Seasonal Changes in Immune Response and Parasite Impact on Hosts

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Submitted May 2, 2002; Accepted September 6, 2002; Electronically published XX

abstract: Seasonal changes in the impact of parasites on hosts should result in seasonal changes in immune function. Since both ectoparasites and endoparasites time their reproduction to that of their hosts, we can predict that hosts have been selected to show an annual peak in their ability to raise an immune response during the reproductive season. We found large seasonal changes in immune function between the breeding and the nonbreeding season for a sample of temperate bird species. These changes amounted to a decrease in spleen mass from the breeding to the nonbreeding season by on average 18% across 71 species and a seasonal decrease in T-cell-mediated immunity by on average 33% across 13 species. These seasonal changes in immune function differed significantly among species. The condition dependence of immune function also differed between the breeding and the nonbreeding season, with individuals in prime condition particularly having greater immune responses during breeding. Analyses of ecological factors associated with interspecific differences in seasonal change of immune function revealed that hole-nesting species had a larger increase in immune function during the breeding season than did open nesters. Since hole nesters suffer greater reduction in breeding success because of virulent parasites than do open nesters, this seasonal change in immune function is suggested to have arisen as a response to the increased virulence of parasites attacking hole-nesting birds.

Keywords: birds, parasite-host interactions, phytohemagglutinin, spleen, T-cell-mediated immune response.

q1 Most parts of the world are characterized by seasonal environments, where seasonal peaks of productivity change with periods of little or no productivity. Organisms often time their reproductive season so that it coincides with seasonal peaks of productivity (e.g., Lack 1954; Wingfield and Kenagy 1991). A number of different studies have shown that parasites time their own reproduction so that it coincides with that of their hosts, and that is the case for ectoparasites, helminths, malaria, and virus (Chernin 1952; Foster 1969; Applegate and Beaudoin 1970; Marshall 1981; Vindervogel et al. 1985; Hughes et al. 1989; Allander and Sundberg 1997; Christe et al. 2000). Depending on duration of the life cycle, parasite reproduction will cause an increase in the abundance of parasites at the end of the reproductive season of the host (references above). q2 Additional factors that increase the abundance of parasites are not only the costs of reproduction in hosts and the associated decrease in immune function below what it would have been in the absence of reproduction (Folstad and Karter 1992; Møller 1993; Deerenberg et al. 1997; Nordling et al. 1998; Moreno et al. 1999; Saino et al. 2002) but also the density-dependent increase in parasite abundance associated with increases in host population density (Anderson and May 1982). Thus, selective pressures arising from multiplication by parasites and damage caused by parasites will tend to peak during the reproductive season of the host, but particularly so at the end of the season.

Host defenses have been hypothesized to evolve in response to selection pressures arising from parasites, with the evolutionarily stable defense strategy of hosts coevolving with the offense strategy of parasites to reach a peak in productive environments (van Baalen 1997; Hochberg and van Baalen 1999). In a similar vein, if seasonal patterns of host defenses have evolved in response to patterns of parasite impact, we should expect peaks in defense to mirror peaks in offense. Therefore, costs of immune function should result in seasonal phenotypic changes in immune function mirroring those of seasonal changes in parasite impact. Several recent reviews have suggested that immune function is costly in terms of time, energy, micronutrients, or autoimmune disease (Råberg et al. 1998; von Schantz et al. 1999; Lochmiller and Deerenberg 2000;

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Am. Nat. 2003. Vol. 161, pp. 000–000. 0003-0147/2003/16103-020182$15.00.

Møller et al. 2000*a*) and that investment in defense should thus mirror peaks of offense.

Seasonal changes in rates of parasitism should affect seasonal changes in the level of antiparasite defenses. Preliminary studies of phenotypic changes in immune function during the breeding season of hosts have shown that T-cell-mediated immune response of nestlings of two species of passerines increases from the first to the second brood of the same parents, even though environmental conditions for reproduction decrease (Merino et al. 2000, 2002). However, parasite-induced mortality increased in the same host species with the progressing breeding season (de Lope and Møller 1993; de Lope et al. 1993). These findings suggest that seasonal changes in immune function during the breeding season parallel changes in parasite attacks.

Several studies of mammals and other vertebrates have suggested that lymphatic organ size peaks in late fall or early winter (reviewed in Isogai et al. 1992; Lochmiller et al. 1994; Nelson and Demas 1996). While these patterns have been suggested to reflect an adaptation to the effects of severe winter conditions on host-parasite interactions (Nelson and Demas 1996), an alternative interpretation is that this seasonal peak in immune function reflects a seasonal peak in parasite-mediated natural selection. However, the number of species studied is limited, and the general pattern of seasonal change in immune function and its causes thus merit further study. For example, there is no clear seasonal pattern of change in spleen size in the different bird species investigated so far. Silverin (1981) presented data on spleen mass of pied flycatchers *Ficedula hypoleuca* during spring migration, breeding, and fall migration. Values peaked during laying, incubation, and the early nestling period, with lower values during migration. In the resident great tit *Parus major*, Silverin (1981) found maximal spleen size during the breeding season but also high values during late summer and early fall. A similar pattern was found for the resident willow tit *Parus montanus* (Silverin et al. 1999). In migratory and nonmigratory populations of white-crowned sparrows *Zonotrichia leucophrys*, Oakesson (1953, 1956) reported peak spleen masses during winter but also a very large size in March and April. Riddle (1928) found enlarged spleens in ring doves *Streptopelia risoria* during spring and summer, and Krause (1922) found a similar pattern in rock doves *Columba livia*. Despite there only being studies of six species, these have produced general claims in the literature concerning seasonality of immune function. Clearly, studies of only six species that do not show a similar seasonal pattern do not justify generalizations. Furthermore, it remains unclear to what extent seasonal changes in immune defense reflect seasonal changes in investment or whether this is confounded by selection. It is possible that a seasonal change in immunity is entirely a result of individuals with a weak immune response dying more often than other individuals.

We suggest four different ways in which seasonal changes in immune function can be assessed. First, a direct assessment of seasonal changes in immune function could be achieved by measurement of phenotypic changes in the same individuals during the annual cycle. This has not been done yet, to the best of our knowledge.

A second way would be to investigate whether seasonal changes are similar in different age classes. Juveniles are likely to have experienced less intense selection than later age classes, and they should thus have greater phenotypic variance than individuals from older age classes. Therefore, we should expect different patterns of seasonal change in immune function among juveniles and adults if selection had played an important role in generating patterns.

A third possible method to resolve the confounding effects of selection and seasonal change is to investigate whether seasonal changes in immune function differ among taxa that are subject to differences in intensity of parasite-mediated selection. Two factors have been hypothesized to result in increased virulence (reviewed in Ewald 1983; Bull 1994; Frank 1996). Parasites may increase in virulence if they are frequently horizontally transmitted, because horizontal transmission does not result in reduced parasite fitness when hosts are severely damaged by parasitism (Ewald 1983). Similarly, when parasites of different genetic strains coexist, they are expected to show greater virulence than when a single strain occurs (Bull 1994; Frank 1996). Thus, hosts that are likely to be affected by horizontally transmitted parasites or by multiple strains of parasites should particularly suffer from parasitism. In accordance with this scenario, colonially breeding birds and hole-nesting birds that reutilize nest sites are particularly negatively affected by parasitism, and they have evolved strong immune responses compared with sister taxa (Møller and Erritzøe 1996; Møller et al. 2001). Direct evidence for differences in level of parasitism between these different categories of hosts exists for ectoparasites and blood parasites (reviewed in Møller and Erritzøe 1996; Møller et al. 2001; Tella 2002). Thus, we should expect seasonal change in immune function to be particularly strong in host species that are affected by virulent parasites.

Fourth, immune function is condition dependent in domestic animals, humans, and a number of wild animals (Chandra and Newberne 1977; Gershwin et al. 1985; Møller et al. 1998; Alonso-Alvarez and Tella 2001). Individuals in prime condition generally have larger immune defense organs and show stronger immune responses to a challenge with a novel antigen than individuals in poor condition. Such condition dependence may have evolved because a large number of different metabolic pathways

contribute to the production of an efficient immune response. However, we can predict that condition dependence will be particularly prominent during the part of the year when selection pressures are the most intense, since individuals in prime condition will benefit the most from shunting limited resources into immune function during that part of the year. Thus, seasonal patterns of condition dependence of immune function will provide important information about the evolution of seasonal change in immunity.

The main objectives of this study were, first, to describe seasonal patterns of change in immune function in a large number of species of birds to investigate the generality of seasonal changes across species. Second, we investigated to what extent patterns of seasonal change were influenced by age, to address whether juveniles and adults showed consistent patterns of seasonal change. Third, we investigated whether ecological factors that were hypothesized to be associated with virulence could explain interspecific patterns of seasonal variation in immune function. Finally, we investigated condition dependence of immune function during the breeding and the nonbreeding season to determine whether condition dependence was greater during the breeding season, as predicted if parasite-mediated selection was more intense during that period. We analyzed data on spleen mass and T-cell-mediated immune response in birds to a challenge with the mitogenic phytohemagglutinin to test for seasonal change in immune function.

Material and Methods

The spleen is an immune defense organ that comprises part of the peripheral lymphoid tissue. It acts as the main site of lymphocyte differentiation (B-cells) and proliferation (B-cells), and these cells are involved in immune responses (reviewed in Arvy 1965; Rose 1981; Keymer 1982; Molyneux et al. 1983; John 1994). We assume throughout this article that a larger spleen provides a better immune defense than a smaller organ for a bird of a given body size. This assumption is likely to be fulfilled since most of the spleen is composed of lymphocytes (Rose 1981; Alberts et al. 1983; Toivanen and Toivanen 1987; John 1994). A comparative study of spleen size has shown that birds with a more species of nematodes have a relatively larger spleen (Morand and Poulin 2000) and that spleen size in snow geese *Anser caerulescens* reflects helminth infection status (Shutler et al. 1999). Spleen size is not a simple consequence of larger exposure to disease, since both disease status and body condition independently affect spleen size (Møller et al. 1998). Hence, a larger spleen implies not only a greater capacity to produce lymphocytes but also a large storage of lymphocytes.

The size of spleens was obtained from postmortem examinations of 1,974 (641 from the breeding season and 1,333 from the nonbreeding season) dead birds brought to a taxidermist, and they were measured blindly with respect to the hypothesis under test by J. Erritzøe. Birds were frozen when received by J. Erritzøe, and any effect of storage on measurements should cause noise in the data set. Although specimens received by a taxidermist may not provide a random sample, we can ensure that a very small fraction of individuals showed signs of death attributed to hunting or direct signs of disease. More than three-quarters of all individuals had died from collision with windows, cars, or overhead wires. Information on body mass was available for all of the specimens. We tested for two potential biases in the data set. First, we tested whether the variance in organ size among species was significantly larger than the variance within species, which is a prerequisite for comparative analyses. There was larger variance among than within species, as shown by one-way ANOVAs of relative organ size measured as the residuals from a linear regression of $\log_{10}$-transformed organ size on $\log_{10}$-transformed body mass ($F = 8.17$, $\mathrm{df} = 20, 309$, $P < .001$). This implies that even a small sample of individuals will provide reliable information on the relative size of the spleen for a given species. That was also the case when the analysis excluded specimens that showed signs of disease or infection. Second, sampling date might influence size estimates of immune organs, since the spleen has sometimes been shown to demonstrate annual fluctuations in size (John 1994). We tested whether date of sampling differed among species but did not find any significant difference (Kruskall-Wallis ANOVA, $P > .40$). The summary data set is reported in appendix A.

Individuals from the breeding season were considered to be from April to August, while individuals from September to March were considered to be from the nonbreeding season. This classification was made to clearly distinguish between birds under the influence of reproductive hormones such as testosterone and estrogen and birds not influenced by their reproductive endocrinological profile.

T-cell-mediated immune response to a challenge with phytohemagglutinin was used as a second measure of immune function. This is a standard estimate from the poultry literature of the ability to produce a T-cell-mediated immune response (Goto et al. 1978; McCorkle et al. 1980; Parmentier et al. 1993; Dietert et al. 1996). Injection with phytohemagglutinin results in local activation and mitogenic proliferation of T-cells, followed by local recruitment of inflammatory cells and major histocompatibility complex molecules (Goto et al. 1978; Abbas et al. 1994; Parmentier et al. 1998). This T-cell-mediated immune response to a challenge of the immune system with an injection with phytohemagglutinin was strongly positively

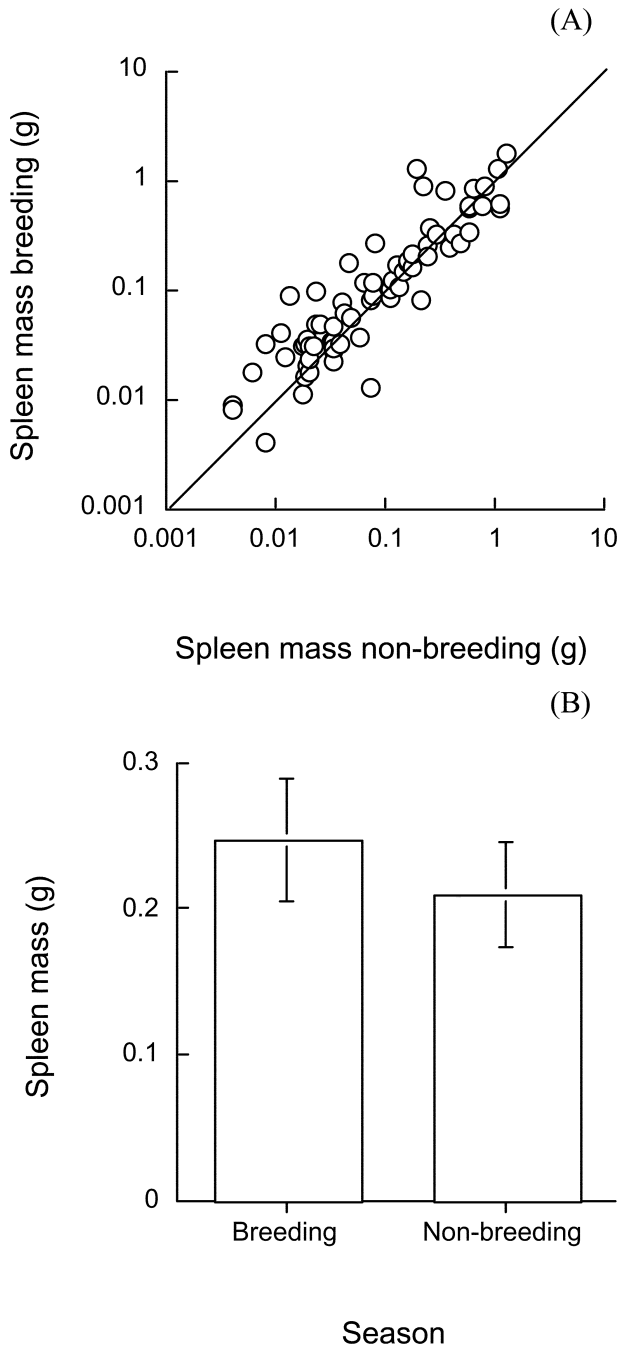

Figure 1: Seasonal change in mean spleen mass (g) of 71 bird species. *A*, Mean values during the breeding season in relation to mean values during the nonbreeding season. The line $y = x$ is included as a reference. *B*, Mean spleen mass (g; ±SE) for the breeding and the nonbreeding season.

correlated with parasite-induced mortality across species of birds (Martin et al. 2001). Birds were injected with 0.05 mL of 0.2 mg phytohemagglutinin (PHA-P) in one wing web and 0.05 mL of physiological water in the other wing web at premarked sites indicated by a mark with a waterproof pen. The dose of PHA used in this study is similar to that used in numerous other studies of free-living or captive birds (Lochmiller et al. 1993; Saino et al. 1997; Christe et al. 1998, 2000, 2001; Birkhead et al. 1999; Brinkhof et al. 1999; González et al. 1999; Hörak et al. 1999; Soler et al. 1999; Merino et al. 2000). We measured the thickness of the patagium injected with PHA and with physiological water before injection and after 6 h, using a pressure-sensitive caliper (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547-301 Japan) with an accuracy of 0.01 mm. Although estimates of T-cell-mediated immune response traditionally have been recorded 24 h postinjection, we measured responses after 3, 6, 12, 24, 36, 48, and 72 h in a study of captive house sparrows *Passer domesticus* and found no significant increase after 6 h (Navarro et al. 2002). Responses of the birds from the nonbreeding season in this study measured after 6 and 12 h were strongly positively correlated (Pearson $r = 0.88$, $N = 134$, $P < .001$), and we found no significant increase in response after 6 h (paired t-test, $t = 0.82$, df = 133, NS), justifying the use of a 6-h period for assessment of T-cell response. A similar finding has been reported by Goto et al. (1978) for chickens. The measure of T-cell response has a very high repeatability, as shown by three independent measurements of both wing webs (first measuring the right

Table 1: Four-way ANOVA with $\log_{10}$-transformed spleen mass as the dependent variable and species, sex, age, and season as factors

Factor	Type III SS	df	*F*	*P*
Species	53.24	13	53.24	<.0001
Sex	.29	1	4.48	.035
Age	.91	1	13.01	.0003
Season	.32	1	4.64	.032
Species × sex	2.92	13	3.22	<.0001
Species × age	2.12	13	2.35	.0046
Species × season	.70	13	.78	.69
Sex × age	.14	1	1.98	.16
Sex × season	.02	1	.32	.57
Age × season	.05	1	.76	.38
Species × sex × age	1.04	13	1.15	.31
Species × sex × season	.80	13	.88	.57
Species × age × season	1.41	13	1.55	.09
Sex × age × season	.01	1	.18	.67
Species × sex × age × season	1.15	13	1.27	.23
Residual	47.64	684		

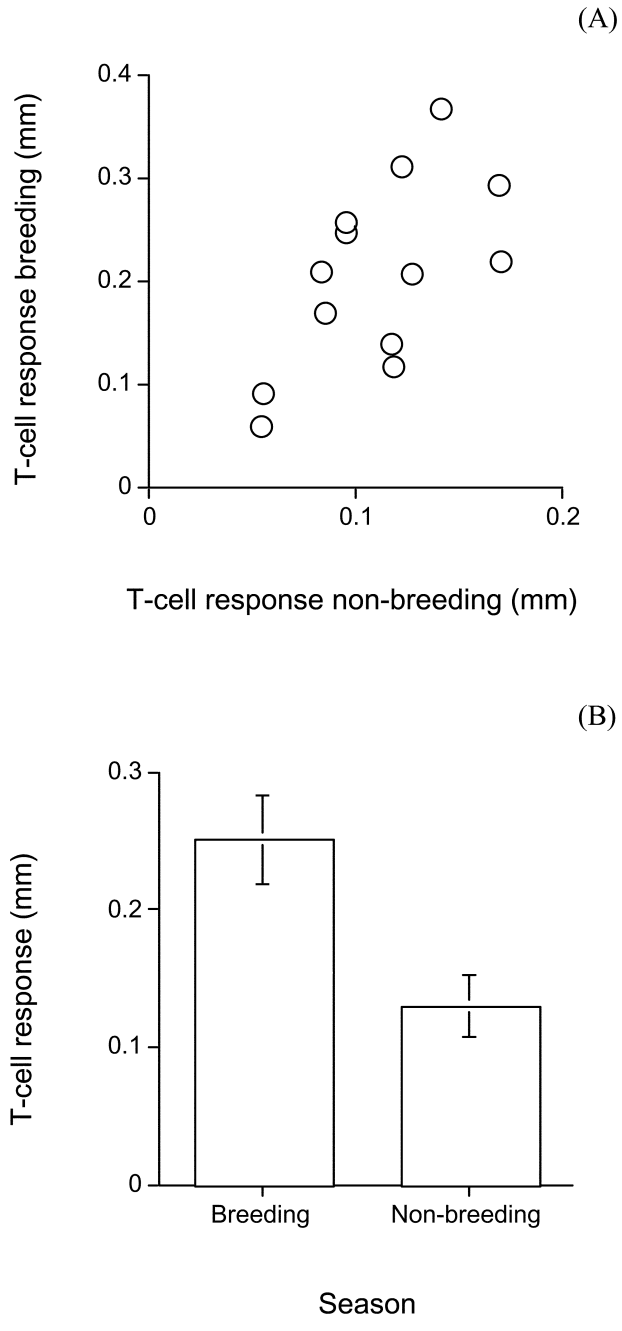

Figure 2: Seasonal change in mean T-cell response (mm) of 13 bird species. *A*, Mean values during the breeding season in relation to mean values during the nonbreeding season. *B*, Mean T-cell response (mm; ±SE) for the breeding and the nonbreeding season.

wing web, then the left wing web, then the right again, etc.; unpublished data). In the subsequent analyses we used the increase in the thickness of the wing injected with PHA minus the increase in the thickness of the wing injected with physiological water as a measure of the intensity of the phytohemagglutinin-induced immune response.

We found highly significant, consistent differences in T-cell-mediated immune response in adults among species when testing these differences with a one-way ANOVA (breeding season: $F = 6.16$, df $= 12, 109$, $P < .0001$; nonbreeding season: $F = 5.51$, df $= 12, 281$, $P < .0001$). Thus, there is considerably more variation among than within species in T-cell response. We captured 416 (122 from the breeding season and 294 from the nonbreeding season) birds in mist nets during the breeding season in May to June 2001 and during the nonbreeding season in December 2001 in Northern Jutland, Denmark. Barn swallows from the nonbreeding season were captured in the winter quarters of the Danish population (as shown by recoveries) near Potchefstrom, South Africa, in January 1995.

All individuals were sexed and aged (yearlings or older) according to criteria listed by Svensson (1995). For species with no external sex criteria during the nonbreeding season, we used laparotomy to distinguish between males and females. The summary data set is reported in appendix B.

When birds were captured, we measured tarsus length (as a measure of skeletal body size) with a digital caliper to the nearest 0.01 mm. Body mass was recorded to the nearest 0.1 g using a Pesola spring balance. Body condition was estimated as the residuals from a regression of body mass on tarsus length raised to the third power. In the analysis of body condition, we could only use 21 species for which a sufficiently large number of individuals were available for both seasons.

Spleen size is not significantly correlated with T-cell-

Table 2: Four-way ANOVA with T-cell response as the dependent variable and species, sex, age, and season as factors

Factor	Type III SS	df	*F*	*P*
Species	.754	12	1.95	.029
Sex	.001	1	.05	.832
Age	.263	1	8.17	.005
Season	.432	1	13.40	.0003
Species × sex	.139	12	.36	.976
Species × age	.103	12	.27	.994
Species × season	.557	12	1.44	.147
Sex × age	.004	1	.14	.710
Sex × season	.008	1	.25	.620
Age × season	.008	1	.24	.623
Species × sex × age	.054	12	.14	.999
Species × sex × season	.195	12	.50	.912
Species × age × season	.096	12	.25	.996
Sex × age × season	.020	1	.23	.428
Species × sex × age × season	.102	12	.27	.994
Residual	9.409	292		

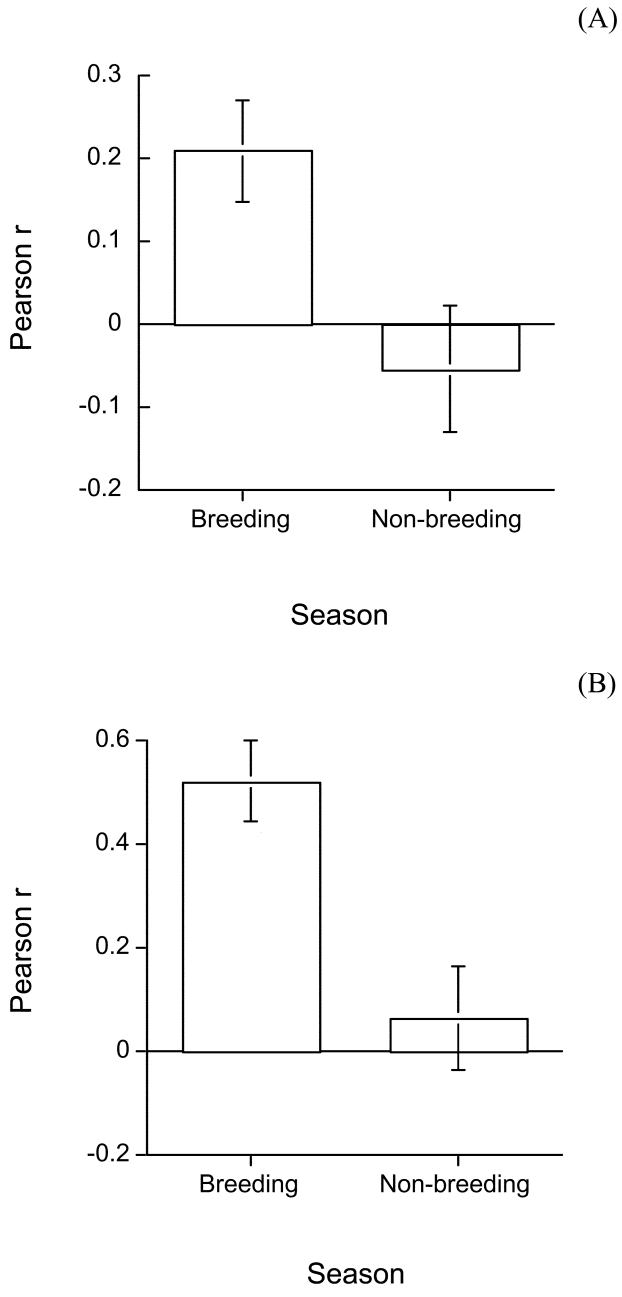

Figure 3: Seasonal change in mean Pearson correlation between (*A*) spleen mass and a body condition index for 21 bird species and (*B*) T-cell-mediated response and a body condition index for 13 bird species. Values are means (±SE).

mediated immune response in 34 species for which both measures of immune function were available for the breeding season ($F = 0.34$, df $= 1, 32$, $r^2 = 0.010$, $P = .565$). Hence, the two measures of immune function are statistically independent, as expected, since the size of the spleen should depend mainly on B-cells. Spleen mass, body mass, and T-cell response were $\log_{10}$ transformed and correlation coefficients were z transformed to meet the requirements for parametric statistical tests.

We compared spleen size and T-cell response between seasons using a paired t-test based on $\log_{10}$-transformed mean values from the two seasons (very similar results were obtained using a nonparametric Wilcoxon matched-pairs signed-ranks test). We controlled for similarity in change in spleen mass due to common descent by comparing change in mass for the closest relative among the species in the data set (Møller and Birkhead 1992). Closest relatives share most phenotypic traits, and any difference in a variable of interest can thus be considered to be relatively independent of confounding variables (Møller and Birkhead 1992). We used phylogenetic information from Sibley and Ahlquist (1990), combined with information from other sources, to identify the closest relatives in our data set.

Results

Seasonal Change in Immune Function

Mean spleen mass for each species during the breeding season was strongly positively correlated with spleen mass during the nonbreeding season (fig. 1*A*; $F = 312.08$, df $= 1, 69$, $r^2 = 0.84$, $P < .001$). Birds had larger spleens during the breeding than during the nonbreeding season (fig. 1*B*; paired t-test, $t = 3.13$, df $= 70$, $P = .0025$). A similar conclusion was reached even if we only included species with more than 30 individuals in the analysis. The difference in mean spleen size between breeding and nonbreeding season for the same 71 species of birds amounted on average to 17.6% or to 0.16 standard deviation units for $\log_{10}$-transformed data. There was no significant difference in mean body mass between breeding and nonbreeding season for the 71 species (paired t-test on $\log_{10}$-transformed data: $t = 1.69$, df $= 70$, $P = .095$). Residual spleen mass during the breeding season was therefore positively related to residual spleen mass during the nonbreeding season (linear regression: $F = 19.86$, df $= 1, 69$, $r^2 = 0.22$, $P < .001$, slope [SE] $= 0.40$ [0.09]), and residual spleen mass differed significantly between the breeding and the nonbreeding season (paired t-test, $t = 3.47$, df $= 70$, $P = .0009$).

For 14 common species with several individuals of each sex, age, and season category, we ran an ANOVA for spleen mass with species, sex, age, and season as factors. We found evidence of a sex difference (males having smaller spleens than females), an age difference (juveniles having larger spleens than adults), and a seasonal difference (with birds from the breeding season having larger spleens than individuals from the nonbreeding season; table 1). Among

Table 3: Seasonal change in spleen mass in relation to ecological factors in 71 species of birds

Factor	F	df	P	0 Mean (SE)	N_0	1 Mean (SE)	N_1
Sexual dichromatism[a]	.26	1, 69	.611	−.107 (.041)	45	−.073 (.050)	26
Migration[b]	.69	1, 69	.411	−.082 (.039)	44	−.139 (.060)	27
Nest site[c]	13.23	1, 69	.0005	−.030 (.035)	52	−.269 (.051)	10
Breeding sociality[d]	.30	1, 69	.585	−.113 (.038)	56	−.069 (.067)	15
Nonbreeding sociality[e]	.50	1, 69	.484	−.075 (.037)	41	−.120 (.055)	30

Note: One-way ANOVA with mean (SE) values reported for categories. Seasonal change was estimated as $\log_{10}$-transformed spleen mass during the nonbreeding season minus $\log_{10}$-transformed spleen mass during the breeding season.

[a] Species were classified as sexually monochromatic (0) or dichromatic (1).

[b] Species were classified as resident (0) or migratory (1).

[c] Species were classified as open nesters (0) or hole nesters (1).

[d] Species were classified as solitary (0) or colonial (1).

[e] Species were classified as solitary (0) or flocking (1).

the two-way interactions, we found evidence of an age difference and a sex difference in spleen mass among species (age × species and sex × species interactions), while there was no significant species difference in seasonal change in spleen mass (species × season interaction; table 1). All other two-way interactions and all three-way and four-way interactions were not statistically significant. Given that seasonal variation in spleen mass was not significantly related to age and sex, we pooled age and sex classes in the following analyses and used mean values for individuals from the breeding and the nonbreeding season as observations.

Bird species with strong T-cell-mediated responses during the breeding season also had strong responses during the nonbreeding season (fig. 2*A*; $F = 12.06$, df $= 1, 11$, $r^2 = 0.52$, $P = .0052$). There was a significant difference in T-cell-mediated immune response between the breeding and the nonbreeding season (fig. 2*B*; paired t-test on $\log_{10}$-transformed data, $t = 5.78$, df $= 12$, $P < .0001$). The nonbreeding season response was on average 57.3% (SE = 6.7) of the breeding season response.

For 13 species with information on T-cell response, we ran an ANOVA with species, sex, age, and season as factors. We found evidence of a significant species difference in T-cell response, a significant age effect (with young birds having stronger responses than older birds), and a significant effect of season (with responses being stronger during the breeding season; table 2). All two-, three-, and four-way interactions were not statistically significant. Given that seasonal variation in T-cell response was not significantly related to age and sex, we pooled age and sex classes in the following analyses and used mean values for individuals from the breeding and the nonbreeding season as observations.

Seasonal Change in Condition Dependence of Immune Function

The correlation between spleen mass and body condition index during the breeding season was significantly positive (fig. 3*A*; one-sample t-test on z-transformed data, $t = 3.34$, df $= 20$, $P = .0033$). During the nonbreeding season, this correlation was not statistically significant (one-sample t-test on z-transformed data, $t = 0.76$, df $= 20$, $P = .455$). The two mean correlation coefficients differed significantly from each other (paired t-test on z-transformed data, $t = 3.02$, df $= 20$, $P = .0068$).

The correlation between T-cell response and body condition index during the breeding season was significantly positive (fig. 3*B*; one-sample t-test on z-transformed data, $t = 3.69$, df $= 12$, $P = .0031$). During the nonbreeding season, this correlation was not statistically significant (one-sample t-test on z-transformed data, $t = 0.71$, df $= 12$, $P = .490$). The two mean correlation coefficients differed significantly from each other (paired t-test on z-transformed data, $t = 2.81$, df $= 12$, $P = .016$).

Ecological Factors Related to Seasonal Change in Immune Function

The only ecological variable that explained a significant amount of variance in change in spleen size between the breeding and the nonbreeding season was nest site (table 3), with hole nesters having a significantly larger change in mean spleen size between the breeding and the nonbreeding season than open nesters (fig. 4*A*). After Bonferroni correction for the number of tests made, nest site still explained a significant amount of variance in spleen mass between the breeding and the nonbreeding season. Colonially breeding species were also predicted to show a clear seasonal change in spleen size because of parasite-

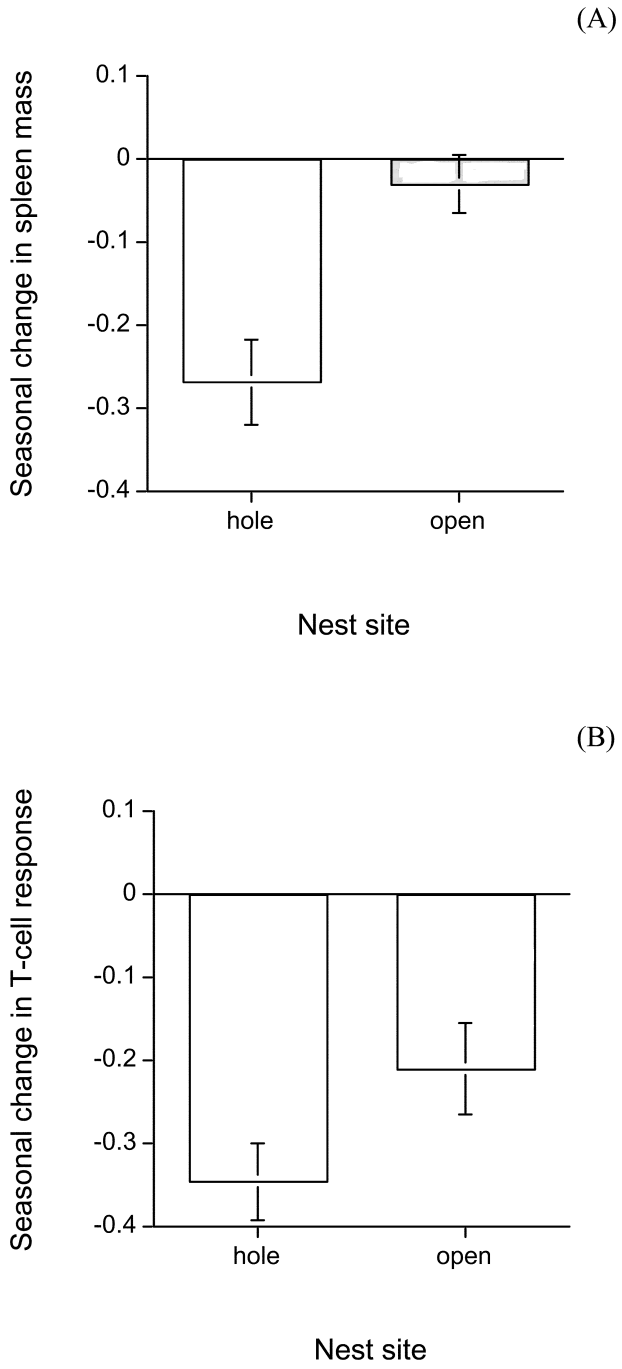

Figure 4: Seasonal change in (*A*) spleen mass and (*B*) T-cell-mediated response for hole-nesting and open-nesting bird species. Values are means (±SE). Data derived from values reported in appendix A.

mediated natural selection (Møller and Erritzøe 1996). However, there was no significant difference in seasonal change in spleen mass between solitary and colonial species (table 3), perhaps because colonial species also are often social during the nonbreeding season. In fact, solitary species did show a significant seasonal change in spleen mass (table 3; mean [SE] change: −0.113 [0.038]; one-sample *t*-test, $t = 2.97$, df = 55, $P < .01$), while colonial species did not show such a significant seasonal change (table 3; mean [SE] change: −0.069 [0.067]; one-sample *t*-test, $t = 1.03$, df = 14, $P = .20$).

A pairwise comparison of change in spleen mass between the breeding and the nonbreeding season for sister taxa differing in nest site also showed a significant difference, with hole nesters changing more than open nesters (table 4; paired *t*-test, $t = 3.60$, df = 8, $P = .007$).

Seasonal change in T-cell-mediated immune response was larger in hole nesters than in open nesters, although not significantly so ($F = 2.32$, df = 1, 11, $P = .156$). Open nesters had a mean seasonal change in T-cell response that was 60% that of hole nesters (fig. 4*B*).

The combined probability from the test of differences in seasonal change in immune function between open nesters and hole nesters on the basis of spleen mass and T-cell response was statistically significant (Fisher's combined probability test, $\chi^2 = 13.64$, df = 4, $P < .01$).

Discussion

Seasonal Change in Immune Function

Analyses of seasonality in immune function in birds revealed seasonal changes in spleen mass and T-cell-mediated immune response (figs. 1, 2), with spleen mass being on average 18% smaller during the nonbreeding than the breeding season and T-cell-mediated immunity being reduced by on average 33% from the breeding to the nonbreeding season. These two measures of immune function are statistically independent, as shown by an absence of correlation between spleen mass and T-cell-mediated immune response during the breeding season in 34 species for which both measures were available (see "Material and Methods"). These statistically significant seasonal patterns of immunity are consistent with detailed studies of two bird species (Zuk and Johnsen 1998; González et al. 1999) but contrast with studies of mammals and other vertebrates, suggesting that lymphatic organ size peaks in late fall or early winter (reviewed in Isogai et al. 1992; Lochmiller et al. 1994; Nelson and Demas 1996). The few studies of seasonal variation in spleen mass in birds showed peaks during the reproductive or the nonreproductive season with no clear general pattern (Krause 1922; Riddle 1928; Oakesson 1953, 1956; Silverin 1981; Silverin et al. 1999). On the basis of this study, we can conclude that both spleen mass and a measure of T-cell-mediated immunity to a challenge with a novel antigen on average

Table 4: Pairwise comparison between avian sister taxa in seasonal change in spleen mass in relation to nest site

Hole nester	Seasonal change in spleen mass	Open nester	Seasonal change in spleen mass
Mergus merganser	−.605	*Somateria mollissima*	.229
Apus apus	−.602	*Asio otus*	−.055
Falco tinnunculus	−.279	*Buteo buteo*	.097
Strix aluco	−.042	*Bubo bubo*	−.153
Sturnus vulgaris	.105	*Turdus philomelos*	−.026
Corvus monedula	−.535	*Corvus corone*	−.051
Phoenicurus phoenicurus	−.144	*Erithacus rubecula*	.176
Oenanthe oenanthe	−.250	*Turdus merula*	.070
Parus major	−.190	*Troglodytes troglodytes*	.046
Passer domesticus	−.067	*Prunella modularis*	−.022

Note: Seasonal change was estimated as $\log_{10}$-transformed spleen mass during the nonbreeding season minus $\log_{10}$-transformed spleen mass during the breeding season.

peaked during the breeding season across a large number of different species.

Seasonal changes in immune function may reflect selection, removing the fraction of the host population with the weakest immune responses (Møller and Erritzøe 2000), or it may reflect phenotypic plasticity. For spleen mass and T-cell response, we could show a seasonal change in size independent of age (tables 1, 2). These findings suggest that seasonal patterns of immunity are independent of selection, since a pattern dependent on selection would require a larger difference in response in juveniles than in adults. The strong age effects shown for spleen size and T-cell response (tables 1, 2) with larger values for juveniles than for adults suggest that young individuals, which encounter novel antigens at a higher rate in their environment than do adults, may invest more in immune function than older individuals of the same species (Møller and Erritzøe 2001). Spleens are larger in juveniles than in adults, independent of infection status (Møller et al. 1998), and the larger spleens in juveniles reported here thus cannot be attributed to simple differences in the probability of being sick.

Seasonal changes in immune function may be caused by seasonal changes in costs and benefits of strong immune responses. The benefits of raising a strong immune response should be most important during the part of the year when parasites are the most abundant, that is, during the breeding season of the host. The immunocompetence hypothesis suggests that testosterone and other reproductive hormones have antagonistic effects on immunity (Folstad and Karter 1992). Indeed, several experiments are consistent with this suggestion (e.g., Casto et al. 2001). Although such an effect might lead to the prediction that immune response should be more depressed by testosterone and estradiol during the breeding season, there might still be stronger immune responses during breeding than during nonbreeding because immunity has been molded to respond to a larger challenge by parasites during the breeding season. Seasonal changes in weather conditions may affect immune responses. Stress arising from cold weather may suppress immune function (reviewed in Apanius 1998). However, this seems an unlikely explanation, at least for the T-cell responses investigated here, since these were obtained during an extremely mild winter with temperatures well above normal and only slightly lower than those during the breeding season. Immunity has been suggested to be costly in terms of time, energy, micronutrients, or autoimmune disease (Råberg et al. 1998; von Schantz et al. 1999; Lochmiller and Deerenberg 2000; Møller et al. 2000*b*). If any of these factors are particularly important during a certain part of the year but not others, this may affect seasonality in optimal levels of immune function. q11

Immune function has been hypothesized to reflect past viability selection imposed by parasites. Martin et al. (2001) showed that interspecific differences in the magnitude of T-cell-mediated immune response in birds paralleled differences in parasite-induced nestling mortality. Møller and Erritzøe (2002) showed a similar pattern for relative spleen mass and nestling mortality in birds that was independent of that found for T-cell response. We hypothesize that seasonal differences in the impact of parasites will parallel seasonal investment in immune function.

Seasonal Change in Condition Dependence of Immune Function

Both spleen mass and T-cell-mediated immune response showed statistically significant condition dependence during the breeding season, with individuals in prime condition having greater responses (fig. 3*A*, 3*B*). However,

there was no significant average positive relationship during the nonbreeding season (fig. 3*A*, 3*B*), and the two sets of correlation coefficients between immunity and condition differed significantly from each other. However, some species did show a significant positive relationship between immune function and condition even during the nonbreeding season. Previous studies of condition dependence of immune function in both domesticated and wild animals and in humans have shown that individuals in prime condition tend to produce stronger immune responses than individuals in poor condition (Chandra and Newberne 1977; Gershwin et al. 1985; Møller et al. 1998; Alonso-Alvarez and Tella 2001). That is the case for spleen mass (Møller et al. 1998) and also for T-cell-mediated immune response (Alonso-Alvarez and Tella 2001). These previous tests were mainly based on birds from the breeding season, and the patterns reported here may thus have been missed in previous studies because only a few studies were conducted outside the reproductive season.

Why should there be a difference in the degree of condition dependence of immune function between the breeding and the nonbreeding season? We hypothesize that individual differences in condition may be more clear-cut during the time of the year that contributes the most to fitness. If parasites impose stronger selection pressures on their hosts during the breeding season than during the nonbreeding season, then hosts should allocate limiting resources to immune function mainly during the part of the year when the risk of parasites exploiting hosts is elevated.

Ecology and Seasonal Change in Immune Function

q12 What are the selective pressures that have molded seasonal changes in immune function? We suggest that seasonal changes in the benefits and costs of strong immune responses have selected for the ability of individuals to phenotypically adjust immune function. This suggestion is supported by our analyses of factors associated with seasonal changes in immune function. Previous studies of ecological correlates of immune function have shown that
q13 immune function correlates with sexual dichromatism, migration, nest site, and breeding sociality (Møller and Erritzøe 1996, 1998; Møller et al. 1999, 2001). Nest site and breeding sociality have been hypothesized to select for increased investment in immune function because both hole nesting and colonial breeding are associated with nest reuse and hence increased probability of horizontal transmission and multiple infection (Møller and Erritzøe 1996). These are mechanisms that have been hypothesized to increase parasite virulence according to several theoretical models (Bull 1994; Frank 1996; van Baalen 1998). Hole nesting should expose avian hosts to virulent parasites during the breeding season, and greater seasonal change in investment in immune function in hole nesters as compared with open nesters is thus consistent with this suggestion (fig. 4; table 4).

Colonially breeding species were expected to show a similar seasonal pattern in spleen mass as hole nesters (Møller and Erritzøe 1996; Møller et al. 2001). However, that was clearly not the case (table 3). An explanation for this apparent inconsistency is that colonially breeding species also tend to be social during the nonbreeding season, selecting for large investment in immune function during both parts of the year. Consistent with this prediction, colonial species did not show a significant seasonal change in spleen mass, whereas solitary species reduced their spleen mass during the nonbreeding season.

Acknowledgments

W. C. Årestrup, N. Cadée, E. Flensted-Jensen, and K. Klarborg kindly helped catch birds. q14

APPENDIX A

Table A1: Seasonal change in spleen mass (g) in birds, sexual dichromatism (0 monochromatic, 1 dichromatic), migration (0 resident, 1 migratory), nest site (0 open nest, 1 hole nest), breeding sociality (0 solitary, 1 colonial), and nonbreeding sociality (0 solitary, 1 flock living)

	Nonbreeding season		Breeding season						
Species	Mean (SE)	*N*	Mean (SE)	*N*	Dichromatism	Migration	Nest site	Breeding sociality	Nonbreeding sociality
Accipiter gentilis	1.101 (.151)	2	.617 (.143)	9	1	0	0	0	0
Accipiter nisus[a]	.145 (.013)	77	.151 (.015)	47	1	0	0	0	0
Alcedo atthis	.019 (.003)	5	.036 (.005)	11	0	0	1	0	0
Alectoris rufa	.211 (.103)	2	.080 (…)	1	1	0	0	0	0
Anthus pratensis	.018 (.006)	5	.016 (…)	1	0	1	0	0	0
Apus apus	.008 (…)	1	.032 (.013)	4	0	1	1	1	1
Asio otus[a]	.157 (.013)	41	.178 (.025)	24	0	0	0	0	0
Bombycilla garrulus	.058 (.006)	17	.038 (.007)	7	0	1	0	0	1
Branta leucopsis	.620 (…)	1	.870 (…)	1	0	1	0	1	1
Bubo bubo	.908 (…)	1	1.290 (…)	1	0	0	0	0	0
Buteo buteo[a]	.740 (.064)	64	.592 (.083)	31	0	0	0	0	0
Calidris alpina	.011 (.003)	5	.040 (…)	1	0	1	0	0	1
Carduelis chloris	.025 (.006)	7	.048 (.016)	12	1	0	0	0	1
Carduelis flammea	.008 (…)	1	.004 (…)	1	1	0	0	0	1
Carduelis spinus	.017 (.004)	3	.011 (.002)	3	1	0	0	0	1
Certhia familiaris	.012 (.003)	2	.024 (…)	1	0	0	1	0	0
Coccothraustes coccothraustes	.040 (.003)	2	.078 (.007)	7	0	0	0	0	0
Columba palumbus	.074 (.026)	2	.091 (…)	1	0	0	0	0	1
Corvus corax	1.040 (…)	1	1.291 (.138)	2	0	0	0	0	0
Corvus corone	.286 (.066)	3	.322 (.050)	2	0	0	0	0	1
Corvus frugilegus	.405 (.029)	4	.330 (…)	1	0	0	0	1	1
Corvus monedula	.077 (.067)	2	.264 (…)	1	0	0	1	1	1
Cuculus canorus	.032 (…)	1	.034 (.008)	3	0	1	0	0	0
Cygnus olor	.797 (.050)	2	.895 (.060)	2	0	0	0	1	1
Dendrocopus major	.023 (.002)	5	.048 (.006)	17	1	0	1	0	0
Emberiza citrinella	.033 (.008)	7	.028 (.007)	12	1	0	0	0	1
Erithacus rubecula	.033 (.007)	9	.022 (.004)	10	0	0	0	0	0
Falco tinnunculus[a]	.061 (.010)	18	.116 (.022)	15	1	0	1	1	0
Fringilla coelebs[a]	.038 (.012)	6	.032 (.005)	28	1	0	0	0	0
Gallinago gallinago	.071 (.012)	3	.013 (…)	1	0	1	0	0	0
Gallinula chloropus	.373 (.098)	7	.247 (.025)	3	0	0	0	0	0
Garrulus glandarius	.159 (.031)	10	.185 (.028)	14	0	0	0	0	0
Gavia stellata	.345 (.205)	2	.799 (.150)	4	0	1	0	0	0
Hirundo rustica	.022 (.005)	7	.031 (.005)	20	1	1	0	1	1
Larus argentatus	1.064 (.572)	2	.554 (.146)	2	0	0	0	1	1
Larus ridibundus	.568 (.251)	5	.334 (.127)	6	0	1	0	1	1
Loxia curvirostra	.023 (.002)	4	.097 (.042)	3	1	0	0	0	1
Mergus merganser	.221 (.119)	2	.889 (.238)	2	1	1	1	0	1
Muscicapa striata	.006 (…)	1	.018 (.005)	5	0	1	1	0	0
Oenanthe oenanthe	.018 (…)	1	.032 (.005)	5	1	1	1	0	0
Parus caeruleus	.004 (…)	1	.009 (.001)	5	1	0	1	0	0
Parus major	.020 (.002)	5	.031 (.006)	10	1	0	1	0	0
Passer domesticus[a]	.048 (.005)	12	.056 (.005)	48	1	0	1	1	1
Passer montanus[a]	.033 (.007)	8	.030 (.004)	22	0	0	1	1	1
Perdix perdix	.124 (.007)	3	.170 (.080)	2	1	0	0	0	1
Pernis apivorus	.780 (…)	1	.876 (…)	1	0	1	0	0	0
Phalacrocorax carbo	1.254 (.302)	7	1.816 (.059)	3	0	1	0	1	1
Phasianus colchicus[a]	.558 (.036)	28	.551 (.090)	4	1	0	0	0	0

Table A1 (*Continued*)

Species	Nonbreeding season Mean (SE)	*N*	Breeding season Mean (SE)	*N*	Dichromatism	Migration	Nest site	Breeding sociality	Nonbreeding sociality
Phoenicurus phoenicurus	.033 (.007)	7	.046 (.024)	6	1	1	1	0	0
Phylloscopus collybita	.004 (.001)	3	.008 (.003)	5	0	1	0	0	0
Pica pica	.253 (.049)	7	.366 (.055)	15	0	0	0	0	0
Picus viridis	.046 (…)	1	.181 (.063)	4	1	0	1	0	0
Pluvialis apricaria	.074 (.025)	2	.120 (…)	1	0	1	0	0	1
Podiceps cristatus	.563 (.057)	2	.586 (.100)	3	0	0	0	1	1
Prunella modularis	.019 (…)	1	.020 (.003)	10	0	0	0	0	0
Pyrrhula pyrrhula[a]	.042 (.010)	23	.063 (.016)	4	1	0	0	0	1
Rallus aquaticus	.130 (…)	1	.108 (.012)	2	0	1	0	0	0
Scolopax rusticola[a]	.172 (.014)	26	.215 (.050)	6	0	1	0	0	0
Sitta europaea	.017 (…)	1	.031 (.004)	5	0	0	1	0	0
Somateria mollissima	.463 (.081)	5	.273 (…)	1	1	0	0	1	1
Strix aluco[a]	.234 (.034)	18	.258 (.087)	16	0	0	1	0	0
Sturnus vulgaris	.107 (.043)	3	.084 (.009)	9	1	1	1	1	1
Sylvia atricapilla	.031 (.006)	2	.034 (.005)	15	1	1	0	0	0
Sylvia borin	.020 (.002)	2	.023 (.004)	5	0	1	0	0	0
Tringa totanus	.013 (…)	1	.089 (…)	1	0	1	0	0	1
Troglodytes troglodytes	.020 (.006)	5	.018 (.004)	6	0	0	0	0	0
Turdus iliacus	.073 (.002)	6	.082 (.002)	2	0	1	0	0	1
Turdus merula[a]	.242 (.013)	78	.206 (.013)	118	1	0	0	0	0
Turdus philomelos[a]	.115 (.010)	10	.122 (.009)	28	0	1	0	0	0
Tyto alba[a]	.109 (.011)	30	.101 (.025)	10	0	0	1	0	0
Vanellus vanellus	.170 (.020)	2	.164 (.121)	4	1	1	0	0	1

[a] Species used in the ANOVA in table 1.

APPENDIX B

Table B1: Seasonal change in T-cell-mediated immune response (mm) in birds, sexual dichromatism (0 monochromatic, 1 dichromatic), migration (0 resident, 1 migratory), nest site (0 open nest, 1 hole nest), breeding sociality (0 solitary, 1 colonial), and nonbreeding sociality (0 solitary, 1 flock living)

Species	Nonbreeding season Mean (SE)	*N*	Breeding season Mean (SE)	*N*	Dichromatism	Migration	Nest site	Breeding sociality	Nonbreeding sociality
Carduelis chloris	.208 (.022)	12	.127 (.033)	6	1	0	0	0	1
Carduelis flammea	.059 (.006)	8	.054 (.014)	6	1	0	0	0	1
Emberiza citrinella	.118 (.011)	7	.118 (.014)	10	1	0	0	0	1
Erithacus rubecula	.392 (.009)	4	.055 (.015)	8	0	0	0	0	0
Fringilla coelebs	.247 (.057)	8	.095 (.017)	8	1	0	0	0	0
Hirundo rustica	.220 (.020)	24	.177 (.020)	160	1	1	0	1	1
Parus caeruleus	.169 (.002)	4	.085 (.014)	4	1	0	1	0	0
Parus major	.257 (.021)	3	.095 (.018)	10	1	0	0	1	0
Passer domesticus	.289 (.044)	6	.169 (.022)	18	1	0	1	1	1
Passer montanus	.368 (.042)	18	.141 (.018)	18	0	0	1	1	1
Prunella modularis	.210 (.030)	9	.083 (.020)	6	0	0	0	0	0
Pyrrhula pyrrhula	.140 (.005)	3	.117 (.028)	8	1	0	0	0	1
Turdus merula	.312 (.031)	16	.122 (.016)	12	1	0	0	0	0

Note: The same populations were tested during the breeding and the nonbreeding season.

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QUERIES TO THE AUTHOR

1 Phytohemagglutinin is spelled in this way by Webster's 10th dictionary, which we try to follow. Is the spelling change OK with you?

2 We need to repeat the references rather than say "references above." Which references do you mean here? (Or maybe you can rewrite these sentences to avoid repeating the references?)

3 Please make sure that Møller et al. 2000*a* is matched to the correct reference.

4 In the sentence beginning "A comparative study," is there a word missing between "more" and "species"?

5 Please check over both appendices very carefully as many numbers dropped out in the translation process.

6 In the literature cited section, you have Birkhead listed as 1998, whereas here it is 1999. Which year is correct?

7 Please provide initials and last name for the unpublished data.

8 In the sentence beginning "Body condition was," I changed "the power 3" to "the third power." Is that OK?

9 We try to avoid one-sentence paragraphs. Is it OK that I included the sentence beginning "Spleen mass" in the previous paragraph?

10 Figure 2 does not have a line, so I deleted "The line $y = x$" sentence.

11 Please make sure Møller et al. 2000*b* matches up with the reference in the literature cited section.

12 I changed "which" to "what" in the beginning of the first sentence of this paragraph. Is that OK?

13 In the sentence beginning "Previous studies," I changed "it correlates" to "immune function correlates." Is this correct? If not, please explain what "it" is referring to.

14 The text on page 10 will be lined up once I get the corrections back from you, so please disregard the shorter length of the second column.

15 Klein 1990 was not referenced anywhere in the text. Please add a citation or delete this reference.

16 Do you have the editor's name for Krause 1922?

17 For Merino et al. 2002, do you have volume and page numbers now?

18 For Møller and Erritzøe 2002, do you have volume and page numbers now?

19 For Navarro et al. 2002, do you have volume and page numbers now?

20 For Saino et al. 2002, do you have volume and page numbers now?

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