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Life history strategies : evolution of developmental periods in birds

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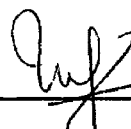
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LIFE HISTORY STRATEGIES:

EVOLUTION OF DEVELOPMENTAL PERIODS IN BIRDS

by

Maria Gabriela Palacios

B.S., Universidad de Buenos Aires, Argentina, 1999

Presented in partial fulfillment of the requirements

for the degree of

Masters of Science

THE UNIVERSITY OF MONTANA
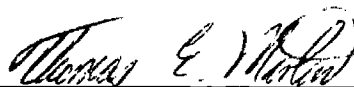
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ABSTRACT

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Life history strategies: evolution of developmental periods in birds.

Advisor: Thomas E. Martin *TEM*

Developmental periods are an integral component of life history strategies that vary enormously among organisms and can have important fitness consequences. However, the ultimate causes and proximate mechanisms underlying their variation are poorly understood. Avian incubation provides an ideal model to study variation in developmental periods, and several selective factors and mechanisms may underlie the broad variation in incubation period among species. Here we focused on the intriguing hypothesis that links selection pressure from parasites with incubation duration through an intrinsic mechanism: development of immunocompetence (the ability of an individual to defend itself from parasites and disease). In particular, we tested the predictions that species with longer incubation periods should have better immunocompetence, and that species subject to greater exposure to parasites should invest more in immunocompetence. We performed a comparative field study among 8 coexisting passerine bird species, and assessed immunocompetence by measuring two different components of the immune system. We found no support for the idea that a longer period inside the egg allows the development of a better immune system to cope with parasites and diseases. Instead, species with relatively longer incubation period mounted weaker cellular immune responses and no relationship was found between incubation period and strength of the humoral immune response. On the other hand, we found a positive relationship between blood parasite prevalence and cellular immune response, providing some support that species facing greater selection pressure from parasites invest more in immunocompetence. In accordance with theoretical expectations and previous empirical studies, we found that nest predation rate might be an important influence on life history evolution. Species facing higher rates of nest predation showed relatively shorter incubation periods, and high nest predation might also favor reduced immunocompetence of the offspring, as suggested by the negative relationship between cellular immune response and nest predation rate. The mechanisms through which nest predation could be influencing these life history traits are not known and we discuss several potential lines for future research.

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PREFACE

Life history strategies vary enormously among organisms and understanding the ultimate causes and proximate mechanisms underlying this variation is a central question in evolutionary biology (Partridge and Harvey 1988, Roff 1992, 2002, Stearns 1992, Martin 1995, 1996, 2002). Life history traits do not vary independently of each other, but are organized into syndromes with species distributed along a slow-fast continuum of life history strategies (Ricklefs 2000). At the slow extreme of this continuum we find organisms having slow development, delayed sexual maturation, small clutch or litter size, high adult survival, and long lifespan; while organisms showing the opposite combination of traits are found at the fast extreme (e.g. Promislow and Harvey 1990, Ricklefs 2000). These syndromes arise because trade-offs among traits, which represent the fitness costs paid when a beneficial change in one trait is linked to a detrimental change in another trait (Stearns 1989), limit the possible combinations of life history characteristics (Roff 1992, Stearns 1992). Many ecological factors have been recognized as potential influences on life history evolution, however the relative importance of each factor and the proximate mechanisms mediating trade-offs among life history traits are not fully understood.

One longstanding question regarding life history evolution is why organisms vary so dramatically in the time required for developing. Particularly intriguing are slow developmental rates (Boersma 1982, Ricklefs 1984, 1992, 1993). Slow development comes at the cost of increased risk of time-dependent mortality to the young, and therefore we would expect selection to favor rapid development in order to decrease this mortality factor. Indeed, most environmental pressures seem to select for fast

development and, hence, short developmental periods (Ricklefs 1993, Ricklefs and Starck 1998, Martin 2002, Remes and Martin 2002, Lloyd and Martin 2003). However, the vast variation in developmental periods observed among organisms suggests that slow development might provide some advantages under certain environmental conditions (Ricklefs 1993, Ricklefs and Starck 1998, Martin 2002). Understanding these conditions and the mechanisms underlying variation in developmental rates were the general questions that inspired the present study.

Variation in developmental period among species is associated with variation in a whole suite of life history traits, such as time of first reproduction, clutch or litter size, survival, and lifespan. Therefore, understanding the evolution of developmental periods can potentially provide new and important insights into the evolution of life history strategies in general.

TABLE OF CONTENTS

Page

vi

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Understanding why organisms vary so dramatically in their life history strategies is a major question in life history theory (Partridge and Harvey 1988, Roff 1992, 2002, Stearns 1992, Martin 1995, 1996, 2002). Developmental rates are an important component of life history strategies that vary enormously among organisms and can have significant fitness consequences (Promislow and Harvey 1990, Roff 1992, 2002, Ricklefs 1993, Benrey and Denno 1997, Gebhart-Heinrich and Richner 1998); yet we do not fully understand the proximate and ultimate causes underlying the observed variation. Extensive variation in developmental periods has been documented for numerous groups of vertebrate as well as invertebrate taxa (e.g. Werner 1986, Fukui 1989, Promislow and Harvey 1990, Ricklefs 1993, Mishashita 1999). For example, among placental mammals, gestation length can vary from 18 to 660 days (Promislow and Harvey 1990), and broad variation remains even after controlling for the allometric effect of body mass. Understanding which environmental pressures and mechanisms (i.e. physiological, behavioral, etc.) determine the rates at which organisms develop is an important end goal, but can also potentially provide important new insights into understanding the evolution of broad life history strategies, where development is one of many correlated characters.

Birds constitute an excellent group in which to study variation in developmental rates because developmental rates vary strongly among species (Ricklefs and Starck 1998), and they are relatively easy to measure. The latter is particularly true for the length of embryonic development, which is manifested as the length of the incubation period. Incubation duration varies widely among bird species, from 9 to about 80 days, 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). Several factors may influence the evolution of incubation periods in birds, including nest predation (Lack 1968, Bosque and Bosque 1995, Martin 1995, 2002), sibling competition (Ricklefs 1993, Lloyd and Martin 2003), adult mortality (Ricklefs 1993, Martin 2002), and parasitism (Ricklefs 1992). However, the relative importance of these selection pressures and the proximate mechanisms through which they cause variation in incubation periods are not fully understood.

Mechanisms underlying variation in incubation period can be broadly divided into those extrinsic or intrinsic to the eggs. Extrinsic mechanisms, such as nest attentiveness (percent of time the parent is on the nest), seem to explain some variation in incubation periods among species; however, considerable residual variation remains unexplained (Martin 2002). Intrinsic mechanisms, such as modification of certain developmental processes in the embryo (Ricklefs 1992, 1993, Ricklefs and Starck 1998), might therefore be important in accounting for this residual variation. A particularly interesting possibility is the potential link between embryonic growth period and development of immunocompetence (the ability of an individual to defend itself from parasites and disease) (Ricklefs 1992).

Ricklefs (1992) found a negative relationship between blood parasite prevalence and incubation period among families of nonraptorial, altricial landbirds; and this pattern led him to propose a direct functional relationship between embryonic growth period and immunocompetence. He suggested that a longer period within the egg could allow the development of a better immune system for fighting parasites and diseases (Ricklefs 1992, 1993). This intriguing hypothesis associating selection pressure from parasites with

incubation period through immunocompetence has not been fully tested and is the focus of this study.

Here we examine the potential relationship between incubation period and immunocompetence, through a comparative field study across coexisting passerine bird species. Specifically, we test the following predictions: 1) species with longer incubation periods should have better immunocompetence (i.e. mount stronger immune responses to novel antigens) than species with shorter incubation periods, and 2) species subjected to greater exposure to parasites (i.e. higher prevalence) should show greater investment in immunocompetence (Moller 1997, 1998; Moller and Erritzoe 1996, 1998; Martin et al. 2001). We also explore potential causes of interspecific variation in immune responses and examine whether variation in parasite prevalence among species is related to ecological variables.

METHODS

Study site and study species

Field work was conducted in snowmelt drainages of a high elevation (2600m) mixed deciduous-conifer forest on the Mogollon Rim in central Arizona. Canopy trees were Quaking Aspen (*Populus tremuloides*), Douglas Fir (*Pseudotsuga menziesii*), White Fir (*Abies concolor*), Ponderosa Pine (*Pinus ponderosa*), White Pine (*Pinus strobiformis*), and Gambel Oak (*Quercus gambellii*), and the understory included Bigtooth Maple (*Acer grandidentatum*), New-Mexican Locust (*Robinia neomexicana*), Golden Pea (*Thermopsis pinetorum*) and various grasses (see Martin 1998 for a more detailed description of the area).

We performed a comparative analysis across 8 passerine bird species that are common breeders in the study site: Red-faced warbler (*Cardelina rubrifrons*; Parulidae), Orange-crowned warbler (*Vermivora celata*; Parulidae), Virginia's warbler (*Vermivora virginiae*; Parulidae), Grey-headed junco (*Junco hyemalis*; Emberizidae), Green-tailed towhee (*Pipilo chlorurus*; Emberizidae), Hermit thrush (*Catharus guttatus*; Turdidae), Cordilleran flycatcher (*Empidonax difficilis*; Tyrannidae), and House wren (*Troglodytes aedon*; Troglodytidae). We searched for nests from the beginning of May to the end of July during the 2001 and 2002 breeding seasons. We located nests based on parental behavior (see Martin and Geupel 1993), and checked them every 2-4 days to record activity and status. Nests found during building or egg laying were monitored more frequently close to hatching events in order to determine exact incubation periods. Incubation period was defined as the interval between laying and hatching of the last laid egg (Nice 1954). Nests constituted the sampling units such that the average measurements across all sampled nests provided the mean measurement for each species.

Assessment of Immunocompetence

The avian immune system is complex and includes different components that interact to defend the organism against parasites and disease (Toivanen and Toivanen 1987). Two main arms are recognized: innate (natural) immunity and acquired (adaptive) immunity. The latter arm can be further subdivided into a humoral and a cell-mediated component (Roitt et al. 1998). A broad scale estimate of these different components of immunocompetence would require performing many different immunological tests, and would likely conflict with practical and ethical considerations (Gonzales et al. 1999, Norris and Evans 2000). This would be particularly true for studies performed on wild

animals. Since the proposed mechanism linking length of the incubation period and immunocompetence involves B- and/or T-lymphocyte proliferation (Ricklefs 1992, Apanius 1998), we focused on the two most relevant components of the immune system: humoral and cell-mediated immunity. We performed two standard immune challenges that have been widely used in birds in the field (e.g. Saino et al. 1997a,b, Christe et al. 1998, Gonzales et al. 1999, Horak et al. 1999, Moller et al. 2001) (described below).

Cell-mediated immunity

We assessed cell-mediated immunity of nestlings using the *in vivo* immune response to the inocuous mitogen phytohaemagglutinin (PHA). We followed the simplified version of this standard test described by Smits et al. (1999). First, we measured the thickness of the wing-web (scapular apterium) using a pressure-sensitive micrometer (Mitutoyo, Japan). Next, we challenged the individual with a subcutaneous injection of 0.2 mg PHA (Sigma L-8754) in 0.04 ml of phosphate buffered saline (PBS). Approximately 24 hours (± 20 min) later we measured the swelling of the wing-web at the site of injection. This swelling represents the immune response to PHA and normally resolves after 48 hours. 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 at a similar developmental stage (i.e. when primary feathers break their sheaths) to standardize for interspecific comparisons. Sample sizes ranged from 1 to 14 nests per species with a median of 6. These sample sizes provided a representative mean response for each species, given that variation among conspecifics was very small compared to variation among species (ANOVA: $F_{7,42}$ = 18.04, P < 0.0001).

Humoral Immunity

We assessed the *in vivo* humoral immune response by immunizing adult females with sheep erythrocytes and quantifying antibody production 7 days after the challenge. This test was not performed on nestlings because at an early age nestlings tend to respond weakly or not at all (Apanius 1998, Moller et al. 2001). Females were captured at their nests during the incubation period using mist nets, banded, and a first blood sample (30-60 μl) was drawn from their brachial vein into microcapillary tubes. Birds were next injected intraperitoneally with a 5% solution of sheep red blood cells (SRBC) in PBS (5 μl/per gram of body mass), and then released. One week later birds were recaptured at their nests and a second blood sample was drawn. All microcapillary tubes were centrifuged, and plasma was isolated and stored at –20 °C until later analysis. Antibody titers were obtained using a microhemagglutination assay (Wegmann and Smithies 1966, Hay and Hudson 1989): serial two-fold dilutions of heat inactivated plasma (56 °C for 30 min.) in PBS were placed into individual wells of a 96-well microtiter plate and mixed with an equal volume of a 2% SRBC solution. Plates were incubated at 37 °C for 1 hour. Antibody titers (SRBC response) were expressed as the $\log_2$ of the reciprocal of the highest dilution of plasma that showed hemagglutination. Pre- and post- immunization plasma samples, as well as negative and positive controls, were assessed in each microtiter plate. None of the pre-immunization plasma samples showed agglutination of SRBC. Post-immunization plasma samples from a few individuals showed no antibody response (i.e. no agglutination), even at the lowest dilution, and were therefore necessarily excluded from the calculations of mean titers (Casto et al. 2001). After the exclusion, sample sizes for calculation of mean titers ranged from 2 to 7 individuals per

species with a median of 4. No data on titers were obtained for the House wren. Variation in titer among conspecifics was small compared to variation among species (ANOVA: $F_{6,18} = 4.032$, $P = 0.01$) and, therefore, even the small samples sizes obtained seemed an adequate representative of the mean response for each species.

Assessment of Parasitism

Birds are host to an incredible variety of parasites (Clayton and Moore 1997). We sampled three main groups of avian parasites in each of the study species: blood parasites, ectoparasites, and intestinal parasites.

Blood Parasites

A broad range of blood parasites infect avian species (Janovy 1997). Among the most common types are flagellated protozoans (i.e. *Trypanosoma*), haematozoan protozoans (i.e. *Haemoproteus*, *Leucocytozoon*, and *Plasmodium*), and juveniles of filarial nematodes (microfilariae) (Janovy 1997). 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. Using a light microscope, we screened each smear under 400x for the presence of *Trypanosoma* spp., *Leucocytozoon* spp., and microfilariae. Whereas *Plasmodium* spp. and *Haemoproteus* spp. were screened using 1000x magnification. The prevalence, mean intensity, and median intensity of blood parasites were quantified for each of the study species. Prevalence was defined as the percentage of infected host individuals in a sample and mean intensity refers to the mean number of individuals of a particular parasite species per infected host individual in a sample (Margolis et al. 1982). Given that parasites usually show an aggregated frequency distribution among hosts (i.e. most hosts having few or no parasites, and few

hosts having many parasites), the median intensity was also calculated to provide the typical level of infection of the hosts in a sample (Rozsa et al. 2000). Intensities were recorded as the total number of parasites per 100 microscope fields and quantification was performed following recommendations by Godfrey et al. (1987).

Ectoparasites

Birds can be parasitized by a vast array of arthropod parasites (Janovy 1997). The main groups of avian ectoparasites are ticks and mites (Acarina), lice (Phthiraptera), true bugs (Hemiptera), fleas (Siphonaptera), and flies and mosquitoes (Diptera) (Janovy 1997). We used a Kilner jar apparatus (Fowler and Cohen 1983) to examine adults birds for ectoparasites. This collection technique has been widely used to delouse live birds (Wheeler and Threlfall 1986, Moller 1990, Poiani 1992,1993, Saino et al. 1998), and is less prone to error and yields a higher fraction of the parasite population than visual examination (Clayton and Walther 1997). The apparatus consisted of a 1 liter jar with a rubber collar placed on the jar opening. The collar had a central hole with a diameter that could be varied according to the size of the bird being treated. A filter paper disk was fitted to the bottom of the jar and a few drops of ethyl acetate were added to the paper. The bird was placed inside the jar with its head held outside by the rubber collar. The body was then exposed for 10 minutes to the ethyl acetate vapor. The head and neck were manually searched for parasites by deflecting the feathers. Anesthetized ectoparasites were then removed from the jar and preserved in 70% ethanol.

Intestinal parasites

The main groups of intestinal parasites found in birds are coccidian protozoans and helminths (worms) (Janovy 1997). Avian intestinal helminths include tapeworms

(Cestoda), roundworms (Nematoda), flukes (Trematoda), and thorny-headed worms (Acanthocephala) (Janovy 1997). Prevalence of intestinal parasites was assessed by analyses of fecal samples. On 2002 we collected fecal samples dropped by birds during their manipulation and preserved them in formalin until later analyses. In the laboratory, we used a standard sedimentation concentration procedure (Garcia and Bruckner 1993) to isolate helminth eggs and protozoan oocysts from the rest of the fecal material. This procedure is easy to perform, allows recovery of the broadest range of organisms, and is least subject to technical error (Garcia and Bruckner 1993). The isolated fraction was stained with Lugol solution and used for preparation of permanent fecal smears. We used a compound microscope to scan the whole fecal smear for the presence of helminth eggs and protozoan oocysts.

Statistical Analyses

We performed multiple regression analyses to test for correlations among the variables of interest (incubation period, immune responses, and parasite prevalence). Selection of variables to be included in the models was performed manually, and confirmed using the standard options (forward selection and backward elimination). Body mass was first forced into the analyses to control for its positive effect on immune responses across species (Moller et al. 2001, Martin et al. 2001, Tella et al. 2001). We used as covariates other variables that may influence incubation period (i.e. nest predation, nest attentiveness, and clutch size), to allow examination of the ability of immunocompetence and parasites to explain primary or residual variation. Given the low sample size in this comparative study (n = 8 bird species) the results from multiple regression analyses should be interpreted with caution since the low ratio of data points to

explanatory variables in the models might lead to decreased precision of parameter estimates. Associations between parasite prevalence and preferred feeding stratum (ground vs. above ground), and nest stratum (ground vs. above ground) were assessed by Mann-Whitney U tests. All statistical analyses were performed with SPSS 11.5.0 (SPSS Inc. 2002).

Comparative Method

Since we were testing for potential evolutionary relationships among species, and species cannot be considered independent data points in statistical analyses, we used phylogenetically independent constrasts (PICs, Purvis and Rambaut 1995) to correct for possible phylogenetic effects. Relationships among independent contrasts of the different variables were estimated using regressions through the origin (Purvis and Rambaut 1995, Harvey and Pagel 1991). The phylogenetic hypothesis used in this study was taken from Martin and Clobert (1996).

RESULTS

Parasites

Blood parasites

Parasites of the genera *Haemoproteus*, *Plasmodium*, *Trypanosoma*, and *Leucocytozoon*, and microfilariae were detected in blood smears. Individuals belonging to 7 of the 8 study species were infected with at least one species of blood parasite; however, the types of parasites found varied considerably across species (Fig. 1). *Haemoproteus* spp. were the most commonly detected parasites, while the remaining parasite types were found in low prevalence (Fig. 1). Total prevalence of blood parasites

(i.e. percentage of individuals infected with at least one parasite type) was greater for species that feed on the ground than for species feeding above the ground (i.e. canopy) (Fig. 2a); while no difference was found between species that nest on the ground and above ground (Fig. 2b). The intensity of *Haemoproteus* infection varied greatly within species (Table 1), and statistical comparison among species was precluded by the low number of infected individuals for most of the species. Intensities of infection by the other blood parasites was very low (range = 1-14).`

Ectoparasites

All probable ectoparasites were sampled, but only mites were collected from adult birds using the Kilner jar apparatus (Table 2). Prevalence of ectoparasitism seems to be very low in our study site; however, the low sample sizes might not allow a precise estimate at present. Intensity of mites (i.e. number of individuals recovered in the jar) in infected birds was very low, indeed only one mite was collected from each of the infected birds (Table 2). No ectoparasites were found in the heads and necks of birds through visual examination; however, magnifying lenses might have been necessary for detecting some microscopic parasites (Clayton and Walther 1997). Given the low sample sizes and the lack of data for three of the study species, these data will not be discussed any further.

Intestinal parasites

Helminth eggs and protozoan oocysts were not detected in any of the fecal smears analyzed. This lack of detection could be due to: 1) absence of infection by these types of parasites in the sampled populations, or 2) low probability of detecting low prevalences with the reduced samples sizes (range = 1 to 6 individuals per species). At present we

cannot discern between the two possible reasons for lack of detection and therefore these data will not be discussed any further.

Incubation Period

The length of the incubation period varied from 11.17 days in Virginia's warbler to 15 days in the Cordilleran flycatcher. Although multiple regression analysis showed that incubation period tended to increase with body mass and to decrease with nest predation rate and with PHA response, these trends were not statistically significant (body mass: $r_p = 0.546$, $P = 0.263$; nest predation: $r_p = -0.442$, $P = 0.380$; PHA response: $r_p = -0.576$, $P = 0.231$). However, one of the study species (Green-tailed towhee) was an outlier in the above relationships. Most importantly, it was a strong and sole outlier in its PHA response relative to its body mass (Fig 3). Because PHA is expected and known to be related to body mass across species (Moller et al. 2001, Martin et al. 2001, Tella et al. 2002) we considered it appropriate to re-examine the relationships with the outlier excluded. When the outlier was excluded from the analysis, all the mentioned relationships became highly significant (Fig. 4). Moreover, these relationships remained significant after potential phylogenetic effects were controlled using independent contrasts (body mass: $r_p = 0.934$, $P = 0.02$; nest predation: $r_p = -0.874$, $P = 0.053$; PHA response: $r_p = -0.957$, $P = 0.011$). No other variable explained significant variation in incubation period among species.

Humoral Immunity

Response to the injection of sheep red blood cells (i.e. SRBC response) increased with body mass across species (Fig. 5). No relationships were found between SRBC response and incubation period or haematozoa prevalence.

Cell-mediated Immunity

To gain insight into the possible causes of variation in PHA response, we conducted multiple regression analyses with it as the dependent variable. Total haematozoa prevalence was the only variable that explained significant variation in PHA response (corrected for body mass) when the 8 study species were included in the analysis ($r_p = 0.748$, P = 0.053). However, when we removed the outlier (Green-tailed towhee, see above) two different models explained significant variation in PHA response across the remaining study species. The first model (Model 1), obtained by forward selection of variables, showed that PHA response was positively associated with body mass ($r_p = 0.975$, P = 0.005) and with haematozoa prevalence (Fig. 6), and negatively associated with incubation period ($r_p = -0.914$, P = 0.03). The second model (Model 2), obtained by backward elimination of variables, showed that PHA response was positively related to body mass ($r_p = 0.995$, P < 0.0001), and negatively related to nest predation rate (Fig. 7) and incubation period ($r_p = -0.982$, P = 0.003). Therefore, both models suggested a positive relationship between PHA response and body mass, and a negative relationship between PHA response and incubation period; while they differed in the third explanatory variable (i.e. haematozoa prevalence or nest predation rate). The latter relationships remained significant when potential phylogenetic effects were controlled using independent contrasts (haematozoa prevalence: $r_p = 0.792$, P = 0.034; nest predation: $r_p = -0.899$, P = 0.038). Given the low sample size we cannot at present decide the relative importance of these two alternative models.

DISCUSSION

Incubation period and immunocompetence

The extensive variation in embryonic developmental rates observed among birds is intriguing given that most selection pressures (e.g. nest predation, harsh weather, sibling competition) seem to favor rapid development and, therefore, short incubation periods (Ricklefs 1993, Martin 2002, Lloyd and Martin 2003). This variation suggests that prolonged incubation might confer some advantages under certain environmental conditions (Ricklefs 1992, 1993, Ricklefs and Starck 1998, Martin 2002). Ricklefs (1992) suggested that a longer period within the egg could allow for the development of a better immune system for fighting parasites and diseases. However, we found no support for enhanced immunocompetence with longer incubation periods. Instead, we found that species with a relatively longer incubation period mounted weaker cellular immune responses (PHA test), and no relationship was found between incubation period and strength of the humoral immune response (SRBC test).

Using data compiled from the literature, Tella et al. (2002) found no relationship between cell-mediated immune response to PHA and incubation period across species, whereas we found a negative relationship between these variables. This difference could be due to the different set of species studied or to the nature of the data used. The dataset gathered from the literature by Tella et al. (2002), included PHA measurements made by different investigators using different protocols (e.g. concentration of PHA solution, volume of PHA solution injected, age/stage at challenge, etc.) and in different environments. All of these variables might introduce "noise" and therefore preclude the detection of existing relationships. Another potential concern with the study by Tella et

al. (2002) is that it included species in zoos that are subjected to artificial feeding and health care, both of which could affect the immune responses. Moreover, data from a field study across 20 coexisting passerine bird species in South Africa also show 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.

The reason for the negative relationship between the length of the incubation period and immunocompetence is unclear. One possible explanation is that both variables might be indirectly correlated by their response to selection from adult mortality. For instance, high adult mortality favors increased parental investment, and two ways in which parents might invest in their young could be a reduction in the length of the incubation period, which reduces the risk of time-dependent mortality, and increased immunocompetence, which reduces the negative effects from parasites and disease. Therefore, species having high adult mortality might show both increased immunocompetence of their offspring and short incubation period. Variation in adult mortality among our study species does not support this explanation, and this result is in accordance with the finding that adult mortality explains variation in incubation period mainly among latitudes (i.e subtropical versus temperate bird species), but not within latitudes (Martin 2002). Nevertheless, independently from the significance of the negative relationship between incubation period and PHA response, our results suggest that a longer time inside the egg does not result in better immunocompetence.

Incubation period and nest predation

Variation in life history traits within latitudes appears to be strongly influenced by juvenile mortality in the form of nest predation (Martin 2002). Here, using a subset of the

species included in Martin's (2002) work, we found that species enduring high nest predation have shorter incubation periods (Fig. 4), as found by others (Bosque and Bosque 1995, Martin 1995, 2002). However, the mechanism through which nest predation could lead to fast growth rates is not understood. Among the possible alternatives, nest predation could be causing fast growth through mechanisms intrinsic to the egg. An interesting possibility is the potential role of hormones of maternal origin influencing embryo growth rates. Females of egg laying vertebrates deposit hormones in their eggs that may influence several embryonic processes, such as growth rates (e.g. Feist et al. 1990, Schwabl 1993, 1996, McNabb et al. 1997, Janzen et al. 1998, McCormick 1999, Lipar and Ketterson 2000, Birkhead et al. 2000, Eising et al. 2001). Experimentally increased levels of two androgens (testosterone and androstenedione) in gull eggs caused eggs to hatch half a day earlier than chicks from control eggs (Eising et al. 2001). Preliminary analyses using data from the literature also show that the level of androstenedione in eggs is negatively correlated with incubation period across passerine birds (H. Schwabl and T. E. Martin *unpublished data*). This seems a fruitful avenue for future research regarding the mechanisms underlying variation in developmental rates among species. During the duration of this study, we collected freshly laid eggs from all our study species and will determine their hormone contents to test whether they explain variation in incubation period in our system.

Immunocompetence and nest predation

Further support for the importance of nest predation pressure on life history variation within latitudes might come from our finding that offspring from species suffering from higher nest predation rates mounted weaker cellular immune responses

(Fig. 7). This result should be viewed with caution as the inclusion of nest predation as an explanatory variable depended on the method used for selection of variables in the statistical analysis (see results). Nevertheless, this is a novel and potentially interesting result that suggests that high juvenile mortality might favor, or come at the cost of, reduced immunocompetence of the young. There are two possible explanations for the latter. The first is that high nest predation selects for fast nestling growth (Lack 1968, Bosque and Bosque 1995, Remes and Martin 2002), which could come at the cost of reduced immunocompetence. Within-species studies have found a trade-off between nestling growth rate and immune response (Merino et al. 2000, Soler et al. 2003, but see Horak et al. 1998), and resolution of this trade-off is likely to differ among species depending on the levels of parasitism and predation pressures suffered by nestlings (Soler et al. 2003). To our knowledge, the negative relationship between nest predation rate and PHA response found in this study is the first to provide empirical support for this hypothesis. Egg testosterone could be a potential mediator of the trade-off between nestling growth and immunocompetence as it enhances nestling growth rate (Schwabl 1996, Eising 2001) but also has immunosupressive effects (Grossman 1985, Duffy et al. 2000, Casto et al. 2001). Therefore, higher levels of egg testosterone could be favored in species with high nest predation rate in order to speed up nestling growth leading to decreased immune responses. The second possible explanation is that high nest predation selects for reduced maternal investment in offspring immunocompetence. Mothers deposit in their eggs components that are directly related to immune function, such as immunoglobulins (reviewed in Apanius 1998, Saino et al. 2002a), carotenoids (Royle et al. 1999, 2000), and lysozyme (Saino et al. 2002b and references therein). Therefore, an

interesting possibility is that mothers might differentially allocate these immune-related resources to eggs according to the reproductive value of their young, in such a way that investment might be reduced in species enduring high juvenile mortality due to nest predation. However, further studies assessing this hypothesis are needed.

Immunocompetence and parasite prevalence

The immune system is one of the main defenses organisms have evolved to cope with parasites and disease and, therefore, we would expect species facing greater selection pressure from parasites to have evolved greater investment in immune defenses (Moller 1997, 1998, Moller and Erritzoe 1996, 1998, Moller et al. 2001, Martin et al. 2001, Tella et al. 2002). In accordance with this expectation, species having higher exposure to parasites have larger immune organs (e.g. bursa of Fabricius and spleen) and mount stronger immune responses (Moller 1997, 1998, Moller and Erritzoe 1996, 1998, Moller et al. 2001). Here, we found that species having a higher prevalence of blood parasites mount stronger immune responses than species with lower prevalences (Fig. 6), but again this model should be viewed with caution as mentioned above for nest predation rate.

An association between prevalence of blood parasites and an immune response was only true for the cell-mediated immune response to PHA, while the humoral immune response to SRBC was not related to blood parasite prevalence. Such differential response may reflect the relative importance of the different immune components. Although several components participate in defense from blood parasites (Roitt et al. 1998), some evidence suggests that the cellular immune response might be more important than the humoral in fighting infections to haematozoans (i.e. *Haemoproteus*

spp., *Plasmodium* spp., and *Leucocytozoon* spp.). For instance, defense against apicomplexan diseases in poultry depends primarily on the cell-mediated 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 (Gonzales et al. 1999, Soler et al. 2003). Evolutionarily, we would then expect that species facing greater pressure from blood parasites invest more in their cellular immune responses, and our results support this prediction.

Tella et al. (2002) did not find a relationship between prevalence of blood parasites and PHA response, but as discussed above, they used data from the literature that might have masked actual relationships. In addition to the potential noise in the PHA data (see above), the data on blood parasite prevalence used in this study is also likely to be very noisy. Prevalence of blood parasites can vary considerably among different populations of a given species and across study years for a given population (Bennet et al. 1995, Yezerinac and Weatherhead 1995, Bauchau 1998), and therefore pooling data from disparate studies might confound analyses of interspecific variation (Yezerinac and Weatherhead 1995). In particular, the study from Greiner et al. (1975), used as a source by Tella et al. (2002), is a compilation of parasite surveys conducted throughout North America over a period of 37 years and, for this reason, its use has been specifically avoided by other researchers conducting comparative studies (Yezerinac and Weatherhead 1995). This suggests that extra caution has to be exercised when choosing data from the literature for comparative studies of parasite levels and highlights the value of performing comparative field studies in which all the variables are measured in the study populations by the same researchers.

Summary

Through a comparative field study among 8 coexisting passerine bird species and using two different measures of immune response we found no support for Ricklefs (1992) hypothesis suggesting that longer incubation periods allow the development of a better immune system. On the other hand, and in accordance with theoretical expectations and previous empirical studies, we found that species facing higher rates of nest predation have relatively shorter incubation periods. Particularly interesting would be to determine the mechanisms through which nest predation pressure leads to fast embryonic development, and we suggest that a fruitful avenue of research in this respect is the levels of hormones mothers deposit in their eggs. High nest predation might also be favoring reduced immunocompetence of the offspring, as suggested by the negative relationship between PHA response and nest predation rate. This interesting possibility deserves further study and we propose two potential mechanisms through which nest predation might be linked to nestling immunocompetence. Evolutionarily we would expect species facing greater selection pressure from parasites to invest more in immune defense. In this study, we show for the first time, that species having higher prevalence of blood parasites mount stronger cellular immune responses, thus providing some support for the above mentioned expectation. An increase in the number of study species would be important for determining the relative importance of nest predation and prevalence of blood parasites in explaining variation in cellular immune response, and for increasing the statistical power of the analyses. Nevertheless, this study provided interesting results and suggests several potentially fruitful lines for future research.

LITERATURE CITED

Apanius, V. 1998. Ontogeny of immune function, pp 203-222. In Starck, J. M. and R. E. Ricklefs [Eds.] Avian growth and development. Oxford University Press, Oxford.

Bauchau, V. 1998. Comparison of parasitism level in two sympatric passerines: the pied flycatcher and the great tit. Ecoscience 5:164-171.

Bennet, G. F., Squires-Parsons, D., Siikamaki, P., Huhta, E., Allender, K., and L. Hillstrom. 1995. A comparison of the blood parasites of three Fenno-Scandian populations of the Pied Flycatcher *Ficedula hypoleuca*. Journal of Avian Biology 26:33-38.

Benrey, B., and R. F. Denno. 1997. The slow-growth-high-mortality hypothesis: a test using the cabagge butterfly. Ecology 78:987-999.

Birkhead, T., Schwabl, H., and T. Burke. 2000. Testosterone and maternal effects-integrating mechanisms and function. Trends in Ecology and Evolution 15:86-87.

Boersma, P. D. 1982. Why some birds take so long to hatch. American Naturalist 120:733-750.

Bosque, C., and M. T. Bosque 1995. Nest predation as a selective factor in the evolution of developmental rates in altricial birds. American Naturalist 145:234-260.

Casto, J. M., Val Nolan, Jr., and E. D. Ketterson. 2001. Steroid hormones and immune function: experimental studies in wild and captive Dark-eyed juncos (*Junco hyemalis*). American Naturalist. 157:408-420.

Christe, P., Moller, A. P., and F. de Lope. 1998. Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. Oikos 83:175-179.

Clayton, D. H., and J. Moore. 1997. Host-parasite evolution: General principles and avian models. Oxford University Press Inc., New York.

Clayton, D. H., and B. A. Walther. 1997. Appendix C: collection and quantification of arthropod parasites in birds, pp. 419-440. In Clayton, D. H., and J. Moore [Eds.] Host-parasite evolution: General principles and avian models. Oxford University Press Inc., New York.

Conway, C. J., and T. E. Martin. 2000. Evolution of passerine incubation behavior: influence of food, temperature, and nest predation. Evolution 54:670-685.

Deeming, D. C. 2002. Functional characteristics of the eggs, pp. 28-42. In D. C. Deeming [Ed.] Avian incubation: behaviour, environment, and evolution. Oxford University Press, Oxford.

Duffy, D. L., Bentley, G. E., Drazen, D. L., and G. F. Ball. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. Behavioral Ecology 11:654-662.

Eising, C. M., Eikenaar, C., Schwabl, H., and T. G. G. Groothuis. 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proceedings of the Royal Society of London B 268:839-846.

Feist, G., Schreck, C. B., Fitzpatrick, M. S., and J. M. Redding. 1990. Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. General and Comparative Endocrinology 80:299-313.

Fowler, J. A., and S. Cohen. 1983. A method for quantitative collection of ectoparasites from birds. Ringing Migration 4:185-189.

Fukui, Y. 1988. Comparative studies on the life history of the grapsid crabs (Crustacea, Brachyura) inhabiting intertidal cobble and boulder shores. Publications of the Seto Marine Biological Laboratory 33:121-162.

Garcia, L. S., and D. A. Bruckner. 1993. Macroscopic and microscopic examination of fecal specimens, pp. 501-540. In Diagnostic medical parasitology. American Society for microbiology, Washington, D. C.

Gebhardt-Heinrich, S., and H. Richner. 1998. Causes of growth variation and its consequences on fitness, pp. 324-339. In Starck, J. M. and R. E. Ricklefs, [Eds.] Avian growth and development. Oxford University Press, Oxford.

Godfrey, R. D., Fedynich, A. M., and D. B. Pence. 1987. Quantification of hematozoa in blood smears. Journal of Wildlife Diseases 23:558-565.

Gonzales, G., Sorci, G., Moller A. P. et al. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). Journal of Animal Ecology 68:1225-1234.

Greiner, E. C., Bennet, G. F., White, E. M., and R. F. Coombs. 1975. Distribution of the avian haematozoa of North America. Canadian Journal of Zoology 53:1762-1787.

Grossman, C. J. 1985. Interactions between the gonadal steroids and the immune system. Science 227:257-261.

Hainsworth, F. R., and M. A. Voss. 2002. Intermittent incubation: predictions and tests for time and heat allocations, pp. 223-237. In D. C. Deeming [Ed.] Avian incubation: behaviour, environment, and evolution. Oxford University Press, Oxford.

Hay, L., and F. C. Hudson 1989. Practical immunology. Blackwell Scientific, Oxford.

Harvey, P., and M. D. Pagel. 1991. The comparative Method in Evolutionary Biology. Oxford University Press, Oxford, UK.

Horak, P., Ots, I., Tegelmann, L., and A. P. Moller. 1998. Health impact of phytohaemagglutinin-induced immune challenge on great tits (*Parus major*) nestlings. Canadian Journal of Zoology 78:905-910.

Horak, P., Tegelmann, L., Ots, I., and A. P. Moller. 1999. Immune function and survival of great tit nestlings in relation to growth conditions. Oecologia 121:316-322.

Janovy, J. Jr. 1997. Protozoa, helminths, and arthropods of birds, pp. 303-337. In Clayton, D. H., and J. Moore [Eds.] Host-parasite evolution: General principles and avian models. Oxford University Press, Oxford.

Janzen, F. J., Wilson, M. E., Tucker, J. K., and S. P. Ford. 1998. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. General and Comparative Endocrinology 111:306-317.

Lack, D.1968. Ecological adaptations for breeding in birds. Methuen, London.

Lillehoj, H. S. 1991. Cell-mediated immunity in parasitic and bacterial diseases, pp. 155-182. In Sharma, J. M. [Ed.] Avian cellular immunology. CRC Press, Boca Raton, Florida.

Lipar, J. L., Ketterson, E. D., and V. Nolan Jr. 1999. Intraclutch variation in testosterone content of Red-winged blackbird eggs. Auk 116:231-235.

Lipar, J. L., and E. D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelaius phoenicus*. Proceedings of the Royal Society of London B 267:2005-2010.

Lloyd, J. D., and T. E. Martin. 2003. Sibling competition and the evolution of prenatal development rates. Proceedings of the Royal Society of London B *On line*.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87-98.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., and G. A. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology 68:131-133.

Martin, T. E. 1995. Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monographs 65:101-127.

Martin, T. E. 1996. Life history evolution in tropical and south temperate birds: What do we really know? Journal of Avian Biology 27:263-272.

Martin, T. E. 1998. Are microhabitat preferences of coexisting species under selection and adaptive? Ecology 79:656-670.

Martin, T. E. 2002. A new view for avian life history evolution tested on an incubation paradox. Proceedings of the Royal Society of London B 269:309-316.

Martin, T. E., and G. R. Geupel. 1993. Nest-monitoring plots: methods for locating nests and monitoring success. Journal of Field Ornithology 64:507-519.

Martin, T. E., and J. Clobert. 1996. Nest predation and avian life-history evolution in Europe versus North America: a possible role of humans? American Naturalist 147:1028-1046.

Martin, T. E., Moller, A. P., Merino, S., and J. Clobert. 2001. Does clutch size evolve in response to parasites and immunocompetence? Proceedings of the National Academy of Sciences 98:2071-2076.

McCormick, M. I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. Oecologia 118:412-422.

McNabb, F. M. A., and C. M. Wilson. 1997. Thyroid hormone deposition in avian eggs and effects on embryonic development. American Zoologist 37:553-560.

Merino, S., Moller, A. P., and F. de Lope. 2000. Seasonal changes in cell-mediated immunocompetence and mass gain in nestling barn swallows: a parasite-mediated effect? Oikos 90:327:332.

Miyashita, T. 1999. Life-history variation in closely related generalist predators living in the same habitat: A case study with three Cyclosa spiders. Functional Ecology 13:307-314.

Moller, A. P. 1990. Effects of parasitism by a haematophagous mite on reproduction in the Barn Swallow. Ecology 71:2345-2357.

Moller, A. P. 1997. Parasitism and the evolution of host life history, pp. 105-127. In Clayton, D. H., and J. Moore [Eds.] Host-parasite evolution: general principles and avian models. Oxford University Press, Oxford.

Moller, A. P. 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, A. P, and J. Erritzoe. 1996. Parasite virulence and host immune defense: host immune response is related to nest reuse in birds. Evolution 50:2066-2072.

Moller, A. P, and J. Erritzoe. 1998. Host immune defense and migration in birds. Evolutionary Ecology 12:945-953.

Moller, A. P., Merino, S., Brown, C. R., and R. J. Robertson. 2001. Immune defense and host sociality: a comparative study of swallows and martins. American Naturalist 158:136-145.

Nice, M. M. 1954. Problems of incubation periods in North American birds. Condor 56:173-197.

Norris, K., and M. R. Evans 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology 11:19-26.

Partridge, L., and P. H. Harvey. 1998. The ecological context of life history evolution. Science 241:1449-1455.

Poiani, A. 1992. Ectoparasitism as a possible cost of social life: a comparative analysis using Australian passerines (Passeriformes). Oecologia 92:429-441.

Poiani, A. 1993. Small clutch sizes as a possible adaptation against ectoparasitism: a comparative analysis. Oikos 68:455-462.

Promislow, D. E. L., and P. H. Harvey. 1990. Living fast and dying young: a comparative analysis of life-history variation among mammals. Journal of zoology, London 220:417-437.

Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analyzing comparative data. Computer and Applied Bioscience 11:247-251.

Rahn, H., and A. Ar. 1974. The avian egg: incubation time and water loss. Condor 76:147-152.

Remes, V., and T. E. Martin. 2002. Environmental influences on the evolution of growth and developmental rates in passerines. Evolution 56:2505-2518.

Ricklefs, R. E. 1984. Prolonged incubation in pelagic seabirds: a comment on Boersma's paper. American Naturalist 123:710-720.

Ricklefs, R. E. 1992. Embryonic development period and the prevalence of avian blood parasites. Proceedings of the National Academy of Sciences 89:4722-4725.

Ricklefs, R. E. 1993. Sibling competition, hatching asynchrony, incubation period, and lifespan in altricial birds, pp. 199-276. In D. M. Power [Ed.] Current Ornithology vol.11. Plenum Press, New York.

Ricklefs, R. E. 2000. Density dependence, evolutionary optimization, and the diversification of avian life histories. Condor 102:9-22.

Ricklefs, R. E., and J. M. Starck. 1998. Embryonic growth and development, pp. 31-58. In Starck, J. M. and R. E. Ricklefs, [Eds.] Avian growth and development. Oxford University Press, Oxford.

Rocza, L., Reiczigel, J., and G. Majoros. 2000. Quantifying parasites in samples of hosts. Journal of Parasitology 86:228-232.

Roff, D. 1992. The evolution of life histories: theory and analysis. New York: Chapman and Hall.

Roff, D. 2002. Life History Evolution. Massachusetts: Sinauer Associates, Inc.

Roitt, I., Brostoff, J., and D. Male. 1998. Immunology. Mosby International Ltd., Philadelphia.

Royle, N. J., Surai, P. F., McCartney, N. J., and B. K. Speake. 1999. Parental investment and egg yolk lipid composition in gulls. Functional Ecology 13:298-306.

Royle, N. J., Surai, P. F., and I. R. Hartley. 2000. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing roles? Behavioral Ecology 12:381-385.

Saino, N., Calza, S., and A. P. Moller. 1997a. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. Journal of Animal Ecology 66:827-836.

Saino, N., Calza, S., and A. P. Moller. 1997b. An experimental test of immunocompetence on nestling barn swallow (*Hirundo rustica*) in relation to brood size and parental effort. Journal of Animal Ecology 66:827-836.

Saino, N., Calza, S., and A. P. Moller.1998. Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. Oikos 81:217-228.

Saino, N., Ferrari, R. P., Martinelli, R., Romano, M., Rubolini, D., and A. P. Moller. 2002a. Early maternal effects mediated by immunity depend on sexual ornamentation of the male partner. Proceedings of the Royal Society of London B 269:1005-1009.

Saino, N., Dall'ara, P., Martinelli, R., and A. P. Moller. 2002b. Early maternal effects and antibacterial immune factors in the egg, nestlings, and adults of the barn swallow. Journal of Evolutionary Biology 15:735-743.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proceedings of the National Academy of Sciences 90:11446-11450.

Schwabl, H. 1996. Maternal testosterone in avian eggs enhances postnatal growth. Comparative Biochemistry and Physiology 114:271-276.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends in Ecology and Evolution 11:317-321.

Smits, J. E., Bortolotti, G. R., and J. L. Tella. 1999. Simplifying the phytohaemagglutinin skin testing technique in studies of avian immunocompetence. Functional Ecology 13:567-572.

Soler, J. J., de Neve, L., Perez-Contreras, T., Soler, M., and G. Sorci. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. Proceedings of the Royal Society of London B 270:241-248.

Stearns, S. C. 1989. Trade-offs in life history evolution. Functional ecology 3:259-268.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford, UK.

Tella, J. L., Scheuerlein, A., and R. E. Ricklefs. 2002. Is cell-mediated immunity related to the evolution of life-history strategies in birds? Proceedings of the Royal Society of London B 269:1059-1066.

Toivanen, A., and P. Toivanen. 1987. Avian Immunology: Basis and Practice. Toivanen, A., and P. Toivanen, [Eds]. CRC Press.

Yezerinac, S. M., and P. J. Weatherhead. 1995. Plumage coloration, differential attraction of vectors and haematozoa infection in birds. Journal of Animal Ecology 64:528-537.

Wegmann, T., and O. Smithies. 1966. A simple haemagglutination system requiring small amounts of red cells and antibodies. Transfusion 6:67-73.

Werner, E. E. 1986. Amphibian metamorphosis, growth rate, predation risk, and the optimal size at transformation. American Naturalist 128:319-341.

Wheeler, T. A., and W. Threlfall. 1986. Observations on the ectoparasites of some Newfoundland passerines (Aves: Passeriformes). Canadian Journal of Zoology 64:630-636.

Table 1. Intensity (parasites/100 fields) of *Haemoproteus* spp. in the study species.

Species (n)	Range	Median	Mean	95% CI of mean
Grey-headed junco (8)	(2-2980)	124	500.13	[37.5-1183.25]
Orange-crowned warbler (1)	NA	5	5	NA
Cordilleran flycatcher (1)	NA	26	26	NA
House wren (1)	NA	42	42	NA
Green-tailed towhee (4)	(3-85)	26	35	[5-64.5]
Hermit thrush (3)	(16-634)	81	243.67	[16-449.67]

Explanation of abbreviations: n = number of individuals infected, NA = not applicable (refers to range and confidence interval in species in which only one individual was infected).

Table 2. Prevalence and intensity of mites in the study species.

Species (n)	# of infected individuals	Prevalence (%)	Mean intensity (SE)
Grey-headed junco (5)	2	20	1 (0)
Orange-crowned warbler (1)	0	0	-
Red-faced warbler (1)	0	0	-
Cordilleran flycatcher (7)	0	0	-
Hermit thrush (8)	1	12.5	1

Note: Individuals of three species, House wren, Green-tailed towhee, and Virginia's warbler, were not sampled for ectoparasites.

Explanation of abbreviations: n = number of individuals examined, SE = standard error of the mean.

FIGURE LEGENDS

Figure 1. Prevalence of blood parasites in the eight study species. Total refers to the percentage of individuals infected with at least one parasite type. Sample sizes are shown for each species: GHJU = Grey-headed junco, OCWA = Orange-crowned warbler, VIWA = Virginia's warbler, RFWA = Red-faced warbler, COFL = Cordilleran flycatcher, HOWR = House wren, GTTO = Green-tailed towhee, and HETH = Hermit thrush.

Figure 2. Total prevalence of blood parasites and ecological variables. Median, 25 and 75 % quartiles are presented. a). Total prevalence is higher in ground feeding species than in above ground feeding species (Mann-Whitney U Test: z = - 1.95, P = 0.05). The star shows an extreme value (House wren) and the circle an outlier (Red-faced warbler). b) Total prevalence does not differ between ground nesting species and species that nest above ground (Mann-Whitney U Test: z = - 0.726, P = 0.468).

Figure 3. Partial regression plot of PHA response relative to body mass across the eight study species (r_p = 0.792, P = 0.06). The outlier species is indicated by GTTO (Green-tailed towhee).

Figure 4. Partial regression plots of incubation period relative to body mass, nest predation, and PHA response across seven bird species (Green-tailed towhee was excluded from the analysis, see text). a) Incubation period increases with body mass (r_p =

0.981, P = 0.003). b) Incubation period decreases with increased nest predation rate (r_p = -0.953, P = 0.012). c) Species having longer incubation periods mounted weaker PHA responses (r_p = -0.982, P = 0.003).

Figure 5. Regression plot of SRBC response relative to body mass across the study species (r = 0.867, P = 0.002).

Figure 6. PHA response increases with blood parasite prevalence (r_p = -0.912, P = 0.028), when the effects of body mass and incubation period are controlled (Model 1).

Figure 7. PHA response decreases with nest predation rate (r_p = -0.959, P = 0.01), when the effects of body mass and incubation period are controlled (Model 2).

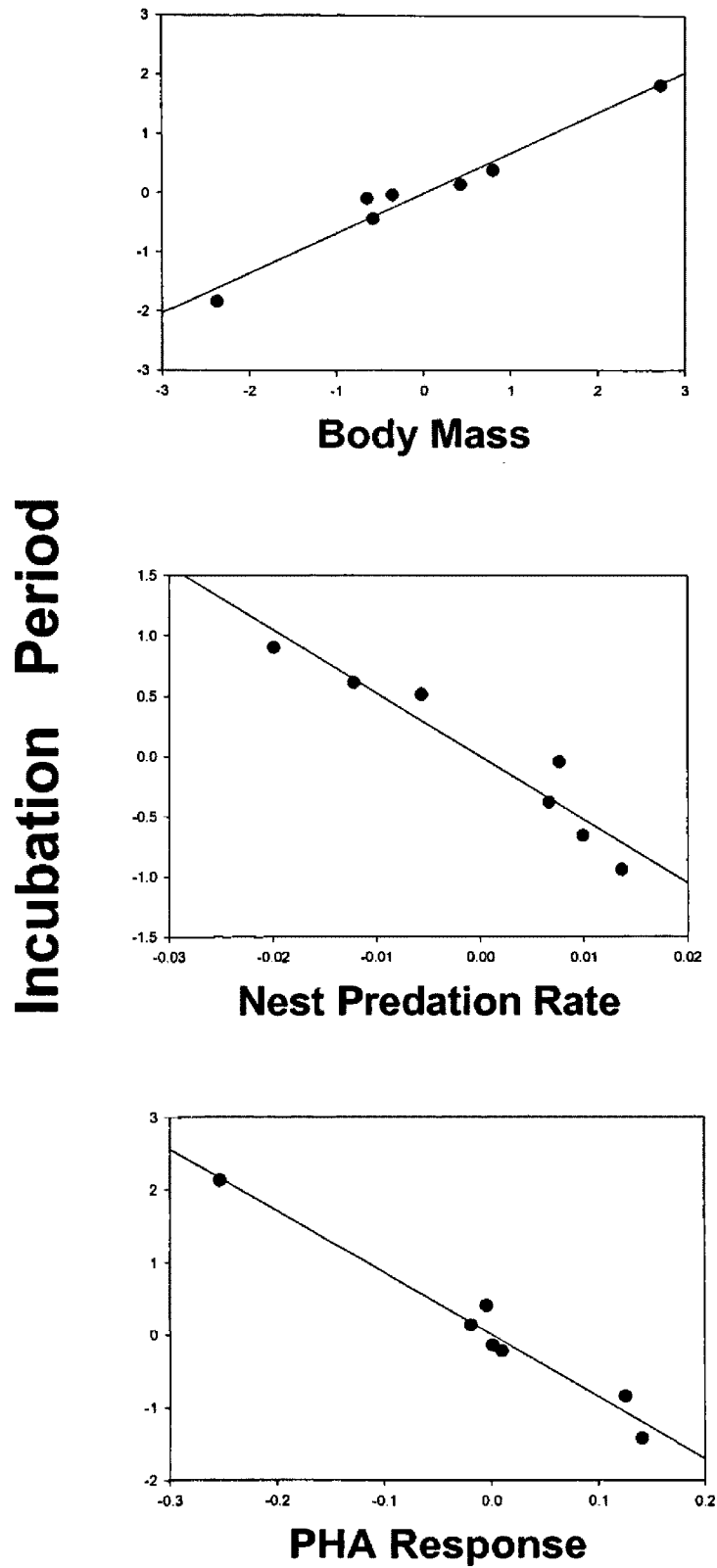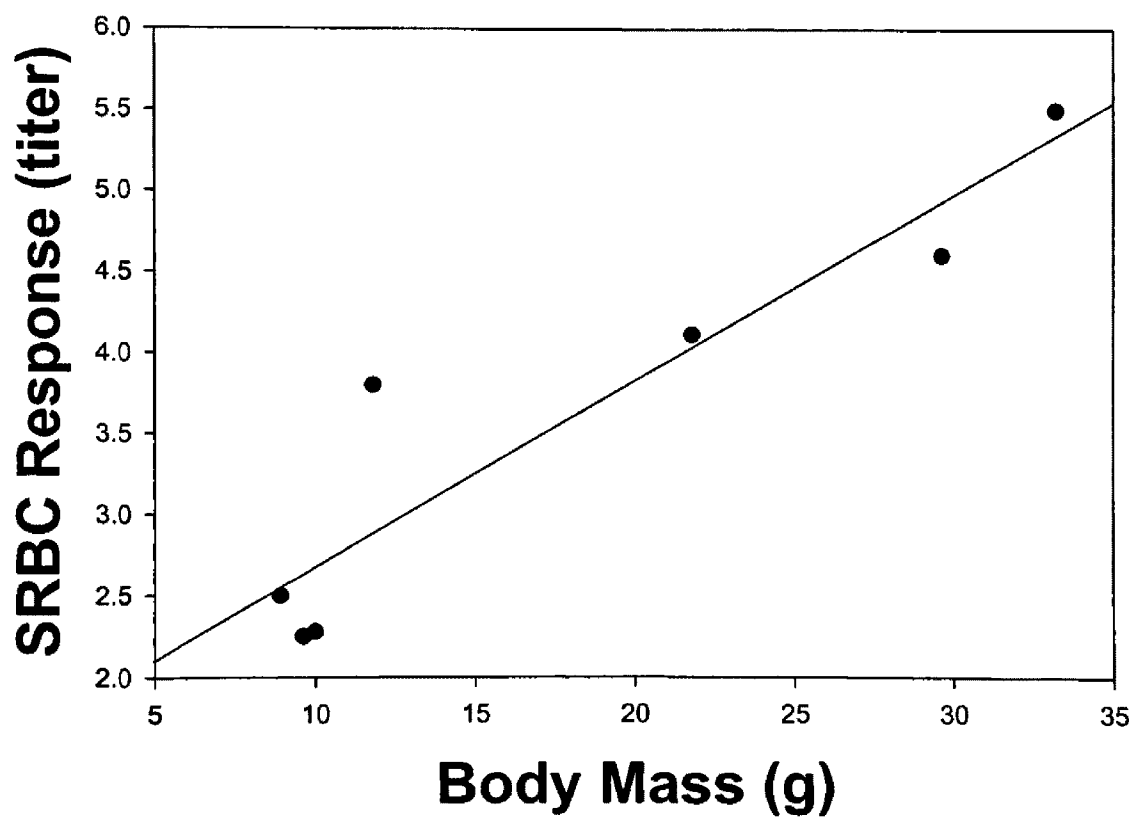Figure 1

A.

Feeding Stratum

B.

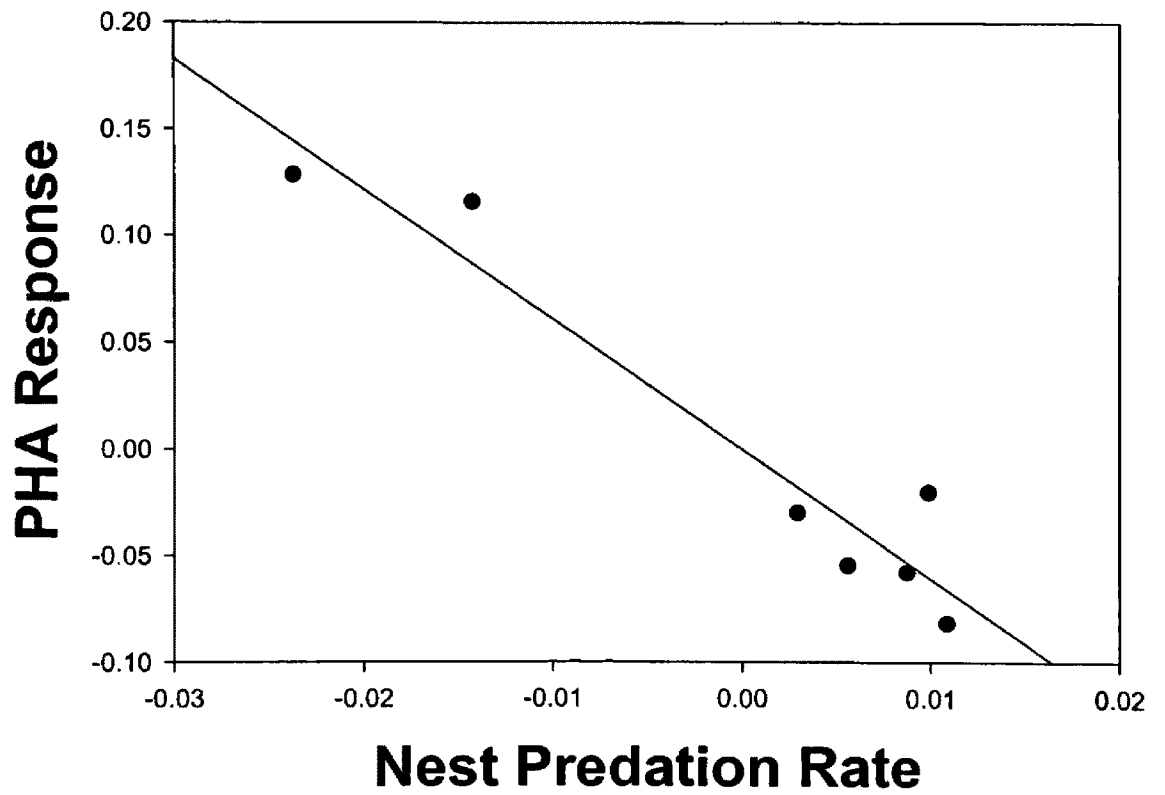Nest Stratum
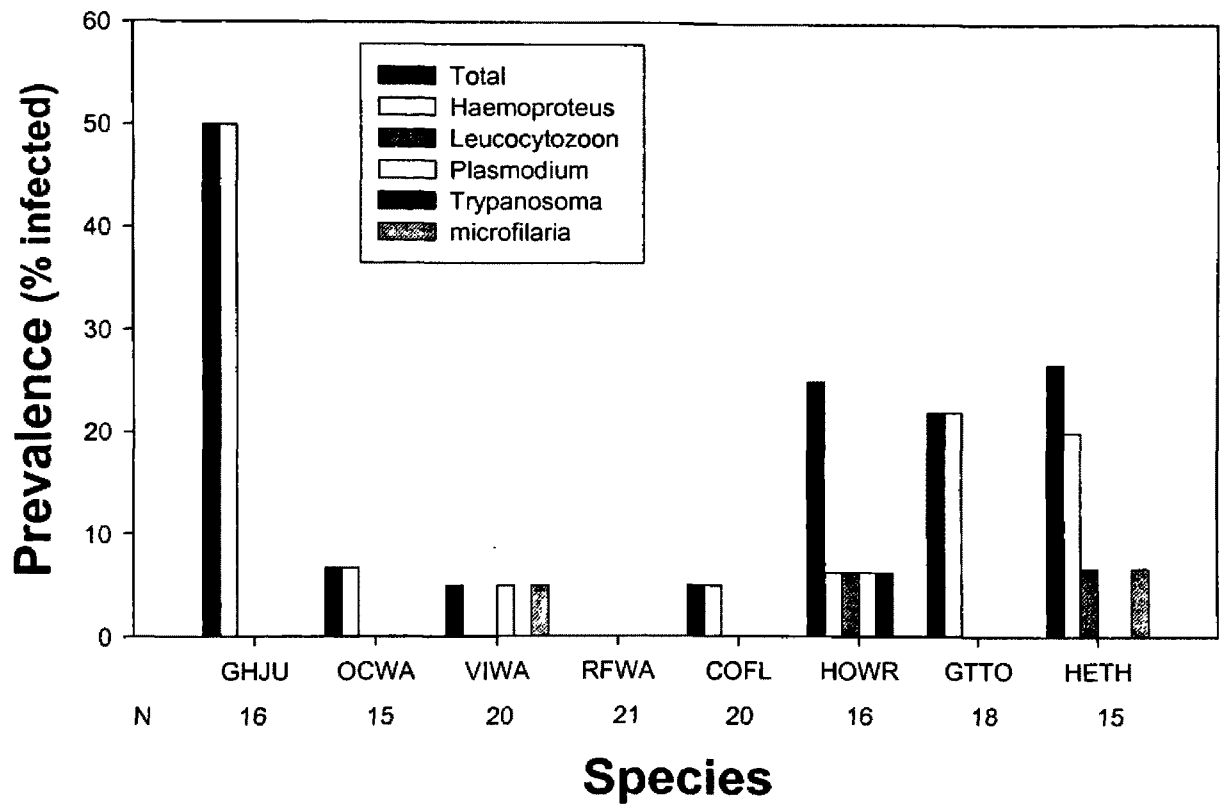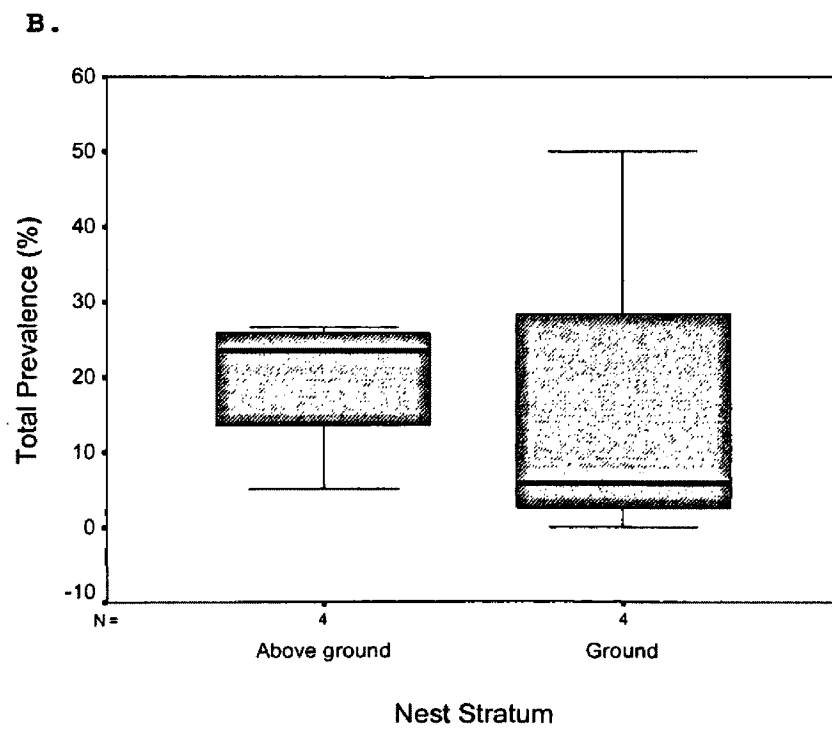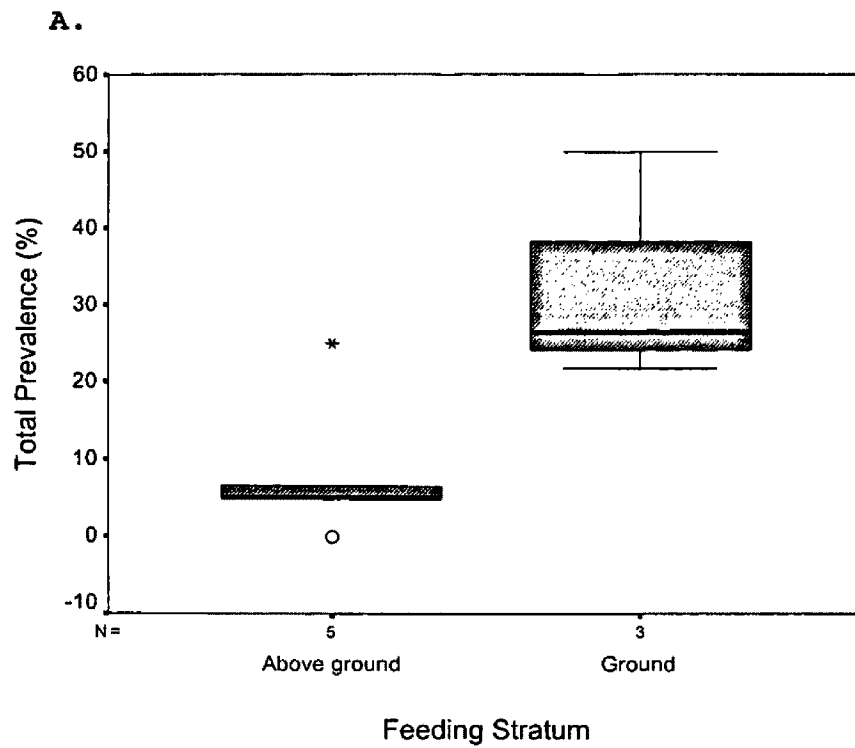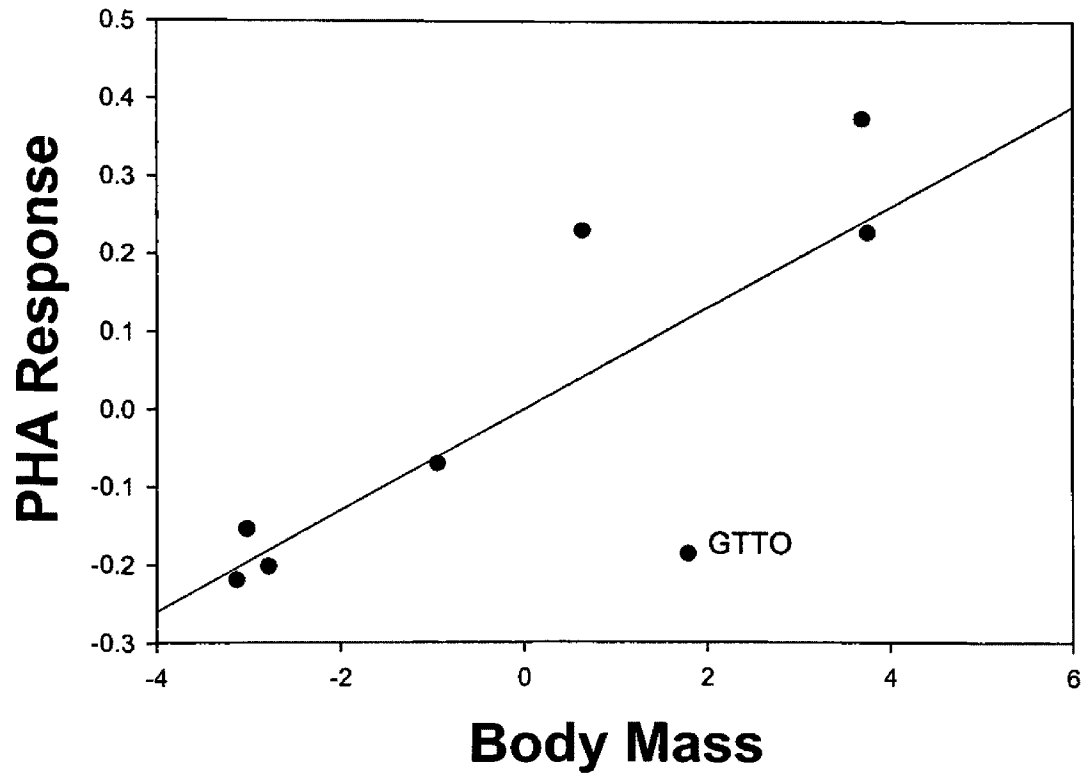
Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7