



Reduced Egg Investment Can Conceal Helper Effects in Cooperatively Breeding Birds

A. F. Russell, *et al.*
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constrained MOC transport does not account for). The presence of the MAR has not been accounted for in our calculations. Including moored-density and bottom-pressure measurements on both flanks of the MAR allows for the computation of $T_{MO}(z)$ below the MAR crest independently for the western and eastern basin (fig. S2). However, the effect of this on the temporal evolution of unconstrained MOC time series is rather small (fig. S3), with the difference between calculations taking into account and neglecting the measurements on the MAR flanks varying by ± 1.1 Sv (SOM).

Fluctuations in $\overline{T_{EK}}$ of ± 3.9 Sv do not dominate MOC variability on subseasonal time scales at 26.5°N . Rather, we observe an equal share of variability between Ekman and density contributions, with the constrained total geostrophic MOC solution (excluding $\overline{T_{EK}}$) displaying ± 4.1 Sv. We have presented evidence that the depth-independent compensation for $\overline{T_{EK}}$ is partly contained in $\overline{T_{EXT}}$. We have demonstrated the validity of our MOC-observing approach and described previously unobserved basic characteristics of the MOC variability near 26.5°N in the Atlantic after 1 year of continuous observations.

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Supporting Online Material

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Reduced Egg Investment Can Conceal Helper Effects in Cooperatively Breeding Birds

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Cooperative breeding systems are characterized by nonbreeding helpers that assist breeders in offspring care. However, the benefits to offspring of being fed by parents and helpers in cooperatively breeding birds can be difficult to detect. We offer experimental evidence that helper effects can be obscured by an undocumented maternal tactic. In superb fairy-wrens (*Malurus cyaneus*), mothers breeding in the presence of helpers lay smaller eggs of lower nutritional content that produce lighter chicks, as compared with those laying eggs in the absence of helpers. Helpers compensate fully for such reductions in investment and allow mothers to benefit through increased survival to the next breeding season. We suggest that failure to consider maternal egg-investment strategies can lead to underestimation of the force of selection acting on helping in avian cooperative breeders.

In cooperative breeding systems, offspring receive food from helpers in addition to their parents. Although parents can reduce the

rate at which they feed their offspring in the presence of helpers, this reduction is usually incomplete, and so offspring receive more food when helpers are present than when they are absent (1). Given that offspring receive more food when also provisioned by helpers, it is currently unclear why many long-term studies have failed to detect helper effects on offspring growth and survival (2) or have detected only weak effects (3). This failure to document the benefits to offspring has prompted hypotheses proposing that helping behavior is an unselected consequence of physiological priming to provide care to begging offspring (4), is contingent on future reciprocity (5), or is a form of “rent payment” (6). These alternatives are problematic because helping has been shown to be costly (7) and strategically

directed to maximize benefits (3, 8), cooperative breeding based on direct reciprocity is inherently unstable (9), and rent payment occurs under conditions that will be seldom met (10).

In cooperative breeding systems, securing and maintaining a breeding position is particularly challenging but offers substantial fitness benefits (11). Consequently, breeders might be expected to be under strong selection to reduce their investment in each reproductive attempt in order to increase the number of attempts that they can have in a lifetime. For example, one of the most commonly reported helper effects in avian cooperative breeding systems is load lightening, where breeding females reduce offspring provisioning with increasing helper numbers (1). Load lightening could also occur at the egg stage (12). However, despite growing evidence from noncooperative species that female birds can adaptively manipulate investment within eggs (13), this possibility has not been explored in cooperatively breeding species. Furthermore, hypotheses regarding such adaptive maternal egg investment typically predict that mothers should increase their level of within-egg investment when breeding in favorable conditions (14, 15). Yet it is also theoretically plausible that mothers breeding in privileged circumstances (in this case, with the benefit of helpers) might also benefit from reductions in egg investment, if the future fitness payoffs from doing so exceed the current fitness payoffs from increasing egg investment. If mothers reduce their investment in eggs when breeding in the presence of helpers, then any benefit that helpers might have on offspring condition and survival will be masked.

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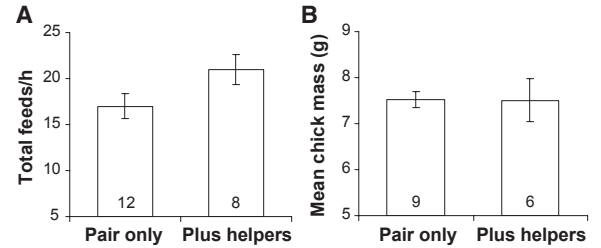
We tested (i) whether helper presence is associated with reductions in maternal investment at the egg stage, (ii) whether these reductions confound estimates of helper contributions to nestling mass, and (iii) whether maternal reductions are adaptive. We investigated these questions in the cooperatively breeding superb fairy-wren *Malurus cyaneus*, a passerine bird endemic to southeastern Australia, whose social mating system comprises both breeding pairs and cooperatively breeding groups (16). Previous work on this species has shown that nestlings receive more food in the presence of helpers (17), but despite this pattern, helpers have no effect on the mass or survival of chicks (18). The same was true in our study population (16). Chicks that were provisioned by a breeding pair with helpers received 19% more food than did those that were tended by an unassisted breeding pair (Fig. 1A). Nevertheless, chicks reared by groups were of similar mass to those reared by pairs alone (Fig. 1B). There were no obvious differences in prey loads or species fed to chicks in pairs and groups (19).

Consequently, we investigated whether mothers reduced their investment in eggs when breeding in groups. Clutch size and mean egg volumes were uncorrelated [linear mixed-effect model (LME) controlling for repeated measures within territories: $\chi_1^2 = 0.01$, $P = 0.91$, $N = 68$ clutches], and clutches were similar in size in both groups and pairs [LME, $\chi_1^2 = 0.29$, $P = 0.59$, $N = 68$ clutches]. In contrast, mothers breeding in groups laid eggs that were 5.3% smaller than eggs from those breeding in pairs (16) (Fig. 2A). Eggs laid by females in groups were also of lower nutritional content than those laid by females in pairs (16). Helpers were associated with a 14% reduction in the wet mass of yolks (Fig. 2B) and a 9% reduction in the dry mass of yolks (Fig. 2C), indicating that the reduction in egg volume was not simply due to reductions in albumen or water content. Furthermore, the dried yolks of eggs laid by females in groups had 12% less lipid and 13% less protein than did those laid by females breeding in pairs (Fig. 2D), suggesting that mothers invest less energy in their eggs when breeding in the presence of helpers.

Further analysis of our observational data suggests that this reduced maternal investment at the egg stage conceals helper effects on chick growth (16). First, small eggs gave rise to small chicks (measured 6 to 8 days after hatching) (Fig. 3A). Second, after controlling for the differences in egg volumes between groups and pairs (Fig. 2A), we found that helpers had a significant positive effect on chick mass (Fig. 3B). The magnitude of this latter helper effect equates to an 18% increase in chick mass, suggestively close to the 19% increase in food that chicks received when provisioned by helpers (Fig. 1A).

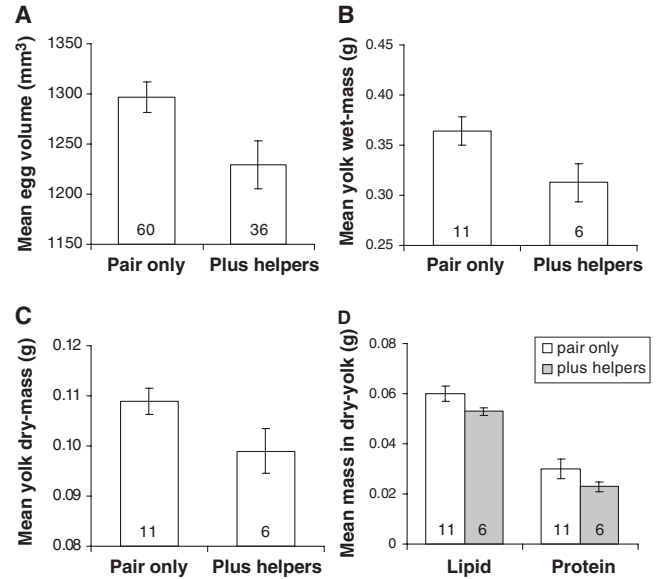
To test fully the possibility that reduced maternal investment obscures helper effects, we conducted a cross-fostering experiment involv-

ing permanent reciprocal translocation of complete clutches between females before hatching (16). There were two experimental treatments: (i) one in which clutches were moved from nests of pairs to nests attended by groups and vice versa (experimental treatment) and (ii) one in which clutches were swapped between nests attended by the same number of adults (control treatment). First, if mothers reduce their egg investment in the presence of helpers, we would predict that chicks from eggs laid in groups and reared in pairs (grp-pr, Fig. 3C) would be significantly lighter than either control chicks or those laid in pairs and reared in groups (pr-grp, Fig. 3C). In support of this prediction, chicks arising from eggs that were laid in groups and reared in pairs were significantly lighter than those from the other two treatments (Fig. 3C). Second, our cross-fostering experiment also confirmed that maternal reductions in egg investment wholly conceal helper effects. We found that the mean mass of nestlings in the foster territory declined as the number of males in the natal territory increased (prenatal-effect size = -0.54 ± 0.18) (Fig. 3D). However, this negative effect was completely offset by the positive influence of the provisioning rate of foster males (postnatal-



effect size = 0.36 ± 0.15) (Fig. 3E), which was itself significantly influenced by the number of foster males contributing (Fig. 3F). Taken together, these results confirm that provisioning frequency is a reliable indicator of chick caloric intake and that the benefits to chicks of receiving food from helpers are concealed by reductions in egg investment by females with helpers.

Given that, in other cooperative breeding systems, offspring of low mass are shown to have reduced chances of recruitment (20) and low future reproductive success (21), why do females breeding in the presence of helpers lay eggs that yield suboptimal offspring? We know that the durations of incubation and chick rearing are inflexible in superb fairy-wrens and so are uninfluenced by helper presence (16, 18). One possibility is that mothers are constrained from optimal allocation of resources into eggs because of competition with helpers for food in their territory. Alternatively, females breeding in groups might exploit helper contributions to nestling mass by reducing their own investment in reproduction to save resources for future breeding attempts. The latter explanation is more strongly supported by our evidence.



the year was fitted as a covariate in (A), and the lay date was fitted as a covariate in (A) to (D). The predicted means \pm SEM (error bars) are shown. The values in each bar are the numbers of independent breeding units.

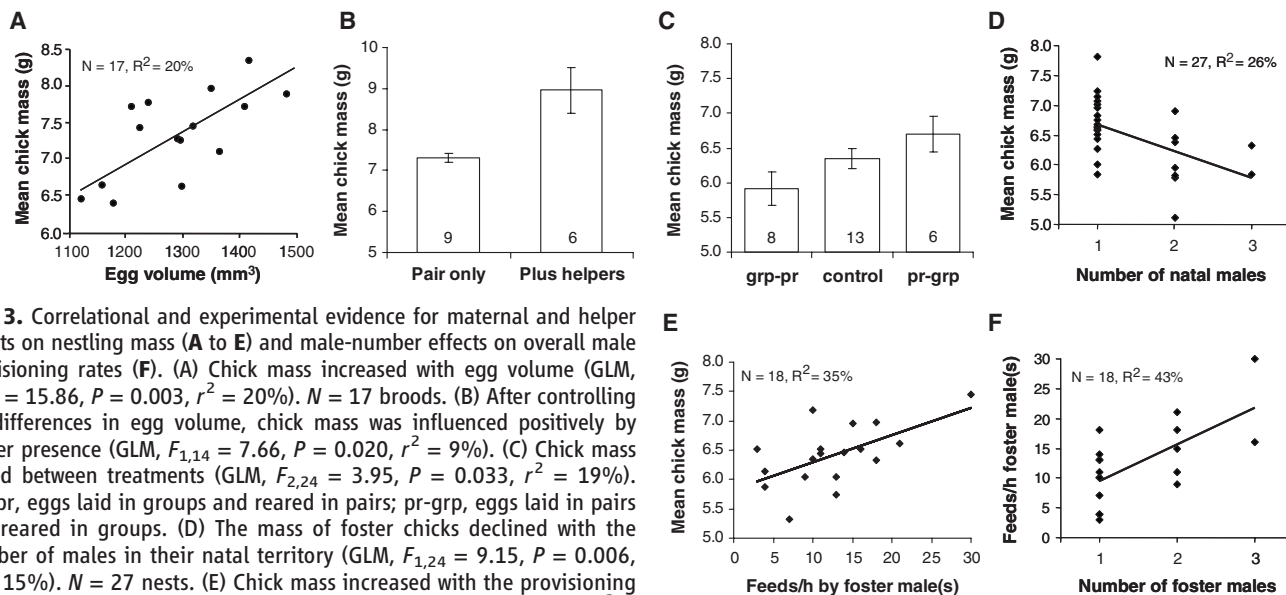


Fig. 3. Correlational and experimental evidence for maternal and helper effects on nestling mass (**A** to **E**) and male-number effects on overall male provisioning rates (**F**). (**A**) Chick mass increased with egg volume (GLM, $F_{1,14} = 15.86$, $P = 0.003$, $r^2 = 20\%$). $N = 17$ broods. (**B**) After controlling for differences in egg volume, chick mass was influenced positively by helper presence (GLM, $F_{1,14} = 7.66$, $P = 0.020$, $r^2 = 9\%$). (**C**) Chick mass varied between treatments (GLM, $F_{2,24} = 3.95$, $P = 0.033$, $r^2 = 19\%$). grp-pr, eggs laid in groups and reared in pairs; pr-grp, eggs laid in pairs and reared in groups. (**D**) The mass of foster chicks declined with the number of males in their natal territory (GLM, $F_{1,24} = 9.15$, $P = 0.006$, $r^2 = 15\%$). $N = 27$ nests. (**E**) Chick mass increased with the provisioning rate of males in their foster territory (GLM, $F_{1,14} = 5.59$, $P = 0.033$, $r^2 = 10\%$). $N = 18$ nests. (**F**) The rate at which chicks received food from foster males increased with the number of foster males (regression, $F_{1,16} = 12.28$, $P = 0.003$, $r^2 = 40\%$). $N = 18$ nests. The tarsus length [(**A**) and (**C** to **E**)], lay-date (**A**), mean egg volume (**B**), and number of males in the

natal territory (**E**) were fitted as covariates. The predicted means \pm SEM (error bars) are shown. The scatter plots show the predicted values, and the values in each bar in (**B**) and (**C**) are the numbers of independent breeding units.

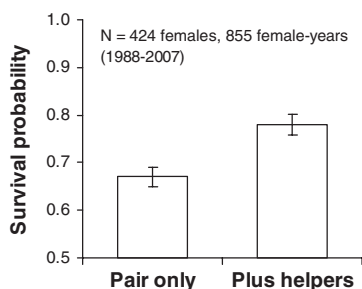


Fig. 4. Relation between helper presence and female survival probability. Females had an increased probability of survival to the following breeding season when they bred in the presence of helpers [GLMM, $\chi^2_1 = 12.13$, $P < 0.001$]. Age and spring rainfall were fitted as covariates, and maternal identity was fitted as a random factor. The predicted means \pm SEM (error bars) are shown.

First, females presented with experimentally enlarged broods increased their provisioning frequency to a similar extent irrespective of whether they were in groups or pairs (16), indicating that extra group members do not constrain females from increasing investment. Second, analysis of data from the 19-year study of an unmanipulated neighboring population (16) revealed that when females bred in the presence of helpers, they had a 22% hazard or mortality until the following year, but when they bred in the absence of helpers, this risk increased to 33%. Helpers are therefore associated with a 30% reduction in mortality risk for mothers (Fig. 4). Relations between helper numbers and adult survival could be spurious, because territories that promote survival are likely to accumulate philopatric helpers (1, 2).

However, we found no evidence that breeding males survive better in the presence of helpers [generalized linear mixed model (GLMM), $\chi^2_1 = 0.02$, $P = 0.89$], suggesting that the effect of helpers on females is causal and operates, in part, through a path primarily accessible to females—in this case, strategic reductions in egg investment.

We have shown that in superb fairy-wrens, mothers reduce egg investment when breeding in the presence of helpers and that the subsequent undernourishment of the young at hatching wholly conceals the positive effect of helper contributions to nestling mass. The critical factor that will select for maternal reductions in egg investment in a cooperative bird is a predictable workforce to assist in provisioning young, for this will allow females to make informed decisions at the egg-laying stage concerning how much food their chicks will receive after hatching (22). This is true of most cooperative birds: The number of helpers present from the onset of egg laying accurately reflects the number of helpers available to feed the offspring after hatching (23). We therefore predict that load lightening of maternal investment at the egg stage will be a general phenomenon in cooperative birds, as is the case with maternal load lightening at the chick-provisioning stage (1, 2). We conclude that all studies conducted on species in which helper numbers are predictable from the onset of breeding have the potential to underestimate the contributions made by helpers to nestling condition and/or maternal survival. Such studies will overlook the significant benefits that helpers stand to gain from breeding cooperatively through kin selection if helpers are related to breeders (24) or group augmentation if they are not (25).

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Supporting Online Material

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A Whole-Genome Association Study of Major Determinants for Host Control of HIV-1

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Understanding why some people establish and maintain effective control of HIV-1 and others do not is a priority in the effort to develop new treatments for HIV/AIDS. Using a whole-genome association strategy, we identified polymorphisms that explain nearly 15% of the variation among individuals in viral load during the asymptomatic set-point period of infection. One of these is found within an endogenous retroviral element and is associated with major histocompatibility allele *human leukocyte antigen (HLA)-B*5701*, whereas a second is located near the *HLA-C* gene. An additional analysis of the time to HIV disease progression implicated two genes, one of which encodes an RNA polymerase I subunit. These findings emphasize the importance of studying human genetic variation as a guide to combating infectious agents.

Humans show remarkable variation in vulnerability to infection by HIV-1 and especially in the clinical outcome after infection. One striking and largely unexplained difference is the level of circulating virus in the plasma during the nonsymptomatic phase preceding the progression to AIDS. This is known as the viral set point and can vary among individuals by as much as 4 to 5 logs (*1–6*). We aimed to identify human genetic differences that influence this variation.

To define a homogeneous phenotype for genetic analyses, a consortium of nine cohorts was formed [termed Euro-CHAVI (Center for HIV/AIDS Vaccine Immunology) (*7*)], and a total of 30,000 patients were screened to identify those most appropriate for analysis. All longitudinal viral-load (VL) data were assessed through a computerized algorithm to eliminate VL not reflecting the steady state and were individually inspected by an experienced infectious-disease clinician (Fellay) to exclude suspicious VL data and patients that do not show a clear set point, leaving 486 patients with a consistent and accurately measured phenotype (*7*). For patients with at least four CD4 cell-count results, we defined a progression phenotype as the time to treatment initiation or to the predicted or observed drop of the CD4 cell count below 350 (*7, 8*).

All samples were genotyped with the use of Illumina's HumanHap550 BeadChip with 555,352 single-nucleotide polymorphisms (SNPs). A series

of quality-control steps resulted in the elimination of 20,251 polymorphisms (*7*). We applied methods to identify deletions and targeted copy-number variations and to assess whether they influenced the phenotype (*7*). Our core association analyses focused on single-marker genotype-trend tests of the quality control-passed SNPs, using linear regression (*7*). To control for the possibility of spurious associations resulting from population stratification, we used a modified EIGENSTRAT method (*7, 9*). We assessed significance with a Bonferroni correction (P cutoff = 9.3×10^{-8}). Analyses incorporating *human leukocyte antigen (HLA)* typing were carried out on a subgroup of 187 patients with available four-digit *HLA* class I allelic determination.

These analyses identified two independently acting groups of polymorphisms, associated with *HLA* loci *B* and *C*, that are estimated to explain 9.6 and 6.5% of the total variation in HIV-1 set point, respectively, and can thus be considered as major genetic determinants of viral set point. A third set located >1 Mb away in the major histocompatibility complex upstream of a gene that encodes an RNA polymerase I subunit explains 5.8% of the total variation in disease progression. Together, the three polymorphisms explain 14.1% of the variation in HIV-1 set point.

One polymorphism located in the *HLA* complex *P5 (HCP5)* gene explains 9.6% of the total variation in set point, despite a minor-allele frequency of 0.05 (Single Nucleotide Polymor-

phism database number rs2395029, $P = 9.36 \times 10^{-12}$). A single copy of the controlling allele was found to result in a reduction in VL of >1 log (Fig. 1); at $P = 9.36 \times 10^{-12}$, this genome-wide association is significant.

The *HCP5* gene is located 100 kb centromeric from *HLA-B* on chromosome 6 (Fig. 2), and the associated variant is known to be in high linkage disequilibrium (LD) with the *HLA* allele *B*5701 (10)* ($r^2 = 1$ in our data set). This allele itself has the strongest-described protective impact on HIV-1 disease progression (*11*) and has been associated with low VL (*12*).

Given the strong functional data supporting a role for *HLA-B*5701* in restricting HIV-1, our first hypothesis is that the association observed

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