



Testosterone and social and reproductive behaviour in *Aphelocoma* jays

CAROL M. VLECK* & JERRAM L. BROWN†

*Department of Zoology and Genetics, Iowa State University

†Department of Biological Sciences, State University of New York, Albany

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When there is a direct relationship between testosterone level and payoff in reproductive success through aggression, testosterone levels should be elevated. Elevated testosterone, however, has fitness costs, particularly a decreased tendency to display parental care. Thus the pattern of testosterone secretion in males should vary with the social and mating system. Western scrub-jays, *Aphelocoma californica woodhouseii*, form monogamous pairs on territories during the breeding season. Mexican jays, *A. ultramarina*, live in large, stable groups and up to five females within a group attempt nesting each spring. In both species, testosterone levels rose rapidly in March and peak levels did not differ. Elevated testosterone levels were only observed for about 3 weeks in the monogamous western scrub-jay, but were observed into May in Mexican jays, a reflection of prolonged opportunity for males to mate with multiple females and continual interaction with other competing males. In Mexican jays, nonbreeding yearlings had lower testosterone levels than all other age groups. Testosterone in males owning nests did not differ from that in other adult males, many of whom engage in extrapair fertilizations. Testosterone was elevated throughout the incubation phase, but was significantly lower when chicks were present in any nest in the group. Nearly all birds in the group fed all chicks. These observations support the hypothesis that testosterone is elevated when male–male competition is frequent and mating opportunities depend on the outcome of that competition, and testosterone is decreased when the necessity for parental or alloparental care would make its effects deleterious.

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In birds, elevated levels of testosterone early in the breeding season drive male reproductive behaviour including territory defence, courtship and mate guarding (reviewed in Wingfield et al. 1994). High plasma testosterone levels, however, also impose physiological and behavioural 'costs'. Manipulative studies suggest that chronically elevated testosterone is associated with decreased survival in birds and other vertebrates (Marler & Moore 1988; Dufty 1989; Moss et al. 1994; Salvador et al. 1996) and lower reproductive success (Silverin 1980; Hegner & Wingfield 1987; Ketterson et al. 1992; but see Raouf et al. 1997). High testosterone is linked to a variety of other deleterious effects, including (1) reduced tendency to provide parental care (Silverin 1980; Hegner & Wingfield 1987; Oring et al. 1989; Ketterson et al. 1992; Vleck & Dobrott 1993; Beletsky et al. 1995; Saino & Moller 1995), (2) immunosuppression (Folstad & Karter 1992; Zuk et al. 1995; Hillgarth et al. 1996; Salvador et al. 1996), (3)

elevated energy turnover (Hogstad 1987; Ketterson et al. 1991; Marler et al. 1995), and (4) disruption of social relationships and increased risk of injury (Dufty 1989; Beletsky et al. 1995). Temporal variation in testosterone may therefore result from a cost–benefit trade-off, with elevated testosterone advantageous for successful territory and mate acquisition, but deleterious outside the immediate mating season.

The trade-off hypothesis grew from comparisons within and between species (Wingfield et al. 1990). Within a bird species, variability between individuals in testosterone levels depends in part on the frequency of male–male challenges (Beletsky et al. 1992). Such agonistic interactions typically elevate testosterone within minutes (Wingfield & Wada 1989). Elevated testosterone may then promote readiness for further agonistic behaviour, a positive feedback system termed the challenge hypothesis (Wingfield et al. 1990). Between species, variation in the duration of elevated testosterone correlates with male social and parental behaviour. In socially monogamous species, plasma testosterone is elevated only briefly during the early part of the breeding season when territory

Correspondence: C. M. Vleck, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011, U.S.A. (email: cvleck@iastate.edu). J. L. Brown is at the Department of Biological Sciences, State University of New York, Albany, NY 12222, U.S.A.

acquisition and pair establishment are in progress. Once territorial boundaries are established, testosterone levels decline because male–male interactions are reduced and parental care becomes more important for fitness than agonistic capability (reviewed in Wingfield & Farner 1993). In polygynous species, males usually sustain high testosterone and generally high levels of male–male conflict for longer periods than in monogamous species and rarely contribute much to care of mates or offspring (reviewed in Beletsky et al. 1995).

The trade-off hypothesis derived from these observations leads to the following predictions (Wingfield et al. 1990). Male birds should have elevated testosterone when male–male competition is frequent and when mating opportunities depend on the outcome of that competition. Testosterone levels should be low if male–male conflict is infrequent, or if the results of such conflict have minimal correlation with fitness, or if other behaviours, like parental care, that have significant impacts on fitness are incompatible with elevated testosterone.

We studied the endocrine physiology of two *Aphelocoma* jays, the social Mexican jay, *Aphelocoma ultramarina*, and a sympatric and closely related, but non-social, species, the interior race of the western scrub-jay, *Aphelocoma californica woodhouseii*. These species overlap spatially in southern Arizona, have similar breeding schedules and are year-round residents. They provide a model system to examine relationships between breeding biology, social structure and testosterone physiology, while confounding effects of genetic and environmental differences that could constrain physiology and behaviour are reduced.

The social biology of Mexican jays in the Chiricahua Mountains of southeastern Arizona is well known (Brown & Brown 1990). The Mexican jay shows communal breeding as defined by Brown (1987): 'a system of breeding that is characterised by the normal presence of helpers at some or all nests'. They live in stable groups of 5–23 birds that defend a common, year-round territory from adjacent groups (Brown et al. 1997). Between zero and five females within a group usually attempt breeding each spring, and two or more are normally successful. Plural breeding is a notable contrast with most species of communal-nesting birds with singular breeding, in which there is only one nesting pair within the group (reviewed in Brown 1987).

In Mexican jays, the nesting pair provides about half of the food to the nestlings; the remainder is contributed by most other members of the group. Nestling care is probably determined by the hormone prolactin and in Mexican jays all group members have elevated prolactin at this time (Brown & Vleck 1998). Nest owners also feed young in nests of other pairs in the group, particularly after young in their own nest have fledged. This mating system has the appearance of monogamy within a season, but is actually more complex (Brown 1994). A male and female cooperate to build a nest. The male defends the female from other males prior to egg laying and feeds her as she alone incubates. Such birds are conventionally termed 'breeders', although 'nest owners' is a better term, because both sexes within a nesting pair may mate with other flock members (Brown 1994), and non-nest-owning

males within the flock routinely father offspring (Li 1997). Here we refer to nest owners and non-nesters to distinguish between birds that own nests and those that do not.

The likelihood that a male Mexican jay will become a nest owner increases with his dominance rank within the group (Brown et al. 1997). Dominance rank is settled by aggressive interactions between males during the prelaying period. Mating opportunity does not, however, end with egg laying. A dominant male may displace a lower-ranking male and begin guarding that bird's fertile female even if the dominant male has another incubating mate. Extrapair fertilizations by non-nesters are common (Bowen et al. 1995; Li 1997), and up to 40% of young are offspring of males other than the putative breeder (Li 1997). Younger, less-dominant group members are responsible for a high proportion of the extrapair fertilizations (Li & Brown 1998). Thus competition (both overt and covert) among males within flocks for females is common.

Western scrub-jays, in contrast, form socially monogamous pairs during the breeding season and defend their territories against other western scrub-jays. Neither helping nor the presence of non-nesters accompanying nest owners has been reported for the western scrub-jay (Carmen 1989). In this paper we compare the temporal pattern of variation in plasma testosterone between Mexican jays and western scrub-jays, then carry out a more detailed analysis of the relationship between hormones and behaviour in Mexican jays.

METHODS

We studied a population of Mexican jays in the Chiricahua Mountains of southeastern Arizona in 1990–1994, and two populations of western scrub-jays, one in the Sierrita Mountains (1993) and the other near the Santa Catalina Mountains (1995) in southern Arizona. In the Mexican jay population, all individuals within several adjacent groups had been colour-banded for individual recognition, ages were known because they were banded as nestlings, and their sex and reproductive status were known from intensive behavioural observations that have been carried out every spring for over 25 years (Brown & Brown 1990). We sexed birds that had not been sexed behaviourally in the field ($N=26$) using the CHD gene as described by (Griffiths et al. 1996). We validated the CHD gene method in 23 other Mexican jays whose sexes were known from behaviour. Sex assignments based on the DNA method agreed completely with assignments from behavioural data.

For the western scrub-jay we trapped and colour-banded birds at baited trapping stations. In a few cases we could assign sex based on behaviour or brood patch, or breeding status based on attendance at a nest. Other birds were sexed based on the length of the wing chord. Males are slightly larger (Pyle 1997), and we considered birds with wing chords greater than 124 mm to be males. This assumption was consistent with birds of known sex (eight females, three males). The breeding biology of the western scrub-jays in southern Arizona is not well known.

At our study sites, the earliest incubating female scrub-jay was found in late March and the latest was found in late May. Most nest-building activity occurred during late March and April, and most feeding of nestlings in May. These dates are similar to those for the Mexican jays we studied (Brown & Li 1995).

We obtained testosterone (T) samples from 27 western scrub-jay males and 53 Mexican jay males. Some birds were caught more than once, but in all statistical tests we used only one sample per individual. We used the first sample obtained for scrub-jays and either the first sample or the first nonyearling sample for Mexican jays when we had samples from 2 different years. We collected blood samples from individuals in the field between February and early July for Mexican jays and in March through May for western scrub-jays. All blood samples were obtained between dawn and noon, immediately after capture of birds in baited traps or mist nets. Mean \pm SE time between trapping and withdrawing a blood sample was 6 ± 0.4 min in Mexican jays and 7 ± 0.6 min in western scrub-jays. Blood was kept on ice for no more than 4 h. It was then centrifuged and separated into plasma and cells which were stored at -20°C until assayed. We determined the level of plasma T using radioimmunoassay and a T antiserum purchased from Wein Laboratories, Inc. (Succasunna, New Jersey). Extraction of T from 50–150 μl of plasma was accomplished with 2.5 ml anhydrous diethyl ether. Testosterone was separated from other steroids by chromatography using celite columns (Wingfield & Farner 1975) and each sample was then assayed in duplicate. Mean recovery after extraction and chromatography was 73% and the least detectable amount was approximately 2 pg/tube (corresponding to an approximate plasma concentration of 25 pg/ml). Intra- and interassay coefficients of variation (SD/mean) were 10.7 and 12.5%, respectively.

We used regression and analysis of variance to compare T concentrations between groups using JMP 3.1 software (SAS Institute 1995). Because the mean and variance of T values within groups were correlated, we log transformed hormone concentrations prior to analysis. In the Mexican jays our samples came from multiple years, and for each sample we knew the bird's age and nesting status, the size of its flock, and the history of nesting attempts within that flock. We used a regression ANOVA to explore the importance of each of these factors in accounting for the variability in testosterone. We selected the regression approach because our data set, like many unplanned (natural) experiments, did not have adequate replication for every combination of factors to permit a complete factorial analysis. First, we listed categories of possible interest for each factor in our analysis, including nesting status, nest stage, age, flock size and sample year. For each T sample, we assigned a category score of 1 if the bird was a member of that category, and 0 if it was not. Each sample had to be a member of one and only one category for a given factor (like nest stage or sample year), so assigning scores for all but one of the categories in a factor determined the score for the last category. To avoid such covariance between categories, we omitted one category from each factor from the regression model. The omitted

categories are represented in the intercept of the regression.

In species of birds in which nesting is not highly synchronous, T is correlated more closely with nesting stage than with calendar date. In our samples, dates for the beginning of incubation ranged from 12 March to 9 June (mean=17 April), so we used nesting stages rather than calendar dates to examine temporal changes in T within the breeding season. We assigned each sample to one of four nest stages. Courting samples were those collected prior to the beginning of incubation, which normally starts on the day the nesting female lays her third egg (Brown 1994). The remaining samples were assigned to nest stages of incubation, nestling care, or fledgling care. Incubation period in Mexican jays is 18 days, and hatchlings remain in the nest for 24–28 days before fledging. Young are fed by adults for several weeks after fledging.

Assigning a nest stage to individuals within a group is more complex than in most bird species. In Mexican jays most or all of the birds in the group (except other incubating females) feed incubating females on the nest and feed the young in the nest after the eggs hatch. A nesting pair can be identified behaviourally for each nest (see above); thus for a nest owner, we assigned nest stage with respect to his own nest. For non-nesters, we assigned nest stage with respect to the most advanced, active nest in the group, excluding nests that failed prior to hatching.

Mexican jays in our sample ranged from 1 to 17 years of age. We divided age into three categories: yearlings, 2-year-olds and older adults. Yearlings do not breed, but older birds of any age may be nest owners, including about 20% of 2-year-olds (Brown 1994). We added a 2-year-old category to test for evidence of transition between yearlings and older adults. Flock size (determined on 1 May each year and excluding young of the year) for the birds we sampled had a mean \pm SE of 13.1 ± 0.4 individuals (range 7–22). We categorized flocks of 10 or fewer individuals as small, and flocks of 16 or more individuals as large.

RESULTS

Comparison of Mexican Jay and Western Scrub-jay Testosterone Levels

Yearling Mexican jays had significantly lower T levels than older Mexican jays, but nest owners and non-nesting adults (2 or more years old) did not differ from each other (see below). In addition, yearling Mexican jays have never been observed to lay eggs or father young (Brown 1994). Consequently we omitted data from yearlings, but included data from all adult Mexican jays in the between-species comparisons that follow.

The temporal pattern of change in T differed between species (Fig. 1). In both species some individuals had elevated T in early March, but in Mexican jays, individuals with elevated T levels were found much later in the breeding season than in scrub-jays. To evaluate statistically the differences between species across time we

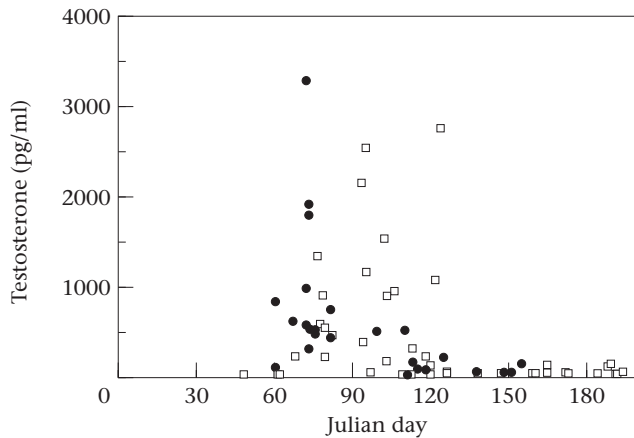


Figure 1. Testosterone in male western scrub-jays (●) and Mexican jays (□) as a function of Julian day.

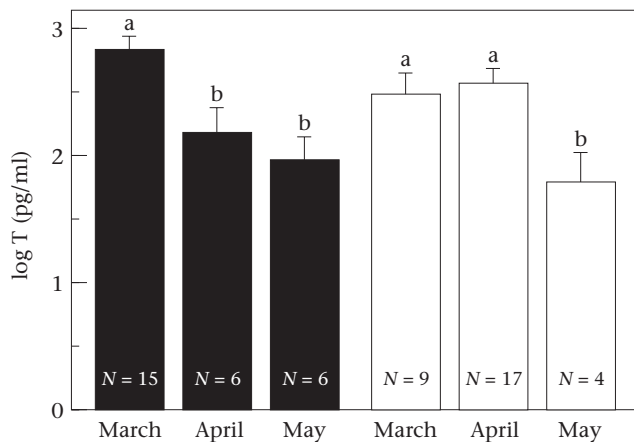


Figure 2. Logarithm of testosterone concentration (pg/ml) in male western scrub-jays (■) and Mexican jays (□) during three 32-day intervals beginning on Julian day 60. Intervals correspond approximately to the months of March, April and May. Values are the least squares mean \pm SE (N =sample size) for the predicted values from the ANOVA model (Table 1). Different letters indicate significant differences between groups (Scheffé test: $P < 0.05$).

restricted the comparison to the time period when we had samples from both species (Julian days 60–155). We divided that period into three equal intervals of 32 days each. Those intervals correspond approximately to the months of March, April and May, respectively (Fig. 2). The results of a model 1 ANOVA with species and interval as main effects and a species–interval interaction are summarized in Table 1. There was no significant difference in T between species across time ($P = 0.79$), but T did vary between time intervals ($P < 0.001$). The species \times time interaction was marginally insignificant ($P = 0.06$).

Following the ANOVA, inspection of the data (Fig. 2) suggested that examining specific hypotheses about the interaction between species and time would be informative. We constructed contrasts to test the general hypothesis that T values were high for both species in March, remained high for Mexican jays but declined for scrub-jays during April, and were low for both species during May (Fig. 2). We used the Scheffé procedure (Sokal

Table 1. Analysis of variance of log testosterone in male Mexican jays and western scrub-jays

Source	df	Sums of squares	F ratio	P
Species	1	0.018	0.073	0.787
Interval*	2	4.118	8.519	<0.001
Species*interval	2	1.432	2.926	0.0607
Model	5	6.047	5.004	<0.001
Error	51	12.326		
Total	56	18.373		

*Time was divided into three 32-day intervals beginning on Julian day 60.

& Rohlf 1981) to evaluate the significance of these post hoc contrasts at an overall significance level of $P < 0.05$. If the 95% confidence limits for a contrast (C) do not include zero, then the contrast is significant. In Mexican jays, T values were not significantly different across the three intervals ($C = 0.739 \pm 0.921$), but T in the western scrub-jay was significantly higher in the first interval than in the second and third intervals ($C = 0.760 \pm 0.659$). Testosterone levels during the first interval in both species and the second interval for the Mexican jay were significantly higher than T levels in the second interval for the western scrub-jay and the third interval for both species ($C = 0.653 \pm 0.514$).

Effect of Age, Nest Stage and Group Size on Testosterone in Male Mexican Jays

Testosterone levels in male Mexican jays were highly variable between individuals during the breeding season (Fig. 3). The complete regression model contained 12 different variables, including 4 sample years (Table 2). The model as a whole accounted for a significant portion of the variance in T levels (ANOVA: $F_{12,52} = 3.09$, $P < 0.004$, adjusted $r^2 = 0.33$). The magnitudes and significance of effects of particular categories are more informative, however, than the whole model statistics. Nest stage and age had significant effects on T in male Mexican jays, but nest ownership status did not ($P = 0.22$), nor did flock size (NS). Males in the courting and incubating nest stages had higher levels of T than males in the fledgling nest stage ($P < 0.03$), but T levels in the nestling and fledgling stages did not differ ($P = 0.11$). Yearlings had lower T than adults ($P = 0.003$), but 2-year-olds did not differ from older adults ($P = 0.11$). There were no significant differences between sample years (NS; Table 2).

This regression ANOVA is computationally similar to a main-effects factorial ANOVA. It does not include interactions. Our data set, however, included enough observations that had the same category scores to provide a direct estimate of the within-groups variance. A lack-of-fit (goodness-of-fit) test (SAS Institute 1995) was not significant ($F_{26,40} = 1.22$, $P = 0.36$), suggesting that our model (Table 2) was appropriate for the data set and that adding interaction terms or power functions of the regressor variables would not provide a significant improvement in explanatory power.

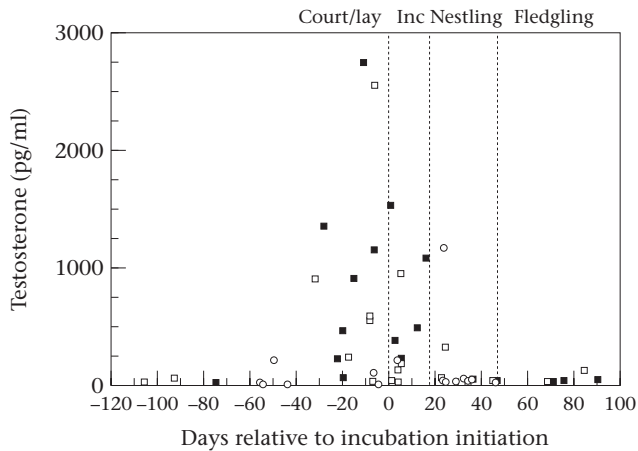


Figure 3. Testosterone in male Mexican jays as a function of time relative to the beginning of incubation (day the third egg was laid). Data for nest owners (■) are plotted relative to their own nest. Data for adult non-nesters (□: 2 years or older) and yearling nonbreeders (○) are plotted relative to the nest stage of the most concurrent, active nest within the group of that individual (see text). The incubation period (Inc) is 18 days and nestlings (Nestling) are fed for 24–28 days before fledgling (Fledgling).

Contrasts between nest owners versus non-nesting adults, nestling stage versus fledgling stage, 2-year-olds versus older birds, small versus medium versus large flocks, and between years were not significant (Table 2). We tested whether these contrasts contributed significantly to the complete regression model (Snedecor & Cochran 1989). The hypothesis that all of these estimates were equal to zero could not be rejected ($F_{9,40}=1.82$, NS), indicating there would be no significant loss of accuracy in the prediction of log T if these terms were omitted. Courtship and incubation stages were both significant and had nearly identical estimates (Table 2). Consequently we reclassified nest stage into two categories, chicks absent (courtship or incubation phases) versus chicks present (nestling or fledgling stages). We reclassified age into yearlings versus adults, omitted flock size, sample year and breeding status and repeated the analysis (Table 3). In the simplified model, nest stage and age were both significant ($P<0.04$). This model also accounted for a significant portion of the variance in T levels ($F_{2,52}=9.13$, $P<0.001$, adjusted $r^2=0.24$). To explore further whether age of adults affected T, we regressed residuals from the model (Table 3) against age for males aged 2–17 years. The regression was not significant (ANOVA: $F_{1,36}=1.55$, $P=0.22$).

DISCUSSION

Interspecific Comparison of the Temporal Pattern of T Elevation

In both Mexican jays and western scrub-jays, testosterone levels rose rapidly in early March at the beginning of the breeding season. Neither basal nor peak levels of testosterone differed between the two species, but the period of time during which T levels could be high was

Table 2. Regression model describing variance in log testosterone concentration (pg/ml) in male Mexican jays during the breeding season

Term	Estimate*	SE†	<i>t</i> ratio	<i>P</i>
Intercept‡	2.01	0.39	5.20	<0.0001
Breeding status				
Non-nester	0.249	0.20	1.25	0.219
Nest owner	0	—	—	—
Nest stage				
Courting	0.648	0.28	2.30	0.026
Incubating	0.734	0.29	2.51	0.016
Nestlings	0.485	0.30	1.61	0.114
Fledglings	0	—	—	—
Age				
Yearling	-0.894	0.29	-3.12	0.003
2-year-old	-0.453	0.27	-1.65	0.106
Older than 2 years	0	—	—	—
Flock size				
Small	-0.303	0.27	-1.13	0.264
Medium	0	—	—	—
Large	-0.008	0.24	-0.33	0.975
Year				
1990	0	—	—	—
1991	-0.324	0.37	-0.88	0.384
1992	-0.180	0.39	0.46	0.649
1993	0.001	0.39	0.00	0.998
1994	-0.235	0.30	-0.79	0.433

*Estimate values are the coefficients in a regression equation describing log T as a function of scores (0 or 1) for each of the terms. Terms with an estimate of zero are included in the intercept, but are listed in the table to indicate their implicit presence in the model.

†Standard errors for each estimate are listed, together with *t* statistics and *P* values testing the hypothesis that the estimate does not differ from zero.

‡The intercept is an estimate of the mean expected log T for a breeder, older than 2 years of age, in a medium-sized flock (11–15 birds), after nestlings have fledged, in 1990.

much longer in Mexican jays than in western scrub-jays. Only during the first 3 weeks of March did we sample western scrub-jays that had T values over ca. 500 pg/ml. In contrast, Mexican jays with values of T this high were found as late as the first week of May. We suggest that this difference is related to the very different social systems of these two closely related species. In western scrub-jays, as in other monogamous species (Wingfield & Farner 1993), there is greatly reduced male–male interaction after territorial establishment and mate bonding. A male and female bond for the entire breeding season and have few interactions with other adult jays while on their territory (Carmen 1989). Thus T levels are elevated only in March, at the beginning of the breeding season when male–male interactions are likely. The pattern in Mexican jays resembles that of polygynous species (Beletsky et al. 1995). Adult males within the group interact daily, and there is competition among males for access to fertile females in the group (Brown et al. 1997). There are also frequent territorial disputes with other adjacent groups of jays. Because there are multiple females in a group, mating opportunities continue even after one female has laid eggs and begun incubation. We suggest that the presence of males with elevated testosterone throughout

Table 3. Simplified regression model describing variance in log testosterone concentration (pg/ml) in male Mexican jays during the breeding season

Term	Estimate*	SE†	t ratio	P
Intercept‡	2.236	0.31	7.22	<0.0001
Nest stage				
Court/incubation	0			
Nestling/fledgling	-0.358	0.17	-2.12	0.039
Age				
Yearling	0			
Adult	0.519	0.18	2.88	0.0058

*Estimate values are the coefficients in a regression equation describing log T as a function of scores (0 or 1) for each of the terms. Terms with an estimate of zero are included in the intercept, but are listed in the table to indicate their implicit presence in the model.

†Standard errors for each estimate are listed, together with t statistics and P values testing the hypothesis that the estimate does not differ from zero.

‡The intercept is an estimate of the mean expected log T for a yearling bird in the courting or incubation stage (no chicks present).

March and into May is a reflection of the prolonged opportunity for these birds to mate with multiple females and the continual interaction with competing males.

Our results from two jay species can also be compared to those for another congener, the Florida scrub-jay, *Aphelocoma coerulescens*. About half of the Florida scrub-jay territories contain only a breeding pair, whereas the other half also contain on average one or two nonbreeding birds, usually offspring of the breeding pair. About 60% of these are yearlings. There is almost always a single nest per group and the breeding pair is truly monogamous (Woolfenden & Fitzpatrick 1990; Quinn et al. 1999). In this species testosterone in breeding males is only elevated for a short time during the nest-building stage when females are receptive (Schoech et al. 1991, 1996). The pattern of T secretion in the Florida scrub-jay resembles that of a typical monogamous species, as does their mating system.

The precision of the match between testosterone levels and social behaviour within *Aphelocoma* jays is a good measure of the strength of the hypothesized linkage between testosterone level and social behaviour. Social systems within the genus range from permanently social groups with plural nesting (Mexican jay) or singular nesting (Florida scrub-jay), to territorial pairs (western scrub-jay). Within the two social species, opportunities for mating differ, and hence the selective importance of male–male interactions and elevated T also differs. Because social systems differs among these congeneric species, the potential for confounding social structure with other historical causes of differences in testosterone is reduced.

Group Structure and Testosterone in Mexican Jays

Nesting status

We found no difference in T in male Mexican jays that were identified as nest owners at nests and those that

were not. In fact, mean T in non-nesting adults was higher (although not statistically so) than T in nest owners when all other factors were held constant (Table 2). Although yearling males did not have elevated T, even 2-year-old birds had levels indistinguishable from older birds. Elevated T in all adult males in the group probably reflects physiological readiness to breed. Although some males have much higher reproductive success than others (Brown et al. 1997), even birds that do not own a nest compete for access to females and often copulate with them, as evidenced by the high levels of extrapair fertilizations recorded in this species (Li 1997).

There are a number of other communal species with helpers in which T levels of breeders and nonbreeders have been measured. In singular breeders (one nest per group), whether or not the nonbreeding males have elevated T during the breeding season varies with the potential opportunity for these nonbreeders to mate with a female. In Florida scrub-jays (Schoech et al. 1991, 1996) and white-browed sparrow weavers, *Plocepasser mahali* (Wingfield et al. 1991), only breeding males have elevated levels of T, and there is one breeding female per group that pairs permanently with that male. In other singular breeders such as pied kingfishers, *Ceryle rudi*, and Harris' hawks, *Parabuteo unicinctus*, T may be elevated in some unrelated 'nonbreeders' which may occasionally mate with the breeding female; T is low in others (usually related birds) that never mate with the breeding female (Reyer 1986; Reyner et al. 1986; Mays et al. 1991).

In communal species with plural nesting behaviour there should be greater opportunity for males to mate with females than in singular breeders. If this is so, and if competition between males is prerequisite to mating, then putative nonbreeders should have T levels as high as those in breeder males. The western form of the Australian magpie, *Gymnorhina tibicen*, lives in groups of up to 20 individuals with multiple breeding pairs per group. Putative nonbreeding adults have levels of T similar to those in the breeders during the egg-laying period (Schmidt et al. 1991). As in Mexican jays, the social status of the male magpie affects his ability to become a breeder, and some putative nonbreeding adult males may be able to obtain copulations with promiscuous females or via forced copulations (Schmidt et al. 1991). The bell miner, *Manorina melanophrys*, lives in loose colonies of 20–200 or more individuals. Breeders have higher levels of androgen (T plus dihydrotestosterone) than sexually mature, nonbreeding adult males, but these nonbreeders do have enlarged gonads and produce spermatozoa (Poiani & Fletcher 1994). Poiani & Fletcher (1994) suggest that these males may 'follow a sneaky strategy to sire offspring without openly challenging the male breeder'. These observations, taken together with our data from the Mexican jay, support the predictions of the testosterone trade-off hypothesis in communal breeders. Male birds have elevated T when mating opportunity depends on the outcome of male–male competition and low T when either male–male aggressive interaction is infrequent or when breeding opportunities are very low under any circumstances.

Age

Yearling Mexican jay males had significantly lower T values than all older males. Young birds in many social species have low reproductive steroid levels during the breeding season (Reyer et al. 1986; Mays et al. 1991; Schmidt et al. 1991; Schoech et al. 1991; Poiani & Fletcher 1994). This has been attributed to reproductive suppression and/or delayed maturation of individuals within the group because some same-aged individuals in each of these species are known to breed. In Mexican jays, yearlings never breed, so we cannot determine whether yearlings are subadults that must age another year before reaching sexual maturity or whether they are reproductively suppressed. In either case the low level of T in these individuals may be beneficial. Yearlings with low T levels do not pay any physiological costs of high T levels and may be tolerated by other males to a greater extent than those males with high T levels. Yearlings are readily identifiable as such because of juvenile bill coloration, and yearlings are given preferential access to food by older birds (Barken et al. 1986). Two-year-old birds do regularly breed, although the average age for first breeding in males is 3.9 years (Brown 1994). We had one 2-year-old nest owner in our sample of males. Except in the case of yearlings, we could distinguish no effect of age on T levels during the breeding season. Similarly, age has no effect on T levels in red-winged blackbirds, *Agelaius phoeniceus*, once they have begun breeding (Beletsky et al. 1992).

Stage

Testosterone was elevated in adult Mexican jay males both before eggs were laid and during the incubation phase, but rarely after the egg hatched and chicks were present (Fig. 3). Importantly, there was no decrease in the level of T between the courting and incubation stages of the nesting cycle. In birds with male parental care, T usually drops to low levels when the male's behaviours switch from those directed primarily towards other adults to those directed towards offspring (reviewed in Wingfield et al. 1990). There are two, not mutually exclusive, hypotheses for this pattern. First, T may drop because paternity issues are settled once the clutch is laid, so the benefits of maintaining elevated T are low. Second, the fitness costs of elevated T may rise because elevated T and parental care of eggs and young are incompatible. Even in species in which males do not incubate, testosterone levels generally fall during incubation (Schoech et al. 1996; Hunt et al. 1997), suggesting that testosterone can be 'allowed' to drop as soon as paternity is settled. This contrasts with Mexican jays in which males have high T during the incubation stage, probably because of continuing interactions with other males and fertile females in the group. Paternity issues in Mexican jays are not settled just because incubation in one nest has begun.

The most striking aspect of the pattern of T levels in male Mexican jays was the paucity of individuals with high T as soon as any nestlings were present in the group. The contrast between high T before any chicks are present (either courtship or incubation phase) and low T when

they are present (either nestling or fledgling phase) supports the hypothesis that high T is incompatible with care of chicks. This incompatibility is probably independent of whether or not the chicks are the offspring of the male in question. In fostering programmes, strong alloparental behaviour in males is inversely correlated with T (Vleck & Dobrott 1993). The drop in T in male Mexican jays when chicks are present in the nest of any group member suggests that fathers, potential fathers and nonfathers all benefit more from caring for chicks than from continued male-male conflict. Sustaining elevated T after hatching of chicks might facilitate a male's access to fertile females in the group, but at the cost of reducing his intensity of feeding of those chicks. Whether or not a male owns a nest, he may have fathered some of the chicks in the group or be closely related to them (Brown & Brown 1990; Li 1997). Because the non-nesters provide about half the feedings to the nestlings, this decrease in alloparental effort could impose a high cost on chick growth and survival, especially in poor years.

It is difficult to tease apart the effects of time per se and presence of chicks on the temporal pattern of T. Testosterone could be low when chicks are present merely because of the passage of time (which will also affect the number of fertile females in the group and thus the benefit to maintaining high T). The incompatibility hypothesis leads to the following prediction: in groups where eggs hatch early, male T should also drop early compared with groups in which eggs hatch later. We can use our data to test this prediction. Among the groups we sampled, the earliest hatching date was Julian day 90, and the mean date when chicks first appeared was Julian day 124. We compared the frequencies of adult males with elevated T (>500 pg/ml) during this calendar interval between males from groups with chicks and males from groups without chicks. The frequency of elevated T was significantly lower in the former (likelihood ratio $\chi^2_{19}=4.02$, $P=0.04$). This is consistent with the prediction that it is the presence of chicks, not the calendar date, that is responsible for the observed drop in T. The presence of young (sight or sound of begging chicks) may trigger an inhibition of T secretion in males, although this has not been tested. Manipulation of the hatching dates by switching eggs between nests in different groups could be used to test this prediction.

The Mexican jay is noteworthy for the complexity of its mating strategies. The mating system contains elements typical of both monogamy (e.g. pair formation and mate guarding, and male parental care) and polygamy (e.g. high variance in reproductive success) and is best described as cryptic polygamy (Johnson & Burley 1998). Recent examination of mating systems in birds in general suggests that mating systems are often quite fluid in ecological time and often condition dependent (Johnson & Burley 1998). Group living in Mexican jays is associated with a situation in which pair bonds can be observed, but females engage in high levels of extrapair copulation, and paired males often compete for access to other fertile females in the group. Males essentially have two strategies available to them: (1) domination of other males with associated nest ownership and mate guarding; and (2)

extrapair copulation with a female even if she is attending a nest with a dominant male. The former strategy is more prevalent in older males, whereas the latter strategy is more prevalent in younger males (Brown et al. 1997). Both strategies, however, are associated with elevated T over a relatively long period. The two key factors for understanding the patterns of T in male Mexican jays are the level of competitive interactions between males, which tends to elevate T, and the presence of chicks in the group, which tends to decrease T.

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References

- Barken, C. P. L., Craig, J. L., Strahl, S. D., Stewart, A. M. & Brown, J. L. 1986. Social dominance in communal Mexican jays *Aphelocoma ultramarina*. *Animal Behaviour*, **34**, 175–187.
- Beletsky, L. D., Orians, G. H. & Wingfield, J. C. 1992. Year-to-year patterns of circulating levels of testosterone and corticosterone in relation to breeding density, experience, and reproductive success of the polygynous red-winged blackbird. *Hormones and Behavior*, **26**, 420–432.
- Beletsky, L. D., Gori, D. F., Freeman, S. & Wingfield, J. C. 1995. Testosterone and polygyny in birds. In: *Current Ornithology*. Vol. 12 (Ed. by D. M. Power), pp. 1–41. New York: Plenum.
- Bowen, B. S., Koford, R. R. & Brown, J. L. 1995. Genetic evidence for undetected alleles and unexpected parentage in the gray-breasted jay. *Condor*, **97**, 503–511.
- Brown, J. L. 1987. *Helping and Communal Breeding in Birds: Ecology and Evolution*. Princeton, New Jersey: Princeton University Press.
- Brown, J. M. 1994. Mexican jay (*Aphelocoma ultramarina*). In: *The Birds of North America*. No. 118 (Ed. by A. Poole & F. Gill), pp. 1–16. Philadelphia: Academy of Natural Sciences.
- Brown, J. L. & Brown, E. R. 1990. Mexican jays: uncooperative breeding. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (Ed. by P. B. Stacey & W. D. Koenig), pp. 269–288. Cambridge: Cambridge University Press.
- Brown, J. L. & Li, S. 1995. Delayed effect of monsoon rains influences laying date of a passerine bird living in an arid environment. *Condor*, **98**, 879–884.
- Brown, J. L. & Vleck, C. M. 1998. Prolactin and helping in birds: has natural selection strengthened helping behavior? *Behavioral Ecology*, **9**, 541–545.
- Brown, J. L., Brown, E. R., Sedransk, J. & Ritter, S. 1997. Dominance, age, and reproductive success in a complex society: a long-term study of the Mexican jay. *Auk*, **114**, 279–286.
- Carmen, W. 1989. Behavioral ecology of the California scrub jay (*Aphelocoma coerulescens californica*): a non-cooperative breeder with close cooperative relatives. Ph.D. thesis, University of California.
- Dufty, A. J. 1989. Testosterone and survival: a cost of aggressiveness? *Hormones and Behavior*, **23**, 185–193.
- Folstad, I. & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist*, **139**, 603–622.
- Griffiths, R., Daan, S. & Dijkstra, C. 1996. Sex identification in birds using two CHD genes. *Proceedings of the Royal Society of London, Series B*, **263**, 1251–1256.
- Hegner, R. & Wingfield, J. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk*, **104**, 462–469.
- Hillgarth, N., Ramenofsky, M. & Wingfield, J. 1996. Testosterone and sexual selection. *Behavioral Ecology*, **8**, 108–109.
- Hogstad, O. 1987. It is expensive to be dominant. *Auk*, **104**, 333–336.
- Hunt, K. E., Hahn, T. P. & Wingfield, J. C. 1997. Testosterone implants increase song but not aggression in male Lapland longspurs. *Animal Behaviour*, **54**, 1177–1192.
- Johnson, K. & Burley, N. T. 1998. Mating tactics and mating systems of birds. *Ornithological Monographs*, **49**, 21–60.
- Ketterson, E. D., Nolan, V., Jr, Wolf, L., Ziegenfus, C., Dufty, A. M. J., Ball, G. F. & Johnsen, T. S. 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. *Hormones and Behavior*, **25**, 489–503.
- Ketterson, E. D., Nolan, V., Jr, Wolf, L. & Ziegenfus, C. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *American Naturalist*, **140**, 980–999.
- Li, S. 1997. The analysis of paternity pattern in a natural population of Mexican jays (*Aphelocoma ultramarina*) (parental effort, female choice, extrapair copulation). Ph.D. thesis, State University of New York at Albany.
- Li, S. & Brown, J. L. 1998. Do extra-pair fertilisations benefit female Mexican jays? *Ostrich*, **69**, 226 (abstract).
- Marler, C. & Moore, M. 1988. Evolutionary costs of aggression revealed by testosterone manipulation in free-living male lizards. *Behavioral Ecology and Sociobiology*, **23**, 21–26.
- Marler, C. A., Walsberg, G., White, M. L. & Moore, M. 1995. Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology*, **37**, 225–231.
- Mays, N. A., Vleck, C. M. & Dawson, J. 1991. Plasma luteinizing hormone, steroid hormones, behavioral role, and nest stage in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk*, **108**, 619–637.
- Moss, R., Parr, R. & Lambin, X. 1994. Effects of testosterone on breeding density, breeding success and survival of red grouse. *Proceedings of the Royal Society of London, Series B*, **258**, 175–180.
- Oring, L., Fivizzani, A. J. & El Halawani, M. E. 1989. Testosterone-induced inhibition of incubation in the spotted sandpiper (*Actitis macularia*). *Hormones and Behavior*, **23**, 412–423.
- Poiani, A. & Fletcher, T. 1994. Plasma levels of androgens and gonadal development of breeders and helpers in the bell miner (*Manorina melanophrys*). *Behavioral Ecology and Sociobiology*, **34**, 31–41.

- Pyle, P. 1997. *Identification Guide to North American Birds. Part I. Columbidae to Ploceidae*. Bolinas, California: Slate Creek Press.
- Quinn, J. S., Woolfenden, G. E., Fitzpatrick, J. W. & White, B. N. 1999. Multi-locus DNA fingerprinting supports monogamy in Florida scrub-jays. *Behavioral Ecology and Sociobiology*, **45**, 1–10.
- Raouf, S. A., Parker, P. G., Ketterson, E. D., Nolan, V., Jr & Ziegenfus, C. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (*Aves: Junco hyemalis*). *Proceedings of the Royal Society of London, Series B*, **264**, 1599–1603.
- Reyer, H. U. 1986. Breeder–helper interactions in the pied kingfisher reflect the costs and benefits of cooperative breeding. *Behaviour*, **90**, 277–303.
- Reyer, H. U., Dittami, J. & Hall, M. 1986. Avian helpers at the nest: are they psychologically castrated? *Ethology*, **71**, 216–228.
- Saino, N. & Moller, A. P. 1995. Testosterone-induced depression of male parental behavior in the barn swallow: female compensation and effects on seasonal fitness. *Behavioral Ecology and Sociobiology*, **35**, 151–157.
- Salvador, A., Veiga, J. P., Martin, J., Lopez, P., Abelenda, M. & Puerta, M. 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioral Ecology*, **7**, 145–150.
- SAS Institute. 1995. *JMP Statistics and Graphics Guide*. Cary, North Carolina: SAS Institute.
- Schmidt, L., Bradshaw, S. & Follett, B. 1991. Plasma levels of luteinizing hormone and androgens in relation to age and breeding status among cooperatively breeding Australian magpies (*Gymnorhina tibicen* Latham). *General and Comparative Endocrinology*, **83**, 48–55.
- Schoech, S. J., Mumme, R. L. & Moore, M. C. 1991. Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub-jays (*Aphelocoma c. coerulescens*). *Condor*, **93**, 354–364.
- Schoech, S. J., Mumme, R. L. & Wingfield, J. C. 1996. Delayed breeding in the cooperatively breeding Florida scrub-jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? *Behavioral Ecology and Sociobiology*, **39**, 77–90.
- Silverin, B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Animal Behaviour*, **28**, 906–912.
- Snedecor, G. W. & Cochran, W. G. 1989. *Statistical Methods*. 8th edn. Ames, Iowa: Iowa State University Press.
- Sokal, R. R. & Rohlf, F. J. 1981. *Biometry*. 2nd edn. San Francisco: W. H. Freeman.
- Vleck, C. M. & Dobrott, S. J. 1993. Testosterone, antiandrogen, and alloparental behavior in bobwhite quail foster fathers. *Hormones and Behavior*, **27**, 92–107.
- Wingfield, J. & Farner, D. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein-binding. *Steroids*, **26**, 311–327.
- Wingfield, J. C. & Farner, D. S. 1993. Endocrinology of reproduction in wild species. In: *Avian Biology. Vol. IX* (Ed. by D. S. Farner, J. R. King & K. C. Parkes), pp. 163–327. New York: Academic Press.
- Wingfield, J. C. & Wada, M. 1989. Changes in plasma levels of testosterone during male–male interactions in the song sparrow, *Melospiza melodia*: time course and specificity of response. *Journal of Comparative Physiology A*, **166**, 189–194.
- Wingfield, J., Hegner, R., Dufty, A. J. & Ball, G. 1990. The ‘challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. *American Naturalist*, **136**, 829–846.
- Wingfield, J., Hegner, R. & Lewis, D. 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Journal of Zoology*, **225**, 43–58.
- Wingfield, J. C., Whaling, C. S. & Marler, P. 1994. Communication in vertebrate aggression and reproduction: the role of hormones. In: *The Physiology of Reproduction* (Ed. by E. Knobil & J. D. Neill), pp. 303–342. New York: Raven Press.
- Woolfenden, G. E. & Fitzpatrick, J. W. 1990. Florida scrub-jays: a synopsis after 18 years. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (Ed. by P. B. Stacey & W. D. Koenig), pp. 241–266. New York: Cambridge University Press.
- Zuk, M., Johnsen, T. S. & Maclarty, T. 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proceedings of the Royal Society of London, Series B*, **260**, 205–210.