

1 **Consistent cooperation in a cichlid fish is caused by maternal and developmental effects rather**  
2 **than heritable genetic variation**

3

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14 **Abstract**

15 Studies on the evolution of cooperative behaviour are typically confined to understanding its  
16 adaptive value. It is equally essential, however, to understand its potential to evolve, requiring  
17 knowledge about the phenotypic consistency and genetic basis of cooperative behaviour. While  
18 previous observational studies reported considerably high heritabilities of helping behaviour in  
19 cooperatively breeding vertebrates, experimental studies disentangling the relevant genetic and non-  
20 genetic components of cooperative behaviour are lacking. In a half-sibling breeding experiment, we  
21 investigated the repeatability and heritability of three major helping behaviours performed by  
22 subordinates of the cooperatively breeding fish *Neolamprologus pulcher*. To experimentally  
23 manipulate the amount of help needed in a territory, we raised the fish in two environments  
24 differing in egg predation risk. All three helping behaviours were significantly repeatable, but had  
25 very low heritabilities. The high within-individual consistencies were predominantly due to maternal  
26 and permanent environment effects. The perceived egg predation risk had no effect on helping, but  
27 social interactions significantly influenced helping propensities. Our results reveal that  
28 developmentally plastic adjustments of provided help to social context shape cooperative  
29 phenotypes, whereas heritable genetic variation plays a minor role.

30

31 **Keywords:**

32 cooperation, variance decomposition, maternal effects, heritability, repeatability, behaviour, cichlid

33 **Introduction**

34

35 Cooperation between individuals is considered a crucial step in evolutionary transitions from solitary  
36 life to complex social organization [1]. Animals cooperate during hunting, anti-predator behaviours  
37 and territory defence, and particularly also during brood care [2,3]. In eusocial societies, in which  
38 assistance with tasks in the colony is obligate, workers are mostly sterile and rarely disperse and  
39 breed on their own. Conversely, in cooperative breeding systems, in which subordinate individuals  
40 help raising offspring that have been produced by dominant breeders [4], the degree to which an  
41 individual assists the dominants with brood care is variable. This necessitates a decision on the part  
42 of the subordinate if and for how long to help and when to disperse. Previous studies on  
43 cooperatively breeding vertebrates have shown limited flexibility in this decision, with intrinsic  
44 differences among individual helpers in the extent to which they carry out different helping tasks,  
45 such as provisioning and protection of young, territory defence, and territory maintenance [5–8].  
46 This raises questions about the role of genetic and environmental factors in shaping such individual  
47 variation in helping effort.

48

49 Potential sources of consistent inter-individual variation in helping propensity include genetic and  
50 developmental differences as well as differences in state [9], such as body size or hunger level.  
51 Furthermore, characteristics of the cooperation partner(s) can influence an individual's helping effort  
52 through social effects [10]. Such effects are expected especially for cooperation, because the  
53 cooperative behaviours of an individual are likely to be influenced by the behaviours of all interaction  
54 partners [11]. While there is evidence that the early social and ecological environment and current  
55 state influence the individual's propensity to help [8,12], the question of whether individuals might  
56 also differ genetically in their propensity to cooperate has received little experimental scrutiny [13–  
57 16]. However, knowledge about among-individual genetic variation is crucial to understand and  
58 predict micro-evolutionary scope and pattern of cooperative behaviour [17]. Estimates of the  
59 amount of genetic variation underlying cooperative behaviour provide information on the

60 evolutionary potential of a population [18], and estimates of genetic correlations among different  
61 helping traits provide insight into whether they have a shared genetic basis and the potential to  
62 coevolve [17].

63

64 To date, few studies have quantified heritable variation underlying cooperative or helping behaviours  
65 in cooperative breeders, and those that did have found remarkably high estimates of heritability. For  
66 instance, in a pedigree-based study of a wild population of western bluebirds (*Sialia mexicana*),  
67 heritable differences have been estimated to account for 76% of the phenotypic variance in  
68 alloparental helping [19]. Similarly, a study on delayed dispersal in red wolves (*Canis rufus*), a  
69 prerequisite for becoming a helper in the pack, was found to have a strong heritable component  
70 (nearly 100%) in males but not in females [20]. The heritabilities for mass of workers, gynes and  
71 males in the ant *Temnothorax curvispinosus*, which can be understood as the outcome of a  
72 cooperative behaviour because workers raise the offspring of a queen, ranges between 37 and 99%.  
73 Finally, studies in human twins suggest significant heritabilities for cooperative behaviour in the trust  
74 game (between 10 and 32%) [21]. Importantly however, observational studies of natural populations  
75 are inherently limited in their ability to tease apart genetic inheritance from non-genetic effects of  
76 the (maternal) environment [22,23]. Adequately controlling for trans-generational environmental  
77 effects might therefore substantially reduce heritability estimates [24]. Carefully planned breeding  
78 experiments control confounding sources of variation. For instance, employing a half-sibling breeding  
79 design under standardized environmental conditions enables a better separation of additive genetic,  
80 maternal and common environment effects shaping variation in helping behaviour.

81

82 Here we present a quantitative genetic analysis of variation in the three major helping behaviours of  
83 the cooperatively breeding cichlid fish *Neolamprologus pulcher* in an experimental setting. In the  
84 wild, groups consist of a dominant breeder pair that monopolizes reproduction, and several  
85 subordinate helpers that delay dispersal. Helpers assist the dominant pair with direct brood care (egg  
86 cleaning), territory maintenance (removal of sand), and with territory defence against fish and egg

87 predators as well as against space competitors [25–27]. We used a paternal half-sibling design and  
88 bred fish in the laboratory from wild-caught parents. We quantified our test fish's effort in three  
89 major alloparental brood care behaviours, the propensity to clean eggs, the propensity to remove  
90 sand from the breeding chamber, and the amount of defence behaviours directed against an egg  
91 predator. First, we investigated whether body size, social interactions, previous exposure to egg  
92 predators or clutch size have an impact on helping behaviour. Second, we disentangle (i) the effects  
93 of additive genetic variation, (ii) the non-genetic effects of the individual environment that are  
94 constant across repeated measurements of this individual, (permanent environment; also includes  
95 non-additive genetic effects), (iii) maternal effects, (iv) social effects of the cooperation partner, (v)  
96 effects of the shared rearing environment (common environment), and (vi) the effect of individual  
97 predators (in case of defence behaviour) on the phenotypic variation in helping behaviours. This  
98 decomposition provides a measure of the fraction of the total phenotypic variance that is  
99 attributable to additive genetic effects, i.e. the narrow-sense heritability ( $h^2$ ). Furthermore, we  
100 provide a measure of individual consistency, i.e. the repeatability (R).

101

## 102 **Material and Methods**

### 103 *Study animals, breeding design and behavioural tests*

104 We used laboratory-bred  $F_1$ -offspring of parents caught shortly before the start of the experiment in  
105 Lake Tanganyika, Zambia. This ensured that our study population captured the full spectrum of  
106 genetic variation found in the lake. All fish were kept under similar temperature, feeding and light  
107 conditions. Experimental families were randomly assigned to tanks (for further methodological  
108 details, see ESM).

109

110 We carried out a nested paternal half-sibling design [28], in which we mated each male (N=39) to a  
111 unique set of two randomly chosen females (N=77) from the same population, which in total resulted  
112 in 3,175 offspring (Fig. 1a, see ESM for further details). One male died after spawning the first clutch,

113 and hence no half-sibling group could be produced. The parents were removed 8-10 days after  
114 spawning, when they no longer provide direct parental care (defined as 'day 0').

115

116 For the following 63 days, a period when *N. pulcher* juveniles manifest significant developmental  
117 plasticity with respect to social and anti-predator skills [29], one randomly chosen half of each clutch  
118 was exposed to the sympatric unspecialized egg predator *Telmatochromis vittatus* [30,31]. Juveniles  
119 in the egg predator treatment group were exposed to an egg predator for 10 min twice a week on  
120 randomly chosen days and times. Juveniles in the control group received the same handling but  
121 without exposure to an egg predator. Between day 64 and day 85, no treatments took place (neutral  
122 phase 1, Fig. 1b). In the final hierarchy phase, selected test fish were housed in the experimental  
123 tanks together with a larger, unrelated territory owner for around 14 days (Fig. 1d upper panel) to  
124 ensure that the test fish adopted a subordinate status, as only subordinate individuals show  
125 alloparental behaviour [32].

126

127 In the behavioural test we recorded egg cleaning, digging, defence and submissive behaviours of the  
128 test fish and the activity of the egg predator according to the ethogram provided in Table S2. Further  
129 details of the experimental procedures are described in the legend to Fig. 1 and in the ESM.

130

### 131 *Statistical Analyses*

132 A visual check of the histograms of the raw data of cleaning and digging suggested high zero inflation  
133 (Fig. S2). Thus we dichotomized the counts of egg cleaning and digging, and modelled these variables  
134 as binary responses 'cleaning propensity' and 'digging propensity' in logit models, where 1 meant  
135 that the individual performed the behaviour at least once during the test and 0 that it did not.

136 Defence had a higher incidence (90%) and was thus less zero-inflated, so we modelled the counts of  
137 defence behaviours ('amount of defence') with a Poisson error family. All models included the full  
138 number of tests (N=454 first tests and N=86 second tests), but we removed 59 individuals that did  
139 not respond to the test stimulus in the behavioural test (see ESM for details). Thus, the models

140 included a total of 481 observations (N=399 first tests and N=82 second tests). All statistical analyses  
141 were carried out in R version 3.2.0 [33] and calculations were performed on UBELIX, the HPC cluster  
142 at the University of Bern.

143

#### 144 *Predictors of helping behaviours*

145 To determine the fixed effects to be included in the quantitative genetic mixed models (see below),  
146 we fitted a series of GLMMs in the 'lme4' package V 1.1-12 in R [34] to test which environmental  
147 factors influenced the three helping behaviours (ESM, Table S3). We subsequently applied a model  
148 selection approach and ranked models with all possible combinations of the predictors (mentioned in  
149 the ESM) according to their AICc value ('dredge' function in R package 'MuMIn' V 1.15.6; [35]).  
150 Following the 'nesting rule' presented in [36], we selected the final model from a 'confidence set'  
151 consisting of all models within a  $\Delta\text{AICc}$  of  $\leq 6$ ). The 'nesting rule' seeks to avoid the selection of overly  
152 complex models by excluding models from the candidate set that contain more parameters than a  
153 model with a lower AIC value. In case of several non-nested models in the confidence set, preference  
154 was given to the model with the lowest AICc. We report the confidence set for each model together  
155 with their marginal  $R^2$  (the variance explained by fixed effects) and the conditional  $R^2$  (the variance  
156 explained by both fixed and random effects) [37] in Table S4.

157

#### 158 *Decomposition of variation in helping behaviour*

159 We estimated genetic and environmental effects on the propensities to clean and dig and the  
160 amount of defence shown towards an egg predator using a Bayesian animal model ('MCMCglmm'  
161 package V 2.22.1 in R, [38]). We assumed that the wild-caught parents were unrelated. We specified  
162 animal models using a probit-link function to analyse the dichotomous variables cleaning and digging  
163 propensity ('threshold' model) and a log-link function for the amount of defence (Poisson model). For  
164 cleaning and digging propensity, we estimated the variance attributable to additive genetic effects  
165 ( $V_A$ ), permanent environment effects (including non-additive genetic effects;  $V_{PE}$ ), maternal effects

166 ( $V_M$ ), common environment effects ( $V_{CE}$ ) and social effects (i.e. identity of the dominant individual in  
167 the helping trials;  $V_S$ ). In the model for the amount of defence, we furthermore included the effect of  
168 the egg predator's identity ( $V_{VID}$ ). Finally, to control for potential differences among populations, we  
169 included the population of parents' origin in the models ( $V_{Pop}$ ). To see whether the inclusion of  
170 covariates as fixed effects had an impact on the estimates of the variance components we  
171 additionally fitted conditional models with the set of mean-centred and scaled predictors that were  
172 contained in the selected models in the previous step (see Tables S5 and S6). Details on model  
173 parameterization are given in the ESM ('Variance components of helping behaviours'). Models were  
174 run with parameter-expanded priors and the residual variation was fixed to 1 for the threshold  
175 models. Models were run for  $2 \times 10^6$  iterations and the first  $10^5$  iterations of the resulting chain were  
176 discarded as burn-in to ensure that the chain had converged. The remaining chain was sampled at an  
177 interval of 1,000 iterations, yielding a posterior distribution of 1,900 estimates. The mixing of the  
178 chain was evaluated by inspecting the trace plots, checking the convergence of the chain  
179 (Heidelberger diagnostic), the autocorrelation of adjacent samples and the effective sample size  
180 (Table S5). To ensure the stability of estimates we ran three additional models with exactly the same  
181 model structure for each helping behaviour (Table S7).

182

183 Estimates derived from a GLMM framework have to be transformed to the original data scale for  
184 inference. Thus, we computed phenotypic means and variances, additive genetic variance ( $V_{A,obs}$ ) and  
185 heritability, on the observed data scale by looping the 'QGparams' function in the 'QGglmm' package  
186 V 0.5 [39] over the posterior distributions of the models. The proportion of variance explained by  
187 each random effect was calculated as the posterior distribution of the respective variance  
188 component (e.g.  $V_M$  or  $V_{PE}$ ) divided by the posterior distributions of the phenotypic variance ( $V_P$ ),  
189 defined as the sum of all variance components, including the residual variance. We assumed a  
190 distribution-specific residual variance on the expected data scale: 1 for threshold models with a  
191 probit-link and  $\log(1/\exp(\text{Intercept}) + 1)$  for the Poisson model [42].  $R^2$  was calculated following [37],  
192 and using R code for non-Gaussian MCMCglmm models (<http://www.i-deel.org/publications.html>).



193 Repeatability, the proportion of variance explained by among-individual variation, was calculated as  
194 the variance explained by the sum of  $V_A$ ,  $V_{PE}$ ,  $V_M$  and  $V_{CE}$  divided by  $V_P$  [43,44]. The permanent  
195 environment effect  $V_{PE}$  refers to the variance in helping behaviours that is due to the unique  
196 environment experienced by individuals, which might lead, together with additive genetic, maternal  
197 and common environment effects, to a consistency in behaviours through time. We present the  
198 modes of the posterior distributions resulting from the models together with the 95% credibility  
199 intervals (highest posterior density intervals). Because variance components are constrained to be  
200 positive, and credibility intervals will hence never include zero, we inspected the shape of the  
201 posterior distributions visually (as described in the ESM).

202

## 203 **Results**

204 We observed egg cleaning, digging and defence behaviours of 454 fish in the behavioural tests.  
205 Approximately half of the test fish did not show egg cleaning and digging behaviours (56% and 46%,  
206 respectively), but 90% defended against the egg predator (Table S1, Fig. S2).

207

### 208 *Predictors of helping behaviours*

209 Model selection (see 'Methods' and Table S4 for potential predictors and procedure) yielded a final  
210 model that contained two predictors of 'cleaning propensity': the size of the clutch a test fish was  
211 exposed to in the helping test, and the amount of submission it displayed towards the dominant  
212 individual (model 18 in Table S4a): Larger clutches were more likely to be cleaned, whereas test fish  
213 that showed more submissive behaviours were less likely to clean. Growing up with or without egg  
214 predators and the size of the test fish relative to its siblings ('relative size') did not influence cleaning  
215 propensity. Random and fixed effects explained 24% of the variance (conditional  $R^2$ ), whereas the  
216 fixed effects alone explained only 7% (marginal  $R^2$ ). The final model for 'digging propensity' contained  
217 'clutch size', 'relative size', 'submission' and 'acceptance status' as predictor variables (model 16 in  
218 Table S4b). The probability to dig increased with clutch size and the test fish's relative body size, and  
219 it decreased with the amount of submissive behaviours. Test fish with acceptance status 'not

220 determined' and 'tolerated' dug less than those with status 'accepted' (Tukey post-hoc comparisons:  
221 'not determined' vs. 'accepted': estimate=-1.13, se=0.39, z=-2.9, p=0.035; 'tolerated' vs. 'accepted':  
222 estimate=-1.57, se=0.50, z=-3.15, p=0.018). This model explained 26% of the variance, of which the  
223 fixed effects explained only 7%. The final model for 'amount of defence' contained 'egg predator  
224 activity', 'submission' and 'acceptance status' as predictors (model 53 in Table S4c). As in the two  
225 previous models, submission had an attenuating effect. Furthermore, active egg predator individuals  
226 were attacked more than inactive ones, and 'fully accepted' fish defended more than 'accepted'  
227 ('fully accepted' vs. 'accepted': estimate=0.58, se=0.18, z=3.24, p=0.013) and 'not determined' ('not  
228 determined' vs. 'fully accepted': estimate=-0.12, se=0.19, z=-3.16, p=0.017). The other pairwise  
229 comparisons for digging propensity and amount of defence were not significant. Most notably, the  
230 'evicted' fish in our dataset were not less likely to clean eggs, dig or defend. The selected model  
231 explained 56% of the variance, and the fixed effects alone explained 4%.

232

### 233 *Variance components of helping behaviours*

234 The variance decomposition of helping behaviours using the animal model showed that repeatability,  
235 i.e. the variance that can be attributed to differences between individuals, was substantial for  
236 cleaning propensity, and moderate for digging propensity and amount of defence (Table 1, Fig. 1).  
237 However, the additive genetic variance, heritability (the proportion of the phenotypic variance that is  
238 explained by additive genetic variation) was very low for all helping behaviours (Table 1). Growing up  
239 in the same social group (common environment effect,  $V_{CE}$ ) and the identity of the dominant fish  
240 (social effect,  $V_S$ ) both explained very little to none of the observed variation. For the number of  
241 defence actions towards the egg predator there was a moderate maternal effect ( $V_M$ ), and the egg  
242 predator's identity (egg predator effect,  $V_{VID}$ ) had a small effect (Fig. 2). These results were reflected  
243 in the large amount of overlap between the real posterior distributions with null distributions  
244 derived from random draws of  $V_A$ ,  $V_{CE}$  and  $V_S$  (see ESM, Table S8). In contrast, the variance  
245 component of individual identity ( $V_{PE}$ ) for egg cleaning and the maternal and egg predator identity

246 ( $V_M$  and  $V_{VID}$ ) for defence had less than 2% overlap with the null distributions, and hence these  
247 effects can be considered as statistically significant. The maternal identity variance component ( $V_M$ )  
248 of digging overlapped only 6% with the null distribution, even though the posterior mode of the  
249 estimate was rather low ( $V_M=0.0014$  ( $2.9 \times 10^{-6}$ , 0.246)). Interestingly, despite the posterior modes of  
250 of  $V_A$ ,  $V_{PE}$ ,  $V_M$  and  $V_{CE}$  all being close to zero for digging, the repeatability estimate, obtained by  
251 adding up the posterior distributions of these variance components, was significant. This is  
252 attributable to the posterior distributions of  $V_A$  and  $V_M$  being negatively correlated (Pearson  
253 correlation:  $r=-0.28$ ,  $CI=(-0.32, -0.24)$ ), suggesting that the model had difficulties in partitioning the  
254 variance between these two components: For each single model of the posterior distribution, the  
255 variance was either attributed to  $V_A$  or  $V_M$ . This indicates that, even though the analysis produced a  
256 reliable and significant estimate of repeatability, the pattern in the data for digging did not allow  
257 disentangling the effects beyond that level, i.e. at the level of single variance components.

258

## 259 **Discussion**

260

### 261 *Low additive genetic variance of helping behaviours*

262 Here we present a measure of the evolutionary potential of vertebrate cooperative behaviour,  
263 variance-standardized heritability ( $h^2$ ), in an experiment controlling for confounding non-genetic  
264 sources of resemblance among relatives. Our results demonstrate that albeit repeatable,  
265 heritabilities of the three major forms of helping behaviours performed by subordinate *N. pulcher* -  
266 egg cleaning, keeping the breeding chamber clear from sand (digging) and defence of the brood  
267 against egg predators - were close to zero. Thus, the standing genetic variation in these behaviours  
268 was very low and hence the response to selection is predicted to be small. The low heritabilities  
269 observed here are in line with the relatively low heritabilities that have been reported for a range of  
270 traits closely associated with fitness (i.e. behaviours and life-history traits) [45,46]. Although this  
271 pattern can arise if fitness-related traits show a disproportionately large amount of variance  
272 attributable to non-additive genetic variance (e.g. dominance or epistasis [47]), indirect genetic

273 effects [48], or residual variance [49], this is an unlikely explanation in our case. Given the posterior  
274 modes of zero for  $V_A$  In this study, low heritabilities are likely to reflect low absolute levels of additive  
275 genetic variance.

276 Our results contrast the few other studies investigating the genetic basis of helping behaviours in  
277 cooperative breeders, which have reported high heritabilities. For example, heritable genetic  
278 variation was reported to have a strong influence on the probability of being a helper and receiving  
279 help in western bluebirds [19] and the age at dispersal in the cooperatively breeding red wolf [20].  
280 This variation in heritability might reflect differences in the relative importance of environmental  
281 variance and thus the degree of flexibility in helping behaviours that different population experience.  
282 In both studies, however, heritability estimates were obtained from observations of field populations  
283 and non-genetic transgenerational effects were not accounted for. For instance, the effects of  
284 inheriting a territory of a certain quality (which potentially entails differences in the ability to attract  
285 helpers or to influence the delay of dispersal) as well as other parental effects could not be separated  
286 from heritable genetic effects. This could also have potentially caused the substantially higher  
287 heritability estimates in those studies compared to our study [23,50].

288

### 289 *Flexible adjustment of helping strategies*

290 As cooperative behaviours need to be fine-tuned to a specific situation, they require a certain degree  
291 of flexibility in response to environmental cues during development. This includes the permanent  
292 environment [51] and information from mothers to offspring about her environment (maternal  
293 effects, [52]), as well as any short-term changes in the costs and benefits of helping versus dispersing  
294 in the current environment [53,54]. Hence, we expect low to moderate heritability, allowing for  
295 selection for flexible rules to adjust helping propensity to the environment.

296

297 Although the lack of additive genetic variation underlying helping behaviour implies a certain inter-  
298 generational plasticity, our finding that helping behaviours in *N. pulcher* are repeatable shows that

299 individuals are consistent in their behaviour throughout their helper stage, and hence show little  
300 flexibility on a short time-scale. This consistency can arise during ontogeny when juveniles integrate  
301 information on their social and ecological environment and their own condition to decide whether to  
302 follow a breeder or a helper strategy. It has been hypothesized that the existence of 'alternative  
303 cooperative phenotypes' promotes the evolution and maintenance of cooperative breeding because  
304 individuals reliably signal their commitment to helping [56]. The existence of such stable helper  
305 personalities has been shown in banded mongooses, *Mungos mungo* [8]. In *N. pulcher*, individuals  
306 that delay dispersal and stay in their natal territory have to act submissively towards the dominant  
307 breeders to remain accepted in their natal group. The dispersing strategy, on the other hand, is  
308 characterized by higher alloparental effort (egg cleaning) but lower investment in submissive displays  
309 [51]. This is consistent with our finding that submissive behaviour is negatively correlated with  
310 helping behaviours in all three cases, and helping behaviour varied consistently between individuals.  
311 We also found that early experience with an egg-predator had no effect on the propensity to help.  
312 Thus, one may speculate that social experiences during ontogeny affecting submissive behaviour may  
313 ultimately influence helping, rather than the direct cues indicating the need of help (e.g. threat of  
314 egg predation).

315

316 Nevertheless, unlike social insects that diverge very early and irreversibly into different  
317 developmental trajectories, cooperatively breeding vertebrates are thought to maintain a certain  
318 degree of flexibility throughout their development because subordinates potentially become  
319 breeders themselves later in life. The degree of this plasticity that enables individuals to switch  
320 between strategies is likely to vary among species [55]. Even though the repeatability estimates of all  
321 helping behaviours were considerable in our study, there was scope for individuals to react to the  
322 challenges posed by their current situation. For instance, the identity of the egg predator in the  
323 behavioural test explained around 15% of the phenotypic variation of defence (Fig. 2), meaning that  
324 fish can flexibly adjust their defence effort to the particular egg predator individual they were  
325 confronted with.

326

327 *Decomposing individual variation*

328 Our repeatability estimate included three different effects apart from additive genetic variance, each  
329 reflecting a different source of information that an individual can base a developmental decision on.

330 First, the permanent environment effect, which we found to be relatively important, includes  
331 experiences that occur during individual ontogeny. This effect is specific to each individual and might  
332 last throughout its life. For instance the social niche [57,58], or, more generally, social interaction  
333 dynamics in a family [59] may influence the specialization into 'cleaner types' that might persist into  
334 adulthood [51]. Second, the common environment of siblings exposed to the same egg predator  
335 treatment group could have resulted in a higher similarity of group members compared to others,  
336 including full siblings in the other treatment group. Since all fish were kept under standardized  
337 conditions, it is unlikely that abiotic factors contributed to these between-group differences. More  
338 likely, social dynamics might result in differences of helping propensity between groups. However,  
339 the common environment effect was not significant for any of the helping behaviours and hence we  
340 did not find evidence for a group-level helping propensity. Instead, maternal full sibs resembled each  
341 other in terms of their defence effort (and probably digging propensity), even if they were in  
342 different treatment groups. This suggests that females influenced levels of helping in their offspring,  
343 possibly either through egg effects or through their behaviour towards the fry. Although we removed  
344 the parents a week after their eggs hatched, mothers could have influenced their offspring in the  
345 period before this removal. For example, mothers can transmit information via the egg by adjusting  
346 the provisioning of the egg with nutrients or hormones [60], or via epigenetic modifications in the  
347 offspring induced by maternal behaviour [61]. The type of information transmitted from the mother  
348 to the offspring could be based on the predation risk and sand load in the mother's current or  
349 previous environment. However, although the mothers of our test fish were caught in the field  
350 before the start of the breeding experiment and were kept under standardized, predator-free  
351 environmental conditions upon arrival in our facility, we did not find evidence for an adaptive  
352 maternal effect concerning fish or egg predation risk or sand load in the populations of origin of our

353 test fish's parents in Lake Tanganyika (see ESM). Furthermore, we found a maternal correlation  
354 between egg cleaning and defence behaviours (Table S9). A potential mechanism that causes this  
355 correlation could be a maternal effect on the expression of a prolactin-like hormone (tiPRL<sub>II</sub>), which  
356 has been suggested to be implicated in parental behaviour in cichlid fish [62] and other teleosts [63].

357

### 358 *Social effects on helper phenotypes*

359 Social interactions are likely to influence cooperative behaviours [16]. If those social effects have a  
360 genetic basis (indirect genetic effects, [64]), they can alter the cooperative trait's response to  
361 selection, also in the absence of direct genetic effects. These indirect genetic effects are expected to  
362 arise when care-takers are sensitive to the helping behaviour of others, for example when parents  
363 negotiate the amount of care they provide [65], when helpers enable breeders to reduce their effort  
364 [66] or when breeders coerce subordinates into helping [67]. Both reduction of maternal effort and  
365 coercion have been demonstrated in *N. pulcher* [68,69]. In this study, we investigated phenotypic  
366 social effects by including the identity of the dominant territory owner in the models, but the  
367 dominant's identity did not explain any of the variation in egg cleaning, digging or defence  
368 behaviours. Still, we found indirect evidence that cooperative behaviours were influenced by the  
369 dominant's phenotype, because the test fish's submissive behaviour, which was highly correlated  
370 with the dominant's aggression (Spearman's rho = 0.69,  $p < 0.0001$ ), was an important predictor for  
371 all helping behaviours.

372

### 373 *Conclusion*

374 This study showed that *N. pulcher* helpers exhibit both long-term plasticity and short-term flexibility  
375 when adjusting their amount of alloparental brood care to environmental conditions. Hence, the  
376 developmental and genetic architecture of cooperative behaviours in this species might be more  
377 complex than previously thought. Likewise, approaches that do not take into account parental and  
378 other indirect genetic effects are unlikely to reflect the actual response to selection for a number of  
379 reasons [70], in particular because of its strong focus on additive genetic variance [71]. These effects

380 have the potential to alter the rate of evolution, especially when their influence spans more than one  
381 generation (i.e. maternal effects [52]).

382

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### 390 **Ethics**

391 All procedures were conducted under the license 52/12 of the Veterinärämamt Bern and adhered to the  
392 guidelines of the Association for the Study of Animal Behaviour.

### 393 **Data accessibility**

394 Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.4b45d>

### 395 **Competing interests**

396 The authors declare no competing interests.

### 397 **Author contributions**

398 C.K., M.K. and B.T. conceived and designed experiments, C.K. performed experiments, C.K. and E.P.  
399 analysed data, and C.K. wrote the first draft of the manuscript. All authors contributed to revisions.

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- 585

586 Tables and figure captions:

587 Tables:

588 Table 1: Quantitative genetics parameters for models including only random effects computed with the  
589 'QGglmm' package in R [39], except for repeatability that was calculated as  $(V_A+V_{PE}+V_M+V_{CE})/V_P$  (see Methods  
590 section for detailed description). Estimates are the modes of the posterior distributions, which are presented  
591 together with their 95% credibility intervals.

	egg cleaning	digging	defence
<b>trait mean</b>	0.42 (0.36, 0.51)	0.54 (0.45, 0.61)	34.94 (17.3, 65.6)
<b>phenotypic variance (<math>V_P</math>)</b>	0.25 (0.23, 0.25)	0.25 (0.24, 0.25)	13144 (1287, 39611)
<b>add. genet. variance (<math>V_A</math>)</b>	$1 \times 10^{-4}$ ( $8 \times 10^{-10}$ , $3 \times 10^{-2}$ )	$3 \times 10^{-4}$ ( $1 \times 10^{-9}$ , 0.05)	7.0 ( $1 \times 10^{-4}$ , 542.0)
<b>heritability (<math>h^2</math>)</b>	$6 \times 10^{-4}$ ( $3 \times 10^{-9}$ , 0.13)	$1 \times 10^{-3}$ ( $1 \times 10^{-9}$ , 0.22)	$4 \times 10^{-4}$ ( $6 \times 10^{-9}$ , 0.05)
<b>permanent environment (<math>V_{PE}</math>)</b>	0.40 (0.13, 0.60)	0.001 ( $8.1 \times 10^{-8}$ , 0.26)	$5.6 \times 10^{-4}$ ( $1.9 \times 10^{-8}$ , 0.08)
<b>repeatability (R)</b>	0.51 (0.29, 0.73)	0.29 (0.14, 0.54)	0.33 (0.18, 0.48)
<b>coefficient of determination (<math>R^2</math>)</b>	0.81	0.70	0.96

592

593 **Figure captions:**

594 **Fig. 1:** The breeding design, experimental timeline and illustrations of the breeding and experimental tanks  
595 used to obtain phenotypic data on helping behaviours in juvenile helpers. **(a)** Fish were bred in a paternal  
596 nested half-sibling breeding design. **(b)** From spawning (sp) onwards until independence, the fry were kept with  
597 their parents (parental care phase, pc). After the removal of the parents on day 0 each clutch was split into two  
598 equal-sized groups that were randomly assigned to one of the egg-predator treatments (see c) that ended at  
599 day 63 (early experience). Between day 85 and 100 test fish were housed in experimental tanks together with a  
600 dominant individual to establish a hierarchy (hierarchy phase, h1, see (d), upper panel), when they were tested  
601 for their cooperative propensity (test1). In order to estimate the permanent environment effect, a component  
602 of the repeatability of helping behaviours, 86 of the 454 individuals were tested again on day 114 (test2), after  
603 a 7-day hierarchy phase (h2). **(c)** During the early experience phase a transparent tube with a live egg predator  
604 individual or an empty tube (control) was inserted into the breeding tank 5 times a week for 10 min. **(d)** Before  
605 testing, we confined the dominant (D) in a vertical transparent tube (hierarchy phase, upper panel). **(e)** At the  
606 beginning of the behavioural test we fixed a portion of a clutch (orange) spawned by unrelated lab-stock pairs



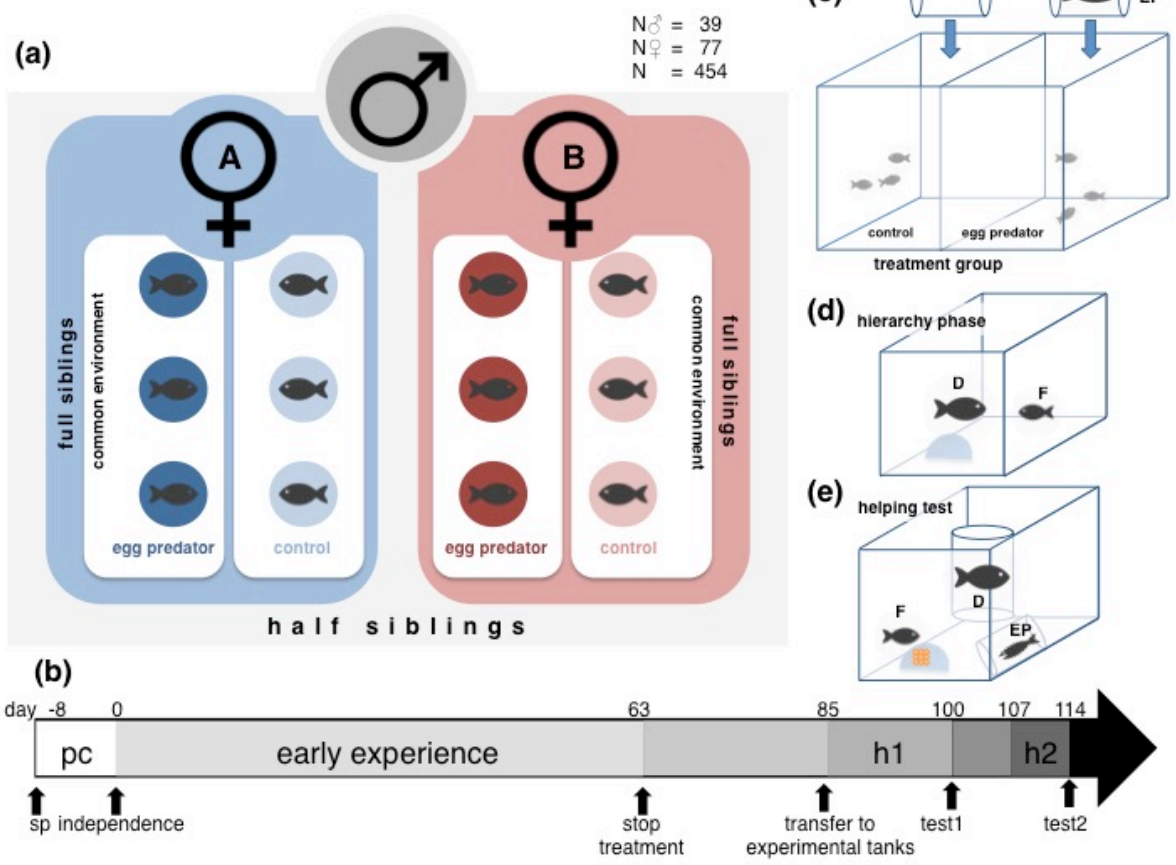
607 on a piece of transparent plastic foil to the inside of the shelter (blue, lower panel). We directly observed all  
608 instances of egg cleaning and digging of the focal fish (F), as well as any interactions with the dominant. After  
609 10 min an egg predator (EP) in a transparent plastic container was introduced, after which all the above-  
610 described behaviours as well as all instances of aggression toward the egg predator were recorded for another  
611 10 min.

612

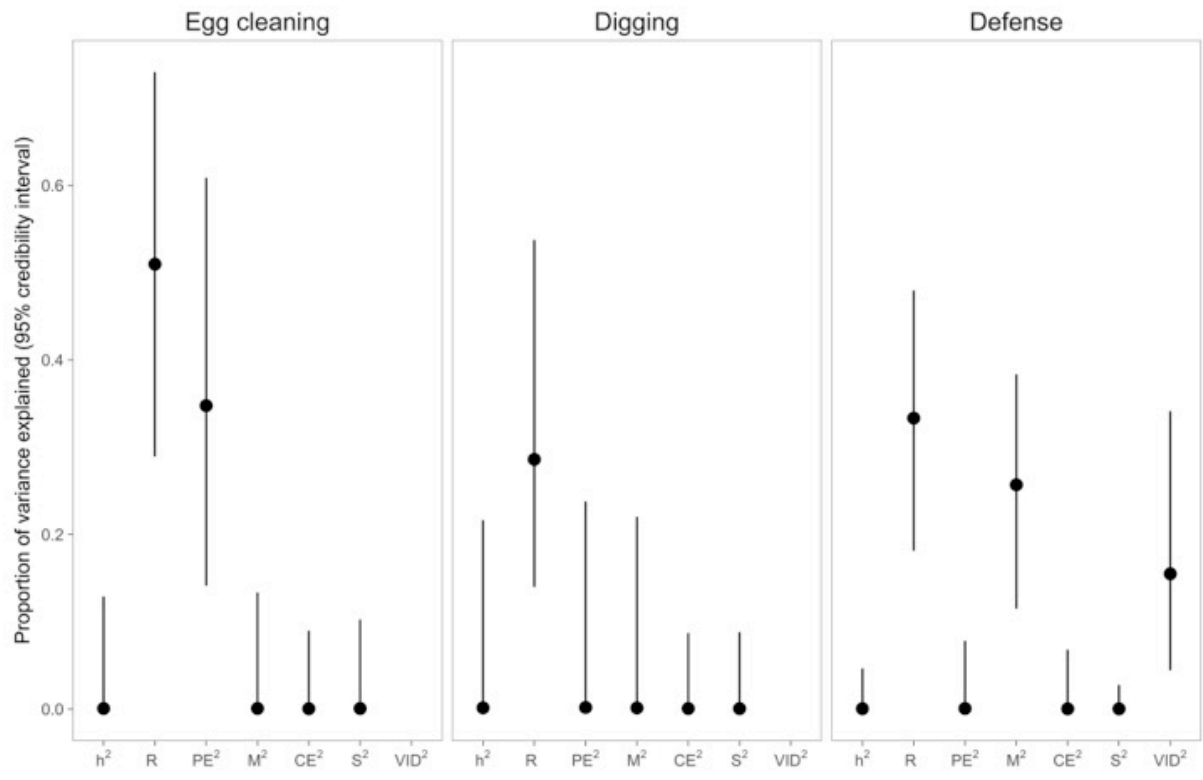
613

614 Fig. 2: The proportion of variance of helping behaviours explained by additive genetic effects (heritability,  $h^2$ ),  
615 by consistent between-individual differences (repeatability,  $R$ ), by the permanent individual environment ( $PE^2$ ,  
616 estimated from the variance component of individual identity of those individuals tested twice), by maternal  
617 identity (maternal effects,  $M^2$ ), by the shared environment during growing up (common environment,  $CE^2$ ), by  
618 the identity of the territory owner (social effects,  $S^2$ ), and by the effect of the egg predator identity ( $VID^2$ ).  
619 Results are presented as point estimates together with their 95% credible interval.

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