THE ROYAL SOCIETY

Received 24 July 2002
Accepted 15 August 2002
Published online 22 October 2002

Competition for male reproductive investment elevates testosterone levels in female dunnocks, *Prunella modularis*

N. E. Langmore[1*], J. F. Cockrem[2] and E. J. Candy[2]

[1]*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*
[2]*Conservation Endocrinology Research Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand*

In many songbirds, females occasionally sing in contexts of high female–female competition. Testosterone may be involved in the activation of song, because testosterone implants elicit female song in many species with rare female song. A possible mechanism for the hormonal control of female song is provided by the challenge hypothesis, which predicts a rise in testosterone in response to aggressive interactions during socially unstable situations. We tested this by comparing faecal testosterone levels in polygynandrous and monogamous female dunnocks. In groups with two to three females (polygynandry and polygyny) males provide less help at each nest than in groups with a single female (monogamy and polyandry). Polygynandrous and polygynous females are aggressive towards one another and attempt to expel rivals. Polygynandrous females had significantly higher testosterone levels than monogamous females. Competition between females that was induced by removal of males caused testosterone levels to rise. Further, female testosterone levels were correlated with the rate of 'tseep' calls, which are produced during aggressive encounters between females. Finally, polygynandrous and polygynous females sang significantly more than monogamous females. To the best of our knowledge, these results provide the first experimental support for the challenge hypothesis in female birds, and suggest that testosterone can regulate facultative female song in songbirds.

Keywords: challenge hypothesis; female–female competition; faecal steroids; testosterone; female song

1. INTRODUCTION

In many birds in which song was thought to be the exclusive preserve of males, females may also sing occasionally (Langmore 1998, 2000). The traditional explanation for occasional female song is that it is a functionless aberration caused by abnormally high androgen levels (Nice 1943; Kern & King 1972; Catchpole & Slater 1995). However, several experimental studies suggest that female song is a facultative trait that occurs under conditions of high female–female competition, particularly in polygynous and polygynandrous species where females may compete for mates or space (reviewed in Langmore 1998). This raises the question of how female song is controlled at the hormonal level; how are females able to 'switch on' song in the appropriate context? One possibility is that aggressive interactions between females cause testosterone levels to rise, which in turn trigger song (Arcese *et al.* 1988; Langmore & Davies 1997).

It is widely known that testosterone increases aggressive behaviour in male vertebrates (Wingfield *et al.* 1990). However, Wingfield (1985) and Wingfield *et al.* (1990) have demonstrated that the causal arrow can also go the other way; in contexts of social instability, territorial aggression between males elevates testosterone levels. This, in turn, increases the frequency and intensity of territorial aggression, creating a positive feedback loop (the challenge hypothesis). This mechanism ensures that testosterone levels increase in the appropriate context (e.g. a territorial challenge), but avoids the potential costs of sustaining high testosterone levels throughout the breeding season (Wingfield *et al.* 1990). The aim of our study was to test whether a similar mechanism occurs in competing female birds.

Few studies have investigated the hormonal control of territorial aggression in free-living females. In a number of species, levels of plasma testosterone were higher in females during times of the year or breeding contexts when they were most aggressive towards other females (western gulls, *Larus occidentalis*: Wingfield *et al.* (1982); spotted sandpipers, *Actitis macularia*: Fivizzani & Oring (1986); European robins, *Erithacus rubecula*: Schwabl (1992); red-winged blackbirds, *Agelaius phoeniceus*: Cristol & Johnsen (1994); moorhens, *Gallinula chloropus*: Eens & Pinxten (2000)). However, Elekonich & Wingfield (2000) found that levels of circulating androgens were no higher in female song sparrows, *Melospiza melodia*, that experienced a simulated intrusion than in control females.

If aggression does elevate testosterone in females, it is plausible that it could also activate female song. Like male songbirds, females have testosterone receptors in the song nuclei of the brain (Brenowitz & Kroodsma 1996), and in several species in which female song is rare, treatment with testosterone induces song (e.g. white-crowned sparrows, *Zonotrichia leucophrys*: Kern & King (1972); canaries, *Serinus canaries*: DeVoogd & Nottebohm (1981); song sparrows: Marler & Peters cited in Arcese *et al.* (1988); starlings *Sturnus vulgaris*: Hausberger *et al.* (1996)) and

*Author and address for correspondence: School of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia (naomi.langmore@anu.edu.au).

can increase the size of the vocal control regions of the brain (e.g. Nottebohm 1980; Brenowitz & Arnold 1990).

We tested whether competition between female dunnocks elevates testosterone, thereby providing a mechanism for the activation of female song. Females may breed in monogamy or polyandry (one or two males defend the territory of one female) and rarely interact with other females, or in polygyny or polygynandry (one or two males defend two to three female territories), in which females are aggressive towards one another and compete for male attention (Davies 1992; Langmore & Davies 1997). Males apportion paternal care between females in relation to the amount of mating access they have obtained, so fertile females compete for paternity to ensure male reproductive investment (Davies 1992). Competition may be direct, in the form of aggressive 'tseep' calls and wing waving displays, which may escalate into chasing and prolonged physical grappling on the ground (Langmore & Davies 1997). Aggression between females can lead to nest desertion, and as a result polygynandrous females have a higher rate of nest failure than monogamous females (Davies 1992). Females also compete indirectly, by using 'trill' calls to attract their mate. Female call rates are higher when alone than when accompanied by a mate-guarding male, and competing females call significantly more than non-competing females (Langmore & Davies 1997). Competing females also occasionally sing at times when their mate has left them to accompany a rival female (Langmore & Davies 1997).

We used non-invasive faecal sampling to test whether aggressive encounters between polygynandrous females cause testosterone levels to increase relative to monogamous females. To control for the possibility that any hormonal differences between polygynandrous and monogamous females were pre-existing, we also experimentally induced female–female competition by temporarily removing males, and compared female testosterone levels during male removals with testosterone levels of the same females at the same breeding stage in non-manipulated groups.

2. MATERIAL AND METHODS

(a) *Behavioural observations*

The study was conducted in the Cambridge University Botanic Garden, Cambridge, UK, from January to mid-June in 1998 and 1999. In January and February dunnocks were colour-ringed and ground-level feeders were established to facilitate locating and observing individuals. Feeders were each supplied with half a cup of Haith's Softbill Mix every day. In January and February 1999, 100 dunnock droppings were collected for use as validation samples for the faecal testosterone radioimmunoassay. From the beginning of March to mid-June, female vocalizations and breeding behaviour were monitored by focal watches on most days (minimum 10 min duration, 230 focal watches). Females were followed for as long as possible (mean + s.e. = 35 + 2 min) and every minute we recorded whether her mate was within 5 m (mate-guarding males usually remain within 5 m; Davies (1992)), and the number of trills, tseeps and songs produced by the female. Any droppings produced by the female in the course of the focal watch were collected immediately, placed in a cool storeroom and frozen at −20 °C within 3 hours. Use of faecal samples rather than blood samples is non-invasive, so hormone profiles are not altered by the stress of capture and handling, and individuals can be sampled repeatedly on a daily basis throughout the breeding cycle (e.g. Cockrem & Rounce 1995).

The breeding cycle of each female was divided into four stages.

(i) 'Pre-breeding' was the period from 1 March until nest building began. During this stage males associate closely with the resident female/s and chase them continuously for periods of up to 10 min around the territory (Davies 1992).
(ii) The fertile period was from the start of nest building until incubation commenced. Throughout this stage females solicit copulations and males mate-guard females closely (Davies 1992).
(iii) 'Incubation' was from the start of incubation until the day before the chicks hatched. Males cease associating with females at this stage.
(iv) 'Chicks' was the period during which the parents provisioned their young and was from the day the chicks hatched until they reached independence, or until the female began building her next nest, whichever came first.

(b) *Male removals*

Males were removed temporarily (2–4 days) to induce competition between females. Competition between females is most intense when they are repeatedly left alone by the male(s) during the pre-breeding and fertile periods (Langmore & Davies 1997). This occurs when another fertile or pre-breeding female attracts the males, or because they are helping another female to feed dependent young. Females are more likely to be left alone if they belong to a group with a female-biased sex ratio. Therefore, we experimentally increased competition between females in two ways; either by removing subordinate males from polygynandrous groups ($N = 4$), or by removal of the mate of monogamous females, inducing the males in a neighbouring group to encompass the female's territory ($N = 1$). Removals were timed so that the competing females were either pre-breeding or fertile. The birds were caught with mist-nets under approval from the Home Office Animals (Scientific Procedures) Act 1986 (ref. PCD 80/2802) and housed alone in a 60 cm × 45 cm × 30 cm cage in a quiet room. They were provided with Haith's Softbill Mix *ad libitum*, water, and a small container of mealworms daily. The first four removed males resumed their position as subordinate males after release and remained on their territories for the duration of the field season. The fifth individual died in captivity of unknown causes, so removal experiments were discontinued thereafter.

(c) *Steroid hormone extraction*

(i) *Faecal sample extraction*

Faecal samples were freeze-dried, transported to the assay laboratory and stored at room temperature. They were ground by hand to a fine powder using a mortar and pestle and 0.01 g of powder was transferred into glass tubes. Ethanol was added (2.5 ml of 90%) (Analar, BDH), the tubes shaken on an orbital shaker for 1 hour, vortexed briefly and then centrifuged for 20 min at 1900 *g*. The supernatants were pipetted into a second tube. A further 1.25 ml of 90% ethanol were added to the pellet, which was vortexed for 60 s, and centrifuged for 20 min at 1900 *g*. The supernatant was added to that from the previous

spin. The ethanol extracts were dried under a stream of air in a heating block at 37 °C, and reconstituted in 500 µl of phosphate-buffered saline with gelatine (PBSG; 0.1 M, pH 7.0). The faecal extracts were then extracted again by adding 5.00 ml of dichloromethane (Analar, BDH). The tubes were vortexed for 30 s, shaken on an orbital shaker for 1 hour and centrifuged for 20 min at 1900 *g*. A 4.50 ml aliquot of dichloromethane was carefully removed, dried under a stream of air in a heating block at 37 °C and the extract reconstituted in 500 µl of PBSG.

(ii) *Recovery of steroid in the faecal buffer extraction process*

The recovery of testosterone following extraction was measured in 9–11 faecal samples in each batch of extractions ($N = 5$) by adding 100 µl of tritiated testosterone (^{3}H-testosterone, Amersham, UK; 5000 cpm) in 90% ethanol at the start of the extraction. At the end of the extraction process 100 µl of the reconstituted extract in PBSG were placed in a scintillation vial. Scintillant (3 ml) (5 g l^{-1} of PPO (2,3-diphenyloxazole, Sigma), 0.3 g l^{-1} of dimethyl POPOP (1,4-bis-[methyl-5-phenyl-2-oxazoly]-benzene, Sigma) in toluene) was added to each vial, the vials were shaken for 1 hour on an orbital shaker, stabilized at room temperature for 1 hour, and counted in a Wallac 1409-411 liquid scintillation counter for 5 min each. The mean recoveries of testosterone in each batch of extractions ranged from 65.8 ± 2.4% to 73.9 ± 1.9%, and the overall mean recovery was 70.1 ± 1.1% ($N = 46$).

(iii) *Calculation of faecal hormone results*

Faecal testosterone concentrations were measured in diluted buffer extracts. Buffer had been added to the faecal samples in proportion to their dry weight, so the hormone concentrations in the buffer were directly proportional to the amount of steroid per unit dry weight of the original faecal sample. The raw assay results were therefore converted to give final values as nanograms of steroid per gram dry weight of faecal sample. The conversion included multiplication factors for the dilution of the buffer and a correction factor for the recovery of labelled steroid in the sample extraction procedure.

(iv) *Radioimmunoassay of testosterone*

Testosterone levels in faecal extracts were measured by radioimmunoassay. One hundred microlitres of diluted faecal extract in PBSG were incubated with 100 µl of antibody (Endocrine Sciences, CA, USA; testosterone antiserum T3-125) and 100 µl of tritiated testosterone (^{3}H-testosterone, Amersham, UK; 5000 cpm) overnight at 4 °C. A 500 µl aliquot of dextran-coated charcoal (2.5 g l^{-1} of charcoal (Sigma), 0.25 g l^{-1} of dextran (Dextran T70, Amersham Pharmacia) in PBSG) was added to each sample, and incubated for 15 min at 4 °C. Samples were then centrifuged at 2000 *g* for 15 min at 4 °C, the supernatant poured off and the vials counted by the same procedure as used for the measurement of extraction efficiencies.

The cross-reactivity of the testosterone antibody with other steroids was tested by Endocrine Sciences and reported as dihydrotestosterone (20%), corticosterone (less than 0.01%), oestradiol (0.14%), D-1-testosterone (52%), 4-androsten-3β-17β-diol (3%), 5α-androstan-3β-17β-diol (1.8%), D-4-androstenedione (0.5%), and others (less than 0.5%).

Serial dilutions of faecal extracts in PBSG were parallel to the testosterone standard curve. The quantitative recovery of testosterone in faecal extracts was measured by adding different amounts of standard testosterone to three faecal extracts in PBSG. The recoveries of added testosterone were 92.9 ± 3.2%, 106.4 ± 4.5% and 93.5 ± 6.1%. The sensitivity of the assay was determined as the hormone concentration at the mean − 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity, expressed as nanograms of steroid per gram dry weight of faecal sample, was 2.45 ng g^{-1}. The intra-assay coefficients of variation were 18.0%, 7.2%, and 3.2% and the inter-assay coefficients of variation were 14.4%, 10.7% and 9.0% for low, medium and high quality control solutions, respectively.

(d) *Statistical analyses*

To control for non-independence of multiple focal watches and multiple faecal samples from the same female, we used mixed models fitted with the restricted maximum-likelihood (REML) procedure (using Genstat 5 for Windows; Genstat Committee 1993). These models allow analysis of unbalanced data by incorporating random effects (in this case 'female' and 'year' as appropriate) as well as the fixed effects of interest. We initially fitted full models, including interaction terms, and progressively eliminated non-significant interactions, and then non-significant main effects, to derive a parsimonious model containing only significant terms. Female call data were binomially distributed with a high proportion of zero values, so we analysed variation in whether or not females called using the generalized linear mixed model (GLMM) procedure with a logit link function and female as a random variable (Genstat 5 for Windows). Testosterone data were log-transformed to normalize residuals. Residual and normal probability plots were used to check model assumptions.

3. RESULTS

A total of 195 faecal samples were obtained from 16 females. The mating system of some females changed during the course of the study owing to the arrival and departure of other males and females in their group. For at least a part of the study, 12 of these females were polygynandrous and 11 were monogamous. Faecal testosterone levels in female samples ranged from 2.45 (lowest detectable dose) to 17.77 ng g^{-1} ($N = 195$, mean + s.e. = 4.95 + 0.20 ng g^{-1}, median = 3.96 ng g^{-1}), compared with a range of 2.80–85.25 ng g^{-1} in male samples ($N = 132$, mean + s.e. = 19.48 + 1.23 ng g^{-1}; median = 15.28 ng g^{-1}; N. E. Langmore *et al.*, unpublished data). Testosterone levels did not vary significantly with respect to time of day ($\chi^2 = 4.45$, d.f. = 10, $p = 0.93$).

(a) *How does testosterone vary in relation to breeding stage and mating system?*

Testosterone levels were significantly higher during the fertile period than at other stages of the breeding cycle (figure 1; $\chi^2 = 60.56$, d.f. = 3, $p < 0.001$), and polygynandrous females had significantly higher testosterone levels than monogamous females (figure 1; $\chi^2 = 12.84$, d.f. = 1, $p < 0.001$). There was no significant effect of female or year, and no significant interaction between breeding stage and mating system ($\chi^2 = 2.5$, d.f. = 3, $p = 0.47$). Within the fertile period, polygynandrous females had significantly higher testosterone levels at times when they were competing (other female pre-breeding, fertile or chicks, $N = 10$) than when they were not competing (other female incubating, $N = 4$; unpaired *t*-test, $t = 3.03$, d.f. = 12, $p = 0.01$).

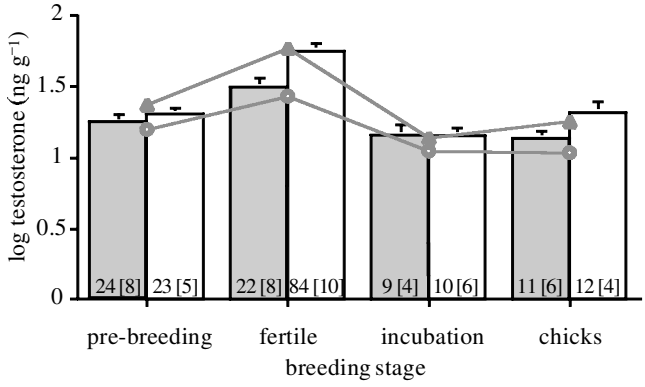

Figure 1. Mean + s.e. log testosterone levels of monogamous (filled bars) and polygynandrous (open bars) female dunnocks at each stage of the breeding cycle. Lines show REML predictions with female identity and year as random effects. Number of testosterone samples is shown at the base of the bars, with number of females in square brackets. Predicted monogamous represented by circles, predicted polygynandrous represented by triangles.

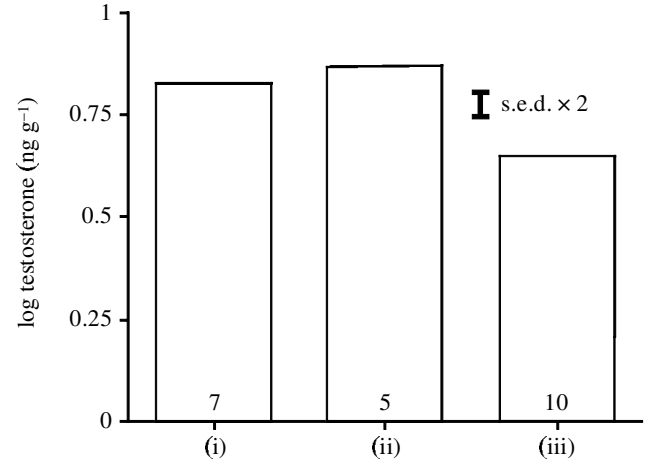

Figure 2. Predicted mean log testosterone levels of fertile female dunnocks that were: (i) competing in non-manipulated groups; (ii) competing following removal of a male, or (iii) not competing with other females. Number of females in each category is shown at the base of the bars. Error bar shows twice the average s.e. of the differences.

(b) *Does experimentally induced competition increase testosterone levels?*

Twenty-three faecal samples were obtained from six females after male removals created female-biased sex ratios in their groups. To test whether an experimental increase in female–female competition resulted in higher testosterone levels, we compared the mean testosterone levels of females following a male removal (experimental) with the mean testosterone levels of the same females at the same stage of breeding in non-manipulated groups (control). Five females were fertile during the removal and we also had faecal samples from four of these females when they were fertile but when no male had been removed (one in the same breeding attempt, three in both the same and different breeding attempts). We had no control samples for the fifth female, so she was excluded from the analysis. The sixth female was pre-breeding during the removal and we also had faecal samples when she was pre-breeding in the absence of a male removal (different breeding attempt). Males were removed in the evening, and the first faecal samples were collected from females the following morning at least 16 hours after the removal. The testosterone levels of these females were significantly higher following the male removals than in controls (Wilcoxon signed-rank test, $N = 5$, $z = -2.02$, $p = 0.04$).

We also tested whether testosterone levels differed during experimentally induced competition from when competition occurred naturally. We restricted the analysis to faecal samples collected during the fertile period to control for breeding stage. We compared the mean testosterone levels of fertile females that were: (i) not competing; (ii) competing following a male removal; and (iii) competing in non-manipulated groups. Testosterone levels were significantly higher in competing females than non-competing females (figure 2, $\chi^2 = 76.15$, d.f. = 2, $p < 0.001$), but there was no significant difference in testosterone levels between females experiencing experimentally induced competition and naturally occurring competition (figure 2).

(c) *Female song*

Three females sang during the study. As has been observed previously (Langmore & Davies 1997), these females all sang during the pre-breeding or fertile period at times when the males in the group were accompanying a rival female. Further, all songs occurred when a new female had just joined the group. One female produced 217 songs in the week after the males in her group encompassed a secondary female (665 min of focal watches), and the secondary female was also heard to sing once during this period (126 min of focal watches). Another primary female sang 12 songs over the five days following the arrival of a secondary female (313 min of focal watches) and she was also observed chasing and grappling with the secondary female during this period. Combining data on all females that were observed during the pre-breeding and fertile periods with those from an earlier part of the same study (Langmore & Davies 1997), the proportion of polygynous and polygynandrous females that sang (6/23) was significantly higher than the proportion of monogamous females that sang (0/17; Fisher's exact test, $p = 0.03$). The number of singing females was too small to test whether testosterone levels were significantly higher in females that sang than in females that did not sing.

(d) *Female calls*

We compared the mean testosterone level of each female, at each breeding stage, with her calling behaviour while alone (because females rarely call when accompanied by a mate-guarding male; Langmore & Davies (1997)). Females mainly called during the pre-breeding and fertile stages of the breeding cycle, so the analysis was restricted to these stages. Tseep calling was significantly more likely to occur at high testosterone levels (figure 3; logit [P tseeping] = (3.75 + 1.70 s.e.) ln testosterone, d.f. = 1, $p < 0.05$), whereas the probability of trill calling was not significantly related to testosterone level ($p > 0.2$).

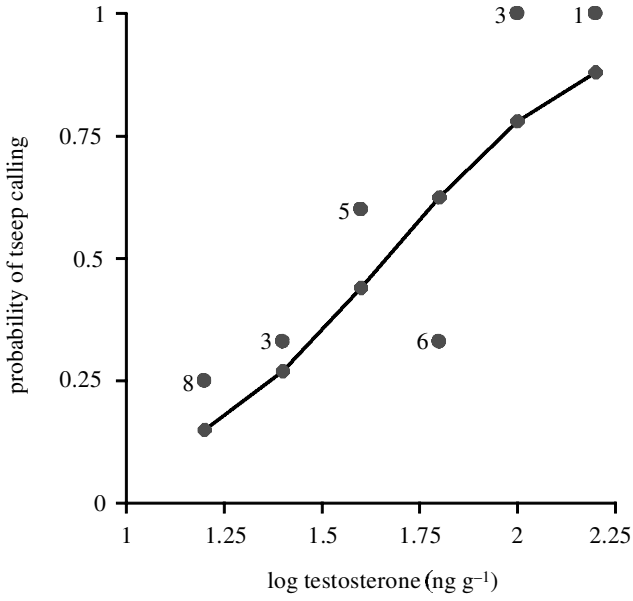

Figure 3. The effect of testosterone level on the probability of tseep calling. The curve shows the logistic regression from the GLMM analysis with female identity fitted as a random effect, and the points depict the actual probabilities.

4. DISCUSSION

The variance in female dunnock testosterone levels was partly explained by breeding stage, with testosterone levels highest during the fertile period and lowest during incubation and chick rearing. However, the mating system also significantly influenced testosterone levels; polygynandrous females had higher testosterone levels than monogamous females, particularly during the fertile period and chick provisioning (figure 1). Competition with a rival female carries the greatest cost in terms of reduced reproductive investment by males at these times (Davies 1992). Competition may have been particularly intense during the fertile period in this study because in four of the six polygynandrous groups the secondary female joined the group during the fertile period of the primary female.

Three sources of evidence suggest that female–female competition caused testosterone levels to rise in polygynandrous females. First, within polygynandrous groups, fertile females that were competing with another female in the group had higher testosterone levels than those that were not competing. Second, the testosterone levels of females that experienced experimentally induced competition caused by removal of males were significantly higher than controls. This indicates that competition between females elevates testosterone levels, rather than the alternative possibility that females with high testosterone levels are more likely to belong to polygynandrous groups. Third, the probability of producing 'tseep' calls, which are used in aggressive interactions between females (Langmore & Davies 1997), was associated with high testosterone levels. This indicates more specifically that direct aggression, rather than indirect competition for males reflected in trill call rates, causes testosterone levels to rise.

These results provide experimental support for the challenge hypothesis in female birds. They also support the proposition that increased testosterone levels mediate the production of song by females in competitive contexts (Arcese *et al.* 1988; Langmore & Davies 1997). Polygynandrous and polygynous females were significantly more likely to sing than monogamous females, indicating that dunnocks, like the congeneric alpine accentor *Prunella collaris* (Langmore *et al.* 1996), use female song to compete for males. Wingfield *et al.* (1990) suggested that an increase in the intensity of reproductive aggression as an effect of testosterone is strongest in situations of social instability. In accordance with this argument, many of the highest female testosterone levels in this study, and all female songs, occurred at times when a secondary female had recently joined a group.

An association between female aggression and elevated testosterone levels has been observed in several other species (Wingfield *et al.* 1982; Fivizzani & Oring 1986; Schwabl 1992; Cristol & Johnsen 1994; Eens & Pinxten 2000). However, the only other experimental test of the challenge hypothesis in female birds failed to find an increase in testosterone as a result of experimentally induced aggression in female song sparrows (Elekonich & Wingfield 2000). These contrasting results are surprising, because in song sparrows, like dunnocks, a minority of females breed in polygynous groups and suffer reduced male parental care, with the result that females compete to prevent polygyny and sing in aggressive contexts (Arcese *et al.* 1988; Arcese 1989). One difference between the two studies was that the female song sparrows were exposed to a single simulated intrusion, whereas the female dunnocks experienced repeated interactions with a rival female over several days. Arcese *et al.* (1988) found in song sparrows that most female song occurred in the context of prolonged conflicts with other females, and they proposed that female song is related to the frequency and intensity of aggressive contests. If so, a greater degree of agonistic stimulation may be necessary to elevate testosterone levels in females than in males.

Wingfield (1994) demonstrated that females tend to have high testosterone levels relative to males when sexual dimorphism is low and when females compete intensely over territories and for access to male investment in paternal care. The data from this study conform to this model and support the argument that sexual selection has promoted a role for testosterone in females that compete for male reproductive investment.

The authors are very grateful to Nick Davies and Rebecca Kilner for help with the fieldwork and comments on the manuscript, to Andrew Cockburn for statistical advice, to Ray Symonds for freeze-drying the samples, and to John Parker, Director of the University Botanic Garden, for permission to work on the study site. This work was supported by a Peterhouse Junior Research Fellowship, a Royal Society research grant and an Australian Research Council Fellowship to N.E.L. and by the Institute of Veterinary, Animal and Biomedical Sciences at Massey University.

REFERENCES

Arcese, P. 1989 Intrasexual competition and the mating system in primarily monogamous birds: the case of the song sparrow. *Anim. Behav.* **38**, 96–111.

Arcese, P., Stoddard, P. K. & Hierbert, S. M. 1988 The form and function of song in female song sparrows. *Condor* **90**, 44–50.

Brenowitz, E. A. & Arnold, A. P. 1990 The effects of systemic

androgen treatment on androgen accumulation in song control regions of the adult female canary brain. *J. Neurobiol.* **21**, 837–843.

Brenowitz, E. A. & Kroodsma, D. E. 1996 The neuroethology of birdsong. In *Ecology and evolution of acoustic communication in birds* (ed. D. E. Kroodsma & E. H. Miller), pp. 283–304. London: Cornell University Press.

Catchpole, C. K. & Slater, P. J. B. 1995 *Bird song: biological themes and variations*. Cambridge University Press.

Cockrem, J. F. & Rounce, J. R. 1995 Non-invasive assessment of the annual gonadal cycle in free-living kakapo (*Strigops habroptilus*) using fecal steroid measurements. *Auk* **112**, 253–257.

Cristol, D. A. & Johnsen, T. S. 1994 Spring arrival, aggression and testosterone in female red-winged blackbirds (*Agelaius phoeniceus*). *Auk* **111**, 210–214.

Davies, N. B. 1992 *Dunnock behaviour and social evolution*. Oxford University Press.

DeVoogd, T. J. & Nottebohm, F. 1981 Gonadal hormones induced dendritic growth in the adult avian brain. *Science* **214**, 202–204.

Eens, M. & Pinxten, R. 2000 Sex-role reversal in vertebrates: behavioral and endocrinological accounts. *Behavioural Processes* **51**, 135–147.

Elekonich, M. M. & Wingfield, J. C. 2000 Seasonality and hormonal control of territorial aggression in female song sparrows (Passeriformes: Emberizidae: *Melospiza melodia*). *Ethology* **106**, 493–510.

Fivizzani, A. J. & Oring, L. W. 1986 Plasma steroid hormones in relation to behavioral sex role reversal in the spotted sandpiper, *Actitis macularia*. *Biol. Reprod.* **35**, 1195–1201.

Genstat Committee 1993 *Genstat 5 release 3 reference manual*. Oxford: Clarendon Press.

Hausberger, M., Henry, L. & Richard, M. A. 1996 Testosterone-induced singing in female starlings (*Sturnus vulgaris*). *Ethology* **99**, 193–208.

Kern, M. D. & King, J. R. 1972 Testosterone-induced singing in female white-crowned sparrows. *Condor* **74**, 204–209.

Langmore, N. E. 1998 Functions of duet and solo songs of female birds. *Trends Ecol. Evol.* **13**, 136–140.

Langmore, N. E. 2000 Why female birds sing. In *Adaptive significance of signalling and signal design in animal communication* (ed. Y. Espmark), pp. 389–399. Trondheim: Transactions of the Royal Norwegian Society of Sciences and Letters.

Langmore, N. E. & Davies, N. B. 1997 Female dunnocks use vocalizations to compete for males. *Anim. Behav.* **53**, 881–890.

Langmore, N. E., Davies, N. B., Hatchwell, B. J. & Hartley, I. R. 1996 Female song attracts males in the alpine accentor *Prunella collaris*. *Proc. R. Soc. Lond.* B **263**, 141–146.

Nice, M. M. 1943 Studies in the life history of the song sparrow. II. The behavior of the song sparrow and other passerines. *Trans. Linn. Soc. NY* **6**, 1–328.

Nottebohm, F. 1980 Testosterone triggers growth of brain vocal control nuclei in adult female canaries. *Brain Res.* **189**, 429–436.

Schwabl, H. 1992 Winter and breeding territorial behaviour and levels of reproductive hormones of migratory European robins. *Ornis Scand.* **23**, 271–276.

Wingfield, J. C. 1985 Short term changes in plasma levels of hormones during establishment and defense of a breeding territory in male song sparrows, *Melospiza melodia*. *Horm. Behav.* **19**, 174–187.

Wingfield, J. C. 1994 Hormone–behaviour interactions and mating systems in male and female birds. In *The differences between the sexes* (ed. R. V. Short & E. Balaban), pp. 303–330. Cambridge University Press.

Wingfield, J. C., Newman, A. L., Hunt, G. I. & Farner, D. S. 1982 Endocrine aspects of female–female pairing in the western gull (*Larus-occidentalis-wymani*). *Anim. Behav.* **30**, 9–22.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. J. & Ball, G. F. 1990 The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. *Am. Nat.* **136**, 829–846.