

Maria G. Palacios · Thomas E. Martin

Incubation period and immune function: a comparative field study among coexisting birds

Received: 26 December 2004 / Accepted: 18 July 2005 / Published online: 11 October 2005
© Springer-Verlag 2005

Abstract Developmental periods are integral components of life history strategies that can have important fitness consequences and vary enormously among organisms. However, the selection pressures and mechanisms causing variation in length of developmental periods are poorly understood. Particularly puzzling are prolonged developmental periods, because their selective advantage is unclear. Here we tested the hypotheses that immune function is stronger in species that are attacked at a higher rate by parasites and that prolonged embryonic development allows the development of this stronger immune system. Through a comparative field study among 12 coexisting passerine bird species, we show that species with higher blood parasite prevalence mounted stronger cellular immune responses than species with lower prevalence. These results provide support for the hypothesis that species facing greater selection pressure from parasites invest more in immune function. However, species with longer incubation periods mounted weaker cellular immune responses than species with shorter periods. Therefore, cellular immune responses do not support the hypothesis that longer development time enhances immunocompetence. Future studies should assess other components of the immune system and test alternative causes of variation in incubation periods among bird species.

Keywords Blood parasites · Development · Immunocompetence · Life history · Passerines

Introduction

Understanding why organisms vary so broadly in their life history strategies is a major question in evolutionary biology (Roff 1992, 2002; Stearns 1992). Rates of development and resulting developmental periods are integral components of life history strategies that can have important fitness consequences (Promislow and Harvey 1990; Ricklefs 1993; Gebhardt-Heinrich and Richner 1998; Martin 2002), and are therefore expected to be optimized within physiological constraints of species facing different environmental pressures (Remes and Martin 2002). Duration of developmental periods shows extensive variation within and among diverse taxa, e.g. birds (Ricklefs 1993), mammals (Promislow and Harvey 1990), snakes (Shine 2003). For example, among placental mammals, gestation length can vary from 18 to 660 days (Promislow and Harvey 1990), and wide variation remains unexplained even after controlling for the allometric effect of body mass. In short, the mechanisms and selection pressures that underlie this extensive variation in duration of developmental periods remain unclear, and need study.

Birds constitute an excellent group in which to study variation in developmental periods because developmental periods vary greatly among species (Ricklefs and Starck 1998; Martin 2002) and are relatively easy to measure in the field. The latter is particularly true for the length of embryonic development, which is manifested as the incubation period. Incubation duration varies from 9 to about 80 days among bird species and can vary over a threefold range even among species of similar body size and developmental state of the neonate (i.e. altricial-precocial spectrum) (Rahn and Ar 1974; Lloyd 2004). Most selection pressures (e.g. nest predation, sibling competition, and harsh weather) are thought to favor short incubation periods in order to

Communicated by Mark Chappell

M. G. Palacios
Montana Cooperative Wildlife Research Unit,
University of Montana, Missoula, MT 59812, USA

T. E. Martin
US Geological Survey, Montana Cooperative Wildlife Research
Unit, University of Montana, Missoula, MT 59812, USA

Present address: M. G. Palacios (✉)
Department of Ecology, Evolution, and Organismal Biology,
Iowa State University, Ames, IA 50010, USA
E-mail: mgp@iastate.edu
Tel.: +1-515-2942759
Fax: +1-515-2948457

reduce the negative effects of time dependent mortality (Lack 1968; Ricklefs 1993; Bosque and Bosque 1995; Martin and Clobert 1996; Martin 2002; Lloyd and Martin 2003). The existence of broad variation in the duration of incubation period and especially long incubation periods, therefore, is puzzling and suggests that a longer embryonic period must provide some advantage (Ricklefs 1993; Martin and Clobert 1996; Ricklefs and Starck 1998; Martin 2002).

A particularly interesting possibility is that longer incubation periods might allow enhanced development of immunocompetence (Ricklefs 1992, 1993), which is defined as the ability of an individual to defend itself from parasites and disease. This hypothesis was proposed based on the finding of a correlation between blood parasite prevalence and incubation period length among families of non-raptorial altricial land birds (Ricklefs 1992); a pattern that was later found for bird of prey species (Tella et al. 1999). Parasites are ubiquitous environmental components that negatively affect the fitness of their hosts and are thought to have played an important role in several aspects of avian life history evolution (e.g. Moller 1997; Fitze et al. 2004). Therefore, the potential role of parasites and immune function in favoring longer incubation periods in birds is intriguing and deserves study.

Under this scenario, bird species facing higher risk from parasites should invest more in development of their immune system (Moller and Erritzoe 1996, 1998; Moller 1998; Martin et al. 2001), which might be achieved by a lengthened incubation period (Ricklefs 1992, 1993). To our knowledge, only one study to date (Tella et al. 2002) has tested the latter hypothesis, and found no support for it. The study by Tella et al. (2002) was performed using data gathered from the literature and therefore included measurements made by different investigators, using different protocols and techniques, and across very different environments. All these factors

introduce extraneous variation in the data, potentially obscuring patterns.

To minimize these potential problems, we conducted a focused comparative field study among passerine bird species coexisting in the same environment (i.e. location and habitat), in which all measurements were performed by the same researchers during a relatively short study period. We examined two main predictions: (1) species subject to greater exposure to parasites should mount stronger immune responses to novel antigens than species facing lower exposure, and in turn (2) species mounting stronger immune responses should have longer incubation periods than species mounting weaker responses. We tested these predictions while controlling for other potential explanatory factors.

Materials and methods

Study site and study species

Field work was conducted in snowmelt drainages of a high elevation (2600 m) mixed deciduous-conifer forest on the Mogollon Rim in central Arizona (see Martin 1998 for habitat details). We performed a comparative study across 12 passerine bird species that are common breeders in the study area and for which we could obtain adequate sample sizes (i.e. ≥ 5 broods per species for immune function sampling and ≥ 10 individuals per species for parasite sampling) (Table 1). We searched for nests from the beginning of May to the end of July during the 2001 and 2002 breeding seasons and during June in the 2003 breeding season. We located nests based on parental behavior (Martin and Geupel 1993). We checked nests every 2–4 days to record activity and status, except that we checked nests more frequently, even daily, during transition events (i.e. hatching and fledging) to quantify period lengths (Martin 1998, 2002).

Table 1 Summary information on PHA response, body mass of nestlings on the day of the PHA test, blood parasite prevalence, and duration of the incubation period for our study species

Species	PHA response (mm) ^a	Body mass (g) ^a	n_1	Blood parasite prevalence (%) ^b	n_2	Incubation period (days)	n_3
American robin (<i>Turdus migratorius</i>)	0.75 (0.106)	43.22 (2.66)	7	31	16	12.70 (0.0303)	180
Western bluebird (<i>Sialia mexicana</i>)	0.68 (0.049)	24.10 (0.73)	6	ND	1	13.70 (0.0306)	37
Green-tailed towhee (<i>Pipilo chlorurus</i>)	0.48 (0.049)	18.32 (0.55)	7	22	18	12.00 (0.1679)	18
Grey-headed junco (<i>Junco hyemalis</i>)	0.90 (0.041)	14.67 (0.30)	15	47	19	12.45 (0.0751)	127
Orange-crowned warbler (<i>Vermivora celata</i>)	0.32 (0.047)	7.55 (0.19)	9	7	15	12.67 (0.0801)	111
Virginia's warbler (<i>Vermivora virginiae</i>)	0.35 (0.049)	6.37 (0.26)	8	5	20	12.30 (0.0936)	85
Red-faced warbler (<i>Cardellina rubrifrons</i>)	0.31 (0.054)	8.20 (0.15)	5	0	20	12.82 (0.0606)	111
Brown creeper (<i>Certhia americana</i>)	0.25 (0.045)	6.67 (0.09)	6	0	10	14.56 (0.0585)	117
House wren (<i>Troglodytes aedon</i>)	0.39 (0.054)	9.23 (0.34)	10	25	16	13.75 (0.0208)	294
White-breasted nuthatch (<i>Sitta canadensis</i>)	1.14 (0.089)	16.62 (0.65)	5	ND	2	12.38 (0.1231)	58
Mountain chickadee (<i>Parus gambeli</i>)	0.44 (0.029)	9.86 (0.42)	6	6	16	13.78 (0.0228)	154
Cordilleran flycatcher (<i>Empidonax occidentalis</i>)	0.24 (0.057)	10.49 (0.24)	6	5	19	14.65 (0.0602)	144

n_1 sample size for PHA response and body mass, n_2 sample size for blood parasite prevalence, n_3 sample size for incubation period, ND no data (see methods)

Data shown are means (SE) except for blood parasite prevalence

^aSampling unit is one brood (see methods)

^bSampling unit is an individual bird

Incubation period was defined as the interval between laying and hatching of the last laid egg (Nice 1954). Data on incubation periods (Table 1) and nest predation rates are from a long-term study of our study populations (Martin 2002) and supplemented with more recent data to ensure robust estimates.

Assessment of parasite prevalence

Birds are host to a wide variety of parasites (Clayton and Moore 1997). We sampled three main groups of avian parasites: blood parasites, ectoparasites, and intestinal parasites. Prevalence of ectoparasites and intestinal parasites was very low in our study site, being zero for many species (M. G. Palacios, unpublished data). Therefore, we focused on the prevalence of blood parasites. Interspecific variation in blood parasite prevalence [i.e. percentage of infected host individuals in a sample (Margolis et al. 1982)] could result from differential exposure or differential resistance to parasites (Read 1991; Yezerinac and Weatherhead 1995). Distinguishing between the two possible causes in wild populations is virtually impossible at present (Yezerinac and Weatherhead 1995). However, some indication that blood parasite prevalence might actually reflect the level of exposure to blood parasites comes from empirical evidence showing that blood parasite prevalence is positively correlated with the abundance and activity of the parasites' vectors (see Yezerinac and Weatherhead 1995). Therefore, we used prevalence of blood parasites as an indicator of the level of exposure to blood parasites in the environment and their potential selection pressure on their hosts.

We estimated the prevalence of blood parasites by scanning blood smears from adult birds. We could not estimate prevalence from nestlings because the latency for detection of blood parasites in peripheral blood is usually longer than the nestling period for many of the study species (Bennett et al. 1995). A droplet of blood from adult birds was used to prepare thin smears using glass microscope slides. Smears were air dried, fixed with absolute methanol, and stained with Giemsa stain. Each whole smear was scanned under 400× for the presence of *Trypanosoma* spp., *Leucocytozoon* spp., and microfilariae; whereas *Plasmodium* spp. and *Haemoproteus* spp. were screened in 100 microscope fields under 1000× magnification. Recommendations by Godfrey et al. (1987) for quantification of blood parasites were followed. Prevalence of blood parasites was calculated for all study species except western bluebird and white-breasted nuthatch (Table 1), for which sample sizes were too low for precise estimation.

Assessment of immune function

The avian immune system includes different components that interact to defend the organism against parasites

and disease (Toivanen and Toivanen 1987). Two main arms are recognized: innate immunity and acquired immunity. The latter arm can be further subdivided into a humoral and a cell-mediated component (Roitt et al. 1998). Given this complexity, a broad scale estimate of immunocompetence would require performing several immunological tests involving different antigens and a large number of individuals, but this is likely to conflict with practical and ethical considerations (Gonzalez et al. 1999; Norris and Evans 2000), especially in studies performed on wild animals in the field. Therefore, since the proposed mechanism linking length of the incubation period and development of the immune system involves mainly the acquired arm (Ricklefs 1992; Apanius 1998), we focused on this component as a first approach for testing the hypothesis of interest.

We assessed immune function of nestlings of all study species using the in vivo immune response to phytohaemagglutinin (PHA), a novel antigen. The use of a novel antigen instead of a real pathogen is necessary to avoid the potential confounding of unknown exposure history to real pathogens by the different species. The PHA skin test is a standard immune challenge that has been widely used as a general index of cell-mediated immunity for birds in the field (Norris and Evans 2000; Fairbrother et al. 2004), and intraspecific studies have shown that stronger immune responses to PHA are associated with increased survival of individuals (e.g. Saino et al. 1997a, b; Christe et al. 1998, 2001; Horak et al. 1999).

We followed the simplified version of the PHA test described by Smits et al. (1999). First, we measured the thickness of the wing-web to the nearest 0.01 mm using a pressure-sensitive micrometer (Mitutoyo, Japan). Next, we injected the wing-web with 0.2 mg PHA (Sigma L-8754) in 0.04 ml of phosphate buffered saline (PBS). This dose of PHA has been widely used in studies of passerine species in the wild (Saino et al. 1997b, 1999, 2003; Soler et al. 1999; Moller et al. 2001). Approximately 24 h later we measured the swelling of the wing-web at the site of injection. The immune response (PHA response) was estimated as the difference between the initial and the final measurements of wing-web thickness. We performed this test on nestlings from all study species (Table 1) at a similar developmental stage (i.e. when primary feathers break their sheaths) to standardize for interspecific comparisons and variation in PHA response among species was significantly larger than variation within species (ANOVA: $F_{11,78} = 23.32$, $p < 0.0001$). Body mass of nestlings was measured to the nearest 0.01 g using an electronic scale. All nestlings in a nest were measured and the average for the brood was calculated. These brood means were then averaged to obtain the mean measurement for each species. Therefore, broods constituted the sampling units for measurements performed on nestlings.

We examined blood parasites in adults because many of the most common blood parasites cannot be detected in circulation at the early age at which the PHA test was

performed (see above) and we assume that differential selection on immune function among species should be appropriately indexed by differences in infection rates as measured in adults. We examined immune function in nestlings because the hypothesis we tested centered on possible effects of developmental period on immune function. However, we assume that a functional relationship exists between nestling and adult immunocompetence such that species having stronger immune responses by nestlings also have stronger responses by adults. This is likely given that most of the ontogeny of the immune system takes place during embryonic and early postnatal development. Indeed, comparative studies show that PHA of nestlings and adults are directly proportional (i.e. do not deviate from isometry) (Moller et al. 2001) and tightly positively correlated among species (e.g. Moller et al. 2001; Tella et al. 2002). Thus, we expect the relationship between immune responses of nestlings and adult parasite infection to reflect adult relationships.

Comparative analyses

We used phylogenetically independent contrasts (Felsenstein 1985) to correct for possible phylogenetic effects. We calculated independent contrasts, assuming equal branch lengths, with the program COMPARE (Martins 2003) and using a phylogenetic hypothesis derived from sources summarized in Martin and Clobert (1996).

Statistical analyses

We performed multiple regression analyses to test for the predicted relationships among the variables of interest (i.e. incubation period, immune response, and parasite prevalence) or their respective independent contrasts. Relationships among contrasts were estimated using regressions through the origin (Harvey and Pagel 1991). Several factors could confound the relationships of interest and we therefore assessed these factors as potential explanatory variables in the multiple regression analyses.

Body mass was used to control for its positive effect on immune responses across species (Martin et al. 2001). We included the rate of nest predation as a covariate that can influence incubation period (Martin 2002) in order to assess the unique relationship between incubation period and immune function. In addition, absolute age at which the PHA test is performed, duration of the nestling period, clutch size, and nest type (i.e. open cup or cavity) were assessed as potential explanatory variables as they have been associated with PHA response in previous studies (Martin et al. 2001; Tella et al. 2002). Absolute age at PHA test (i.e. age at pin break in our case) was known for all species except the western bluebird.

All variables were \log_{10} transformed to achieve normality before analyses, except for nest predation rate and clutch size that were normally distributed. All statistical analyses were performed using SPSS 11.5.0.

Results

Blood parasite prevalence and immune function

All major types of avian blood parasites (i.e. *Haemoproteus* spp., *Plasmodium* spp., *Trypanosoma* spp., *Leucocytozoon* spp., and microfilaria) were present in the study species. Total blood parasite prevalence (i.e. percentage of individuals infected with any type of blood parasite) varied considerably among species (Table 1), and increased with body mass ($r=0.682$, $p=0.030$, $n=10$), although not when independent contrasts were used to control for possible phylogenetic effects ($r=0.524$, $p=0.148$, $n=9$). There was no relationship between total blood parasite prevalence and incubation period among species, either in a simple regression ($r=-0.422$, $p=0.225$, $n=10$) or in multiple regression models controlling for potential confounding variables (i.e. body mass and/or nest predation rate) ($r_p < -0.433$, $p > 0.244$, $n=10$). The strength of the cellular immune response assessed by the PHA test increased with body mass ($r=0.703$, $p=0.011$, $n=12$), as seen in previous comparative studies (e.g. Martin et al. 2001; Tella et al. 2002); and there was a marginally significant trend when possible phylogenetic effects were controlled with independent contrasts ($r_p=0.614$, $p=0.079$, $n=11$). After controlling for allometric effects, PHA response increased with total blood parasite prevalence among species ($r_p=0.923$, $p < 0.0001$, $n=10$, Fig. 1), even after controlling for possible phylogenetic effects ($r_p=0.954$, $p < 0.0001$, $n=9$). Thus, the results provide support for

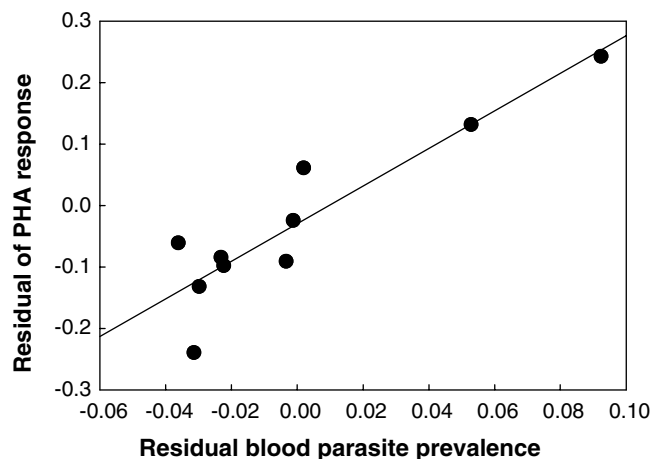


Fig. 1 Scatterplot of the residuals from the multiple regression model showing increasing PHA response with increasing prevalence of blood parasites when controlling for body mass among ten passerine bird species (see Table 1 and methods). Linear regression line: $y = 0.164 + 0.278x$ ($r_p = 0.923$, $p < 0.0001$)

the prediction that species facing greater exposure to parasites show stronger cellular immune responses to novel antigens.

Developmental periods and immune function

Age of nestlings on the day of the PHA challenge (i.e. pin break) was strongly positively related to total length of the nestling period among species ($r=0.830$, $p=0.002$, $n=11$; independent contrasts: $r=0.827$, $p=0.003$, $n=10$). However, neither age of nestlings nor length of the nestling period explained significant variation in PHA response among the study species when controlling for body mass (age at test: $r_p=0.096$, $p=0.791$, $n=11$; independent contrasts: $r_p=-0.015$, $p=0.970$, $n=10$; nestling period: $r_p=0.011$, $p=0.974$, $n=12$; independent contrasts: $r_p=0.013$, $p=0.972$, $n=11$). Furthermore, lengths of the nestling period and incubation period were not correlated among species ($r=0.498$, $p=0.100$, $n=12$; independent contrasts: $r=0.032$, $p=0.925$, $n=11$). Therefore, length of the nestling period is not likely to confound the analyses involving length of the incubation period and immune response. Similarly, clutch size and nest type did not explain variation in PHA response (after controlling for allometry) among our study species and are therefore not discussed any further (clutch size: $r_p=0.353$, $p=0.286$, $n=12$; nest type (ANCOVA): $F_{1,10}=0.713$, $p=0.420$, $n=12$).

Given the observed allometric effect on PHA responses in this and other studies (e.g. Moller et al. 2001; Tella et al. 2002), we used residual PHA response after correcting for body mass in the remaining analyses. Both nest predation rate and residual PHA responses explained significant variation in incubation period among species (whole model: $r^2=0.718$, $p=0.003$, Fig. 2). As found in other studies (e.g. Martin 2002), species suffering higher rates of nest predation had shorter incubation periods than species with lower nest predation rates ($r_p=-0.807$, $p=0.003$, $n=12$, Fig. 2a). However, contrary to our initial prediction, we found a negative relationship between length of the incubation period and strength of the PHA immune response ($r_p=-0.761$, $p=0.007$, $n=12$, Fig. 2b); that is, species with longer

incubation periods mounted weaker rather than stronger cellular immune responses. The latter result remained significant when we controlled for phylogeny using independent contrasts ($r_p=-0.700$, $p=0.036$, $n=11$), while the relationship between incubation period and nest predation rate was no longer significant ($r_p=-0.302$, $p=0.439$, $n=11$). Thus, our results do not provide support for the prediction that species with longer incubation periods are able to develop and mount stronger cellular immune responses to novel antigens.

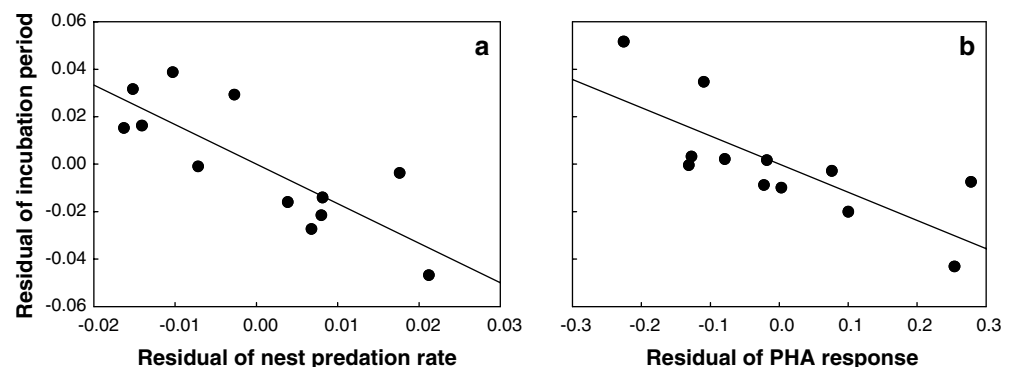
Discussion

Blood parasite prevalence and immune function

The immune system is one of the main defenses organisms have evolved to cope with parasites and disease, and therefore, we expect species facing greater selection pressure from parasites (either at the nestling or adult stage) to have evolved greater investment in immune defenses. In accordance with this expectation, species likely to face higher exposure to parasites (e.g. colonial species and tropical species) have larger immune organs and/or mount stronger immune responses than their counterparts (e.g. solitary species and temperate species) (Moller and Erritzoe 1996, 1998; Moller 1997, 1998; Moller et al. 2001). Moreover, parasite abundance can predict immunological investment among populations within a species (Lindstrom et al. 2004).

Our field study provides additional evidence that exposure to parasites can influence investment in immune function. We show for the first time that species having higher prevalence of blood parasites mount stronger cellular immune responses than coexisting species with lower prevalences (Fig. 1). This result follows theoretical predictions that an evolutionary arms race should cause species that are attacked by parasites at a higher rate to evolve stronger immune defense, but still be attacked at a higher rate, causing a positive relationship between parasite abundance and immune function (Martin et al. 2001). Indeed, our results are opposite that expected if immune function influenced parasite prevalence (i.e. a proximate effect); in this case, greater immune function should, if anything, yield

Fig. 2 Scatterplots of the residuals from the multiple regression model of incubation period relative to **a** nest predation rate among species and **b** PHA response (mass corrected)



reduced parasite prevalence and immune function should show a negative relationship with parasite prevalence rather than the positive one that we observed.

On the other hand, it could be argued that a positive relationship between blood parasite prevalence and immune response could arise if species facing current infection with parasites had enhanced overall immune responses to fight the ongoing infection. Although we do not know the infection history of our populations, as is the case of most studies of ecological immunology, evidence from intraspecific studies suggests that this is not the case. That is, individuals infected with parasites actually have lower immune responses to PHA than healthy individuals (e.g. Johnsen and Zuk 1999; Navarro et al. 2003). Thus, the positive relationship we found between blood parasite prevalence and immune response to PHA across species suggests that greater exposure to blood parasites favors increased investment in cell-mediated immunity.

Our finding is also in accordance with evidence suggesting that the cellular immune response might be of main importance in fighting infections by haematozoans (i.e. *Haemoproteus* spp., *Plasmodium* spp., and *Leucocytozoon* spp.), which were the most common blood parasites in our study populations. For instance, defense against apicomplexan parasites in poultry depends largely on the cellular component of the immune system (Lillehoj 1991), and experimental studies have shown an association between blood parasite infection and cellular immune response, but not with humoral immune response (Gonzalez et al. 1999; Soler et al. 2003).

To our knowledge, only Tella et al. (2002) assessed the relationship between prevalence of blood parasites and PHA response among avian species. In contrast to expectations and our results, they found no relationship between prevalence of blood parasites of adults and PHA response of adults or nestlings among 27 small altricial species using data compiled from the literature. The discrepancy between these results potentially reflects the different set of species included in the studies and/or the different nature of the data used. Data gathered from the literature are inherently more variable than data collected by the same researchers in a planned field study. For example, PHA responses gathered from the literature usually include measurements made by different investigators using critically different protocols (e.g. concentration of PHA solution, volume of PHA solution injected, use of controls, age/stage at challenge) and on species that live in very different environments. The same is probably the case for the data on blood parasite prevalence gathered from the literature. Prevalence of blood parasites can vary considerably among environments (i.e. habitats) and across study years for a given population (Bennett et al. 1995; Yezerinac and Weatherhead 1995; Bauchau 1998). Pooling data from disparate studies might, therefore, confound analyses of interspecific variation (Yezerinac and Weatherhead 1995).

As a consequence, the study by Tella et al. (2002) and other studies using data gathered from the literature might fail to detect existing patterns due to inherent extraneous variation in the dataset. This highlights the value of performing comparative field studies in the same habitat and location and in which all the variables are measured by the same researchers using the same methodology.

Incubation period and immune function

Our results from cellular immune responses did not support the hypothesis that longer incubation periods allow enhanced immune function (Ricklefs 1992, 1993), even after assessing (and when necessary controlling) for potential confounding factors such as allometry, rate of nest predation, age of nestlings at PHA challenge, duration of the nestling period, clutch size, nest type, and phylogeny. Indeed, opposite to the prediction, our field study across coexisting passerines shows that species with relatively longer incubation periods mounted weaker cellular immune responses than species with shorter periods. At present, the reason for the negative relationship between incubation period and PHA response among the study species is unclear. However, a field study of 24 coexisting passerine bird species in South Africa also found a strong negative relationship between incubation period and PHA response (P. Lloyd and T. E. Martin, unpublished data), suggesting that the pattern observed here may be robust.

In addition, we did not find the negative relationship between blood parasite prevalence and incubation period that inspired the hypothesis linking immune function and duration of embryonic development (Ricklefs 1992; Tella et al. 1999). This disparity could be due to the different set of species analyzed or could be the result of the taxonomic level at which the analyses were performed (e.g. family level in Ricklefs (1992) while species level in our study). Thus, our results suggest that longer embryonic development in birds does not result in better immune function, at least as measured by the PHA test, but that immune function measured by the PHA test is responsive to parasite attack rates and thus a reasonable index of immune function responsiveness to environmental selection.

The conclusion that longer embryonic development does not result in better immune function was also reached by Tella et al. (2002) using data from the literature. They found a positive correlation between PHA response and incubation period, but this relationship did not hold when other explanatory variables were included in a multiple regression analysis (Tella et al. 2002). On the other hand, we found a negative relationship between incubation period and PHA response after accounting for potential confoundings. Again, comparative field studies such as ours might be more likely to detect existing patterns than studies that use data from the literature due to the reduction in extraneous varia-

tion that might obscure relationships. Moreover, the significant relationships that we observed occurred across a relatively narrow range of incubation periods, as typical of north temperate passerines (Martin 2002). Incubation periods vary much more extensively among latitudes (Martin 2002), and comparisons of related species among latitudes might provide further insights. In addition, future tests of this hypothesis involving immunological tests that assess other components of the immune system would be important to evaluate the generality of our results. Our understanding of why birds vary so widely in the length of the incubation period would also benefit from assessment of other proposed physiological trade-offs such as development of a more complex nervous system or enhanced general quality of tissues with slower development (Ricklefs 1993; Ricklefs and Starck 1998). Finally, consideration of alternative hypotheses that do not depend on physiological trade-offs are also important; in particular, prolonged incubation could result from reduced nest attentiveness (Martin 2002). If longer incubation periods are the result of time spent at suboptimal embryo temperatures due to reduced attentiveness (i.e. Martin 2002), then development of all systems might be retarded (Martin et al. in review), potentially explaining our observations of reduced immune function in such species.

Acknowledgements We thank S. Pearson, M. Agudelo, A. Evans, R. Ton, and T.J. Fontaine for important assistance in the field, and C. Vleck and M. Haussmann for valuable comments on previous versions of this manuscript. We also thank Mark Chappell and Jose Luis Tella for thoughtful reviews that helped improve our manuscript. This work was supported by a National Science Foundation grant (DEB-9707598 and DEB-9981527) to TEM. PHA measurements of nestlings were made in accordance with standard animal care protocols and approved by The University of Montana Animal Care and Use Committee.

References

- Apanius V (1998) Ontogeny of immune function. In: Starck JM, Ricklefs RE (eds) *Avian growth and development*. Oxford University Press, New York, pp 203–222
- Bauchau V (1998) Comparison of parasitism level in two sympatric passerines: the pied flycatcher and the great tit. *Ecoscience* 5:164–171
- Bennett GF, Squiresparsons D, Siikamaki P, Huhta E, Allander K, Hillstrom L (1995) A comparison of the blood parasites of 3 Fenno-Scandian populations of the pied flycatcher *icedula-hypoleuca*. *J Avian Biol* 26:33–38
- Bosque C, Bosque MT (1995) Nest predation as a selective factor in the evolution of developmental rates in altricial birds. *Am Nat* 145:234–260
- Christe P, De Lope F, Gonzalez G, Saino N, Moller AP (2001) The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* 126:333–338
- Christe P, Moller AP, De Lope F (1998) Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos* 83:175–179
- Clayton DH, Moore J (1997) Introduction. In: Clayton DH, Moore J (eds) *Host-parasite evolution: general principles and avian models*. Oxford University Press, New York, pp 1–6
- Fairbrother A, Smits J, Grasman KA (2004) Avian immunotoxicology. *J Toxicol Environ Health B: Crit Rev* 7:105–137
- Felsenstein J (1985) Phylogenies and the comparative method. *Am Nat* 125:1–15
- Fitze PS, Clobert J, Richner H (2004) Long-term life-history consequences of ectoparasite-modulated growth and development. *Ecology* 85:2018–2026
- Gebhardt-Heinrich S, Richner H (1998) Causes of growth variation and its consequences on fitness. In: Starck JM, Ricklefs RE (eds) *Avian growth and development*. Oxford University Press, New York, pp 324–339
- Godfrey RD, Fedynich AM, Pence DB (1987) Quantification of hematozoa in blood smears. *J Wildl Dis* 23:558–565
- Gonzalez G, Sorci G, Moller AP, Ninni P, Haussy C, De Lope F (1999) Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). *J Anim Ecol* 68:1225–1234
- Greiner EC, Bennet GF, White EM, Coombs RF (1975) Distribution of avian haematozoa of North America. *Can J Zool* 53:1762–1787
- Harvey P, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford University Press, Oxford
- Horak P, Tegelmann L, Ots I, Moller AP (1999) Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia* 121:316–322
- Johnsen TS, Zuk M (1999) Parasites and tradeoffs in the immune response of female red jungle fowl. *Oikos* 86:487–492
- Lack D (1968) *Ecological adaptations for breeding in birds*. Methuen, London
- Lillehoj HS (1991) Cell-mediated immunity in parasitic and bacterial diseases. In: Sharma J (ed) *Avian cellular immunology*. CRC Press, Boca Raton, FL, pp 155–182
- Lindstrom KM, Foufopoulos J, Parn H, Wikelski M (2004) Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proc R Soc Lond Ser B-Biol Sci*; DOI 10.1098/rspb.2004.2752
- Lloyd JD, Martin TE (2003) Sibling competition and the evolution of prenatal development rates. *Proc R Soc Lond Ser B-Biol Sci* 270:735–740
- Lloyd P (2004) Eight- to ten-day incubation and nestling periods among Eremopterix sparrow-larks. *Ibis* 146:347–350
- Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (Report of an ad hoc Committee of the American-Society of Parasitologists). *J Parasitol* 68:131–133
- Martin TE (1998) Are microhabitat preferences of coexisting species under selection and adaptive? *Ecology* 79:656–670
- Martin TE (2002) A new view of avian life-history evolution tested on an incubation paradox. *Proc R Soc Lond Ser B-Biol Sci* 269:309–316
- Martin TE, Clobert J (1996) Nest predation and avian life-history evolution in Europe versus North America: a possible role of humans? *Am Nat* 147:1028–1046
- Martin TE, Geupel GR (1993) Nest-monitoring plots – methods for locating nests and monitoring success. *J Field Ornithol* 64:507–519
- Martin TE, Moller AP, Merino S, Clobert J (2001) Does clutch size evolve in response to parasites and immunocompetence? *Proc Natl Acad Sci USA* 98:2071–2076
- Martin TE, Auer SK, Bassar RD (in review) Maternally induced embryonic temperature explains global variation in avian incubation periods. *Proc Natl Acad Sci*
- Martins EP (2003) COMPARE, version 4.5. Computer programs for the statistical analysis of comparative data
- Moller AP (1997) Parasitism and the evolution of host life history. In: Clayton DH, Moore J (eds) *Host-parasite evolution: general principles and avian models*. Oxford University Press, New York, pp 105–127
- Moller AP (1998) Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos* 82:265–270

- Moller AP, Erritzoe J (1996) Parasite virulence and host immune defense: host immune response is related to nest reuse in birds. *Evolution* 50:2066–2072
- Moller AP, Erritzoe J (1998) Host immune defence and migration in birds. *Evol Ecol* 12:945–953
- Moller AP, Merino S, Brown CR, Robertson RJ (2001) Immune defense and host sociality: a comparative study of swallows and martins. *Am Nat* 158:136–145
- Navarro C, Marzal A, de Lope F, Moller AP (2003) Dynamics of an immune response in house sparrows *Passer domesticus* in relation to time of day, body condition and blood parasite infection. *Oikos* 101:291–298
- Nice MM (1954) Problems of incubation periods in North American birds. *Condor* 56:173–197
- Norris K, Evans MR (2000) Ecological immunology: life history trade-offs and immune defense in birds. *Behav Ecol* 11:19–26
- Promislow DEL, Harvey PH (1990) Living fast and dying young—a comparative-analysis of life-history variation among mammals. *J Zool* 220:417–437
- Rahn H, Ar A (1974) Avian egg—incubation-time and water-loss. *Condor* 76:147–152
- Read AF (1991) Passerine polygyny—a role for parasites. *Am Nat* 138:434–459
- Remes V, Martin TE (2002) Environmental influences on the evolution of growth and developmental rates in passerines. *Evolution* 56:2505–2518
- Ricklefs RE (1992) Embryonic-development period and the prevalence of avian blood parasites. *Proc Natl Acad Sci USA* 89:4722–4725
- Ricklefs RE (1993) Sibling competition, hatching asynchrony, incubation period, and lifespan in altricial birds. In: Power DM (ed) *Current ornithology*, vol 11. Plenum Press, New York, pp 199–276
- Ricklefs RE, Starck JM (1998) Embryonic growth and development. In: Starck JM, Ricklefs RE (eds) *Avian growth and development*. Oxford University Press, New York, pp 31–58
- Roff D (1992) *The evolution of life histories: theory and analysis*. Chapman and Hall, New York
- Roff D (2002) *Life history evolution*. Sinauer Associates, MA
- Roitt I, Brostoff J, Male D (1998) *Immunology*. Mosby, London
- Saino N, Bolzern AM, Moller AP (1997a) Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proc Natl Acad Sci USA* 94:549–552
- Saino N, Calza S, Moller AP (1997b) Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J Anim Ecol* 66:827–836
- Saino N, Calza S, Ninni P, Moller AP (1999) Barn swallows trade survival against offspring condition and immunocompetence. *J Anim Ecol* 68:999–1009
- Saino N, Ferrari R, Romano M, Martinelli R, Moller AP (2003) Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. *Proc R Soc Lond Ser B-Biol Sci* 270:2485–2489
- Shine R (2003) Reproductive strategies in snakes. *Proc R Soc Lond Ser B-Biol Sci* 270:995–1004
- Smits JE, Bortolotti GR, Tella JL (1999) Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct Ecol* 13:567–572
- Soler JJ, de Neve L, Perez-Contreras T, Soler M, Sorci G (2003) Trade-off between immunocompetence and growth in magpies: an experimental study. *Proc R Soc Lond Ser B-Biol Sci* 270:241–248
- Soler M, Martin-Vivaldi M, Marin JM, Moller AP (1999) Weight lifting and health status in the black wheatear. *Behav Ecol* 10:281–286
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, Oxford
- Tella JL, Blanco G, Forero MG, Gajon A, Donazar JA, Hiraldo F (1999) Habitat, world geographic range, and embryonic development of hosts explain the prevalence of avian hematozoa at small spatial and phylogenetic scales. *Proc Natl Acad Sci* 96:1785–1789
- Tella JL, Scheuerlein A, Ricklefs RE (2002) Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proc R Soc Lond Ser B-Biol Sci* 269:1059–1066
- Toivanen A, Toivanen P (1987) *Avian immunology: basis and practice*. CRC Press, Boca Raton, FL
- Yezerinac SM, Weatherhead PJ (1995) Plumage coloration, differential attraction of vectors and hematozoa infections in birds. *J Anim Ecol* 64:528–537