



Cost of Female Intrasexual Aggression in Terms of Offspring Quality: A Cross-Fostering Study

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Abstract

Growing evidence that female ornaments and armaments may be important for female reproductive success suggests that a reevaluation of the costs of these potentially sexually selected traits is also necessary. Here, I examine whether intrasexual aggression, a trait favored during direct female–female competition for nesting sites in tree swallows (*Tachycineta bicolor*), is costly in terms of the quantity or quality of offspring. I compared measures of female aggressiveness to clutch size, and I also cross-fostered offspring just after hatching to explore a possible causal link between female aggression and nestling mass, an established proxy for offspring quality. High levels of aggression in females were not associated with the quantity of offspring, but instead more aggressive females had offspring of lower quality. While several causal factors appear to influence offspring quality, the mechanism most consistent with this cost of aggression is a trade-off between female aggression and aspects of maternal care. Site differences may create variation in how selection shapes female aggression, but the finding that more aggressive females had lower-quality control offspring indicates that this cost may work counter to selection favoring aggressive behavior in the context of competition over nestboxes.

Introduction

Aggression is a key component of intrasexual competition in both sexes; however, our understanding of the relative costs and benefits of this behavior is heavily biased toward males (Andersson 1994; Searcy & Nowicki 2005). In spite of the assumption that high levels of intrasexual female aggression are costly to females, there is little empirical evidence quantifying the fitness costs of female aggressiveness, with a few exceptions. Experimental elevation of testosterone suggests several potential costs (Ketterson et al. 2005), including delayed breeding (Clotfelter et al. 2004), decreased parental care (O'Neal et al. 2008), or reduced fecundity (Rutkowska et al. 2005); however, these manipulations do not necessarily reflect the magnitude of these costs in nature.

A rare example of natural variation in the costs of female aggressive behavior comes from research on the white-throated sparrow (*Zonotrichia albicollis*), where females of the more aggressive white-striped morph provide less parental care than females of the less aggressive tan-striped morph (Knapton & Falls 1983; Kopachena & Falls 1993). To my knowledge, however, no study has directly measured the costs of female–female aggression in terms of natural variation in female reproductive success.

Here, I investigate the cost of natural levels of intrasexual aggression in free-living female tree swallows (*Tachycineta bicolor*). Tree swallows are obligate secondary cavity nesters, and both sexes compete aggressively for access to limited nesting sites (Holroyd 1975; Chek & Robertson 1991; Robertson et al. 1992). With the high frequency of intruding female

floaters (Stutchbury & Robertson 1985, 1987) that occasionally kill residents during territorial challenges (Leffelaar & Robertson 1985), increased aggressiveness may be particularly advantageous in this system. Furthermore, highly aggressive females are more likely than less aggressive females to obtain a nesting cavity (Rosvall 2008), meaning increased aggression is favored during female–female competition for nestboxes, much like aggressiveness and dominance are favored in intrasexual competition among males (Andersson 1994). While more aggressive females are thus more likely to breed, the potential fitness costs of this behavior are not well understood.

In this study, I tested the hypothesis that females who are more aggressive experience a fitness cost in terms of the number or quality of offspring. First, I asked whether female aggressiveness predicts natural variation in offspring quantity by comparing female behavior to clutch size, a measure of fecundity. Next, I measured whether aggressiveness is associated with the quality of offspring produced, using a well-established index of offspring quality: nestling mass prior to fledging. Collectively, these two measures provide reliable proxies of quantity and quality, because they predict the number of offspring produced and the likelihood of those offspring to survive into the future, respectively (see Materials and Methods for details). I explore the causal link between female phenotype and offspring quality by cross-fostering offspring, swapping half-broods on the day after hatching to disentangle pre- and post-hatch influences on offspring quality. I combine these results with focal feeding observations, morphological data, and aggression scores obtained from genetic and foster mothers to determine how female aggressive behavior may influence the quantity or quality of offspring.

Materials and Methods

Studies were conducted in 2005 and 2006 using a population of tree swallows breeding in nestboxes near Linesville, PA (41°40' N, 80°26' W). The population consists of two primary sites: the Linesville State Fish Hatchery (hereafter "Hatchery") and Pennsylvania State Gamelands no. 214 (hereafter "Gamelands"). Approximately 40 pairs of tree swallows breed annually at each site. The two sites are separated by 3 km, but I consider them part of the same population because both juveniles and breeders regularly disperse between sites.

Early in the season, nests were checked at least every three days to determine clutch initiation date,

clutch size, and estimated hatch date. Nests were checked daily beginning the day before the estimated hatch date. Nearly all males and females breeding in the population were banded with one US Fish and Wildlife metal band and one plastic color band (red for females and blue for males), and they were marked with dabs of non-toxic acrylic paint on the wings and rump for individual identification (Dunn et al. 1994).

I focused on clutch size as a measure of the number of young. Clutch size is highly correlated with both brood size and number of young fledged in this population (Pearson correlation for $n = 65$ nests: *clutch* to *brood*: $r = 0.65$, $p < 0.0001$, *clutch* to *fledge*: $r = 0.55$, $p < 0.001$). Further, all nests were partially cross-fostered at the point of hatching, thus confounding any relationship between a female's level of aggression and the number of young fledged from a given nest, because half of those offspring were laid and incubated in a different nest.

I used offspring mass on day 12 post-hatch as a measure of offspring quality because offspring that develop more slowly, or those that are smaller prior to fledging, are considered lower quality in many songbird species (Starck & Ricklefs 1998), including tree swallows (McCarty 2001). Slower development and smaller mass have been linked with lower survival (Perrins 1965; Tinbergen & Boerlijst 1990; Magrath 1991; McCarty 2001; Schwagmeyer & Mock 2008), lower fecundity (Alatalo & Lundberg 1986; Haywood & Perrins 1992), reduced expression of sexually selected traits (Nowicki et al. 2002), and smaller adult body size (Richner 1992). While this proxy for quality does not identify the underlying causes of large mass (e.g. greater structural size and increased fat or muscles reserves), each of these potential sources of large mass is likely to be beneficial.

Because aspects of female size could influence offspring mass in a few ways, I collected morphological data from breeding females during banding, measuring mass to the nearest 0.1 g with a spring-loaded Pesola scale. Females who are structurally larger may have larger offspring, owing to heritability of size (Boag 1983; Alatalo & Lundberg 1986). Likewise, females who are in better condition may invest more in yolk or egg size, thus promoting offspring growth (Styrsky et al. 1999; Whittingham et al. 2007). While distinguishing among these possibilities is outside of the purview of this study, I controlled for the mass of genetic and foster mothers in the cross-fostering study to improve the power of the statistical models in predicting potential relationships between female aggressiveness and offspring mass.

Aggression Assay

All females were assayed for aggressiveness using a simulated territorial intrusion with a live subadult female decoy. Decoys were captured from at least 1 km away and placed in a cage (30 × 30 × 25 cm) mounted on a tripod situated 1.5 m in front of the focal female's nestbox. During each 5-min trial, I recorded aggressive behaviors that the female directed at the live decoy, namely diving at or hovering within 0.75 m of the cage (i.e. half the distance to the nestbox), perching on the cage, and attempted pecking at the decoy. Aggression scores were calculated by summing the number of 5-s intervals during which the focal female aggressively responded to the decoy (range: 0–60 intervals). In previous work, I have shown that this measure of aggression is highly repeatable among individuals and does not depend on the identity of the decoy ($r = 0.79$, Rosvall 2008). All assays were performed between 0600 and 1200 h during the nestling phase of breeding. Immediately after aggression trials, decoys were released at their capture site, where they resumed normal breeding activities. Decoy females and their offspring were not used in any other analyses in this study.

Cross-Fostering Design

To distinguish between pre-hatch and post-hatch influences on offspring mass, I cross-fostered half-broods the day after hatching ($n = 23$ nests in 2005 and $n = 42$ nests in 2006). For odd-numbered broods (i.e. five offspring), I selected either two or three offspring to swap. I made an effort to evenly divide the brood by offspring size on day 1 post-hatch, such that control (unswapped) and experimental (swapped) offspring were similar in starting mass at the time of swapping. Brood size was not manipulated. Broods were matched for cross-fostering if (1) the nests hatched on the same day and (2) the difference in brood size between nests was ≤ 1 . Half-broods were swapped between either two or three nests (i.e. either offspring from nest A and B were swapped reciprocally or offspring were swapped among three nests as follows: $A \rightarrow B \rightarrow C \rightarrow A$). Thus, all 65 nests contained both control and experimental chicks. To distinguish between control and experimental offspring, I either (1) marked the toes of control offspring with a permanent marker or (2) trimmed natal down into unique patterns that differed between treatment groups. These identifying features lasted long enough to reliably distinguish between control and experimental offspring until

they were large enough to be banded with a US Fish and Wildlife band (day 6–7 post-hatch). On day 12 post-hatch, all offspring were weighed to the nearest 0.1 g using a portable digital scale (Ohaus HH120). I averaged masses of control chicks within a nest to create one variable, and I averaged masses of experimental chicks within a nest to create a second variable. These two mass measures were used as the dependent variable in statistical models. While most tree swallows do not fledge for another 7–10 days after day 12 post-hatch, offspring are already at adult mass (offspring: 21.4 ± 0.1 g; breeding adults: 20.6 ± 0.2 g) and size hierarchies typically remain unchanged for at least a few days following day 12 (Ardia 2006). Therefore, I did not measure mass after this point to avoid causing premature fledging.

Focal Observations of Provisioning

Feeding observations were made on either day 8 or 9 post-hatch in 2006. Each feeding watch consisted of 60 min of uninterrupted observation (following Winkler & Allen 1995), beginning with the first female provisioning trip to the nestbox. Most feeding watches were performed from inside a parked vehicle, which acted as a blind. Any remaining feeding watches were performed from at least 30 m away, and the birds were given at least 30 min to acclimate to the presence of the observer before observation began. As a measure of maternal provisioning, I used the total number of female feeds performed per chick per hour, as well as the proportion of total feeds performed by the female. Previous work has demonstrated that measuring the number of parental visits to the nest during feeding is an accurate measure of the amount of food delivered by parents (McCarty 2002) and that parental provisioning predicts aspects of nestling quality and growth in tree swallows (Quinney et al. 1986; Ardia 2007).

Statistical Methods

All statistical analyses were carried out using JMP 9.0 (SAS Institute, Cary, NC, USA). The relationship between female aggression and clutch size was evaluated using a least-squares linear regression. I examined the relationship between female aggression and female mass using a multiple regression that included female age and relative hatch date as covariates, and the interaction between hatch date and female mass. In all models, aggression scores were square-root transformed to achieve normality.

Next, I used restricted maximum likelihood (ReML) nested linear mixed models to examine the effects of several variables on offspring mass in the cross-fostering experiment. I averaged the masses for all natal offspring within a nest and again for all fostered offspring within a nest, and I used these two averages (one for control offspring and another for experimental offspring) as the dependent variable in all ReML models ($n = 115$ half-broods). The original models included independent variables central to the hypothesis that female aggression influences offspring mass (aggression scores of genetic and foster mothers), as well as several other variables with the potential to influence offspring mass, including mass of genetic and foster mothers, relative hatch date, brood size, female age, nest of rearing, nest of origin, chick treatment (cross-fostered versus control), year, and site of rearing. Nestbox of origin was nested within box of rearing, and, together with box of origin, year and site of rearing were entered as random effects, with all other variables entered as fixed effects. To test whether cross-fostering itself influences offspring mass, I also added treatment group as a fixed effect in the original model.

I focused on two particular terms in the statistical models. First, to test the critical prediction that aggressiveness is associated with a cost in terms of offspring quality, the key statistical test is whether there is a relationship between female aggression and offspring mass. To test the second critical prediction – that aggressiveness predicts offspring mass differentially in control and experimental offspring – the key statistical test is whether there is an interaction between treatment group and aggression score of either mother. This statistical interaction would indicate that cross-fostering at the point of hatching changed the effect of female aggressiveness on offspring mass, thus disentangling the contribution of pre- and post-hatch factors on offspring mass. In cases where the interaction terms were significant, but singular terms were not meaningful, I excluded those singular effects from the models, thus eliminating their contribution to the least-squares mean, following Howell (2009) and Ratner (2003). Thus, chick fostering status was excluded as a main effect in all models, even when interactions were included, because preliminary analysis showed that cross-fostered offspring were not significantly different in mass from offspring that remained in their natal nest (control offspring mass = 21.4 ± 0.2 g, experimental offspring mass = 21.5 ± 0.2 g, $t = -0.06$, $n = 124$ half-broods, $p = 0.54$).

Preliminary models also showed no detectable relationships between either female aggression or offspring mass and brood size, female age, and relative hatch date. Thus, with the exception of relative hatch date, I excluded these terms from the candidate models, to prevent overfitting the data (Burnham & Anderson 2002). Because hatch date showed a marginal interaction with one of the independent variables (female mass), hatch date was included as a covariate in all ReML models to control for minor influences of date on other predictor variables.

Finally, identical variables from both genetic and foster mothers were not included within the same models, to avoid issues of collinearity (Johnson & Wichern 2007). For example, mass of rearing mother and mass of genetic mother are the same exact values for half of the data points (i.e. control offspring are also reared by their genetic mother), and so including both variables within the same model creates statistical redundancies that yield unreliable estimates of the collinear parameters. Fortunately, multi-model averaging allows for this approach, because the relative weight of each parameter is determined by the weight of models in which that parameter is present, not the co-occurrence of each parameter within the same models (Burnham & Anderson 2002).

This process yielded 18 possible models, which were then ranked according to corrected Akaike information criterion (AICc), where the model with the lowest AICc suggests the best fit (Burnham & Anderson 2002). Because multiple models demonstrated a good fit (defined conservatively as $\Delta\text{AICc} < 5$), I used Akaike model weights and parameter weights to evaluate the relative importance of various models and independent variables. Because models with $\Delta\text{AICc} > 10$ have effectively no support (Burnham & Anderson 2002), I limited my analyses of parameter weights to only models with $\Delta\text{AICc} < 10$. From these seven models, I calculated Akaike model weights (w) to determine the relative support for each model. Values of w range from 0 to 1, with those closer to 1 indicating that the model is a better fit to the data. To determine the relative explanatory power of each variable, I calculated parameter weights as the sum of Akaike weights from each model in which the variable was found. Therefore, variables found in all models have a parameter weight of 1, whereas variables found only in one model have the weight of only that model.

Next, I used multi-model averaging based upon the confidence set of candidate models (i.e. those models with $w \geq 10\%$ of the top model weight; listed in Table 1). This process summarizes parameter

Table 1: Confidence set of candidate models

Parameter	Model A		Model B		Model C		Model D		Model E	
	F ratio	p								
Aggression (genetic mother)							4.45	0.039	4.41	0.040
Aggression (rearing mother)	7.20	0.0087	6.11	0.015	7.19	0.0087				
Mass (genetic mother)	6.35	0.014	6.29	0.015	5.09	0.027	9.26	0.0033	9.39	0.0031
Aggression (genetic) × treatment							10.8	0.0018	8.98	0.0041
Aggression (rearing) × treatment	4.52	0.037	4.44	0.039						
Mass (genetic mother) × treatment			0.26	0.61					0.888	0.35
Relative hatch date	1.11	0.30	1.13	0.29	1.27	0.26	1.93	0.17	2.00	0.16
R^2_{adj}		0.795		0.796		0.738		0.794		0.808
AICc		425.75		427.03		427.15		429.30		429.89
$\Delta AICc$		0		1.28		1.40		3.55		4.14
Akaike weight (w) ^a		0.431		0.227		0.214		0.073		0.054

AICc, Akaike information criterion.

^aAkaike model weights shown for the candidate set of models (all models with weights $\geq 10\%$ of the best fit model). All models also include nestbox of rearing [nestbox of origin], nestbox of origin, year, and site as random effects.

estimates and errors for each variable, weighed by the model-averaged Akaike weights. I report model-averaged unconditional parameter estimates, standard errors, and 95% confidence intervals based upon this composite model (Burnham & Anderson 2002). Finally, to visually represent the bivariate relationships within these models, I plot leverage values for each key variable, which correct for other significant variables within each treatment group (Sall 1990).

To characterize the nature of the relationship between female aggressiveness and provisioning parameters, I first used an ANCOVA to test for a significant interaction based on site. Next, I used Spearman rank correlations to characterize the rank-order relationship between aggressive and provisioning behaviors separately at each site. Samples sizes for some analyses differ from the original 65 nests that were cross-fostered for the following reasons: (1) nest failure because of predation or other unknown reasons, (2) feeding observations were conducted only in 2006, and (3) not all females were captured for morphological measurements.

Results

I found no detectable relationship between female aggression and clutch size (Fig. 1, linear regression: $R^2_{adj} = -0.0096$, $F = 0.44$, $n = 63$ nests, $p = 0.51$). A *post hoc* power analysis revealed that a sample size of 524 nests would be required for $\alpha = 0.05$ (raw effect size (δ) = 0.069, power = 0.10), suggesting that this finding is unlikely to be a result of type II error.

Female aggression positively covaried with female mass (Fig. 2; whole model, predicting female aggress-

sion: $R^2 = 0.13$, $F = 3.32$, $p = 0.016$; female mass: $F = 6.63$, $p = 0.013$; relative hatch date: $F = 1.08$, $p = 0.30$; female age: $F = 2.17$, $p = 0.15$; female mass × relative hatch date: $F = 3.00$, $p = 0.09$), demonstrating that females that are more aggressive are generally heavier than less aggressive females. Neither relative hatch date nor age had a significant effect on this relationship, although there was a marginal effect of the interaction between hatch date and female mass, reflecting that larger, more aggressive females tend to breed slightly earlier in the season.

The model selection process indicated multiple models that were a good fit for offspring mass (Table 1). Parameter weights demonstrate a strong negative effect of the rearing mother’s aggressiveness on offspring mass ($w = 0.857$) and a strong positive effect of the genetic mother’s mass on offspring

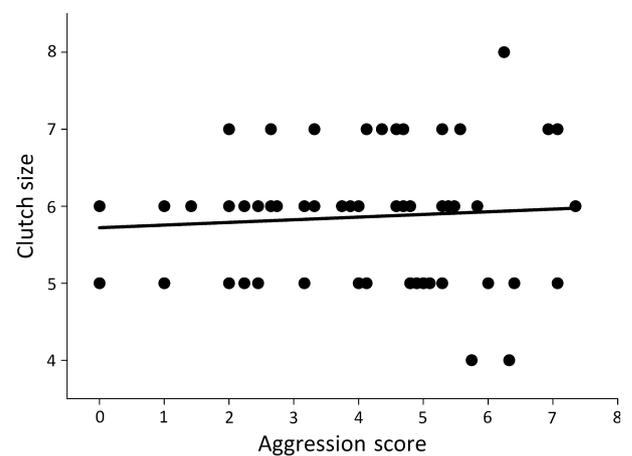


Fig. 1: Linear regression of clutch size on aggression score. Aggression scores were square-root transformed to achieve normality.

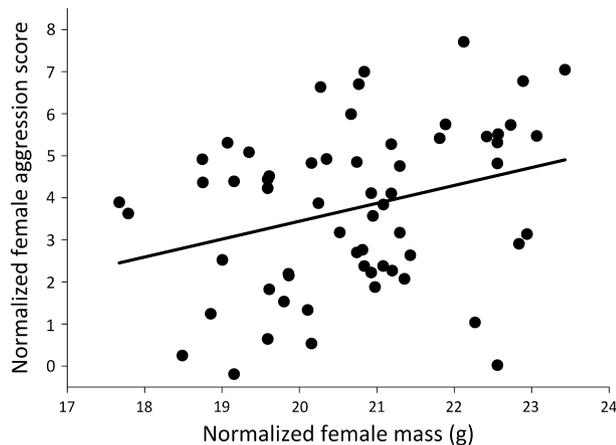


Fig. 2: Visual representation of the bivariate relationship between female aggression score (square-root transformed) and female body mass. Values presented have been normalized to correct for the other predictor variables in the multiple regression (female age, relative hatch date, and the interaction between mass and hatch date).

mass ($w = 0.982$; see Table 2 of parameter weights). Because the genetic and rearing mothers are one and the same for control chicks, the interaction between each of these variables and chick treatment group is also revealing. Parameter weights indicate relatively strong support for the interaction between chick treatment group and the aggressiveness of the rearing mother as well ($w = 0.647$), indicating that the extent of covariation between female aggressiveness and offspring mass varied between control and experimental chicks.

Model averaging produced a composite model in which the aggressiveness of each mother, mass of the genetic mother, and interactions between mother's

Table 2: Summary of parameter weights (w) for variables of interest, rank ordered. w can range from 0 to 1, with parameters closer to 1 being found in more models, and models with more support. Parameter weights were calculated from all models with some amount of support ($\Delta AICc \leq 10$). Note that relative hatch date was also included in all models as a covariate (hence, $w = 1$), but it did not significantly predict offspring mass in any models (see also Table 3)

Parameter	w
Mass (genetic mother)	0.982
Aggression (rearing mother)	0.857
Aggression (rearing) \times treatment	0.647
Mass (genetic) \times treatment	0.277
Aggression (genetic mother)	0.143
Aggression (genetic) \times treatment	0.143
Mass (rearing mother)	0.018
Mass (rearing) \times treatment	0.006

AICc, Akaike information criterion.

aggressiveness and chick treatment group all predict variance in nestling mass (Table 3). The composite model also included relative hatch date and the interaction between the genetic mother's mass and treatment; however, neither of these last two variables explained significant variance in offspring mass. Nestlings that were larger (i.e. higher quality) had larger but less aggressive mothers. Because more aggressive females were also larger than less aggressive females (Fig. 2), these findings together suggest that there is a cost of being aggressive, manifest as smaller offspring mass, but this cost is partly mitigated by a positive effect of female mass on offspring mass. The interaction between female aggressiveness and chick treatment group indicates that the relationship between female aggressiveness and offspring mass differs in control (unswapped) and experimental (cross-fostered) offspring, meaning that this cost of aggression varied in the two treatment groups.

Visual examination of the data (Fig. 3) clarifies the statistical patterns captured by the parameter weights and composite model. Females that were more aggressive have smaller control offspring, and heavier females have heavier control chicks. Thus, in a

Table 3: Model-averaged parameter estimates, unconditional standard error, and 95% confidence intervals for each fixed effect. The composite model therefore was: Offspring mass = $21.27 - 0.77 \times \text{Aggression (genetic)} - 0.78 \times \text{Aggression (rearing)} + 3.33 \times \text{Mass (genetic)} + 0.68 \times \text{Aggression (genetic)} \times \text{treatment} + 0.55 \times \text{Aggression (rearing)} \times \text{treatment} + 0.39 \times \text{Mass (genetic)} \times \text{treatment} + 0.50 \times \text{Hatch Date} + \text{Error}$. The models also include nestbox of rearing [nestbox of origin], nestbox of origin, year, and site as random effects

Parameter	Model-averaged parameter estimate	Unconditional SE	Upper 95	Lower 95
Intercept ^a	21.27	0.46	22.18	20.36
Aggression (genetic mother) ^a	-0.77	0.36	-0.05	-1.48
Aggression (rearing mother) ^a	-0.78	0.30	-0.20	-1.36
Mass (genetic mother) ^a	3.33	1.35	5.98	0.68
Mass (rearing mother) ^b				
Aggression (genetic) \times treatment ^a	0.68	0.22	1.11	0.26
Aggression (rearing) \times treatment ^a	0.55	0.26	1.05	0.04
Mass (rearing) \times treatment ^b				
Mass (genetic) \times treatment	0.39	0.66	1.69	-0.91
Relative hatch date	0.50	0.45	1.37	-0.38

Estimates for which 95% confidence intervals do not cross zero are considered to be strong effects, marked with (^a). Not all variables of interest were included in the confidence set of candidate models marked with (^b), and thus, they are likewise not present in the composite model.

natural setting (i.e. control offspring), larger females produce larger, higher-quality chicks (Fig. 3a), but more aggressive females produce smaller, lower-quality chicks (Fig. 3b). In experimental offspring, however, the strength of these correlations breaks down. For chicks that were cross-fostered, the mass of the genetic mother predicts offspring quality: larger females still bear larger, higher-quality chicks, even if those offspring are reared by a different mother (Fig. 3c). While the composite model demonstrates a statistical effect of each mother's aggressiveness on chick mass, the interactions of these parameters with chick treatment group demonstrate that these relationships are significantly stronger in one treatment group than the other. Visual inspection of these bivariate relationships suggests that any relationship between the aggressiveness of either genetic or foster mother on nestling mass in experimental nestlings is weak (Fig. 3d,e), but this relationship is strong in control nestlings (Fig. 3b) that were reared by their genetic mother.

Focal feeding observations indicated that the relationship between female aggressive and provisioning behaviors varies between the two study sites (ANCOVA: $R_{\text{adj}}^2 = 0.24$, $n = 36$ nests, site: $F = 3.16$, $p = 0.085$; proportion of feeds: $F = 1.51$, $p = 0.23$; site \times prop. of feeds: $F = 8.19$, $p = 0.0074$). At the Gamelands, more aggressive females perform significantly lower percent of the feeding visits to chicks than do less aggressive mothers ($r_s = -0.51$, $n = 22$ nests, $p = 0.016$) and feed marginally less per chick per hour (Fig. 4; $r_s = -0.39$, $n = 22$ nests, $p = 0.075$). At the Hatchery, the trend was in the opposite direction, although not significantly so ($n = 14$ nests, proportion of feeds: $r_s = 0.50$, $p = 0.069$; female feeds per chick per hour: $r_s = 0.35$, $p = 0.22$; Fig. 4). A *post hoc* Levene's test revealed that the variance in the number of female feeds per chick per hour is significantly higher at the Gamelands than at the Hatchery ($F = 5.94$, $p = 0.020$), suggesting that detecting these behavioral correlations may be statistically more difficult at the Hatchery site.

Discussion

Cost of Aggression: Quality, Not Quantity, of Offspring

Taken together, these results indicate a cost of high levels of aggressive behavior in terms of an established measure of quality in control offspring: more aggressive females naturally have smaller offspring relative to less aggressive females. I did not find a relationship

between female aggression and clutch size, indicating that more aggressive females do not have smaller offspring as a by-product of having a large brood. Given the strong correlations between clutch size and the number of young fledged, the lack of a relationship between female aggressiveness and clutch size suggests that highly aggressive females do not suffer a cost in terms of the number of young produced; rather, these costs are only seen in terms of offspring quality, and they are most striking in control offspring instead of experimental (cross-fostered) offspring.

A negative association between female aggressiveness and nestling mass likely represents a reproductive cost of aggression. Nestling birds may have reduced mass because of the number of environmental and genetic factors (e.g. depressed growth rates, lower fat reserves, and smaller structural size). While this study does not distinguish among these possibilities, each is likely to influence the future success of those offspring because offspring that are smaller, or those that develop more slowly, have been shown to experience a suite of fitness costs in a number of avian species, including tree swallows. Smaller nestlings are less likely to survive (Tinbergen & Boerlijst 1990; Gebhardt-Henrich & Richner 1998), and they may become smaller adults (Richner 1992), resulting in negative effects on behavior or life history (Starck & Ricklefs 1998). Furthermore, females that are smaller as offspring may be less fecund as adults (Alatalo & Lundberg 1986; Haywood & Perrins 1992) and smaller male offspring may have reduced expression of sexually selected traits as adults (reviewed in Searcy & Nowicki 2005). While the proximate causes of increased growth or size in songbirds are not always easy to predict, the general effect that larger mass or faster growth predicts success later in life remains relatively robust in many species (Nowicki et al. 1998; Starck & Ricklefs 1998), including this species (McCarty 2001). Accordingly, the finding that more aggressive females have offspring that are smaller on day 12 post-hatch constitutes a probable fitness cost of aggressive behavior. Notably, this cost of aggression is significant despite likely short-term fluctuations in offspring mass because of a recent feeding or defecation.

Insights into the Mechanisms of the Cost of Aggression

The costs of aggression varied depending on the mother's mass and whether or not a chick was cross-fostered. These patterns point to possible mechanisms by which this cost of aggression may operate. Model

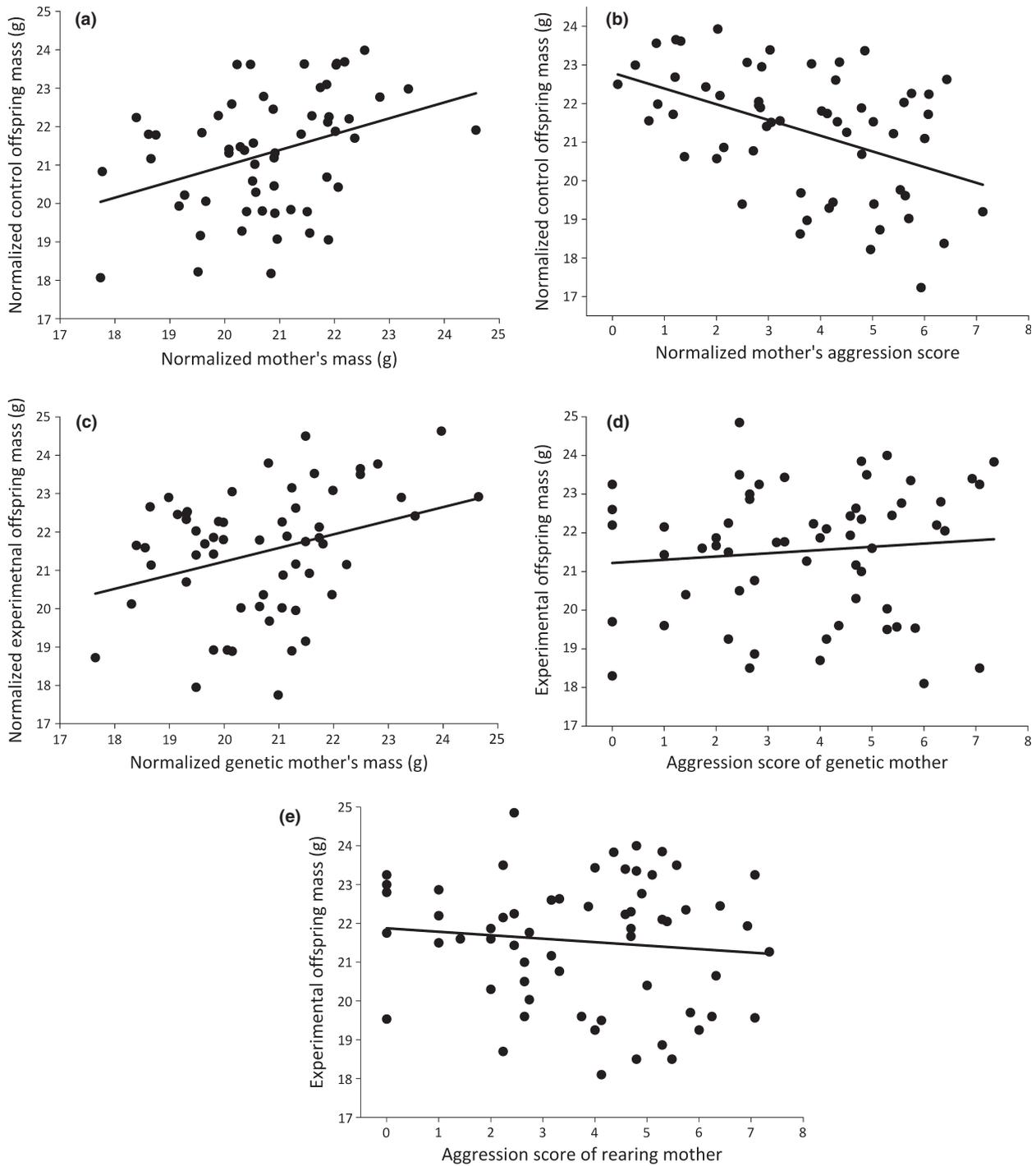


Fig. 3: Visual representation of bivariate relationships from the mixed models, showing the relationship between control offspring mass and (a) mother's mass and (b) mother's aggressiveness, and the relationships between experimental (cross-fostered) offspring mass and (c) genetic mother's mass, (d) genetic mother's aggressiveness, and (e) rearing mother's aggressiveness. Note that variables that significantly predict offspring mass within each treatment group (a, b, and c) are adjusted using leverage plots to control for other variables in the model, while uncorrected, raw values are shown for non-significant variables (d and e). See Tables 1, 2, and 3 for the detailed statistics describing these relationships.

averaging and parameter weights showed that offspring were larger if their genetic mother was larger, consistent with heritability of size (Boag 1983).

On the other hand, larger females were, on average, more aggressive than smaller females, meaning that more aggressive females have smaller (control) off-

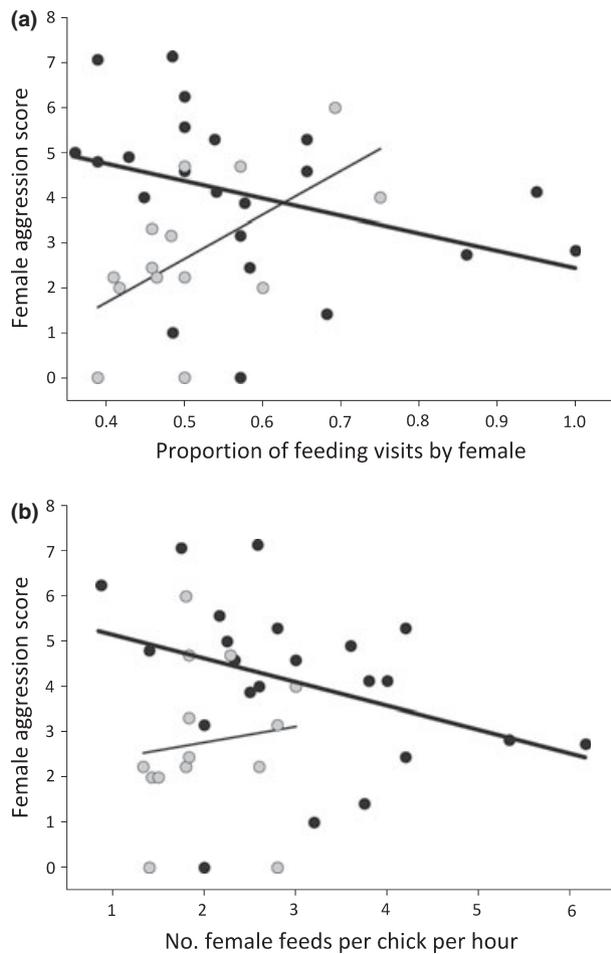


Fig. 4: Spearman rank correlations between aggressive behavior and two measures of female provisioning: (a) the proportion of feeding visits performed by females and (b) the number of female feeds per chick per hour. Females at the Gamelands site are shown in black circles with a heavy line, and females at the Hatchery site are shown in gray circles with a light line. Aggression scores were square-root transformed to achieve normality.

spring in spite of their mother's large size. Stated another way, the cost of aggression is more severe for females with reduced mass than for females with greater mass. It is not clear exactly how this compensatory effect of female mass may reduce the negative effect of female aggression, but heritability of size, or other maternal effects associated with larger females may contribute and should be explored in the future (e.g. larger yolks producing offspring with larger mass, Williams 1994; Styrsky et al. 1999; Christians 2002; Whittingham et al. 2007). The finding that more aggressive females are relatively larger, yet their unmanipulated offspring are smaller, minimizes the likelihood that heritable aspects of female size explain

this cost of aggression (e.g. if more aggressive females had been smaller). Instead, this cost is more likely to be mediated by a maternal or environmental effect.

The interaction between chick treatment group and female aggressiveness, which indicates that the cost of aggression differs between control and cross-fostered chicks, also provides a window into the possible mechanisms mediating this cost of aggression. Because control and experimental offspring only differ in whether or not they were reared (post-hatch) by their genetic mothers, this interaction helps to disentangle pre- and post-hatch influences on offspring mass. The negative effect of female aggressiveness on offspring mass (i.e. the cost of aggression) was stronger in control offspring than in experimental offspring (Fig. 3, in combination with treatment \times aggression interactions in Table 3). Thus, whatever pre-hatch and post-hatch mechanisms link female aggressiveness with smaller offspring must typically work in a concerted manner (i.e. in control offspring). However, when decoupled by cross-fostering at the point of hatching (i.e. in experimental offspring), there is no longer a detectable cost of aggression in terms of offspring quality. Cross-fostering appears to have masked the negative relationship between female aggression and offspring mass that is observed in control offspring (contrast Fig. 3b with Fig. 3d,e), meaning that the cost of aggression is primarily evident when offspring are both conceived and fully reared by an aggressive female.

What might explain different relationship between female aggressiveness and offspring mass in the two treatment groups? If aggressive behavior is negatively associated with both pre- and post-hatch maternal care, experimentally swapping offspring after hatching may decouple mechanisms that normally work together to cause more aggressive females to have poorer-quality offspring. While not tested here, a likely *pre-hatch* mechanism that may partly account for this cost of aggression is a trade-off between female incubation and aggressive behaviors. When induced to be more aggressive because of experimental testosterone elevation, female tree swallows do incubate less, significantly decreasing nest temperatures (Rosvall, in preparation), and reduced nest temperatures are known to impede offspring growth and development (Winkler 1993; Perez et al. 2008). Evidence supporting a *post-hatch* behavioral trade-off comes from the Gamelands site, where more aggressive females provision offspring relatively less (Fig. 4). Previous work on tree swallows suggests that parental feeding is the largest predictor of offspring growth (Quinney et al. 1986; McCarty 2002), and so a nega-

tive relationship between female aggression and provisioning may well explain the reproductive cost of aggression observed here. Moreover, this result agrees with several correlative and experimental studies demonstrating a negative relationship between aggression and provisioning in male and female songbirds (Wingfield et al. 1987, 2001; Ketterson et al. 1992, 2005; Tuttle 2003; Møller et al. 2005; Duckworth 2006).

If this cost were completely mediated by a trade-off between aggressiveness and *post-hatch* maternal care, then experimental chicks would also have shown decreased mass if they were reared by an aggressive mother; however, this was not the case (Fig. 3e). Likewise, if the cost of aggression were mediated only by *pre-hatch* factors, then experimental chicks would have shown lower mass if their genetic mothers were highly aggressive, regardless of the aggressiveness of their foster mothers; this prediction also was not supported (Fig. 3d). Instead, the cost of aggression was primarily seen in control chicks, suggesting that both pre- and post-hatch behavioral trade-offs likely account for some degree of the measured cost of aggression.

An essential caveat to this interpretation is that the trade-off hypothesis is not supported at the Hatchery site. Because the two sites in this study are linked by gene flow, with juveniles and breeders dispersing between sites (Rosvall, pers. obs.), local genotypic adaptation is unlikely to create different behavioral correlations at the two sites (e.g. Lande & Arnold 1983). Instead, variable behavioral correlations are more likely to be caused by either phenotypic plasticity or different norms of reaction in the two sites (Carroll & Corneli 1999; Thompson 1999). Different relationships between female aggression and provisioning may have arisen if females adjust either of these behaviors in response to ecological conditions that differ among the sites, such as the potential for polygyny (Dubois et al. 2006), the density of competitors (Magurran & Seghers 1994), or the availability of food (Ardia 2006; Bretagnolle et al. 2008). While I have not directly tested these alternatives, the two sites do differ in nestbox density (distance to nearest neighbor, Gamelands: 180 ± 9 m, Hatchery: 56 ± 2 m), amount of water in the habitat (Hatchery groundcover is mostly water, while the Gamelands is mostly agricultural fields), and variance in feeding rates (see Results).

Regardless of its cause, variation in behavioral trade-offs may dampen the strength of selection (Grant & Grant 2002; Dingemanse et al. 2004; Chaine & Lyon 2008; Gosden & Svensson 2008). If

aggressive behavior is associated with poor-quality offspring, as I have shown here in control offspring, and females vary in the extent to which aggressiveness is negatively associated with provisioning those young, the costs of aggression may vary geographically or ecologically, thus slowing the overall effect of these costs on the evolution of behavior.

Implications for the Evolution of Intrasexual Aggression in Females

The results presented here demonstrate a fitness cost associated with female aggression in control offspring; more aggressive female tree swallows appear to suffer a cost in terms of offspring quality in natural situations (i.e. in control offspring), but they do not appear to suffer a cost in terms of reduced number of offspring. Although the mechanism by which this cost operates is complex and probably multidimensional, behavioral trade-offs with maternal incubation and provisioning behavior are consistent with these patterns. Finding a reproductive cost associated with female intrasexual aggression agrees with previous work demonstrating that male sexual traits, including ornaments, weaponry, and aggressive displays, are costly to produce or maintain (reviewed in Andersson 1994; Searcy & Nowicki 2005). The costs of female–female aggressive behavior have rarely been explored, despite the prevalence of intrasexual aggression in females (e.g. Slagsvold & Lifjeld 1994; Wolff & Peterson 1998). The study described here is unique in quantifying the magnitude of these costs in a natural setting in females, and it provides the first evidence that more aggressive females suffer a cost in terms of offspring quality.

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