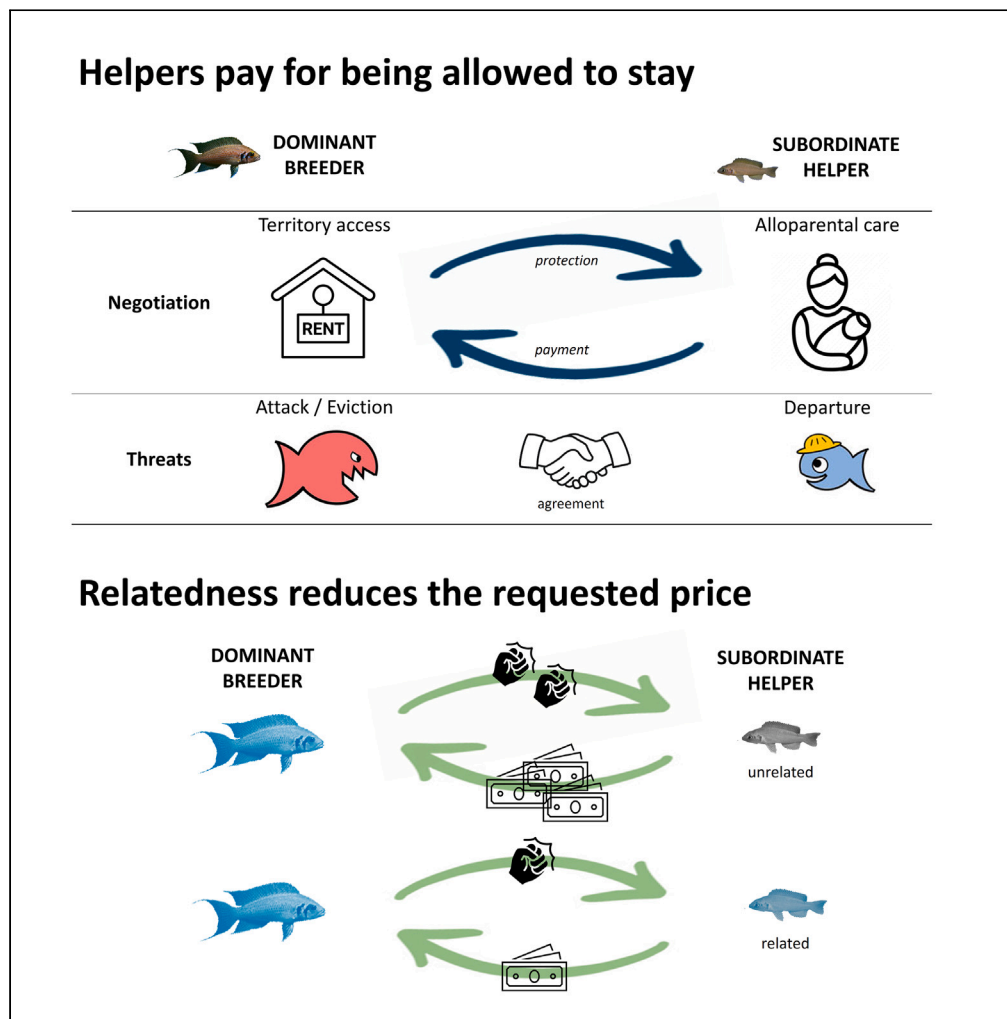


Article

Nepotism mediates enforced cooperation in asymmetric negotiations



Irene García-Ruiz,
Michael Taborsky

igaru.13@gmail.com

Highlights

Experimental manipulation of behavior reveals bargaining among asymmetric partners

Helpers must pay for being allowed to stay in the territory of dominant breeders

Related helpers are allowed to pay a lower price

Cooperatively breeding fish illustrate how correlated payoffs affect negotiation

García-Ruiz & Taborsky,
iScience 27, 110334
July 19, 2024 © 2024 The
Authors. Published by Elsevier
Inc.
[https://doi.org/10.1016/
j.isci.2024.110334](https://doi.org/10.1016/j.isci.2024.110334)



Article

Nepotism mediates enforced cooperation
in asymmetric negotiationsIrene García-Ruiz^{1,2,5,*} and Michael Taborsky^{1,3,4}

SUMMARY

In cooperative societies, group members typically exchange different commodities among each other, which involves an incessant negotiation process. How is the conflict of fitness interests resolved in this continual bargaining process between unequal partners, so that maintaining the cooperative interaction is the best option for all parties involved? Theory predicts that relatedness between group members may alleviate the conflict of fitness interests, thereby promoting the evolution of cooperation. To evaluate the relative importance of relatedness and direct fitness effects in the negotiation process, we experimentally manipulated both the relatedness and mutual behavioral responses of dominant breeders and subordinate helpers in the cooperatively breeding cichlid fish *Neolamprologus pulcher*. Results show that coercion by breeders is crucial for the performance of alloparental egg care by helpers, but that kinship significantly decreases the need for coercion as predicted by theory. This illustrates the relative importance of kinship and enforcement in the bargaining process.

INTRODUCTION

In societies characterised by high levels of cooperation, different commodities are typically traded among social partners,^{1–8} such as help in brood care in exchange for protection and access to vital resources, reproductive opportunities or territory inheritance.^{9,10} As conflicts of interest typically exist between group members, such trading involves a negotiation process.^{11–20} For example, negotiation about cooperative investment occurs commonly among partners caring for offspring,²¹ which may reduce conflict,^{22–29} but not necessarily so.^{30–32} Social partners negotiating about cooperative effort usually involve asymmetries, for example in capability, resource holding power, and the costs and benefits from cooperation.^{19,33–35} This may allow one side of the interaction to demand more from the other than the latter would voluntarily give.^{36–44} Subordinate group members, for instance, may be enforced to provide service to the benefit of dominants just to be tolerated in the group and get access to vital resources.^{13,14,45–49} Subordinates may hence pay to stay within the territory of dominants, which represents one cause for alloparental care in cooperatively breeding animals.^{7,50–59}

The conflict of interest between group members is reduced by the degree to which they are related to each other.⁶⁰ Therefore, kinship is expected to strongly affect the negotiation process among social partners.^{2,17,19,61–64} The influence of relatedness as a mediator in negotiation processes is apparent when scrutinizing helpers' brood care decisions in Seychelles warblers (*Acrocephalus sechellensis*⁶⁵), placid greenbuls (*Phyllastrephus placidus*⁶⁶) and Lake Tanganyika cichlids (*Neolamprologus pulcher*⁶⁷), as well as in intragroup interactions among cooperative banded mongooses (*Mungos mungo*⁶⁸) and polyembryonic wasps (*Copidosoma floridanum*⁶⁹), and in the reciprocal food provisioning of Norway rats (*Rattus norvegicus*⁸). The relative influence of negotiation and relatedness in interactions involving asymmetries in bargaining power is however yet unclear.

Cooperative breeders pose a highly suitable model to study negotiation among unequal social partners with mixed relatedness.^{67,70} By helping to rear the offspring of dominant breeders, subordinate group members bear costs and delay their own reproduction.^{71–74} In these systems the breeders benefit from alloparental care received from their subordinate helpers, while the latter benefit from indirect fitness gains if raising kin, and generally from the safety in the group, access to resources, reproductive opportunities, or an enhanced probability to inherit the territory, breeding position or help in raising their own offspring in the future.^{9,75–79} Naturally, dominant individuals possess a higher bargaining power than subordinates and may enforce help by attacking idle group members.⁵⁶ Nevertheless, subordinates may leave for good if the net benefits of staying recede below those accruing from dispersing elsewhere.^{15,46,47,80,81} On the other hand, dominant individuals may evict subordinates when the costs of their presence, such as competition for resources or reproduction, outweigh the benefits.^{50,82–90} Consequently, the threat of eviction may prompt subordinates to provide help as payment of rent to be tolerated in the territory.^{13,14,45,52,56,91} The conflict over maximizing each party's payoff can be solved through negotiations in which individuals adjust their behavior in response to the

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

²Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY 10027, USA

³Institute for Advanced Study (Wissenschaftskolleg zu Berlin), 14193 Berlin, Germany

⁴Department of Collective Behavior, Max Planck Institute of Animal Behavior, 78467 Konstanz, Germany

⁵Lead contact

*Correspondence: igaru.13@gmail.com

<https://doi.org/10.1016/j.isci.2024.110334>



behavior of the other party.^{16,19,92,93} Outside options as well as biological market effects of offer and demand may define the level of tolerance on whether to maintain the cooperative association or not.^{46,81,94–98} In essence, for cooperative associations to form and remain stable, participating individuals must find an equilibrium affording that each party obtains a net fitness benefit, which applies if the payoffs of social partners are correlated.^{9,99–101}

Disentangling the relative importance of negotiation, coercion and kinship for cooperation among group members requires the manipulation of the response of social partners to each other's behavior to determine how they react to transgressions, while at the same time experimentally controlling the relatedness between partners. How do dominants respond if their expectations of help from subordinates are violated, and how do subordinates respond to being coerced by dominants? Theory predicts that the underlying negotiation process should be strongly influenced by the degree of relatedness between the involved parties, as this modulates their genetic conflict of interest,^{17,19,61,62,64} but this has not yet been tested. A model system in which this negotiation process and the relative importance of relatedness, trading, and coercion can be investigated is the cooperatively breeding cichlid fish *Neolamprologus pulcher*. In this species, groups are composed of a mixture of related and unrelated individuals that differ in their bargaining power due to size-based rank differences, which determine the power asymmetry among group members.^{9,102,103} If subordinates are experimentally prevented from helping, they receive more aggression from dominants and may be evicted from the territory.^{52,56} In response to such behavioral manipulation, helpers increase submission and cooperation,^{7,48,49,56} as predicted by pay-to-stay models.^{13,47} In addition, larger helpers which impose a higher risk of reproductive competition to the dominant pair,^{104–106} receive more aggression when help is needed and respond by helping more readily than smaller helpers.^{53,54} Subordinates are accepted in the territory dependent on the need of help^{50,107} and they adjust cooperation levels to their outside options,⁴⁶ which corresponds with predictions from biological market theory.^{33,94} Furthermore, unrelated subordinates provide more alloparental care than related ones,⁶⁷ suggesting that kin selection may hamper the evolution of enhanced cooperative investment, as expected if cooperation is driven by enforcement or reciprocal exchange.^{19,61}

Here we use the unique opportunity to concurrently manipulate in highly social animals both kinship and behavior of all parties involved under semi-natural conditions. In a full factorial design, we experimentally test how the interaction between enforcement and relatedness controls alloparental care in *N. pulcher*. The idea is to simulate transgression by altering the reaction of social partners to the social conditions, and to measure the mutual responses (Figure 1). First, the experimental groups consisting of a pair of dominant breeders with either a related or unrelated subordinate are habituated to a mesh cage put over one of the shelters (pre-experimental phase). Then, we put the mesh cage over the breeding shelter so that only the helper can care for the eggs and measure their amount of egg care, and the levels of the aggression and submission of all group members (baseline phase). At the next stage (experience phase), we either prevent the subordinate from providing alloparental brood care by using a finer mesh for the cage (treatment group) or let it care for the eggs by using a traversable net (control group). The resulting omission of help in the treatment group is predicted to cause punishment by the dominants, which is either allowed or prevented in the final phase of the experiment (test phase), where the breeders are confined in clear plexiglass tubes or can freely move and physically interact with their helper. According to the pay-to-stay hypothesis, the expected punishment of idle helpers by dominants in the "interaction allowed condition" is predicted to elicit a compensatory response from the subordinate, i.e., enhanced levels of egg care (Table 1). According to the kin selection hypothesis, both coercion of help and appeasement by cooperation are predicted to be mitigated by relatedness between dominants and subordinates (Table 1).

RESULTS

Experience phase

We used hurdle models to assess the significance of the presence or absence of the response behaviors (binary data) together with their quantitative significance (non-zero count data; see STAR Methods for further details). Confirming predictions from the pay-to-stay hypothesis (Table 1), when helpers were prevented from helping during the experience phase, both kin and non-kin subordinates were exposed to enhanced aggression by the breeders (binary data: 1.17 ± 0.34 , $Z = 3.47$, $p < 0.001$; non-zero count data: 1.04 ± 0.41 , $Z = 2.54$, $p = 0.011$; Figure 2A; Table S1). In addition, breeders also used overt aggressive behaviors more often than restrained aggression when charging "idle" helpers (1.70 ± 0.81 , $Z = 2.11$, $p = 0.034$; Figure 2B; Table S2). Coinciding with the increased breeder aggression, helpers increased submissive displays when prevented from helping (binary data: 2.05 ± 0.46 , $Z = 4.48$, $p < 0.001$; non-zero count data: 0.91 ± 0.17 , $Z = 5.52$, $p < 0.001$; Figure 2C; Table S1) and they showed more avoidance behaviors against breeders (binary data: 2.69 ± 0.91 , $Z = 2.94$, $p = 0.003$; non-zero count data: 1.01 ± 0.17 , $Z = 6.07$, $p < 0.001$; Table S1).

Confirming predictions from the kin selection hypothesis (Table 1), relatedness alleviated dominant aggression as related helpers were attacked less in the experience phase than unrelated helpers (binary data: -1.26 ± 0.47 , $Z = -2.67$, $p = 0.007$; non-zero count data: -1.44 ± 0.49 , $Z = -2.92$, $p = 0.003$; Figure 2A; Table S1). The ratio between overt and restrained aggression did not differ between related and unrelated helpers (-0.77 ± 0.75 , $Z = -1.04$, $p = 0.30$; Figure 2B; Table S2). Coinciding with the lower aggression levels received by breeders, related helpers showed less submission compared to unrelated helpers (binary data: -1.36 ± 0.53 , $Z = -2.56$, $p = 0.011$; non-zero count data: -0.43 ± 0.20 , $Z = -2.17$, $p = 0.030$; Figure 2C; Table S1), and they showed less avoidance behaviors toward breeders when prevented from helping than unrelated helpers (binary data: -2.18 ± 1.16 , $Z = -1.88$, $p = 0.060$; non-zero count data: -0.58 ± 0.22 , $Z = -2.62$, $p = 0.009$; Table S1).

Aggression toward helpers differed between male and female breeders, with males being aggressive less often than females (binary data: -0.88 ± 0.32 , $Z = -2.72$, $p = 0.006$; Table S1), whereas males exhibited higher aggression levels than females when they did charge helpers (non-zero count data: 0.62 ± 0.17 , $Z = 3.67$, $p < 0.001$; Table S1).

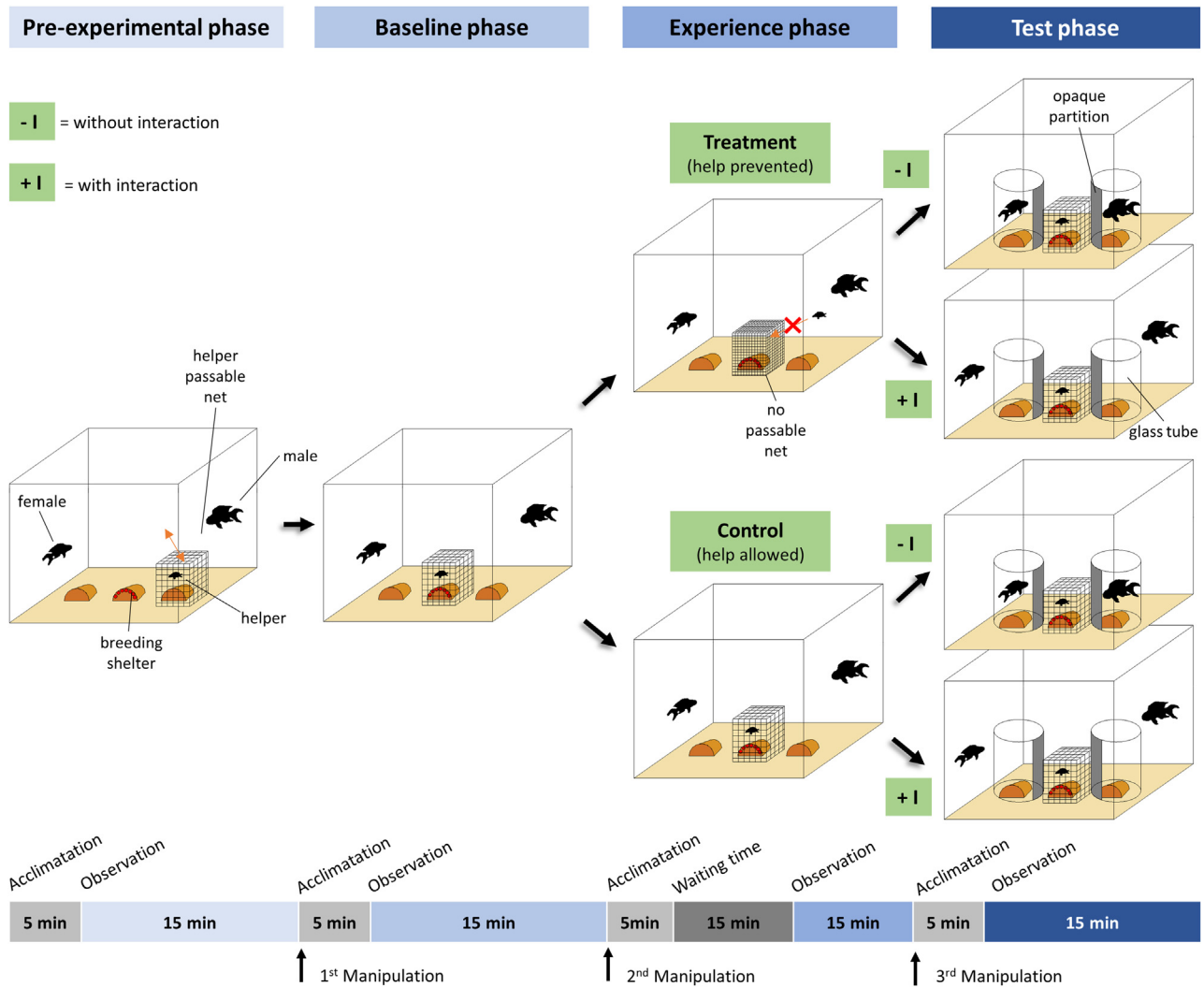


Figure 1. Schematic of the experimental set-up

The experiment started the day after the dominant female spawned. *Pre-experimental phase*: the recording device was placed in front of the aquarium, and both breeders and helper were able to access the breeding shelter. *Baseline phase*: a mesh-cage only passable by the helper was placed over the breeding shelter so that only the helper was able to access the eggs. *Experience phase*: either a non-passable net was placed over the breeding shelter preventing the helper from alloparental care during 30 min (*treatment*), or the passable net was replaced over the breeding shelter (*control*). *Test phase*: the breeders were either prevented to interact with the helper by a half transparent/half opaque cylinder (-I), or they were allowed to freely 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. To test for kinship effects, groups consisted of a pair with a related helper or a pair with an unrelated helper.

These results suggest that both kin and non-kin are enforced to help, but relatedness moderates coercion and submissive responses.

Test phase

As predicted by the pay-to-stay hypothesis (Table 1), in the test phase helpers provided higher levels of egg care when previously prevented from helping, which pertained only when breeders were enabled to physically interact with helpers, allowing them to enforce help (non-zero count data, Prevention x Interaction with breeders: 0.50 ± 0.11 , $Z = 4.38$, $p < 0.001$; Figure 3A; Table S3). Helpers provide alloparental care also in the form of digging out sand from the breeding shelter. This behavior 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. As with alloparental egg care, helpers also showed higher levels of digging out the breeding shelter after they had been prevented from helping before (non-zero count data: 0.56 ± 0.19 , $Z = 2.94$, $p = 0.003$; Figure 3B; Table S3) and when breeders were able to interact with them (non-zero count data: 0.58 ± 0.23 , $Z = 2.47$, $p = 0.014$; Figure 3B; Table S3).

Table 1. Predictions evaluated in this study

	Pay-to-stay	Kin selection	PS + KS	Results
Experience phase				
Aggression by breeders	T > C	None	NK > K	T > C, NK > K
Submission by subordinates	T > C	None	NK > K	T > C, NK > K
Test phase				
Alloparental care by subordinates	T > C (when +I)	T ≥ C K > NK +I = -I	K > NK NK ≥ K (when +I) +I > -I	T > C (when +I) K > NK NK > K (when +I) +I > -I
Baseline phase				
Alloparental care by subordinates	NK > K	K > NK	NK ≥ K	NK = K
Aggression by breeders	NK = K	None	NK > K	NK > K
Submission by subordinates	NK = K	None	NK > K	NK > K

PS: pay-to-stay hypothesis, KS: kin selection hypothesis, T: help prevention, C: control, +I: interaction (punishment) allowed between breeder and helper, -I: interaction (punishment) prevented between breeder and helper. NK: non-kin, K: kin.

In general, during the test phase related helpers provided egg care more often than unrelated helpers (88.75% vs. 70.37% of the trials; binary data: 3.25 ± 1.43 , $Z = 2.28$, $p = 0.023$) as predicted by the kin selection hypothesis (Table 1). However, related helpers provided brood care less frequently than unrelated helpers when they had been previously prevented from helping (binary data, Prevention x Relatedness: -2.52 ± 1.21 , $Z = -2.08$, $p = 0.038$). This is in accordance with the pay-to-stay hypothesis suggesting that related helpers have to pay a lower price than unrelated helpers. Related helpers also showed lower levels of digging out the breeding shelter than unrelated helpers (non-zero count data: -0.80 ± 0.31 , $Z = -2.56$, $p = 0.011$).

Baseline condition

Data obtained in the baseline condition corroborated the experimental results from the experience and test phases regarding relatedness effects on breeder aggression, and they provided information on the brood care of helpers supporting the pay-to-stay hypothesis. Aggression levels of breeders were lower toward kin than toward non-kin also in the baseline condition (non-zero count data: -1.46 ± 0.61 ,

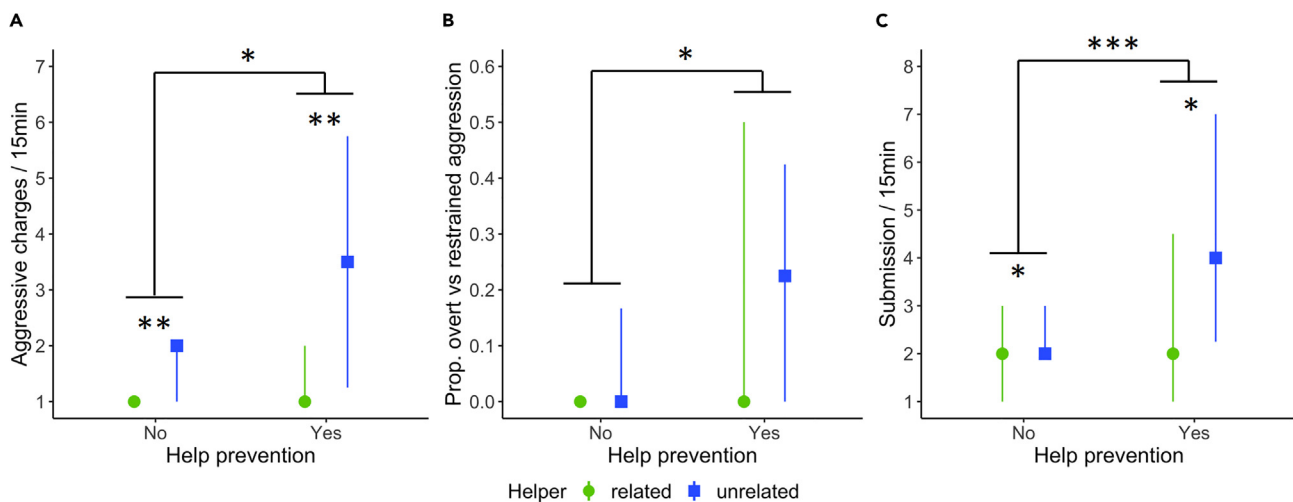


Figure 2. Amount of aggression by breeders and submission by helpers performed during the experience phase

(A) The frequency of the breeders' overt and restrained aggressive behaviors 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 are given in Table S1.

(B) The breeders used more overt than restrained aggression when helping was prevented than in the control condition; detailed statistics are given in Table S2.

(C) Both related and unrelated helpers increased submissive displays when they were prevented from helping, and unrelated helpers showed overall more submission than related helpers; detailed statistics are given in Table S1. Depicted are medians \pm interquartile ranges of behaviors when they occurred (i.e., zero occurrences are omitted). Significant differences are denoted with asterisks: one asterisk (*) indicates $p < 0.05$, two asterisks (**) $p < 0.01$, and three asterisks (***) $p < 0.001$.

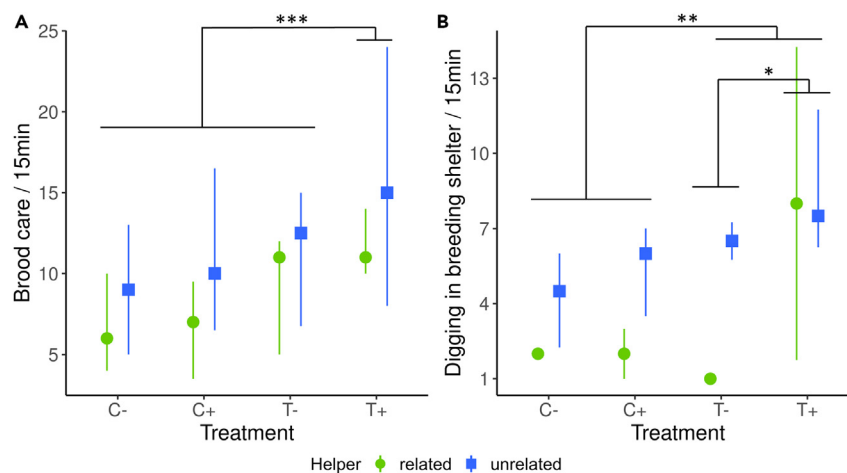


Figure 3. Allopaparental care provided during the test phase

(A) Both related (green circles) and unrelated (blue squares) helpers provided more brood care when they had been previously prevented from helping (T: treatment vs. C: control), but only if the breeders could physically interact with them (“+”: breeders could interact with helpers, vs. “-”: breeders were prevented to interact with helpers).

(B) Helpers dug out sand from the breeding shelter more often when they had been previously prevented to help, and they increased their digging frequency when breeders could physically interact with them; overall, more digging was performed by unrelated helpers. Depicted are medians \pm interquartile ranges of behaviors when they occurred (i.e., zero occurrences are omitted). Significant differences are denoted with asterisks: one asterisk (*) indicates $p < 0.05$, two asterisks (**) $p < 0.01$, and three asterisks (***) $p < 0.001$. The overall significant difference between digging levels of related and unrelated helpers ($p = 0.011$) is not indicated by asterisks in the graph. For all statistical details see Table S3.

$Z = -2.39$, $p = 0.017$; Table S4), and females attacked the helpers more frequently than males (binary data: -6.15 ± 1.38 , $Z = -4.46$, $p < 0.001$; Table S4). Related helpers tended to spend more time in the breeding shelter than unrelated helpers (LMM, $t = 2.09$, $p = 0.051$; Table S5), and the amount of allopaparental investment (egg care and sand digging) increased with the time spent in the breeding shelter (LMM, $t = 3.52$, $p = 0.003$; Table S5). Nevertheless, the time spent in the breeding shelter was not related to the amount of aggression shown by the breeders (LMM, $t = -0.67$, $p = 0.51$; Table S5), which indicates that breeders were not limited in their attacks on helpers by the latter’s inaccessibility when being in the brood shelter.

Overall, the quantity of allopaparental care did not differ between related and unrelated helpers in the baseline condition (binary data: 0.31 ± 0.89 , $Z = 0.35$, $p = 0.72$; non-zero count data: -0.13 ± 0.15 , $Z = -0.87$, $p = 0.38$; Table S4), but allopaparental investment seemed to increase with the need for help, as more egg care was provided for larger clutches (non-zero count data: 0.01 ± 0.002 , $Z = 2.49$, $p = 0.013$; Table S4). Digging in the brood shelter showed the opposite tendency (non-zero count data: -0.02 ± 0.01 , $Z = -2.21$, $p = 0.027$; Table S4), which might indicate a trade-off between egg care and sand digging while spending time in the brood shelter; larger clutches require more egg care, but not more removal of sand. Larger helpers provided more allopaparental care both in the form of egg care (non-zero count data: 0.41 ± 0.19 , $Z = 2.23$, $p = 0.026$; Table S4) and digging in the breeding shelter (non-zero count data: 1.21 ± 0.47 , $Z = 2.56$, $p = 0.011$; Table S4), which corroborates the pay-to-stay hypothesis as larger helpers cause higher costs to breeders than smaller helpers and hence need to pay a higher price.^{50,54,82,105}

DISCUSSION

The threat of punishment has been invoked as a key factor promoting the evolution of cooperation among non-relatives,^{15,19,44,50,91,108,109} but few studies have demonstrated the link between punishment and cooperation in animal societies.^{48,49,110,111} Here, we find support for the evolution of allopaparental care as a result of a negotiation processes involving unequal partners in which each group member tries to maximize their own pay-off.

Consistent with predictions from pay-to-stay models,^{13,14,45} we found that help-prevention increased the dominants’ aggression toward their helpers both in number and intensity. Crucially, punishment increased cooperation (direct egg care and digging out sand from the breeding shelter), and this increase in cooperation was realized by the ability of breeders to enforce help by physical interactions. In addition, subordinates responded by increased submissive displays, corroborating previous field observations.⁵⁴ Helpers exert submission as a direct response to received aggression by the breeders,⁷⁵ which involves considerable energy costs.¹¹² Submissive displays of helpers may also serve as a pre-emptive appeasement to reduce aggression and increase tolerance in the territory.^{7,50}

Enforcement was also involved if helpers were related to breeders, but as predicted¹⁹ relatedness clearly reduced coercion in the negotiation process between helpers and breeders. Dominant breeders attacked related helpers less than unrelated helpers during experimental help prevention. In response to reduced aggression levels by the breeders, related helpers displayed less submission and exerted less compensatory helping after being idle than unrelated helpers. It seems that even if related helpers also pay rent like unrelated subordinates, the breeders demand less from kin as reflected by (i) the reduced levels of aggression and submission, and (ii) the fact that related helpers

compensated previous help prevention by enhancing egg care to a lesser degree than unrelated helpers. This clearly indicates a moderating effect of relatedness on the negotiation between breeders and helpers.

Unrelated helpers invested more in shelter digging than related helpers, which is the most energy-demanding form of help, causing a 6-fold increase in routine metabolic rate.¹¹³ This is in accordance with theory predicting that higher helping efforts should result from enforcement (corresponding to the pay-to-stay hypothesis) than from relatedness (corresponding with the kin selection hypothesis.¹⁹ Nevertheless, it is worth stressing that the alloparental care of related subordinates is apparently also mediated by the aggression of dominant breeders and not a completely voluntary activity; negotiation and indirect fitness benefits seem to interactively influence the subordinates' helping decisions.

Breeders may not only benefit from the presence of subordinates but also suffer costs, for instance through the risk of egg cannibalism,^{67,114} reproductive parasitism^{82,90} and the competition for resources (reviewed in⁹). Breeder males suffer higher costs from reproductive parasitism than breeder females, which might explain why males showed higher aggression levels than females when attacking a helper in the experience phase, even if in our study helpers were still sexually immature. In contrast, females charged helpers on more occasions than males, which might have been due to the reproductive stage (egg care phase) in which the experiment was conducted. The main risk during this period is egg cannibalism^{67,114} and females are mainly responsible for guarding the eggs.¹¹² Clarifying the sex-specific negotiation between breeders and sexually mature helpers seems a promising goal for future studies.

Understanding the evolution of decision rules that underlie the negotiation processes between asymmetric social partners is one of the key challenges in the study of social behavior.^{2,11,16,23} Social partners are bound to vary in many respects, including their resource holding potential, individual needs, genetic makeup, experience, cognitive abilities, and so forth, which affects their qualification, skills and motivation when interacting with one another.^{2,35} This intriguing diversity of influencing factors entails boundless possibilities for the evolution of decision rules, which have rarely been explored. Our study is a first attempt to test the influence of asymmetries between social partners in a negotiation process by simultaneously controlling both relatedness and the behavioral responses of interaction partners toward each other. Capitalizing on the unique possibility to manipulate at our discretion both group composition and behavior in a cooperatively breeding species under semi-natural conditions, the results show that dominants enforce from subordinates' altruistic investment in their broods, while the scale of enforcement depends on the relatedness between the involved parties. Hence, our study highlights the importance of using an integrative approach when attempting to understand the negotiation processes involved in cooperative interactions among group members in highly social animals.

The evolution of alloparental care behavior in cooperative breeders is commonly attributed to the effect of kin selection,^{79,115} but evidence is accumulating that other selection mechanisms are often crucially involved.^{10,116–122} Our study highlights that kin selection should not be automatically assumed to be the sole or major force selecting for cooperation, even if breeders and helpers are closely related. Theoretical models suggest that direct fitness benefits can constitute the original force selecting for group formation, which may then enable kin selection to take effect.¹²³ By adding an important parameter, the enforcement of help in dependence of relatedness, the present study may help to resolve the ongoing debate about the importance of direct and indirect fitness benefits driving the evolution of cooperation and altruism (e.g.,^{2,77,124–129}).

To recapitulate, in *N. pulcher*, group membership is essential for survival and reproduction.^{9,75,130–133} Natural groups consist of a mixture of related and unrelated males, females and immatures, which inevitably differ in resource holding power due to associated size differences, as teleost fishes grow interminably.⁹ This prompts asymmetric interactions between group members, where larger, more dominant individuals can demand a higher price for what they can offer in return. Hence subordinate helpers must pay by caring for the dominants' offspring to be allowed to stay in the territory and benefit from safety and the use of its resources. They are only tolerated when needed,^{50,107} and if their contribution falls short of demand they are punished through breeder attacks,⁵⁶ which elicits enhanced helping levels.^{48,49,54,57} Our results show that their own offspring are not exempt from paying for tolerance, but obviously they benefit from price deduction.

Limitations of the study

A limitation of our methodology is that the mesh cage used to prevent the breeders from accessing the breeding shelter could have served also as a protected shelter for the subordinates during the control conditions. However, we did not find a correlation between the time subordinates spent in the breeding shelter and the agonistic behaviors 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 retreat instead of the mesh cage. Likewise, breeders could have increased agonistic behavior due to the prevention of access to the breeding shelter. This effect should be higher for the female than for the male breeder, as female breeders typically contribute much more to direct brood care. In fact, breeders did show aggressive displays when the helper was inside the breeding shelter, but the same behavior was shown when the helper was in a shelter covered with the mesh cage other than the breeding shelter during the pre-experimental phase, so this behavior did not seem to be related to the access of the helpers to the eggs. Regardless, this potential effect is the same for all treatment conditions, since the breeders were not allowed to access the breeding shelter ever during the duration of the experiment. Another limitation of the study was that we did not provide digging challenges to the helpers by adding sand into the breeding shelter, as our main aim was to test alloparental egg care. However, the helpers still engaged in digging behavior in the breeding shelter. Even if digging behavior was not experimentally manipulated, the results of digging still support our findings for egg care. Lastly, we were unable to determine the sex of the helpers, as it is only possible to do so reliably once they reach sexual maturity. Therefore, we could not assess any sex-related effects. However, it is unlikely that such effects are important during the immature stages.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
 - Lead contact
 - Materials availability
 - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
 - Study species
 - Experimental set-up
- **METHOD DETAILS**
 - Experimental design and procedure
 - Behavioral observations
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2024.110334>.

ACKNOWLEDGMENTS

We thank Lorenzo Arduini for his participation with the data collection and Evi Zwygart for the help in taking care of the fish. We also thank Daniel Zobrist for his help drawing [Figure 1](#). Funding was provided by SNSF-grant 31003A 176174 to Michael Taborsky.

AUTHOR CONTRIBUTIONS

Conceptualization IGR and MT, methodology IGR and MT, investigation IGR, visualization IGR, supervision MT, writing—original draft IGR, and writing—review and editing MT.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: March 27, 2024

Revised: May 7, 2024

Accepted: June 18, 2024

Published: June 20, 2024

REFERENCES

1. Taborsky, M., Frommen, J.G., and Riehl, C. (2016). Correlated pay-offs are key to cooperation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371, 20150084. <https://doi.org/10.1098/rstb.2015.0084>.
2. Taborsky, M., Cant, M.A., and Komdeur, J. (2021). *The Evolution of Social Behaviour* (Cambridge University Press), p. 410. <https://doi.org/10.1017/9780511894794>.
3. Tiddi, B., Aureli, F., and Schino, G. (2010). Grooming for infant handling in tufted capuchin monkeys: a reappraisal of the primate infant market. *Anim. Behav.* 79, 1115–1123. <https://doi.org/10.1016/j.anbehav.2010.02.008>.
4. Tiddi, B., Aureli, F., Polizzi di Sorrentino, E., Janson, C.H., and Schino, G. (2011). Grooming for tolerance? Two mechanisms of exchange in wild tufted capuchin monkeys. *Behav. Ecol.* 22, 663–669. <https://doi.org/10.1093/beheco/arr028>.
5. Borgeaud, C., and Bshary, R. (2015). Wild Vervet Monkeys Trade Tolerance and Specific Coalitionary Support for Grooming in Experimentally Induced Conflicts. *Curr. Biol.* 25, 3011–3016. <https://doi.org/10.1016/j.cub.2015.10.016>.
6. Carne, C., Wiper, S., and Semple, S. (2011). Reciprocation and interchange of grooming, agonistic support, feeding tolerance, and aggression in semi-free-ranging Barbary macaques. *Am. J. Primatol.* 73, 1127–1133. <https://doi.org/10.1002/ajp.20979>.
7. Bergmüller, R., and Taborsky, M. (2005). Experimental manipulation of helping in a cooperative breeder: Helpers “pay to stay” by pre-emptive appeasement. *Anim. Behav.* 69, 19–28. <https://doi.org/10.1016/j.anbehav.2004.05.009>.
8. Schweinfurth, M.K., and Taborsky, M. (2018). Relatedness decreases and reciprocity increases cooperation in Norway rats. *Proc. Biol. Sci.* 285, 20180035. <https://doi.org/10.1098/rspb.2018.0035>.
9. Taborsky, M. (2016). Cichlid fishes: A model for the integrative study of social behavior. In *Cooperative Breeding in Vertebrates*, W.D. Koenig and J.L. Dickinson, eds. (Cambridge University Press), pp. 272–293. <https://doi.org/10.1017/1017107338357.017>.
10. Kingma, S.A. (2017). Direct benefits explain interspecific variation in helping behaviour among cooperatively breeding birds. *Nat. Commun.* 8, 1094. <https://doi.org/10.1038/s41467-017-01299-5>.
11. McNamara, J.M., Gasson, C.E., and Houston, A.I. (1999). Incorporating rules for responding into evolutionary games. *Nature* 401, 368–371. <https://doi.org/10.1038/43869>.
12. Taylor, P.D., and Day, T. (2004). Stability in negotiation games and the emergence of cooperation. *Proc. Biol. Sci.* 271, 669–674. <https://doi.org/10.1098/rspb.2003.2636>.
13. Kokko, H., Johnstone, R.A., and Wright, J. (2002). The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behav. Ecol.* 13, 291–300. <https://doi.org/10.1093/beheco/13.3.291>.
14. Hamilton, I.M., and Taborsky, M. (2005). Unrelated helpers will not fully compensate for costs imposed on breeders when they

- pay to stay. *Proc. Biol. Sci.* 272, 445–454. <https://doi.org/10.1098/rspb.2004.2961>.
15. Cant, M.A., and Johnstone, R.A. (2006). Self-serving punishment and the evolution of cooperation. *J. Evol. Biol.* 19, 1383–1436. <https://doi.org/10.1111/j.1420-9101.2006.01151.x>.
 16. Binmore, K. (2010). Bargaining in biology? *J. Evol. Biol.* 23, 1351–1363. <https://doi.org/10.1111/j.1420-9101.2010.02011.x>.
 17. Johnstone, R.A. (2011). Load lightening and negotiation over offspring care in cooperative breeders. *Behav. Ecol.* 22, 436–444. <https://doi.org/10.1093/beheco/arq190>.
 18. van de Waal, E., Spinelli, M., Bshary, R., Ros, A.F.H., and Noë, R. (2013). Negotiations over Grooming and appeasement can be more effective drivers of sociality than kin selection. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371, 20150089. <https://doi.org/10.1098/rstb.2015.0089>.
 19. Ito, K., McNamara, J.M., Yamauchi, A., and Higginson, A.D. (2017). The evolution of cooperation by negotiation in a noisy world. *J. Evol. Biol.* 30, 603–615. <https://doi.org/10.1111/jeb.13030>.
 20. Houston, A.I., Székely, T., and McNamara, J.M. (2005). Conflict between parents over care. *Trends Ecol. Evol.* 20, 33–38. <https://doi.org/10.1016/j.tree.2004.10.008>.
 21. Houston, A.I., and Davies, N.B. (1985). The evolution of cooperation and life history in the dunnock *Prunella modularis*. In *Behavioural Ecology*, R.M. Sibley and R.H. Smith, eds. (Blackwell Scientific Publications), pp. 471–487.
 22. Johnstone, R.A., and Hinde, C.A. (2006). Negotiation over offspring care—how should parents respond to each other's efforts? *Behav. Ecol.* 17, 818–827. <https://doi.org/10.1093/beheco/arl009>.
 23. Hinde, C.A., and Kilner, R.M. (2007). Negotiations within the family over the supply of parental care. *Proc. Biol. Sci.* 274, 53–60. <https://doi.org/10.1098/rspb.2006.3692>.
 24. Johnstone, R.A., Manica, A., Fayet, A.L., Stoddard, M.C., Rodríguez-Gironés, M.A., and Hinde, C.A. (2014). Reciprocity and conditional cooperation between great tit parents. *Behav. Ecol.* 25, 216–222. <https://doi.org/10.1093/beheco/art109>.
 25. Bebbington, K., and Hatchwell, B.J. (2016). Coordinated parental provisioning is related to feeding rate and reproductive success in a songbird. *Behav. Ecol.* 27, 652–659. <https://doi.org/10.1093/beheco/arv198>.
 26. Johnstone, R.A., and Savage, J.L. (2019). Conditional Cooperation and Turn-Taking in Parental Care. *Front. Ecol. Evol.* 7, 335. <https://doi.org/10.3389/fevo.2019.00335>.
 27. Smiseth, P.T. (2019). Coordination, Cooperation, and Conflict Between Caring Parents in Burying Beetles. *Front. Ecol. Evol.* 7, 397. <https://doi.org/10.3389/fevo.2019.00397>.
 28. Storey, A.E., Wilhelm, S.I., and Walsh, C.J. (2020). Negotiation of Parental Duties in Chick-Rearing Common Murre (*Uria aalge*) in Different Foraging Conditions. *Front. Ecol. Evol.* 7, 506. <https://doi.org/10.3389/fevo.2019.00506>.
 29. Iserbyt, A., Farrell, S., Eens, M., and Müller, W. (2015). Sex-specific negotiation rules in a costly conflict over parental care. *Anim. Behav.* 100, 52–58. <https://doi.org/10.1016/j.anbehav.2014.11.014>.
 30. McNamara, J.M. (2003). Should young ever be better off with one parent than with two? *Behav. Ecol.* 14, 301–310. <https://doi.org/10.1093/beheco/14.3.301>.
 31. Lessells, C.M., and McNamara, J.M. (2012). Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. *Proc. Biol. Sci.* 279, 1506–1514. <https://doi.org/10.1098/rspb.2011.1690>.
 32. Noë, R., van Schaik, C.P., and van Hooff, J.A.R.A.M. (1991). The Market Effect: an Explanation for Pay-off Asymmetries among Collaborating Animals. *Ethology* 87, 97–118. <https://doi.org/10.1111/j.1439-0310.1991.tb01192.x>.
 33. Raihani, N.J., Thornton, A., and Bshary, R. (2012). Punishment and cooperation in nature. *Trends Ecol. Evol.* 27, 288–295. <https://doi.org/10.1016/j.tree.2011.12.004>.
 34. Phillips, T. (2018). The concepts of asymmetric and symmetric power can help resolve the puzzle of altruistic and cooperative behaviour. *Biol. Rev.* 93, 457–468. <https://doi.org/10.1111/brv.12352>.
 35. Tebbich, S., Taborsky, M., and Winkler, H. (1996). Social manipulation causes cooperation in keas. *Anim. Behav.* 52, 1–10. <https://doi.org/10.1006/anbe.1996.0147>.
 36. Reeve, H.K., and Gamboa, G.J. (1987). Queen Regulation of Worker Foraging in Paper Wasps: a Social Feedback Control System (*Polistes fuscatus*, Hymenoptera: Vespidae). *Beyond Behav.* 102, 147–167. <https://doi.org/10.1163/156853986X00090>.
 37. Gamboa, G.J., Wacker, T.L., Scope, J.A., Cornell, T.J., and Shellman-Reeve, J. (1990). The Mechanism of Queen Regulation of Foraging by Workers in Paper Wasps (*Polistes fuscatus*, Hymenoptera: Vespidae). *Ethology* 85, 335–343. <https://doi.org/10.1111/j.1439-0310.1990.tb00412.x>.
 38. Steinegger, M., and Taborsky, B. (2007). Asymmetric sexual conflict over parental care in a biparental cichlid. *Behav. Ecol. Sociobiol.* 61, 933–941. <https://doi.org/10.1007/s00265-006-0322-x>.
 39. Madden, J.R., and Clutton-Brock, T.H. (2009). Manipulating grooming by decreasing ectoparasite load causes unpredicted changes in antagonism. *Proc. Biol. Sci.* 276, 1263–1268. <https://doi.org/10.1098/rspb.2008.1661>.
 40. Melis, A.P., Hare, B., and Tomasello, M. (2009). Chimpanzees coordinate in a negotiation game. *Evol. Hum. Behav.* 30, 381–392. <https://doi.org/10.1016/j.evolhumbehav.2009.05.003>.
 41. Inglis, R.F., West, S., and Buckling, A. (2014). An experimental study of strong reciprocity in bacteria. *Biol. Lett.* 10, 20131069. <https://doi.org/10.1098/rsbl.2013.1069>.
 42. Leighton, G.M., and Vander Meiden, L.N. (2016). Sociable Weavers Increase Cooperative Nest Construction after Suffering Aggression. *PLoS One* 11, e0150953. <https://doi.org/10.1371/journal.pone.0150953>.
 43. Ågren, J.A., Davies, N.G., and Foster, K.R. (2019). Enforcement is central to the evolution of cooperation. *Nat. Ecol. Evol.* 3, 1018–1029. <https://doi.org/10.1038/s41559-019-0907-1>.
 44. Gaston, A.J. (1978). The Evolution of Group Territorial Behavior and Cooperative Breeding. *Am. Nat.* 112, 1091–1100. <https://doi.org/10.1086/283348>.
 45. Bergmüller, R., Heg, D., and Taborsky, M. (2005). Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proc. Biol. Sci.* 272, 325–331. <https://doi.org/10.1098/rspb.2004.2960>.
 46. Hellmann, J.K., and Hamilton, I.M. (2018). Dominant and subordinate outside options alter help and eviction in a pay-to-stay negotiation model. *Behav. Ecol.* 29, 553–562. <https://doi.org/10.1093/beheco/ary006>.
 47. Naef, J., and Taborsky, M. (2020). Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *R. Soc. Open Sci.* 7, 191808. <https://doi.org/10.1098/rsos.191808>.
 48. Naef, J., and Taborsky, M. (2020). Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Anim. Behav.* 168, 137–147. <https://doi.org/10.1016/j.anbehav.2020.08.006>.
 49. Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Beyond Behav.* 95, 45–75. <https://doi.org/10.1163/156853985X00046>.
 50. Mulder, R.A., and Langmore, N.E. (1993). Dominant males punish helpers for temporary defection in superb fairy-wrens. *Anim. Behav.* 45, 830–833. <https://doi.org/10.1006/anbe.1993.1100>.
 51. Balshine-Earn, S., Neat, F.C., Reid, H., and Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behav. Ecol.* 9, 432–438. <https://doi.org/10.1093/beheco/9.5.432>.
 52. Brintjes, R., and Taborsky, M. (2008). Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Anim. Behav.* 75, 1843–1850. <https://doi.org/10.1016/j.anbehav.2007.12.004>.
 53. Heg, D., and Taborsky, M. (2010). Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS One* 5, e10784. <https://doi.org/10.1371/journal.pone.0010784>.
 54. Reyer, H.-U. (1986). Breeder-Helper-Interactions in the Pied Kingfisher Reflect the Costs and Benefits of Cooperative Breeding. *Beyond Behav.* 96, 277–302. <https://doi.org/10.1163/156853986X00522>.
 55. Fischer, S., Zöttl, M., Groenewoud, F., and Taborsky, B. (2014). Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proc. Biol. Sci.* 281, 20140184. <https://doi.org/10.1098/rspb.2014.0184>.
 56. Zöttl, M., Schreier, T., and Taborsky, M. (2023). Coercion promotes alloparental care in cooperative breeders. *Behav. Ecol.* 34, 363–372. <https://doi.org/10.1093/beheco/arac125>.
 57. de Souza, A.R., Lino-Neto, J., and do Nascimento, F.S. (2017). Pushing Wasps to Work: Decentralized Aggression Induces Increased Activity in the Paper Wasp *Polistes versicolor*. *J. Insect Behav.* 30,

- 360–373. <https://doi.org/10.1007/s10905-017-9624-2>.
59. Trapote, E., Canestrari, D., and Baglione, V. (2021). Female helpers signal their contribution to chick provisioning in a cooperatively breeding bird. *Anim. Behav.* 172, 113–120. <https://doi.org/10.1016/j.anbehav.2020.12.011>.
 60. Hamilton, W.D. (1964). The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7, 17–52. [https://doi.org/10.1016/0022-5193\(64\)90039-6](https://doi.org/10.1016/0022-5193(64)90039-6).
 61. Marshall, J.A.R., and Rowe, J.E. (2003). Kin selection may inhibit the evolution of reciprocity. *J. Theor. Biol.* 222, 331–335. [https://doi.org/10.1016/S0022-5193\(03\)00039-0](https://doi.org/10.1016/S0022-5193(03)00039-0).
 62. Johnstone, R.A. (2008). Kin selection, local competition, and reproductive skew. *Evolution* 62, 2592–2599. <https://doi.org/10.1111/j.1558-5646.2008.00480.x>.
 63. Johnstone, R.A. (2003). Sibling negotiation. *Behav. Ecol.* 14, 780–786. <https://doi.org/10.1093/beheco/arg024>.
 64. Savage, J.L., Russell, A.F., and Johnstone, R.A. (2013). Intra-group relatedness affects parental and helper investment rules in offspring care. *Behav. Ecol. Sociobiol.* 67, 1855–1865. <https://doi.org/10.1007/s00265-013-1595-5>.
 65. Richardson, D.S., Komdeur, J., and Burke, T. (2003). Avian behaviour: Altruism and infidelity among warblers. *Nature* 422, 580. <https://doi.org/10.1038/422580a>.
 66. Cousseau, L., Van de Looek, D., Apfelbeck, B., Githiru, M., Matthyssen, E., and Lens, L. (2022). Kin do not always help: testing multiple hypotheses on nest feeding in a cooperatively breeding bird. *Behav. Ecol.* 33, 1080–1092. <https://doi.org/10.1093/beheco/ara073>.
 67. Zöttl, M., Heg, D., Chervet, N., and Taborsky, M. (2013). Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nat. Commun.* 4, 1341. <https://doi.org/10.1038/ncomms2344>.
 68. Thompson, F.J., Cant, M.A., Marshall, H.H., Vitikainen, E.I.K., Sanderson, J.L., Nichols, H.J., Gilchrist, J.S., Bell, M.B.V., Young, A.J., Hodge, S.J., and Johnstone, R.A. (2017). Explaining negative kin discrimination in a cooperative mammal society. *Proc. Natl. Acad. Sci. USA* 114, 5207–5212. <https://doi.org/10.1073/pnas.1612235114>.
 69. Dunn, J., Dunn, D.W., Strand, M.R., and Hardy, I.C.W. (2014). Higher aggression towards closer relatives by soldier larvae in a polyembryonic wasp. *Biol. Lett.* 10, 20140229. <https://doi.org/10.1098/rsbl.2014.0229>.
 70. Donaldson, L., Thompson, F.J., Field, J., and Cant, M.A. (2014). Do paper wasps negotiate over helping effort? *Behav. Ecol.* 25, 88–94. <https://doi.org/10.1093/beheco/art089>.
 71. Brown, J.L. (1987). *Helping and Communal Breeding in Birds: Ecology and Evolution* (Princeton University Press), p. 384.
 72. Taborsky, M. (1994). Sneakers, Satellites, and Helpers: Parasitic and Cooperative Behavior in Fish Reproduction. *Adv. Study Behav.* 23, 1–100. [https://doi.org/10.1016/S0065-3454\(08\)60351-4](https://doi.org/10.1016/S0065-3454(08)60351-4).
 73. Solomon, N.G., and French, J.A. (1997). *Cooperative Breeding in Mammals* (Cambridge University Press), p. 408.
 74. Dickinson, J.L., and Hatchwell, B.J. (2004). Fitness consequences of helping. In *Ecology and Evolution of Cooperative Breeding in Birds*, W.D. Koenig and J.L. Dickinson, eds. (Cambridge University Press), pp. 48–66.
 75. Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Anim. Behav.* 32, 1236–1252. [https://doi.org/10.1016/S0003-3472\(84\)80241-9](https://doi.org/10.1016/S0003-3472(84)80241-9).
 76. Cockburn, A. (1998). Evolution of Helping Behavior in Cooperatively Breeding Birds. *Annu. Rev. Ecol. Syst.* 29, 141–177. <https://doi.org/10.1146/annurev.ecolsys.29.1.141>.
 77. Clutton-Brock, T. (2002). Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296, 69–72. <https://doi.org/10.1126/science.296.5565.69>.
 78. Kingma, S.A., Santema, P., Taborsky, M., and Komdeur, J. (2014). Group augmentation and the evolution of cooperation. *Trends Ecol. Evol.* 29, 476–484. <https://doi.org/10.1016/j.tree.2014.05.013>.
 79. Koenig, W.D., and Dickinson, J.L. (2016). Introduction. In *Cooperative Breeding in Vertebrates*, W.D. Koenig and J.L. Dickinson, eds. (Cambridge University Press), pp. 1–5. <https://doi.org/10.1017/CBO9781107338357>.
 80. Bergmüller, R., Taborsky, M., Peer, K., and Heg, D. (2005). Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. *Beyond Behav.* 142, 1643–1667. <https://doi.org/10.1163/156853905774831800>.
 81. Jungwirth, A., Walker, J., and Taborsky, M. (2015). Prospecting precedes dispersal and increases survival chances in cooperatively breeding cichlids. *Anim. Behav.* 106, 107–114. <https://doi.org/10.1016/j.anbehav.2015.05.005>.
 82. Dierker, P. (1999). Reproductive parasitism of broodcare helpers in a cooperatively breeding fish. *Behav. Ecol.* 10, 510–515. <https://doi.org/10.1093/beheco/10.5.510>.
 83. Mitchell, J., Heg, D., Taborsky, M., and Jutzeler, E. (2009). Dominant members of cooperatively-breeding groups adjust their behaviour in response to the sexes of their subordinates. *Beyond Behav.* 146, 1665–1686. <https://doi.org/10.1163/000579509X12459328580026>.
 84. Mitchell, J.S., Jutzeler, E., Heg, D., and Taborsky, M. (2009). Gender Differences in the Costs that Subordinate Group Members Impose on Dominant Males in a Cooperative Breeder. *Ethology* 115, 1162–1174. <https://doi.org/10.1111/j.1439-0310.2009.01705.x>.
 85. Skubic, E., Taborsky, M., McNamara, J.M., and Houston, A.I. (2004). When to parasitize? A dynamic optimization model of reproductive strategies in a cooperative breeder. *J. Theor. Biol.* 227, 487–501. <https://doi.org/10.1016/j.jtbi.2003.11.021>.
 86. Johnstone, R.A., and Cant, M.A. (1999). Reproductive skew and the threat of eviction: a new perspective. *Proc. R. Soc. Lond. B.* 266, 275–279. <https://doi.org/10.1098/rspb.1999.0633>.
 87. Thompson, F.J., Marshall, H.H., Vitikainen, E.I., Young, A.J., and Cant, M.A. (2017). Individual and demographic consequences of mass eviction in cooperatively banded mongooses. *Anim. Behav.* 134, 103–112. <https://doi.org/10.1016/j.anbehav.2017.10.009>.
 88. Cant, M.A., Hodge, S.J., Bell, M.B.V., Gilchrist, J.S., and Nichols, H.J. (2010). Reproductive control via eviction (but not the threat of eviction) in banded mongooses. *Proc. Biol. Sci.* 277, 2219–2226. <https://doi.org/10.1098/rspb.2009.2097>.
 89. Hellmann, J.K., Reddon, A.R., Ligocki, I.Y., O'Connor, C.M., Garvy, K.A., Marsh-Rollo, S.E., Hamilton, I.M., and Balshine, S. (2015). Group response to social perturbation: impacts of isotocin and the social landscape. *Anim. Behav.* 105, 55–62. <https://doi.org/10.1016/j.anbehav.2015.03.029>.
 90. Hellmann, J.K., Ligocki, I.Y., O'Connor, C.M., Reddon, A.R., Garvy, K.A., Marsh-Rollo, S.E., Gibbs, H.L., Balshine, S., and Hamilton, I.M. (2015). Reproductive sharing in relation to group and colony-level attributes in a cooperative breeding fish. *Proc. Biol. Sci.* 282, 20150954. <https://doi.org/10.1098/rspb.2015.0954>.
 91. Cant, M.A. (2011). The role of threats in animal cooperation. *Proc. Biol. Sci.* 278, 170–178. <https://doi.org/10.1098/rspb.2010.1241>.
 92. Cant, M.A., and Shen, S.-F. (2006). Endogenous timing in competitive interactions among relatives. *Proc. Biol. Sci.* 273, 171–178. <https://doi.org/10.1098/rspb.2005.3132>.
 93. Van Doorn, G.S., Riebli, T., and Taborsky, M. (2014). Coaction versus reciprocity in continuous-time models of cooperation. *J. Theor. Biol.* 356, 1–10. <https://doi.org/10.1016/j.jtbi.2014.03.019>.
 94. Hammerstein, P., and Noë, R. (2016). Biological trade and markets. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371, 20150101. <https://doi.org/10.1098/rstb.2015.0101>.
 95. Branconi, R., Barbasch, T.A., Francis, R.K., Srinivasan, M., Jones, G.P., and Buston, P.M. (2020). Ecological and social constraints combine to promote evolution of non-breeding strategies in clownfish. *Commun. Biol.* 3, 649. <https://doi.org/10.1038/s42003-020-01380-8>.
 96. Rueger, T., Barbasch, T.A., Wong, M.Y.L., Srinivasan, M., Jones, G.P., and Buston, P.M. (2018). Reproductive control via the threat of eviction in the clown anemonefish. *Proc. Biol. Sci.* 285, 20181295. <https://doi.org/10.1098/rspb.2018.1295>.
 97. Nelson-Flower, M.J., Flower, T.P., and Ridley, A.R. (2018). Sex differences in the drivers of reproductive skew in a cooperative breeder. *Mol. Ecol.* 27, 2435–2446. <https://doi.org/10.1111/mec.14587>.
 98. Nelson-Flower, M.J., Wiley, E.M., Flower, T.P., Ridley, A.R., Nelson-Flower, M.J., Wiley, E.M., Flower, T.P., and Ridley, A.R. (2018). Individual dispersal delays in a cooperative breeder: ecological constraints, the benefits of philopatry and the social queue for dominance. *J. Anim. Ecol.* 87, 1227–1238. <https://doi.org/10.1111/1365-2656.12814>.
 99. Cant, M.A., and Johnstone, R.A. (2009). How Threats Influence the Evolutionary Resolution of Within-Group Conflict. *Am. Nat.* 173, 759–771. <https://doi.org/10.1086/598489>.
 100. Buston, P.M., and Zink, A.G. (2009). Reproductive skew and the evolution of conflict resolution: a synthesis of transactional and tug-of-war models. *Behav. Ecol.* 20, 672–684. <https://doi.org/10.1093/beheco/arp050>.
 101. Roberts, G. (2005). Cooperation through interdependence. *Anim. Behav.* 70, 901–908. <https://doi.org/10.1016/j.anbehav.2005.02.006>.
 102. Bender, N., Hamilton, I.M., and Heg, D. (2005). Size differences within a dominance

- hierarchy influence conflict and help in a cooperatively breeding cichlid. *Beyond Behav.* 142, 1591–1613. <https://doi.org/10.1163/156853905774831846>.
103. Reddon, A.R., Balk, D., and Balshine, S. (2011). Sex differences in group-joining decisions in social fish. *Anim. Behav.* 82, 229–234. <https://doi.org/10.1016/j.anbehav.2011.04.017>.
 104. Heg, D., and Hamilton, I.M. (2008). Tug-of-war over reproduction in a cooperatively breeding cichlid. *Behav. Ecol. Sociobiol.* 62, 1249–1257. <https://doi.org/10.1007/s00265-008-0553-0>.
 105. Heg, D., Jutzeler, E., Bonfils, D., and Mitchell, J.S. (2008). Group composition affects male reproductive partitioning in a cooperatively breeding cichlid. *Mol. Ecol.* 17, 4359–4370. <https://doi.org/10.1111/j.1365-294X.2008.03920.x>.
 106. Heg, D., Bergmüller, R., Bonfils, D., Otti, O., Bachar, Z., Burri, R., Heckel, G., and Taborsky, M. (2006). Cichlids do not adjust reproductive skew to the availability of independent breeding options. *Behav. Ecol.* 17, 419–429. <https://doi.org/10.1093/beheco/arj056>.
 107. Zöttl, M., Frommen, J.G., and Taborsky, M. (2013). Group size adjustment to ecological demand in a cooperative breeder. *Proc. Biol. Sci.* 280, 20122772. <https://doi.org/10.1098/rspb.2012.2772>.
 108. Clutton-Brock, T.H., and Parker, G.A. (1995). Punishment in animal societies. *Nature* 373, 209–216. <https://doi.org/10.1038/373209a0>.
 109. Cant, M.A., and Young, A.J. (2013). Resolving social conflict among females without overt aggression. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368, 20130076. <https://doi.org/10.1098/rstb.2013.0076>.
 110. Bshary, R., and Grutter, A.S. (2005). Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol. Lett.* 1, 396–399. <https://doi.org/10.1098/rsbl.2005.0344>.
 111. Wong, M.Y.L., Buston, P.M., Munday, P.L., and Jones, G.P. (2007). The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. *Proc. Biol. Sci.* 274, 1093–1099. <https://doi.org/10.1098/rspb.2006.0284>.
 112. Taborsky, M., and Grantner, A. (1998). Behavioural time–energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Anim. Behav.* 56, 1375–1382. <https://doi.org/10.1006/anbe.1998.0918>.
 113. Grantner, A., and 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). *J. Comp. Physiol. B* 168, 427–433. <https://doi.org/10.1007/s003600050162>.
 114. von 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. <https://doi.org/10.1111/j.1439-0310.1990.tb00785.x>.
 115. Hatchwell, B.J. (2009). The evolution of cooperative breeding in birds: Kinship, dispersal and life history. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 3217–3227. <https://doi.org/10.1098/rstb.2009.0109>.
 116. Sumner, S., Kelstrup, H., and Fanelli, D. (2010). Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proc. Biol. Sci.* 277, 1721–1728. <https://doi.org/10.1098/rspb.2009.2289>.
 117. Kingma, S.A., Hall, M.L., and Peters, A. (2011). Multiple benefits drive helping behavior in a cooperatively breeding bird: An integrated analysis. *Am. Nat.* 177, 486–495. <https://doi.org/10.1086/658989>.
 118. Wright, J., McDonald, P.G., te Marvelde, L., Kazem, A.J.N., and Bishop, C.M. (2010). Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proc. Biol. Sci.* 277, 437–445. <https://doi.org/10.1098/rspb.2009.1360>.
 119. Magrath, R.D., and Whittingham, L.A. (1997). Subordinate males are more likely to help if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. *Behav. Ecol. Sociobiol.* 41, 185–192. <https://doi.org/10.1007/s002650050378>.
 120. Sherley, G.H. (1990). Co-operative breeding in rifleman (*Acanthisitta chloris*) benefits to parents, offspring and helpers. *Beyond Behav.* 112, 1–22. <https://doi.org/10.1163/156853990X00653>.
 121. Reyer, H.-U. (1980). Flexible helper structure as an ecological adaptation in the pied kingfisher (*Ceryle rudis rudis* L.). *Behav. Ecol. Sociobiol.* 6, 219–227. <https://doi.org/10.1007/BF00569203>.
 122. Jungwirth, A., and Taborsky, M. (2015). First- and second-order sociality determine survival and reproduction in cooperative cichlids. *Proc. Biol. Sci.* 282, 20151971. <https://doi.org/10.1098/rspb.2015.1971>.
 123. García-Ruiz, I., Quiñones, A., and Taborsky, M. (2022). The evolution of cooperative breeding by direct and indirect fitness effects. *Sci. Adv.* 8, eabl7853. <https://doi.org/10.1126/sciadv.abl7853>.
 124. Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature* 462, 51–57. <https://doi.org/10.1038/nature08366>.
 125. Dickinson, J.L., and Hatchwell, B.J. (2004). Fitness consequences of helping. In *Ecology and evolution of cooperative breeding in birds* (Cambridge University Press), pp. 48–66.
 126. Heinsohn, R.G. (2004). Parental care, load-lightening, and costs. In *Ecology and evolution of cooperative breeding in birds*, W.D. Koenig and J.L. Dickinson, eds. (Cambridge University Press), pp. 67–80.
 127. Lehmann, L., and Keller, L. (2006). The evolution of cooperation and altruism - A general framework and a classification of models. *J. Evol. Biol.* 19, 1365–1376. <https://doi.org/10.1111/j.1420-9101.2006.01119.x>.
 128. West, S.A., Gardner, A., and Griffin, A.S. (2006). Altruism. *Curr. Biol.* 16, R482–R483. <https://doi.org/10.1016/j.cub.2006.06.014>.
 129. West, S.A., Griffin, A.S., and Gardner, A. (2007). Evolutionary Explanations for Cooperation. *Curr. Biol.* 17, 661–672. <https://doi.org/10.1016/j.cub.2007.06.004>.
 130. Heg, D., Bachar, Z., Brouwer, L., and Taborsky, M. (2004). Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proc. Biol. Sci.* 271, 2367–2374. <https://doi.org/10.1098/rspb.2004.2855>.
 131. Taborsky, M., Brouwer, L., Heg, D., and Bachar, Z. (2005). Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. *Beyond Behav.* 142, 1615–1641. <https://doi.org/10.1163/156853905774831891>.
 132. Brouwer, L., Heg, D., and Taborsky, M. (2005). Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behav. Ecol.* 16, 667–673. <https://doi.org/10.1093/beheco/ari042>.
 133. Groenewoud, F., Frommen, J.G., Josi, D., Tanaka, H., Jungwirth, A., and Taborsky, M. (2016). Predation risk drives social complexity in cooperative breeders. *Proc. Natl. Acad. Sci. USA* 113, 4104–4109. <https://doi.org/10.1073/pnas.1524178113>.
 134. Friard, O., and Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7, 1325–1330. <https://doi.org/10.1111/2041-210X.12584>.
 135. R Core Team, and Team, R.C. (2020). R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing). <https://www.R-project.org>.
 136. Hartig, F. (2021). DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models. <https://CRAN.R-project.org/package=DHARMA>.
 137. Brooks, M., Kristensen, K., Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Mächler, M., and Bolker, B. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *R J.* 9, 378. <https://doi.org/10.32614/RJ-2017-066>.
 138. Taborsky, M., and Limberger, D. (1981). Helpers in fish. *Behav. Ecol. Sociobiol.* 8, 143–145. <https://doi.org/10.1007/BF00300826>.
 139. Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M., and Werner, N. (2001). Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behav. Ecol. Sociobiol.* 50, 134–140. <https://doi.org/10.1007/s002650100343>.
 140. Brintjes, R., Bonfils, D., Heg, D., and Taborsky, M. (2011). Paternity of Subordinates Raises Cooperative Effort in Cichlids. *PLoS One* 6, e25673. <https://doi.org/10.1371/journal.pone.0025673>.
 141. Stiver, K.A., Dierkes, P., Taborsky, M., Gibbs, H.L., and Balshine, S. (2005). Relatedness and helping in fish: examining the theoretical predictions. *Proc. Biol. Sci.* 272, 1593–1599. <https://doi.org/10.1098/rspb.2005.3123>.
 142. Dierkes, P., Heg, D., Taborsky, M., Skubic, E., and Achmann, R. (2005). Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecol. Lett.* 8, 968–975. <https://doi.org/10.1111/j.1461-0248.2005.00801.x>.
 143. Brintjes, R., and Taborsky, M. (2011). Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Anim. Behav.* 81, 387–394. <https://doi.org/10.1016/j.anbehav.2010.10.004>.
 144. Hert, E. (1985). Individual Recognition of Helpers by the Breeders in the Cichlid Fish *Lamprologus brichardi* (Poll, 1974). *Z. Tierpsychol.* 68, 313–325. <https://doi.org/10.1111/j.1439-0310.1985.tb00132.x>.

145. Le Vin, A.L., Mable, B.K., and Arnold, K.E. (2010). Kin recognition via phenotype matching in a cooperatively breeding cichlid, *Neolamprologus pulcher*. *Anim. Behav.* 79, 1109–1114. <https://doi.org/10.1016/j.anbehav.2010.02.006>.
146. Stiver, K.A., Fitzpatrick, J., Desjardins, J.K., and Balshine, S. (2006). Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. *Anim. Behav.* 71, 449–456. <https://doi.org/10.1016/j.anbehav.2005.06.011>.
147. Jungwirth, A., Zöttl, M., Bonfils, D., Josi, D., Frommen, J.G., and Taborsky, M. (2023). Philopatry yields higher fitness than dispersal in a cooperative breeder with sex-specific life history trajectories. *Sci. Adv.* 9, eadd2146. <https://doi.org/10.1126/sciadv.add2146>.
148. Wong, M., and Balshine, S. (2011). The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biol. Rev.* 86, 511–530. <https://doi.org/10.1111/j.1469-185X.2010.00158.x>.
149. Brooks, M., Kristensen, K., Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Mächler, M., and Bolker, B. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400. <https://doi.org/10.32614/RJ-2017-066>.
150. Heinsohn, R., and Legge, S. (1999). The cost of helping. *Trends Ecol. Evol.* 14, 53–57. [https://doi.org/10.1016/S0169-5347\(98\)01545-6](https://doi.org/10.1016/S0169-5347(98)01545-6).
151. Emlen, S.T., and Wrege, P.H. (1988). The role of kinship in helping decisions among white-fronted bee-eaters. *Behav. Ecol. Sociobiol.* 23, 305–315. <https://doi.org/10.1007/BF00300577>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Repository data	This paper	https://doi.org/10.6084/m9.figshare.26018287
Experimental models: organisms/strains		
<i>Neolamprologus pulcher</i>	Captive descendants from individuals caught in the wild near Kasakalawe point at the southern end of Lake Tanganyika (Zambia).	N/A
Software and algorithms		
Boris v.7.9	Friad & Gamba ¹³⁴	https://www.boris.unito.it/
R v.3.6.3	R Core Team ¹³⁵	https://www.R-project.org/
R package DHARMA v.0.4.1	Hartig ¹³⁶	https://github.com/florianhartig/DHARMA
R package glmmTMB v.1.1.2	Brooks et al. ¹³⁷	https://github.com/glmmTMB/glmmTMB

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Irene García-Ruiz (igaru.13@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All original data can be accessed in Figshare. DOI is listed in the [key resources table](#).
- This paper does not report original code.
- Additional information required to reanalyse the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

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 sizes.^{129,133,138,139} The reproductive skew is high, with mature subordinates of both sexes producing between 5 and 15 per cent of offspring in the group.^{9,82,90,104,140} Overall relatedness between subordinates and dominants is low and decreases with helper age, mainly due to the replacement of the breeders.^{141,142} Both related and unrelated subordinates participate in brood care of the dominants' broods by cleaning and fanning the eggs,^{67,75} digging out sand from the breeding chamber,^{138,143} and defending the territory against predators of eggs and young.^{7,49,50,54,75,132,138,139} Individual and kin recognition have been demonstrated in this species.^{144,145} 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.^{46,81,146,147} Predation risk is the major environmental factor constraining dispersal^{75,130}; see⁹ 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). Experimental groups were established of a pair and an immature subordinate of unknown sex; reliable sexing of immature subordinates is impossible in this species, but there are no apparent sex differences in helping behaviors.⁵⁰ Groups were established following the natural group structure, where dominant males are the largest individual in the group (mean: 64.4mm, range: 54-74mm of standard body length (SL)) followed by the dominant female (mean: 54.5mm, range: 45-61mm SL) with a minimum size difference of at least 5mm⁷⁵ and an average of 10mm. Unrelated (mean: 26.4mm, range: 19-32mm SL) and related subordinates (mean: 25.0mm,

range: 19–33mm SL) were added, which 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 own 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 SL, all offspring but one were removed from the tank. The subordinate chosen was never the biggest, to avoid inadvertent rank effects. For unrelated helpers, first the prospective 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 after the aggressor was again released, 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 respective helper's presence. Therefore, the helpers already had experience in egg care before the experiment started. No egg cannibalism was observed for any of the experimental subordinates, and all subordinates were observed taking care of the eggs previous to the start of the experiment. Related and unrelated helpers were chosen of a similar size to avoid helper 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 an air-driven 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 later in discussion for a description of the experimental procedure), while the subordinates could freely pass through the mesh. Right after group formation, 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°C–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.⁷⁵ The fish were fed with dry food five times a week and with defrosted 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.

METHOD DETAILS

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 simulated idleness of helpers by preventing them from helping and measured the response by the breeders. In the test phase, we assessed 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. The helpers used in this experiment were either related or unrelated to the dominant pair to assess relatedness effects on the interaction between breeders and subordinates. We also recorded a pre-experimental phase before any manipulations happened in which the breeders could access the breeding shelter to assess by comparison with later phases of the experiment the impact of restraining the breeders from accessing the eggs in 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 behavior (egg cleaning and fanning), because it reflects altruistic behavior⁶⁷ causing considerable opportunity and energy costs without immediate direct fitness benefits to the actor.^{54,112,113,148} We also scored 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 (10 × 10mm). 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 had a finer mesh size which allowed water flow but prevented fish from accessing the covered brood chamber.

During all experimental phases, breeders were excluded from brood care by the experimental cage described above (Figure 1). 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 behaviors described above for 15min. In the "experience phase" lasting 30 min groups were divided into treatment groups (T), in which the whole group was prevented from brood care by the fine-meshed cage for 30min, and control groups (C), in which the coarse-meshed cage was placed over the breeding shelter like in the baseline condition, allowing the subordinate to care for the eggs. Behaviors as described were scored for the last 15min of the 30min experience phase in both treatment and control conditions. Subsequently, during the "test phase" 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 them that was covered with a fine mesh on top to allow water exchange (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 and chemically 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 of them and hence unconstrained in their ability to punish the subordinate. During this phase, the mesh-cage was placed over the breeding chamber like in the baseline condition. We scored the helper's behavior for 15min after a 5min acclimatisation period.

After each phase, we manipulated the mesh-cage to control for manipulation between control and treatment. Each replicate group was exposed to all different experimental treatments. We performed a full factorial experimental design with randomised order of the treatment conditions. After finishing each trial, a gap of 2h was kept before starting the next trial.

Behavioral observations

We scored two aspects of direct brood care behavior: the frequency of micro nipping of the eggs, which serves a hygienic function and is referred to as "egg cleaning", and the frequency of generating water flow to the clutch, which increases the oxygen supply and is referred to as "egg fanning".^{112,113} Additionally, we measured sand digging separately inside the breeding chamber and in other shelters. 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 behaviors see.⁷⁵ The time spent in the breeding chamber or in other shelters was also scored. The experimental phases were video recorded and encoded for blind scoring purposes where possible. The recordings were manually scored using the Boris 7.9 software.¹³⁴

QUANTIFICATION AND STATISTICAL ANALYSIS

During the "test phase" we assessed the pay-to-stay hypothesis predicting that helpers increase alloparental care after having been prevented to help during the "experience phase" only if the breeders can interact with the helper (T/+I). In contrast, the kin selection hypothesis does not predict an influence of the presence of breeders during the test phase (+I = -I), (Figure 1; Table 1), while voluntary compensatory help may occur. We used brood care (egg cleaning and fanning), and sand digging in the breeding chamber as response variables. The fixed effects variables of the statistical model were prevention to access the eggs during the previous phase (T vs. C), interaction with the breeders (+I vs. -I), relatedness status between helper and breeders (related vs. unrelated), helper size, and the number of eggs. We also included the interaction between the factors for the response variable brood care but not for digging behavior, as the low frequency of digging events did not allow to perform interaction analyses. Group ID was added as a random effect in all analyses to account for repeated use of the same groups for the different treatment conditions.

We ran zero-altered generalised linear mixed-effects models using the R package glmmTMB.¹⁴⁹ Hurdle models were used to test the significance of the presence or absence of a behavior together with the quantitative significance. The probability of occurrence can be a more reliable factor as the amount of a given behavior can be affected by many uncontrolled factors, such as the costs that the behavior imposes in a given context.^{150,151} For the presence/absence of the behavior 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.¹³⁶ 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 enhance aggression toward subordinates when they were prevented from helping. Enforcement was predicted by the pay-to-stay hypothesis but not by the kin selection hypothesis. We ran 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 status between helper and breeders, and breeder sex, as well as the interactions between 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 status or breeder sex. For that, we ran 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 behaviors (avoid and flee) toward the breeders during this phase. An increase in submission and avoidance behaviors of the subordinate would be expected for the prevention treatment only when enforcement occurs. We ran ZAP-GLMMs as described above for both response variables. The fixed effects variables for both analyses were prevention to access the eggs and relatedness status 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 ran ZAP-GLMMs for levels of alloparental care and breeding chamber digging behaviors, using relatedness as a fixed factor and helper size and clutch size as covariates. For aggression levels, we ran a ZAP-GLMM using relatedness and breeder sex as fixed effects. To check whether helpers used the breeding chamber to avoid being attacked by the breeders, we run linear mixed models (LMM) to analyze the time the helper spent in the breeding chamber in relation to the level of aggression received by the breeders, while controlling for the effect of helping and relatedness.

All simulations and statistical tests were performed in R 3.6.3.¹³⁵