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Correlated evolution of male and female testosterone profiles in birds and its consequences

Abstract Circulating levels of testosterone in adults have mainly evolved as a consequence of selection on males for increased levels, while levels of circulating testosterone in females may be an indirect consequence of selection on males. A review of the literature revealed that intense directional selection for high levels of circulating testosterone in birds is likely to be mainly due to direct selection on males. A comparative study of testosterone levels in birds revealed a strong positive relationship between peak testosterone levels in adult females and peak levels in males. The slope of this relationship was significantly less than unity, implying that the testosterone levels in females have been reduced in species with high levels in males. An analysis of the order of evolutionary events suggested that peak concentration of testosterone in females changed after peak concentrations of testosterone in males. Females in colonial species of birds had significantly higher circulating peak testosterone levels compared to females of solitary species, and relative levels after controlling for the effects of peak levels in males were also larger, suggesting that any costs of high testosterone levels in females are particularly likely in colonial birds. Direct selection on male circulating testosterone levels may increase the costs that females incur from high testosterone titers. For example, high female levels may negatively affect ovulation and laying and may also affect the levels of testosterone that females deposit in their eggs and hence the exposure of pre- and post-hatching offspring to testosterone. This in turn may affect not only offspring behavior, but also offspring development and the trade-offs between growth, development of immune function, and behavior in offspring.

Keywords Birds · Correlated evolution · Cost of hormones · Immune suppression · Maternal testosterone

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Introduction

Testosterone is one of the prime hormones that affect male reproductive behavior in vertebrates (e.g., Balthazart 1983). Experimental studies have shown that testosterone affects male reproductive behavior of birds in a number of different ways. First, testosterone enhances male production of song mainly through an increase in song rate (e.g., Arnold 1975; Nottebohm et al. 1987; Marler et al. 1988; Rost 1990, 1992; DeVoogd 1991; Dittami et al. 1991; Schwabl and Kriner 1991). Second, testosterone is associated with increased intensity of mate guarding (Moore 1984; Saino and Møller 1995). Third, testosterone is associated with reduced male incubation (Oring et al. 1989; Ketterson and Nolan 1999) and male food provisioning of offspring (e.g., Silverin 1980; Wingfield 1984; Hegner and Wingfield 1987; Dittami et al. 1991). Fourth, testosterone causes an increase in male mating success (e.g., Silverin 1980; Watson and Parr 1981) and extra-pair paternity (Raouf et al. 1997). Thus, there is considerable evidence that male testosterone levels are under strong direct selection, and

that males with high levels of testosterone enjoy a selective advantage in terms of increased reproductive success. This interpretation is also supported by a recent comparative analysis that showed a positive relationship between circulating testosterone levels, polygyny, and extra-pair paternity across species of birds (Garamszegi et al. 2005).

However, there is much less evidence concerning the costs of high testosterone levels. These potentially include immuno-suppression and the increased risks of parasitism and disease (Folstad and Karter 1992). An experimental study of starlings (*Sturnus vulgaris*) showed that elevated testosterone in both adult males and females reduced the strength of immune responses to a similar extent (Duffy et al. 2000). Numerous other experiments on males have shown negative effects of testosterone on immunity (Casto et al. 2001; Mougeot et al. 2004; Owen-Ashley et al. 2004; review in Roberts et al. 2004). Exposure to androgens and estrogens early in life may have long-lasting effects on immunity, and these effects differ between daughters and sons (Martin 2000). Other potential costs of high testosterone levels are high risks of injury during male–male interactions (Beletsky et al. 1995) and effects on down-regulation of immunity during sperm production (Folstad and Skarstein 1997; Hillgarth et al. 1997). Additionally, high levels of testosterone may affect levels of activity (Wada 1986) and costly sexual display (e.g., Arnold 1975; Nottebohm et al. 1987; Marler et al. 1988; Rost 1990, 1992; DeVoogd 1991; Dittami et al. 1991; Schwabl and Kriner 1991), thereby increasing the production of free radicals. Free radicals are known to have effects on somatic and germline mutation rates (e.g., Leffler 1993; Ahmad 1995; Gregus and Klaassen 1996).

There is considerably less information on the costs and benefits of high testosterone levels in females. Androgens in female birds are mainly secreted by pre-ovulatory follicles (Harvey et al. 1986). The function of these hormones in females remains unclear, apart from their biochemical role in the synthesis of estrogens (Staub and De Beer 1997). Androgens are also passed from mother to offspring via egg yolk, as first shown by Schwabl (1993), and it has been shown that females may vary their androgen investment in the offspring as a function of male ornamentation (Gil et al. 1999, 2003). Given that there is considerable variation in concentration of testosterone in egg yolk (range from 2.4 to 51.1 pg/mg across 101 species (D. Gil et al. unpublished information)), we could also imagine that there is scope for direct selection on female testosterone levels. For example, mothers may allocate testosterone to eggs to give their sons a head start in life, and this may particularly be the case in species with high levels of extra-pair paternity, which generally have high circulating levels of testosterone (Garamszegi et al. 2005). There are few empirical studies of female testosterone levels in the wild. A study by Cristol and Johnsen (1994) on female red-winged blackbirds (*Agelaius phoeniceus*) showed that female testosterone profile peaked when aggression rates peaked during the breeding cycle. A second study by Wingfield et al. (1980) of the highly colonial Western gull (*Larus occidentalis*) showed very high levels of testosterone in females com-

pared to other species. Third, in the moorhen (*Gallinula chloropus*), a sexually monomorphic monogamous bird, both sexes have similar testosterone levels during the reproductive season and the size and other characteristics of the red frontal shield respond very quickly to experimental changes in testosterone in both males and females (Eens et al. 2000; Eens and Pinxten 2000). Fourth, a study of the dark-eyed junco (*Junco hyemalis*) revealed that testosterone implants delayed start of egg laying relative to termination of nest building (Clotfelter et al. 2004). Finally, an experimental study of zebra finches (*Taeniopygia guttata*) showed that injection of testosterone in females during laying disrupted ovulation and reduced clutch size (Rutkowska et al. 2005). This may indicate that high T levels are incompatible with normal female reproduction. Thus, there is some albeit limited information suggesting that circulating levels of testosterone in females are under direct selection. However, numerous studies of testosterone in males provide evidence of direct selection, suggesting that selection for high circulating levels of testosterone may mainly act on males rather than females.

Selection on individuals of one sex often results not only in responses to selection in that sex, but also to correlated responses in the other sex (Roff 1997). If the genetic correlation is perfect (i.e., the genetic correlation between sexes equals one), we would expect a similar response to selection in the two sexes (Roff 1997). Such strong genetic correlations may eventually after a very large number of generations and/or extreme selection pressures be broken up by different patterns of selection in the two sexes. However, even in this situation it is possible to imagine correlated responses to selection because genetic correlations rarely decrease to zero. Thus, selection on males may impose strong selection pressures on females, and this may have fitness consequences for females, which is the cause of further divergence in phenotype between individuals of the two sexes.

The aim of the present study was to document evolutionary covariation in testosterone levels between the sexes, using birds as a model system. This was done by investigating the independent evolution of testosterone profiles during the reproductive season in males and females while considering similarity in phenotypic values among species due to common ancestry. The second aim of the study was to analyze patterns of coevolution by investigating to which extent the hypothesis that changes in male state of peak testosterone concentration precede changes in female state of peak testosterone concentration. Furthermore, we tested whether the slope between circulating testosterone levels in males and females differed significant from unity. We would expect that to be the case since selection against circulating testosterone in females should be particularly intense in species with very high levels in males. The third aim was to test the prediction that females have particularly high levels of testosterone in colonial species of birds. In colonial species, females play a crucial role in defense of breeding territories (Burger et al. 1980), as has been shown intraspecifically in the cliff swallow (*Petrochelidon pyrrhonota*) (Smith et al. 2005). In the

Discussion we consider the potential consequences for females of indirect selection due to selection on males.

Materials and methods

Data set

Information on testosterone was obtained from an extensive search of the literature combined with unpublished information. Information was obtained for a total of 56 species. Garamszegi et al. (2005) provide a list of references, while the references for *Dolichonyx oryzivorus*, *Fregata magnificens*, *Hemignathus virens*, *Himatione sanguinea*, *Sula nebouxii* and *Vestiaria coccinea* are Wingfield et al. (1999), Chastel et al. (2005) and J. Wingfield unpublished data.

Circulating levels of testosterone were used as reported in the original sources and expressed as ng/ml. We used average peak levels for males and females estimated as the maximum natural level during the annual cycle. The estimate for males was significantly repeatable among the studies for species with more than a single estimate, as shown by one-way analysis of variance ($F=13.30$, df$=22,46$, $P<0.0001$, $R=0.80$), while there were too few data to calculate repeatability for females. Thus, we assume that the highly significant repeatability recorded for males also applies to females.

We classified the species as solitarily or colonially breeding depending upon whether they had all-purpose breeding territories, or just small territories with a nest site based on the information obtained from handbooks, in particular Cramp and Perrins (1977–1994) and Glutz and Bauer (1966–1997). The data set is presented in the appendix.

Comparative methods

We analyzed the predictions posed in the Introduction by statistical tests controlling for similarity in variables of interest due to common ancestry. This was done by relying on statistically independent standardized linear contrasts, as originally suggested by Felsenstein (1985). We used the computer program CAIC for the analyses (Purvis and Rambaut 1995). Branch lengths were generated in either of two ways according to Purvis and Rambaut (1995): either all branches in the phylogeny are considered to have the same length (similar to a punctuational model of evolution), or the ages of taxa are proportional to the number of species they contain (similar to a gradual model of evolution). However, the results generated from the two models were qualitatively similar, and we hence only present the results for the models based on branch lengths proportional to the number of species that they contain. The variables were log$_{10}$-transformed to obtain normal distributions. We used coloniality as a continuous variable despite it being a variable with states of either zero or one. The reason for this approach is that intermediate states are biologically meaningful, and the approach is akin to the use of dummy variables in standard regression analysis (Sokal

and Rohlf 1995). All regressions were forced through the origin (Garland et al. 1992), and standardization of contrast values was checked by examination of absolute values of standardized contrasts versus their standard deviations (Garland 1992; Garland et al. 1992).

We calculated the slopes of the relationship between peak female and peak male testosterone levels using the original log-transformed data and the linear contrasts. Since both the variables were measured with error, we estimated the reduced major axis, which in this situation provides an unbiased estimate of the slope (Kermack and Haldane 1950). We calculated this slope by standardizing the dependent and the independent variable to a mean of zero and a standard deviation of one and then calculated the slope of the regression (Sokal and Rohlf 1995, p 550). This procedure has the advantage that it can readily be adopted for contrasts as well.

We tested for the order of evolutionary events in the coevolution of male and female testosterone levels using Pagel's discrete variable method available in the software Discrete (Pagel 1994). For this approach we categorized both male and female testosterone levels as being larger or smaller than the mean of the distribution. Collapsing continuous variables into two-state categories might reduce the power of the analysis due to loss of information. The discrete method applies a continuous-time Markov model to characterize evolutionary changes along each branch of a phylogenetic tree. Two models are fitted to the data, one allowing only for independent evolution, the other for correlated evolution of the two characters. Hypothesis testing in this program relies on likelihood ratio test statistics. This compares the log-likelihood of the model corresponding to a null hypothesis (H_0) over the model for an alternative hypothesis (H_1), where the likelihood ratio (LR) $= -2$ log$_e$[H_0/H_1]. Then the LR statistic for the discrete model (omnibus test) compares the fit of the independent model as H_0 to the fit of the dependent or correlated evolution model as H_1. This test is asymptotically chi-square distributed with df$=4$, and a Monte Carlo simulation procedure can be used to derive the null hypothesis distribution of significance. The advantage of the discrete model is that it enables one to ask not only whether the two variables tend to evolve together, but also whether changes in one variable make changes in another more or less likely than expected by chance. We used the discrete variables method to test the temporal ordering and direction of evolutionary change of testosterone levels in males and females. The method allows various tests, with df$=1$, of whether specified character transitions are significantly different from zero or from each other. Transition rate parameters, q_{ij}, denote the rate of change from state i to state j. The subscripts refer to the start and the end character states for each particular transition, where 1$=$0,0, 2$=$0,1, 3$=$1,0, and 4$=$1,1. We tested models of evolution in which certain types of transitions are excluded a priori, by forcing the relevant parameters (q_{ij}) to zero. The fit of the reduced model in which one parameter is constrained is then compared to the full model of eight parameters. To test whether evolutionary transitions in female testosterone levels are contingent upon the

testosterone levels in males, we compared parameters for parallel evolutionary routes. If q_{12} and q_{34} or q_{21} and q_{43} differ then this is evidence that changes in female testosterone levels are more likely given the particular state of male testosterone levels. Similar hypotheses were tested by forcing the relevant parameters to take the same values, thereby creating a model with seven parameters. We assumed that the ancestral state of male and female peak testosterone was low. This seems reasonable since the initial evolution of endocrine mechanisms must have started out with low concentrations of testosterone. In addition, the evolution of polygyny and extra-pair paternity preceded the evolution of high peak testosterone levels in males (Garamszegi et al. 2005).

The general topology of the phylogenetic tree (Fig. 1) for our comparative analyses was derived from a number of sources using molecular techniques for which references are given in Garamszegi et al. (2005). We applied branch lengths from the tapestry tree of Sibley and Ahlquist (1990) for higher taxonomic levels. Within families the distance between different genera was set to 3.4 $\Delta T_{50}H$ units, and between species within genera to 1.1 $\Delta T_{50}H$ units (Bennett and Owens 2002; Sibley and Ahlquist 1990). We resolved the phylogeny with complete bifurcation as required for the chosen phylogenetic method.

Results

We recorded female testosterone estimates from 56 species belonging to ten orders, with 25 belonging to Passeriformes, 8 to Charadriiformes, 7 to Ciconiiformes, 4 to Sphenisciformes, 3 to Galliformes and Anseriformes, 2 to Struthioformes and Columbiformes and 1 each to Accipitriformes and Psittaciformes. Thus, there was a huge taxonomic diversity. Female peak circulating levels of testosterone ranged from 0.05 to 2.45 ng/ml across the 56 species, with a mean value of 0.64 ng/ml (SE=0.08). Thus, the range of concentration across species varied by a factor of 49. The values for males of the same species ranged from 0.33 to 7.87 ng/ml, with a mean value of 3.17 ng/ml (SE=0.27). Thus, the range of concentration across species varied by a factor of 24.

A paired t-test revealed a highly significant difference between peak levels of testosterone for males and females (t-test based on log-transformed values: t=13.75, df=55, $P<0.0001$). Since the mean logged value for males was 0.40 (SE=0.33) and the mean logged value for females was -0.35 (SE=0.39), females differed from males by 2.2 standard deviation units. Even for the log-transformed data the variance for females, 0.16, was larger than the variance for males, which was 0.11.

Female peak circulating levels of testosterone were positively correlated with male peak levels when based on log-transformed concentrations for the 56 species (Fig. 2; F=9.38, df=1,55, r^2=0.15, P=0.003). The slope of the reduced major axis was 0.39 (SE=0.16). The reduced

major axis slope for the transformed variables was significantly less than unity for the regression between female and male peak levels of circulating testosterone. That was the case when the calculations were based on species-specific values (t=3.81, df=54, $P<0.001$), and when based on statistically independent linear contrasts (t=4.30, df=54, $P<0.001$). Thus female testosterone increased less than expected from a predicted isometric relationship. Female peak circulating levels of testosterone were positively correlated with male peak levels in an analysis of independent contrasts that controlled for similarity in values among taxa due to common ancestry (F=9.31, df=1,52, r^2=0.15, P=0.004), with the reduced major axis slope 0.27 (SE = 0.17).

Females of colonial species had significantly larger peak levels of circulating testosterone than females of solitary species (Fig. 3a; F=10.54, df=1,54, P=0.002). Likewise, there was a significant difference in the relative peak level of circulating testosterone in females with respect to coloniality, after controlling statistically for peak level in males (Fig. 3b; F=10.76, df=1,53, P=0.002). These conclusions remained qualitatively similar when based on statistically independent contrasts (absolute level in females: F=6.65, df=1,52, P=0.013; residual levels in females: F=7.09, df=1,52, P=0.010). Polygyny, extra-pair paternity, mating system, latitude and testes size, that have all been shown to correlate with peak testosterone levels in males (Garamszegi et al. 2005), did not explain significant variation in female peak testosterone (results not shown).

We investigated the casual relationship between peak levels of testosterone in males and females by using the phylogenetic method developed for discrete variables. We found significant evidence for correlated evolution of male and female testosterone levels on a categorical scale (LR=6.783, $P<0.01$ after 100 simulations). Hence, we proceeded with hypothesis testing corresponding to the temporal order of changes by using discrete models with significant evidence for correlated evolution.

We characterized the most likely route of evolution from the ancestral state, in which both males and females had low testosterone levels. Table 1 shows the log-likelihood values for the alternative models. The coupled evolution of testosterone levels in males and females can be summarized by the following major events (Fig. 4). First, testosterone levels in males increased without an increase in females (transition rate q_{13}). Second, female peak testosterone levels augmented (transition rate q_{34}). However, testosterone levels in both sexes can decrease (transition rates q_{31} and q_{43}), and can vary independently of each other from the state where males have high and females have low testosterone levels. Testing for the dependence of testosterone levels of females on that of males, we found that the evolution of peak testosterone levels in females are significantly more likely, when males have high levels of testosterone (Table 1). On the other hand, there was no significant evidence that male testosterone levels were contingent upon female testosterone levels.
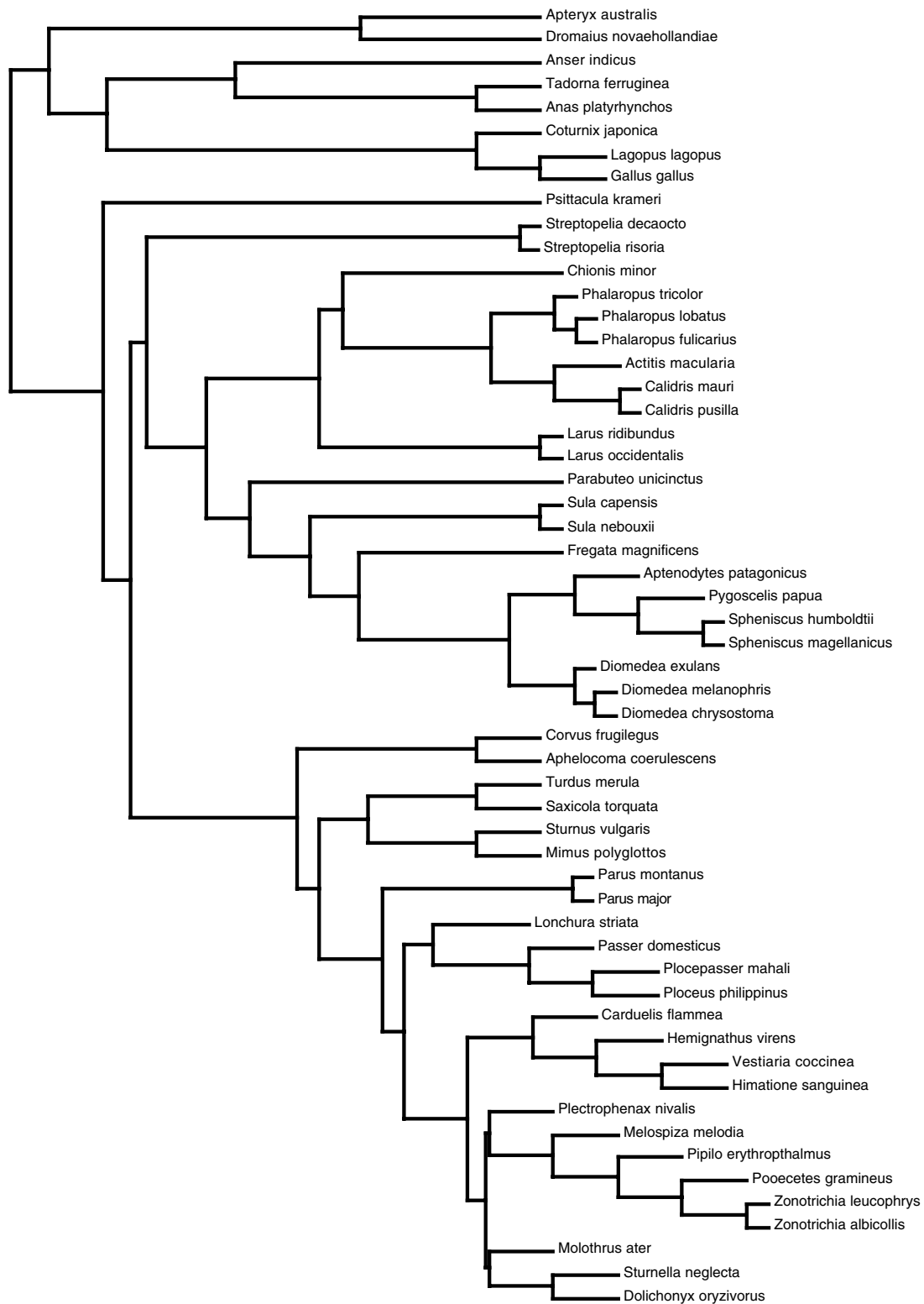
Apteryx australis
Dromaius novaehollandiae
Anser indicus
Tadorna ferruginea
Anas platyrhynchos
Coturnix japonica
Lagopus lagopus
Gallus gallus
Psittacula krameri
Streptopelia decaocto
Streptopelia risoria
Chionis minor
Phalaropus tricolor
Phalaropus lobatus
Phalaropus fulicarius
Actitis macularia
Calidris mauri
Calidris pusilla
Larus ridibundus
Larus occidentalis
Parabuteo unicinctus
Sula capensis
Sula nebouxii
Fregata magnificens
Aptenodytes patagonicus
Pygoscelis papua
Spheniscus humboldtii
Spheniscus magellanicus
Diomedea exulans
Diomedea melanophris
Diomedea chrysostoma
Corvus frugilegus
Aphelocoma coerulescens
Turdus merula
Saxicola torquata
Sturnus vulgaris
Mimus polyglottos
Parus montanus
Parus major
Lonchura striata
Passer domesticus
Plocepasser mahali
Ploceus philippinus
Carduelis flammea
Hemignathus virens
Vestiaria coccinea
Himatione sanguinea
Plectrophenax nivalis
Melospiza melodia
Pipilo erythropthalmus
Pooecetes gramineus
Zonotrichia leucophrys
Zonotrichia albicollis
Molothrus ater
Sturnella neglecta
Dolichonyx oryzivorus

Fig. 1 Phylogenetic relationships between the species included in this study. The scale on the phylogeny is one $\Delta T_{50}H$ unit

Discussion

The first main finding of the present study was that peak levels of circulating testosterone in female birds were strongly positively correlated with peak levels of testosterone in males (Fig. 2). Furthermore levels in females were much smaller than in males, but despite this mean difference of 2.2 standard deviations, the variance in levels in females was still greater than the variance in males. The positive
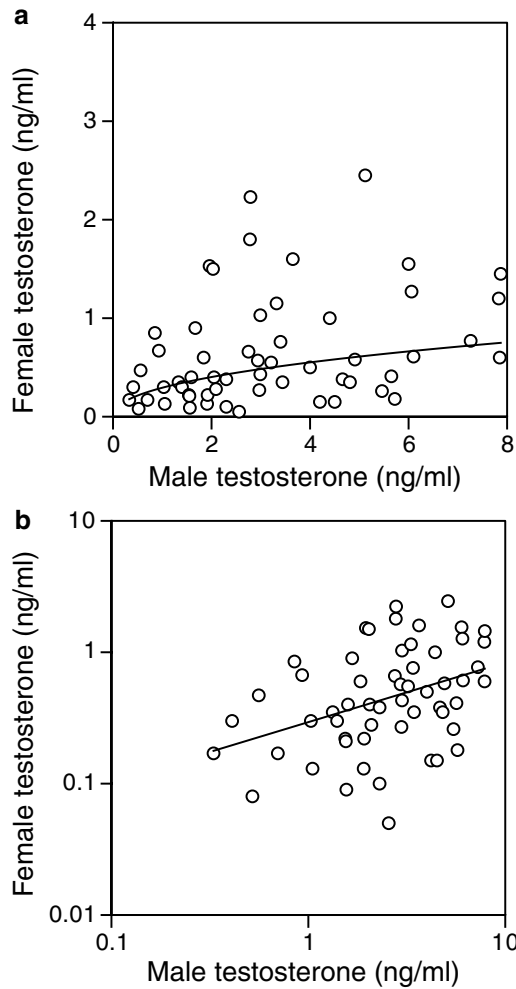
Fig. 2 Peak female circulating testosterone (ng/ml) in relation to peak male circulating testosterone (ng/ml) in birds on (**A**) ordinary and (**B**) log-transformed axes. The line is the linear regression line with the equation log(Peak female testosterone) = −0.53 (SE=0.08)+0.45 (SE=0.15) log(Peak male testosterone)

Fig. 3 (**A**) Mean (±SE) peak female testosterone (ng/ml) and (**B**) residual mean (±SE) peak female testosterone after controlling statistically for peak male testosterone in solitarily and colonially breeding species

relationship between peak levels in females and in males was also present when based on statistically independent linear contrasts that take similarity in phenotype among taxa due to common ancestry into account. An analysis of the order of evolutionary events implied that evolution of high levels of male testosterone preceded the evolution of high levels in females, and that levels of testosterone in males were the main cause of changes in levels in females. This finding implies that selection for high testosterone in males also causes a correlated response to selection in females. Female peak testosterone levels were lower than male levels. In addition, female circulating peak testosterone levels were relatively lower in species with very high levels of male peak testosterone, as shown by the slope of the relationship being significantly smaller than one. This latter observation is consistent with the hypothesis that selection for increased testosterone in males is associated with a relative decrease in females. We hypothesize that this decrease in females of species in which males have very high circulating levels of testosterone is associated
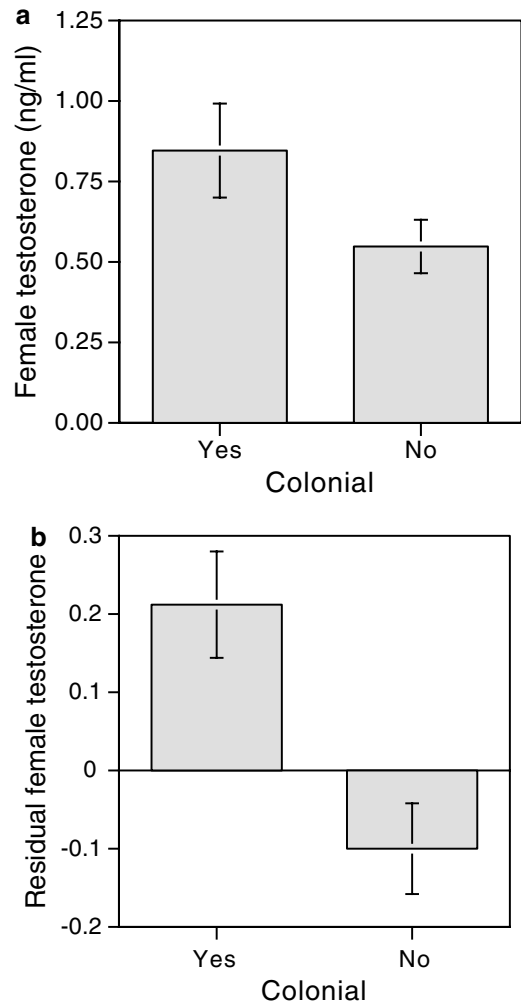
with the costs of high testosterone levels in females. We briefly reviewed the literature on selection on male and female testosterone in birds in the Introduction. We will briefly discuss the implications of our findings in the following paragraphs.

The second major finding of this study was that peak levels of circulating testosterone in females were significantly higher in colonial as compared to solitarily breeding species. This relationship was as predicted, since females play a crucial role in defense of scarce nest sites in colonial species (Burger et al. 1980). Furthermore, an intraspecific study of the cliff swallow showed a positive relationship between circulating testosterone and colony size in both sexes (Smith et al. 2005). The finding that females have absolutely higher levels of peak testosterone in colonial species, but also relatively higher levels after taking into account the levels in males of the same species, suggest that colonial species of birds are the most likely species in which the costs of high testosterone levels can be estimated. Colonial species are generally exploited by
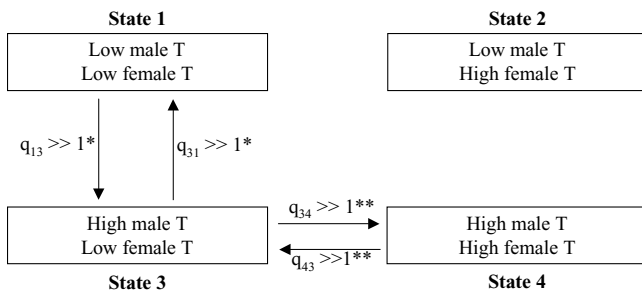
Fig. 4 Flow diagram tracing the most likely evolutionary scenario for the coupled evolution of male and female peak testosterone levels in birds. Only significant pathways are shown. The q_{ij}s are the transition rate parameters, with subscripts referring to the beginning and end character states for each particular transition, where 1=0,0, 2=0,1, 3=1,0, and 4=1,1. *$P<0.05$,**$P<0.01$. See also Table 1

many virulent parasites that cause considerable parasite-induced mortality among nestlings (Møller and Erritzøe 1996). Colonial species have therefore evolved strong immune defenses compared to solitary species, with the size of immune defense organs and the magnitude of T- and B-cell mediated immunity increasing with colony size (Møller and Erritzøe 1996; Møller et al. 2001). Androgens have been implicated to have not only dual functions of stimulation of secondary sexual characters and sexual displays, but also of immuno-suppression (Folstad and Karter 1992). A large number of empirical studies are consistent with this immuno-competence handicap hypothesis (Casto et al. 2001; Mougeot et al. 2004; Roberts et al. 2004; Owen-Ashley et al. 2004). We hypothesize that high levels of testosterone in females of colonial bird species are particularly harmful because colonial species suffer disproportionately from parasite-induced natural selection.

High levels of circulating testosterone in females may also have implications for reproduction and immunity. First, high testosterone is usually considered to suppress reproduction in females (Balthazart 1983), as shown experimentally for the zebra finch (Rutkowska et al. 2005).

Table 1 Comparisons of likelihood values for alternative discrete models of evolution, in which one transition is excluded or one parameter is restricted to be equal with another, versus an eight-parameter model of dependent evolution of male and female peak testosterone levels. The log-likelihood of the full, eight-parameter model was −67.40

Alternative models	$L(D_7)$	LR	P
$q_{12}=0$	−67.40	0.00	1.000
$q_{13}=0$	−70.17	5.54	0.019
$q_{21}=0$	−67.40	0.00	1.000
$q_{24}=0$	−67.91	1.02	0.312
$q_{31}=0$	−69.81	4.82	0.028
$q_{34}=0$	−71.43	8.06	0.005
$q_{42}=0$	−67.77	0.74	0.390
$q_{43}=0$	−71.51	8.22	0.004
$q_{12}\neq q_{34}$	−70.46	6.13	0.013
$q_{21}\neq q_{43}$	−69.18	3.57	0.059
$q_{13}\neq q_{24}$	−67.72	0.64	0.800
$q_{31}\neq q_{42}$	−69.02	3.23	0.072

Thus, females may experience a trade-off between benefits and costs of high testosterone titers. However, an elevated level of testosterone in females may be compensated by physiological mechanisms although the development and the maintenance of these mechanisms may also impose costs on females. These mechanisms include androgen-metabolizing enzymes, changes in receptor sensitivity and changes in degradation of testosterone. There is an extensive literature on aromatase activity in specific tissues that convert testosterone into estrogen (e.g., Baillien and Balthazart 1997; Balthazart 1997; Balthazart et al. 2001; Riters et al. 2001; Soma et al. 2000). However, it remains unknown whether aromatase activity varies among species in relation to the costs and benefits of testosterone. Second, there are numerous studies suggesting that a decreasing number of testosterone receptors in females compared to males may modulate the effects of testosterone (e.g., Dewil et al. 1998; Gahr 2003). For instance, the territorial behavior of females found in some sex-role reversed shorebirds is not caused by increased testosterone levels (Fivizzani et al. 1986), but possibly brought about by changes in androgen receptivity. Third, degradation of testosterone can be regulated, and females that produce detrimental levels of testosterone may be able to regulate such levels by increased rates of degradation. Again, there is no information available suggesting that this is the case.

A second major implication is that testosterone has been considered to have immuno-suppressive effects, thereby enforcing reliability on signaling systems that involve testosterone (Folstad and Karter 1992). Several recent studies have suggested that testosterone indeed may have such an effect (e.g., Casto et al. 2001; Mougeot et al. 2004; review in Roberts et al. 2004). If this effect proves to be common, we can predict that females in species of birds with high testosterone levels will suffer more from immuno-suppression than females in species with low testosterone titers. Indeed, Duffy et al. (2000) have shown that implanting starling females with high male T levels elicits a suppression of humoral (but not cell mediated) immunity. Similarly, De Ridder et al. (2002) recently investigated the effects of testosterone implantation on female starlings to evaluate short- and long-term costs. Testosterone-treatment strongly suppressed tail feather re-growth after experimental plucking, and also delayed onset of molt of wing feathers and slowed its progress. T-females were significantly more infected with *Staphylococcus aureus* than control females. Thus, they demonstrated several different costs of high testosterone levels in females. If such immuno-suppression turns out to be particularly common in colonial species, in which females have disproportionately high circulating levels of peak testosterone, we would predict that females are trading the testosterone-dependent benefits arising from territory defense against the testosterone-dependent costs of immuno-suppression.

Covariation in circulating testosterone levels between adult males and females may have consequences for the deposition by female birds of testosterone in their eggs (e.g., Schwabl 1993), since the concentrations in eggs have been shown to reflect circulating levels in the plasma of

the mother (Schwabl 1996a). Egg testosterone has been shown to affect begging and growth of nestlings (Eising et al. 2001; Lipar and Ketterson 2000; Schwabl 1996b), as well as subsequent development of dominance in adults (Schwabl 1993). Apart from immune suppression, high female testosterone levels may impose costs in nestlings through increased begging intensity that may cause non-adaptive brood reduction (Mock and Parker 1997) and increased nest predation (Haskell 1994). It is also fully imaginable that maternal testosterone deposited in eggs may affect not only offspring behavior, growth and immunity, but also influence the trade-offs between these variables. Thus, correlated responses in females to selection on male circulating titers of testosterone may have important consequences for the performance of offspring in the next generation, mediated through maternal effects.

In conclusion, we found evidence of coevolution of circulating testosterone levels in adult male and female birds. Circulating testosterone was lower in females than in males, but relatively lower in species with high male peak testosterone. Females of colonially breeding species had particularly high levels of peak testosterone levels compared to females of solitarily breeding species. This decrease in female titers may have arisen as a consequence of fitness costs incurred by females, including female immuno-suppression and effects of immuno-suppression on offspring performance through maternal testosterone in eggs. Alternatively, there may be less parental care in species with high male testosterone levels, and females may therefore adjust investment in offspring by boosting development through hormone deposition.

Appendix

Table A Information on species, coloniality (0 – solitary, 1 – colonial), maximum peak male testosterone (ng/ml) and maximum peak female testosterone (ng/ml). References are given in Garamszegi et al. (2005)

Species	Coloniality	Male testosterone (ng/ml)	Female testosterone (ng/ml)
Actitis macularia	0	6.10	0.61
Anas platyrhynchos	0	3.44	0.35
Anser indicus	1	3.21	0.55
Aphelocoma coerulescens	0	1.56	0.09
Aptenodytes patagonicus	1	7.26	0.77
Apteryx australis	0	2.30	0.10
Calidris mauri	0	1.59	0.40
Calidris pusilla	0	2.99	1.03
Carduelis flammea	0	4.50	0.15
Chionis minor	1	1.67	0.90
Corvus frugilegus	1	3.40	0.76
Coturnix japonica	0	7.85	0.60
Diomedea chrysostoma	1	1.96	1.53
Diomedea exulans	1	5.12	2.45
Diomedea melanophris	1	2.03	1.50
Dolichonyx oryzivorus	0	1.40	0.30
Dromaius novaehollandiae	0	6.06	1.27
Fregata magnificens	1	1.03	0.30
Gallus gallus	0	7.83	1.20
Hemignathus virens	0	4.81	0.35
Himatione sanguinea	0	2.94	0.57
Lagopus lagopus	0	1.91	0.13
Larus occidentalis	1	0.41	0.30
Larus ridibundus	1	4.66	0.38
Lonchura striata	0	1.54	0.22
Melospiza melodia	0	7.87	1.45
Mimus polyglottos	0	2.30	0.38
Molothrus ater	0	1.92	0.22
Parabuteo unicinctus	0	0.52	0.08
Parus major	0	1.05	0.13
Parus montanus	0	1.33	0.35
Passer domesticus	1	5.65	0.41
Phalaropus fulicarius	0	6.00	1.55
Phalaropus lobatus	0	5.46	0.26

Table A Continued

Species	Coloniality	Male testosterone (ng/ml)	Female testosterone (ng/ml)
Phalaropus tricolor	0	4.91	0.58
Pipilo erythropthalmus	0	5.72	0.18
Plectrophenax nivalis	0	4.20	0.15
Plocepasser mahali	1	0.33	0.17
Ploceus philippinus	0	1.55	0.21
Pooecetes gramineus	0	4.40	1.00
Psittacula krameri	0	0.93	0.67
Pygoscelis papua	1	2.97	0.27
Saxicola torquata	0	1.84	0.60
Spheniscus humboldtii	1	3.32	1.15
Spheniscus magellanicus	1	2.75	0.66
Streptopelia decaocto	0	2.09	0.28
Streptopelia risoria	0	0.70	0.17
Sturnella neglecta	0	4.00	0.50
Sturnus vulgaris	1	2.78	1.80
Sula capensis	1	0.85	0.85
Sula nebouxii	1	0.56	0.47
Tadorna ferruginea	0	3.65	1.60
Turdus merula	0	2.56	0.05
Vestiaria coccinea	0	2.05	0.40
Zonotrichia albicollis	0	2.79	2.23
Zonotrichia leucophrys	0	2.99	0.43

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