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Are there differences in immune function between continental and insular birds?

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Generally, immune system architecture varies with different environments, which presumably reflect different pathogen pressures. Specifically, populations from relatively disease-free, oceanic islands are expected to exhibit reorganized immune systems, which might be characterized by attenuated responses, given the costs of immune function. Some insular animals exhibit an 'island syndrome,' including increased susceptibility to disease, and some insular populations have declined when they failed to resist infection by introduced pathogens. I measured eight indices of immune function (haemolysis, haemagglutination, concentration of haptoglobin and concentration of five leukocyte types) in 15 phylogenetically matched pairs of bird populations from North America and from the islands of Hawaii, Bermuda and the Galápagos. Immune responses were not attenuated in insular birds, and several indices, including the concentration of plasma haptoglobin, were elevated. Thus, I find no support for the specific hypothesis that depauperate parasite communities and the costs of immune defences select for reduced immune function. Instead, I suggest that life on islands leads to an apparent reorganization of immune function, which is defined by increases in defences that are innate and inducible. These increases might signal that systems of acquired humoral immunity and immunological memory are less important or dysfunctional in island populations.

Keywords: comparative immunology; oceanic islands; leukocytes; haptoglobin; natural antibodies

1. INTRODUCTION

Biologists widely believe that insular avifaunas are particularly vulnerable to introduced diseases, and numerous examples of increased susceptibility to specific diseases exist in both wild and captive populations of insular animals (Jarvi *et al.* 2001; Van Riper & Scott 2001; Wikelski *et al.* 2004). In the Hawaiian Islands, for example, populations of many native bird species have declined, in some cases to extinction, owing to introductions of two pathogens (avian pox virus, *Avipoxvirus* spp., and the malaria parasite *Plasmodium relictum*) and a vector (southern house mosquito, *Culex quinquefasciatus*; Jarvi *et al.* 2001; Van Riper & Scott 2001). Recent reports from the Galápagos Archipelago documenting the establishment of *C. quinquefasciatus* (Whiteman *et al.* 2005) and characterizing avian pox there (Thiel *et al.* 2005), raise concerns that the Galápagos avifauna could meet a similar fate (Wikelski *et al.* 2004).

The immunological and evolutionary foundations of reduced resistance and increased susceptibility in insular populations are poorly understood and have only recently begun to be investigated (e.g. Jarvi *et al.* 2001; Lindström *et al.* 2004). Ostensibly, these changes represent one aspect of an 'island syndrome' of reduced interspecific (in this case, host–parasite) interactions in the simplified ecological communities of islands (Hochberg & Moller 2001; Blumstein & Daniel 2005). Attenuated parasite and pathogen pressure on islands might weaken selective pressures that maintain immune system function (Frankham 1997; Jarvi *et al.* 2001; Van Riper & Scott 2001), leading to diminished immune function as an evolutionary response to the energetic (Martin *et al.* 2002), autoimmunological (Råberg *et al.* 1998), growth (Klasing *et al.* 1987) and survival (Hanssen *et al.* 2005) costs of immune system development, maintenance and use. Even without these fitness costs, reduced benefits of immunologically relevant genetic diversity might result in the loss of this genetic diversity through mutation or drift in small insular populations (Frankham 1997).

Some studies suggest that parasite communities on small, isolated islands are depauperate (Wikelski *et al.* 2004), although the extent of this phenomenon is unclear. Positive taxa–area relationships have been found in microbial communities (Horner-Devine *et al.* 2004; Bell *et al.* 2005), and avian blood parasites are reduced on, or even absent from, some islands (Steadman *et al.* 1990; Super & van Riper1995). In any case, on distant islands, small populations of potential hosts could suffer immunological consequences of founder effects and inbreeding depression (Frankham 1997) independently of both disease threats and immune system costs. Links between indices of immune function and inbreeding at the individual (Reid *et al.* 2003) and population levels (Whiteman *et al.* 2006) are known, and genetic consequences associated with small, introduced populations have been implicated in increased disease susceptibility (Hawley *et al.* 2006).

The immune system has many components, each with its own inherent costs and protective value. The mix of

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components likely reflects an array of factors, including the nature of the disease threat (e.g. epidemic interval; Harding *et al.* 2005). For instance, frequent and repeated exposure to several common antigens might favour highly specific immunological strategies that maximize pathogen control and minimize collateral damage (Segel & Bar-Or 1999). Conversely, infrequent exposure to a broader range of rarer antigens might favour responses that maximize the speed of the initial response. Thus, the equilibrium between costs and benefits of immune responses should produce varied immunological strategies both within and among species. Indeed, numerous indices of immune function have been shown to vary among individuals of the same species (e.g. with stress: Råberg *et al.* 1998) and among species (Matson *et al.* 2005, 2006; Tieleman *et al.* 2005; Mendes *et al.* 2006). Moreover, in waterfowl, a multivariate analysis of immune function revealed that the measured indices vary independently and inconsistently among species (Matson *et al.* 2006). Such variation likely reflects some level of environmental optimization via phenotypic plasticity (Ricklefs & Wikelski 2002), but the common garden designs in both Martin *et al.* (2004) and Matson *et al.* (2006) suggest that evolutionary differences due to genotype–environment interactions are important as well. A limited, but growing, number of comparative studies have investigated environmental effects on immune responses simultaneously in multiple species of free-living vertebrates (cf. Mendes *et al.* 2006). Such studies provide insights into the cost–benefit balance of immune function if individual responses vary consistently in relation to disease threat.

In this study, comparisons were made between members of 15 phylogenetically matched pairs that contrast birds in North America with close relatives in Hawaii, Bermuda and the Galápagos Islands. The insular taxa included endemics, natives and recent introductions. Blood plasma was used in two assays to measure agglutination and lysis of rabbit red blood cells (RBCs) and baseline concentrations of the acute phase protein haptoglobin (Hp). Blood smears were used to estimate concentrations of five types of leukocytes. These indices were selected to probe a variety of protective functions that have a range of costs of use. Agglutination titres are indicative of levels of natural antibodies (NAbs), which facilitate initial pathogen recognition and initiate acquired immune responses. Lysis titres are indicative of complement and other circulating lytic enzymes. Hp offers protection against harmful end products of the immune response, namely haem from damaged host cells and free radicals from phagocytes. Among leukocytes, lymphocytes mediate the acquired antibody and the cell-mediated responses, which are pathogen-specific but of little value in early defence against novel pathogens; the other cell types mediate innate immunity, the primary defence against novel pathogens.

I sought to use these indices to examine how life on distant, oceanic islands has moulded the evolution of immune defences. Assuming that immune defences incur costs, birds with evolutionary histories on oceanic islands would exhibit reduced immune function if the relative threats of disease-causing micro-organisms were reduced. Accordingly, compared to continental populations, insular populations would exhibit reduced haemolysis and haemagglutination titres, lower plasma concentrations of Hp and depressed circulating concentrations of leukocytes. However, because the relationships among different measures of immune function are complex and poorly understood (Matson *et al.* 2006), measurable reductions might be limited to indices of functions that are costly or under strong genetic influence, and, hence, those that are most responsive to selection or other evolutionary forces, such as mutation and drift. Then again, this high degree of complexity might result in general reorganization in immune system architectures, which might or might not be consistent across species. Finally, it should be pointed out that, because immune function has been proposed to be related to invasiveness (Lee & Klasing 2004) and because of the disparity in time spent adapting to island life, differences in immune function between insular and continent populations might depend on population status (i.e. introduced versus native/endemic).

2. MATERIAL AND METHODS

(a) *Subjects and samples*

I collected small blood samples from 516 individual birds representing 25 species in 17 genera (appendix, table 1—electronic supplementary material). In some cases, samples from multiple populations (e.g. on different islands or from different seasons) were collected within species (appendix, table 1—electronic supplementary material).

After using a needle to puncture the brachial vein, blood was drawn into heparinized microcapillary tubes. At collection, I used several drops of blood to make smears for leukocyte enumeration. The remaining blood was centrifuged and the plasma collected. After centrifugation, plasma samples were frozen at $-20\,^{\circ}\mathrm{C}$ or below until analysis. All work was approved by the Animal Care Committee at the University of Missouri, St Louis (#W01-12).

(b) *Immune assays*

Innate humoral immunity was assessed by using a haemolysis–haemagglutination assay to characterize NAb-mediated agglutination and lysis of exogenous RBCs, as described by Matson *et al.* (2005). Both lysis and agglutination are recorded as the negative $\log_2$ of the last plasma dilution exhibiting each function (i.e. a dilution of 1 : 8 is scored as 3). Hp, an acute phase protein found in a wide range of taxa including birds (Delers *et al.* 1988), was quantified (mg ml^{-1}) by following the 'manual method' instructions provided with a commercially available assay kit (#TP801; Tri-Delta Diagnostics, Inc., Morris Plains, NJ). A single blood smear from each bird was evaluated blindly to species by an individual veterinary diagnostic laboratory technician (AVL Veterinary Clinical Laboratory, St Louis, MO). From differential counts (percentage of heterophils, lymphocytes, monocytes, eosinophils and basophils) and estimations of overall leukocyte concentration (Bounous & Stedman 2000), concentrations of each leukocyte type were estimated. In addition to the original methodological publications, all assays used in the present study have been summarized previously (Matson *et al.* 2006) and extended methodologies are appended.

Table 1. Paired *t*-tests of the effect of island–continent status on three indices of immune function: agglutination titre, lysis titre and haptoglobin concentration. (Mean values for each location within each genus are weighted by the square root of the sampled population sizes. The larger value in each of the pairwise comparisons is in bold type.)

	agglutination (titres)		lysis (titres)		[haptoglobin] (mg ml^{-1})	
genus	isl	cont	isl	cont	isl	cont
Anas	**8.1**	7.7	4.4	**4.8**	**0.23**	0.15
Branta[a]	**7.5**	7.4	4.2	**4.9**	—	—
Buteo[b]	**8.7**	7.6	**0.3**	0.1	—	—
Cardinalis	6.4	**6.5**	**0.8**	0.3	**0.23**	0.19
Carpodacus/Hemignathus[a]	**3.4**	3.3	0.0	0.0	**0.12**	0.07
Columba	**6.5**	4.1	0.0	0.0	—	—
Columbina	**4.9**	4.1	0.0	0.0	**0.13**	0.04
Dendroica[b]	**4.3**	3.7	0.0	**0.6**	**0.17**	0.11
Dumetella[c]	**4.6**	4.4	**3.3**	3.1	**0.45**	0.42
Mimus/Nesomimus[b]	**5.0**	4.7	**1.6**	1.0	—	—
Passer	**6.4**	5.7	**0.7**	0.0	**0.09**	0.08
Sialia[c]	3.4	**4.2**	0.0	**1.2**	**0.16**	0.12
Sturnus	3.9	**5.1**	0.4	**3.1**	—	—
Vireo[b]	5.7	**5.8**	**2.5**	0.6	—	—
Zenaida[a]	4.8	4.8	0.0	0.0	**0.12**	0.08
mean	5.6	5.3	1.2	1.3	0.19	0.14
s.d.	1.7	1.5	1.6	1.8	0.11	0.11
t	1.4		−0.4		5.7	
d.f.	14		14		8	
p	0.2		0.7		<0.0005	

[a] Some populations endemic to islands; included in analysis limited to *in situ* island natives/endemics the weighted means of only those populations: agglutination, *Branta* = 7.3, *Hemignathus* = 4.8, *Zenaida* = 3.6; lysis, *Branta* = 3.9; haptoglobin, *Hemignathus* = 0.12, *Zenaida* = 0.12.
[b] Endemic to islands; included in analysis limited to *in situ* island natives/endemics.
[c] Native to islands; included in analysis limited to *in situ* island natives/endemics.

(c) *Statistical analyses*

I summarized the raw data for each immune variable by calculating means and standard deviations for each population and used both univariate parametric (general linear model, GLM) and non-parametric (Kruskal–Wallis, KW) tests (SPSS v. 13.0) to investigate the effects of population (samples collected from a species in a specific place and at a specific time) within each genus. All GLMs identifying a significant effect of population were followed with Tukey's *post hoc* tests to identify homogenous subsets (SPSS v. 13.0).

Separately within each genus, I calculated means of insular and continental populations. This process resulted in single insular and continental units, eliminated pseudo-replication, and provided a conservative estimation of island–continent differences. Simple means and means weighted by the square root of the sampled population sizes (i.e. the number of individuals sampled per population) were generated. These means were used to test by pairwise comparisons (paired samples *t*-test and Wilcoxon signed-ranks test), the effect of island status on immune function. Coefficients of variation (CVs) were used to summarize variation among populations and genera; all CVs were corrected for sample size (Sokal & Rohlf 1995).

3. RESULTS

(a) *Haemagglutination/haemolysis*

I measured agglutination and lysis titres in all 516 individuals, which belonged to 59 populations (mean = 8.7 indiv/pop, s.d. = 5.8), representing species, island–continent status, location and time (month/breeding stage) of sample collection and captivity status of the individuals (appendix, tables 1, 2A and 2B—electronic supplementary material). Comparisons were made within and among genera. No significant effects of season were detected in agglutination or lysis in the four genera that were sampled during different seasons (continental *Cardinalis*, *Dumetella*, *Vireo*, *Zenaida*). Similarly, no effects of captivity were seen in the two genera for which samples were collected from both wild and captive individuals (continental *Cardinalis* and *Dumetella*). Overall, univariate tests identified significant effects of population on agglutination in four genera (*Anas*, *Buteo*, *Columba* and *Sturnus*; appendix, table 2A—electronic supplementary material) and on lysis in seven genera (*Anas*, *Branta*, *Dendroica*, *Passer*, *Sialia*, *Sturnus* and *Vireo*; appendix, table 2B—electronic supplementary material). With the exceptions of *Sturnus* agglutination and *Vireo* lysis, the results of the GLM and KW tests were similar. These effects of population suggested the need to use weighted means when collapsing populations within each genus; however, in all cases, weighted and unweighted means were highly correlated (all $r > 0.95$) and did not differ significantly (all $p > 0.5$). Nonetheless, I use weighted means in further analyses.

When all populations and all genera were included, pairwise tests indicated no significant difference in agglutination (paired samples *t*-test, $t = 1.4$, d.f. = 14, $p = 0.2$; Wilcoxon signed-ranks test $p = 0.1$) or lysis (paired samples *t*-test, $t = -0.4$, d.f. = 14, $p = 0.7$; Wilcoxon signed-ranks test $p = 0.8$) between insular and continental forms (table 1). Limiting the assessment to comparisons between *in situ* native continental populations and *in situ* native or endemic insular populations did not change this result for agglutination (paired samples *t*-test, $t = 0.6$,

Table 2. Paired *t*-tests of the effect of island–continent status on heterophil, lymphocyte, monocyte, eosinophil, basophil and total leukocyte concentration. (Mean values for each location within each genus are weighted by the square root of the sampled population sizes. The larger value in each of the pairwise comparisons is in bold type.)

	[heterophil] (no. $\times 10^3$ μl^{-1})		[lymphocyte] (no. $\times 10^3$ μl^{-1})		[monocyte] (no. $\times 10^3$ μl^{-1})		[eosinophil] (no. $\times 10^3$ μl^{-1})		[basophil] (no. $\times 10^3$ μl^{-1})		[total leukocyte] (no. $\times 10^3$ μl^{-1})	
genus	isl	cont	isl	cont	isl	cont	isl	cont	isl	cont	isl	Cont
Cardinalis	**3.10**	0.95	**4.70**	3.64	**0.55**	0.27	**0.037**	0.027	**0.005**	0.000	**8.39**	4.92
Dumetella	**2.48**	1.93	3.32	**4.25**	**0.52**	0.29	**0.125**	0.083	**0.034**	0.006	6.49	**6.55**
Zenaida	**2.63**	2.59	4.16	**4.97**	0.27	**0.30**	**0.073**	0.000	0.000	0.000	7.12	**7.85**
mean	2.74	1.82	4.06	4.29	0.45	0.29	0.078	0.037	0.013	0.002	7.33	6.44
s.d.	0.32	0.83	0.70	0.67	0.15	0.02	0.044	0.042	0.018	0.003	0.97	1.47
t	1.4		−0.4		1.7		2.3		1.3		0.7	
d.f.	2		2		2		2		2		2	
p	0.3		0.8		0.2		0.1		0.3		0.6	

d.f. = 8, $p = 0.6$; Wilcoxon signed-ranks test $p = 0.5$) or lysis (paired samples *t*-test, $t = 0.0$, d.f. = 8, $p = 1$; Wilcoxon signed-ranks test $p = 0.9$).

(b) *Haptoglobin*

I measured Hp concentration in 209 individuals, which were divided, in a similar manner as above, into 33 populations (mean = 6.3 indiv/pop, s.d. = 4.8; appendix, tables 1 and 3—electronic supplementary material). Within genera among continental populations, effects of captivity and season were examined. No effects of captivity were seen in *Cardinalis*, but captive *Dumetella* had higher plasma Hp concentrations than free-living ones ($p < 0.05$). In Zenaida, no effects of season were observed. Overall, univariate tests identified significant effects of population on Hp concentration in four genera (*Anas*, *Columbina*, Dendroica and *Dumetella*; appendix, table 3—electronic supplementary material). With *Anas* and *Columbina*, the results of the GLM and KW tests were similar; with *Dendroica* and *Dumetella*, only the GLM identified significant effects (both $p < 0.04$).

As with the agglutination and lysis variables, I conducted pairwise analyses using the weighted means. When all populations and all genera were included, pairwise tests revealed significantly higher plasma Hp concentrations in insular forms (paired samples *t*-test, $t = 5.7$, d.f. = 8, $p < 0.0005$; Wilcoxon signed-ranks test $p = 0.007$; table 1). Limiting this analysis to comparisons between *in situ* native continental populations and *in situ* native or endemic insular populations did not change this result (paired samples *t*-test, $t = 7.2$, d.f. = 4, $p = 0.002$; Wilcoxon signed-ranks test $p = 0.04$).

(c) *Leukocyte concentrations*

Concentrations of five leukocyte types were estimated from 107 blood smears from individuals in three genera, which were subdivided into 14 populations (mean = 7.6 indiv/pop, s.d. = 6.5; appendix, tables 1 and 4—electronic supplementary material). About 40% of the smears ($n = 44$) were reported to have some smudged cells. Smudged cells can affect the estimation of leukocyte concentrations; however, because the presence of smudged cells did not significantly affect overall leukocyte concentration (*Cardinalis*, $F_{1,35} = 0.7$, $p = 0.42$; *Dumetella*, $F_{1,33} = 2.2$, $p = 0.15$; *Zenaida*, $F_{1,14} = 1.8$, $p = 0.21$) or create a significant interaction between smudge status and population (*Cardinalis*, $F_{4,35} = 0.2$, $p = 0.96$; *Dumetella*, $F_{2,33} = 2.4$, $p = 0.11$; *Zenaida*, $F_{2,14} = 0.9$, $p = 0.42$), data from all smears were included in the analysis.

No significant effects of population were detected for any of the five types of leukocytes in *Zenaida* doves. In *Cardinalis*, a significant effect of population was found in heterophil concentration using both GLM and KW (both $p < 0.02$). A Tukey's *post hoc* test revealed that this effect was driven by island–continent status rather than captivity status or seasonal differences. Among *Dumetella* populations, significant effects of population appeared in concentrations of heterophils, lymphocytes and monocytes, with GLM and KW tests agreeing in the cases of lymphocytes and monocytes (all $p < 0.04$). With heterophils and monocytes, the highest concentrations were in the Bermuda and captive St Louis populations; with lymphocytes, the highest concentration was in the wild, autumn St Louis population.

As in the case of the other measures, weighted and unweighted means of cellular concentrations were similar for insular and continental populations within each genus, but significant effects of population necessitated the use of weighted means when pooling samples for the overall island–continent comparisons, which revealed no significant pattern in any of the five leukocyte types (all $p \geq 0.1$, table 2). Despite the lack of significance, consistently across all three genera of birds surveyed for leukocytes, insular populations had higher circulating concentrations of heterophils (by an average of 50%) and eosinophils (by an average of 114%) than continental populations. Both leukocyte types are involved in innate immunity. Heterophils are phagocytes that are important in early control of bacterial infection; eosinophils are thought to be involved with both parasitic infection and allergic reaction. On average, monocyte and basophil concentrations were also higher in insular populations, but elevations were only observed in *Cardinalis* and *Dumetella*. Overall concentration of lymphocytes, the leukocytes most involved in acquired immunity, averaged 5% lower in insular populations, with only *Cardinalis* exhibiting a higher concentration.

4. DISCUSSION

The hypothesis that immune function might be attenuated in insular faunas is rooted in the ideas that islands have

impoverished parasite communities and that immune functions incur physiological costs. The results of this study do not point to any overall attenuation in immune responses associated with island life. Instead, the results identify significantly higher concentrations of plasma Hp and suggest elevations in two leukocyte types in insular birds. Overall, the observed patterns provide novel perspectives on, and raise new questions about, the evolutionary lability of immune function.

(a) *Advantages and limitations of immune indices*

The measurements used here require only a single blood sample collected upon capture. While this approach is ideal for comparative immunological studies where large sample sizes are needed, measurements made before and after immunological challenges (e.g. non-specific cellular response to phytohaemagglutinin, Martin *et al.* 2002, or specific antibody response to vaccination, Hasselquist *et al.* 1999) are required to completely characterize immune systems and, accordingly, to definitively identify any changes in immune functions associated with island life. These challenge assays, however, require repeated capture or holding of birds over periods ranging from 1 to 30+ days, increasing the likelihood of confounding effects from stress responses and other physiological consequences of handling and captivity. In fact, most measures of immune function, including the ones used in this study, depend on and reflect health status (e.g. stress, body condition, disease exposure) to an extent; this caveat is especially relevant to comparative studies where confounding factors can be difficult to control.

Among challenge assays, the specificity of acquired humoral responses to vaccination complicates broad characterization of island–continent differences. Circumventing this specificity by instead characterizing the acute phase response (APR) through measurement of changes in acute phase protein concentrations, basal metabolic rates and behaviour represents one alternative. Macromolecular or particulate antigens with abundant epitopes (e.g. whole killed bacteria) can trigger APRs which are energetically costly (Martin *et al.* 2002), ensuring significant impacts on fitness. Measuring the *in vitro* ability of blood to kill a range of micro-organisms is another alternative, given this index's broad relevancy to innate immunity, simple interpretation and known associations with metabolism (Tieleman *et al.* 2005).

In addition to comparative immunology, a broad understanding of parasite-driven evolution of immune function will also require investigations in population genetics and immunogenetics. A better characterization of communities of disease-causing organisms, including the diversity and abundance of unicellular and multicellular pathogens and parasites, among different environments is also essential.

(b) *Evidence of reduced genetic variability?*

NAbs react with various affinities to a wide variety of epitopes on bacteria, viruses and toxins (Ochsenbein & Zinkernagel 2000). Evolutionarily, NAbs are encoded directly by the germ line genome (Ochsenbein & Zinkernagel 2000) and respond to selection (Parmentier *et al.* 2004). Developmentally, the presence of NAbs does not require previous antigenic exposure and they have been described in immunologically naive animals. An important role of these molecules is early resistance against infection (Ochsenbein & Zinkernagel 2000). In contrast, acquired antibodies are highly specific, require antigenic stimulation and depend on somatic gene rearrangement. Haemagglutination and haemolysis revealed no overall differences between insular and continental birds. The absence of a reduction in these indices in insular birds suggests that the broad benefits of NAbs and lytic enzymes, regardless of parasite environment, outweigh the costs of maintenance. This absence could also suggest that no overarching differences exist between the parasite communities of islands and continents.

Haemagglutination and haemolysis titres appear to be relatively stable within species, regardless of short-term health status (Matson *et al.* 2005). The titres, however, are far from invariant—differing significantly among bird species (Matson *et al.* 2005, 2006) and in some cases significantly (e.g. up to 2.4 $\log_2$ units in *Columba*, see appendix, table 2A—electronic supplementary material) within individual island–continent pairs. Compared to continental titres, insular agglutination titres were higher in some comparisons and lower in others. The lack of a consistent result suggests that within-pair differences result from population-specific genetic differences (e.g. lack of diversity from founder effects, inbreeding, or drift in insular populations; Frankham 1997) rather than weakened natural selection, physiological costs of immune function, or simplified insular pathogen communities. Haemagglutination titres have been shown to differ significantly, but not predictably, among populations of a naturally inbred species of bird exhibiting different levels of heterozygosity (Whiteman *et al.* 2006). Additionally, on average, more-inbred populations show less within-population variability in agglutination than less-inbred populations (Whiteman *et al.* 2006). And similarly, on average and compared to continental populations, insular populations show lower within-population CVs for both agglutination (island = 34%; continent = 43%) and lysis (island = 68%; continent = 86%).

(c) *A shift in the balance of immune function?*

Interactions between the innate and acquired branches of the immune system can affect the evolution of immune function. Models of the evolution of interacting immune responses suggest that acquired immunity (i.e. specific antibodies to a disease) can reduce selection pressure on the evolution of innate resistance traits (Harding *et al.* 2005). Consequently, where the loss of genetic variability impairs one or more components of immunity, a shift in the functional balance could result in greater reliance on other components.

In the case of Hp, the island–continent analysis showed significantly higher levels in insular populations. Hp works to complex and remove haem, thereby preventing the haem from serving as a nutrient for pathogens and from initiating deleterious oxidation reactions. Dobryszycka (1997, p. 647) concludes 'probably the most important biological function of Hp consists in the host defence responses to infection and inflammation, acting as a natural antagonist for receptor–ligand activation of the immune system.'

Hp normally circulates at low levels, but concentrations increase during inflammatory responses, which result

from infection or trauma. Increased Hp means that insular populations have (i) higher baseline levels, (ii) higher response levels, or (iii) larger proportions of individuals responding to challenges at any one time. Although all three options suggest a more intense or more prominent APR in insular populations, with non-repeated measurements the cause cannot be distinguished definitively. However, because the first and third causes both result in less within-population variation, the slightly higher mean within-population CV of Hp in islands (island = 49%; continent = 45%) combined with the higher mean Hp levels suggests that higher response levels are, at least in part, the cause.

Given the energetic costs of APRs (e.g. anorexia and hyperthermia, but also increased resting metabolic rates, cf. Martin *et al.* 2002) and the direct relationship between energetic and fitness costs (Deerenberg & Overkamp 1999), an APR intensification would be unusual, but elevated Hp might signify an impairment of other components and an associated shift in immune defence strategy. Indeed, Hp appears to have a modulatory role in the T-helper-1 (generally cell-mediated immunity) and T-helper-2 (generally humoral immunity) balance (Arredouani *et al.* 2003). With this hypothesized shift, tradeoffs between increases in one component and decreases in another are expected; however, island–continent differences in Hp and differences in agglutination or lysis were not correlated (both $p > 0.3$). Comparing additional measures, such as cytotoxic lymphocyte responses and antibody or MHC diversity, between insular and continental populations might reveal such tradeoffs. An alternative explanation of higher Hp concentrations in insular birds is related to the molecule's 'anti-endotoxin effects', with the protective effects of higher levels actually counteracting systemic inflammation (e.g. by dampening monocyte responses, Arredouani *et al.* 2005). Experimental elicitation of systemic inflammation combined with baseline and response measurements of Hp could be used to determine if such a relationship exists.

The significantly higher concentrations of Hp in insular birds (and their tendency to have increased innate leukocytes) suggest a shift in immune defence strategy, which seemingly favours innate as opposed to acquired responses. Innate responses might dominate if systems of acquired humoral immunity and immunological memory are less important (e.g. due to epidemiological properties of islands) or dysfunctional. Insular populations might be compensating for some aspects of reduced genetic diversity or immune system quality through the upregulation of these innate markers. Additionally, these elevations raise the possibility that islands have intensified, rather than reduced, disease risks.

(d) *Increased disease susceptibility on islands and exceptions to the 'rule'*

The extent to which insular taxa, as a whole, exhibit increased disease susceptibility compared to continental taxa is unclear. Sustaining longer lasting and more lethal infections, various native Hawaiian birds are particularly susceptible to malaria (Jarvi *et al.* 2001; Van Riper III & Scott 2001). However, at least one Hawaiian native species—a thrush (*Myadestes obscurus*)—is able to produce antibodies against and survive *Plasmodium* infection (Atkinson *et al.* 2001). Conversely, continental taxa can also suffer the effects of introduced or newly emergent diseases. For example, following the 1999 arrival of West Nile Virus to North America, some corvid and owl populations declined as the result of their high susceptibility, the causes of which are poorly understood (Gancz *et al.* 2004). As for other examples, in a summary of 30 emergent infectious diseases affecting wildlife (Daszak *et al.* 2000), all but one (avian malaria in Hawaii) primarily affect continental areas, and the three diseases found in continental birds are associated with high mortality.

This present study of phylogenetically matched pairs of bird populations from North America and from oceanic islands provides evidence of an apparent shift in the mix of immune function towards components that are innate and inducible. Significantly higher concentrations of Hp and the tendency of heterophils and eosinophils to circulate at higher concentrations in insular populations provide no obvious support for the notion that islands have impoverished parasite communities. The precise causes of the observed immunological shift are unknown and require further investigation. Moreover, a number of other wide-ranging questions remain unanswered as well. For example, do generalizable differences in disease susceptibility between continental and insular faunas exist and can these differences be measured? Or is each insular population uniquely defended against disease threats as a result of genetic and stochastic processes related to small population sizes and limited geographical ranges? By documenting systematic differences in immune function between insular and continental birds, the present study should serve as a foundation and a catalyst for addressing these questions.

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