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Immunological investments reflect parasite abundance in island populations of Darwin's finches

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The evolution of parasite resistance can be influenced by the abundance of parasites in the environment. However, it is yet unresolved whether vertebrates change their investment in immune function in response to variation in parasite abundance. Here, we compare parasite abundance in four populations of small ground finches (*Geospiza fuliginosa*) in the Galapagos archipelago. We predicted that populations exposed to high parasite loads should invest more in immune defence, or alternatively use a different immunological defence strategy. We found that parasite prevalence and/or infection intensity increased with island size. As predicted, birds on large islands had increased concentrations of natural antibodies and mounted a strong specific antibody response faster than birds on smaller islands. By contrast, the magnitude of cell-mediated immune responses decreased with increasing parasite pressure, i.e. on larger islands. The data support the hypothesis that investments into the immune defence are influenced by parasite-mediated selection. Our results are consistent with the hypothesis that different immunological defence strategies are optimal in parasite-rich and parasite-poor environments.

Keywords: host–parasite coevolution; immunocompetence; *Geospiza fuliginosa*

1. INTRODUCTION

The evolution of parasite resistance has been the focus of many studies in evolutionary biology over the past decade (Zuk & Stoehr 2002; Schmid-Hempel 2003; Schmid-Hempel & Ebert 2003). It is now becoming increasingly clear that immune defences are coupled with costs and investments are adjusted according to the availability of resources (Sheldon & Verhulst 1996; Norris & Evans 2000). For example, in birds it has been shown that the immune system is suppressed during reproduction when workloads are heavy (Nordling *et al.* 1998; Moreno *et al.* 1999; Lochmiller & Deerenberg 2000). However, not only the costs of immunity can influence resource allocation decisions but also the potential benefits. Taken to the extreme, any immune investment will be wasted in an environment without parasites. Thus, the abundance of parasites in the environment is also expected to influence an individual host's investments in immunological defences (Zuk & Stoehr 2002; Schmid-Hempel & Ebert 2003). We still know very little about how parasite abundance affects immune investments and in most studies, the abundance of parasites is unfortunately an unknown variable. In this study, we make use of an environmental gradient in parasite abundance to investigate the relationship between parasite abundance and host immunity. We measured parasite abundance and immunity in small ground finches (*Geospiza fuliginosa*) in a well-characterized island ecosystem, the Galapagos archipelago. Based on classic island biogeography models, we predicted the number of parasite species to increase with the size of the study island (MacArthur & Wilson 1967). The relationship between biodiversity and island size has been demonstrated to apply to taxonomically diverse groups such as plants, vertebrates and insects, but has so far only rarely been used to predict the distribution of parasites (Janzen 1973; Poulin 1997). According to island biogeography models, hosts living on large islands could be exposed to a higher diversity of parasites. To test this assumption we selected four study populations within a large range of island sizes. We also expected the force of infection to increase with population size (Anderson & May 1979; May & Anderson 1979). Such a relationship could be expected if many susceptible hosts are present in a large population at any given time, and the number of contacts between infected and susceptible individuals is increased (de Jong *et al.* 1995; McCallum *et al.* 2002). Thus, because large islands would hold small ground finch populations of larger size, we could expect transmission rates to be increased on larger islands. Also, a large host population can allow for the persistence of parasite species with low reproductive rate (R_o) that are unable to persist in smaller populations (Dobson & Carper 1996).

If parasite diversity or abundance varies between populations we also expected hosts to respond evolutionarily to this variation by increasing their immunological investments or by adopting different immunological defence strategies (Zuk & Stoehr 2002; Schmid-Hempel 2003; Schmid-Hempel & Ebert 2003). The vertebrate immune system consists of two main arms of defence that provide protection against infections. In this study, we measured the magnitude of a cell-mediated and humoral immune response and the concentrations of natural antibodies. We

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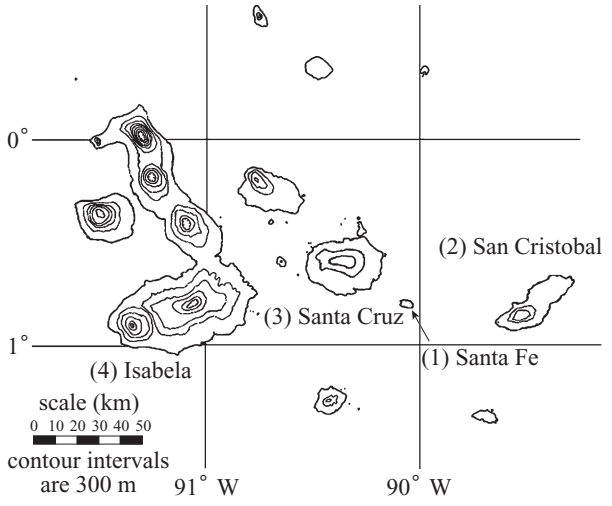

Figure 1. Map of the Galapagos archipelago, located *ca*. 1000 km west of South America and mainland Ecuador. The islands included in this study were (1) Santa Fe, 24 km^2; (2) San Cristobal, 558 km^2; (3) Santa Cruz, 986 km^2; and (4) Isabela, 4588 km^2.

had two alternative hypotheses to explain host differences in immune function: (i) if immunological defences are coupled with low costs, we expected investments in immune defence to increase with parasite abundance. According to this prediction, the magnitude of all types of immune response should be greater in parasite-rich communities; alternatively (ii) if immune responses are costly, hosts should optimize their defence in a way that minimizes the combined cost of parasite defence and parasite-inflicted damage (Frank 2000; Shudo & Iwasa 2001, 2002). In this case we expect an internal immunological trade-off where one type of immune defence is replaced by another as the abundance or diversity of parasites changes (Frank 2000; Shudo & Iwasa 2001). To test our predictions we quantified parasite diversity and abundance as well as the magnitude of selected immune responses in Darwin's finch populations on four islands in the Galapagos archipelago.

2. MATERIAL AND METHODS

We studied small ground finches on the islands of: (1) Santa Fe, 24 km^2; (2) San Cristobal, 558 km^2; (3) Santa Cruz, 986 km^2; and (4) Isabela, 4588 km^2 (figure 1). These islands are located *ca*. 1000 km from the Ecuadorian mainland and isolated from each other by at least 16 km of ocean. Small ground finches are seed-eating passerines (13 g) that are endemic to the Galapagos islands (Grant 1999). On all study islands, small ground finches were common and occurred in most habitats. Using identical sampling efforts and techniques, we examined 100 finches from each population for parasites between 1 October and 4 December 2002. On all islands, birds in non-breeding condition were captured in mist nets in comparable dry bush habitat at low altitude (less than 50 m). The four islands were sampled in a randomized order for their size.

(a) *Parasite abundance*

Each captured bird was examined for 5 min for ectoparasites as described in Clayton & Walther (1997). Samples of parasites were collected in alcohol-containing vials. A sub-sample of 20 birds per island was additionally treated with powder containing pyrethroid and dusted over a paper for collection of ectoparasites (Clayton & Walther 1997). The identification of ectoparasites was performed by Barry O'Connor, University of Michigan, USA. The most common ectoparasites were *Trouessartia* sp. nov., and *Proctophyllodes* sp. (Mirnov & Perez 2002). The parasite loads of *Trouessartia* sp. nov. were categorized into the classes: 0, 1–50, 51–100, 101–500 and 501–1000, whereas loads of *Proctophyllodes* sp. were categorized as being 0, 1–10, 11–50, 51–100, 101–500 or 501–1000. Birds were also examined for symptoms of avian pox infections including swollen lesions and missing digits. Birds showing these symptoms were categorized as pox-positive. Although diagnosis of avian pox usually requires histological examination, our approach should be valid on the Galapagos because no other avian disease with similar symptoms has been recorded (H. Vargas, personal communication). The presence of avian pox is well documented, and the virus has been isolated from several species of endemic birds (Duffy & Harcourt 1981). It should be noted our prevalence data include birds that recovered from the infection.

(b) *Immune tests*

We performed immune tests on 12 or 13 randomly selected small ground finches on each of the four islands. The birds were captured in mist nets and housed in aviary cages (0.5 m × 0.5 m × 0.5 m). Cages contained water, perches and protective bushes. Birds were fed chicken starter pellets supplemented with eggs and vegetables. Shortly after capture (1–3 days), we took an initial blood sample before injecting all birds subcutaneously with a small volume (50 µl) of the immunostimulatory protein keyhole limpet haemocyanin (KLH) (Sigma-Aldrich, St Louis, Missouri, USA) (1 mg ml^{-1}) dissolved in saline and emulsified in Freund's incomplete adjuvant. The KLH antigen has been used extensively in medical research and immunizations result in a strong specific antibody response in most vertebrates (Harris & Markl 1999). Plasma for quantification of the specific antibody titres was obtained from a sample of 50–100 µl blood taken from the jugular vein 10 and 12 days after KLH injection. All blood samples were centrifuged (5 min at 5000 r.p.m.) and the plasma was collected within 1 h. Samples were stored cold (4 °C) until frozen (−20 °C). Initial samples were frozen after 14 days, whereas samples taken on day 10 and 12 were frozen after 6 and 4 days, respectively. The antibody concentration of each sample was quantified in duplicates in an enzyme linked immunosorbent assay (ELISA) performed on a small (10 µl) volume of plasma diluted 1 : 1000 (Hasselquist *et al.* 1999). Antibody concentrations were measured with a kinetic reader and are reported as titres of optical densities (ODs) at 405 nm. The detection limit for OD was 0.2. Intra-assay variation was 2% and inter-assay variation was 5%. In the naive samples that were taken before the antigen injection, we detected low levels of antigen binding with ODs ranging between 0.4 and 5.0. These concentrations of antibodies in the initial samples are reported as the concentrations of natural antibodies. To separate between natural and specific antibody responses in later samples, we treated all birds that had antibody titres below OD 5.0 as non-responders. To measure cell-mediated immune responses, we used the simplified protocol of the phytohaemagglutinin (PHA) skin test (Smits *et al.* 1999). This test provides a measure of the proliferative response potential of circulating T lymphocytes to an injected mitogen. We injected a small (50 µl) volume of saline solution containing 1 mg ml^{-1} of PHA (Sigma-Aldrich, St Louis, Missouri, USA)

subcutaneously into one wing web to elicit a cell-mediated immune response at the injection site. PHA was injected in the afternoon, 4 days after the injections with KLH. At this time we also took measurements of body mass (to the nearest 0.1 g) using a pesola scale and measured tarsus length (to the nearest 0.1 mm) using digital callipers. The response to the PHA injection was defined as the increase in wing-web thickness at the injection site 24 h after injection. The wing web was measured (to the closest 0.01 mm) three times with a thickness gauge (Mitutoyo, Tokyo, Japan), and we used the average as the response. The repeatability of the measurements was very high ($r = 0.99$, $p < 0.001$).

(c) *Statistical analyses*

We used the free software Quantitative Parasitology, v. 2.0 to calculate the mean infection intensity, prevalence and 95% confidence intervals for each population and to test for differences in parasite abundance between islands (Rózsa *et al.* 2000). Because our hypothesis was that immune parameters would change in relation to parasite abundance, which in turn would be related to the relative size of the study island, we had an *a priori* expectation of how the immune responses of the island populations should be ordered. Therefore, we used an ordered heterogeneity test to examine this hypothesis (Rice & Gaines 1994; Sokal & Rohlf 1995). This composite test takes both the variance between populations (p-value from ANOVA), and the ordering of the data (r_s from a non-parametric correlation) into account. In the ANOVA we included island as an independent factor and immune parameters (cellular immunity, natural or specific antibody concentration) as dependent factors. We used r_s values from non-parametric correlations between island size and the mean of immune parameters to calculate the test statistics ($r_s P_c$). The presented p-values are from two-tailed tests. We used an ANCOVA to test if the effect of island size on cell-mediated immunity was independent of the effect of body mass. In this model we included cell-mediated immune response as the dependent variable, island size as the independent variable and body mass as a covariate. Specific antibody titres on day 10 and 12 were not normally distributed, thus we used log-transformed values in the analyses.

3. RESULTS

(a) *Parasite abundance*

Three types of infection were commonly found in visual inspections: avian pox (*Poxvirus avium*) and feather mites (Arachnida, Acari) either in the crown (*Trouessartia* sp. nov.) or on the wings (*Proctophyllodes* sp.). These parasites were present on all islands, revealing no difference in parasite diversity between islands. The prevalence of avian pox symptoms differed between islands ($\chi^2 = 110$, d.f. = 3, $p < 0.000$) and increased from the smallest to the largest island: (1) 3%; (2) 20%; (3) 32%; and (4) 69% (figure 2*a*). For the feather mites found in the crown, the prevalence of infection was significantly different between islands ($\chi^2 = 51.9$, d.f. = 3, $p < 0.000$), being low on the smallest island (1) with 52% infected birds, and higher on all larger islands: (2) 93%; (3) 73%; and (4) 84%. The variance-per-mean ratio (of mite load) increased with island size: (1) 261; (2) 315; (3) 487; and (4) 1680; thus the distribution of mites became more aggregated with increased island size. The mean intensity of infection (number of crown mites per bird) differed between islands

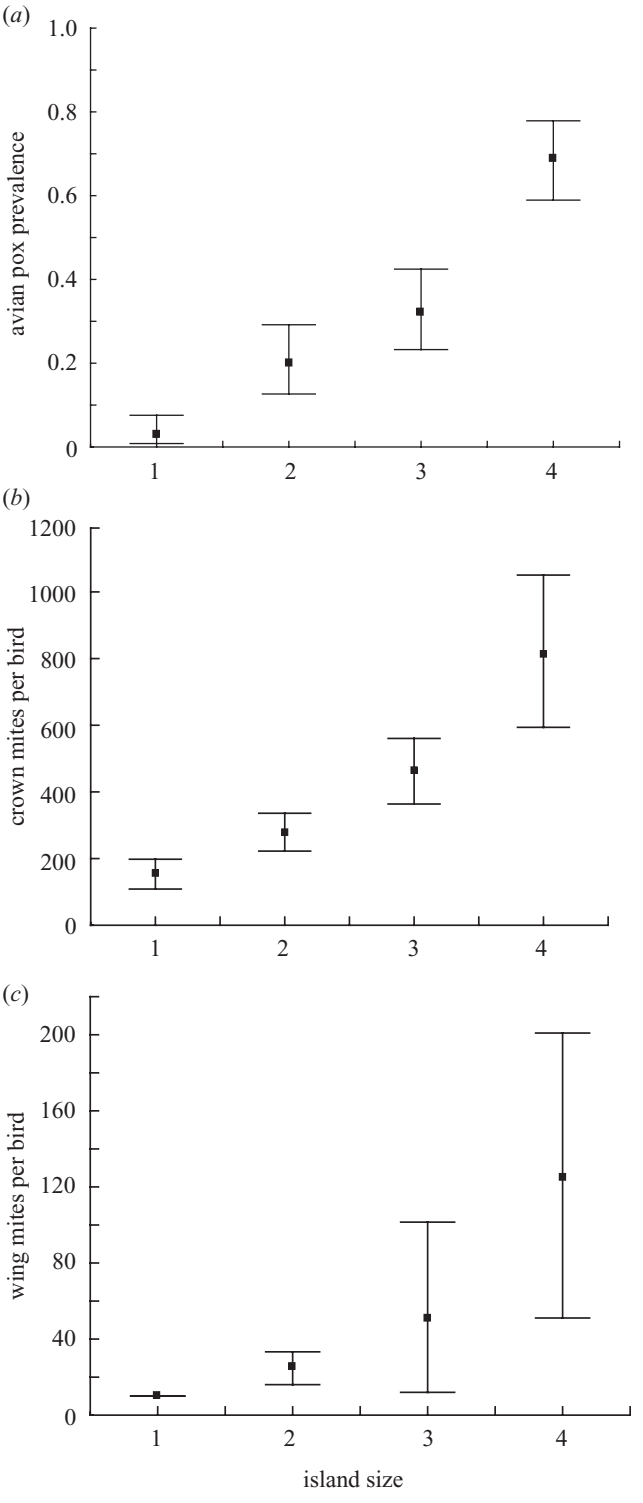

Figure 2. Parasite abundance in four populations of small ground finches in relation to the relative size of the study island. The mean prevalence of (*a*) avian pox (*Poxvirus avium*) infections and 95% confidence intervals (Clopper–Pearson); (*b*) mean infection intensity of feather mites (*Trouessartia* sp.) found in the crown; and (*c*) the mean infection intensity of wing mites (*Proctophyllodes* sp.). In (*a*) and (*b*) bars represent 95% bootstrap confidence intervals. (For wing mites on Santa Fe, the confidence interval could not be reliably calculated.) Sample sizes are 100 individuals per island.

(Mood's median test: d.f. = 3, $p < 0.000$) and increased with island size from an average of (1) 156; (2) 275; (3) 417; to (4) 813 mites per bird (figure 2*b*). A similar pattern was found for the mite species found on the wing feathers. Here, the prevalence of infection did not differ significantly between islands ($\chi^2 = 5.63$, d.f. = 3, $p = 0.13$) but tended to increase with island size: (1) 31%; (2) 33%; (3) 40%; and (4) 46%. Again, the variance-per-mean ratio increased from: (1) 7; (2) 43; (3) 604; to (4) 629; with wing mites having a more aggregated distribution on larger islands. The mean intensity of infection differed between islands (Mood's median test: d.f. = 3, $p = 0.002$) and increased with island size from an average of: (1) 10; (2) 25; (3) 51; to (4) 124 mites per bird (figure 2*b*).

(**b**) *Immune tests*

We found that the strength of cellular immune responses significantly decreased with increasing island size ($r_sP_c = 0.77$, $k = 4$, $p = 0.03$; figure 3*a*). The strength of the cell-mediated immune response was also related to body mass ($r = 0.48$, $n = 48$, $p = 0.001$). To examine if effect of island size was dependent of variation in body mass, we performed an ANCOVA with island size included as an independent factor and body mass as a covariate. In this test we found that there was a significant effect of island ($F_{4,43} = 3.38$, $p = 0.04$) independent of the effect of body mass ($F_{1,43} = 9.00$, $p = 0.004$). The concentrations of natural antibodies significantly increased with island size ($r_sP_c = 0.85$, $k = 4$, $p = 0.001$; figure 3*b*). Not all birds produced specific antibodies within the time course of our study. Ten days after the antigen injection, 36% of all birds had produced antibody titres that exceeded the highest titre observed in the zero samples. Twelve days after antigen injection 64% of all birds had responded and antibody titres at this day were significantly increased compared with those at day 10 (paired *t*-test, $t = 5.11$, d.f. = 18, $p < 0.000$). On both days, the proportion of non-responders was randomly distributed between islands ($\chi^2 < 1.19$, d.f. = 3, $p < 0.75$). When comparing specific antibody titres of birds that responded, there was a significant effect of island size for specific antibody titres at day 10 ($r_sP_c = 0.71$, $k = 4$, $p = 0.048$; figure 3*c*) but not at day 12 after challenge ($r_sP_c = 0.31$, $k = 4$, $p = 0.32$). There were no significant relationships between body mass and natural antibody titres ($r = 0.05$, $n = 49$, $p = 0.71$), or specific antibody titres at day 10 ($r = 0.20$, $n = 20$, $p = 0.39$) or at day 12 ($r = 0.07$, $n = 30$, $p = 0.97$). The titres of natural antibodies were not significantly related to the magnitude of cell-mediated responses ($r = -0.14$, $n = 49$, $p = 0.33$), the titres of specific antibodies at day 10 ($r = 0.09$, $n = 20$, $p = 0.71$), or at day 12 ($r = 0.13$, $n = 30$, $p = 0.50$). There were no relationships between the strength of the cell-mediated and specific antibody response either at day 10 ($r = -0.07$, $n = 20$, $p = 0.75$) or at day 12 ($r = -0.03$, $n = 30$, $p = 0.85$).

We tested for an effect of avian pox infection status on immune parameters for birds on islands (2)–(4), where some (16 out of 34) of the birds in the immune study had been infected. Among these birds, there was no significant effect of infection status on any of the immune parameters (*t*-tests, infected versus uninfected, all tests, $p > 0.15$). For birds from all islands, there was no significant effect of wing mite infection status (*t*-tests, infected versus uninfected, all tests: $p > 0.17$) or crown mite load (*t*-tests, heavily infected versus less than 100 mites, all tests: $p > 0.18$). We found no significant correlation between the

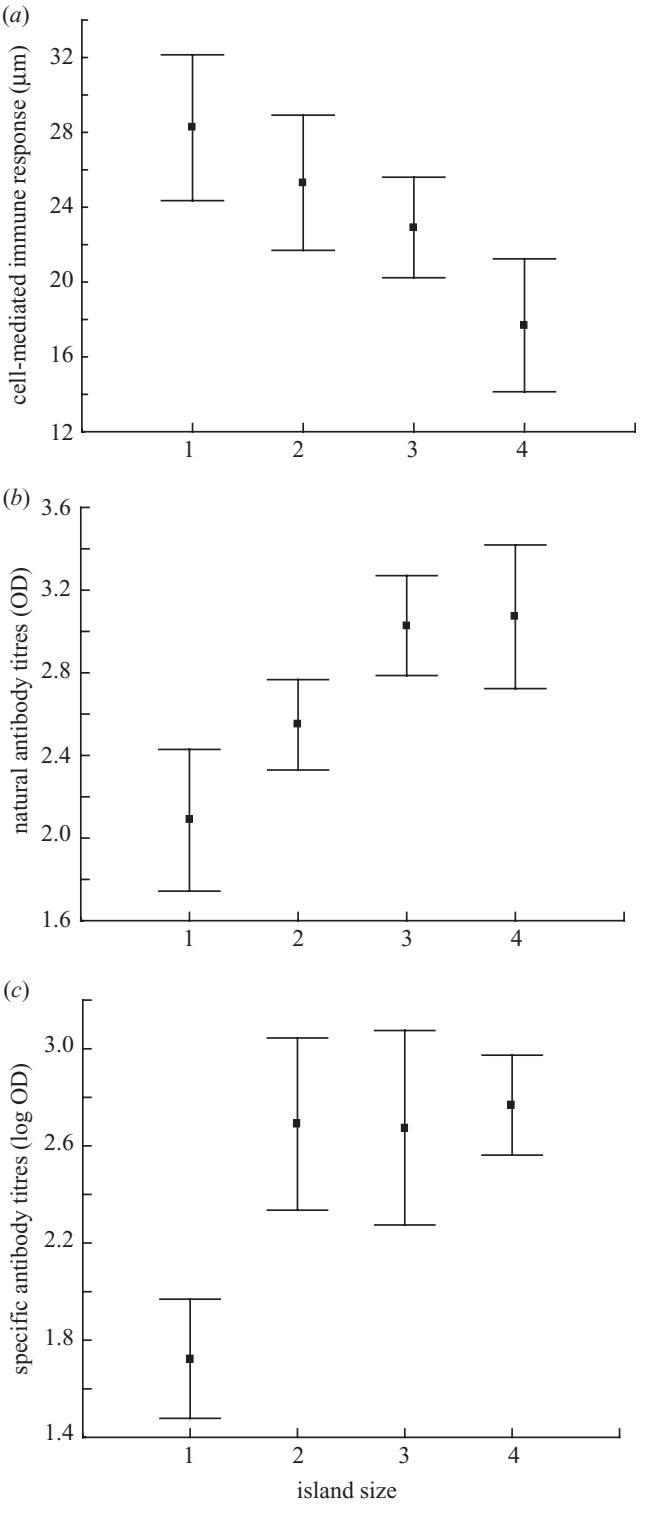

Figure 3. Immunity of small ground finches in relation to relative island size. (*a*) The magnitude of cell-mediated immune responses; (*b*) the concentrations of natural antibodies; and (*c*) specific humoral immune responses (log transformed) of responding birds 10 days post-injection. The values are means and bars represent standard error of means. Sample sizes for each island are: (*a*) 1, 12; 2, 12; 3, 13; and 4, 13; (*b*) 1, 12; 2, 12; 3, 13; and 4, 3; (*c*) 1, 5; 2, 5; 3, 5; and 4, 5.

size of the human population on the islands and the prevalence of avian pox ($r_s = 0.4$, $n = 4$, $p = 0.6$).

4. DISCUSSION

The prevalence and/or intensity of parasitic infections in Darwin's finches increased with island size, at least for the three most common types of infection investigated here. These results are, to our knowledge, the first empirical evidence documenting a positive effect of island area on parasite abundance in wildlife. We found no support for our prediction that parasite diversity should be higher on larger islands. However, because these populations were not examined for the presence of many other usually abundant parasites (e.g. haematozoa, intestinal parasites, bacteria, viruses), our measurement of parasite diversity was incomplete and it remains unclear if such a pattern exists. Previous studies have found no effect of island size on parasite abundance. For example, in a study where parasites were collected from birds in the Antilles, there were no differences between islands in the prevalence of *Haemoproteus* sp. infections, whereas the presence of a protozoan parasite was restricted to one island (Apanius *et al.* 2000). However, the size-range of the Antillean islands was much smaller than used in the present study. Also, the predictions made here are not expected to apply for vector-borne infections like *Haemoproteus* sp. Similarly, in a parasite study of *Anolis* lizards, island size did not influence the distribution of helminth intestinal parasites (Dobson *et al.* 1992). Instead, the moisture of the habitat was the most important predictor for parasite distribution. In the present study, we collected parasites in environments selected to be as similar as possible to disentangle island-size-related factors from other environmental factors (Blaustein *et al.* 1983; Poulin 1997; Moyer *et al.* 2002). However, we cannot exclude the possibility that the distribution of parasites could also have been influenced by environmental factors.

We classified the two species of feather mites found in this study as parasites, although very little is known about their biology or their effect on host immunity. In fact, only some species of avian feather mites are true parasites and many species live as commensals on their hosts (Proctor & Owens 2000; Blanco *et al.* 2001). Nevertheless, it has been shown that high mite loads can be associated with a reduction of body condition or plumage coloration in birds (Thompson *et al.* 1997; Harper 1999; Figuerola *et al.* 2003). Such observations suggest either that mites can cause direct damage to their hosts when present in high numbers, or that high mite loads are associated with high loads of other more virulent parasites. In this study, populations with high mite loads also had high infection prevalence of avian pox, which is an avian pathogen with high virulence (Van Riper *et al.* 2002).

The most likely mechanism to explain the increase of parasite abundance with island size is the increasing size of the avian host population. The three parasites investigated here can all be transmitted through direct contacts (Duffy & Harcourt 1981; Proctor & Owens 2000). Therefore, an increased number of contacts between infected and susceptible hosts will increase parasite transmission (Anderson & May 1979; May & Anderson 1979; de Jong *et al.* 1995; Dobson & Carper 1996). In our study, the absolute sizes of the islands, and presumably also the sizes of the host populations, differed dramatically among islands, and high prevalence and infection intensities suggested that infection rates were indeed higher in larger populations. This pattern could be either a result of a direct effect on transmission dynamics of population size, or by an indirect effect where the size of the study populations affects the density. Alternatively, differences in dispersal patterns of birds could have influenced disease transmission. The large islands in this study also contained moist highlands and if infected birds were migrating down from the highlands on larger islands, this could influence the parasite load of the bird at lower altitudes. Differences in host susceptibility and parasite virulence can also affect disease dynamics (Poulin 1997). This possibility can currently not be studied in Darwin's finches because it would require a transfer of hosts or parasites between study populations. Current National Park regulations do not allow such procedures.

The clear gradient in parasite abundance found between the bird populations enabled us to examine how variation in parasite abundance relates to immunological investments. We found that island size was related to immune investments, a finding that is consistent with the hypothesis that immunity was influenced by the abundance of parasites. Immune investments changed with island size in several ways: birds on larger islands had higher initial concentrations of antibodies capable of binding to the novel antigen (KLH). This result is most probably a result of bindings by natural antibodies that are part of the non-specific innate immune response, or by cross-reacting specific antibodies that were originally produced against antigens that were similar to the one used in the experimental challenge. The function of natural antibodies has only recently been studied in mammals confirming that natural antibodies indeed form an important first line of defence against both bacterial and viral infections (Ochsenbein *et al.* 1999). For birds, natural antibodies with low specific antigen affinity have recently been detected in chickens (Lammers *et al.* 2004). Chicken lines selected for high specific antibody production capacity also obtained high levels of natural antibodies, suggesting that these two traits are genetically linked (Parmentier *et al.* 2004). Similarly, in our study we found that birds on larger islands produced a strong antibody response faster. Ten days after an exposure to an antigen, birds on larger islands had higher antibody titres. Two days later, when specific antibody production had been initiated in more birds, island size no longer predicted the magnitude of response. Thus birds on larger islands were able to mount a strong response faster. In contrast to the pattern found for natural and specific antibodies, the strength of cell-mediated immune responses was negatively associated with island size. In birds, the magnitude of the cell-mediated immune response is often related to an individual's body mass or body condition (Alonzo-Alvarez & Tella 2001). In our study, birds with a high body mass produced stronger cell-mediated immune responses. However, when the effect of body mass was taken into account, island size explained an independent and significant proportion of the variation in cell-mediated immune responsiveness.

We found evidence that the small ground finches had increased levels of natural antibodies, faster produced a

strong specific antibody response and decreased magnitudes of cellular immune responses in populations where parasites were more abundant. We suggest that this variation in immunological investments was influenced by the variation in parasite abundance (Moret 2003; Schmid-Hempel 2003). An influence of parasite abundance on immunity could have both environmental and genetic components (Alonzo-Alvarez & Tella 2001). Because the birds used in our study had previous experience of the environments on the study island, past or present parasite exposure could have affected immune responsiveness. However, we found no clear effect of parasites on immune responsiveness in the birds in this study.

The opposite patterns found for cell-mediated versus natural and specific immunity gave support to the second of our initial hypotheses, that different arms of the immune system were subject to internal trade-offs (Frank 2000; Moret 2003). The existence of trade-offs between different arms of the immune system has been demonstrated previously in bumble-bees where strains with strong non-specific responses showed reduced specific resistance to infections (Mallon *et al.* 2003). A trade-off between specific and non-specific arms of immunity is the predicted outcome in the theoretical model presented by Frank (2000). In this model, immune responses are coupled with costs. Hosts that are protected by one type of immunity can benefit by reducing their investments in other defences. Further evidence for internal immune trade-offs comes from the fact that the two arms of the vertebrate immune system (humoral and cellular) are known to be inversely regulated. The activation of the immune system takes place when immature T cells encounter an antigen and mature into either Th1 or Th2 cells that specifically activate either the cell-mediated (Th1) or the humoral (Th2) arm of the immune defence (Infante-Duarte 1999). The type of immune response that is elicited is thought to depend on the type of invader as well as the infective dose. Within individuals, we found no evidence of internal trade-offs to occur between the different arms of the immune system. Still, such a trade-off was apparent at the population level.

The fitness costs as well as benefits of maintaining and using different types of immune defence need to be evaluated to understand how immunological investments are influenced by natural selection (Zuk & Stoehr 2002; Mallon *et al.* 2003). Recent ecological studies have shown that an activation of the immune system can be costly for the organism and cause reductions of host fitness (Moret & Schmid-Hempel 2000; Schmid-Hempel 2003). Thus for infection clearance, hosts need to choose the most efficient type of response to minimize both the damage inflicted by the pathogen but also by the immune reaction itself (Frank 2000; Shudo & Iwasa 2001, 2002). Our results imply that the benefit of using different arms of the immune system varies with the abundance of parasites in the environment. Specifically, our results suggest that the presence of natural antibodies combined with a rapid production of specific antibodies provides a more cost-efficient protection than cellular immunity in environments where parasites are more abundant.

Today the small ground finches on the Galapagos, as well as many other endemic island species, are facing an increased risk of encountering new pathogens that are introduced through human activities (Wikelski *et al.* 2004). The increasing problem of emerging diseases is not restricted to island populations: globally, emerging diseases have been classified as the second most important threat to biodiversity after habitat degradation (Daszak *et al.* 2000). A major challenge for future researchers will be to understand which immunological factors make an animal population more susceptible to pathogens. More empirical work is needed to understand the functional significance for the variation in immune responsiveness between populations. We suggest that island ecosystems where animal populations are distinctly separated in space would provide an excellent setting to address this type of question.

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