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Is cell-mediated immunity related to the evolution of life-history strategies in birds?

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According to life-history theory, the development of immune function should be balanced through evolutionary optimization of the allocation of resources to reproduction and through mechanisms that promote survival. We investigated interspecific variability in cell-mediated immune response (CMI), as measured by the phytohaemagglutinin (PHA) assay, in relation to clutch size, longevity and other life-history traits in 50 species of birds. CMI exhibited significant repeatability within species, and PHA responses in chicks were consistently stronger than in adults. Univariate tests showed a variety of significant relationships between the CMI of both chicks and adults with respect to size, development period and lifespan, but not clutch size or prevalence of blood parasites in adults. Multivariate analyses confirmed these patterns but independent variables were too highly correlated to isolate unique influences on CMI. The positive relationship of chick CMI to nestling period is further complicated by a parallel relationship of chick CMI to the age at testing. However, multivariate analysis showed that chick CMI varies uniquely with length of the nestling period. Adult CMI was associated with a strong life-history axis of body size, development rate and longevity. Therefore, adult CMI may be associated with prevention and repair mechanisms related to long lifespan, but it also may be allometrically related to body size through other pathways. Neither chick CMI nor adult CMI was related to clutch size, contradicting previous results linking parasite-related mortality to CMI and the evolution of clutch size (reproductive investment) in birds.

Keywords: birds; cell-mediated immunocompetence; comparative analysis; life-history traits; reproductive investment; phytohaemagglutinin assay

1. INTRODUCTION

The phytohaemagglutinin (PHA) skin test has become a standard method of assessing cell-mediated components of the immune response of birds (Smits *et al.* 1999). This assay consists of injecting, subcutaneously, a novel mitogen (PHA) and measuring the amount of swelling after a period of 24 h. The response to PHA involves macrophages, basophils, heterophils and B lymphocytes, and it is orchestrated by cytokines secreted by T lymphocytes (Stadecker *et al.* 1977). Briefly, heterophils and macrophages infiltrate the injection site in the acute phase (1–2 h after injection), and about 30% of T-cell lines proliferate after mitogenic stimulation by PHA (Klein 1993; Elgert 1996). These produce the cytokines interleukin-2 and interferon-γ that attract basophils and macrophages (McCorkle *et al.* 1980), which in turn orchestrate an inflammatory response. The final stage of the response consists of a swelling at the injection site, caused by dense infiltration of macrophages and lymphocytes, as well as basophils and heterophils, in postcapillary venules (Stadecker *et al.* 1977; Goto *et al.* 1978). This process is a typical cutaneous basophil hypersensitivity response and provides a general index of cell-mediated immunity (CMI).

A growing literature describing studies within species shows that CMI is related to an array of factors such as sex (Moreno *et al.* 2001; Fargallo *et al.* 2002), diet (González *et al.* 1999), body condition (Alonso-Alvarez & Tella 2001), parental clutch size (Tella *et al.* 2000), exposure to parasites (Christe *et al.* 2000), sexual ornaments (González *et al.* 1999; Velando *et al.* 2001), within-brood hierarchy (Tella *et al.* 2001) and paternity (Johnsen *et al.* 2000) of chicks, and survival (González *et al.* 1999; Horak *et al.* 1999). More recently, CMI has been compared among species to examine the relationship between immune function and some life-history traits. In particular, Martin *et al.* (2001) related clutch size in birds to parasite-induced juvenile mortality, which was assessed by the PHA response. Their focus was primarily the evolution of clutch size as an expression of parental investment. They concluded that parental investment in offspring is directly related to adult parasite-induced mortality, and inversely related to chick parasite-induced mortality, as judged by adult and chick CMI, respectively. These results are consistent with life-history theory, and suggest that CMI may be an important life-history trait.

Immune response represents an investment in the sense that it incurs costs, although the nature and size of these costs are poorly known (Klasing *et al.* 1987; Klasing 1998; Fair *et al.* 1999; Lochmiller & Deerenberg 1999). Besides the allocation of tissue, energy and nutrients to immune function, these costs are evident in observed negative relationships between reproductive investment, body condition, stress and immune responsiveness (Dhabhar *et al.* 1994; Raberg *et al.* 1998; Svensson *et al.* 1998; Dhabhar & McEwen 1997; Braude *et al.* 1999), and between antigen challenge and growth rate (Fair *et al.* 1999), although the mechanisms are not well understood (Raberg *et al.* 2000).

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To the extent that investment in immune function is costly, its development is presumably balanced through evolutionary optimization of the allocation of resources to reproduction and mechanisms that promote survival. This trade-off gives rise to a number of predictions from simple hypotheses based on the theory of life-history evolution (Stearns 1992; Charlesworth 1994).

(i) Immune function should increase with increasing adult survival rate or, more generally, length of life, which favours maintenance mechanisms to further enhance survival at the expense of reproduction (Hamilton 1966; Charlesworth 1994).
(ii) Immune function should be directly related to length of the development period if time is required for the maturation of critical components of the immune system (Ricklefs 1992).
(iii) Immune function should be directly related to prevalence of parasites and disease as a means to control infection (Martin *et al.* 2001).
(iv) Immune function may exhibit an allometric relationship to body size, which is characteristic of many aspects of physiological function, independently of size-dependent relationships linking immune function to other life-history or environmental characteristics (Calder 1984).

Because each individual faces the same immunological challenges regardless of body size, the 'diversity' of the immune response should not scale to body mass (Cohn & Langman 1990). However, titres of immunologically relevant units (e.g. T cells, macrophages and antibodies) may well be size dependent.

We use comparisons among species to test these hypotheses by examining the relationship of the PHA response to several life-history traits, including body size, egg size, clutch size, incubation and nestling periods, postnatal development rate, adult mortality rate, lifespan and prevalence of blood parasites.

2. MATERIAL AND METHODS

We compiled PHA responses of 50 species of birds (see electronic Appendix A available on The Royal Society's Publications Web site). Injected doses of PHA varied both between and within species, ranging from 20 to 500 μg diluted in phosphate-buffered saline (PBS). Most studies injected the same volume of PBS in the opposite wing or foot web as a control following the original protocol (Goto *et al.* 1978), but others have recently abandoned the PBS injection after the recommendation of Smits *et al.* (1999). Both kinds of study were included in our comparative analysis, since the two methods produce nearly identical results (Smits *et al.* 1999). Therefore, CMI was calculated as the difference in web thickness prior to, and 24 h after, injection, controlled or not controlled for the insignificant effect of PBS injection in the opposite web. Measurement error within studies is low (Smits *et al.* 2001). Many factors influence variability in CMI within species (see examples above). To examine variation among studies within species, we compiled a total of 101 results for 27 species (range of two to nine per species). We then calculated the within-species repeatability of CMI, using the intraclass correlation coefficient (r_i), to determine whether the PHA response varies significantly between species, as required for comparative analyses. Because repeatability was highly significant (see § 3), for further analyses we averaged PHA responses within species for which several sets of results were available.

Life-history attributes were obtained from a variety of sources, including handbooks and data compilations of the authors. These attributes included body mass, clutch size, egg volume, incubation and nestling periods, the growth rate constant (K) of the logistic growth model, annual adult mortality rate, oldest reported age (Ricklefs 1983, 1993; Starck & Ricklefs 1998; Carey & Judge 2000) and prevalence of blood parasites (Peirce 1981; Greiner *et al.* 1975; R. E. Ricklefs, unpublished data). All variables, except clutch size, were $\log_{10}$-transformed. Following Martin *et al.* (2001), we distinguished species from the Northern and Southern Hemispheres. We also distinguished hole-nesting and open-nesting species (Martin & Li 1992), but used different criteria from those of Martin *et al.* (2001). We defined hole nesting as any use of a cavity or enclosed space for breeding, given the implications of cavity nesting for nest reuse, i.e. the horizontal transmission of parasites and evolution of the immune systems of their avian hosts (Møller & Erritzøe 1996).

Statistical analyses were performed with SAS 6.12 (SAS Institute 1996). All statistics in multivariate analyses of variance (ANOVA) and covariance (ANCOVA) are based on type III sums of squares. Hence, they test the unique contribution of each factor to variation in the response variable. Stepwise regressions used the FORWARD option. Factor analysis was based on the principal components of the correlation matrix and used the Varimax method of factor rotation. We did not use phylogenetic correction (e.g. phylogenetic independent contrasts; PIC) in our analyses because the deeper branches of the avian phylogeny are not well resolved, PIC methods introduce variation through the estimation of ancestral trait values, and PIC and species comparisons generally give similar results (Ricklefs & Starck 1996). Indeed, the results of phylogenetically corrected and uncorrected analyses in the study of Martin *et al.* (2001) are virtually indistinguishable.

3. RESULTS

(a) *Within-species consistency and age differences in CMI*

Despite the great variability in CMI, within-species repeatability was highly significant ($r_i = 0.78$, $F_{26,74} = 13.5$, $p < 0.001$). However, chicks tend to have higher CMI than adults (figure 1). A Wilcoxon matched-pairs signed-ranks test showed higher responses in chicks than in adults in 16 of 20 pairwise comparisons within species ($Z = 3.21$, $p < 0.001$). Consequently, chicks and adults were separated for further analyses.

Adult and chick CMI were significantly correlated with each other among species ($r = 0.57$, $p = 0.009$, $n = 20$). To determine whether this relationship is independent of correlations with additional life-history variables, we compared the residuals of adult CMI from its regression on body mass (see below; $F_{1,18} = 20.3$, $p < 0.001$) and of chick CMI from its regression on nestling period (see below; $F_{1,18} = 12.3$, $p = 0.003$). The residuals were significantly correlated ($r = 0.52$, $p = 0.018$, $n = 20$), supporting the suggestion that there is a generalized component of CMI that varies among species and affects both chicks and adults.

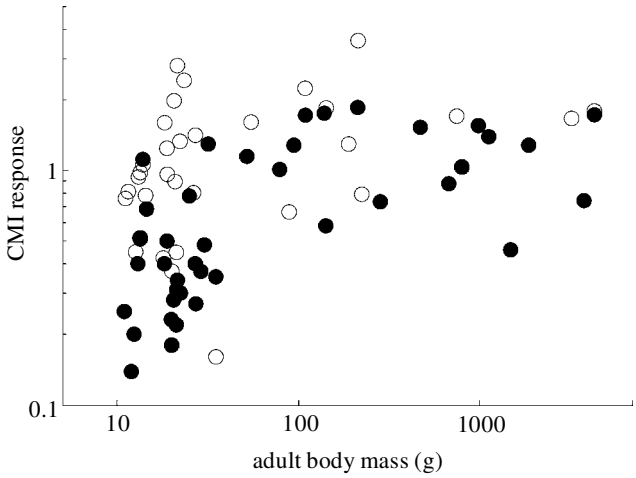

Figure 1. The CMI response of adults (black circles) and chicks (open circles) as a function of adult body mass.

(b) *Correlates of chick CMI*

Simple correlations of other variables with the CMI of chicks were strongest for incubation period, nestling period and adult mortality rate, marginally significant for body mass and egg volume, and insignificant for all other variables (table 1). The relationship of CMI to mass appears to be nonlinear (figure 1) and the sample of species is heterogeneous with respect to development mode. To obtain a set of species with more uniform life-history traits, we analysed separately altricial species weighing less than 300 g ($n = 28$ species with chick CMI and 29 species with adult CMI). Most of these were passerines. The pattern of correlation for this subset of species was similar to that of the larger sample (table 1).

Because of the high correlation among significant variables, no single variable contributed uniquely to variation in chick CMI. Among 18 species for which all variables were available, clutch size entered first in a stepwise regression ($F_{1,16} = 6.8$, $p = 0.019$, $r^2 = 0.30$) and no other variable was significant. When nestling growth rate, adult mortality rate and maximum reported lifespan were dropped from the analysis to increase sample size to 30 species, nestling period entered as the first variable ($F_{1,28} = 17.4$, $p = 0.0003$, $r^2 = 0.38$) with no other significant effects. For the subset of small altricial species, the same analysis also revealed nestling period as the single strongest predictor of chick CMI ($F_{1,24} = 25.7$, $p = 0.0001$, $r^2 = 0.52$).

The age at which CMI is tested is directly correlated with the length of the nestling period ($r = 0.77$, $p = 0.0001$, $n = 21$) because investigators conduct the assay as close to fledging as possible. Thus, it is possible that the correlation between CMI and the length of the nestling period represents the age at which the assay is made, and therefore the developmental course of CMI. Among the 21 species for which we could determine the test age, it was correlated with CMI ($r = 0.51$, $p = 0.017$), possibly indicating a generalized increase in CMI with age (figure 2). However, when CMI was tested in a multiple regression against nestling period and test age, nestling period was a significant effect ($F_{1,19} = 8.70$, $p = 0.008$) but test age was not ($F_{1,19} = 0.10$, $p = 0.759$). A quantitatively similar result was obtained for small altricial species. Thus, CMI appears to respond to selection associated with nestling period and does not increase with development through the range of ages tested.

(c) *The relationship between clutch size and chick CMI*

Martin *et al.* (2001) specifically tested the hypothesis that clutch size should decrease with increasing CMI, which was positively related to parasite-induced mortality of the chicks. They reasoned that greater immune responsiveness was correlated with lower probability of chick survival, and thus weaker selection on adults to invest in reproduction. They observed a negative relationship between clutch size and CMI in their data, which was significant when adult CMI, adult mass, nest type and hemisphere were entered as covariates. Following Martin *et al.* (2001), we distinguished between northern and southern species to account for the generally higher clutch sizes of birds in the temperate regions of the Northern Hemisphere (Martin *et al.* 2000). An analysis of covariance relating clutch size to CMI and hemisphere had an insignificant interaction effect ($F_{1,29} = 0.00$, $p = 0.97$). With the interaction term removed, hemisphere was a highly significant effect ($F_{1,30} = 20.7$, $p < 0.0001$) but clutch size was not related to CMI ($F_{1,30} = 2.9$, $p = 0.1$).

The trend relating clutch size to CMI in the previous analysis was negative (slope = -1.25 ± 0.73 s.e., eggs per $\log_{10}$ CMI). Because this relationship may have been obscured by other factors, we included adult mass, adult CMI, hemisphere, and hole versus open nesting in a further multiple regression analysis. In this case, the relationship of clutch size to chick CMI was not significant ($F_{1,14} = 3.8$, $p = 0.071$). Finally, residuals from the regressions of both clutch size and chick CMI on adult CMI, adult mass, nest type and hemisphere were not significantly correlated ($r = -0.376$, $p = 0.102$, $n = 20$). A similar result was obtained for the subset of small altricial birds. Thus, our results do not support an inverse relationship between clutch size and chick CMI using a somewhat larger sample of species than Martin *et al.* (2001).

(d) *Variation in adult CMI*

Adult CMI was significantly correlated with most size and development variables, and marginally for annual adult mortality, but not for clutch size and parasite prevalence (table 1). Because of the high degree of correlation among the independent variables, the pattern of variation associated with adult CMI was examined by factor analysis of CMI and nine additional variables. Three eigenvalues of the correlation matrix exceeded 1.0 (6.28, 1.49 and 1.06: 88.3% of the total variance) and these were retained for a Varimax rotation to produce three factors that maximized the contrasts between the factor loadings of the variables (table 2). The rotated factor pattern associates CMI ($r = 0.83$) directly with size, development period and lifespan, and inversely with nestling growth rate and adult mortality (Factor 1). Factor 2 associates clutch size ($r = 0.95$) and adult mortality ($r = 0.64$); clutch size, in particular, is largely independent of other life-history variables, including CMI. Factor 3 represents, primarily, variation in blood parasite prevalence, which is unrelated to other life-history and demographic variables.

Among the subset of small altricial species, adult CMI

Table 1. Correlations of the CMI response of chicks and adults with other life-history traits and prevalence of blood parasites. (Significant correlations ($p < 0.05$) are in bold.)

	all species			small altricial species		
variable	*n*	*r*	*p*	*n*	*r*	*p*
chick CMI response						
mass	33	**0.367**	0.035	28	0.332	0.084
egg volume	33	**0.349**	0.046	28	0.281	0.148
incubation period	32	**0.458**	0.008	27	**0.436**	0.023
nestling period	33	**0.605**	0.001	28	**0.718**	0.001
growth rate (K)	24	−0.258	0.224	19	−0.350	0.142
reported lifespan	26	0.337	0.092	21	0.222	0.334
adult mortality	22	**−0.525**	0.012	17	**−0.615**	0.009
clutch size	33	−0.275	0.121	28	−0.176	0.371
parasite prevalence	31	0.026	0.890	27	0.190	0.343
adult CMI response						
mass	37	**0.680**	0.001	29	**0.717**	0.001
egg volume	37	**0.669**	0.001	29	**0.653**	0.001
incubation period	36	**0.576**	0.001	28	**0.485**	0.009
nestling period	37	**0.543**	0.001	29	0.339	0.072
growth rate (K)	28	**−0.636**	0.001	20	**−0.615**	0.004
reported lifespan	30	**0.456**	0.011	24	0.374	0.072
adult mortality	28	**−0.379**	0.047	20	**−0.488**	0.029
clutch size	37	0.212	0.207	29	0.146	0.451
parasite prevalence	34	−0.086	0.628	26	0.027	0.897

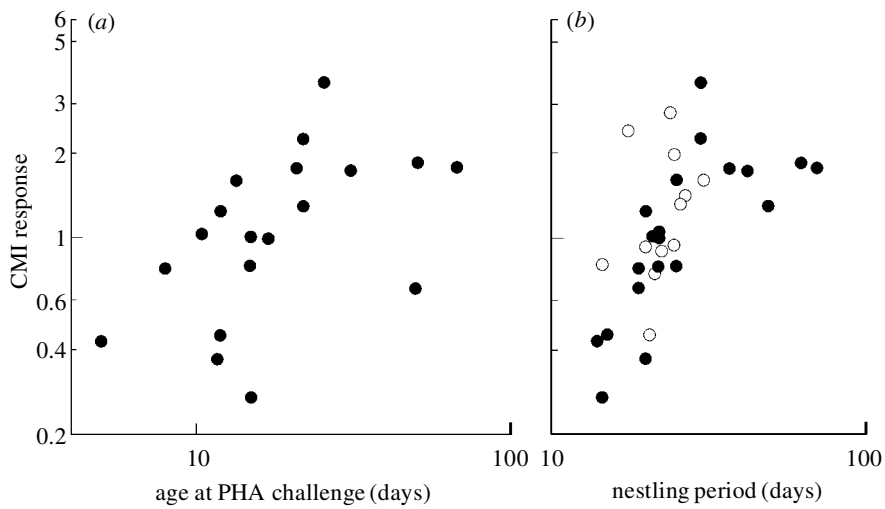

Figure 2. Relationship between the CMI response of chicks and the age post-hatch at which the PHA assay was administered (*a*) and the length of the nestling period (*b*). Open symbols represent species for which age at PHA test was not reported.

was significantly correlated with mass, egg volume, incubation period, nestling growth rate (inverse) and adult mortality rate (inverse) (table 1). Stepwise regression of adult CMI on mass, egg volume, incubation and nestling periods, clutch size and parasite prevalence ($n = 25$ species) entered adult mass first ($F_{1,23} = 53.3$, $p < 0.001$, $r^2 = 0.699$) followed by parasite prevalence ($F_{1,22} = 5.5$, $p = 0.028$, total $R^2 = 0.759$) and no other significant effects. A factor analysis including all the independent variables (table 2; $n = 15$) showed that adult CMI in this sample is associated with factor 1 ($r = 0.81$) along with body mass (0.87), adult mortality rate (−0.56) and longevity (0.76), and independently of clutch size (−0.01), nestling period (0.17) and prevalence of blood parasites (0.19). When growth rate, lifespan and adult mortality were dropped from the analysis to increase the sample of species to 25 (results not shown), CMI remained associated with factor 1 ($r = 0.92$), along with mass (0.93), egg volume (0.93), incubation period (0.86) and nestling period (0.65), but to the exclusion of clutch size (0.14) and parasite prevalence (0.13).

(e) *Clutch size and adult CMI*

Martin *et al.* (2001) found a strong positive relationship between clutch size and adult CMI. They interpreted adult CMI as an indicator of disease-related mortality, and suggested that higher adult mortality favours greater parental investment in offspring and hence large clutch size.

Table 2. Correlation coefficients of adult CMI response and each life-history variable with each of the axes derived by Varimax rotation of the original principal components calculated from the correlation matrix.
(Significant correlations ($p < 0.01$) are in bold.)

	all species			small altricial species		
variable	factor 1	factor 2	factor 3	factor 1	factor 2	factor 3
adult CMI response	**0.832**	0.113	0.154	**0.813**	**0.447**	0.174
mass	**0.966**	−0.017	0.108	**0.871**	0.297	0.300
egg volume	**0.951**	−0.085	0.223	**0.838**	0.304	0.358
incubation period	**0.939**	−0.124	0.056	**0.420**	**0.819**	0.229
nestling period	**0.936**	0.063	−0.141	0.174	**0.954**	−0.125
growth rate (K)	**−0.885**	−0.214	0.073	−0.332	**−0.876**	−0.050
reported lifespan	**0.783**	−0.281	−0.052	**0.758**	0.165	**−0.405**
adult mortality	**−0.719**	**0.640**	−0.067	**−0.556**	**−0.422**	**−0.598**
clutch size	0.133	**0.951**	−0.016	−0.009	−0.029	**−0.782**
parasite prevalence	0.055	−0.029	**0.989**	0.188	−0.022	**−0.878**
variance explained	6.225	1.503	1.100	3.356	2.935	2.223

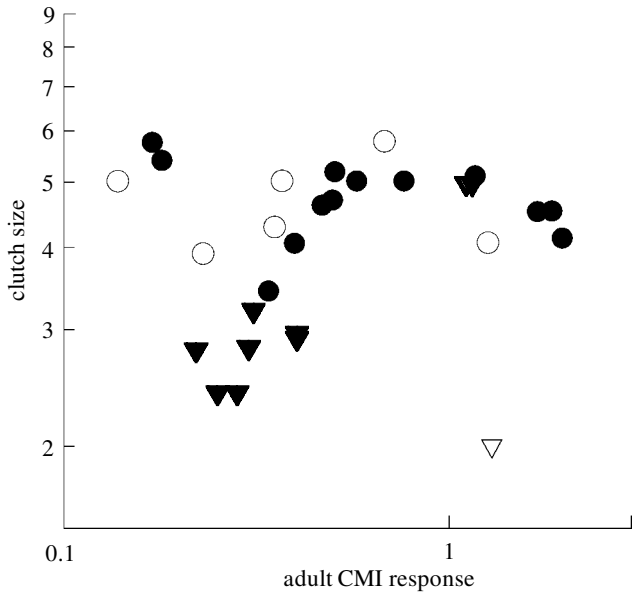

Figure 3. The relationship between clutch size and the CMI response of adults, with hemisphere and nest type distinguished. Only small altricial species ($n = 29$) are shown to make our sample comparable to that of Martin *et al.* (2001). Martin *et al.*'s criterion for hole nesting included only the two enclosed-nesting species at the upper left of the data distribution. The point at the lower right of the graph is the diamond dove, *Geopelia cuneata*, of Australia. Open circles, Northern open; filled circles, Northern enclosed; open triangles, Southern open; filled triangles, Southern enclosed.

To make our analyses comparable with that of Martin *et al.* (2001), we used the sample of small altricial species. In an ANCOVA relating clutch size to CMI, adult mass and nest type, neither the effect nor the covariates were significant ($p > 0.5$, $r^2 = 0.04$). In a second ANCOVA relating clutch size to CMI and hemisphere, the CMI × hemisphere interaction was marginally significant ($F_{1,25} = 5.1$, $p = 0.034$). With the interaction term removed, clutch size was greater in the Northern Hemisphere by 1.51 eggs ($F_{1,26} = 23.3$, $p = 0.0001$) but CMI was not a significant covariate ($F_{1,26} = 0.28$, $p = 0.60$) (figure 3). For the Northern Hemisphere species only, clutch size was not significantly related to CMI ($F_{1,17} = 0.6$, $p = 0.44$). For a smaller sample of Southern Hemisphere species, clutch size was also unrelated to CMI ($F_{1,8} = 3.2$, $p = 0.11$), primarily owing to diamond dove, *Geopelia cuneata*, which exhibited a strong PHA response and has a clutch of two eggs. Without this species, which was absent from the dataset of Martin *et al.* (2001), clutch size among Southern Hemisphere species bears a strong relationship to CMI ($F_{1,7} = 75.7$, $p < 0.0001$). Finally, the same tests applied to our whole dataset of species ($n = 37$) did not support a relationship between adult CMI and clutch size (all $p > 0.30$).

4. DISCUSSION

(a) *CMI as a life-history trait*

The within-species repeatability of CMI, and its significant correlation between adults and chicks, are consistent with the idea that CMI is, at least in part, a generalized, evolved attribute of species with considerable evolutionary lability. Even when the influence of nestling period (chick) and adult body mass (adult) on CMI were taken into account, residual variation in chick and adult CMI was correlated.

Simple correlations indicate that the CMI of chicks is directly related to the length of the nestling period, and inversely related to adult mortality. The results of stepwise regressions are more ambiguous but generally support the relationship of CMI to the nestling period. The relationship between chick CMI and nestling period was potentially confounded by a strong correlation between the nestling period and the age at which the PHA test was performed. However, multiple regression showed that only nestling period was a significant effect. The distinction between nestling period and test age is important because age dependence would mean that most of the variation observed among species reflected the developmental course of CMI (see § 1, proposition (ii)). If, however, the chick CMI varied among species but was correlated with the nestling period, then some factor

associated with nestling period would presumably exert selection on this component of the immune system. Long periods in nests, especially enclosed nests, many of which are reused over the season or even in subsequent years, would potentially lead to heavy parasite loads and selection for a strong immune response (proposition (iii); Møller & Erritzøe 1996). Alternatively, selection for a strong immune system could lead to the evolution of long incubation and nestling periods, if the development of a strong CMI response required time (proposition (ii)). This does not appear to be the case.

Adult CMI is strongly correlated with a number of life-history traits associated with size, development period and lifespan, but notably excluding clutch size and prevalence of blood parasites. In the sample of small altricial birds, nestling period and growth rate were not associated with adult CMI in a factor analysis. Clearly, adult CMI is associated with a strong life-history axis of body size and longevity, which may or may not be independent of development rate. Adult CMI is plausibly responsive to selection for prevention and repair mechanisms related to long lifespan (proposition (i)). However, the strong allometric relationship of CMI to body size as well as to mortality rate might arise through unspecified physiological mechanisms or through spurious variation in results caused by size itself, for example by the thickness of the skin affecting the magnitude of the PHA response (proposition (iv)).

Adult and chick CMI responses were unrelated to the proportion of individuals infected by blood parasites (primarily intracellular haemosporidians) in most of our analyses. Defence against apicomplexan diseases in domestic poultry depends primarily on the cell-mediated immune system (Lillehoj 1991), and presumably results from selective proliferation of T cells sensitive to parasite antigens. This suggests that the PHA response should be a good index for generalized resistance to infection by blood parasites (see also González *et al.* 1999). However, Cheng *et al.* (1991) found mostly negative correlations between strength of the PHA response and other measures of immune system function, indicating that defence against parasites may involve complex reactions that might not be adequately assessed by single assays. Moreover, although CMI cannot be ruled out, it is still not clear which arm of the immune system predominates to defend against blood parasite infections in birds (K. Klasing, personal communication). Thus, the PHA response could be independent of blood parasite prevalence because it does not reflect components of the immune system responsible for disease resistance, or because parasite prevalence does not reflect the interaction of the immune system and these particular parasites. Moreover, prevalence of blood parasites may be affected primarily by ecological factors such as habitat and geographical distribution of avian hosts (Tella *et al.* 1999), which could mask their relationships with host CMI.

(b) *Clutch size, immune response and demographically sensitive parental investment*

The hypothesis of Martin *et al.* (2001) was based on the premise that CMI is directly related to parasite-induced mortality. Their direct measurement of this relationship in the nestlings of 12 species supported the relationship strongly. It is more difficult to distinguish whether variation in CMI reflects an evolutionary response to selection applied by parasite infection, or is a phenotypically plastic response to the level of infection experienced. Studies on the direct effects of parasitism on PHA response remain equivocal. Gwinner *et al.* (2000) found higher PHA responses in nestling starlings (*Sturnus vulgaris*) from nests with high ectoparasite loads, a result confirmed experimentally by Christe *et al.* (2000) in other bird species, indicating that CMI can be primed by exposure. Alternatively, given that CMI has been found to be negatively correlated with antibody response (Cheng *et al.* 1991), these results could mean that birds with a high PHA response have low antibody responses and are poorly defended against ectoparasites. In contrast, Saino *et al.* (1998) and Brinkhof *et al.* (1999) found no effect of ectoparasites on the PHA response in experimentally infected chicks of the barn swallow (*Hirundo rustica*).

Assuming that the PHA response provides an index to nestling mortality and the strength of selection on adults to invest in offspring, Martin *et al.* (2001) expected an inverse relationship between clutch size and immunocompetence. We could find no statistical support for such a relationship, using a larger sample and also controlling for other variables, although the trend was negative, as expected.

No analysis similar to that of Martin *et al.* for chicks has related parasite-induced mortality to CMI in adults. Simple correlations show that adult CMI is positively related to body size, development period, survival rate and longevity. In multivariate analyses, the relationship between CMI and longevity remained strong. Whether larger organisms are inherently more vulnerable to parasite infection is not known. In our sample of species, prevalence of blood parasites was not related to body mass ($r = 0.030$, $p = 0.86$, $n = 35$), adult CMI ($r = 0.028$, $p = 0.87$, $n = 35$), maximum reported age ($r = -0.074$, $p = 0.71$, $n = 27$) or adult mortality rate ($r = 0.009$, $p = 0.97$, $n = 26$). The evidence currently available does not adequately address whether the PHA response might indicate parasite-induced mortality in adults.

With regard to the hypothesis of Martin *et al.* (2001), this is a moot point because clutch size and adult CMI are not related. The positive relationship reported by Martin *et al.* appears to have resulted, in part, from the criteria they adopted for hole nesting, which was used as a covariate in their analysis, and in part from their particular sample of species. Although not explicitly stated by the authors, we infer that they included only secondary hole nesters (*Ficedula hypoleuca* and *Tachycineta bicolor*), which do not excavate their own cavities or burrows. Such birds may be nest-site limited, which could keep populations low relative to food resources and allow pairs to raise larger broods. Thus, it is reasonable to group secondary hole nesters separately from other species, owing to the effect of nest type on clutch size. For our analyses, we reasoned that birds using any cavity or enclosure for nesting would potentially be exposed to higher parasite burdens because of the associated long nesting periods and frequent reuse of nest sites by cavity nesters. Thus, we also included as hole nesters *Merops* (bee-eaters), *Petrochelidon*, *Delichon*, *Riparia* (swallows and martins), and *Passer domesticus* (house sparrow). Our sample also included species

with CMI greater than 0.7, which were absent from Martin *et al.*'s analysis.

The connection between immune response and parasite-induced mortality is tenuous, at least for adults, and the expected relationships between clutch size and CMI were not significant for either chicks or adults when using larger samples than Martin *et al.* (33 species for chicks and 37 for adults, versus 22 and 17, respectively). Thus, we find little empirical support for Martin *et al.*'s hypothesis that clutch size represents demographically sensitive parental investment. The basic premise of the argument should also be questioned, because the relative value of present and future reproduction depends on relative rates of overall adult and juvenile mortality, not just components resulting from parasites and disease. To fully assess the relationship between parental investment and mortality, one would have to know the probability of recruitment of individual offspring into the breeding population, compared with the probability of survival of an adult from one breeding season to the next (Ricklefs 1983). CMI is correlated only with a small component of that mortality. However, components of the life table are so strongly intercorrelated in birds (Ricklefs 2000) that, in the absence of estimates of pre-reproductive survival, adult mortality rate provides a reasonable approximation of the axis of presumed parental investment in present reproduction. Indeed, clutch size is positively related to the logarithm of annual adult mortality rate in our sample ($r = 0.59$, $p = 0.0002$, $n = 35$), which would be in accordance with life-history theory if clutch size measured parental investment.

In conclusion, although the PHA index responds to experimental treatments and other sources of variation within species, demonstrating the phenotypic plasticity of this component of the immune system, its application to understanding variation, evolved or otherwise, in the immune response among species is not yet certain. Future comparative studies should include a wider variety of assays of immune function (Norris & Evans 2000), and species should be selected to provide statistical independence, to the extent possible, of variables potentially influencing the immune response. Moreover, more information is needed on which components of the immune system are involved in protection against different types of disease-causing organism, to assess correctly the relationships between parasites and the immunocompetence and life-history traits of their hosts.

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