Behavioral Ecology Advance Access published March 1, 2007

Behavioral Ecology
doi:10.1093/beheco/arm004

Maternal immune factors and the evolution of secondary sexual characters

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Secondary sexual characters have been hypothesized to reveal the ability of males to resist debilitating parasites. Although such reliable signaling of parasite resistance may be maintained by parasite–host coevolution, maternal effects potentially provide a previously neglected factor that could affect the level of genetic variation in resistance to parasites. That could be the case because maternal effects have an entirely environmental basis, or because they can maintain considerable amounts of genetic variation through epistatic effects, even in the presence of strong directional selection. Maternal effects have been shown to occur as maternal allocation of immune factors to offspring, and such allocation may depend on the mating prospects of sons, causing mothers to differentially allocate maternal effects to eggs in species subject to intense sexual selection. Here we show that a maternal effect through innate antibacterial immune defense, lysozyme, which is transferred from the mother to the egg in birds, is positively associated with the evolution of secondary sexual characters. Previous studies have shown that females differentially allocate lysozyme to their eggs when mated to attractive males, and elevated levels of lysozyme are associated with reduced hatching failure and superior health among neonates and adults. In this study, comparative analyses of lysozyme from eggs of 85 species of birds showed a strong positive relationship between brightness of male plumage and egg lysozyme, even when controlling for potentially confounding variables. These findings suggest that maternal immune factors may play a role in the evolution of secondary sexual characters. *Key words:* birds, comparative analyses, egg, immunity, lysozyme, maternal effects. *[Behav Ecol]*

A distinguishing feature of many models of sexual selection is that females make mate choices for genetic benefits from their partners, even though genetic variation for such benefits may become depleted, because beneficial alleles should go to fixation (Taylor and Williams 1982; Pomiankowski and Møller 1995). This so-called lek paradox may be resolved if more metabolic pathways, and hence their underlying genetic variation, affect the expression of condition-dependent secondary sexual characters compared with other traits (Rowe and Houle 1996). A second scenario is based on parasite–host coevolution maintaining genetic variation in parasite resistance, with only resistant males being able to develop the most exaggerated characters (Hamilton and Zuk 1982; Folstad and Karter 1992). A third scenario relying on mutational input affecting the expression of secondary sexual characters is based on the observation that males generally have higher mutation rates than females (Bartosch-Harlid et al. 2003; Møller and Cuervo 2003; Kirkpatrick and Hall 2004). Although the relative merits of these hypotheses remain unexplored, they may on their own or in combination be sufficient to explain the small amounts of genetic benefits typically found in studies of sexual selection (Møller and Alatalo 1999).

Despite a number of different explanations potentially accounting for the lek paradox, we cannot exclude the possibility that as yet unexplored factors may be important. Here we suggest that maternal effects may constitute one such factor. Maternal effects occur when the mother, or the environment in which she lives, directly affects the phenotype of her offspring (Mousseau and Fox 1998). Maternal effects may be entirely environmental or may be affected by genes, and they can maintain considerable amounts of genetic variation through epistatic effects, even in the presence of strong directional selection (Wade 1998). The reason for this fact is that selection on maternal effects depends on a combination of within- and among-family components (Wade 1998). Maternal effects may have long-lasting consequences for offspring (Mousseau and Fox 1998; Martin 2000; Metcalfe and Monaghan 2001) and thereby affect adult phenotype.

Female oviparous animals allocate many different kinds of components to their eggs including hormones, antioxidants, and immune factors (Rose and Orlans 1981; Schwabl 1993; Mousseau and Fox 1998; Gil et al. 1999; Blount et al. 2000; Kudo 2000). Studies of maternal effects of birds have shown that females differentially allocate substances to eggs depending on sexual attractiveness of their partner (Gil et al. 1999, 2004; Saino, Bertacche, et al. 2002; Saino, Ferrari, et al. 2002; Saino, Martinelli, et al. 2002). Early maternal effects can have

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Received 21 February 2005; revised 16 January 2007; accepted 17 January 2007.

important consequences for the development of immune function in adults (Martin 2000). Thus, there is scope for maternal effects affecting performance of offspring when these reach adulthood.

In all vertebrates, lysozyme is a fundamental component of innate antibacterial immune defense that is transferred from the mother to the egg in birds, digesting the peptoglycanes that are major components of bacterial cell walls (Tizard 1991; Trziszka 1994; Braun and Fehlhaber 1996; Roitt et al. 1996; Pastoret et al. 1998; Kudo 2000). Concentration of egg lysozyme reflects circulating levels in mothers, and females may trade allocation to eggs against the use of lysozyme for their own immune protection. Egg lysozyme may be carried over into the embryo and nestling (Board and Fuller 1974). Egg lysozyme enhances hatching success and may facilitate offspring survival (Melek 1977; Prusinowska and Jankowski 1996; Prusinowska et al. 2000; Saino, Martinelli, et al. 2002). However, information is unavailable for the duration of the effects of maternal lysozyme transmission to offspring.

The aim of this comparative study was to investigate whether lysozyme as a maternal effect is positively related to the expression of a secondary sexual character, plumage coloration. Females have been shown to allocate lysozyme to their eggs depending on the attractiveness of the mates in one species, the barn swallow *Hirundo rustica* (Saino, Martinelli, et al. 2002). The positive association between lysozyme concentration in the eggs and paternal ornamentation may occur as a result of selection for larger maternal allocation to eggs laid for high-quality males. We may therefore expect that as secondary sexual characters become exaggerated across species, females would be selected to allocate an ever-larger amount of lysozyme to eggs, which is a potentially very important immune factor that would allow further exaggeration of secondary sexual characters. This is the case because exaggerated male plumage brightness may reflect the long-term effects of intense sexual selection. The possible cost for females of doing so in terms of reduction in their own immune defense would be balanced by an increased benefit in terms of mating success of their sons due to the greater variance in male than in female reproductive success. We tested this prediction by determining whether male and female coloration were positively related to lysozyme concentration of eggs, assuming that a brighter coloration of males compared with females would reflect an increase in the intensity of sexual selection. In fact, sexual dichromatism has been shown to reflect interspecific variation in sperm competition and in the level of polygyny (e.g., Andersson 1994; Møller and Birkhead 1994). We controlled for similarity in phenotype among taxa due to common descent by calculating standardized linear contrasts that reveal convergent evolution rather than similarity among species due to common descent (Felsenstein 1985).

METHODS

Field procedures

We collected nonincubated eggs of 85 species of birds under permit in Europe ($n = 60$), North America ($n = 1$), and South Africa ($n = 21$) (plus *Gallus gallus*, *Numida meleagris*, and *Phasianus colchicus* eggs collected from captive stocks) during 2000–2003. Sample sizes were kept to a minimum for ethical reasons, while still allowing for tests of variance among and within species. For 14 species, some eggs could not be univocally assigned to their original clutch at the time of lysozyme analysis. For the remaining 71 species, the mean number of eggs collected per clutch was 1.58 (0.07 standard error [SE], range 1–6). We based our analyses on the general mean lysozyme concentration for each species computed on all eggs available for that species, irrespective of the clutch of origin. For the 71 species for which all eggs available to lysozyme analysis could be assigned to their original clutch, there was an extremely high correlation between the mean lysozyme concentration computed over all eggs of each species, irrespective of their clutch of origin, and the mean of the clutch means for that species ($r = 0.996$, $n = 71$, $P < 0.0001$). Because the mean values computed in the 2 ways were highly positively correlated, and the adoption of the general mean allowed us to include the 14 species for which assignment of some eggs was uncertain, we decided to adopt this approach.

Quantifying lysozyme in eggs

Eggs were separated into egg white and yolk, and the egg white was used for lysozyme analysis. Both components of the eggs were frozen as soon as possible after separation (usually within 1 day) and then transferred into a −20 °C freezer until analysis. Lysozyme activity was determined using the lysoplate method on egg white (Osserman and Lawlor 1966), modified in order to carry out the assay using a micromethod. The test was performed on an agar gel with a dried strain of *Micrococcus lysodeikticus* (M-3770, Sigma Chemical Co., Milano, Italy), which is particularly sensitive to lysozyme activity, by inoculating each test hole with 12.5 µl albumen. Crystalline hen egg white lysozyme (L-6876, Sigma Chemical Co.) was used to prepare a standard curve in each plate. Agar gels were incubated at 27 °C for 20 h, and the area of the gel surrounding the plasma inoculation site where bacterial growth was inhibited was measured using an ad hoc ruler and converted into hen egg lysozyme equivalents (HEL equivalents, expressed in µg/ml) according to the standard curve. Hence, lysozyme activity is expressed as HEL per milliliter throughout this study (µg/ml) and assumed to reflect concentration (Brightman et al. 1991). Intra-assay coefficient of variation for the albumen was 9.6%. Interassay coefficient of variation was 10.3%. Lysozyme activity was $\log_{10}$ transformed to achieve normality. There was considerably more variance among than within species in lysozyme content of eggs ($F_{84,583} = 72.42$, $P < 0.001$, $r^2 = 0.90$) based on $\log_{10}$-transformed data. The data set is reported in the Appendix.

Color scores of adult birds

We scored the brightness of the plumage of adult males and females in full breeding plumage using color plates in field guides (Peterson et al. 1988; National Geographic Society 1992; Sinclair et al. 1993). Color scores on a scale from 1 to 6, where 1 is uniform and drab and 6 is conspicuous and bright, were highly repeatable among 3 independent scorers, who were unaware of the purpose of the study (male scores: $F_{84,170} = 15.30$, $P < 0.0001$, $r^2 = 0.825$; female scores: $F_{84,170} = 8.70$, $P < 0.0001$, $r^2 = 0.718$; sex difference in scores: $F_{84,170} = 29.58$, $P < 0.0001$, $r^2 = 0.904$). Color scores were only assessed for the part of the spectrum that is visible to humans.

Confounding variables

Breeding habitat was classified as either aquatic, including marshes, meadows, and other humid habitats (score of 0) or terrestrial (score of 1). Mating system was scored as monogamous (score of 0) or nonmonogamous (score of 1). Breeding sociality was scored as 0 for solitary species, 1 for species breeding in colonies with 2–10 pairs, 2 for species breeding in colonies with 11–100 pairs, 3 species breeding in colonies with 101–1000 pairs, and 4 species breeding in colonies with more than 1000 pairs. We used maximum colony size. Information was obtained from Glutz von Blotzheim (1966–1997),

Cramp et al. (1982–1994), and Fry et al. (1982–2004) combined with our own field experience.

Comparative methods

We constructed a composite phylogeny of the species based on Sibley and Ahlquist (1990), Sheldon and Winkler (1993), Crochet et al. (2000), and Barker et al. (2001) (Figure A1). The phylogeny is presented in the Appendix.

We calculated statistically independent linear contrasts for each variable according to the method developed by Felsenstein (1985) using the software CAIC (Purvis and Rambaut 1995). All branches were assigned the same length, although a second set of analyses based on uneven branch lengths, assuming a gradual evolution model as implemented in the software, produced qualitatively similar results. We tested for violations of statistical assumptions by regressing standardized contrasts against their standard deviations (Garland et al. 1992). None of these tests revealed any significant deviations, after Bonferroni adjustment for multiple tests. Contrasts with extreme residuals were deleted from analyses to test for robustness of results (Jones and Purvis 1997), and this did not change any of the conclusions presented here. Similarly, tests using ranked independent variables in cases with extreme residuals did not produce qualitatively different results. Contrasts were analyzed by forcing regressions through the origin because the dependent variable a priori is expected not to have evolved when the independent variable has not shown any evolutionary change (Harvey and Pagel 1991).

Regression analyses require that models not be overparameterized relative to the number of observations in the data set (Neter et al. 1989; Sokal and Rohlf 1995; Zar 1996). Therefore, we attempted not to increase the number of predictors in order to strike a balance between having exhaustive models and having models with a low number of predictor variables. We scrutinized multiple regression analyses for multicollinearity of independent variables. However, in no case did the condition index indicate that parameter estimates were substantially biased by multicollinearity problems, as also suggested by the low values of the pairwise correlations between independent variables ($r < 0.56$ in all cases). Residuals of lysozyme concentration on independent variables were normally distributed.

RESULTS

Mean concentration of lysozyme was 295.18 μg/ml, range 0.03–8719.35 μg/ml, $n = 85$ species and 668 eggs, or on average 7.9 eggs per species. If maternal effects through lysozyme in eggs affected the evolution of plumage brightness, we should expect a positive association between plumage brightness and lysozyme across taxa. Indeed, there was a strong positive relationship for male plumage brightness across 85 species of birds (Figure 1A; $F_{1,83} = 39.40$, $r^2 = 0.32$, $P < 0.0001$, slope [SE] = 0.45 [0.07]), as predicted, whereas the relationship was not statistically significant for plumage brightness of females (Figure 1B; $F_{1,83} = 3.72$, $r^2 = 0.04$, $P = 0.06$), which can be considered to act as an internal control group. When we excluded from the analysis 7 data points (i.e., those corresponding to *Anas platyrhynchos*, *Euplectes orix*, *G. gallus*, *Merops hirundineus*, *Nectarinia chalybea*, *P. colchicus*, and *Ploceus velatus*) in the upper right part of Figure 1A that subjectively appeared to stretch the relationship between lysozyme and male plumage brightness, the relationship was still positive and highly significant ($F_{1,76} = 23.00$, $r^2 = 0.23$, $P < 0.001$). The difference in plumage brightness between males and females (dichromatism) was positively associated with lysozyme concentration in eggs (Figure 1C; $F_{1,83} = 24.01$, $r^2 = 0.22$, $P < 0.0001$, slope [SE] = 0.44 [0.09]). A multiple regression showed no significant interaction between male and female brightness, whereas the main effect of male brightness remained significant (Table 1). Based on sum of squares reported in Table 1, the effect of the interaction would be significant in an analysis of as many as approximately 170 species.

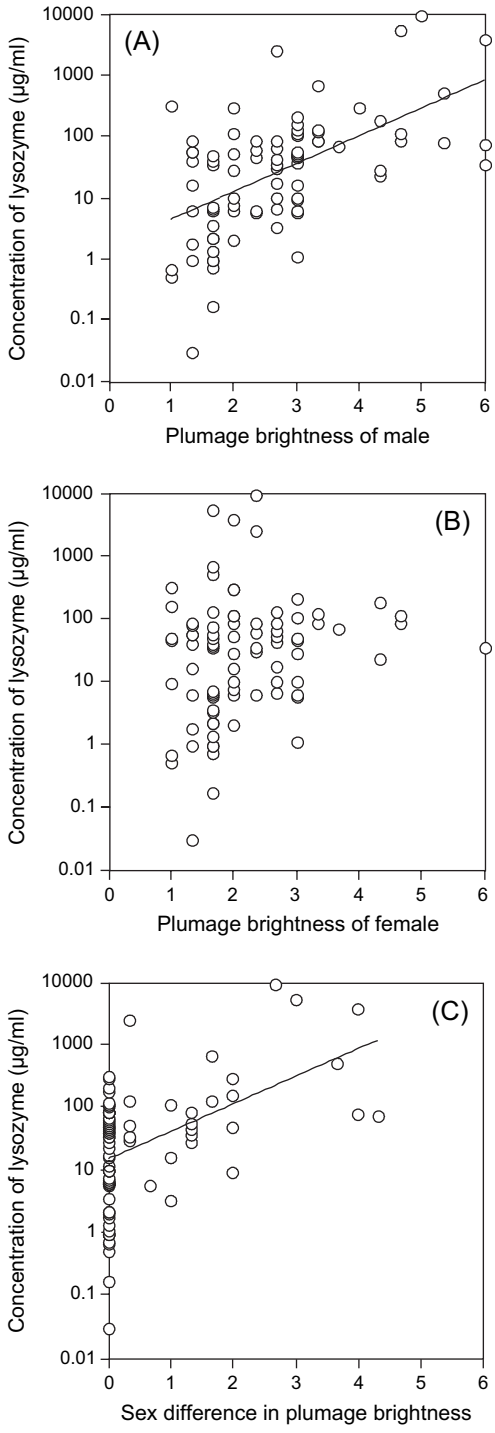

Figure 1
Mean egg lysozyme concentration (μg/ml) in relation to plumage brightness of bird species. (A) Brightness of male plumage, (B) brightness of female plumage, and (C) sex difference in brightness of plumage (male minus female brightness). The regression lines are shown for significant relationships.

Table 1

Multiple linear regressions between $\log_{10}$-transformed lysozyme concentration (μg/ml) and plumage brightness of males, females, and their interaction

Parameter	Sum of squares	df	Coefficient (SE)	t	P
Species					
Intercept		1	−0.33 (0.54)	−0.62	0.54
Male brightness	13.75	1	0.69 (0.15)	4.68	<0.001
Female brightness	0.34	1	0.22 (0.29)	0.74	0.46
Male brightness × female brightness	1.21	1	−0.09 (0.07)	−1.39	0.17
Error	50.86	81			
Independent contrasts					
Male brightness	0.65	1	0.22 (0.08)	2.64	0.01
Female brightness	0.17	1	−0.15 (0.11)	−1.35	0.18
Male brightness × female brightness	0.29	1	0.22 (0.12)	1.78	0.08
Error	7.11	77			

The 2 models had the following statistics when based on species: $F_{3,81} = 14.76$, $r^2 = 0.35$, $P < 0.0001$, and on independent contrasts: $F_{3,77} = 3.96$, $r^2 = 0.13$, $P = 0.011$. df, degrees of freedom.

Table 2

Multiple linear regressions between $\log_{10}$-transformed lysozyme concentration (μg/ml) and plumage brightness of birds, breeding habitat, and breeding sociality

Parameter	Sum of squares	df	Coefficient (SE)	t	P
Species					
Intercept		1	−0.25 (0.54)	−0.46	0.65
Male brightness	9.37	1	0.58 (0.13)	4.42	<0.001
Female brightness	0.09	1	0.11 (0.26)	0.43	0.68
Male brightness × female brightness	0.88	1	−0.08 (0.06)	−1.36	0.18
Breeding habitat	4.69	1	0.66 (0.21)	3.13	0.002
Breeding sociality	2.50	1	−0.18 (0.08)	−2.29	0.03
Error	37.89	79			
Independent contrasts					
Male brightness	0.60	1	0.22 (0.08)	2.58	0.01
Female brightness	0.24	1	−0.18 (0.11)	−1.63	0.11
Male brightness × female brightness	0.28	1	0.21 (0.12)	1.76	0.08
Breeding habitat	0.01	1	0.07 (0.28)	0.24	0.81
Breeding sociality	0.32	1	−0.16 (0.09)	1.88	0.06
Error	6.71	75			

The 2 models had the following statistics when based on species: $F_{5,79} = 17.01$, $r^2 = 0.49$, $P < 0.001$, and on independent contrasts: $F_{5,75} = 3.33$, $r^2 = 0.18$, $P = 0.009$. df, degrees of freedom.

Analysis of contrasts revealed similar conclusions, with a significant effect of male plumage brightness and no effect of the interaction with female plumage brightness, indicating that the slope of the relationship between lysozyme concentration and plumage brightness of either sex did not vary according to plumage brightness of the other sex (Table 1). However, in this case, only approximately 100 data points (i.e., contrasts) would make the effect of the interaction significant. These conclusions are robust with respect to the topology of the phylogeny because any uncertainty in the phylogeny will be reflected in estimates of rates of evolution for both males and females.

Regression analyses of lysozyme concentration in eggs based on species-specific data where we also included body mass as a regressor revealed that levels were elevated in species from terrestrial habitats ($F_{1,82} = 12.56$, $P = 0.001$, $r^2 = 0.17$, slope [SE] = 0.95 [0.27]), solitary breeders ($F_{1,82} = 18.26$, $P < 0.001$, $r^2 = 0.16$, slope [SE] = −0.33 [0.08]), and monogamous species ($F_{1,82} = 8.17$, $P = 0.005$, $r^2 = 0.08$, slope [SE] = 0.71 [0.25]). A multiple regression based on species-specific values where we included the effects of male and female plumage brightness together with their interaction showed that only habitat and coloniality retained their significant effects (Table 2), whereas the effect of mating system could be excluded by step-down selection of nonsignificant regressors (Crawley 1993). In this analysis, although the effect of plumage brightness on lysozyme concentration was still highly significant and positive after controlling for the concomitant effect of female plumage brightness and other confounding effects, female plumage brightness did not predict lysozyme concentration (Table 2). Removal of nonsignificant terms presented in the raw data model in Table 2 did not alter the results concerning significant predictors.

A multiple regression approach for the linear contrasts only showed significant effects of male plumage brightness, with the effects of female brightness and its interaction with male plumage brightness, breeding habitat, and breeding sociality not being statistically significant (Table 2). Thus, our conclusions of a relationship between lysozyme concentration and male plumage brightness based on contrasts were unaffected by potentially confounding variables (details not shown). Step-down exclusion of nonsignificant terms from the contrasts-based model in Table 2 disclosed a marginally significant negative effect of coloniality ($F_{1,78} = 3.95$, $P = 0.050$, $r^2 = 0.08$, slope [SE] = 0.71 [0.25]).

In an analysis of species-specific data, lysozyme was predicted by sexual dichromatism, expressed as the within-species difference between male and female plumage brightness scores ($F_{1,83} = 24.01$, $P < 0.001$, $r^2 = 0.22$, slope [SE] = 0.44 [0.09]). A multiple regression model of species-specific data with lysozyme as the dependent variable and sexual dichromatism, habitat, and coloniality as predictors produced a model that explained 47% of the variance ($F_{3,81} = 23.99$, $P < 0.001$). The partial regressions were significant for sexual dichromatism ($F_{1,81} = 24.62$, $P < 0.001$, slope [SE] = 0.38 [0.08]), habitat ($F_{1,81} = 9.68$, $P = 0.003$, slope [SE] = 0.67 [0.22]), and coloniality ($F_{1,81} = 9.64$, $P = 0.003$, slope [SE] = −0.24 [0.08]).

An analysis of contrasts with lysozyme as the dependent variable and sexual dichromatism as the independent variable showed a significant, positive effect ($F_{1,79} = 5.60$, $P = 0.020$, slope [SE] = 0.18 [0.08]).

A multiple regression of contrasts including lysozyme as the dependent variable showed a positive effect of sexual dichromatism ($F_{1,77} = 5.75$, $P = 0.02$, slope [SE] = 0.18 [0.08]) and a negative effect of coloniality ($F_{1,77} = 4.13$, $P = 0.046$, slope [SE] = −0.18 [0.09]).

An analysis of species-specific lysozyme data with ranked values of the same independent variables included in the model presented in Table 2 led to qualitatively identical results with, in particular, a significant positive effect of male plumage brightness on lysozyme concentration ($F_{1,79} = 15.91$, $P < 0.001$, slope [SE] = 0.02 [0.01]). Similarly, an analysis of ranked contrasts of independent variables with the same model as in Table 2 confirmed a significant positive effect of male plumage brightness on lysozyme concentration ($F_{1,75} = 9.76$, $P = 0.003$, slope [SE] = 0.005 [0.002]).

We also repeated these analyses with ranked values of sexual dichromatism, rather than plumage brightness of the 2 sexes separately, and found that lysozyme concentration was

positively predicted by species-specific sexual dichromatism ($F_{1,81} = 21.00$, $P < 0.001$, slope [SE] = 0.02 [0.004]) as well as by the linear contrasts in sexual dichromatism ($F_{1,78} = 6.82$, $P = 0.01$, slope [SE] = 0.003 [0.001]).

Exclusion of the 3 species from captivity (*G. gallus*, *P. colchicus*, and *N. meleagris*) did not change the conclusions (results not shown).

DISCUSSION

Our main findings were that 1) females allocated more lysozyme to their eggs in species with bright male plumage; 2) therefore, eggs contained more lysozyme in sexually dichromatic species than in monochromatic species; and 3) this was the case even when taking potentially confounding variables into account and when controlling for similarity among species due to common descent. However, the statistical effect of a unit change in plumage brightness of either sex on lysozyme concentration did not depend on the concomitant effect of plumage brightness of the other sex, as indicated by the nonsignificant interaction. We will discuss the implications of these observations.

Sexual dichromatism explains variation in egg lysozyme concentration even after controlling for other variables that were independently related to lysozyme concentration. None of the potentially confounding variables accounted for additional variation in lysozyme content in the contrast analyses, and apparent relationships based on analyses of species-specific data must therefore be ascribed to similarity due to common descent rather than convergent evolution. Although we cannot infer causality in this comparative study, this observation can be interpreted in at least 2 different ways. First, mothers affect the ability of offspring to cope with the parasite environment during early development, providing sons with a benefit when they encounter the costs of developing and maintaining their costly secondary sexual characters. Second, susceptibility to parasitism is often elevated in species with sexual dichromatism, in particular in sons, and mothers may compensate for this susceptibility by allocation of lysozyme to eggs. Adult males are often differentially susceptible to parasitism (Alexander and Stimson 1989; Zuk 1990; Poulin 1996; Møller, Sorci, and Erritzøe 1998), and empirical studies have generally shown that males with the most exaggerated secondary sexual characters tend to have fewer parasites and stronger immune response or larger immune defense organs than less adorned males (Møller et al. 1999). In addition, species with more exaggerated sexual dichromatism tend to have larger immune defense organs (Møller, Dufva, and Erritzøe 1998). Finally, adult but not juvenile males tend to have smaller immune defense organs than females, and this reduction in size of immune defense organs of adult males increases with the intensity of sexual selection as reflected by the frequency of extrapair paternity (Møller, Sorci, and Erritzøe 1998). These findings imply that males in species of birds with exaggerated sexual coloration indeed may be more susceptible to parasitism than females. If this is generally the case, then we can interpret the elevated lysozyme levels in eggs of more sexually dichromatic species we demonstrated in the analyses based on the within-species difference in brightness between the sexes as a maternal mechanism to compensate for immunosuppression in sons when sons reach adulthood. Both of these interpretations are based on the assumption that mothers benefit from differential allocation of maternal effects to eggs when sons have greater variance in reproductive success than daughters because such sons will enjoy an additional probability of reproducing.

There is a dearth of information on the effects of lysozyme on free-living birds, with almost all knowledge being attributed to studies of domesticated chickens and turkeys. The only extensive study of lysozyme in birds is on the barn swallow *H. rustica* (Saino, Martinelli, et al. 2002). Female barn swallows differentially allocate lysozyme to eggs of long-tailed males. This is allocation in the strict sense because the concentration of lysozyme in eggs is considerably higher than in maternal plasma, as also reported for other species (Bizzarri et al. 1999).

The range of lysozyme concentration spanned 6 orders of magnitude across 85 species of birds. We can only speculate about the function of this enormous amount of variation. We suggest that lysozyme functions to protect eggs and neonates from bacterial infection, as shown for chickens and turkeys (Melek 1977; Prusinowska and Jankowski 1996; Prusinowska et al. 2000). Very little is known about the importance of bacteria as a cause of mortality in natural populations of birds, with studies by Cook et al. (2003, 2005) being a notable exception. Lysozyme allocation to eggs may be costly to mothers because lysozyme allocated to eggs is unavailable to mothers, thus potentially trading maternal viability against egg viability (Saino, Martinelli, et al. 2002). The selection pressures that have resulted in the evolution of lysozyme concentrations are likely to be caused by bacteria, and, therefore, we can use data from the present study to make predictions about the species of birds that are likely to suffer particularly from health problems due to bacteria. It could be argued that, because parasite load is known to increase with colony size (e.g., Loye and Zuk 1991), lysozyme concentration should be higher in colonial than in solitary species. The effect of coloniality on lysozyme concentration, however, was found to be negative rather than positive. A possible interpretation of this finding is that a trade-off may exist between allocation of lysozyme to the eggs and maternal immune defense, whereby mothers of colonial species, being more exposed to virulent bacteria, tend to allocate more to own immune defense at the expense of their eggs.

Finally, we suggest that the priming effects of early maternal effects on the development of immunity may be important. Many studies have suggested that the early embryonic environment, which is strongly influenced through maternal effects, may have important implications for the development of immunity (reviewed in Martin 2000).

The results presented here have several implications. First, the lek paradox, which arises from the puzzling observation that directional preferences for genetic benefits of mate choice appear to be common despite the fact that genetic variation in such benefits should disappear, may be resolved if a major source of variation in male quality derives as a consequence of genetic and nongenetic maternal effects, as suggested here. Second, the evolution of sexual dichromatism is linked to maternal innate immunity, suggesting that maternal effects may have played an important role in the evolution of such characters. Third, the interspecific patterns of phenotypic variation observed here also have implications for the evolution of immunity. Studies of chickens, turkeys, and barn swallows have shown positive correlations between lysozyme concentrations in maternal plasma and in eggs and offspring (Melek 1977; Prusinowska et al. 2000; Saino, Martinelli, et al. 2002), suggesting that patterns of phenotypic variation in lysozyme concentration among adults should parallel the patterns observed for eggs. Thus, the importance of this component of immunity varies considerably among species with potential consequences for sexual selection and life history.

In conclusion, we have shown that in species with more sexual dichromatism, eggs receive more maternally derived lysozyme. This suggests that maternal effects may have played a role in the evolution of sexual dichromatism and hence in interspecific variation in sexual selection.

APPENDIX

Information on lysozyme concentration (μg/ml) (SE), sample size (number of eggs), male color, female color, breeding habitat (0, aquatic; 1, terrestrial), and breeding sociality (0, solitary; 1, colonies 2–10 pairs; 2, 11–100 pairs; 3, 101–1000 pairs; 4, more than 1000 pairs).

Species	Lysozyme concentration (μg/ml) (SE)	*N*	Male color	Female color	Breeding habitat	Breeding sociality
Accipiter nisus	5.47 (1.95)	3	2.333	1.667	1	0
Aegithalos caudatus	43.35 (8.44)	7	3.000	3.000	1	0
Anas platyrhynchos	75.45 (7.42)	16	5.333	1.333	0	0
Apus affinis	1.30 (0.35)	4	1.667	1.667	1	2
Apus apus	0.48 (0.26)	3	1.000	1.000	1	2
Apus caffer	0.91 (0.44)	2	1.333	1.333	1	1
Apus melba	0.95 (0.39)	2	1.667	1.667	1	2
Athene noctua	6.02 (–)	1	2.000	2.000	1	0
Bostrychia hagedash	5.79 (–)	1	2.333	2.333	1	0
Carduelis carduelis	22.28 (3.46)	4	4.333	4.333	1	1
Carduelis chloris	81.83 (38.82)	7	3.333	2.000	1	1
Cettia cetti	39.73 (–)	1	1.333	1.333	1	0
Clamator glandarius	51.55 (6.65)	6	2.000	2.000	1	0
Colius colius	62.26 (9.94)	3	2.667	2.667	1	0
Columba guinea	9.47 (5.98)	2	2.667	2.667	1	0
Columba livia	6.45 (–)	1	2.667	2.667	1	1
Corvus cornix	54.35 (8.40)	7	1.333	1.333	1	0
Cossypha caffra	83.69 (40.57)	2	3.333	3.333	1	0
Cyanopica cyana	66.46 (7.37)	6	3.667	3.667	1	0
Delichon urbica	39.52 (4.53)	17	1.667	1.667	1	3
Egretta garzetta	0.64 (0.61)	3	1.000	1.000	0	2
Emberiza citrinella	26.37 (2.52)	4	4.333	3.000	1	0
Euplectes orix	70.29 (3.96)	6	6.000	1.667	1	2
Falco tinnunculus	3.17 (0.30)	4	2.667	1.667	1	1
Ficedula albicollis	35.25 (3.66)	6	3.000	1.667	1	0
Ficedula hypoleuca	54.38 (22.50)	2	3.000	1.667	1	0
Fulica atra	6.04 (1.06)	11	1.333	1.333	0	0
Galerida cristata	82.64 (–)	1	1.333	1.333	1	0
Gallinula chloropus	7.37 (0.93)	4	2.000	2.000	0	0
Gallus gallus	8719.35 (2245.81)	4	5.000	2.333	1	0
Gelochelidon nilotica	0.91 (0.06)	9	1.667	1.667	0	2
Haematopus ostralegus	16.41 (5.67)	5	2.667	2.667	0	0
Hirundo cucullata	118.73 (47.33)	6	3.333	3.333	1	0
Hirundo fuligula	111.90 (–)	1	2.000	2.000	1	1
Hirundo rustica	32.93 (1.06)	184	2.667	2.333	1	2
Hirundo semirufa	80.24 (10.08)	7	4.667	4.667	1	0
Hirundo spilodera	200.65 (58.84)	5	3.000	3.000	1	3
Larus cachinnans	5.89 (0.40)	20	1.667	1.667	0	3
Larus genei	2.10 (0.10)	11	1.667	1.667	0	3
Larus ridibundus	6.43 (1.01)	3	1.667	1.667	0	4
Luscinia megarhynchos	312.90 (50.07)	3	1.000	1.000	1	0
Merops hirundineus	32.98 (7.64)	3	6.000	6.000	1	0
Motacilla alba	2460.91 (450.37)	5	2.667	2.333	1	0
Nectarinia chalybea	503.63 (–)	1	5.333	1.667	1	0
Numida meleagris	50.43 (5.51)	5	3.000	2.667	1	0
Nycticorax nycticorax	3.34 (–)	1	1.667	1.667	0	3
Panurus biarmicus	126.65 (10.60)	28	3.000	2.667	0	1
Parus caeruleus	107.03 (8.23)	17	4.667	4.667	1	0
Parus cinerascens	82.22 (4.41)	10	2.667	2.667	1	0
Parus major	177.71 (31.54)	20	4.333	4.333	1	0
Parus palustris	27.05 (9.31)	4	2.000	2.000	1	0
Passer domesticus	9.14 (1.21)	3	3.000	1.000	1	1
Passer hispaniolensis	48.66 (7.37)	9	3.000	1.000	1	4
Passer italiae	151.90 (43.54)	4	3.000	1.000	1	1
Passer melanurus	110.44 (14.36)	4	3.000	2.000	1	1
Passer montanus	30.13 (4.34)	21	2.667	2.333	1	1
Phalacrocorax carbo	0.03 (0.00)	13	1.333	1.333	0	3
Phasianus colchicus	3591.41 (138.31)	3	6.000	2.000	1	0
Philetairus socius	9.92 (–)	1	2.000	2.000	1	2
Phoenicurus ochruros	657.34 (39.25)	2	3.333	1.667	1	0
Phoenicurus phoenicurus	275.89 (34.88)	4	4.000	2.000	1	0
Pica pica	47.64 (5.79)	8	3.000	3.000	1	0
Platalea leucorodia	1.73 (0.42)	2	1.333	1.333	0	3
Ploceus velatus	5095.89 (2839.70)	5	4.667	1.667	1	2
Podiceps cristatus	5.42 (1.21)	8	3.000	3.000	0	1
Recurvirostra avosetta	1.08 (0.08)	6	3.000	3.000	0	2
Saxicola torquata	124.33 (77.88)	4	3.333	1.667	1	0
Serinus serinus	15.46 (1.29)	5	3.000	2.000	1	0
Sitta europaea	102.87 (11.63)	3	3.000	3.000	1	0
Sterna albifrons	2.13 (0.21)	12	1.667	1.667	0	2
Sterna hirundo	0.69 (0.10)	8	1.667	1.667	0	3
Sterna sandvicensis	0.16 (0.14)	5	1.667	1.667	0	4
Streptopelia decaocto	6.99 (–)	1	1.667	1.667	1	0
Streptopelia senegalensis	1.95 (0.25)	3	2.000	2.000	1	0
Sturnus vulgaris	281.65 (46.84)	5	2.000	2.000	1	1
Sylvia atricapilla	33.54 (–)	1	1.667	1.667	1	0
Tachycineta bicolor	57.05 (3.48)	8	2.333	2.333	1	0
Troglodytes troglodytes	15.79 (6.11)	3	1.333	1.333	1	0
Turdus merula	43.04 (4.47)	9	2.333	1.000	1	0
Turdus olivaceus	83.80 (29.84)	3	2.333	2.333	1	0
Turdus philomelos	56.08 (7.83)	5	1.333	1.333	1	0
Turdus pilaris	47.52 (46.56)	2	1.667	1.667	1	2
Vanellus armatus	9.38 (7.07)	2	3.000	3.000	1	0
Vanellus coronatus	5.96 (0.78)	4	3.000	3.000	1	0
Vanellus vanellus	41.28 (22.35)	4	2.667	2.667	0	1

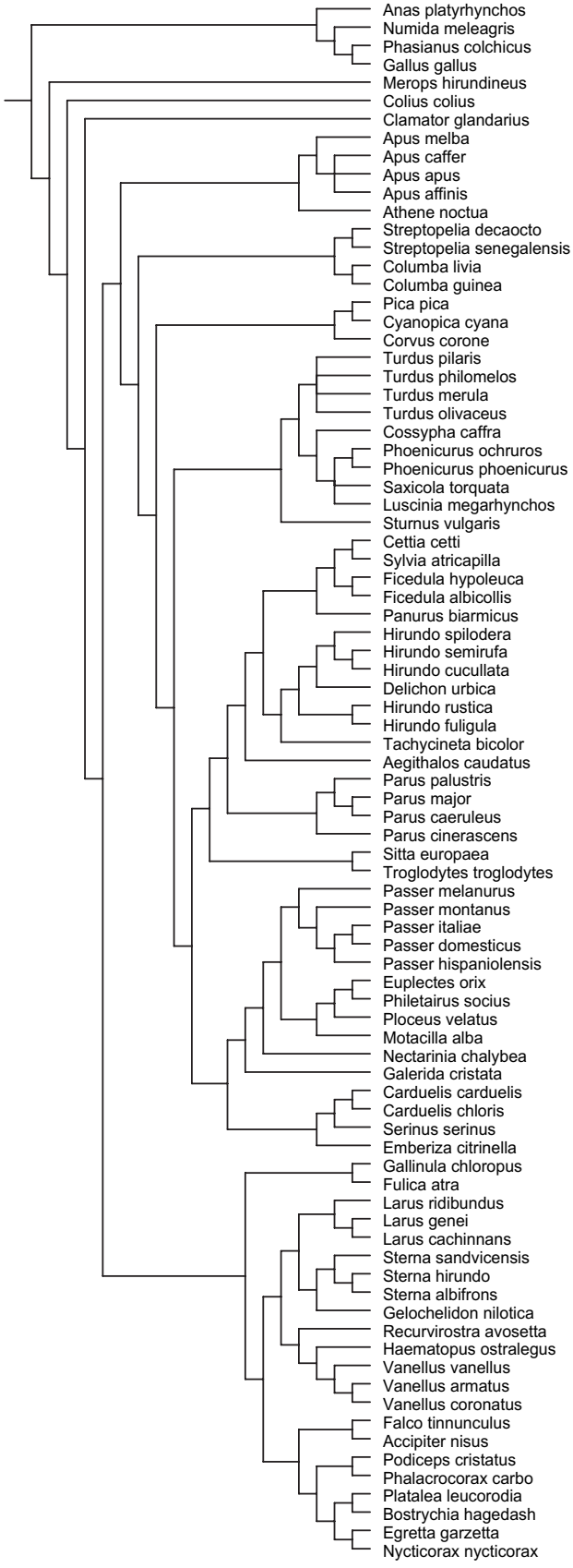

Figure A1
Phylogeny of the 85 bird species included in the study of maternal effects and sexual selection, based on Sibley & Ahlquist (1990), Barker et al. (2003), Crochet et al. (2000) and Sheldon & Winkler (1993).

We are thankful to N. Baccetti, M. Caffi, M. Fasola, R. Visagie for help in collecting the eggs. We are also grateful to the Northern Cape Province's Department of Tourism, Environment, and Conservation for egg-collecting/research permits. Two referees provided constructive comments to earlier drafts of the paper.

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