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Animal behaviour

The couple that sings together stays together: duetting, aggression and extra-pair paternity in a promiscuous bird species

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When individuals mate outside the pair bond, males should employ behaviours such as aggression or vocal displays (e.g. duetting) that help assure paternity of the offspring they care for. We tested whether male paternity was associated with aggression or duetting in the red-backed fairy-wren, a species exhibiting high rates of extra-pair paternity. During simulated territorial intrusions, aggression and duetting were variable among and repeatable within males, suggesting behavioural consistency of individuals. Males with quicker and stronger duet responses were cuckolded less often than males with slower and weaker responses. In contrast, physical aggression was not correlated with male paternity. These results suggest that either acoustic mate guarding or male–female vocal negotiations via duetting lead to increased paternity assurance, whereas physical aggression does not.

1. Introduction

The majority of socially monogamous bird species exhibit extra-pair mating [1]. In species with paternal care, males should evolve behaviours that help assure paternity of offspring produced by their social mates [2]. In territorial species, aggression in response to intruders may serve to repel rivals [3] and decrease the likelihood that the male is cuckolded [4]. Duetting, the synchronous combination of male and female vocalizations [5], may also decrease extra-pair mating by signalling commitment between pair members or by directly guarding against extra-pair copulations [6–8]. However, the relative influence of aggression and vocal displays on paternity has never been experimentally tested and is key to understanding the evolution of promiscuity.

We tested whether behavioural responses to simulated territorial intrusions were correlated with genetic paternity in a population of red-backed fairy-wrens (*Malurus melanocephalus*), an Australian passerine. Males foray onto neighbouring territories pursuing extra-pair females [9,10], eliciting a response from the resident male including physical aggression and song. We used artificial mounts and song playback to simulate intrusions and recorded the aggressive and duetting responses of territory holders. We predicted a negative association between the proportion of extra-pair young (EPY) in a male's nest and either aggression, duetting or both, depending on which behaviours affect paternity allocation.

2. Material and methods

(a) Simulated territorial intrusions

We conducted these experiments in Samsonvale, Queensland (GPS = 27°16' S, 152°51' E). We presented an artificial mount and song playback on territories

Table 1. Two PCAs used to quantify aggression and duetting. A dash indicates that the behaviour was not included in that PCA.

	Aggression PC1	Duet PC1
eigenvalue	6.72	2.45
per cent variation	67.2	81.5
time spent within 10 m	0.35	—
time spent within 5 m	0.37	—
time spent in mount bush	0.36	—
time spent within 0.5 m	0.33	—
time spent attacking mount	0.19	—
latency to 10 m	−0.26	—
latency to 5 m	−0.31	—
latency to mount bush	−0.35	—
latency to 0.5 m	−0.34	—
latency to attack mount	−0.27	—
total duets	—	0.56
ratio duets : solos	—	0.59
latency to duet	—	−0.58

and video recorded the behaviour of the territorial pair (for details, see [11] and the electronic supplementary material). We reviewed the videos and quantified attacks, songs and latency to/duration of approach to various distances (table 1). Because there are two subspecies that differ in plumage (red versus orange) and song [11–14], we presented six different treatments representing combinations of local, foreign and heterospecific plumage and song. We used principal components analysis (PCA) of two separate subsets of behaviours to create one composite score representing physical aggression towards the mount ('Aggression PC1') and one representing duetting behaviour ('Duet PC1', table 1). Red-backed fairy-wrens are facultative cooperative breeders, but we only tested older males (i.e. after-second-year) exhibiting orange–black nuptial plumage, with no auxiliaries.

(b) Repeatability of aggression and duetting

We used three subsets of the data to analyse repeatability across multiple trials to confirm that behaviours were representative of a natural response and to determine temporal consistency. Because the PCA data were non-Gaussian, we calculated repeatability using an overdispersed binomial generalized linear mixed model in the R v. 3.1.1 [15] package *rptR* [16], followed by randomization tests. First, we chose the subset of males that received all six treatments ($N = 44$ males) and calculated repeatability in both aggression and duetting. Then, because previous analyses revealed that response was strongest to the local song type but equivalent between red and orange mount colour [11], we calculated repeatability in aggression again on the subset of males that received local song with red or orange mount ($N = 51$ males). Finally, we estimated repeatability in duetting again, using this same restricted subset, plus the female present ($N = 18$ males). In summary, we estimated repeatability of aggression and duetting twice: once with a large dataset including responses to all six trials, and once with a narrower subset including only the two trials eliciting the strongest responses most representative of a typical territorial intrusion.

(c) Aggression, duetting and cuckoldry

We assessed paternity using seven microsatellite loci ([17], electronic supplementary material). To explore relationships between aggression, duetting, and cuckoldry, we ran two binomial generalized linear models with logit-link functions using the R v. 3.1.1 [15] package *lme4*. For aggression, proportion of EPY in a male's nest was the response variable, and average Aggression PC1 and trial date were fixed effects. This model used the two-trial dataset for males with at least one nest ($N = 35$). For duetting, proportion of EPY in a male's nest was the response variable, and average Duet PC1 and trial date were fixed effects. This model used the two-trial, female-present dataset for males with at least one nest ($N = 13$).

3. Results

We assigned genetic paternity to 97% (181/186) of offspring, and the five unassigned offspring were confirmed EPY due to low assignment probability with their social father. Forty-seven per cent of offspring (88/186) were EPY, and 60% (44/73) of nests contained at least one EPY.

Both male aggression and duetting behaviour were significantly repeatable across the six- and two-trial datasets (table 2). Both behaviours were marginally, but non-significantly, more repeatable when analysed across the two trials expected to elicit strong, comparable responses.

There was a significant negative relationship between Duet PC1 and proportion of EPY in a male's nest (estimate = -4.44 , $z = -2.37$, $p = 0.018$, figure 1a). In contrast, there was no significant association between Aggression PC1 and proportion of EPY in a male's nest (estimate = -0.08 , $z = -0.11$, $p = 0.913$, figure 1b). Trial date had no significant effect on either model (duet model: estimate = 0.04 , $z = 0.83$, $p = 0.406$; aggression model: estimate = -0.01 , $z = -0.60$, $p = 0.547$).

4. Discussion

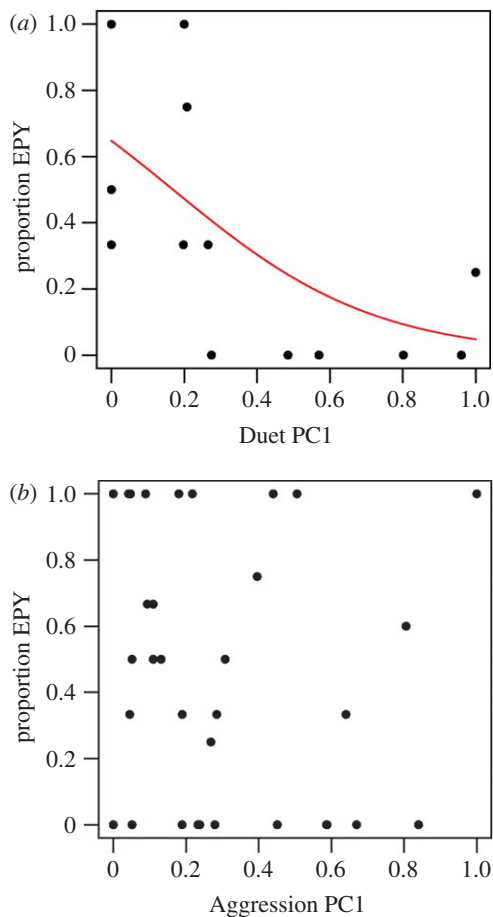
When presented with simulated territorial intrusions, male red-backed fairy-wrens responded with physical aggression and duetting, both of which were repeatable across variable contexts, including heterospecific mounts and songs. This pattern suggests that although males modify their aggressive and vocal responses to some extent, there are consistent among-individual differences in both of these behaviours [18]. Here, we examined the hypothesis that they are a behavioural strategy to increase paternity assurance.

Aggression was not significantly associated with cuckoldry (figure 1b), suggesting that aggressive males were not particularly successful at deterring rivals from copulating with their mates. This pattern could arise if extra-pair copulations are initiated by females off-territory or occur pre-dawn [19,20]. Aggression may be more effective at defending resources on the territory rather than mates or may maintain territory boundaries.

In contrast, males with a fast and strong duet response were cuckolded significantly less often by their social mates (figure 1a). There are several reasons a strong duet response could relate to high paternity assurance. First, if males acoustically mate guard, those with stronger duet responses may more effectively mask their female's acoustic signal that would otherwise attract rivals [6–8]. Because we did not have data on duet initiation, we could not determine if females initiated

Table 2. Repeatability of aggression and duetting in both the six-trial (all combinations of mount and song) and two-trial (red or orange mount and local song) datasets.

behaviour	dataset	N (males)	R	95% CI	p
Aggression PC1	six-trial	44	0.29	0.09–0.44	0.001
Aggression PC1	two-trial	51	0.32	0.00–0.61	0.001
Duet PC1	six-trial	44	0.24	0.00–0.47	0.001
Duet PC1	two-trial	18	0.29	0.00–0.72	0.03

**Figure 1.** Relationships between duetting (a), aggression (b) and proportion of EPY in a male's nest. *x*-values were scaled 0–1 for visualization only; scaling did not transform the data. (Online version in colour.)

duets that were joined by males, as would be predicted by this hypothesis. Nonetheless, previous work on another population of red-backed fairy-wrens failed to demonstrate acoustic mate guarding [21,22]. Another possibility is that duetting is a

general 'keep out' signal produced by the pair to prevent usurpation of a partner or pair bond position [23]. However, previous work suggested that duetting functions mostly to establish and maintain the territory rather than deter intruding males *per se* [21]. Finally, duetting may strengthen the pair bond, especially if the female joins the male's song to signal her commitment [24–26]. These hypotheses are not mutually exclusive, and duetting may serve multiple functions. A forthcoming study of male versus female duet initiation/joining and paternity will help distinguish among these hypotheses. In red-backed fairy-wrens, there is support for territory establishment and defence [21,22], but the results presented here suggest that strong duetting response is also associated with increased paternity assurance. This pattern has been inferred previously from behavioural evidence or life-history trends [27–29], but this study is, we believe, the first to demonstrate a clear link based on within-population variance in duetting and genetic paternity. Finally, this result contrasts with a recent study on crimson-breasted shrikes that found a positive relationship between duetting and cuckoldry [30], further supporting multiple functions of duetting across species.

Ethics. We secured approval from animal ethics and permitting agencies (Cornell IACUC no. 0105, James Cook University Ethics no. A1340, Queensland SPP no. WISP07773610).

Data accessibility. Data are available in the electronic supplementary material.

Authors' contributions. All authors designed the study, revised and approved the manuscript, and are accountable for this work. D.T.B. and E.I.G. collected the data. D.T.B. analysed the data and wrote the manuscript.

Competing interests. We have no competing interests.

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