky 2011), and in territory defence against predators, space competitors and conspecific intruders (Taborsky and Limberger 1981; Taborsky 1984, 1985; Balshine et al. 2001; Bergmüller and Taborsky 2005; Naef and Taborsky 2020). Subordinates show a size-dependent polyethism in task specialization (Bruintjes and Taborsky 2011). Individual and kin recognition have been demonstrated in this species (Hert 1985; Le Vin et al. 2010). Subordinates remain in their natal group queuing for dominance, or they disperse into other groups either as subordinates or by taking over a vacant breeding position, usually long after sexual maturity (Bergmüller et al. 2005; Stiver et al. 2006; Jungwirth et al. 2015). Predation risk is the major environmental factor constraining dispersal (Taborsky 1984; Heg et al. 2004); see (Taborsky 2016) for review).

Experimental set-up

Test fish used in this experiment were captive descendants from *N. pulcher* individuals caught in the wild near Kasakalawe point at the southern end of Lake Tanganyika (Zambia). Fish were kept in non-reproductive aggregations in separate tanks.

Experimental groups were established of a pair and a subordinate of unknown sex; reliable sexing of immature subordinates by external anatomical features is impossible in this species, but there are no apparent sex differences in helping behaviours (Taborsky 1985). Groups were established following the natural group structure, where dominant males are the largest animals in the groups, followed by the dominant female. Subordinates were unrelated to the dominant pair and ranged between 19 and 32mm standard length (mean: 26.4mm).

Groups were housed in a 100L compartment of a 200L aquarium subdivided by a glued opaque partition. Each experimental compartment contained three clay flowerpot-halves serving as shelters and as breeding substrate, a semi-transparent tube and an opaque tube suspended below the water surface to serve as refuge, and a filter. The bottom of the aquarium was covered with a mixture of fine gravel and sand about 3cm deep. An experimental cage made of coarse-mesh was constructed to prevent the breeders from accessing a shelter over which the cage was placed (see below for a description of the experimental procedure), while the subordinates could freely pass through the mesh. After group formation and before the experiment started, this cage was put over one of the clay shelters in the tank to habituate group members to the object. The water temperature was held constant at 26-28°C and the light regime was set at a 13h-light and 11h-dark cycle including dawn and dusk, simulating natural conditions in Lake Tanganyika. The fish were fed with dry food five times a week and with de-frozen food on one day. An additional provision of krill was provided once a week to promote egg-laying.

Groups were checked for clutches every day. Experiments took place during the egg phase on the first and second day after spawning. We obtained a total sample size of 22 groups (3 groups had missing data).

The experiments were conducted from February 2019 to June 2020 at the Ethological station Hasli at the University of Bern, Switzerland under the ethical approval license of Veterinaermt Bern 74/15.

Experimental design and procedure

The experiment involved two phases: a help prevention phase and a help compensation phase. In the help prevention phase, we simulate idle helpers by preventing them from helping for 0, 30 or 60 min. and measured the response by the breeders. In the help compensation phase, we assess whether helpers compensate for the lack of help in the previous phase by increasing alloparental care, and whether compensation is proportional to the time of help prevention and aggression by the breeders.

Previous studies have shown that helpers increase their brood care investment after time periods where they were unable to conduct alloparental care (Zöttl et al. 2013a), but whether this increase was voluntary or due to enforcement was unclear. In another experiment, helpers provided increased levels of compensatory help when breeders could interact with them (Chapter 3). If punishment mediates help provisions, we would expect that an increase in help prevention leads to higher frequency of attacks or to eviction, and that help would escalate accordingly.

We started the experiment when the group was stable and after they produced their second clutch, to ensure helpers were accepted in the group and were allowed to provide brood care. We used alloparental egg care as the target behaviour (egg cleaning and fanning), because it reflects truly altruistic behaviour (Zöttl et al. 2013b) that causes considerable opportunity and energy costs without immediate direct fitness benefits to the actor (Grantner and Taborsky 1998; Taborsky and Grantner 1998; Heg and Taborsky 2010; Wong and Balshine 2011). We also scored other cooperative behaviours such as digging out sand from the breeding shelter, as well as submission and agonistic interactions between the group members. Before the start of the experiment, the fish were habituated to the experimental equipment (the mesh-cage) and the subordinates learned that they could pass freely through the mesh net. The coarse-meshed cage consisted of a transparent plastic front for visualization purposes, and a mesh covering the other sides and the top with a mesh width of (10x10mm). In all instances, subordinates were observed accessing the covered shelter through the mesh without difficulties. The experimental cage used for help prevention during the experience phase consisted of a transparent isolation chamber inverted which allowed water flow but prevented fish from accessing the covered shelter.

During all experimental phases, breeders were excluded from brood care by the experimental cage described above. During the “help prevention phase” groups were divided into two treatment groups, in which the whole group was prevented from brood care by a transparent cage for 30min (short help prevention, *SP*) or for 60 min (long help prevention, *LP*), and control groups (*C*), in which the meshed cage was placed over the breeding shelter allowing the subordinate to care for the eggs (Figure 1). The last 15min of the experimental period in both treatments and control conditions were scored. Subsequently, during the “help compensation phase”, helpers were allowed to provide alloparental care (Figure 1). We scored the helper behaviour for 15min after a 5min acclimatisation period. In this last phase, breeders were not able to access any shelter due to part of the experimental set-up being shared with another experiment.

After each phase, we manipulated the mesh-cage to control for manipulation between control and treatment. We performed a full factorial experimental design with randomised order of the three trials. After finishing each trial, a gap of at least 2h was kept before starting the next trial. After the long prevention treatment, a gap of 12h was kept before the next trial to avoid interference between trials.

For the “help compensation phase”, we used brood care (egg cleaning and fanning), and sand digging in the breeding chamber as response variables. The fixed effects variables of the model were time prevention to access the eggs during the previous phase (C vs SP vs LP), helper size, clutch size and the aggression received during the previous phase.

Group ID was added as a random effect in all analyses to account for repeated use of the same groups for the different treatments. When analysing aggression by the breeders, we also included the trial identifier as a random factor.

We ran zero-altered generalised linear mixed-effects models using the R package glmmADMB (Zuur et al. 2009; Fournier et al. 2012). Huddle models allows us to test the significance of the presence or absence of a behaviour together with the quantitative significance. The probability of occurrence can be a more reliable factor as the amount of a given behaviour can be affected by many uncontrolled factors, such as the costs that the behaviour imposes in a given context (Emlen and Wrege 1988; Heinsohn and Legge 1999). For the presence/absence of the behaviour we fitted a binomial distribution, and a Poisson distribution for the count data (ZAP-GLMMs). To check for model fit and overdispersion, we used the function simulateResiduals from the R package DHARMA. All simulations and statistical tests were performed in R 3.6.3. (R Core Team 2020).

Preliminary results

Aggression by the breeders

Confirming predictions from the pay-to-stay hypothesis, when helpers were prevented from helping during the help prevention phase, subordinates were exposed to more aggression by the breeders. However, this difference was only significant after long help prevention (ZAP binomial: $Z = 2.55$, $p = 0.01$; Figure 2a). Under short help prevention, the frequency of aggression tended to also be higher compared to the control (ZAP count: $Z = 1.93$, $p = 0.05$; Figure 2a). In addition, breeders tended to use overt aggressive behaviours more often than restrained aggression when charging the helpers after long help prevention compared to the control ($Z = 1.89$, $p = 0.06$; Figure 2b). When comparing aggressive responses towards helpers between male and female breeders, we found that male breeders were aggressive less often than female breeders (ZAP binomial: $Z = -1.19$, $p = 0.01$), but their aggression levels were higher when they did charge helpers (ZAP count: $Z = 0.89$, $p < 0.001$).

Coinciding with the increased breeder aggression in the prevention treatment, helpers increased submissive displays when prevented from helping, both in short (ZAP binomial: $Z = 2.13$, $p = 0.03$; count: $Z = 3.25$, $p = 0.001$) and long help prevention (ZAP binomial: $Z = 1.88$, $p = 0.06$; count: $Z = 2.93$, $p = 0.003$; Figure 2c) compared to the control. No difference in submission between short and long help prevention was detected (ZAP binomial: $Z = -0.74$, $p = 0.5$; count: $Z = -0.35$, $p = 0.7$). Likewise, helpers also showed more avoidance behaviours against breeders after short (ZAP binomial: $Z = 2.10$, $p = 0.04$; count: $Z = 2.11$, $p = 0.04$) and long prevented from helping (ZAP binomial: $Z = 1.94$, $p = 0.05$; count: $Z = 3.11$, $p = 0.002$) than in the control condition.

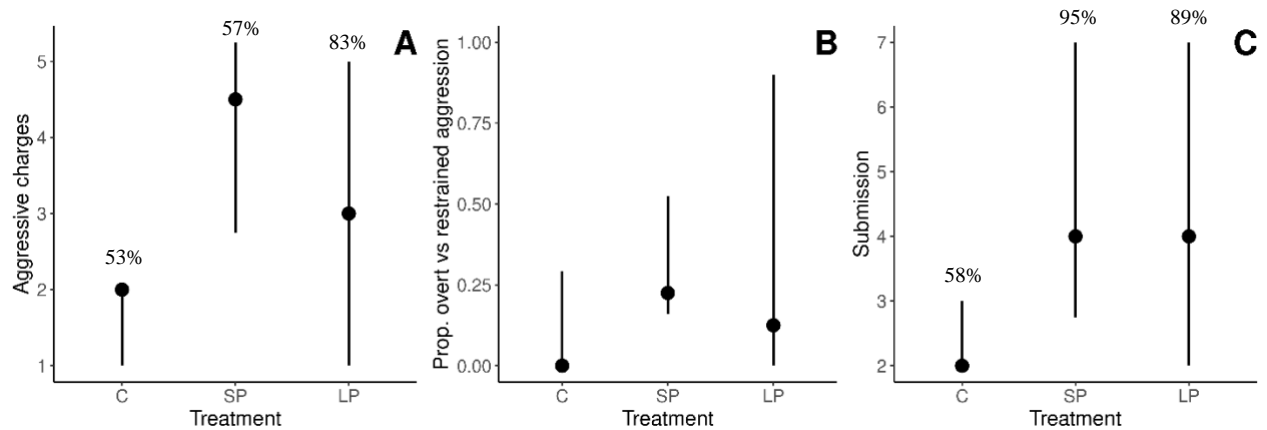


Figure 2. Overt aggression and restrained aggressive displays by breeders and submission by helpers are shown during the *help prevention phase*. (a) Helpers received overt and restrain aggressive behaviours more often after a long help prevention (C vs LP) compared to the control. Detailed statistics given in Table S1. (b) The breeders tended to use more overt aggressive displays than restrained aggression after long help prevention compared to control. Detailed statistics given in Table S2. (c) Helpers increased submissive displays when they were prevented from helping. Detailed statistics given in Table S3. Depicted are medians \pm interquartile ranges of behaviours when they occurred (i.e., zero occurrences are omitted). Numbers indicate the percentage of cases in which the behaviours were shown.

Help provisions

As expected, help prevention elicited a compensatory response from the helpers that escalated with time (Figure 3). Brood care increased after short time help prevention compared to the control (ZAP count: $Z = 7.17$, $p < 0.001$), and from short to long time prevention (ZAP count: $Z = 4.28$, $p < 0.001$; Figure 3A). Larger, although immature, helpers provided more alloparental care in the form of direct brood care (ZAP count: $Z = 3.41$, $p < 0.001$). However, contrary to expected under pay-to-stay, aggression received during the previous phase reduced brood care provisions (ZAP count: $Z = -2.71$, $p = 0.007$). Conversely, digging in the breeding chamber escalated positively with the aggression received during the help prevention phase (ZAP count: $Z = 3.06$, $p = 0.002$; Figure 3B).

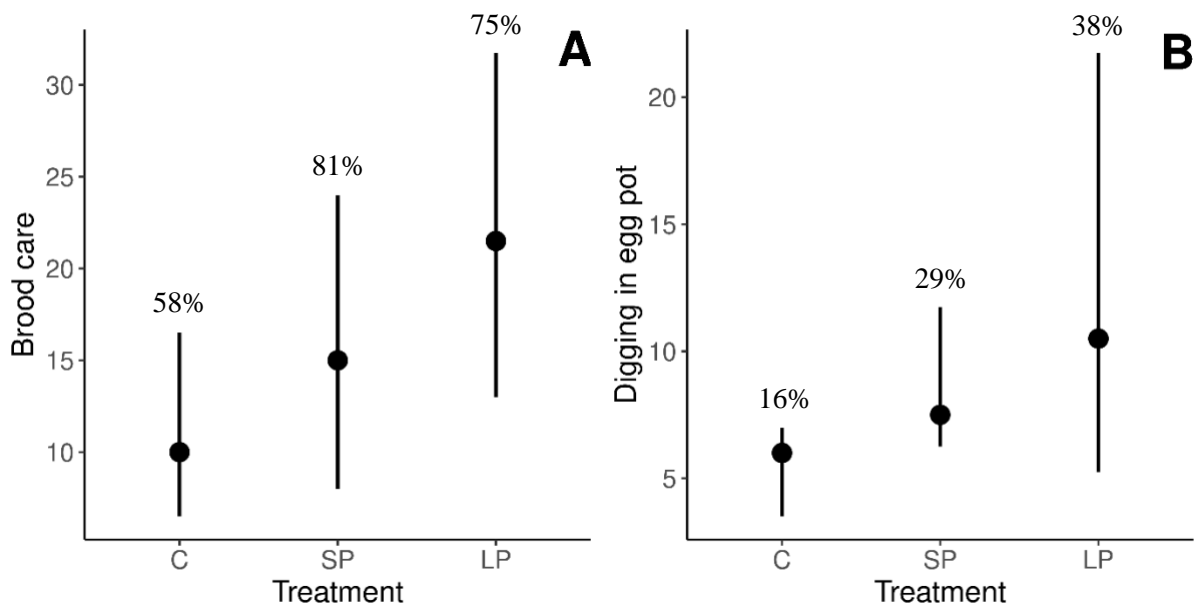


Figure 3. Alloprenatal care during the *help compensation phase*. (a) Helpers increase brood care proportional to help prevention time. (b) Helpers dug in the breeding shelter proportional to aggression received in the help prevention phase. Depicted are medians \pm interquartile ranges of behaviours when they occurred (i.e., zero occurrences are omitted). Numbers indicate the percentage of cases in which the behaviours were shown. Detailed statistics given in Table S4.

Brief discussion

Consistent with predictions from pay-to-stay models (Gaston 1978; Kokko et al. 2002; Hamilton and Taborsky 2005), we found that help-prevention increased the dominants' aggression, and this effect was stronger for longer time periods of help prevention. Breeders also showed a tendency for more aggressive forms of aggression, suggesting that they may increase their threats from warning attacks to eviction. As a result of the increase in punishment, subordinates raised submissive displays, corroborating previous findings (Heg and Taborsky 2010; Chapter 3).

Decisively, increased aggression led to higher frequency of digging in the breeding chamber, supporting previous findings (Chapter 3). However, contrary to expectations of pay-to-stay, brood care increased with time of help prevention, but decreased as aggression escalated. This result may hint that helpers provided, at least partly, voluntary brood care as the need of help increased, by instance, to increase direct benefits derived from increased group size. Previous results, nevertheless, suggested that brood care was regulated by pay-to-stay processes as the increase of alloprenatal egg care was mediated by the ability of breeders to interact with the helpers (Chapter 3). Therefore, more research is needed to understand alternative, non-mutually, selective pressures in the evolution of cooperative breeding. Altogether, this study provides further evidence that different commodities and services may be under different selective pressures (Taborsky and Limberger 1981; Bruintjes and Taborsky 2011; Chapter 2).

References

- Balshine, S., B. Leach, F. Neat, H. Reid, M. Taborsky, and N. Werner. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* 50:134–140.
- Bergmüller, R., D. Heg, and M. Taborsky. 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences* 272:325–331.
- Bergmüller, R., and M. Taborsky. 2005. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- Bruintjes, R., D. Bonfils, D. Heg, and M. Taborsky. 2011. Paternity of subordinates raises cooperative effort in cichlids. *PLoS One* 6:e25673.
- Bruintjes, R., and M. Taborsky. 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* 81:387–394.
- Dierkes, P., D. Heg, M. Taborsky, E. Skubic, and R. Achmann. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* 8:968–975.
- Dierkes, P., M. Taborsky, and U. Kohler. 1999. Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behavioral Ecology* 10:510–515.
- Emlen, S. T., and P. H. Wrege. 1988. The role of kinship in helping decisions among white-fronted bee-eaters. *Behavioral Ecology and Sociobiology* 23:305–315.
- Fournier, D. A., H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. N. Maunder, A. Nielsen, et al. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* 27:233–249.
- Friard, O., and M. Gamba. 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.
- Gaston, A. J. 1978. The Evolution of Group Territorial Behavior and Cooperative Breeding. *The American Naturalist* 112:1091–1100.
- Grantner, A., and M. Taborsky. 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 168:427–433.
- Groenewoud, F., J. G. Frommen, D. Josi, H. Tanaka, A. Jungwirth, and M. Taborsky. 2016. Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences* 113:4104–4109.
- Hamilton, I. M., and M. Taborsky. 2005. Unrelated helpers will not fully compensate for costs imposed on breeders when they pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 272:445–454.
- Heg, D., Z. Bachar, L. Brouwer, and M. Taborsky. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences* 271:2367–2374.

- Heg, D., L. Brouwer, Z. Bachar, and M. Taborsky. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg, D., and I. M. Hamilton. 2008. Tug-of-war over reproduction in a cooperatively breeding cichlid. *Behavioral Ecology and Sociobiology* 62:1249–1257.
- Heg, D., and M. Taborsky. 2010. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- Heinsohn, R., and S. Legge. 1999. The cost of helping. *Trends in Ecology and Evolution* 14:53–57.
- Hellmann, J. K., I. Y. Ligoeki, C. M. O'Connor, A. R. Reddon, K. A. Garvy, S. E. Marsh-Rollo, H. L. Gibbs, et al. 2015. Reproductive sharing in relation to group and colony-level attributes in a cooperative breeding fish. *Proceedings of the Royal Society B: Biological Sciences* 282:20150954.
- Hert, E. 1985. Individual recognition of helpers by the breeders in the cichlid fish *Lamprologus brichardi* (Poll, 1974). *Zeitschrift für Tierpsychologie* 68:313–325.
- Jungwirth, A., J. Walker, and M. Taborsky. 2015. Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Animal Behaviour* 106:107–114.
- Kokko, H., R. A. Johnstone, and J. Wright. 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behavioral Ecology* 13:291–300.
- Le Vin, A. L., B. K. Mable, and K. E. Arnold. 2010. Kin recognition via phenotype matching in a cooperatively breeding cichlid, *Neolamprologus pulcher*. *Animal Behaviour* 79:1109–1114.
- Naef, J., and M. Taborsky. 2020. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* 168:137–147.
- Stiver, K. A., P. Dierkes, M. Taborsky, H. Lisle Gibbs, and S. Balshine. 2005. Relatedness and helping in fish: examining the theoretical predictions. *Proceedings of the Royal Society B: Biological Sciences* 272:1593–1599.
- Stiver, K. A., J. Fitzpatrick, J. K. Desjardins, and S. Balshine. 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Animal Behaviour* 71:449–456.
- Taborsky, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour* 32:1236–1252.
- . 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* 95:45–75.
- . 2016. Cichlid fishes: a model for the integrative study of social behavior. Pages 272–293 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*. Cambridge University Press, Cambridge.
- Taborsky, M., and A. Grantner. 1998. Behavioural time–energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Animal Behaviour* 56:1375–1382.
- Taborsky, M., and D. Limberger. 1981. Helpers in fish. *Behavioral Ecology and Sociobiology* 8:143–145.
- Wong, M., and S. Balshine. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biological Reviews* 86:511–530.

Zöttl, M., S. Fischer, and M. Taborsky. 2013*a*. Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour* 85:1471–1478.

Zöttl, M., D. Heg, N. Chervet, and M. Taborsky. 2013*b*. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* 4:1341.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Zero-truncated and zero-inflated models for count data. Pages 261–293 *in* *Mixed effects models and extensions in ecology with R*. Springer.

Supplementary material

Table S1. Effect of time of help prevention on the levels of overt and restrained aggression by the breeders, during the help prevention phase. The analysis scrutinised the presence/absence of aggression and the amount of aggression performed. Bold numbers despite significant main effect. C: control, SP: short prevention (30min), LP: long prevention (60min).

	Presence/absence				Count			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
C vs SP	0.75	0.60	1.25	0.211	0.90	0.47	1.93	0.054
C vs LP	1.70	0.67	2.55	0.011	0.31	0.45	0.69	0.490
SP vs LP	0.96	0.60	1.59	0.111	-0.59	0.36	-1.63	0.102
Sex (male)	-1.19	0.48	-2.48	0.013	0.89	0.16	5.56	<0.001

Table S2. Effect of time of help prevention on the levels of overt vs restrained aggression by the breeders, during the help prevention phase. The analysis scrutinised the proportion of overt aggression vs the total of aggression performed. Bold numbers despite significant main effect. C: control, SP: short prevention (30min), LP: long prevention (60min).

	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
C vs SP	0.74	0.91	0.81	0.416
C vs LP	1.66	0.88	1.89	0.059
SP vs LP	0.92	0.71	1.31	0.191
Sex (male)	-0.69	0.61	-1.14	0.255

Table S3. Effect of time of help prevention on the levels of submission and breeders' avoidance by the helper, during the help prevention phase. The analysis scrutinised the presence/absence of aggression and the amount of aggression performed. Reference categories are no prevention (for prevention), unrelated (for relatedness) and female (for breeder's sex). Bold numbers despite significant main effect. C: control, SP: short prevention (30min), LP: long prevention (60min).

	<i>Submission</i>							
	Presence/absence				Count			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
C vs SP	4.02	1.88	2.13	0.033	0.85	0.26	3.25	0.001
C vs LP	2.93	1.56	1.88	0.060	0.79	0.27	2.93	0.003
SP vs LP	-1.09	1.46	-0.74	0.458	-0.06	0.17	-0.35	0.727
	<i>Avoidance</i>							
C vs SP	2.55	1.22	2.10	0.036	0.44	0.21	2.11	0.035
C vs LP	2.40	1.23	1.94	0.052	0.66	0.21	3.11	0.002
SP vs LP	-0.16	1.46	-0.11	0.915	0.22	0.14	1.56	0.120

Table S4. Effect of time of help prevention and aggression on the levels of brood care (egg cleaning and fanning) and digging in the brood chamber, during the help compensation phase. The analysis

scrutinised the presence/absence and the amount of alloparental care performed. Bold numbers despite significant results. C: control, SP: short prevention (30min), LP: long prevention (60min).

	<i>Brood care</i>				<i>Count</i>			
	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>	<i>Estimate</i>	<i>SE</i>	<i>z</i>	<i>p</i>
C vs SP	2.58	1.47	1.76	0.08	0.78	0.11	7.17	<0.001
C vs LP	2.12	1.45	1.46	0.14	1.13	0.11	10.14	<0.001
SP vs LP	-0.46	1.18	-0.39	0.70	0.34	0.08	4.28	<0.001
Aggression	-0.04	0.05	-0.96	0.34	-0.03	0.01	-2.71	0.007
Helper size	1.24	2.72	0.46	0.65	1.26	0.37	3.41	<0.001
Clutch size	-0.02	0.034	-0.64	0.52	-0.002	0.004	-0.45	0.650
	<i>Digging in the breeding chamber</i>							
C vs SP	1.75	1.44	1.22	0.224	0.26	0.36	0.74	0.461
C vs LP	2.47	1.57	1.58	0.115	0.43	0.35	1.22	0.224
SP vs LP	0.71	1.20	0.59	0.553	0.16	0.22	0.74	0.456
Aggression	0.02	0.09	0.22	0.829	0.25	0.08	3.06	0.002
Helper size	3.12	3.62	0.86	0.389	2.08	1.39	1.49	0.135
Clutch size	-0.08	0.06	-1.22	0.224	0.02	0.02	1.21	0.226

General discussion

In this thesis, we explored the hypothesis of kin selection, group augmentation and pay-to-stay as drivers for the evolution of cooperative breeding. We chose the cooperative breeding cichlid fish *Neolamprologus pulcher* as a model species since many preconditions and assumptions regarding the named evolutionary mechanism are met in this species. Results of this thesis further our understanding of the validity of these alternative mechanisms and their interplay across different ecological scenarios.

In Chapters 1 and 2 we investigated the validity of the group augmentation hypothesis from a theoretical and empirical perspective, respectively. Even though a positive correlation is found in many cooperative breeders between group size and survival (Clutton-Brock et al. 1999; Balshine et al. 2001; Taborsky et al. 2005; Covas et al. 2008; Cant et al. 2016; Ridley 2016), evidence of this hypothesis is scarce (Clutton-Brock 2002; Kingma et al. 2011; Kingma 2017).

In **Chapter 1** we found that group living fitness benefits are the main driver of philopatry to evolve, a prerequisite for the emergence of alloparental care. Therefore, in contrast to the common view, our model suggests that indirect fitness benefits alone are unlikely to select for the evolution of cooperative breeding, seemingly because kin competition prevents the evolution of the relatedness structure that generates sufficient indirect fitness benefits to build up. Additionally, we also found that the prevalence of direct and indirect fitness benefits selecting for alloparental care varies according to the ecological context. In benign environments close to saturation, the model predicts that alloparental care evolves only under kin selection once groups are formed as a result of direct fitness benefits. In harsh unsaturated habitats, however, alloparental care can also evolve under group augmentation benefits. This results helps to solve the paradox of environmental quality and sociality, as both benign and harsh environments seem to promote the evolution of sociality, and supports previous predictions on the duality of different selective benefits depending on the harshness of the environment (Emlen 1982; Shen et al. 2017; Lin et al. 2019).

In **Chapter 2**, we tested empirically whether helpers adjust help provisions to group size in *N. pulcher*. This species has been shown previously to benefit from increased survival due to reduced predation risk in large groups (Balshine et al. 2001; Heg et al. 2004; Taborsky et al. 2005). In the field, helpers visited breeding shelters more often after group size reduction (Brouwer et al. 2005), which is a proxy for direct brood care (Balshine-Earn et al. 1998; own unpubl. data). In addition, group members benefit also from the vicinity of other groups, as joint effort in antipredator defence allows them to reduce their own expense, revealing a significant benefit of coloniality in addition to group living (Jungwirth et al. 2015). Our model also predicted that group augmentation can be an important factor in harsh environments as exposed earlier. *N. pulcher* lives in habitats remain unsaturated (Heg et al. 2011), and predation risk linked to dispersal seems to be the main ecological factor driving delayed dispersal (Heg et al. 2004; Heg and Taborsky 2010; Groenewoud et al. 2016). Therefore, our model predicts that group augmentation could be an important factor driving

the evolution of cooperation in this species. In addition to these preconditions that are met in *N. pulcher*, lower levels of alloparental care in large groups are predicted under the group augmentation hypothesis because the need to increase the group size is greater. However, load-lightening produces equivalent lower levels of help in large groups, because additional help has no or little further effect on increasing group size or in providing a service to the breeders, as also expected under hypotheses like pay-to-stay. Therefore, in our experiment we controlled for alternative selective pressures like load-lightening, coercion and kinship. We found that relatively large helpers provided more defence against an egg predator in small vs large group sizes. This difference was, however, commodity-specific, as helpers in different group sizes performed similar digging effort in the breeding chamber. A previous study found that experimental prevention of defence against an egg predator elicited a compensatory response without enforcement, while prevention of territory maintenance by digging sand outside of shelters triggered punishment by the breeders (Naef and Taborsky 2020a). Hence, egg predator defence seems to be controlled by group augmentation benefits while shelter digging seems to be controlled by coercion, corresponding to the pay-to-stay mechanism regulating altruistic help in this species (Taborsky 2016). This study is the first to validate the short-term group augmentation predictions against alternative hypotheses. Our study remarks the importance of considering different commodities when studying the evolution of cooperative breeding.

In **Chapter 3** we experimentally analysed the evolution of alloparental care under enforcement following pay-to-stay predictions. Even though the threat of punishment has been invoked as a key factor promoting the evolution of cooperation among non-relatives (Taborsky 1985; Clutton-Brock and Parker 1995; Cant and Johnstone 2006; Cant 2011; Cant and Young 2013; Quiñones et al. 2016; Ågren et al. 2019; Engelhardt and Taborsky 2020), few studies have demonstrated the link between punishment and cooperation in animal societies (Bshary and Grutter 2005; Wong et al. 2007; Naef and Taborsky 2020a, 2020b). To investigate the link between aggression and cooperation, we experimentally simulated transgression by the helpers by preventing them from help provision and manipulated the possibility of breeders to punish idle helpers. Concurrently, we manipulated relatedness between helpers and breeders to unravel the role that enforcement plays between related interacting partners, as common indirect fitness benefits could weaken effective punishment while subordinates have incentives to provide voluntary help (Kokko et al. 2002; Quiñones et al. 2016). Previous experiments in *N. pulcher* have shown that help prevention resulted in higher levels of aggression by the breeders and helpers increasing their submission (Fischer et al. 2014; Naef and Taborsky 2020a, 2020b), as expected under pay-to-stay models. Our experiment corroborates these results. In addition, helpers were shown to increase helping behaviour after being prevented from helping (Bergmüller and Taborsky 2005; Naef and Taborsky 2020a). In this experiment, we show that this increase in help (direct brood care and digging in the breeding chamber) was a result of enforcement and not a voluntary increase due to the augmented need for care. This further supports previous results that territory maintenance is mainly driven by coercion as exposed earlier. Another study showed that unrelated subordinates provided more alloparental care than related ones under enforcement (Zötzel et al. 2013). However, it was unclear whether help in related individuals is

primarily driven by kin selection or enforcement. Our experiment is the first to show that related helpers can also provide help under coercion, but kin selection reduces the required provision of help demanded by the breeders.

So far, we demonstrated that kin selection, group augmentation and pay-to-stay mechanisms may vary in importance depending on the commodity analysed and the ecological context. However, other variables like dominance, age, sex and other life-history traits linked to resource holding potential may also influence the strength of different mechanisms driving the provisions of alloparental care. In our model, we allow individuals to evolve age-dependent plasticity in their decisions to disperse and help according to the ecological context. In highly saturated habitats in which it pays-off to queue for the breeding position in the natal or another territory, our model predicts early dispersal to reduce kin competition for the breeding position. Empirical data in a range of taxa are in accordance with the prediction that early dispersal is triggered by avoidance of local competition with kin for resources and breeding opportunities (Zack and Rabenold 1989; Field et al. 1999; Clutton-Brock and Lukas 2012). Under this scenario, we also expect alloparental care to decrease with age as the degree of relatedness declines due to time-dependent breeder replacement and dispersal dynamics (Dierkes et al. 2005). Our model further predicts delayed dispersal in low-quality habitats with high mortality rates, because the natal territory serves as a safe haven. Offspring that delay dispersal to benefit from group protection may help to raise kin, thereby gaining indirect fitness benefits while waiting for a safe opportunity to leave for independent reproduction elsewhere. Empirical results from a wide range of animal taxa conform with this prediction, including birds (Hunter 1987), mammals (Mares et al. 2012) and insects (Korb and Schmidinger 2004). Predictions of your model on dispersal patterns in harsh environments are also met in *N. pulcher*, as their dispersal propensity increases with age (Wong and Balshine 2011; Taborsky 2016). However, our model also predicts a decrease in alloparental care with age as levels of relatedness plunge, but we observe the opposite pattern in this species (Bruitjes and Taborsky 2008; Heg and Taborsky 2010). In *N. pulcher*, breeders punish larger (i.e. older) helpers more seemingly because they impose a higher risk of reproductive competition (Bruitjes and Taborsky 2008; Heg and Taborsky 2010). Therefore, this discrepancy may emerge because we did not include enforcement and eviction by dominants in our model to account for pay-to-stay negotiation processes (Bergmüller et al. 2005; Quiñones et al. 2016). Altogether, it seems likely that in harsh environments kin selection is a stronger evolutionary force for alloparental care in young low ranked helpers, while direct benefits like group augmentation and pay-to-stay have a higher influence in driving help in later life stages. Therefore, age-based task specialization observed for different commodities may stem not only from distinct capabilities and efficiency that vary along the lifetime of the helpers, but also from different selective pressures (Taborsky and Limberger 1981; Bruitjes and Taborsky 2011; Chapter 2).

To conclude, we found evidence for a conjunction interplay of kin selection, group augmentation and pay-to-stay driving the evolution of alloparental care. The relative importance of these selective forces seems to vary across different ecological scenarios and along the lifetime of individuals. Therefore, future research should consider both direct and

indirect fitness benefits when studying the evolution of cooperative breeding, while considering social and environmental conditions.

Future directions

In **Chapter 1**, we build a model to better understand the role of group size fitness benefits and the interplay of kinship in the evolution of cooperative breeding. Extensions to the model would provide further insight into the evolutionary forces driving cooperation in nature. First, the inclusion of negotiation between breeders and helpers, and the possibility of breeders to enforce help. Previous models exist on the pay-to-stay mechanism (Kokko et al. 2002; Quiñones et al. 2016), but no model explores this mechanism in conjunction with group augmentation and kin selection. Secondly, our model assumed asexual reproduction for simplicity. Expanding the model to include sexual reproduction would not only draw more meaningful predictions on the role of kin selection and kin competition, but could also help to make predictions on sex-biased dispersal patterns that remarkably differ between mammals and birds. Third, we assumed complete reproductive skew. It would be interesting to see how different degrees of reproductive skew, driven by the breeders' lack of control over the helper's reproduction or due to breeders' reproductive concessions in exchange for help, affect our results. This could prove useful to expand the applicability of our results to a wider range of both cooperative breeders and communal breeders. Lastly, we assumed that dispersed individuals join random groups. However, this is unlikely to be the case in nature, as dispersers may benefit from joining groups according to their size or territory quality. Providing individuals with the option to choose according to these parameters may change dispersal propensities shown in our model. Additionally, differences in territory quality through space and time are likely to produce similar results to environmental harshness, but this is still to be tested.

In **Chapter 2**, we focus on validating predictions of short-term group augmentation benefits. The addition of relatedness to this experimental set-up could help us understand the interplay of kin selection and group augmentation. To our knowledge, this is first study testing for short-term group augmentation benefits experimentally in a cooperatively breeding vertebrate. Research on other cooperative species on this topic is necessary to elucidate how spread this evolutionary mechanism is in nature, and to further validate our results.

In **Chapter 3**, we studied the prediction of pay-to-stay by manipulating both helpers and breeders, together with the influence of relatedness. Future experiments may consider a wider range of helper and group sizes to investigate the robustness of the results.

References

- Ågren, J. A., N. G. Davies, and K. R. Foster. 2019. Enforcement is central to the evolution of cooperation. *Nature Ecology and Evolution* 3.
- Balshine, S., B. Leach, F. Neat, H. Reid, M. Taborsky, and N. Werner. 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* 50:134–140.
- Balshine-Earn, S., F. C. Neat, H. Reid, and M. Taborsky. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology* 9:432–438.
- Bergmüller, R., D. Heg, and M. Taborsky. 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society B: Biological Sciences* 272:325–331.
- Bergmüller, R., and M. Taborsky. 2005. Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour* 69:19–28.
- Brouwer, L., D. Heg, and M. Taborsky. 2005. Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behavioral Ecology* 16:667–673.
- Bruintjes, R., and M. Taborsky. 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour* 75:1843–1850.
- . 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* 81:387–394.
- Bshary, R., and A. S. Grutter. 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters* 1:396–399.
- Cant, M. A. 2011. The role of threats in animal cooperation. *Proceedings of the Royal Society B: Biological Sciences* 278:170–178.
- Cant, M. A., and R. A. Johnstone. 2006. Self-serving punishment and the evolution of cooperation. *Journal of Evolutionary Biology* 19:1383–1385.
- Cant, M. A., H. J. Nichols, F. J. Thompson, and E. Vitikainen. 2016. Banded mongooses: Demography, life history, and social behavior. Pages 318–337 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Cant, M. A., and A. J. Young. 2013. Resolving social conflict among females without overt aggression. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368:20130076.
- Clutton-Brock, T. H. 2002. Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72.
- Clutton-Brock, T. H., D. Gaynor, G. M. McIlrath, A. D. C. Maccoll, R. Kansky, P. Chadwick, M. Manser, et al. 1999. Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *Journal of Animal Ecology* 68:672–683.
- Clutton-Brock, T. H., and D. Lukas. 2012. The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology* 21:472–492.
- Clutton-Brock, T. H., and G. A. Parker. 1995. Punishment in animal societies. *Nature* 373:209–216.

- Covas, R., M. A. du Plessis, and C. Doutrelant. 2008. Helpers in colonial cooperatively breeding sociable weavers *Philetairus socius* contribute to buffer the effects of adverse breeding conditions. *Behavioral Ecology and Sociobiology* 63:103–112.
- Dierkes, P., D. Heg, M. Taborsky, E. Skubic, and R. Achmann. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters* 8:968–975.
- Emlen, S. T. 1982. The evolution of helping. I. An ecological constraints model. *The American Naturalist* 119:29–39.
- Engelhardt, S. C., and M. Taborsky. 2020. Broad definitions of enforcement are unhelpful for understanding evolutionary mechanisms of cooperation. *Nature Ecology & Evolution* 4:322.
- Field, J., G. Shreeves, and S. Sumner. 1999. Group size, queuing and helping decisions in facultatively eusocial hover wasps. *Behavioral Ecology and Sociobiology* 45:378–385.
- Fischer, S., M. Zöttl, F. Groenewoud, and B. Taborsky. 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences* 281:20140184.
- Groenewoud, F., J. G. Frommen, D. Josi, H. Tanaka, A. Jungwirth, and M. Taborsky. 2016. Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences* 113:4104–4109.
- Heg, D., Z. Bachar, L. Brouwer, and M. Taborsky. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences* 271:2367–2374.
- Heg, D., L. Brouwer, Z. Bachar, and M. Taborsky. 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142:1615–1641.
- Heg, D., S. Rothenberger, and R. Schürch. 2011. Habitat saturation, benefits of philopatry, relatedness, and the extent of co-operative breeding in a cichlid. *Behavioral Ecology* 22:82–92.
- Heg, D., and M. Taborsky. 2010. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS ONE* 5.
- Hunter, L. A. 1987. Acquisition of territories by floaters in cooperatively breeding purple gallinules. *Animal behaviour* 35:402–410.
- Jungwirth, A., D. Josi, J. Walker, and M. Taborsky. 2015. Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Functional Ecology* 29:1218–1224.
- Kingma, S. A. 2017. Direct benefits explain interspecific variation in helping behaviour among cooperatively breeding birds. *Nature Communications* 8:1–7.
- Kingma, S. A., M. L. Hall, and A. Peters. 2011. Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *American Naturalist* 177:486–495.
- Kokko, H., R. A. Johnstone, and J. Wright. 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behavioral Ecology* 13:291–300.
- Korb, J., and S. Schmidinger. 2004. Help or disperse? Cooperation in termites influenced by food conditions. *Behavioral Ecology and Sociobiology* 56:89–95.

- Lin, Y.-H., S.-F. Chan, D. R. Rubenstein, M. Liu, and S.-F. Shen. 2019. Resolving the paradox of environmental quality and sociality: the ecological causes and consequences of cooperative breeding in two lineages of birds. *The American Naturalist* 194:207–216.
- Mares, R., A. J. Young, and T. H. Clutton-Brock. 2012. Individual contributions to territory defence in a cooperative breeder: weighing up the benefits and costs. *Proceedings of the Royal Society B: Biological Sciences* 279:3989–3995.
- Naef, J., and M. Taborsky. 2020a. Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *Royal Society Open Science* 7:191808.
- . 2020b. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Animal Behaviour* 168:137–147.
- Quiñones, A. E., G. S. van Doorn, I. Pen, F. J. Weissing, and M. Taborsky. 2016. Negotiation and appeasement can be more effective drivers of sociality than kin selection. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150089.
- Ridley, A. R. 2016. Southern pied babblers: The dynamics of conflict and cooperation in a group-living society. Pages 115–132 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Shen, S. F., S. T. Emlen, W. D. Koenig, and D. R. Rubenstein. 2017. The ecology of cooperative breeding behaviour. *Ecology Letters* 20:708–720.
- Taborsky, M. 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* 95:45–75.
- . 2016. Cichlid fishes: A model for the integrative study of social behavior. Pages 272–293 in W. D. Koenig and J. L. Dickinson, eds. *Cooperative Breeding in Vertebrates*. Cambridge University Press, Cambridge.
- Taborsky, M., and D. Limberger. 1981. Helpers in fish. *Behavioral Ecology and Sociobiology* 8:143–145.
- Wong, M., and S. Balshine. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biological Reviews* 86:511–530.
- Wong, M. Y. L., P. M. Buston, P. L. Munday, and G. P. Jones. 2007. The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. *Proceedings of the Royal Society B: Biological Sciences* 274:1093–1099.
- Zack, S., and K. N. Rabenold. 1989. Assessment, age and proximity in dispersal contests among cooperative wrens: field experiments. *Animal Behaviour* 38:235–247.
- Zöttl, M., D. Heg, N. Chervet, and M. Taborsky. 2013. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Communications* 4:1341.

Acknowledgments

I would like to express my warmest gratitude to Tobias Zobrist, for his unconditional support, love, encouragement, and patience, who also taught me so much about proper coding. This thesis would not have been possible without your help.

My especial thanks go to Andrés Quiñones for his invaluable advice and help on the model development, as well as to Michael Taborsky. Thanks Michael for making this thesis possible, for your interesting discussions and help with the design of the experiments and the writing of the three chapters.

I also thank Lorenzo Arduini for helping to carry out the long and exhausting experiments in Chapter 3 and Appendix 2, and to my student Alexis Perret who did a wonderful job carrying out the experiment of Appendix 1. Also, I extend a general thanks to the Hasli team for their advice in the early experimental design period.

I would like to thank Michael Cant for agreeing to be the external expert for this thesis, and to Claudia Bank, to act as chair of the defence.

I want to thank SaraPaint for the gorgeous drawings in this thesis and for her friendship.

And lastly, I would also like to thank my parents. Gracias por vuestro apoyo y cariño.

Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: García Ruiz, Irene

Registration Number: 17-143-611

Study program: PhD in Ecology and Evolution

Bachelor Master Dissertation

Title of the thesis: Mechanisms that shape the evolution of cooperative breeding

Supervisor: Prof. Dr. Michael Taborsky

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, 4th April 2022

Place/Date

Signature



