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Evolutionary epidemiology of endemic Gal¿pagos birds and their parasites

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Dissertation Abstract

In order to better understand parasite diversification, I went to the Galápagos Islands to study the ecology and evolution of a model bird-parasite system, which included four phylogenetically independent ectoparasite lineages infecting the Galápagos Hawk (Aves: *Buteo galapagoensis*). The parasites comprised two lice (Insecta: Phthiraptera: Amblycera, Ischnocera), a louse fly (Diptera: Hippoboscidae) and an avian skin mite (Acari: Epidermoptidae). Ultimately, my goal was to examine ectoparasite evolutionary epidemiology and disease susceptibility in relation to the host's ecological and colonization history.

At the outset, I hypothesized that parasite natural history was key in influencing the coalescent process. Accordingly, I found differences in prevalence, abundance and degree of aggregation among each hawk ectoparasite species. I proposed using parasite population genetics to infer host history as a new rationale for parasite conservation. In that context, a DNA barcoding approach revealed predictable differences in transmission rates of two Galápagos dove (*Zenaida galapagoensis*) louse genera to hawks during predation events. A 'generalist' mite species from Galápagos hawks and Flightless cormorants (*Phalacrocorax harrisi*) comprised two cryptic species, one of which was structured genetically between two hawk island populations. The hawk amblyceran and louse fly harbored less population genetic structure than the ischnoceran, which was more differentiated than the host, although isolated populations of both lice contained unique, fixed haplogroups, illuminating cryptic parasite diversity and restricted host gene flow among islands. This variation, however, was only related to host genealogy in the

ischnoceran and the rate of molecular evolution was faster in the ischnoceran than in the host. Among islands, hawk inbreeding explained louse infection intensity and natural antibody levels, and the latter was inversely related to amblyceran louse abundance, which encounters the host immune system.

Separately from the ectoparasite work, I collaborated on a characterization of *Avipoxvirus* isolates from Galápagos birds, showing significant recombination among pox strains, and we recovered *Haemoproteus*-like parasites from multiple seabird species on Genovesa. Finally we showed that a vector of avian disease was established on Isla Santa Cruz (*Culex quinquefasicatus*). This study was the first to examine host-parasite evolutionary epidemiology within the Galápagos avifauna, one of the most intact and threatened island bird communities.

Table of Contents

Chapter I: General Introduction……..……**10**

Chapter II: Using Parasites to Infer Host History……..…….…….**22**

Chapter III: Body Condition and Parasite Load Predict Territory Ownership in the Galápagos Hawk……..……..…….**48**

Chapter IV: Effects of Host Sociality on Ectoparasite Population Biology**.....72**

Chapter V: Differences in Straggling Rates Between Two Genera of Dove Lice (Insecta: Phthiraptera) Reinforce Population Genetic and Phylogenetic Patterns……..….**109**

Chapter VI: Disease Ecology in the Galápagos Hawk (*Buteo galapagoensis*): Host Genetic Diversity Parasite Load and Natural Antibodies……..**134**

Chapter VII: Establishment of the Avian Disease Vector *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador……..**164**

Chapter VIII: Characterization of Canarypox-like Viruses Infecting Endemic Birds in the Galápagos Islands…… **177**

Chapter IX: Population Genetics of the Galápagos Hawk (*Buteo galapagoensis*): Genetic Monomorphism within Isolated Populations……**209**

Chapter X: Phylogeography of the Galápagos Hawk (*Buteo galapagoensis*): A Recent Arrival to the Galápagos Islands……**242**

Chapter XI: Health Assessment of Seabirds on Isla Genovesa, Galápagos Islands…… **281**

Chapter XII: Cryptic Host Specificity and Genetic Epidemiology of a Generalist Avian Skin Mite (Acari: Astigmata) from Two Sympatric and Threatened Galápagos

Endemic Birds**……310**

Chapter XIII: Comparative Population Genetic and Phylogeography of a Model Host-Parasite System from the Galápagos Islands**…….358**

Please note that nine chapters (I through VII, XII and XIII) are manuscripts on which I was first author and for which I was primarily responsible and four chapters (VIII through XI) are manuscripts on which I was a co-author but are directly related to my first-authored chapters.

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which led to several successfully-funded grant proposals and gave me a clear springboard into the remaining years of my dissertation research. Dr. Kellogg benefited my training in many ways, including by serving as an excellent Director of Graduate Studies during my first year at UM-St. Louis. Dr. Kellogg gave us a helpful and clear academic plan during our Ph.D. student-orientation, which I tried to follow closely. Dr. Kellogg also helped train me in the theory of systematics and evolutionary biology more generally, and encouraged me to find and pursue my interests. Dr. Kevin Johnson visited campus early during my dissertation research, and along with Dr. Vince Smith, provided support of my ideas and research plan, which was very important to me since they are students of louse biology. Dr. Johnson provided crucial information in a collaboration that led to the publication of our straggling paper. Dr. Johnson has given me many specimens, data from which have been included in this dissertation. Dr. Marquis has helped ensure that my data collection, analyses and questions were as rigorous as possible. I am especially thankful of his support during the peer-review process. Dr. Marquis also challenged me to think about the importance of host-defenses as a driver of parasite diversification. Finally, I am thankful of Dr. Ricklefs' early encouragement and support of my research. A question Dr. Ricklefs asked after the oral presentation of my proposal defense led to the collaboration with Kevin Matson that examined natural antibodies in the hawks (e.g., "Is there a mechanism for your predictions about how host inbreeding affects louse abundance?"). Moreover, the intellectual dialogue that resulted from my interactions with many invited guests of this department helped to shape several chapters of this dissertation. In particular, Dr. Dale Clayton (University of Utah) gave a seminar very early on in my tenure at UM-St. Louis, which led to my idea oft using the hawk's

parasites to infer host history (since they evolve more quickly than the host) in the archipelago. Dr. Cliff Cunningham (Duke University) gave me a reference that led to the use of the COI primer set that has yielded much of the population genetic data in this dissertation. He was very encouraging of my ideas, as was Dr. Paul Ewald (University of Louisville), who helped me to think about the broader implications of my work in the context of the evolution of infectious disease. I am also thankful for meeting Dr. Jim Hunt, who encouraged me to apply to UM-St. Louis and to follow my dream of studying in the tropics.

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Chapter I.

General Introduction

"Parasites may provide useful characters for tracing the evolution of vertebrate groups" (Ayala and Hutchings 1974).

The observation that the evolutionary history of parasites and their hosts is linked, and therefore the patterns emerging from host-parasite interactions could be used as evolutionary inferential tools, was central to the development of evolutionary biology. Therefore, a short review of the development of coevolutionary biology is helplful in understanding the potential pitfalls and promise of studies of host-parasite evolution.

Studies of host-parasite evolution began by using parasite distributions to infer host and geological history. The logic of inferring host phylogeny from parasite distribution was based on the observation that morphologically, parasites evolve more slowly than their hosts (Klassen 1992). Thus, parasites may still possess characters useful in the elucidation of their relationships, which the hosts no longer have (see Brooks and McLennan 1993) and the presence or absence of particular parasites was therefore genealogical information itself (Ronqvist 2003). von Ihering (1891, 1902), Kellogg (1896a, b) and Fahrenholz (1913) may have arrived at the general conceptual framework (that host and parasite evolution were correlated) independently and contemporaneously, although the mechanisms hypothesized to underlie the patterns they observed varied (Klassen 1992).

Although there are numerous examples, two studies, separated by over a century, are illustrative of how the methodology was used. In two papers that arguably form the foundation of modern coevolutionary biology, von Ihering (1891, 1902) used the

distribution of parasitic worms to infer that two host-parasite systems were likely in place before the breakup of Gondwanaland. von Ihering inferred that, given the similarities between parasitic flatworms (Platyhelminthes: Temnocephalidae) from their freshwater crayfish hosts in South America and New Zealand, the two land masses must have been connected previously. Similarly, Gardner and Campbell (1992) inferred that *Lintowia* tapeworms (Platyhelminthes: Cestoda) and their marsupial hosts from South America and Australia were interacting evolutionarily before the breakup of Gondwanaland using a phylogenetic tree (but the close phylogenetic relationship between their hosts was obscured by morphological divergence). Implicit in von Ihering's argument, was that isolation was the driving force behind speciation and linked to that view was his belief that the landmasses of the southern hemisphere were once connected (e.g., continental drift). Wallace and Darwin, rejected the idea of isolation (and were, obviously, in favor of natural selection as the driving force behind speciation), and the former argued in favor of the "fixity of continents" (Klassen 1992). However, Darwin, in a letter to Henry Denny toyed with the idea that host-parasite studies might inform evolutionary thinking when he remarked: "Some of the species of birds in Europe and North America appear certainly identical; many form very closely related species or as some would think races; what an interesting investigation would be the comparison of the parasites of the closely allied and representative birds of the two countries" (in Hoberg et al. 1997).

Kellogg and Faherenholz views' of parasite speciation were somewhere in between conceptually (either explicitly or implicitly acknowledging the importance of both isolation and natural selection; Klassen 1992). Rothschild and Clay (1952) proposed that using multiple parasite species would be most informative when respect to inference of host relationships, which was later formalized by Brooks (1981). Emphasis

then shifted away from the study of biogeography in a coevolutionary context and towards an orthogenetic framework, which posited that high host specificity led to coevolution between parasites and their hosts (at least according to Brooks and Ferrao 2005). Brooks and Ferrao (2005) argued that the modern "maximum cospeciation" school (*sensu* Page 2003) resulted from this orthogenetic view (developed in the 1930s) of host-parasite evolution and host specificity. Alternatively, Ehlich and Raven's (1964) synthesis argued that reciprocal natural selection was the driving force behind insect and plant diversification, which was complemented by Brooks (1979; 1981) and Brooks and McLennan (2002), who argued that it was inappropriate to link host-specificity *per se*, with an expectation of cospeciation or host-switching. This hypothesis was subsumed under Janzen's (1985) notion of ecological fitting, which posited that interactions of parasites with their hosts are resource-driven (e.g., host specificity may be an artifact of geography; specialization on one host may simply be due to local or temporal constraints and host switching should be expected to have occurred in the past and the future, depending on resource availability in space and time). Brooks and Ferrao (2005) contend that there does appear to be an intellectual consolidation between the 'maximum cospeciation' school and the 'ecologcal fitting' school of parasite diversification research.

Clearly, many researchers have used parasite distribution to infer host and geological history. Of particular interest are two papers that advocated using parasite distribution to infer host history within the Galápagos fauna. Ayala and Hutchings (1974) describe protozoan blood parasites from *Tropidurus* and *Amblyrhynchus* reptiles endemic to Galápagos and stated:

> The hemogregarines may prove exceptionally helpful in tracing the evolution of lava lizards within the Galapagos Islands complex. Each island has a distinct species of lava lizard and it is not yet clear whether

> this is the result of interisland dispersal and speciation of a single colonial stock, or repeated colonization from mainland sources (Carpenter, 1966b). If lava lizards on all the islands carry the same parasite, this suggests evolution from a common island source. The change of migration of infected intermediate hosts alone from island to island seems unlikely, especially if they are ectoparasitic acarines.

Is this method of inference now outdated? The answer is no, but the rationale has changed (Whiteman and Parker 2005). The advent of polymerase chain reaction (PCR), DNA sequencing and realistic phylogenetic and population genetic analytical tools (Avise et al. 1987), has allowed evolutionary biologists to estimate genealogies and gene flow using organismal genes themselves. This has largely obviated the need for parasites in evolutionary inference. However, recent studies have shown that the rate of DNA nucleotide substitution is typically faster and the amount of genetic variance is greater within parasite lineages and populations relative to their hosts (see Whiteman and Parker 2005). Rather than abandoning their use as an evolutionary inferential tool, Funk et al. (2000) suggested that this characteristic be exploited to infer host phylogeny (subsequently, Page 2003 also suggested a reappraisal of their use). This logic would seem especially useful when applied to cases in which the host's genealogy is difficult to estimate directly. Whiteman and Parker (2005) present a review of and rationale for studies using parasite population genetic structure and phylogeography to infer host history (ecological or evolutionary). In line with this logic they also present a new rationale for conserving parasites given their potential utility as markers of host ecology and evolutionary history. An empirical example of this methodological approach (where parasite genealogy was used to infer host genealogy because the latter was difficult to estimate directly), was provided by Whiteman and Parker (in prep.). Interactions

between hosts can also be inferred using DNA sequences of parasites, over evolutionary and ecological timescales (Reed et al. 2004; Whiteman et al. 2004; Ricklefs et al. 2005).

Factors Governing Parasite Population Genetic Structure

Despite the appeal of using parasites to infer host history, important consideration must be given to the natural history of the parasite, since many factors may cloud inference of host history (Rannala and Michalakis 2003). Although generalizations regarding parasite natural history abound, they are highly problematic conceptually and practically (Clayton and Moore 1997). Parasite natural history is extremely important in determining parasite population genetic structure and therefore coevolution and cospeciation more generally (Nadler 1995; Clayton et al. 2004; Huyse et al. 2005). Many authors lump parasites into the convenient 'microparasite' and 'macroparasite' groupings, the former often exhibiting direct reproduction within the host, often requiring a vector, and the latter exhibiting an 'indirect' reproductive strategy often involving a free-living stage or requiring more than one host species for development. However, such groupings are inadequate for taxa such as lice, which are permanent parasites, can complete their entire life cycle on one host individual and are capable of both horizontal and vertical direct transmission and transmission via phoresis on other insects (Marshall 1981; Keirans 1975). Similarly, viruses in the family Poxviridae do not require a vector for transmission, although mechanical transmission may occur, and virions may remain viable in the environment for years (Nuttall 1997). Furthermore, even some ectoparasites of vertebrates, such as *Myialges* and *Microlichus* avian skin mites (Whiteman et al. in prep.), require a developmental vector (e.g., hippoboscid fly) to complete their life cycle (Fain 1965). Fain (1965) thus argued that host specificity of these mites should mirror that of their fly hosts, a hypothesis supported by a molecular genetic study of sympatric

populations of *Myialges* occurring on two different hippoboscid host species that were in turn each associated with a different avian host species (hawk and cormorant). Whiteman et al. (in prep.) showed that at least two cryptic *Myialges* species, one for each fly (and bird) species, existed in sympatry. However, previous authors believed that the *Myialges* from the two fly and two avian hosts were conspecific, a testament to the pitfalls of using morphological characters (which in this case were often continuously variable) to differentiate parasite species.

Marshall (1981) cautioned against making life history generalizations within ectoparasites, and Whiteman and Parker (2004a, b) further argued against lumping species from the two major clades of lice (Amblycera and Ischnocera) in ecological studies, given the basic differences in their natural history that may also influence coevolution generally, including louse-host arms races (Møller and Rózsa 2005). This is underscored by Johnson et al.'s (2004) finding that the Amblycera and Ischnocera likely arose independently from two free-living common ancestors within the Psocodea. Several recent reviews discuss the factors governing parasite population genetic structure, which includes life cycle, host sociality, parasite dispersal abilities, effective population size and other variables (Criscione and Blouin 2005a, b; Huyse et al. 2005; Whiteman and Parker 2005). Whiteman and Parker (in prep.) showed that differences in parasite natural history (e.g., differences in population size and dispersal ability) correlated with degree of population genetic structure within and among island populations of the avian host (*sensu* Johnson et al. 2002). Whiteman et al. (2005) also showed that while both suborders of lice appeared to take advantage of genetically depauperate host populations, only the amblyceran's abundance was related to the degree of the host's innate antibody response, which is consistent with the finding of a potential coevolutionary arms race

between amblycerans and their hosts' immune response at the macroevolutionary scale (Møller and L. Rózsa 2005). Thus, studies of microevolutionary processes are being recognized as key for understanding macroevolutionary patterns of parasites.

What began over a century ago as an exercise in inferring host history via parasites using the language of 'Parascript' (Brooks and McLennan 1993) has emerged as a rapidly growing field, which now includes subfields ranging from disease ecology to a phylogenetics, which use the latest conceptual and methodological tools. However, much still remains to be understood with respect to parasite diversification and how they interact with their hosts, which was the impetus for the present study.

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Whiteman, N. K. and P. G. Parker. In prep. Comparative population genetic and phylogeography of a model host-parasite system in the Galápagos Islands.

Chapter II.

Using Parasites To Infer Host History: A New Rationale For Parasite Conservation

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ABSTRACT

Only one of the 5,000 extant louse species (Phthiraptera), and no species of flea (Siphonaptera), parasitic helminth (Platyhelminthes), parasitic nematode (Nemata), mite or tick (Acari) is listed as threatened by the IUCN, despite impassioned pleas for parasite conservation beginning more than a decade ago. Though they should be conserved for their own sake, past arguments, highlighting the intrinsic and utilitarian value of parasites, have not translated into increased attention by scientists or conservation managers, at least by the standard of listing for protection. Here, the use of estimated genealogies and population genetic patterns of parasites to illuminate their hosts' evolutionary and demographic history is advocated. Parasite DNA generally evolves more rapidly than their hosts', which renders it an underexploited resource for conservation biologists, particularly in cases where the hosts' genealogy or degree of population genetic structure is difficult to measure directly. Moreover, parasite gene flow may occur during host dispersal irrespective of host gene flow, revealing host movement through space and time. Parasite ecology and evolution may thus become another tool for the management of endangered vertebrate populations. This will result in the

recognition of new host records, parasite species, and cryptic lineages, which will help lift the veil of ignorance with respect to parasite biodiversity.

Introduction

Parasites are the most diverse metazoan group on Earth. Despite the passing of more than a decade since the first articulation of impassioned pleas for parasite conservation (e.g., Windsor, 1990, 1995; Rózsa, 1992; Holmes, 1993; Stork & Lyal, 1993; Durden & Keirans, 1996; Gompper & Williams, 1998; Koh et al., 2004), few are presently listed on the IUCN Red List of Threatened Species (IUCN, 2003). For example, only one of the 5,000 species of louse (Insecta: Phthiraptera; Price, Hellenthal & Palma, 2003) is currently listed. While the listing of the pygmy hog sucking louse (Hematopinidae: *Haematopinus oliveri*) represents a victory for parasite conservation, no other lice have been given this designation, despite there being another 2,323 potential host species (among the mammals and birds) listed (IUCN, 2003). Other parasites of vertebrates are similarly neglected, as no species of flea (Siphonaptera), parasitic helminth (parasitic Platyhelminthes), parasitic nematode (Nemata), mite (Acarina), or tick (Acari) is listed despite the fact that 3,524 vertebrate species are listed. Though Durden & Kerians (1996) identified 48 species of tick as candidates for endangered status, none are listed by the IUCN. Similarly, Perez & Palma (2001) suggested listing of the newly described host-specific louse *Felicola isidoroi* (Trichodectidae) from the Iberian lynx (*Lynx pardinus*), yet it presently remains unlisted. For some parasites, such as a potentially unique louse lineage (*Neotrichodectes minutus*) from the black-footed ferret (*Mustela nigripes*), or host-specific lice (*Colpocephalum californici*) of the California Condor (*Gymnogyps californianus*), it is too late, as parasites were

intentionally killed during population management and captive breeding efforts (Gompper & Williams, 1998; Koh et al., 2004). These examples underscore the fact that formal protection of a host does not necessarily assure protection of its parasites or other symbionts, which is not a novel observation. One study estimated that 200 "affiliate" species are now extinct due to their hosts' demise and that 6,300 other affiliate species are coendangered with their hosts' (though most affiliates remain unlisted; Koh et al., 2004). Invertebrates may be particularly prone to extinction risk (Hadfield, 1993; Clark & May, 2002; Stein, Master & Morse, 2002), and since parasites are distributed in a negative binomial fashion among hosts (most hosts have few parasite individuals and few hosts have many parasite individuals; Crofton, 1971), they are particularly vulnerable to extinction when host populations are small or when natural dispersal is disrupted (*sensu* Templeton et al, 2001).

Animal Conservation published no comparative or theoretical papers on invertebrate conservation in its first five years, during which it published 50 such studies on vertebrates (Reynolds et al., 2003). It seems that past arguments, highlighting the intrinsic and utilitarian value of invertebrate parasites, have not translated into increased attention by scientists or conservation managers, at least by the standards of publication or listing. This problem is not specific to the IUCN or *Animal Conservation*. It is the result of our general ignorance of invertebrate biology and diversity, and we recognize that part of the problem is simply not knowing what to conserve. We urge funding agencies worldwide to increase the amount of monies available for cataloging biodiversity. Those who would argue for parasite conservation must address the fact that "in order to care deeply about something important it is first necessary to know about it"

(Wilson, 2000), yet we still know so little. Here, in this context, we propose a novel and pragmatic rationale for conserving parasites and pathogens, which may help to address all of these problems.

Parasites as inferential tools

Understanding the historical and contemporary relationships among fragmented vertebrate populations is important to conservation managers, for a variety of reasons (Avise, 1994, 1996; Templeton et al., 2001). Unfortunately, low genetic variability within and among populations of many vertebrate taxa obscures our ability to infer these historical genetic and contemporary demographic processes (e.g., cheetahs, Kieser, 1991; northern elephant seals, Hoelzel et al., 1993; killer whales, Hoelzel et al., 2002; Hainan Eld's deer, Pang et al., 2003). Population genetics of parasites of these vertebrates may offer another avenue for illuminating their hosts' evolutionary history and current demographic processes, which buttresses arguments for conserving such host-parasite systems (if parasites contain more population genetic information than their hosts').

Parasitologists have long used parasites to infer a host's evolutionary history (von Ihering, 1891, 1902; Fahrenholz, 1913; Eichler, 1942; Brooks, 1977; Brooks, Thorson & Mayes, 1981; Brooks, 1993; Hoberg, 1997; Hugot, 1999, 2003). The key assumption is that parasites are transmitted vertically across generations, and from parental to daughter lineages, in an ancestor-descendent fashion (Clay, 1949; Page, 2003). The root of this practice lies in the observation that morphological evolution within parasites proceeds more slowly than in their hosts' (Klassen, 1992). Parasites may thus possess a "conservative tendency that makes them useful as biological tags" (Ayala & Hutchings, 1974). Over the same time interval, while a pair of host sibling species may have

undergone extensive morphological change since divergence from their common ancestor, the pair's parasites should have retained characters useful in the elucidation of their (and by extension, the hosts') evolutionary history. The presence (or absence) on a host of a parasite taxon is therefore genealogical information itself (Ronquist, 2003). For example, Gardner & Campbell (1992) used a phylogeny based on morphological characters of marsupial and monotreme cestodes (*Lintowia* spp.) to infer that this host-parasite system was in place before the breakup of Gondwanaland. The hosts' phylogeny was obscured by "morphological divergence of marsupials in the Neotropical and Australian regions." Thus, a monophyletic origin of the host lineages was recapitulated via phylogenetic data from their parasites, which were "phylogenetic relicts" (*sensu* Brooks and Bandoni, 1988).

However, the advent of PCR, DNA sequencing and realistic phylogenetic and population genetic analytical tools (Avise, 1994; Templeton, 1998, 2004), has allowed evolutionary biologists to estimate genealogies and gene flow using organismal genes themselves. This has largely obviated the need for parasites in evolutionary inference. Here we argue, on other logical grounds, that this route of deduction still has conceptual merit and practical conservation application at the microevolutionary level, particularly in cases where the host's genealogy or population genetic structure is difficult to estimate directly.

There is growing evidence, across taxonomic boundaries, that the rate of molecular evolution is faster in parasite DNA relative to that within the homologous loci of their hosts' (Hafner et al., 1994; Downton & Austin, 1995; Moran, van Dohlen & Baumann, 1995; Page et al., 1998; Clark et al., 2000; Funk et al., 2000; Paterson et al.,

2000; cf. Ricklefs & Fallon, 2002). For example, Clayton and Johnson (2003) have shown that the rate of evolution in the mitochondrial DNA of avian lice is 10 times faster than the hosts'. It is this property that has led several biologists to propose a new look at the use of parasites and other symbionts for inferring host evolutionary history (Funk et al., 2000; Page, 2003). Funk et al. (2000) noted that parasites' more rapid evolutionary rate, relative to their hosts', yields DNA sequence data that are "comparatively informative sources of phylogenetic data." Moreover, beyond consideration of mutation rates, the difference in generation time alone between most hosts-parasite pairs allows for the coalescent process to proceed much more rapidly in the latter all else being equal (Rannala & Michalakis, 2003). Thus, not only can one expect more genetic variance to be present in the DNA or RNA of pathogens and parasites relative to their hosts', the analysis of how this variance is partitioned among host populations could reveal the hosts' evolutionary history before the host DNA has coalesced (Rannala & Michalakis, 2003). This is a powerful inferential tool indeed.

This logic was used to attack the difficult problem of characterizing evolutionary relationships among human populations and historical human migration patterns. Genealogical relationships and gene flow patterns were inferred with success within and among populations of persistent human pathogens such as the ulcer-causing bacterium *Helicobacter pylori* (Ghose et al., 2002; Falush et al., 2003; Wirth et al., 2004) and urinary JC virus (Sugimoto et al., 1997). Moreover, comparisons between *H. pylori* DNA sequences could "distinguish between closely related human populations and are superior in this respect to classical human genetic markers" (Wirth et al., 2004).

Inferring Host Genealogy

Rannala & Michalakis (2003) provide a superb theoretical framework relating population genetic processes to co-phylogenetic patterns between hosts and parasites via coalescent theory, which is a useful context for the present discussion. Their analysis of host-tracking by parasites through time was split into three components 1) within-population, 2) between-population, and 3) between-species.

Regardless of the level of analysis, inference of host genealogical history will be strongest when genetic data from vertically transmitted parasites or pathogens are used: "The gene genealogy of a parasite with vertical transmission carries potential information about the genealogical relationships of infected hosts" (Rannala & Michalakis, 2003). However, some parasites and pathogens are transferred horizontally among host species and populations (host-switching). This may cloud the inference of host genealogy, causing problems analogous to those caused by horizontal transfer of genes (Page, 2003). These horizontally-transferred host-parasite pairs are useful in other contexts (Rannala & Michalakis, 2003; see below). Close attention should be paid to life history differences among parasite lineages when they are used as evolutionary inferential tools.

In theory, N_e (effective population size) of hosts and parasites is extremely important in determining the level of population genetic structure in parasites (Nadler, 1995), and the degree of congruence between host and parasite lineages (Rannala & Michalakis, 2003). Moreover, the lineages of larger populations will arrive at reciprocal monophyly more slowly than smaller populations (Avise, 1994); lineage sorting may distort inferences of host history and result in host lineages coalescing before the parasite's, assuming equal generation times (Rannala & Michalakis, 2003).

Specifically, lineage sorting is a problem if "surviving lineages in the parasite trace to phylogenetic splits either predating or postdating nodes in the host phylogeny" (Avise, 1994). Thus, as Rannala & Michalakis (2003) showed, lineage sorting can easily lead to incongruence between host and parasite trees within populations. Only if the parasite's N_e is "very small" or if the hosts sampled "are relatively distantly related, the parasite gene genealogy should provide a good estimate" of the ancestral infection graph (the actual history of parasite transmission, from host individual to individual, whether vertical or horizontal). Specifically, Rannala & Michalakis suggest that a parasite gene genealogy will reflect the history of transmission among hosts (and thus the host's history) if the number of generations (or parasite transfer events) between the hosts is > 10 times the parasite's N_e. Between populations, variance in migration rate, internal branch lengths within gene trees and N_e emerge as important determinants of whether host and parasite gene trees will accurately reflect population history. Simply stated, parasite species typified by relatively small population sizes, and persistence on hosts separated by relatively longer periods of time, will yield more accurate information about host ecology or evolutionary history than the converse.

Given this, and the important influences of life-history factors such as host range (Nadler et al., 1990), host sociality (Whiteman & Parker, 2004), parasite dispersal abilities (Johnson et al., 2002), and life cycle (Criscione & Blouin, 2004) on parasite population genetic structure, the examination of multiple parasite lineages within a particular host species may prove most useful, just as multiple loci should be used to increase the accuracy of phylogeny or population genetic structure estimates (Nadler, 1995; Johnson et al., 2002; Constantine, 2003; Criscione & Blouin, 2004). Hierarchical,

comparative population genetic (Jarne & Theron, 2001) and phylogeographic approaches (Avise et al., 1996; Templeton, 1998, 2004) can then be used to infer both distant and recent population histories of multiple and phylogenetically independent parasite populations. Objectively differentiating among the various population genetic processes, such as range expansion, fragmentation, or isolation by distance is now at least technically feasible, and hundreds of articles in the past few years alone have implemented statistical phylogeography, which is a testament to its broad appeal (Templeton, 2004). Such studies deepen our understanding of the vast interrelationships and interdependencies among taxonomically diverse lineages.

Focusing on permanent, directly transmitted parasites (those that generally depend on host-host contact for transfer, e.g., Phthiraptera) or pathogens that produce chronic and persistent infections (e.g., *Helicobacter*, helminths), may be one of the best strategies for implementing applied parasite population genetics. For example, host specificity of lice on birds and mammals is high, with each species occurring on an average of only 2 bird and 2.6 mammal species (Price et al., 2003). Lice are relatively easy to collect (Walther & Clayton, 1997; Clayton & Drown, 2001), and genotyping of large numbers of individuals is now routine (e.g., Johnson et al., 2002). Nadler et al. (1990), studying lice of pocket gophers (*Thomomys bottae*), found that significant population genetic structure existed among louse populations and that this structure was broadly correlated with host gene flow. Barker et al. (1991) and Barker, Close & Briscoe (1991) also found significant structure among lice (*Heterodoxus octoseriatus*) from different colonies of their wallaby hosts and this was more broadly correlated with latitude, which in turn was correlated with the ranges of two different wallaby subspecies.

In our own preliminary work estimating the genealogical relationships among the nine extant island populations of the threatened Galapagos Hawk (Aves: Falconiformes: *Buteo galapagoenesis*), its ectoparasites (Insecta: Phthiraptera) have served as excellent markers of host population differentiation. Generally, we found much more population genetic structure in the parasite's (Philopteridae: *Degeeriella regalis*) mtDNA (~1.5% maximum divergence within Galapagos) relative to that within the host's (~0.2% maximum divergence within Galapagos). Moreover, there was a greater degree of geographic partitioning of this variance among parasite populations than among their host's (Whiteman and Parker, unpublished data). This approach may be useful for inferring the population histories of other endemic Galapagos vertebrates, which, like other taxa inhabiting oceanic archipelagoes, are relatively genetically invariant (Tye et al., 2002).

Inferring Host Population Dynamics

On the other hand, population genetic studies of horizontally transmitted parasites and pathogens can provide information such as past host dispersal events that resulted in gene flow for the pathogen, but not the host (Criscione & Blouin, 2004; Whiteman et al., 2004). In other words, "[The gene genealogy] of a parasite with horizontal transmission carries potential epidemiological information about the patterns of parasite transmission among hosts" (Rannala & Michalakis, 2003). Tabor et al. (2001) advocated the use of this logic in a wildlife management context by suggesting using viral genetics as a means of inferring metapopulation dynamics of their lynx and mountain lion hosts. This, the authors argued, would help managers determine the location of natural corridors and areas where wild populations interact with domesticated animals. Host dispersal and

demographic processes were illuminated via population genetics of lemming (*Dicrostonyx* spp.) cestodes (Wickström et al., 2003). The authors found evidence that population genetics of these parasites could serve "as indicators of fine-scaled (temporal and geographical) events that are not (or not as clearly) apparent in the assessments of the biogeographical history of the hosts." Similarly, dating the genealogical split between human head (*Pediculus humanus capitis*) and body lice (*P. h. corporis* or *humanus*) has given insight into when humans first started to wear clothing, since body lice require it for survival (Kittler, Kayser & Stoneking 2003). Reed et al. (2004) have used parasite genealogies to infer that direct contact occurred between modern and archaic lineages of *Homo* (and corrected an error in Kittler, Kayser & Stoneking's, 2003 study). From a wildlife perspective, Weckstein (2004) showed that louse lineages of sympatric, but unrelated toucan hosts, were often each others closest relatives, indicating, perhaps, historic inter-specific host behavioral interactions (e.g., two species serially nesting in the same tree cavity hole) generated the observed patterns. At the population level, Whiteman et al. (2004) used a DNA barcoding approach in a simplified ecological setting to show that dispersal of lice from Galápagos doves (*Zenaida galapagoensis*) to Galápagos hawks (*Buteo galapagoensis*) occurred as a result of hawks feeding on doves.

Disease transmission within and among individuals *within* a population can reveal interactions among hosts. For example, population genetic data incriminated a physician who allegedly infected another person with an HIV-1 strain obtained from one of his patients (Metzker et al., 2002). A phylogenetic analysis revealed that the source of the strain could be identified, provided that the horizontal transmission event from source to recipient was recent enough for a paraphyletic relationship to remain between some of

the source viral isolates and the recipient isolates (since the recipient often receives a genetic subset of the source's total number of genetic HIV isolates; Metzker et al., 2002). This logic could easily be applied to conservation management context as well.

Impact on Parasite Conservation

How exactly, will this benefit parasite conservation? The careful genetic characterization of parasite populations requires extensive sampling within and across host populations. Such basic distributional data of parasites themselves will begin to lift the veil of ignorance with respect to parasite biodiversity. Parasites comprise most of earth's species (Windsor 1998) and most of the species within the Insecta (Price 1980), the most species-rich taxon on earth (Stork 1988; Samways 1994). Thus, examining fine-scale patterns of divergence among populations will help to unravel the processes responsible for the diversification of most of earth's species. New host records will accumulate and new host-specific parasites will be discovered and named. The degree of fine-scale parasite population structure within hosts may be astoundingly high (e.g. Nadler et al., 1990; Johnson et al., 2002; McCoy et al., 2003); its description will invariably illuminate the presence of a multitude of cryptic evolutionary lineages within classically defined species of parasite or pathogen (e.g. Barker et al., 1991, Barker, Close & Briscoe, 1991; Hung et al., 1999; Jousson, Bartoli & Pawlowski, 2000; Perkins, 2000; Criscione & Blouin, 2004). The use of DNA barcoding approaches (Hebert et al., 2003; Hebert, Ratnasingham, & deWaard, 2003) may further facilitate identification and classification of these lineages and provide insight into how parasites disperse between host individuals.

In light of the pragmatic value of parasites, managers of captive vertebrate populations may be encouraged to screen and genetically characterize the parasite populations of the vertebrates they manage, which will allow for a more informed discussion of host-parasite management options. If a population's parasites are eradicated before genetic characterization can take place, a great deal of information, much of it of possible management value for the host, will be lost forever. Results from many studies generally support the argument that parasite population genetics can reveal host population biology (e.g., Mulvey et al., 1991; Blouin, 1995; Dybdahl & Lively, 1996; Demastes et al., 1998; McCoy et al., 2003; Wickström et al., 2003).

Specific Recommendations for Managers

Do we suggest that managers indiscriminately sample parasites from small, threatened vertebrate populations? Obviously, this could result in the loss of a parasite population or species. Managers should consult with entomologists, microbiologists, parasitologists or other specialists before proceeding with large-scale sampling and genotyping. A large part of the problem of parasite conservation simply stems from not knowing what kind of diversity exists, given that parasites are the most diverse group on earth. Thus, partnerships between managers/conservation biologists and parasite specialists will help to fill in this gap in our knowledge while also alerting parasitologists to the presence of rare species.

When animals for captive rearing are first brought into captivity, or while being given wildlife health exams, managers should not rush to control parasites. Instead we recommend they (in consultation with the appropriate specialists) make every reasonable effort to sample (e.g., through physical examinations by veterinarians, blood smears,

faecals, pelage brushing, dust-ruffling of a limited number of hosts) parasites and then send such samples to experts for identification. Protocols for sampling parasites of mammals (Gardner, 1996), birds (appendices in Clayton & Moore, 1997), amphibians, reptiles, and fish (available online, from the Ecosystem Monitoring and Assessment Network of Environment Canada; http://www.eman-rese.ca/eman/ecotools/protocols) are all available. If unique parasites or other symbionts are found, they may be cultured in captivity on tissues of other host species (e.g., lice on feathers), or actually on other, more common host species ('purgatory hosts'), since many parasites are less host-specific in captivity. Though this may sound difficult to implement, researchers have developed this capability for some parasite taxa (e.g., lice; Clayton, Al-Tamini, & Johnson, 2003). For smaller parasites (e.g., trypanosomes), cryopreservation of live samples is a viable option (Ndao et al., 2004). Such samples could be cultured and captive animals infected prior to release. Could wildlife biologists and veterinarians establish a parasite bank for endangered species? It has been done for parasites of human importance. The Malaria Parasite Bank of India, established in 1992 accumulates, identifies, and cultures these parasites. Lice of the California Condor now appear to be extinct (Koh et al., 2004); perhaps a culturing attempt may have saved them.

Will there be more parasites on the IUCN Red List of Threatened species a decade from now? Perhaps, if conservation managers begin to view applied parasite population genetics as another tool under the broader rubric of vertebrate conservation genetics. This could bring a revolution to the field of conservation biology because parasite conservation will become directly relevant to vertebrate conservation. To reiterate, however, we believe that parasites have intrinsic value and should be conserved

for their own sake, not merely because they can be used as inferential tools. What we hoped to have accomplished presently is to illustrate what will be lost if vertebrate conservation biologists are not empowered to conserve parasites (Koh et al., 2004). It is through this new pragmatism, perhaps, that we may finally begin to live up to Wilson's (2000) lofty assertion that our conservation ethic is without taxonomic bias: "The conservation biologist knows that each imperiled species is a masterpiece of evolution, potentially immortal except for rare chance or human choice, and its loss a disaster." Lice and fleas, just like the lions and birds of paradise on which they live, are masterpieces of evolution, too. However, human taxonomic bias seems to fault even conservationists (Clark and May, 2002). Hopefully, the limelight will begin to shine on parasites and other symbionts, but it is up to us to make room for them on the stage.

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Chapter III.

Body Condition And Parasite Load Predict Territory Ownership In The Galápagos Hawk

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ABSTRACT

We tested for associations between body condition, territory ownership, and permanent parasite load of Galápagos hawks (*Buteo galapagoensis*) (Gould) on Isla Marchena, Galápagos. Two louse species were collected from most of the 26 hosts sampled: the amblyceran *Colpocephalum turbinatum* and the ischnoceran *Degeeriella regalis*. Nonterritorial hawks were in significantly poorer body condition than territorial hawks. Body condition was negatively correlated with the abundance of *C. turbinatum*. Nonterritorial hawks had significantly higher mean abundances, mean intensities, and median intensities of both louse species than territorial hawks. The amblyceran's mean abundance and intensity were significantly higher than the ischnoceran's. Abundances of the two lice were positively related when the population size of *C. turbinatum* was <100 individuals, and negatively related when >100 individuals. Parasite load and body condition both predicted territory ownership well.

Key words: body condition, ectoparasite, Falconiformes, Galápagos, Phthiraptera, territoriality.

SPANISH ABSTRACT

La Condición Corporal y la Carga Parasitaria Predicen la Propiedad de los Territorios en el Gavilán de Galápagos

Resumen. Probamos la relación entre la condición corporal, la propiedad de los territorios y la carga parasitaria permanente de *Buteo galapagoensis* en la Isla Marchena, Galápagos. En la mayoría de los 26 hospederos muestreados se colectaron dos especies de piojo: el ambliceránido *Colpocephalum turbinatum* y el ischnoceránido *Degeeriella regalis*. Los hospederos no territoriales se encontraron en condiciones corporales significativamente más pobres que los hospederos territoriales. Encontramos una correlación negativa y significativa entre la condición corporal y la abundancia de *C. turbinatum*. Los hospederos no territoriales tuvieron significativamente mayor abundancia, intensidad e intensidad mediana de las dos especies de piojo que los hospederos territoriales. La abundancia promedio y la intensidad de los ambliceránidos fueron significativamente mayores que las de los ischnoceránidos. Las abundancias de las dos especies de piojo estuvieron positivamente correlacionadas cuando el tamaño poblacional de *C. turbinatum* fue < 100 individuos, y negativamente correlacionada cuándo > 100 individuos. Tanto la carga parasitaria como la condición corporal predijeron bien la propiedad de los territorios.

INTRODUCTION

Nonterritorial birds occur within wild populations of many bird species (Brown 1969, Krebs 1971, McCrary et al. 1992, Blanco 1997, Newton 1998), including the Galápagos Hawk (*Buteo galapagoensis*) (Faaborg et al. 1980, Faaborg 1986). Generally, non-territorial birds are in poorer body condition (Fretwell 1969, Hogstad 1987) and suffer from higher parasite loads than territorial birds (Jenkins et al. 1963). However, the relationships among these variables are not well understood (Jenkins et al. 1963, Halvorsen 1986, Potti and Merino 1995, Harper 1999, Darolova et al. 2001, Calvete et al. 2004). Moreover, reports linking these factors are scarce. In this study, we examined the interrelationships between host territoriality, body condition, and parasite load.

The Galápagos Hawk is endemic to nine islands within the Galápagos archipelago, Ecuador (de Vries 1975). This species has been of particular interest to biologists due to its unusual mating system, cooperative polyandry (Faaborg et al. 1995). Polyandrous groups are composed of two to five males and one female on Marchena, the study island (Bollmer et al. 2003). These individuals form permanent all-purpose territories, which both sexes defend throughout the year (de Vries 1975). Territorial birds rarely leave the occupied territory (de Vries 1975, Faaborg and Bednarz 1990, Donaghy Cannon 2001). Individuals do not attain group membership while retaining juvenile plumage (de Vries 1975).

Nonterritorial hawks live in poorer quality areas and do not breed (de Vries 1975, Faaborg et al. 1980, Faaborg 1986, Donaghy Cannon 2001). Non-territorial hawks also suffer higher mortality than territorial hawks (Faaborg et al. 1980, Faaborg 1986, Faaborg

and Bednarz 1990). Given this information, it is reasonable to predict that non-territorial birds will be in poorer physical condition and suffer higher parasite loads than territorial birds.

Lice (Phthiraptera) comprise the largest number of ectoparasitic insect species (Marshall 1981). The chewing/biting lice (paraphyletic Mallophaga) are grouped into two monophyletic lineages: the Amblycera and Ischnocera (Marshall 1981, Cruickshank et al. 2001, Johnson and Whiting 2002). Amblycerans consume most epidermal tissues and blood, are generally less host specific, less restricted to a particular region of the host's body, and are more vagile than feather-feeding ischnocerans (Ash 1960, Marshall 1981). Data from other studies indicate that when these suborders co-occur on hosts, amblycerans are more abundant than ischnocerans (Nelson and Murray 1971, Lindell et al. 2002). Thus, it is reasonable to predict that amblycerans should be more abundant than ischnocerans on an individual host and (if the two are competitive or if the former depredates the latter, Nelson 1971) negatively affect the population size of the latter when their population sizes are large.

Two louse species were previously collected from the Galápagos Hawk (de Vries 1975): the amblyceran *Colpocephalum turbinatum* Denny, and the ischnoceran *Degeeriella regalis* (Giebel). Forty-seven host species within the Falconiformes and the domestic pigeon (*Columba livia*) are known hosts of *C. turbinatum* (Price and Beer 1963, Price et al. 2003). The known hosts of *D. regalis* are typically limited to the Galápagos Hawk and the Swainson's Hawk (*B. swainsoni*) in the New World (Clay 1958). Both louse species are probably restricted to Galápagos hawks in the Galápagos, as they have never been reported from any other host there. Given this limited louse fauna, and the

differences in their evolutionary and life histories, the opportunity exists to examine the degree to which these two dissimilar species coexist and vary with host territorial status. Terminology with regard to parasite load follows Bush et al. (1997).

METHODS

The Galápagos Archipelago is approximately 1000 km west of mainland Ecuador, South America. We studied the hawk population of Isla Marchena (00˚ 18'54'' N, 090˚31'89'' W; 130 km^2 in area, 343 m elevation; Black 1973), which is situated in the northern portion of the archipelago (Thornton 1971).

Territorial Galápagos hawks were characterized by at least two of the following criteria: (1) they defended territories against foreign hawks; (2) they gave a distinct warning call when humans or foreign hawks crossed the territorial bounds (de Vries 1975); (3) when nesting, they defended the nest when we approached; or (4) they performed aerial displays (with soaring-circling-spiral flight, de Vries 1975). All nonterritorial adults were captured on an area of southeastern coastline not defended by territorial adults and were not observed in any territorial group thereafter, nor were any territorial birds ever seen within this area.

Hawks were captured using a pole and noose from 4-15 June 2001. Mature adults were identified by uniform dark-brown plumage; and juveniles/immatures had distinct light brown mottled plumage (de Vries 1975). To calm each bird after capture, we placed a loose cloth hood over the head during handling. To avoid cross-contamination, the hood was visually inspected and thoroughly cleaned between handlings. All birds were banded with aluminum alphanumeric colored bands or numeric aluminum bands. Mass was measured with a Pesola scale (to the nearest 5 g) and wing chord was measured to

the nearest mm (unflattened length from the tip of the longest primary feather to the wrist).

To quantify ectoparasite loads, birds were sampled via dust ruffling (Walther and Clayton 1997) with pyrethroid insecticide (derived from the chrysanthemum, and nontoxic to birds; Zema® Z3 Flea and Tick Powder for Dogs, St. John Laboratories, Harbor City, CA) composed of 0.10% pyrethrins and 1.00% of the synergist piperonyl butoxide. A small amount (~2 g) of insecticide was evenly applied to each bird's plumage. This was followed by four to six 30-sec bouts of feather ruffling to dislodge the parasites. Ruffling was ended when the last bout yielded <5% of the total number of lice collected during all previous bouts combined (Whiteman and Parker 2004 Whiteman et al. 2004). Our louse removal efficiencies were congruent with other studies attempting to quantify such loads (Clayton et al. 1992). Each bird was held over a clean plastic tray during ruffling to collect dislodged ectoparasites (stored in 95% ethanol). Ectoparasites were examined in the laboratory using a stereo microscope and identified to species.

STATISTICAL ANALYSES

In order to calculate the overall body condition of territorial and non-territorial Galápagos hawks, a linear regression of body mass against wing length was performed in SPSS (1997). The residuals of this analysis were used as the index of body condition (Brown 1996). To determine if data from adult nonterritorial and juvenile/immature nonterritorial birds could be combined to increase statistical power we first tested for differences in body condition between them (independent samples *t*-tests in SPSS 1997). The average body condition of nonterritorial birds was then compared to that of the territorial birds using independent samples *t*-tests. To test for a general relationship between host body

condition and louse abundance, louse abundance data were first transformed ln(louse abundance + 1) transformed for each species due to the high variance in louse abundance. A bivariate two-tailed Pearson's correlation was then performed for host condition vs. louse abundance, for each parasite species.

Rózsa et al. (2000) cautioned that misleading results are easily obtained when using nonparametric statistical tests to compare parasite populations. Thus, where possible, we used the Quantitative Parasitology 2.0 program, which employs distribution-free tests (Rózsa et al. 2000, Reiczigel and Rózsa 2001). Using bootstrapped *t*-tests, mean abundances (a metric that includes uninfested birds) and intensities (a metric that includes only infested birds) were compared within a parasite species, between territorial and nonterritorial hosts, and between parasite species (Rózsa et al. 2000). Prevalences (the percentage of birds infected out of the total number sampled) were also compared between these hosts using Fisher's exact tests (Rózsa et al. 2000). Median intensities were compared using Mood's test of medians (Rózsa et al. 2000). To determine if data from adult nonterritorial and juvenile/immature non-territorial hosts could be combined to increase statistical power, we first tested for differences in these parameters between them.

We expected the amblyceran to negatively influence the population size of the ischnoceran, if the two are competitive or if the former depredates the latter. Thus, we performed a linear regression analysis in SAS (1997) to test their degree of coexistence. In the model, dummy variables separated territorial from nonterritorial hosts. Abundance of *D. regalis* was the dependent variable, and the abundance of *C. turbinatum* and the product of this value and the dummy variable were the independent variables.

Note that the relationship between territorial hawk group size (which varies on many islands in the Galápagos) and louse abundance is treated elsewhere (Whiteman and Parker 2004).

RESULTS

We captured and sampled 26 Galápagos hawks, over one-third of the total estimated host population size on Marchena. Of the 26 hawks, 21 were adults (17 territorial, four nonterritorial) and five were nonterritorial juveniles/immatures.

Average body condition did not differ between adult nonterritorial and juvenile/immature nonterritorial birds (independent samples *t*-test; $t_7 = 0.8$, $P = 0.43$). Thus, condition data for the two groups were pooled. Nonterritorial birds were in significantly poorer body condition than territorial hawks (equal variances not assumed, independent samples $t_{23} = 2.9$, $P < 0.01$; Fig. 1).

We collected 3186 lice from 25 infested Galápagos hawks. Of these, 2872 were *C. turbinatum* and 314 were *D. regalis*. Most *D. regalis* specimens were collected from wing and tail feathers, whereas *C. turbinatum* was collected from all body regions.

Host body condition and louse abundance were significantly negatively related for *C. turbinatum* ($r = -0.43$, $P = 0.03$, but not for *D. regalis* ($P > 0.05$; Fig. 2).

There were no significant differences between adult nonterritorial ($n = 4$) and juvenile/immature nonterritorial hawks ($n = 5$) for any of the parasite load metrics (*C. turbinatum*: all $t \leq 0.8$, all $P > 0.4$; *D. regalis*: all $t \leq 0$, all $P > 0.4$. Thus, parasite data were pooled for adult and juvenile/immature nonterritorial hawks. Mean abundances (Table 1) and mean and median intensities (Fig. 3) of both louse species were

significantly higher within nonterritorial hawks when compared to territorial hawks; thus, parasite load was indicative of hawk territorial status.

Within both nonterritorial and territorial hawks, mean abundance (Table 1) and mean and median intensity of *C. turbinatum* were significantly higher than that of *D. regalis* (nonterritorial hawks: mean intensities, $t_9 = 4.1$, $P < 0.01$; median intensities, $P < 0.01$; territorial hawks: mean intensities, $t_{17} = 2.7$, $P = 0.03$); except for median intensities, $P > 0.05$).

In the regression *D. regalis* abundance on *C. turbinatum* abundance, the intercepts and slopes for territorial and nonterritorial hawks differed significantly and SAS (1997) separated the two data sets (both $t_1 > -4.5$, $P \leq 0.02$; Fig. 4). The regression equation for territorial hawks was $y = 0.187x + 2.006$ and for nonterritorial hawks was $y = -0.043x + 33.747$. The slopes for territorial hawks was significantly positive (= 0.41, $t_1 = 3.2$, $P < 0.01$) and that of the nonterritorial hawks was significantly negative (= 0.25, $t_1 = -2.3$, $P = 0.04$; Fig. 4).

DISCUSSION

We found a strong relationship between louse load, host body condition, and territorial status in a population of the Galapagos Hawk. Nonterritorial birds were in significantly poorer body condition and had higher loads of both louse species than did territorial hosts. Some studies have found similar results (Jenkins et al. 1963), although others have not (Blanco et al. 2001, Darolova et al. 2001).

We also found a negative relationship between host body condition and the abundance of *C. turbinatum*.

Thus, the abundance of the amblyceran louse, *C. turbinatum*, had a stronger correlation with body condition than the ischnoceran. Are these lice directly reducing host body condition? This seems possible, given that they feed on blood and vector endoparasites, and are at least in part transmitted by physical contact other than the parent-offspring route (Whiteman and Parker, 2004), each of which may correlate with increased virulence (Seegar et al. 1976, DeVaney et al. 1980, Clayton and Tompkins 1994). Ischnoceran lice, however, can influence host fitness as well, usually by damaging feathers, which compromises thermoregulatory ability, and reduces survivorship and male mating success (Booth et al., 1993, Clayton et al. 1999). Notably, Calvete et al. (2003) found that in contrast to our findings, the relationship between body condition and the abundances of both amblyceran and ischnoceran lice (each louse species was analyzed separately) were significantly inversely related, thus generalizations on the effects of these two suborders are not yet possible.

Alternatively, parasite populations may respond to changes in host behavior that independently affect host body condition. For example, preening rate is perhaps the most important regulator of ectoparasite load (Clayton 1991). However, preening consumes time and energy (Giorgi et al. 2001). It is reasonable to assume that resource-stressed hosts (nonterritorial) preen less than non-resource-stressed hosts (territorial). Thus, preening rate and body condition may be linked, which would release constraints on parasite population growth rates, resulting in higher parasite loads in nonterritorial hosts, which are also in poorer body condition. Generally, hosts with better nourishment are more resistant to parasites (Nelson et al. 1975, Marshall 1981, Nelson 1984, cf. Kartman 1949), which may be directly linked with immunocompetence (Christe et al. 1998). The

relationship may also be more complicated than any of these scenarios. The association between condition and parasite load may instead generate a feedback loop (poor condition leading to increased parasitism leading to poorer condition).

We found that the mean abundance and median and mean intensity of the amblyceran, *C. turbinatum*, were significantly higher than in the ischnoceran, *D. regalis*, within nonterritorial and territorial hawks. The abundance of *D. regalis* appeared to be negatively affected by the abundance of *C. turbinatum*, in excess of 100 individuals. For territorial hawks, the relationship between the abundances of the two louse species was positive and linear, whereas the population sizes of the two louse species for nonterritorial hawks was negative and linear. Possible mechanisms to explain this pattern include interspecific predation or competition (Gotelli 1998). There is evidence to suggest that *C. turbinatum* is predaceous on lice (Nelson 1971). When its abundances are relatively high, it may begin feeding on other lice. Alternatively, competition may begin when the abundance of *C. turbinatum* is above a threshold and individuals begin to invade microhabitats typically occupied only by *D. regalis* (Nelson 1972). Clayton (1991) found that *Columbicola columbae* lice were more resistant to host preening than *Campanulotes bidentatus*, suggesting that preening regulated the latter's abundance. Hopkins (1949) also demonstrated that louse coexistence was mediated by grooming behavior in guinea pigs (*Cavia porcellus*). We speculate that once a Galápagos Hawk becomes territorial, the abundances of the two louse species equilibrate and become positively related instead of being negatively related as occurs when *C. turbinatum* abundances become large.

Future research should focus on decoupling the degree to which parasite load drives host territoriality and the degree to which territorial status drives parasite load (and parasite coexistence).

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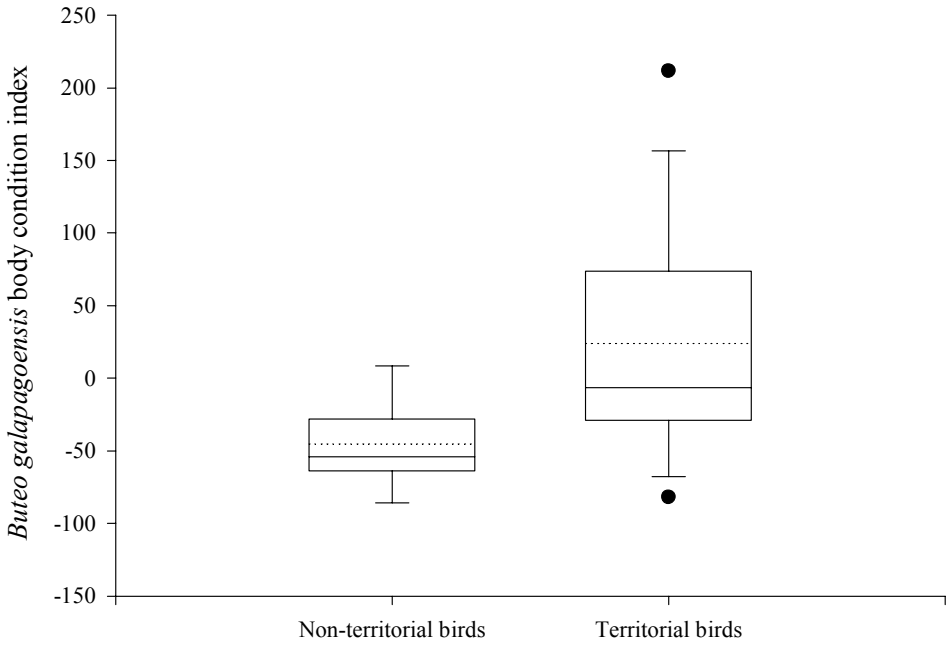
FIGURE 1. Nonterritorial Galápagos hawks (n = 9) were in significantly poorer physical condition than territorial hawks (n = 17). Body condition is the residuals of a regression of body mass on wing chord. Box and whisker plots show means (dotted lines), medians (solid lines) and 5th and 95th percentiles.

FIGURE 2. Scatterplots of body condition vs. abundance of two louse species for territorial and nonterritorial Galápagos hawks. (A) *Colpocephalum turbinatum* (significant correlation); (B) *Degeeriella regalis* (nonsignificant).

FIGURE 3. Infestation intensity by two louse species on territorial and nonterritorial Galápagos hawks. Box and whisker plots show mean (dotted lines), median (solid lines) and 5th and 95th percentiles. (A) *Colpocephalum turbinatum*; (B) *Degeeriella regalis*. Intensity is a measure of parasite abundance calculated from infested hosts only. Numbers below plots are numbers of hawks sampled. Note difference in y-axis scales.

FIGURE 4. Scatterplot of total abundances of the lice *Colpocephalum turbinatum* vs. *Degeeriella regalis* for territorial (n = 17) and nonterritorial (n = 9) Galápagos hawks. Slopes of both regression lines were significantly different from zero.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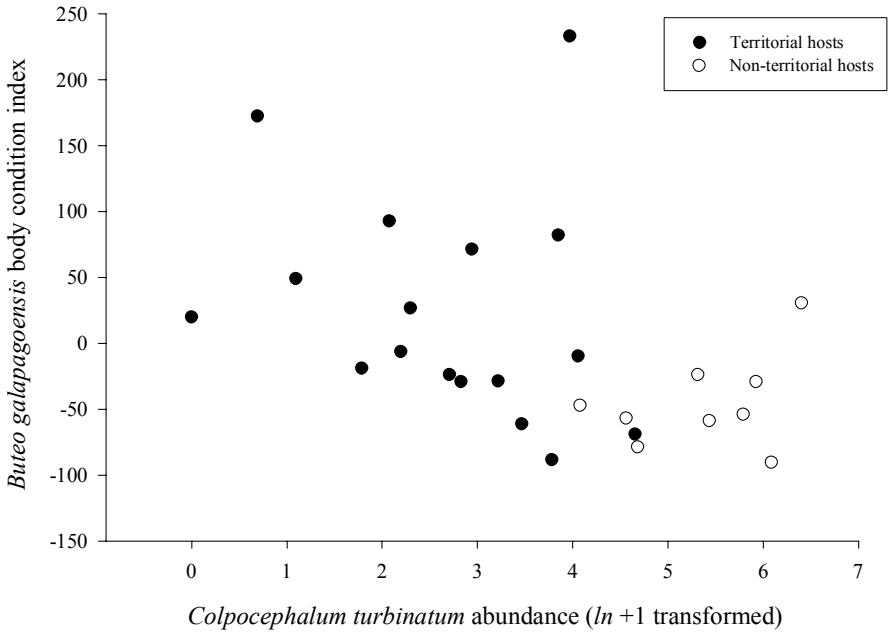
Buteo galapagoensis body condition index
Non-territorial birds
Territorial birds

A


Territorial hosts
Non-territorial hosts
Buteo galapagoensis body condition index
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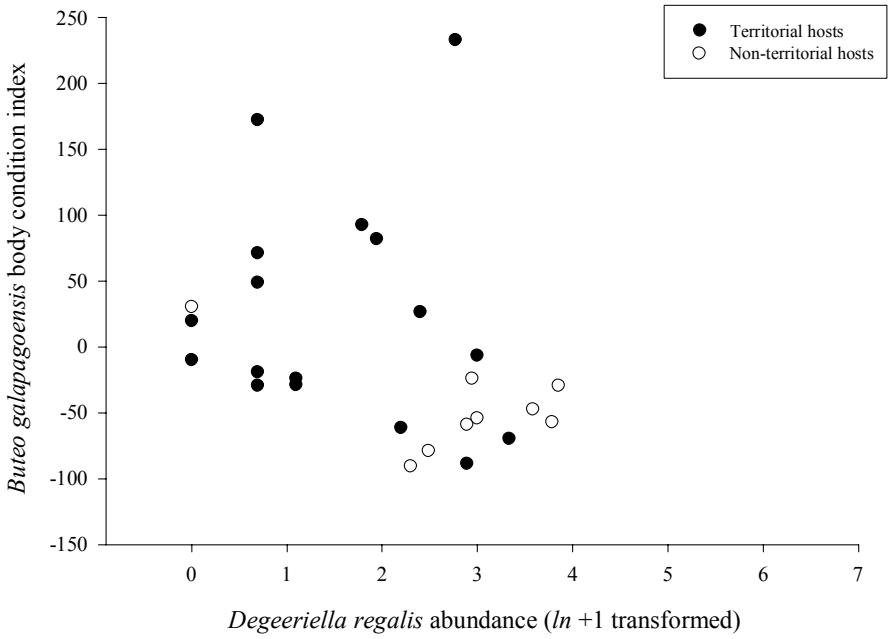
200
150
100
50
0
-50
-100
-150
0
1
2
3
4
5
6
7
Colpocephalum turbinatum abundance (ln +1 transformed)

B


Territorial hosts
Non-territorial hosts
Buteo galapagoensis body condition index
250
200
150
100
50
0
-50
-100
-150
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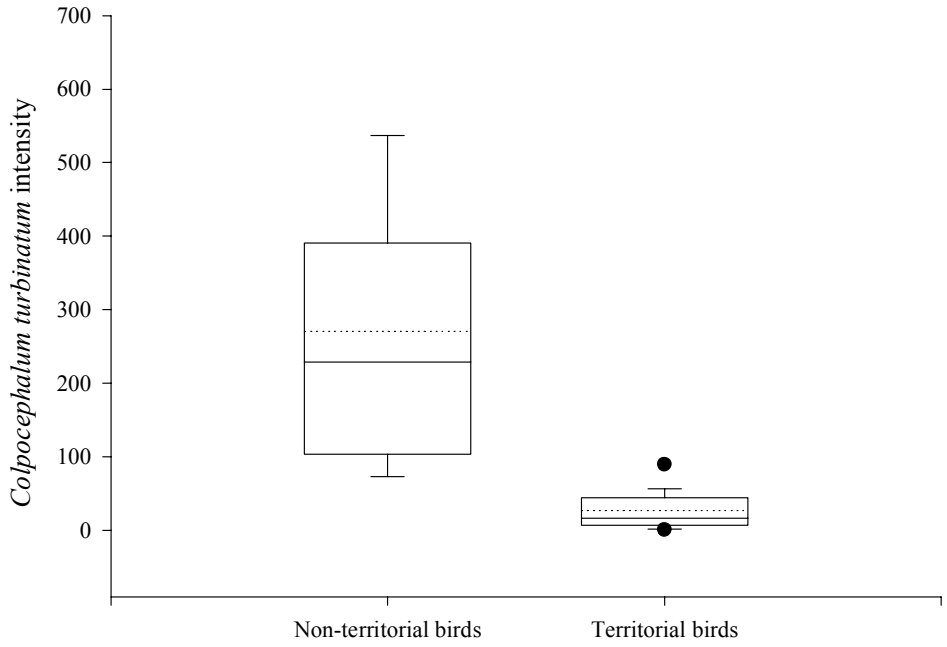
6
7
Degeeriella regalis abundance (ln +1 transformed)

A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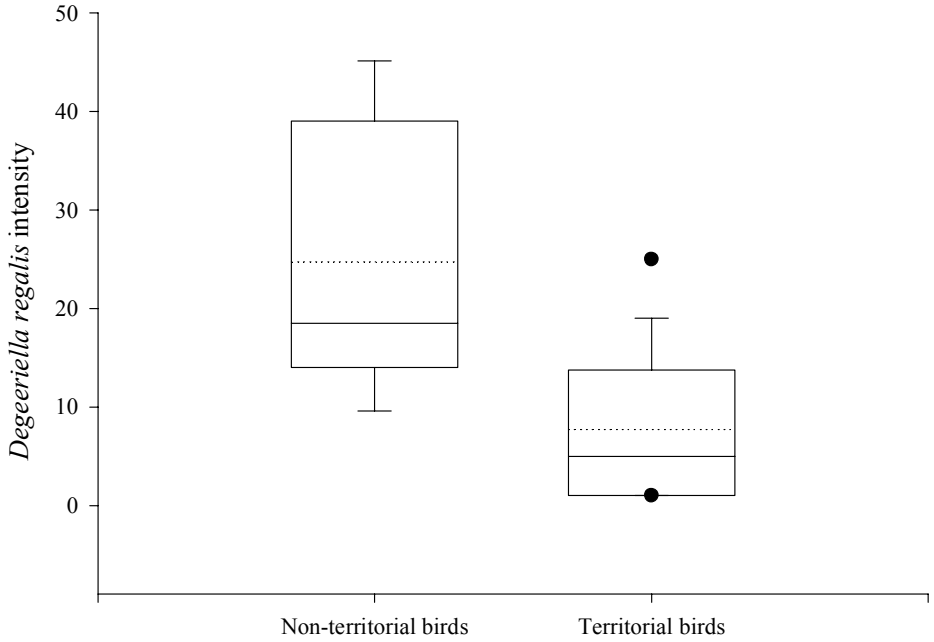
Colpocephalum turbinatum intensity
Non-territorial birds
Territorial birds

B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40
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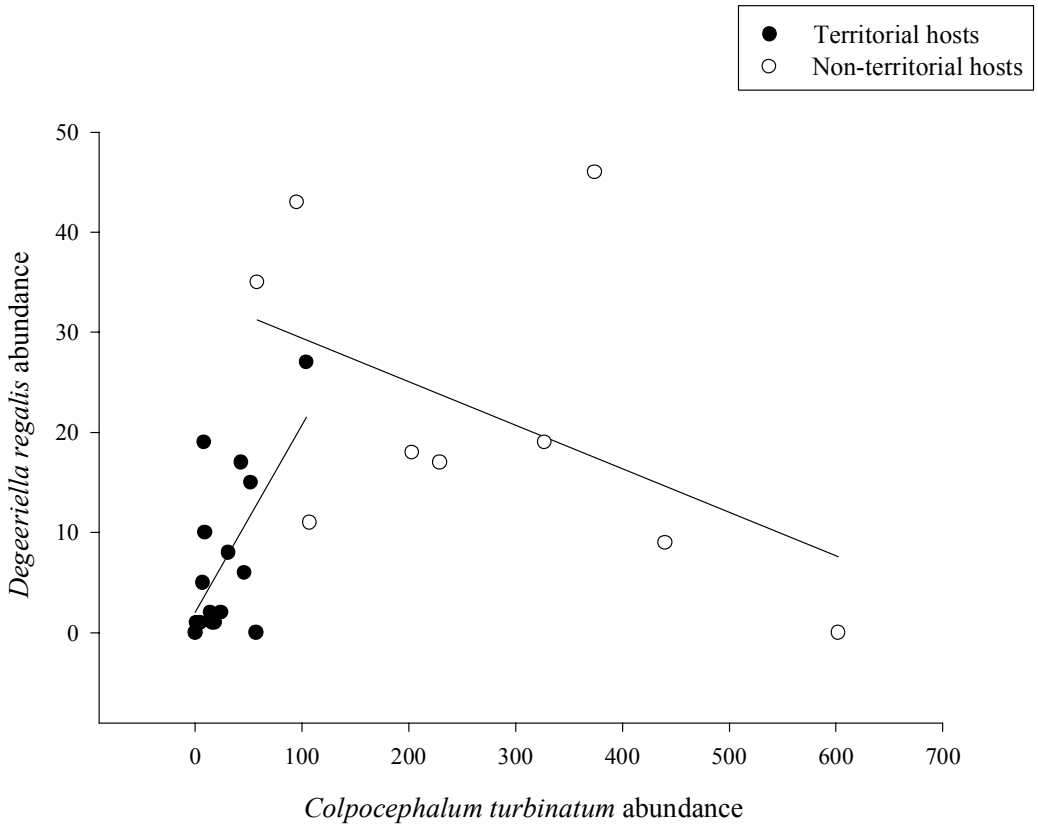
0
Degeeriella regalis intensity
Non-territorial birds
Territorial birds


Territorial hosts
Non-territorial hosts
50
40
30
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10
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Degeeriella regalis abundance
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100
200
300
400
500
600
700
Colpocephalum turbinatum abundance

TABLE 1. Prevalences and mean abundances of the lice *Colpocephalum turbinatum* (Amblycera) and *Degeeriella regalis* (Ischnocera) for nonterritorial (n = 9) and territorial (n = 17) Galápagos hawks from Isla Marchena, Galápagos, Ecuador.

	Prevalence[a]			Mean abundance[b]	
	Non-territorial	Territorial	*P*	Non-territorial	Territorial
C. turbinatum	100%	94%	1.0	270.6 (162.3-385.2)	25.7 (13.7-38.4)
D. regalis	89%	88%	1.0	22.0 (12.1-31.6)	6.8 (3.2-10.5)

[a]All prevalence comparisons were significant with a Fisher's exact test. Prevalence is the percentage of individuals infested with lice out of the total number of hawk sampled. Values in parentheses are 95% bootstrap confidence limits around the mean abundance (2000 replications). [b]All abundance comparisons were significant (all $t \geq 2.7$, all $P \leq 0.03$).

Chapter IV.

Effects of Host Sociality on Ectoparasite Population Biology

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ABSTRACT

Theory predicts a positive relationship between parasite infection intensity and host density. However, this generalization is complicated in natural systems by differences in life history among parasite taxa (e.g., transmissibility). Accordingly, predictions relating host density to parasite load should be specific to each parasite taxon. To illustrate, we studied parasites that differed greatly in life history in the context of the Galapagos Hawk's (*Buteo galapagoensis*) variably cooperative mating system. Two louse (Phthiraptera) species were collected: *Colpocephalum turbinatum* (Amblycera), with 53 host species, and *Degeeriella regalis* (Ischnocera), with 10 host species, although *B. galapagoensis* was the only known Galapagos host. Sixty territorial adult male hawks from 26 groups of 1 to 6 males were quantitatively sampled for lice. Average abundance and intensity of *C. turbinatum*, but not *D. regalis*, were significantly larger in large groups of hawks than small groups. Males from the same polyandrous group harbored significantly correlated abundances of *C. turbinatum*, but not *D. regalis*. Prevalence, average abundance, and intensity of *C. turbinatum* were significantly higher than *D. regalis*. These are the first results to demonstrate significant differences in a suite of population responses between these louse suborders in the context of host sociality.

INTRODUCTION

There are several presumed benefits and costs to group living (Alexander, 1974). The benefits, which may include direct and inclusive fitness returns and enhanced access to resources, have been the focus of intense investigation (e.g., Stacey and Koenig, 1990; Queller and Strassmann, 2002). However, the costs, such as increased risk and intensity of parasite infection, are less well studied (Anderson and May, 1978; May and Anderson, 1978; Dobson, 1990; Brown et al., 1995; Krasnov et al., 2002). Although theoretical models predict a positive relationship between parasite infection intensity and host density, this generalization is complicated in natural systems by basic life history differences among parasite taxa (e.g., transmissibility). Accordingly, predictions relating host density to parasite abundance should be specific to each parasite taxon.

Investigations into the parasite-host sociality nexus have focused on colonially breeding host species (Brown and Brown, 1986; Rózsa et al., 1996; Avilés and Tufiño, 1998; Hoi et al., 1998), or those that form non-breeding aggregations (Moore et al., 1988; Blanco et al., 1997). However, little attention has been given to cooperative breeders despite abundant data on other aspects of their biology (Brown, 1987; Stacy and Koenig, 1990; Ligon, 1999; Bennett and Owens, 2002). Well-documented intraspecific variation in sociality (de Vries, 1975; Bollmer et al., 2003), a characteristic of some cooperative breeders, is a key advantage when relating host density to parasite abundance (Rózsa et al., 1996).

Populations of parasites that are at least partially horizontally transmitted (mediated through contact other than parent-offspring) should be affected by changes in the size of their host's reproductive coalitions since parasite infrapopulation size is

partially controlled by transmission rate (Anderson and May, 1978; May and Anderson, 1978; Dobson, 1990). Conversely, species that are more dependent on the vertical transmission (parent-offspring) route should be less responsive to such changes in host group size. We tested these predictions within the context of the Galapagos Hawk's (Aves: Falconiformes: *Buteo galapagoensis*) (Gould) variably cooperative mating system.

Galapagos Hawks establish social groups that vary in size from monogamous pairs to cooperatively polyandrous groups (de Vries, 1973, 1975; Faaborg et al., 1980, 1995; Faaborg, 1986; Bollmer, 2000; Donaghy Cannon, 2001; Bollmer et al., 2003). Polyandrous groups are comprised of 1 female and from 2 to 8 males. Each social unit (monogamous or polyandrous) occupies an all-purpose territory, which is defended yr-round (de Vries, 1975; Faaborg et al., 1980, 1995). Adults within groups are not offspring that have delayed their dispersal and are not close relatives (de Vries, 1973, 1975; Faaborg et al., 1995). Hawks rarely leave the territorial boundary, which, along with group composition, is generally stable over time (de Vries, 1975; Faaborg, 1986; Faaborg and Bednarz, 1990; Donaghy Cannon, 2001). For example, of 62 male birds marked on Isla Santiago in 1999, 95% were present on the same territory 1 yr later (Donaghy Cannon, 2001). This relative stability increases the likelihood that parasite populations will track the host's social system (Moore et al., 1988; Rózsa et al., 1996). In 1999-2000 on Isla Santiago, territory sizes were statistically independent of the number of males within a territory (Donaghy Cannon, 2001). If this pattern is general, group size and host density should increase concomitantly and parasites, if horizontally transmitted, should also respond positively to this increase in host density. Within territorial groups,

opportunities for parasite transfer occur with regularity. Each male repeatedly copulates with the female (5 to 10 copulations per day), male-male copulations occur within polyandrous groups (N.K. Whiteman, pers. obs.), group members use communal roosts, and all birds within the breeding group brood the young (de Vries, 1975; Faaborg et al., 1995; Donaghy Cannon, 2001).

Lice (Insecta: Phthiraptera) comprise the largest number of ectoparasitic insect species (Marshall, 1981) and along with their hosts, have emerged as model systems in which a variety of ecological and evolutionary theories have been tested (Borgia, 1986; Brown and Brown, 1986; Hafner and Nadler, 1988; Borgia and Collis, 1989, 1990; Clayton and Tompkins, 1994; Rózsa et al., 1996; Rékasi et al., 1997; Hoi et al., 1998; Clayton et al., 1999; Poiani et al., 2000; Page, 2003).

The 2 most species-rich lineages within the chewing/biting lice (the paraphyletic Mallophaga) are the Amblycera and Ischnocera, which are each monophyletic (Marshall, 1981; Cruickshank et al., 2001; Johnson and Whiting, 2002). Amblycerans feed on epidermal tissues and blood, and are generally less host-specific, less restricted to a particular region of the host's body, and more vagile than feather-feeding ischnoceran lice (Ash, 1960; Askew, 1971; Marshall, 1981). Horizontal transmission may be a more important dispersal route in amblyceran lice than ischnoceran lice (DeVaney et al., 1980; Marshall, 1981; Clayton and Tompkins, 1994; cf. Hillgarth, 1996). Such general parasite life-history differences may interact with host social behavior to generate predictable differences in parasite population parameters. For example, an amblyceran was less aggregated among hosts within a population of social crows (Corvidae), relative to its distribution within a population of an asocial species, where they were more aggregated

within a few members of the population (Rózsa et al., 1996). This is in contrast to the distributions of the ischnocerans, which were similarly aggregated between the 2 host populations (Rózsa et al., 1996). Several studies have shown that when these suborders co-occur on hosts, amblycerans were more abundant than ischnocerans (Nelson and Murray, 1971; Eveleigh and Threlfall, 1976), which, among many other factors, may indicate that parasite population growth is constrained by rate of transmission among hosts (Arneberg et al., 1998).

Two louse species, the amblyceran *Colpocephalum turbinatum* Denny (Menoponidae) and the ischnoceran *Degeeriella r. regalis* (Giebel) (Philopteridae), were previously collected from *B. galapagoensis* (de Vries, 1975). The 2 species are at opposite ends of the host-range spectrum. *Degeeriella r. regalis* (referred to hereafter as *D. regalis* sensu Price et al. [2003]) occurs on 10 hosts worldwide, only 2 of which are found in the New World, *B. galapagoensis* and *B. swainsoni* (Clay, 1958; Price et al., 2003). This is in contrast to the hosts of *C. turbinatum* (although considered to be a single species by Price and Beer [1963] and Price et al. [2003], it was given *sensu lato* status by the former), which include 53 species according to Price et al. (2003). In Galapagos, these species have only been reported from *B. galapagoensis*. However, Price et al. (2003) reported *C. turbinatum* from the Barn Owl (*Tyto alba*), a subspecies of which (*T. alba punctatissima*) occurs on Santiago (but not Marchena). Whether it occurs on *Tyto* in Galapagos is not known, although given that Barn Owls are nocturnal (and hawks diurnal), roost during the day in lava tubes out of reach of hawks (Kircher, 2003), and are not preyed upon by *B. galapagoensis*, we consider the direct interaction between

hawks and owls unlikely. Therefore we assumed that any effect on *C. turbinatum*'s population size or distribution on the hawks from parasite transfer is negligible.

We assumed that host range correlated positively with parasite dispersal abilities, consistent with other authors (e.g., Johnson et al., 2002a; Clayton and Johnson, 2003). Thus, we formed a series of predictions of louse population responses to host social behavior. Prevalence was defined as the number of infected hosts/total number of hosts sampled, mean abundance was the average number of parasites on hosts, including uninfected hosts, and mean and median intensity were the average and typical number of parasites on infected hosts, respectively (Margolis et al., 1982; Bush et al., 1997).

First, given that parasite load should increase with host density, the importance of dispersal ability in determining transmission rates among hosts (Anderson and May, 1978; May and Anderson, 1978; Dobson, 1990), and other basic life history differences, prevalence, mean abundance, and mean and median intensity of amblyceran (*C. turbinatum*) lice (more mobile), but not ischnoceran (*D. regalis*) lice (less mobile), are positively related to size of social groups (number of males/group) of the Galapagos hawk.

Second, given that almost all parasites display a lumped distribution among hosts (a negative binomial distribution) and differences in parasite dispersal abilities, infrapopulations (1 infrapopulation = the number of parasite individuals occurring on 1 host individual) of amblyceran (*C. turbinatum*), but not ischnoceran (*D. regalis*) lice, are less aggregated among males from large groups than those from small groups, sensu Rózsa et al. (1996).

Third, given that, in other bird species, inter-sex pair members have significantly

correlated louse infrapopulation sizes (Potti and Merino 1995; Hoi et al.,1998), abundances of amblyceran (*C. turbinatum*), but not ischnoceran (*D. regalis*) lice, are more similar among polyandrous territorial group-mates than among polyandrous territorial males randomly paired with males from other groups, after controlling for the effects of group size.

Finally, given higher inferred dispersal rates, the importance of transmission in constraining parasite population growth (Arneberg et al., 1998), and other basic life history differences, prevalence, abundance, and intensity of amblyceran (*C. turbinatum*) lice are always greater than those of ischnoceran (*D. regalis*) lice, regardless of host group size.

MATERIALS AND METHODS

Study site

The Galapagos Archipelago is located on the equator, ~1,000 km west of mainland Ecuador, South America. *Buteo galapagoensis* is endemic to 9 main islands within the archipelago (de Vries, 1973, 1975). It is listed in the I.U.C.N. Red List of Threatened Species (I.U.C.N., 2002), and has been anthropogenically extirpated from at least 5 islands within the archipelago (de Vries, 1973, 1975). Data are presented from 2 island populations in the archipelago, Santiago and Marchena. Isla Santiago, located in the center of the archipelago, is 585 km^2 in area and rises to a height of 907 m (Kricher, 2002). Hawks were sampled from 2 locations on Santiago: James (Espumilla) Bay, along the western coastline (~00˚20'S, 090˚82'W), and Sullivan Bay, along the eastern shore (~00˚30'S, 090˚58'W). The locations of most hawk territories studied on Santiago were determined previously (DeLay, 1992; Donaghy Cannon, 2001). Isla Marchena,

located in the northern part of the archipelago, is 130 km^2 in area, and rises to a height of 343 m (Kricher, 2002). The study site was located along and inland from a beach situated on the south-southwestern coastline (~00˚18'N, 090˚32'W).

Host capture

Territorial Galapagos Hawks were characterized by at least 2 of the following criteria. First, all group members defended territories against foreign hawks. Second, territorial birds gave a distinct warning call when humans or foreign hawks crossed the territorial bounds (de Vries, 1973). Third, if nesting, group members actively defended the nest when approached by us. Or, fourth, both sexes were seen in aerial display (soaring-circling-spiral flight, de Vries, 1973). Group sizes were recorded for each territory.

Territorial adult male hawks from Marchena were captured over a 12-day period from 4-15 June 2001. Hawks on Santiago were captured over a 45-day period from 14 May-29 June 2002. Females were not included in this study because of the requirements of an ongoing and unrelated experiment. Individuals were live-captured by pole noosing or Bal-chatri traps (Santiago only) baited with live rats introduced to the island previously (Berger and Mueller, 1959). To calm each bird after capture, a loose cloth hood was placed over the head and neck region during handling, until release (to avoid contamination, the hood was thoroughly cleaned between handlings and visually inspected). Unless banded previously, all birds were marked with aluminum alphanumeric colored and/or numeric aluminum bands.

Collection and quantification of louse load

To quantify ectoparasite loads, birds were sampled via dust ruffling (Walther and Clayton, 1997) with pyrethroid insecticide (derivatives of the chrysanthemum flower and non-toxic to birds; Zema® Z3 Flea and Tick Powder for Dogs, St. John Laboratories, Harbor City, California) comprised of 0.10% pyrethrins and 1% of the synergist piperonyl butoxide. Although other methods, e.g., body washing, may remove more parasites (but require dead birds), the number of lice removed after 1, 60-sec dust-ruffling bout adequately predicted total louse abundance of feral rock doves (*Columba livia*; Clayton and Drown, 2001).

In this study, a small amount (~2g) of insecticide was applied to each bird's plumage (all feather tracts except the head). This was followed by 5 to 8 timed bouts (30 sec each) of feather ruffling to dislodge the parasites. Ruffling was stopped when the last bout yielded <5% of the total number of lice collected during all previous bouts combined. Our louse removal efficiencies were congruent with other studies attempting to quantify such loads (Clayton et al., 1992). Each bird was held over a clean plastic tray during ruffling to collect dislodged ectoparasites, which were stored in 95% ethanol.

Statistical analyses

Due to the aggregated nature of their distributions, many authors have utilized non-parametric statistics when comparing parasite populations. However, Rózsa et al. (2000) cautioned that using such statistics yields misleading results. Thus, to the extent possible, we used distribution-free statistical tests designed specifically for such data sets (Rózsa et al., 2000; Reiczigel and Rózsa, 2001). All of our analyses included both adult and nymphal lice.

To test if the frequency distributions of lice among hosts could be described by the negative binomial distribution (an expectation of parasite distributions; Crofton, 1971), expected negative binomial distributions were calculated using the program Ecological Methodology (Krebs, 1989). Frequency classes were pooled to increase the expected number of hosts to ≥ 3. The observed frequency distributions were then tested to determine if they differed from the expected distributions using chi-square tests.

Prevalence, mean and median intensity, and mean abundances (sensu Margolis et al., 1982) were calculated using the program Quantitative Parasitology 2.0 (Rózsa et al., 2000; Reiczigel and Rózsa, 2001). Fisher's exact tests were used to compare parasite prevalences between host populations. Distribution-free 2-sample bootstrap *t*-tests were used to compare mean intensities and abundances (each with 2,000 replicates). Mood's median tests were used to compare median (typical) intensities. Because standard deviations are uninformative in aggregated distributions (Rózsa et al., 2000), here we report bootstrap confidence intervals for mean abundance and intensity. We also calculated the moment '*k*,' which is inversely related to the degree of aggregation of parasite abundances among members of the host population (Crofton, 1971), and the index of discrepancy '*D*,' which is directly related to the degree of aggregation of parasite abundances among members of the host population (Poulin, 1993). The index of discrepancy is the degree to which the observed distribution of parasites among the host population differs from a hypothetical one in which each host harbors the same number of parasites (Poulin, 1993). We employed 2-sample Kolmogorov-Smirnov tests to compare distributions within large groups of hawks to those within small groups for each parasite species.

We tested if louse infrapopulation abundances were more similar among polyandrous group-mates than among pairs of birds drawn from different groups. Louse infrapopulation sizes from dyads of males from the same or different polyandrous groups were tested for similarity using Pearson's correlation in SPSS (1 analysis for each louse species). In this analysis the effect of group size was controlled as a partial correlation. However, these data represented a fraction of the total available since we used only 2 males from each polyandrous group rather than using each available male. Thus, Mantel tests (Mantel, 1967) were employed using PC-ORD, to test if louse infrapopulation abundances were more similar among polyandrous group-mates than non-group mates. In the Mantel analysis, louse abundance data from dyads of 53 polyandrous males of 60 total males were used (7 males were not used because 6 were from monogamous pairs or in the case of 1 male, the other polyandrous group members were not captured). Thus, 1 matrix was comprised of the differences in louse abundances between 2 birds (the difference was calculated by first adding 1 to each count to eliminate zero values and then employing the formula: [larger abundance – smaller abundance]/smaller abundance) for all possible dyad combinations. A second matrix was constructed in which the same dyads were given a 'zero' (indicating that the paired males were from the same group) or a 'one' (indicating that the paired males were from different groups). A Monte Carlo randomization approach was then used to test if the matrices were independent.

RESULTS

On Marchena, a total of 14 territorial adult male Galapagos Hawks from 6 territories were sampled for ectoparasites. On Santiago, a total of 46 territorial adult male Galapagos Hawks from 20 territories were sampled for ectoparasites. Thus, in total, 60

males from 26 territorial groups were sampled. The number of territorial adult male hawks per group ranged from 1 to 6 individuals; thus, males from both monogamous pairs and polyandrous groups were sampled (mean number of males/group for both islands combined = 2.85 ± 1.49 SD). We were unable to sample 14 of the males present in nine of the territorial groups.

A total of 1,510 lice was collected from the 60 male hawks. Of these, 1,229 were *C. turbinatum* and 281 were *D. regalis*. In addition, 8 *Columbicola macrourae* (Wilson) (Philopteridae) lice were collected from 4 hosts and were not considered in our analyses (they were presumed stragglers from *Zenaida galapagoensis* hosts, which are preyed on by *B. galapagoensis*; de Vries, 1973; Donaghy Cannon, 2001). Most *D. regalis* specimens were collected from wing and tail feathers, whereas specimens of *C. turbinatum* were collected from throughout the body. Individuals of *C. turbinatum* were seen actively crawling on the skin and feathers of the host, and would often crawl upon our hands; *D. regalis* individuals were never seen actively crawling on the host or our hands.

To facilitate analyses, parasite counts from hosts were grouped into 'small groups' (those from groups comprised of 1 to 3 males) and 'large groups' (those from groups comprised of 4 to 6 males). Each measure of parasite load, including prevalence, mean abundance, mean intensity, and median intensity, did not differ significantly between Marchena and Santiago for either parasite species within either large or small groups (Table 1). Thus, to increase sample sizes, data from Marchena and Santiago were combined. With the exception of *D. regalis* within small groups, frequency distributions of either species within each class did not differ from an expected negative binomial

distribution (small groups: *C. turbinatum*, $\chi^2 = 8.3664$, $P = 0.593$; *D. regalis*, $\chi^2 = 11.5824$, $P = 0.021$; large groups: *C. turbinatum*, $\chi^2 = 4.089$, $P = 0.665$; *D. regalis*, $\chi^2 = 6.2367$, $P = 0.397$).

Prediction 1

Given that parasite load should increase with host density, the importance of dispersal ability in determining transmission rates among hosts (Anderson and May, 1978; May and Anderson, 1978; Dobson, 1990), and other basic life history differences, prevalence, mean abundance, and mean and median intensity of amblyceran (*C. turnbinatum*) lice (more mobile), but not ischnoceran (*D. regalis*) lice (less mobile), are positively related to size of social groups (number of males/group) of the Galapagos hawk.

Mean intensity, abundance and median intensity of *C. turbinatum* were significantly higher among hosts from larger social groups than smaller social groups (Table II, Fig. 1a). The same measures were not statistically different for *D. regalis* between small or large groups(Table II, Fig. 1b). Infected males from large groups had an average of 2.68 times as many *C. turbinatum* individuals as males from smaller groups (Fig. 1a). Likewise, the typical (median) level of infestation within infected males from large groups was 4.57 times greater than that of males from small groups (Fig. 1a). There was no overlap in the 95% confidence limits of mean *C. turbinatum* abundance between small and large groups, while those limits overlapped by 56.83% between average abundances of *D. regalis* from small and large groups (Table II). Prevalence of *C. turbinatum* or *D. regalis* did not differ statistically between small and large groups.

Prediction 2

Given the lumped distribution of parasites among hosts (a negative binomial distribution) and differences in parasite dispersal abilities, infrapopulations of amblyceran (*C. turbinatum*), but not ischnoceran (*D. regalis*) lice, are less aggregated among males from large groups than those from small groups, sensu Rózsa et al. (1996).

We compared the distributions within a louse species between small and large groups. Those distributions differed significantly between these groups for *C. turbinatum* (Fig. 2a), but not for *D. regalis* (Fig. 2b). The *C. turbinatum* population was less aggregated among hosts from larger social groups than those from smaller social groups, as is indicated by differences in their respective '*k*' and '*D*' values (Table II). The magnitude of '*k*' within large groups was over 2.5 times higher than its magnitude within small groups. The same is not true for *D. regalis*, where the magnitude of '*k*' differed by 0.02 between the louse populations from small and large groups (Table II).

Prediction 3

Given that, in other bird species, inter-sex pair members have significantly correlated louse infrapopulation sizes (the number of lice on an individual host; Potti and Merino 1995; Hoi et al., 1998), abundances of amblyceran (*C. turbinatum*), but not ischnoceran (*D. regalis*) lice, are more similar among polyandrous territorial group-mates than among territorial males randomly paired with males from other groups, after controlling for the effects of group size.

Correlational analyses using only two males/group resulted in a significantly positive relationship between the pair-wise abundances of *C. turbinatum* (Fig. 3; Pearson's $r = 0.771$, $P = 0.000$ [1-sided], $n = 19$; after controlling for group size, Pearson's $r = 0.663$, $P = 0.001$ [1-sided]), but not *D. regalis* (Pearson's $r = -0.23$, $P =$

0.462 [1-sided] n = 19; after controlling for group size, Pearson's $r = 0.0087$, $P = 0.486$ [1-sided]). In the expanded Mantel analysis, dyads of males from the same polyandrous group were more similar in *C. turbinatum* infection abundance than dyads from different groups (1,000 randomized runs, standardized Mantel $r = 0.0592$, observed Z =17,382, average Z = 16,997, $P = 0.011$). The same relationship was true for abundances of *D. regalis* (1,000 randomized runs, standardized Mantel statistic $r = 0.0551$, observed Z = 8,064, average Z = 7,927, $P = 0.023$).

Prediction 4

Given higher overall dispersal rates, and the importance of transmission in constraining parasite population growth (Arneberg et al., 1998), and other basic life history differences, prevalence, abundance, and intensity of amblyceran (*C. turbinatum*) lice are always greater than those of ischnoceran (*D. regalis*) lice, regardless of host group size.

Within the small groups, mean abundance (Table II), intensity (*C. turbinatum* = $12.55^{(7.10\text{-}18.61)}$, *D. regalis* = $3.92^{(1.96\text{-}6.00)}$, $P = 0.0465$, $t = 2.690$) and median intensity (*C. turbinatum* = 7, *D. regalis* = 1, $P = 0.007$), were significantly higher in *C. turbinatum* than *D. r regalis*. The same pattern was observed within large groups for mean abundance (Table II), intensity (*C. turbinatum* = $33.60^{(25.64\text{-}41.48)}$, *D. regalis* = $6.79^{(4.00\text{-}9.79)}$, $P = 0.0000$, $t = 5.872$), and median intensity (*C. turbinatum* = 32, *D. regalis* = 6, $P = 0.000$). Prevalence of *C. turbinatum* was higher than *D. regalis* within both small and large groups, but only significantly so in large groups (Table II).

DISCUSSION

"One of the most intimate of biological relationships is that which exits between a parasite and its host. This closeness is strikingly illustrated by the Mallophaga or avian biting lice. . ." (Foster, 1969).

Regardless of the metric used, birds from larger groups harbored significantly larger numbers of lice (both species combined) than birds from smaller groups (Table II). This result is largely consonant with epidemiological theory (Anderson and May, 1978; May and Anderson, 1978; Dobson, 1990; Arneberg et al., 1998), and corroborates findings of similar studies that have examined parasite population sizes in relation to host density and group size (e.g., Hoogland, 1979; Brown and Brown, 1986; Moore et al., 1988; Côté and Poulin, 1995; Arneberg et al., 1998; Avilés and Tufiño, 1998; Hoi et al., 1998; Krasnov et al., 2002; cf. Rózsa 1997). However, the situation here was more complex than this generalization. When the two louse suborders are considered separately, our study was the first, to our knowledge, to document significant differences in abundance and intensity between these louse clades, in the context of host sociality. Thus, differences in parasite natural history should be considered when such comparisons are made because disparate responses by each species to host sociality may yield misleading results and incorrect interpretations thereof.

Galapagos Hawks within small groups harbored similarly aggregated (where most hosts harbored few parasites and few hosts harbored many parasites) distributions of both louse species. As host group size increased, however, the more mobile amblyceran (*C. turbinatum*) was less aggregated among hosts than the less mobile ischnoceran (*D. regalis*). Rózsa et al. (1996) found similar patterns between colonial vs. territorial crow

(*Corvus* spp.) host species. The amblycerans (*Myrsidea* spp.) were less aggregated within the colonial host species' population relative to those found within the territorial host's, whereas the ischnocerans (*Philopterus* spp.) were similarly aggregated in a negative binomial fashion between the 2 host populations. Thus, in both cases only the populations of amblyceran lice responded positively to an increase in degree of host sociality. Similarly, a more general comparative study of louse distributions in the context of sociality (where both louse suborders were pooled) found that lice were less aggregated among social hosts than asocial hosts (Rékáksi et al., 1997). However, these studies were interspecific in nature, and our results, although similar, are novel because we compared intraspecific variance in host sociality.

Our bivariate correlational analysis revealed significant similarities in abundances of *C. turbinatum*, but not *D. regalis*, between individuals of the same sex within a reproductive coalition. To our knowledge, this is the first report demonstrating such differences between these louse lineages. In a separate analysis that used data from more individuals within polyandrous groups, the differences between individuals in their louse abundances were significantly smaller in magnitude among polyandrous group members than non-group members, for both louse species, although a much stronger relationship was found for *C. turbinatum* than *D. regalis*.

The most parsimonious explanation for these results is that repeated horizontal transfer of lice occurs between individual group-members during sexual contact, provisioning of the young at the nest, or while roosting communally, as others have argued for similar findings within inter-sex breeding pairs (Potti and Merino, 1995; Blanco et al., 1997; Darolova et al., 2001). Other general avenues for louse transmission

include loose feathers, shared dust baths, and phoresis via hippoboscid flies, which are present on Galapagos Hawks (de Vries, 1973, 1975; Keirans, 1975; Clayton et al., 2003). Transmission via phoresy by hippoboscid flies as a source of dispersal between hosts seems unlikely to explain the patterns observed here given that ischnocerans, not amblycerans, are most commonly observed attached thereto (Keirans, 1975). Specifically, morphological constraints prevent most amblycerans, e.g., *C. turbinatum,* from effectively dispersing via hippoboscids. If phoresy was a driving force in the dispersal of *D. regalis*, patterns in abundance between host classes should have been similar to those of *C. turbinatum*, which they were not.

We expected and found that differences in both host and parasite biology reflected differences in parasite infection abundance, intensity and their distribution among hosts. This prediction was formulated in part because Amblycera are generally "more mobile than Ischnocera" (Clayton and Tompkins, 1995). In particular, *C. turbinatum* individuals run rapidly on the host's body surface (Nelson and Murray, 1971) and amblycerans in general are readily horizontally transmitted to humans who have handled their hosts (Ash, 1960; Eveleigh and Threlfall, 1976; N. K.Whiteman, pers. obs.); *C. turbinatum* has an unusually large host-range (Price and Beer, 1963; Askew, 1971; Marshall, 1981' Price et al., 2003) relative to the host-restricted ischnoceran *D. regalis* (Clay, 1958; Price et al., 2003).

Within the ecologically simplified setting of the Galapagos Islands, where no other known host is present, *C. turbinatum* was both more widespread among Galapagos Hawk hosts, and more abundant, than *D. regalis*, regardless of the degree of host sociality. Similar logic derived from observations on basic differences in parasite life

histories, e.g., inferred dispersal abilities, has been used to formulate hypotheses and to interpret results regarding studies of micro- and macro-evolutionary processes and patterns within the Phthiraptera (Johnson et al., 2002a, b; Clayton and Johnson, 2003).

Although parasite populations with negligible effects on host fitness will increase in size with increasing host density/group size (Arneberg et al., 1998), it is worthwhile to consider the implications if the parasites actually had an impact on host fecundity or mortality. First, host infestation by lice may lead to decreased fitness (Derylo, 1974; DeVany, 1976; Richner et al., 1993), feeding efficiency (DeVaney, 1976), survivorship (Brown et al., 1995; Clayton et al.,1999), thermoregulatory abilities (Booth et al., 1993), male courtship displays (Clayton, 1990), male mating success (Borgia and Collis, 1989), and increased mortality (Ash, 1960; Eveleigh and Threlfall, 1976; Marshall, 1981). Second, parasite transmission modes and virulence are linked (Ewald, 1994, 1995; Clayton and Tompkins, 1994, 1995). Horizontal transmission allows parasites to evolve increased virulence relative to those vertically transmitted, because the fitness of horizontally transmitted parasites is not tied to the hosts', unlike that of vertically transmitted parasites (Ewald, 1994, 1995; Clayton and Tompkins, 1994, 1995). Thus, the formation of larger groups of Galapagos Hawks, which have higher overall loads of *C. turbinatum*, may be disfavored if such parasites are at least partially horizontally transmitted and negatively affect host fitness. The relatively low level of aggregation (and high *k* value of 1.95) of *C. turbinatum* within hosts from large groups may correlate with high virulence. Hudson and Dobson (1995) observed that *k* values for macroparasites typically ranged from 0.1- 1.0 and values above this were correlated with parasite populations that regulated host populations. The hypothesized mechanism lies in

the observation that aggregation of parasites within a small proportion of the host population stabilizes host-parasite interactions and departure from this distribution destabilizes them (Anderson and May, 1978; May and Anderson, 1978; Hudson and Dobson, 1995). These factors may explain why polyandrous groups of 2 to 3 males are the most typical size within the populations of Galapagos Hawks considered in this study, and why larger groups are more rare (Donaghy Cannon, 2001).

The breadth of influence imposed by pathogens on the evolution of breeding systems extends beyond the classical parasite-mediated sexual selection paradigm of Hamilton-Zuk (1982), particularly if pathogens are capable of horizontal transmission (Antonovics et al., 2002). Thus, more general ecological phenomena, e.g., classic density dependence of parasite population size on host density, may continue to influence the evolution of the host's reproductive tactics (e.g., Brown and Brown, 1996, 2000). This study shows that emergent phenomena, such as host-parasite interactions, only make sense in the context of the basic life-history characteristics of each participant.

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Figure 1

A. Box and whisker plots of mean (solid line within box) and median (dotted line within box) intensity (uninfected hosts are not considered by these measurements) of *Colpocephalum turbinatum* among territorial adult male Galapagos Hawks (*Buteo galapagoensis*) from small (1-3 males/group; n = 31) and large (4-6 males/group; n = 25) breeding groups. Hosts from small groups yielded significantly lower mean ($t = -4.002$, two-sided $P = 0.0005$) and median ($P = 0.000$) intensities of *C. turbinatum* than those from large groups at the 95% level. Dots above and below whiskers are 5th and 95th percentiles.

B. Box and whisker plots of mean (solid line within box) and median (dotted line within box) intensity (uninfected hosts are not considered by these measurements) of *Degeeriella regalis* among territorial adult male Galapagos Hawks (*Buteo galapagoensis*) from small (1-3 males/group; n = 25) and large (4-6 males/group; n = 19) breeding groups. Hosts from small groups yielded mean ($t = -1.541$, two-sided $P = 0.1275$) and median ($P = 0.066$) intensities of *D. regalis* intensities that, at the 95% level, were equal to those from large groups. Dots above and below whiskers are 5th and 95th percentiles.

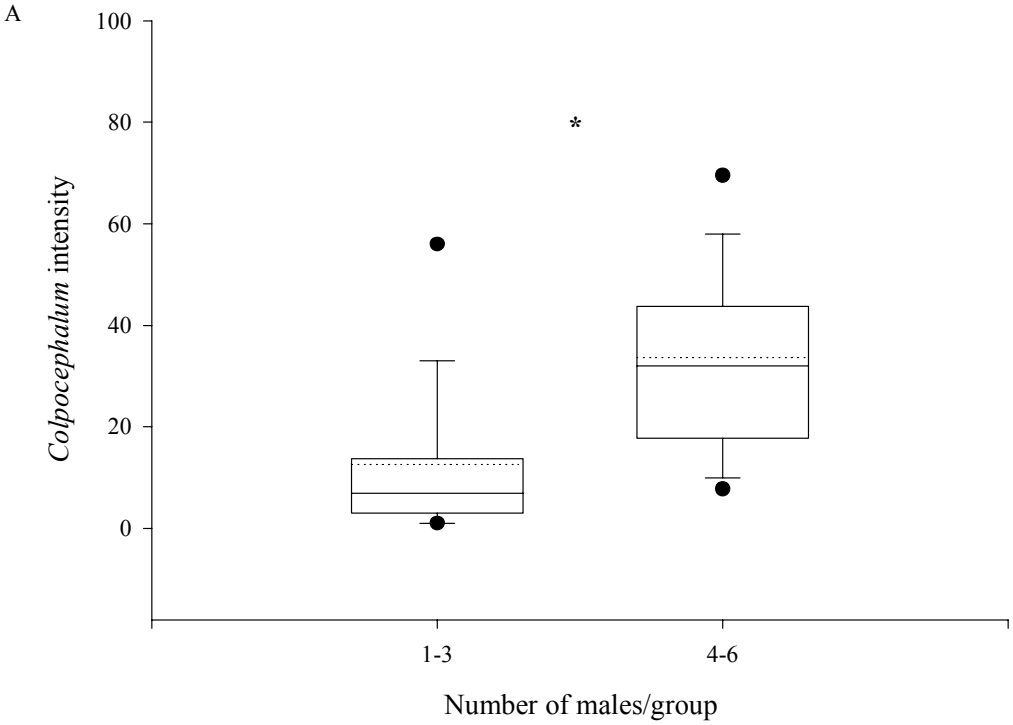
Figure 2

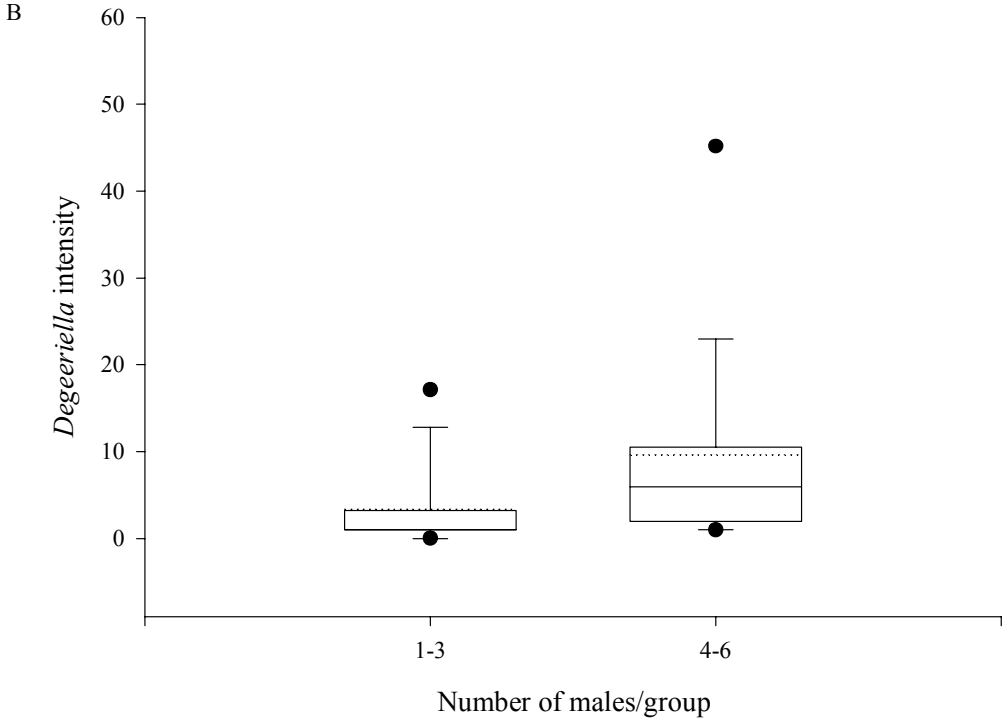
A. Frequency distributions of *Colpocephalum turbinatum* among territorial male Galapagos Hawks (*Buteo galapagoensis*) from small (1-3 males/group; n = 34) and large (4-6 males/group; n = 26) breeding groups. The 2 distributions were significantly different from each other (most-extreme absolute differences = 0.670, Kolmogorov-Smirnov $Z = 2.571$, $P = 0.000$).

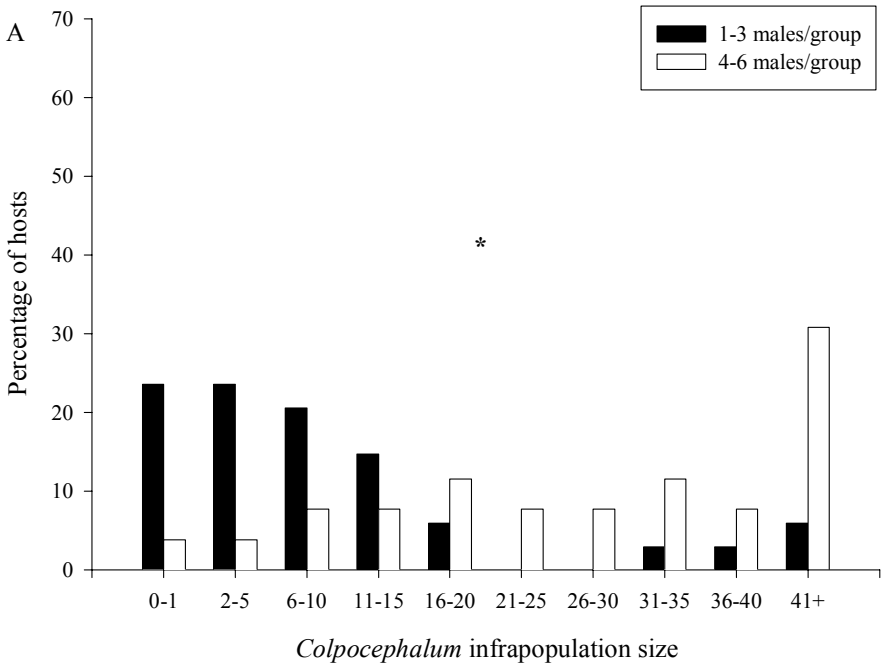
B. Frequency distributions of *Degeeriella regalis* among territorial male Galapagos Hawks (*Buteo galapagoensis*) from small (1-3 males/group; n = 34) and large (4-6 males/group; n = 26) breeding groups. In order to test if the 2 distributions differed, frequency classes were pooled such that each class contained ≥ 3% of the total. The 2 distributions were not significantly different from each other (most-extreme absolute differences = 0.267, Kolmogorov-Smirnov Z = 1.025, $P = 0.244$).

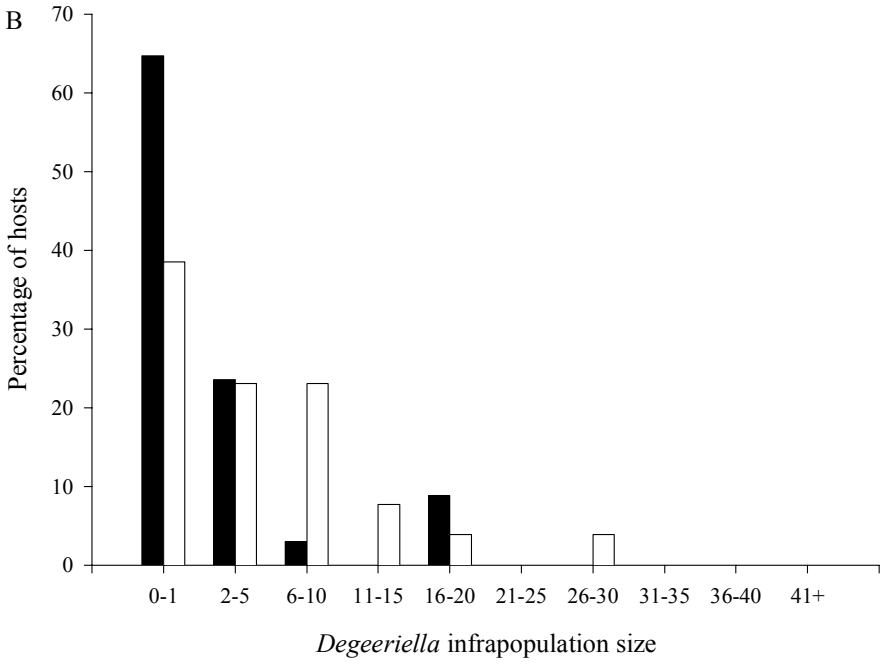
Figure 3

Scatterplot of correlation between *Colpocephalum turbinatum* abundances (*ln* transformed) from 19 dyads of male Galapagos Hawks (*Buteo galapagoensis*). Dyads represent louse abundances of 2 males from the same polyandrous group. In cases where the number of males sampled/group > 2, dyads are comprised of 2 randomly chosen males from the same polyandrous group. Groups were sampled only once. Pearson's $r = 0.711$, $P = 0.000$ (1-tailed); after controlling for the effect of group size, Pearson's $r = 0.66$, $P = 0.001$ (1-tailed).


A
100
80
60
40
20
0
*
Colpocephalum intensity
1-3
4-6
Number of males/group


B
60
50
40
30
20
10
0
Degeeriella intensity
1-3
4-6
Number of males/group


A
1-3 males/group
4-6 males/group
Percentage of hosts
70
60
50
40
30
20
10
0
*
0-1
2-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
41+
Colpocephalum infrapopulation size


B
Percentage of hosts
70
60
50
40
30
20
10
0
0-1
2-5
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16-20
21-25
26-30
31-35
36-40
41+
Degeeriella infrapopulation size

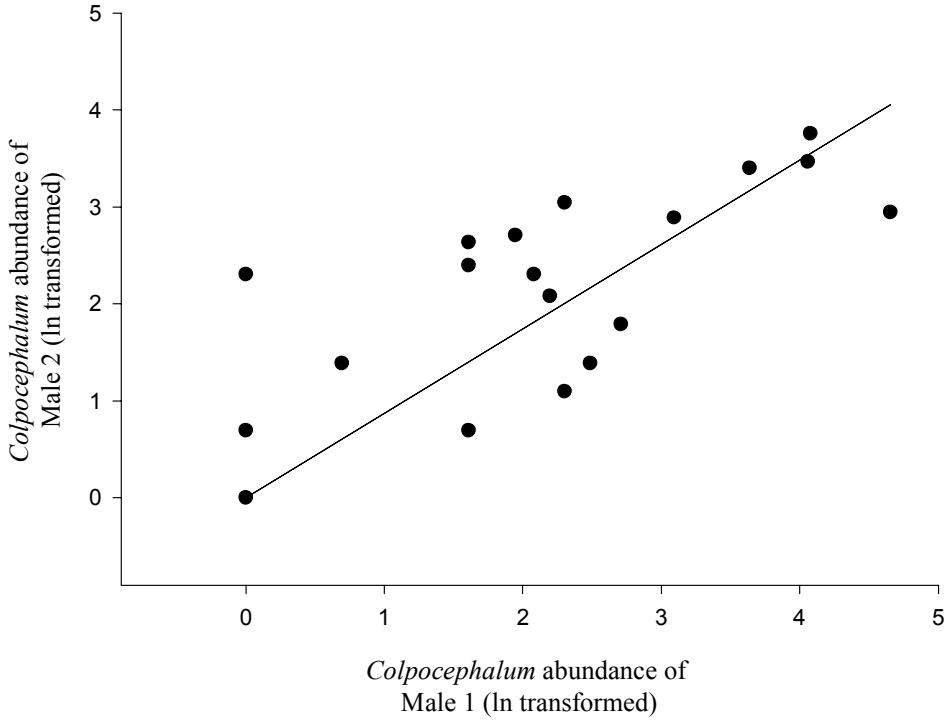
5
4
3
2
1
0
0
1
2
3
4
5
Colpocephalum abundance of Male 2 (ln transformed)
Colpocephalum abundance of Male 1 (ln transformed)

Table I

Comparisons of *Colpocephalum turbinatum* and *Degeeriella regalis* (Insecta: Ph hiraptera) loads between Marchena and Santiago island populations of *Buteo galapagoensis* (Aves: Falconiformes).

Metric	**Parasite**	**1-3 males Marchena (n = 9)**	**1-3 males Santiago (n = 25)**	***P***	***t***	**4-6 males Marchena (n = 5)**	**4-6 males Santiago (n = 21)**	***P***	***t***
Prevalence*	*C. turbinatum*	88.9%	92.0%	1.000	-----	100%	95.2%	1.000	-----
	D. regalis	77.8%	68.0%	0.692	-----	100%	66.7%	0.278	-----
Mean abundance†	*C. turbinatum*	13.889	10.560	0.6460	0.475	47.000	28.810	0.3665	1.158
	D. regalis	4.111	2.160	0.4275	0.889	10.600	3.619	0.2550	1.378
Mean intensity‡	*C. turbinatum*	15.625	11.478	0.5940	0.545	47.000	30.250	0.3870	1.068
	D. regalis	5.286	3.176	0.4665	0.780	10.600	5.429	0.3570	1.015
Median intensity§	*C. turbinatum*	7.5	7.0	1.000	-----	43.0	30.5	0.623	-----
	D. regalis	2.0	1.0	0.659	-----	6.0	5.0	1.000	-----

FOOTNOTE

*Prevalences were compared with Fisher's exact tests. †Abundances and ‡intensities were each compared with two-sample bootstrap *t*-tests. §Medians were compared with Mood's test of comparing medians. All *p* values are 2-sided.

Table II

Prevalences, mean infection abundances, and degree of aggregation (*k* and D) of the lice *Colpocephalum turbinatum* and *Degeeriella regalis* (Insecta: Phthiraptera) within and between small and large groups of *Buteo galapagoensis* (Aves: Falconiformes) from Galapagos, Ecuador.

	Prevalence			**Mean abundance***				***k***		***D***	
	1-3 males	4-6 males	*P*‡	1-3 males	4-6 males	*t*‡	*P*‡	1-3 males	4-6 males	1-3 males	4-6 males
Both species	91.2%	96.2%	0.626	15.71(9.58-22.81)	38.76(28.76-48.60)	-3.682	0.0000	0.78	1.91	0.571	0.350
C. turbinatum	91.2%	96.2%	0.626	11.44(5.94-16.53)	32.31(24.15-40.15)	-4.036	0.0000	0.71	1.95	0.597	0.344
D. regalis	73.5%	73.1%	1.000	2.88(1.29-4.44)	4.96(2.65-7.31)	-1.374	0.1760	0.59	0.61	0.667	0.595
t†	------	------	------	2.908	6.061						
P†	0.109	0.050		0.0400	0.0000						

FOOTNOTE

n = 34 hosts from groups with 1-3 males/group and n = 26 hosts from groups with 4-6 males/group. Fisher's exact tests were used to compare prevalences; 2-sample bootstrap *t*-tests were used to compare mean abundances; all *p* values are 2-sided. *Values in parentheses indicate 95% bootstrap confidence limits around the mean abundance (2,000 replications). †Values represent separate comparisons between *C. turbinatum* and *D. regalis* within small and large host groups. ‡Values represent comparisons within a taxonomic class (both species, *C. turbinatum* only and *D. regalis* only).

Chapter V.

Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylogenetic patters.

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ABSTRACT

Differences in dispersal abilities have been implicated for causing disparate evolutionary patterns between *Columbicola* and *Physconelloides* lice (Insecta: Phthiraptera). However, no study has documented straggling (when lice are found on atypical hosts) rates within these lineages. We used the fact that the Galapagos Hawk, *Buteo galapagoensis* (Gould) (Falconiformes) feeds on the Galapagos Dove *Zenaida galapagoensis* Gould (Columbiformes) within an ecologically simplified setting. The Galapagos Dove is the only typical host of *Columbicola macrourae* (Wilson) and *Physconelloides galapagensis* (Kellogg and Huwana) in Galapagos. We quantitatively

sampled and found these lice on both bird species. A DNA barcoding approach confirmed that stragglers were derived from Galapagos doves. We also collected a *Bovicola* sp. louse, likely originating from a goat (*Capra hircus*). On hawks, *C. macrourae* was significantly more prevalent than *P. galapagensis*. On doves, the two lice were equally prevalent and abundant. Differences in prevalence on hawks was a function of differences in straggling rate between lice, and not a reflection of their relative representation within the dove population. This provides further evidence that differences in dispersal abilities may drive differences in the degree of cospeciation in *Columbicola* and *Phyconelloides* lice, which have become model systems in evolutionary biology.

Key words: Cospeciation, DNA barcoding, Dove, Galapagos, Lice, Straggling.

1. Introduction

Since lice are the most species-rich lineage of ectoparasite, understanding the ecological processes driving their evolution is of general interest to evolutionary biologists (Marshall, 1981; Clayton et al., 2003 a, b). The dispersal (and by some, establishment) of a louse species from the typical host species to an atypical one has variably been referred to as host transfer (Kethley and Johnston, 1975), host switching (Clayton et al., 2003 a), 'straggling' (Rózsa, 1993) and secondary interspecific infestation (Clay, 1949). Straggling and subsequent host-switching is accepted as a powerful force in phthirapteran evolution (Clay, 1949; Rózsa, 1993; Tompkins and Clayton, 1999; Johnson et al., 2002 a, b, c; Clayton and Johnson, 2003). Natural 'straggling' and host-switching are not synonyms (Rózsa, 1993; Clayton et al., 2003 a). Straggling is the antecedent of host-switching (Rózsa, 1993). The interpretation of straggling as a window into the development of host-switching merits further study.

Differing interspecific rates of louse straggling between hosts may influence long-term evolutionary outcomes (Johnson et al., 2002 a; Clayton and Johnson, 2003). Those louse species that tend to have fidelity to a particular host species over ecological time should have a higher probability of cospeciation, whereas those taxa prone to straggling should show less evidence of cospeciation. "Thus straggling may be of considerable significance, particularly given the expanse of evolutionary time over which repeated dispersal events can eventually yield a successful host switch" (Clayton et al., 2003 a). However, little information is currently available on the ecological processes underpinning phthirapteran evolution (Johnson et al., 2002 a).

Parasite life-history characteristics must be considered when examining coevolutionary and ecological interactions between lice and their hosts (e.g., Johnson et al., 2002 a, b; Clayton and Johnson, 2003; Whiteman and Parker, 2004). For example, a spectacular coevolutionary similarity has been revealed between the phylogenies of *Physconelloides* (Ischnocera: Philopteridae) lice and their New World dove hosts (Aves: Columbiformes: Columbidae). In contrast, no significant cospeciation was found between less host-specific *Columbicola* (Ischnocera: Philopteridae) lice on the same hosts (Clayton and Johnson, 2003). *Columbicola* lice are probably more dispersive than *Physconelloides* lice, which has driven the differing degrees of host-specificity and, eventually, cospeciation in these lineages. This assertion was based on a suite of evidence, including experimental (Dumbacher, 1999), observational (Keirans, 1975), and population genetic (Johnson et al., 2002 a) data on louse biology. The population genetic data showed that *Columbicola* populations harbored significantly less population genetic structure than *Physconelloides* populations. However, no quantitative behavioral or ecological study has unequivocally shown that *Columbicola* lice have a higher straggling rate than *Physconelloides* lice between two populations of hosts in nature. If such ecological data were available, they would have bearing on the macro- and micro-evolutionary evidence that louse dispersal ability is a key influence on the evolutionary trajectories of these particular lineages, which have emerged as a model system in evolutionary biology (Johnson et al., 2002 a, b; Clayton and Johnson, 2003; Clayton et al., 2003 a, b).

A prey-predator host system is a good candidate system in which to evaluate the relative rates of straggling between these louse genera. Clay (1949) postulated that prey

to predator straggling and subsequent host-switching has been important in the evolutionary history of lice, followed by allopatric speciation between lineages on old and new hosts. Johnson et al. (2002 b) have given molecular evidence supporting this notion. Louse species within the *Degeeriella* complex found on the Falconiformes are, in general, more closely related to lice found on non-falconiform birds than they are to each other (Johnson et al., 2002 b).

One potential avenue for exploring dispersal rate differences within a predator-prey system is to use an ecologically simplified natural setting. The low α diversity and high population densities of many species of the Galapagos avifauna renders it a good natural laboratory for studies examining the ecology of host-parasite dynamics. We used the fact that Galapagos hawks, *Buteo galapagoensis* (Gould) (Falconiformes) prey on Galapagos doves, *Zenaida galapagoensis* Gould (Columbiformes) (de Vries, 1975; Donaghy Cannon, unpublished M.Sc. thesis, 2001, Arkansas State University, Jonesboro, Arkansas) within the Galapagos.

In this study, we found that the rate of prey-predator straggling of *Columbicola* and *Physconelloides* lice from doves to hawks was observable and predictable in nature. Moreover, the Galapagos Dove is the only typical host of *Columbicola macrourae* (Wilson) and *Physconelloides galapagensis* (Kellogg and Huwana) in the archipelago. Rock doves (*Columba livia*) occur on islands other than those used in this study; but are not typically host to either of these louse species. Thus, straggling to the predator via a host other than the Galapagos Dove, the only resident columbiform on these islands is unlikely. Similar studies within more diverse communities are likely confounded by the presence of multiple suitable host species.

2. Materials and methods

2.1. Sampling

From 14 May-29 June 2001 and 12-23 June 2002 Galapagos hawks (*B. galapagoensis*) were live captured on Santiago and Pinta islands, respectively, in the Galapagos National Park, Ecuador, using either a pole-noose, baited balchatri-trap (Berger and Mueller, 1959), or by hand from the nest as is described elsewhere (Bollmer et al., 2003; Whiteman and Parker, 2004). From 15 May-29 June 2001, Galapagos doves *(Z. galapagoensis*) were captured on Santiago using hand or mist nets as is described in detail elsewhere (Santiago-Alarcon, unpublished M.Sc. thesis, 2003, University of Missouri-St. Louis, St. Louis, Missouri). Sampling of lice was not carried out on doves from Pinta, due to logistical constraints. Dove and hawk sampling on Santiago was conducted in two general areas: James (Espumilla) Bay, along the western coastline (~00˚20'S, 090˚82'W), and Sullivan Bay, along the eastern shore (~00˚30'S, 090˚58'W). Sampling of hawks on Pinta was conducted near a base camp on the southern shore (~00˚33'N, 090˚44'W). Ectoparasites were quantitatively sampled from the birds via dust-ruffling (Walther and Clayton, 1997) with pyrethroid insecticide (Zema® Flea and Tick Powder for Dogs, St. John Laboratories, Harbor City, California, U.S.A.). The particular quantitative sampling procedure used by us is described in detail elsewhere (Whiteman and Parker, 2004). To avoid human-caused transfer of lice, doves and hawks were handled on separate days and sampling for each involved separate equipment.

2.2 DNA barcoding

Some individuals of *C. macrourae* and *P. galapagensis* are morphologically indistinguishable from some mainland congeners (Clayton and Price, 1999; Price et al.,

1999), and *C. macrourae* from Galapagos doves is indistinguishable from conspecifics collected from Mourning doves (*Z. macroura*) (Clayton and Price, 1999). Thus, to assure our species identifications were correct, we used a DNA barcoding approach (Besansky et al., 2003; Hebert et al., 2003 a, b) to diagnose these louse species and geographical origin (Galapagos vs. mainland). Specifically, mitochondrial DNA from two *Columbicola macrourae* representatives (one each from Galapagos hawk hosts on islas Santiago and Pinta, GenBank accession numbers **AY594662**, **AY594663**), 1 *Physconelloides galapagensis* individual (from a Galapagos Hawk host on Isla Pinta, GenBank accession number **AY594666**) and the *Bovicola* sp. individual (from a Galapagos Hawk host on Isla Santiago, GenBank accession number **AY594667**) was extracted and a 379-bp portion of subunit I of the cytochrome *c* oxidase gene (COI) amplified and sequenced using primers L6625 and H7005, following Johnson et al. (2002 a). For the dove lice, two sequence alignments were created, one each for sequences from *Columbicola* and *Physconelloides*. Specifically, alignments were comprised of straggling louse sequences from Galapagos hawks (using sequences from this study), and of conspecific or congeneric sequences of lice collected from Galapagos doves (using sequences from GenBank and Johnson and Clayton 2003) and their closest relatives (using sequences from Johnson et al., 2002 a), Mourning doves and White-winged doves (*Z. asiatica*). In both phylogenies, louse sequences from White-winged dove hosts were used as outgroups (Clayton and Johnson, 2003). These alignments were subjected to phylogenetic parsimony analysis using Paup* version 4.0b10 (Swofford, 2002) (Fig. 1). The sequences from the nymphal *Bovicola* sp. were compared to those from other trichodectid lice previously sequenced (Johnson et al., 2003).

2.3 *Statistical Analyses*

Prevalence, mean and median intensity, and mean abundances (Margolis et al., 1982; Bush et al., 1997) of the two louse species within each host species were compared using the program Quantitative Parasitology 2.0 (Rózsa et al., 2000; Reiczigel and Rózsa, 2001). Fisher's exact tests were used to compare prevalences of each parasite species (*C. macrourae* vs. *P. galapagensis*) within each host species. Distribution-free two-sample bootstrap *t*-tests were used to compare mean intensities and abundances (each with 2,000 replicates). Mood's median tests were used to compare median (typical) intensities. We report 95% bootstrap confidence intervals (2,000 replications each) for mean abundance and intensity (Rózsa et al., 2000). Since only one *P. galapagensis* individual was collected from the 91 Galapagos hawk hosts sampled, only prevalence and mean abundance were calculated. We also calculated the moment '*k*' of the negative binomial distribution, which is inversely related to the degree of aggregation of parasite abundances among members of the host population (Crofton, 1971), and the index of discrepancy '*D*,' which is directly related to the degree of aggregation of parasite abundances among members of the host population (Poulin, 1993). The index of discrepancy is the degree to which the observed distribution of parasites among the host population differs from a hypothetical one in which each host harbors the same number of parasites (Poulin, 1993).

3. Results

A total of 60 individuals of the Galapagos hawk, including two nestlings, were live captured on Isla Santiago, and a total of 31 individuals were captured on Isla Pinta. On Santiago, a total of 1,602 lice were collected from the 60 hawks, of which 10 lice on

six hawks represented stragglers for which hawks are atypical hosts (Table 1). On Pinta, a total of 3,306 lice were collected from the 31 hawks, of which four lice on four hawks represented stragglers for which hawks are atypical hosts (Table 1). In total, straggling lice were collected from 10 different Galapagos hawk host individuals out of the 91 sampled (Table 1). Eight Galapagos hawks harboured 12 individuals of *C. macrourae*. Notably, two hosts from different hawk social groups on Santiago harbored individuals of both sexes (Table 1). In two cases, two hawks from the same social group each harbored a *C. macrourae* individual (from one territory on Santiago and one on Pinta) (Table 1). Only one *P. galapagensis* individual was collected from a single hawk host on Pinta (Table 1). For both islands combined, prevalence of *C. macrourae* on hawks was significantly higher than that of *P. galapagensis* (Table 2). Only one nymphal *Bovicola* sp. was collected from a hawk on Santiago, where its prevalence was 1.67% (1/60 hosts infected) (Table 1). Thus, for both islands combined, its prevalence on hawks was 1.1% (1/91 hosts infected). All stragglers were deposited in the Phthiraptera collection of the Illinois Natural History Survey, Champaign, Illinois.

A total of 28 individuals of the Galapagos Dove were live captured on Isla Santiago. A total of 851 *C. macrourae* and 863 *P. galapagensis* were collected from these hosts. Most hosts (>90%) harbored *C. macrourae* and *P. galapagensis* (Table 2). Prevalence, mean abundance, intensity and median (typical) intensity of the two louse species were not significantly different within the Santiago dove population. The populations of *C. macrourae* and *P. galapagensis* were similarly aggregated among members of the dove population (Table 2).

The two *C. macrourae* COI sequences obtained from Galapagos hawks were identical to each other and identical to a sequence from an individual collected from a Galapagos Dove on Isla Santa Fe, Galapagos (Fig. 1). These sequences differed by about 0.5 % from two sequences from *C. macrourae* from Galapagos doves on Isla Genovesa (Fig. 1). In contrast, the difference between sequences of *C. macrourae* from Galapagos doves and Mourning doves (Johnson et al., 2002 a) is about 3.3%, indicating that the COI gene provides a "barcode" to identify the host of origin. *Columbicola macrourae* from White-winged doves (Johnson et al., 2002 a) is even more divergent, about 19% from the populations on the Galapagos doves and Mourning doves. The single *P. galapagensis* COI sequence from a Galapagos Hawk was identical to one *P. galapagensis* sequence collected from Galapagos Dove from Isla Genovesa, Galapagos (Johnson and Clayton, 2003) (Fig. 1).

Since the *Bovicola* individual was a nymph, identification to species based on morphology is not possible. Neighbor joining analysis using Paup* version 4.0b10 (Swofford, 2002) involving 380 species of lice (Johnson et al., 2003, unpublished data) indicated the *Bovicola* sp. individual from a Galapagos hawk was most genetically similar to *Bovicola bovis* from a domestic cow (*Bos taurus*), but differing by 21.8%, clearly indicating it is a different species. Although, COI sequences from *Bovicola* from goats were not available for comparison, this was likely the original host based on possible hosts for *Bovicola* on Isla Santiago.

4. Discussion

We found three straggling louse species on 10 different Galapagos Hawk hosts. These stragglers are species normally associated with Galapagos doves and goats. Given

that some lice from Galapagos doves cannot be morphologically distinguished from lice on other hosts (e.g., Mourning doves), a DNA barcoding approach was necessary to clearly identify the host of origin (Besansky et al., 2003; Hebert et al., 2003 a, b). We were able to do this in the case of *C. macrourae* and *P. galapagensis*. The ability to determine the source host for the straggling parasites demonstrates the utility of using ecologically simplified settings in which to examine host-parasite ecology. We found that *C. macrourae* were significantly more prevalent than *P. galapagensis* among Galapagos hawks, though our sample sizes were small. In contrast, the prevalence, average abundance, intensity and typical intensity of these species did not differ within the sympatric dove prey population sampled simultaneously. Thus, the difference in prevalence on hawks was likely a function of louse biology, and not an artifact of differences in louse population ecology within the source host's population.

To our knowledge, this is the first report of the straggling rate of *Columbicola* or *Physconelloides*, and the first report of a trichodectid louse straggling to a falconiform host. Previously, two *Buteo b. buteo* specimens were found to be host to one specimen each of *Columbicola columbae columbae* (L.) (Pérez et al., 1988; *C. columbae*, Price et al., 2003). However, the hosts were captive specimens, thus human contamination or the artificial conditions of captivity may have facilitated transfer. Other reports from the Old World include *C. columbae* from *Falco aesalon* Tunstall, (Séguy, 1944), *Aviceda l. leuphotes* Dumont, and *Haliastur i. indus* Boddaert (Tendeiro, 1965), and *C. columbae bacillus* (*C. bacillus*, Price et al., 2003) from *Milvus milvus* (Mocci Desmartis and Restivo de Miranda, 1978). Our study, which included New World louse species studied in population genetic and phylogenetic studies, is germane to the finding that

Columbicola species have less population genetic structure within species, and less evidence for cospeciation with their hosts than *Physconelloides* species (Johnson et al., 2002 a; Clayton and Johnson, 2003).

Galapagos hawks routinely feed on and provision their young with Galapagos doves and goats, which they have killed or scavenged in the case of goats (de Vries, 1975; Donaghy Cannon, unpublished M.Sc. thesis, 2001). For example, on Santiago in 2000, a total of 69 Galapagos Dove individuals were brought to 11 nests where prey deliveries were observed (nests were monitored from 36.0–64.2 hours each; Donaghy Cannon, unpublished M.Sc. thesis, 2001). Hawks were also observed depredating on Galapagos doves on Pinta during this study (T. de Vries, personal communication). Thus, the presence of *C. macrourae* and *P. galapagensis* on Galapagos hawks is most parsimoniously explained by horizontal transfer of these lice from Galapagos doves to hawks after hawks captured them as prey. That a *C. macrourae* individual was collected from a nestling hawk was probably the result of transfer at the nest from a dove killed by one of its parents. Similarly, two territorial adult female hawks successfully killed newborn goats and goat parts were brought to nests on Santiago in three instances each in 1999 and 2000 (Donaghy Cannon, unpublished M.Sc. thesis, 2001). Horizontal transfer also most parsimoniously explains the presence of a Bovicola individual on a hawk host from a goat host after hawk depredation.

Galapagos hawks are not known to share nests or dust baths with doves, which were two other mechanisms proposed for straggling (Clay, 1949; Timm, 1983; Clayton et al., 2003 a). However, another reasonable dispersal avenue for these lice is horizontal transfer of *C. macrourae* and *P. galapagensis* via hippoboscid flies from doves to hawks

(Keirans 1975). The hippoboscid fly *Microlynchia pusilla* (Speiser), typically found on columbiforms, was collected from a Galapagos Hawk host on Española Island, Galapagos in 1929 (Bequaert, 1933). Thus, transient *M. pusilla* with phoretic *C. macrourae* or *P. galapagensis* individuals attached, could have contacted a Galapagos Hawk host followed by subsequent dispersal of the louse or lice.

Straggling is a combination of "variables influencing dispersal" and "variables influencing establishment" (Clayton et al., 2003 a). In this case, prevalence of both *C. macrourae* and *P. galapagensis* on their typical Galapagos dove hosts is high (>90%). Our finding that ~9% of hawk hosts harbored at least one *C. macrourae* individual may indicate that these hawks are not as effective as other doves are in killing *Columbicola* lice by preening. Galapagos hawks do not harbor their own "wing" lice such as *Falcolipeurus* species, which normally take refuge between feather barbs. It is reasonable to assume that efficiency of wing feather preening is relaxed in the absence of such parasites and that straggling wing lice may be able to survive on these hosts. *Columbicola* lice can establish populations on doves that are an order of magnitude different in body size, but only when host defenses are impaired (Clayton et al. 2003 a, b). Thus, the greater dispersal abilities of *Columbicola* lice combined with the absence of a typical "wing" louse and host defenses, may account for its surprisingly high rate of straggling. The low rate of straggling in *P. galapagensis* is unsurprising given that it does not take refuge between feather barbs, and it is less likely to disperse than *Columbicola*. Experimental transfers of these lice would clarify the importance of these and other variables in determining success of straggling (*sensu* Tompkins and Clayton,

1999), but are not especially feasible considering the threatened status of *B. galapagoensis*.

In conclusion, predictable differences in straggling rates between two louse lineages were observed in a sympatric avian prey-predator system within a simplified ecosystem. This study adds to the accumulating evidence indicating the importance of basic differences in life history in creating evolutionary patterns between these louse lineages, which are quickly becoming a model system in ecology and evolutionary biology. It is also notable that dove lice have the potential to transmit other parasites to hawks (e.g., Harmon et al., 1987; Hong et al., 1989; McQuistion, 1991; Mete et al., Padilla et al., 2004).

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Table 1

All straggling lice (Insecta: Phthiraptera) collected after sampling from 91 Galapagos hawk (*Buteo galapagoensis*) (Aves: Falconiformes) hosts captured on Santiago and Pinta islands, Galapagos (during 2002 and 2003 respectively). Abbreviations are: A = adult, M = male, F = female, T = territorial, L= non-territorial (floating), N = nymph.

Straggler species	**Island**	***Buteo galapagoensis* data (band, age, sex, territorial status)**	**Straggler data (abundance, age, sex)**
I. *Columbicola macrourae*	Santiago	Red 24, AMT	3 AF, 1 AM
	Santiago	Red 26, AMT	1 AF, 1 AM
	Santiago	Blue 2M, JM (nestling)	1 AM
	Santiago	Green 2R, AMT	1 AM
	Santiago	Black 35, AMT (in same territory as Green 2R)	1 AF
	Pinta	Black 49, AMT	1 AM
	Pinta	Red OX, AFT	1 AF
	Pinta	Black 36, AMT (in same territory as Red OX)	1 AF
II. *Physconelloides galapagensis*	Pinta	Black 41, JML	1 AF
III. *Bovicola* sp.	Santiago	Blue 4P, JFL	1 N

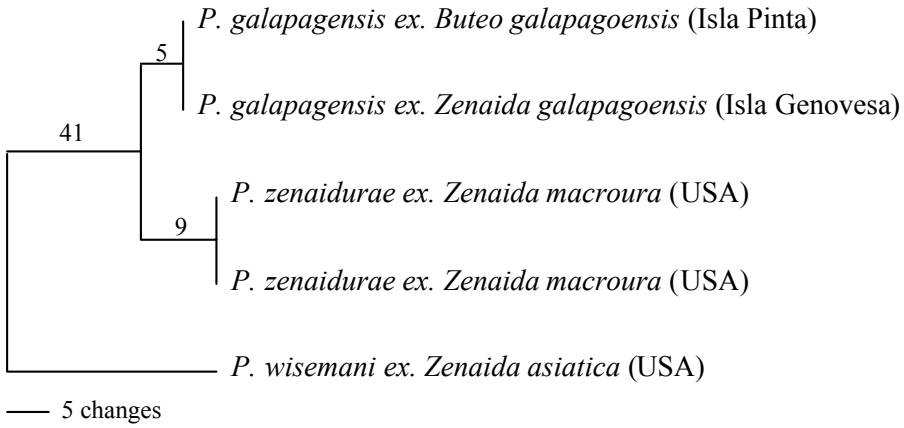
Table 2

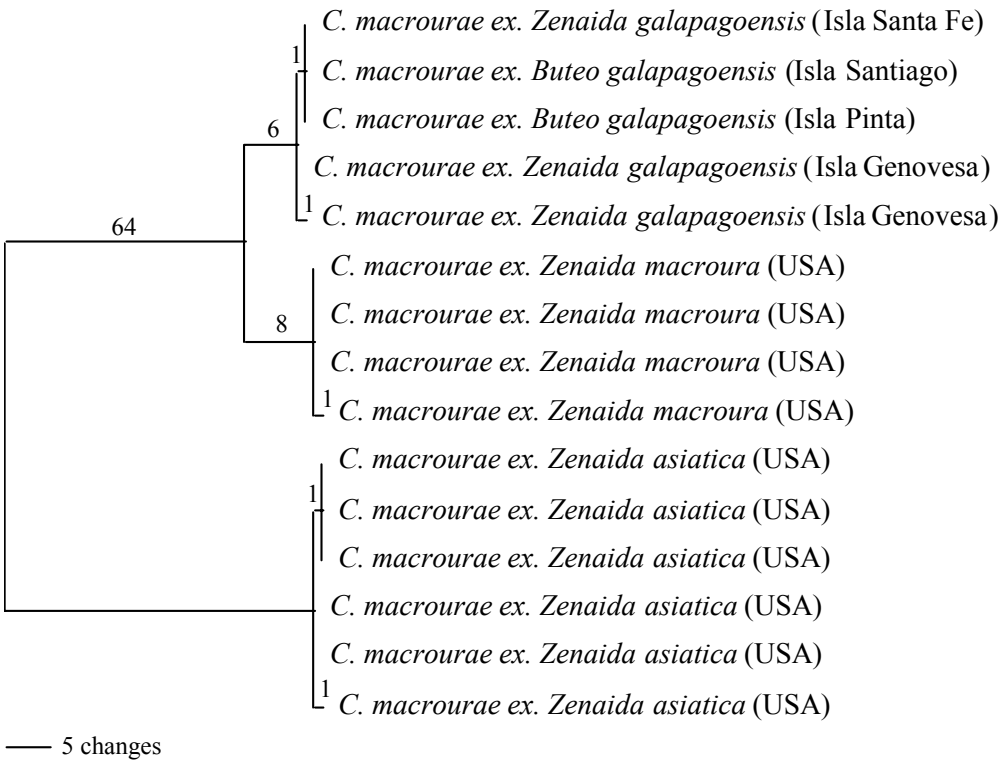
Prevalences, mean infection abundances, and degree of aggregation (*k* of the negative binomial and *D*, the index of discrepancy) of 12 *Columbicola macrourae* lice (Insecta: Phthiraptera) collected from 91 Galapagos hawks (*Buteo galapagoensis* (Aves: Falconiformes) host individuals from Santiago (in 2002) and Pinta (in 2003) islands (values are in first row in each category), and 851 *C. macrourae* and 863 *Physconelloides galapagensis* lice collected from 28 Galapagos Dove (*Zenaida galapagoensis*) (Aves: Columbiformes) host individuals from Santiago Island, Galapagos, Ecuador in 2002 (values are in second row in each category). Fisher's exact tests were used to compare prevalences of each parasite species (*C. macrourae* vs. *P. galapagensis*) within each host species and the associated *P* values are reported below. Distribution-free two-sample bootstrap *t*-tests were used to compare mean intensities and abundances (each with 2,000 replicates) of each parasite species within each host species; the *t* values and associated *P* values of which are reported below. Similarly, mood's median tests were used to compare median (typical) intensities; the *P* values of which are reported below. Because only one *P. galapagensis* individual was collected from the 91 Galapagos hawk hosts sampled, only prevalence and mean abundance were calculated for this louse species. Numbers in parentheses are 95% bootstrap (2,000 replications) confidence intervals.

Louse Species	*C. macrourae*	*P. galapagensis*	t	P
Prevalence	8.8% (3.87-16.59) 96.4% (81.65-99.91)	1.1% (0.02-5.0) 92.9% (76.49-99.13)	N/A N/A	0.034 1.000
Mean Adundance	0.132 (0.02-0.22) 30.393 (22.43-38.39	0.011 (0.00-0.02) 30.821 (20.75-41.57)	2.170 -0.062	0.0990 0.9485
Mean Intensity	1.5 (1-2) 31.519 (23.07-39.63)	N/A 33.192 (23.00-44.31)	N/A -0.237	N/A 0.8030
Median Intensity	1.0 (1-2) 30.0 (19-41)	N/A 24.5 (10-36)	N/A N/A	N/A 0.414
k	0.13 1.34	N/A 0.93		
D	0.926 0.384	N/A 0.477		

Legend to Figure

Fig. 1. Most parsimonious phylogenetic tree generated in Paup* version 4.0b10 (Swofford, 2002) based on 379 bp of the mitochondrial cytochrome *c* oxidase subunit I gene for *Physconelloides* (*P.*) and *Columbicola* (*C.*) lice from doves in the genus *Zenaida*. Trees include sequences for "stragglers" of these genera on Galapagos hawks (*Buteo galapagoensis*). Each louse sequence was derived from a different host individual; each terminus represents one louse sequence from (*ex*) a unique host individual, followed by the collection locality (USA or islands within the Galapagos). Branch lengths appear as numerals along branches and are proportional to reconstructed changes using maximum parsimony; the branch length scale is indicated below each tree.


P. galapagensis ex. Buteo galapagoensis (Isla Pinta)
5
P. galapagensis ex. Zenaida galapagoensis (Isla Genovesa)
41
P. zenaidurae ex. Zenaida macroura (USA)
9
P. zenaidurae ex. Zenaida macroura (USA)
P. wisemani ex. Zenaida asiatica (USA)
5 changes


C. macrourae ex. Zenaida galapagoensis (Isla Santa Fe)
1
C. macrourae ex. Buteo galapagoensis (Isla Santiago)
C. macrourae ex. Buteo galapagoensis (Isla Pinta)
6
C. macrourae ex. Zenaida galapagoensis (Isla Genovesa)
1
C. macrourae ex. Zenaida galapagoensis (Isla Genovesa)
64
C. macrourae ex. Zenaida macroura (USA)
C. macrourae ex. Zenaida macroura (USA)
8
C. macrourae ex. Zenaida macroura (USA)
1
C. macrourae ex. Zenaida macroura (USA)
C. macrourae ex. Zenaida asiatica (USA)
1
C. macrourae ex. Zenaida asiatica (USA)
C. macrourae ex. Zenaida asiatica (USA)
C. macrourae ex. Zenaida asiatica (USA)
C. macrourae ex. Zenaida asiatica (USA)
1
C. macrourae ex. Zenaida asiatica (USA)
5 changes

Chapter VI.

Disease Ecology in the Galápagos Hawk (*Buteo galapagoensis*): Host genetic diversity, parasite load and natural antibodies

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ABSTRACT

Erosion of genetic diversity is one factor increasing extinction risk in island endemics and threatened species, but causal mechanisms remain poorly understood. An increased susceptibility to disease is one hypothesis explaining how inbreeding hastens extinction in these populations. Experimental studies show that disease resistance declines as inbreeding increases, but data from *in situ* wildlife systems are scarce. Genetic diversity varies positively with island size across the entire range of an extremely inbred Galápagos endemic bird, providing the context for a natural experiment examining the effects of inbreeding on parasite load and innate, constitutively produced natural antibody (NAb) levels. Extremely inbred populations of Galápagos hawks had higher parasite abundances than relatively outbred populations. We found a significant island effect on NAb levels and inbred populations generally harboured lower average and less variable NAb levels than relatively outbred populations. Furthermore, NAb levels explained abundance of amblyceran lice, which encounter the host immune system. This is the first study linking inbreeding, innate immunity and parasite load in an endemic, *in situ* wildlife

population. By demonstrating that variation in innate immunity may underly the vulnerability of small, natural populations to pathogens, this provides a clear framework for assessment of disease risk in a Galápagos endemic.

KEY WORDS: disease, Galápagos Islands, genetic diversity, immune function, natural antibodies.

1. INTRODUCTION

Extinctions of island endemics account for 75% of animal extinctions and 90% of bird extinctions (Myers 1979; Reid & Miller 1989). Several synergistic key factors may be responsible for this high extinction rate, including introduction of exotic animal and human predators (Blackburn *et al.* 2004), habitat destruction (Rolett & Diamond 2004), demographic stochasticity (Drake 2005), and inbreeding in island endemics and threatened species (Frankham 1998; Spielman *et al.* 2004a).

The interaction of disease agents with genetically depauperate (Pearman & Gamer 2005) and isolated populations is one hypothesis explaining how inbreeding facilitates extinction in small populations (de Castro & Kolker 2005). Parasites evolve more quickly than hosts, so host antiparasite adaptations are perpetually obsolete (Hamilton *et al.* 1990; Lively & Apanius 1995). Consequently, genetically uniform host individuals (Acevedo-Whitehouse *et al.* 2003) and populations (Spielman *et al.* 2004b) are more susceptible to parasitism than genetically diverse hosts. Studies of model laboratory systems (Arkush *et al.* 2002), captive wild (Cassinello *et al.* 2001; Hedrick *et al.* 2001; Hawley *et al.* 2005; Pearman & Gamer 2005), and free-ranging domesticated animal populations (Coltman *et al.* 1999) support this claim, although other studies do not (Trouvé *et al.* 2003) or were equivocal (Wiehn *et al.* 2002). Scant evidence of this phenomenon exists from *in situ* native wildlife populations (Meagher 1999; Reid *et al.* 2003), and no study has

examined the effects of inbreeding on parasite load and innate, humoral immunity across bird populations in the wild (Keller & Waller 2002). The intact endemic avifauna of the Galápagos Islands provides a unique opportunity to examine disease ecology and will provide insight into the impact of invasive disease agents that may enter the ecosystem (Lindström *et al.* 2004; Wikelski *et al* 2004; Gottdenker *et al.* 2005; Thiel *et al.* 2005; Whiteman *et al.* 2005).

The Galápagos hawk (*Buteo galapagoensis*), an endemic raptor threatened with extinction (2004 IUCN Red List), breeds on eight islands within the Galápagos National Park, and has been extirpated from several others (Fig. 1). Island size and genetic diversity are positively related and between-island population structure is high, rendering it an appealing model system in which to examine the effects of inbreeding on disease severity (Bollmer *et al.* 2005). The basic biology of its two chewing louse species (Insecta: Phthiraptera), an amblyceran (*Colpocephalum turbinatum*) and an ischnoceran (*Degeeriella regalis*), has been described (Whiteman & Parker 2004a, b). Thus, we examined the response of each parasite lineage to variance in host inbreeding, using population-level heterozygosity values from the eight island populations of *B. galapagoensis* and one population of the sister species (*B. swainsoni*; Reising *et al.* 2003).

We also examined the relationship between immunological host defences, island-level inbreeding effects, and parasite abundance. To assess immunological host defences, we quantified non-specific natural antibody (NAbs) titres within seven populations of *B. galapagoensis*. Of the several methods available to assess comparative immune response in vertebrates, quantification of NAbs has several conceptual and methodological advantages (Matson *et al.* 2005). NAbs are a product of the innate, humoral immune system and their production is constitutive (stable over

time and generally not induced by external antigenic stimulation). Encoded by the germ-line genome, NAbs are present in antigenically naïve vertebrates (Boes 2000; Oschsenbein & Zinkernagel 2000; Baumgarth *et al.* 2005), form a large percentage of the serum immunoglobulin (Kohler *et al.* 2003), and are capable of recognizing any antigen (Adelman *et al.* 2004). In chickens, NAbs reacting to ectoparasite-derived antigens have been identified (Wikel *et al.* 1989) and in lines artificially selected for either high or low levels of specific antibodies, specific and natural antibody levels covary (Parmentier *et al.* 2004). NAb response is hypothesized to predict the strength of the adaptive immune response (Kohler *et al.* 2003). Indeed, NAbs enhance the specific antibody response by providing pre-existing reactivity to novel antigens, which successfully controls early infections by a wide range of parasites during adaptive response activation, and by priming the adaptive immune response through antigen presentation (Adelman *et al.* 2004; Baumgarth *et al.* 2005). Thus, NAbs form a functional link between the innate and acquired parts of the humoral immune system (Lammers *et al.* 2004).

Inbreeding may negatively impact phytohemagglutinin (PHA) induced swelling within wild bird populations (Reid *et al.* 2003), and reductions in population size reduce overall within-population genetic variation, including variation at loci of immunological import in vertebrates (Sanjayan *et al.* 1996; Aguilar *et al.* 2004; Hedrick 2004; Miller & Lambert 2004). Since variation in NAb levels responds to artificial selection in chickens (Parmentier *et al.* 2004), it is reasonable to predict that variation in NAb levels will covary with variation in wild bird population genetic diversity. However, the impact of natural microevolutionary processes on circulating levels of NAbs is unknown in wild vertebrates.

Amblyceran lice (e.g., *C. turbinatum*) directly encounter host immune defences because they feed on epidermal and subepidermal tissues, including blood and living skin (Marshall 1981; Møller & Rózsa 2005). Conversely, bird ischnocerans (e.g., *D. regalis*) generally feed on the keratin of feathers and dead skin (Marshall 1981) and mainly encounter the mechanical host defences (e.g., preening; Møller & Rózsa 2005). Feeding by lice and other ectoparasites on skin and blood elicits immune responses (Wikel 1982) that vary from cell-mediated (Prelezov *et al.* 2002) to humoral (i.e., antibodies; Wikel *et al*. 1989; Ben-yakir *et al.* 1994; Pfeffer *et al.* 1997) and from innate (Wikel *et al.* 1989; Prelezov *et al.* 2002) to acquired (Wikel *et al*. 1989; Minnifeld *et al.* 1993; Ben-yakir *et al.* 1994; Pfeffer *et al.* 1997). Host antibodies reduce louse fecundity and survivorship, and regulate population growth rate (Ben-yakir *et al.* 1994). Across birds species, variation in PHA induced swelling was directly related to amblyceran but not ischnoceran species richness (Møller & Rózsa 2005). However, whether NAbs regulate ectoparasite populations, and louse populations in particular, is unknown.

We measured host inbreeding, parasite abundance and NAb response, and made three predictions: (1) at the island-level, higher inbreeding results in lower average humoral immune response relative to outbred populations; (2) also at the island-level, higher inbreeding results in reduced variation in humoral immune response relative to outbred populations; and (3) birds with high humoral immune responses harbour fewer parasites (amblyceran lice) relative to birds with lower immune responses.

2. METHODS

(a) *Host sampling*

We live-captured a total of 211 *Buteo* hawk individuals on eight of the Galápagos Islands (*n* =202 *B. galapagoensis*; Fig. 1) and near Las Varillas, Córdoba, Argentina (n =9 *B. swainsoni*; Whiteman & Parker 2004a), from May-August 2001 (Islas Española, *n* =8; Isabela, *n* =25; Marchena, *n* =26; Santa Fe, *n* =13), May-July 2002 (Isla Santiago, *n* =58), January 2003 (Argentina, *n* =9), and May-July 2003 (Islas Fernandina, *n* =28; Pinta, *n* =31; Pinzón, *n* =10). Birds were sampled following Bollmer *et al.* (2005), from multiple locations throughout each island. The University of Missouri-St. Louis Animal Care Committee and the appropriate governmental authorities approved all procedures and permits.

(b) *Parasite sampling*

We quantitatively sampled parasites from birds via dust ruffling with pyrethroid insecticide (non-toxic to birds; Zema® Z3 Flea and Tick Powder for Dogs, St. John Laboratories, Harbor City, California; Whiteman & Parker 2004a, b). Dust-ruffling provides excellent measures of relative louse intensity (Clayton & Drown 2001).

(c) *Blood Collection*

From each bird, we collected two 50 µl blood samples via venipuncture of the brachial vein for genetic analyses. Samples were immediately stored in 500 µl of lysis buffer (Longmire *et al.* 1988). For immune assay, whole blood samples were collected from a subsample of birds (*n* = 46) in heparinized tubes, centrifuged in the field and plasma was stored in liquid nitrogen. Due to logistical constraints, no plasma was collected from the Pinzón population of *B. galapagoensis* or from *B. swainsoni*.

(d) *Innate humoral immunity*

We used the general hemolysis-hemagglutination assay protocol (Matson *et al.* 2005) with two minor modifications (we used plates from Corning Costar #3798, instead of #3795 and Dulbecco's PBS, #D8662, Sigma, St Louis, MO). Sample sizes from Galápagos hawk island populations were as follows: Española, n =3; Fernandina, n =15; Isabela, n =3; Marchena, n =5; Pinta, n =7; Santa Fe, n =5; Santiago, n =8. In each plate, we ran the assay on six hawk samples and two positive controls (pooled chicken plasma, #ES1032P, Biomeda, Foster City, CA). Using digitized images of the assay plates, all samples were blindly scored twice to individual, plate number and position. To demonstrate positive standard reliability, assay variation never exceeded 6.8% and 5.6% coefficient of variation (CV=the sample standard deviation/sample mean) for agglutination titres among and within plates, respectively. Mean NAb agglutination titres and CV were calculated for each island population from which plasma was collected. CV is a useful measure in studies such as these, since island population means varied widely and CV is dimensionless and relatively stable compared to standard deviation (Snedecor & Cochran 1989). We also calculated standard deviations for comparative purposes (Fig. 2b).

(e) ***DNA fingerprinting***

To determine island-level population genetic diversity, we performed phenol-chloroform DNA extraction on a subset of hawks from each population comprising a total of 118 individuals (Galápagos hawks: Española, n =7; Fernandina, n =20; Isabela, n =10; Marchena, n =20; Pinta, n =10; Pinzón, n =10; Santa Fe, n =10; Santiago, n =23; Swainson's hawks: n =8), followed by multi-locus minisatellite (VNTR) fingerprinting using the restriction endonuclease *Hae III* and Jeffreys' probe 33.15 (Jeffreys *et al.* 1985) and following procedures described elsewhere for birds

generally (Parker *et al.* 1995) and Galápagos hawks (Bollmer *et al.* 2005). Estimates of island-level population genetic diversity were obtained by calculating multilocus VNTR heterozygosity values (referred to as *H*; Stephens *et al.* 1992) for each island population and for the population of Swainson's hawks using GELSTATS v.2.6 (Rogstad & Pelikan 1996). These markers yield an excellent measure of relative genetic diversity in small, isolated vertebrate populations (Gilbert *et al.* 1990; Stephens *et al.* 1992; Parker *et al.* 1998; Bollmer *et al.* 2005) but do not measure individual heterozygosity values.

A large study on Galápagos hawk population genetics (Bollmer *et al.* 2005) used the same multilocus minisatellite markers to estimate population genetic diversity (and included all of the individuals genotyped here). Bollmer *et al.* (2005) strongly supports the pattern of genetic diversity that we found among these hawk populations. Nearly 90% of the variation in hawk population genetic diversity was explained by island area, and the latter correlates with hawk population size (Bollmer *et al.* 2005). The four smallest islands with hawk populations had the highest reported levels of minisatellite uniformity of any wild, relatively unperturbed bird species.

As in Bollmer *et al.* (2005), we randomly selected individuals sampled within each population to assess the relative amount of genetic diversity within each population. We prioritized samples from adults in territorial breeding groups (groups are comprised of unrelated adults; Faaborg *et al.* 1995). On Isla Pinzón, we sampled only from non-territorial birds from multiple geographic locales because we were unable to capture adults there. However, these birds were likely offspring of multiple breeding groups given that many were of the same age cohort (based on plumage characteristics), and that hawks usually produce only one offspring per breeding attempt. Moreover, marked, non-territorial birds disperse from the natal territory

following fledging and roam over their entire natal islands (de Vries 1975; Faaborg 1986; Bollmer *et al*. 2005). To ensure that our sampling of birds was not biased by the possible presence of within-island population genetic structure, we sampled and multilocus genotyped birds from multiple geographic locales. For example, on Islas Española and Santiago (which harbour hawk populations with among the lowest and highest genetic diversity, respectively), we sampled territorial birds from the extreme eastern and western portions of the islands (Fig. 1). On the smaller islands, we sampled birds from a greater proportion of island area than on the larger islands (Fig. 1). Due to the low genetic diversity within the four smallest hawk populations (Española, Santa Fe, Pinzón, and Marchena), sampling from relatively fewer individuals on the smallest islands was sufficient to characterize their population genetic diversity (Bollmer *et al.* 2005). Bollmer *et al.* (2005) found only four multilocus genotypes within Isla Santa Fe in the 15 birds sampled from both multiple years and geographic locations throughout the island (the entire population of hawks on Santa Fe is likely to be ~30 birds). Bollmer *et al.* (2005) further found that populations from Islas Santa Fe, Española, Pinzón, and Marchena were all relatively inbred compared to more variable (but still inbred) populations from Islas Pinta, Fernandina, Isabela and Santiago. Our samples from Swainson's hawks (n =8) and from Isla Isabela (n =10) were small relative to the larger Galápagos hawk population sample sizes, yet both were relatively outbred based on H estimated from the minisatellites. Given this, our estimation of relative genetic diversity within each hawk population sampled is representative of the standing genetic diversity within each population and is not an artifact of sampling bias or within-population genetic structure.

(f) ***Statistical analyses***

For all statistical analyses except the overall comparison of prevalence between louse species which utilized Quantitative Parasitology v.2.0 (Reiczigel & Rózsa 2001), louse abundance data were *ln* + 1 transformed and Stephen's heterozygosity values were arcsine square root transformed to meet assumptions of normality.

We performed a Pearson's correlation analysis in SPSS v.11.0 (2004) to assess the strength of the relationship between host population genetic diversity (*H*) and average host population parasite abundance from nine hawk populations (eight *B. galapagoensis* and one *B. swainsoni*). The correlation analyses were one-tailed given our *a priori* predictions about the direction of the relationship between the variables. We then examined the relationship between average louse abundance and *H* for the eight Galápagos hawk populations to determine if the relationship was being driven by the relatively outbred Swainson's hawks.

Next, we examined the relationship between innate humoral immunity (NAb agglutination titres) and *H* on the entire subset of individuals (n =46) for which plasma was collected. The relationship between average island NAb agglutination titres and *H* was not linear. Thus, we used the GLM procedure in SPSS to determine if there was a significant effect of island-level *H* (a fixed factor) on NAb agglutination titres (the dependent variable) instead (Española, n =3; Fernandina, n =15; Isabela, n =3; Marchena, n =5; Pinta, n =7; Santa Fe, n =5; Santiago, n =8).

Finally, we performed a GLM analysis in SPSS using a subset of data that included all 43 birds sampled for both plasma and parasites to determine if antibodies and louse abundances were correlated. In order to control for the effect of island inbreeding we used the GLM procedure as in the preceding analysis (NAb agglutination titres of the 43 hawks dependent on island as a fixed factor) except that

louse abundance for each of the 43 individuals was included as a covariate in the model (Española n =3; Fernandina n =14; Isabela n =3; Marchena n =5; Pinta n =7; Santa Fe n =4; Santiago n =7). One analysis was performed for each louse species. A scatterplot of the louse abundance data and NAb agglutination titres was created to show the relationships between the two variables before the analyses and individuals were labeled as either inhabiting a relatively inbred (Española, Marchena or Santa Fe) or outbred (Fernandina, Isabela, Pinta or Santiago) island (Fig. 3).

3. RESULTS

(a) *Parasite collections*

We collected a total of 14,843 individuals of the louse *C. turbinatum* and 2,858 individuals of the louse *D. regalis* from 199 Galápagos hawks sampled for lice. These lice typically occur on no other birds in the Galápagos, but have been reported from mainland *Buteo swainsoni* (Whiteman & Parker 2004a). Overall prevalence (across islands) of *C. turbinatum* (97.5%) was higher than that of *D. regalis* (85.4%; Fisher's exact test, $p < 0.001$); both louse species occurred in all 8 host populations.

We collected a total of 17 individuals of *C. turbinatum*, 22 individuals of *Laemobothrion maximum* and 11 individuals of a *Kurodaia* sp. from the nine Swainson's hawks. These three species abundances were pooled and constitute the amblyceran lice from Swainson's hawks; *C. turbinatum* was the only amblyceran collected from Galápagos hawks. No *Degeeriella* were collected from the nine Swainson's hawks.

(b) *Assessment of population genetic diversity*

Untransformed values of H for each host population are shown in Figure 1. Individuals from the smallest island-populations of the Galápagos hawk had the highest reported levels of minisatellite uniformity of any wild, unperturbed bird

species and these results are consistent with those of Bollmer *et al.* (2005). As in Bollmer *et al.* (2005), we found >50% of all bands were fixed within these populations (Santa Fe, 13/16 bands fixed; Española, 10/16 bands fixed; Pinzón, 11/20 bands fixed; Marchena, 11/18 bands fixed). The four most inbred populations contained multiple individuals or sets of individuals that were genetically identical at all loci, whereas no identical individuals were found within the four larger islands populations or within Swainson's hawks (Bollmer *et al.* 2005).

(c) ***Effects of genetic diversity and other host factors on parasite load***

Among *Buteo* populations (n =208 total individuals sampled for lice by population: Española, n =8; Fernandina, n =28; Isabela, n =25; Marchena, n =26; Pinta, n =31; Pinzón, n =10; Santa Fe, n =13; Santiago, n =58; Swainson's hawks n =9), average amblyceran louse abundance within populations and H were significantly and negatively related across populations (Fig. 2A; *C. turbinatum*; Pearson's $r = -0.949$, n =9, $p < 0.0001$; *D. regalis*; $r = -0.854$, n =9, $p < 0.01$). When limited to the eight Galápagos hawk island populations only, similar negative relationships were found: *C. turbinatum* ($r = -0.875$, n =8, $p < 0.01$) and *D. regalis* ($r = -0.69$, n =8, $p < 0.05$).

(d) ***Innate antibody levels, genetic diversity and parasite load***

We found a significant (and non-linear) effect of island on average NAb agglutination titres (Fig. 2B; one-way ANOVA; n =46, $F_{6, 39} = 3.41$, $p < 0.01$). The Marchena population, the third most inbred population, exhibited the highest average titre and Española and Santa Fe, the most inbred populations, exhibited the lowest (Fig. 2B). The more outbred island populations had intermediate NAb titres. The variance in NAb titres was lower within the inbred populations than the more outbred populations (Fig. 2B). The CV of the inbred populations (Santa Fe, Española,

Marchena) was 11.5% within and 24.2% among islands, whereas the CV of the more outbred islands (Fernandina, Isabela, Pinta, Santiago) was 17.2% within and 4.6% among islands. There was no significant relationship between sample size and CV (Pearson's $r = 0.397$, $n = 7$, $p > 0.05$) or between sample size and standard deviation (Pearson's $r = 0.522$, $n = 7$, $p > 0.05$) across islands, indicating that variation in sample size did not bias these results. Furthermore, *C. turbinatum* abundance was significantly and negatively related to NAb agglutination titres when individual birds were considered (controlling for the effects of island in a GLM; corrected model $F_{7, 35} = 4.05$, $p < 0.01$; island effect $F = 2.50$, $p < 0.05$, *C. turbinatum* abundance parameter estimate $\beta = -0.342$, $F = 4.10$, $p = 0.05$; Fig. 3A). The scatterplot yielded a triangular pattern whereby birds with low NAb titres consistently harboured high *C. turbinatum* abundances, but birds with high NAb titres harboured both low and high louse abundances. As predicted, no significant relationship was found between the ischnoceran, feather-feeding *D. regalis* and NAb agglutination titres, although a trend indicates a negative relationship between these variables (controlling for the effects of island in a GLM; corrected model $F_{7, 35} = 3.01$, $p < 0.05$; island effect $F = 2.60$, $p < 0.05$, *D. regalis* abundance parameter estimate $\beta = -0.259$, $F = 1.68$, $p > 0.05$; Fig. 3B).

4. DISCUSSION

We have shown that variation in host population genetic diversity is correlated negatively with average parasite load and positively with variation in NAb levels across populations of an unmanipulated, *in situ* threatened wildlife species. Smaller, more inbred host populations generally had higher parasite loads, lower average immune responses and lower variation in within-population immune response than more outbred populations. NAb levels were negatively correlated with the abundance

of a skin and blood feeding amblyceran louse, further linking inbreeding, immune response and parasite burden in this system.

As a result of lower within-population genetic variability and lower and less variable within-population NAb levels, most of the peripheral, inbred and highly differentiated island populations of the Galápagos hawk are vulnerable to disease agents. These populations contained more among-island variability in NAb levels than the larger island-populations, possibly due to the strong effects of genetic drift (Rowe & Beebee 2003; Bollmer *et al.* 2005; Pearman & Garner 2005) or local coevolutionary dynamics (Thompson 1999). Replicate inbred lines of *Drosophila* varied considerably in disease resistance and stress response, indicating that stochasticity also influences immunocompetence in small, inbred populations (Spielman *et al.* 2004b; Kristensen *et al.* 2005). Protection of the highly differentiated peripheral hawk populations should be prioritized as the variation they contain is essential for the long-term viability of this species (Lesica & Allendorf 1995). Conversely, the large amount of within-population genetic and immunological variation within the largest hawk island populations is also important from a conservation perspective. Since tradeoffs exist between the humoral and cellular immune response (Lindström *et al.* 2004), these populations may be better able to respond to multiple invasions of pathogens than the smaller, more isolated populations.

As a potential mechanism underlying the relationship between host genetic diversity and average parasite load, we showed that NAb agglutination titres were negatively related to abundance of native parasites that fed on skin and blood (*C. turbinatum*). Conversely, there was a relatively weak relationship between NAb levels and abundances of an ischnoceran (*D. regalis*), mainly feather-feeding louse, as

expected. Amblycerans and their avian hosts' immune systems are engaged in a coevolutionary arms race (Møller & Rózsa 2005) across deep evolutionary scales; we have revealed components of the system interacting at the individual and population levels. Although other host factors affect louse abundances (Whiteman & Parker 2004a, b), our results suggest that NAb levels regulate chronic infections of relatively permanent parasites (Marshal 1981). Although feeding by ectoparasites on avian skin or blood invokes an innate cellular immune response (Szabó *et al.* 2002; Prelezov *et al.* 2002), our study differs by reporting a relationship between the innate humoral response and ectoparasite load in wild birds. Generalized inbreeding depression may also lead to physical and behavioral changes that affect preening efficiency and this may be particularly germane for *D. regalis*, which mainly encounters mechanical host defences (Clayton *et al.* 1999; Whiteman & Parker 2004b).

Our use of population-level *H* to compare parasite loads among relatively inbred and outbred populations is a comparative framework similar to that used by other key disease ecology studies (Liersch & Schmid-Hempel 1998; Pearman & Garner 2005). However, the influence of another unmeasured factor correlating with population genetic diversity may also explain the results, although we know of no such factor. Nearly 90% of the variation in hawk genetic diversity is explained by island size, and these hawk populations are genetically isolated from one another and underwent rapid range expansion after colonizing the archipelago (Bollmer *et al.* 2005; Bollmer *et al.* in press). Thus, loss of genetic diversity and inbreeding likely underlie the relationship between *H* and parasite load found in this study. Specific mechanisms may include the exposure of deleterious recessive alleles (Keller & Waller 2002), the fixation of slightly deleterious alleles through genetic drift (Johnson

& Seger 2001), other microevolutionary processes associated with founder events and maintenance of small population sizes over time, or a combination of these.

Recent theoretical work has extended the foundational deterministic models of host-parasite dynamics to include disease-caused extinction of host populations (de Castro & Bolker 2005). Although only a few confirmed examples of disease-induced extinction or population reductions of naturally occurring or captive wildlife species with low population sizes exist, many similar extinctions or population reductions were likely caused by disease (de Castro & Bolker 2005). While several of the causal disease agents were novel infectious diseases to the hosts, we have shown that parasites that likely co-colonized the Galápagos archipelago with their hosts, have exploited genetically depauperate host populations.

Extinction and disease ecology are "by their nature cryptic and difficult to study in natural communities" (de Castro & Bolker 2005). Clearly, however, studies of disease ecology reveal the importance of demographic and population history in mediating the outcome of host-parasite interactions (McCoy *et al.* 2002). This information is of basic biological interest and offers insight into how populations will respond to invasions of alien pathogens, which is underway in most previously isolated ecosystems. Future studies examining host immunogenetics, parasite population genetics and transmission dynamics are necessary for fully assessing the threat of pathogens to this island endemic.

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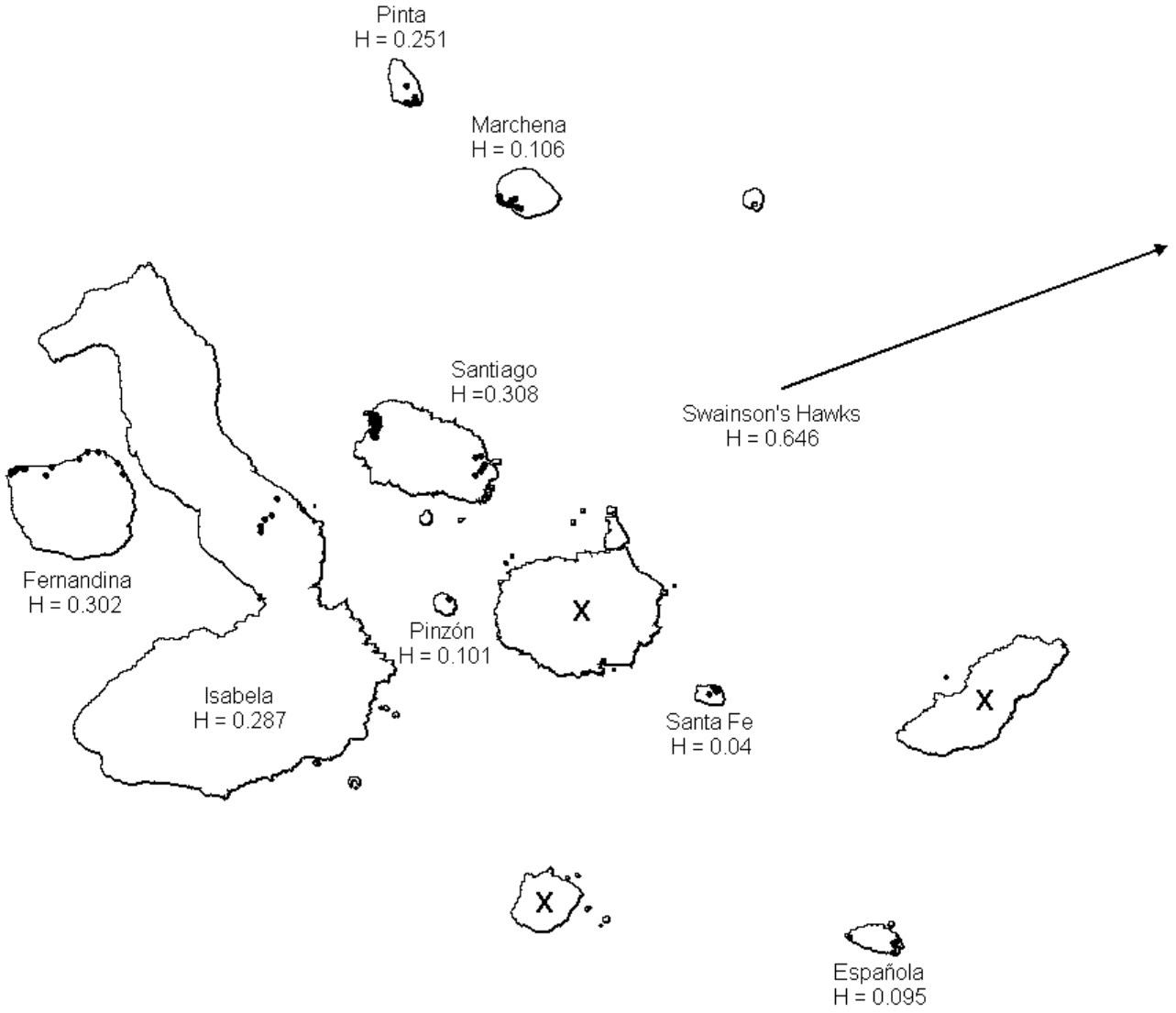
FIGURE LEGENDS

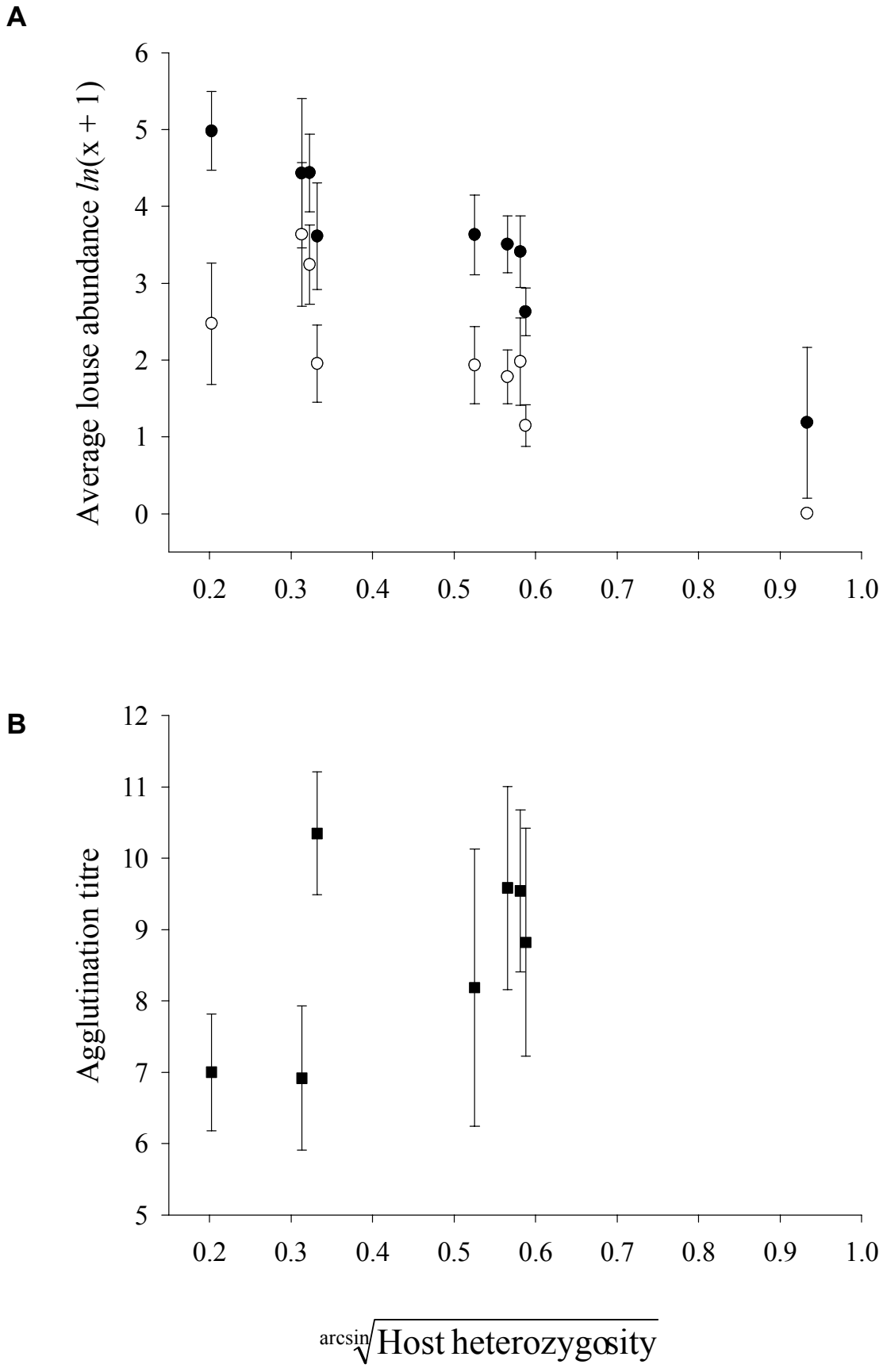
Figure 1. Map of the Galápagos Archipelago, located ~1000 km west of mainland Ecuador, South America. Extant breeding island populations of the Galápagos hawk (*Buteo galapagoensis*) are named, followed by estimates of island population genetic diversity (*H*; Stephens heterozygosity values) calculated from multilocus minisatellite data. Small black dots within islands indicate sampling localities. An estimation of *H* from the mainland Swainson's hawk (the putative sibling species of *B. galapagoensis*) was included for comparative purposes. Extinct island populations of *B. galapagoensis* are indicated by an "X" (there is no evidence indicating hawks have ever inhabited Isla Genovesa located in the northeastern part of the archipelago).

Figure 2. Scatterplot of two disease susceptibility variables vs. estimated host population genetic diversity (heterozygosity) values. **(A)** Louse abundance vs. host population genetic diversity. Closed circles = average amblyceran abundance ± 95% confidence intervals (*Colpocephalum turbinatum*, *Laemobothrion maximum*, and *Kurodaia* sp.; $r = -0.949$, $n = 9$, $p < 0.0001$). Open circles = average ischnoceran abundance ± 95% confidence intervals (*Degeeriella regalis*; $r = -0.854$, $n = 9$, $p < 0.01$). Dyads with heterozygosity values > 0.9 represent a mainland *B. swainsoni* population and the remaining values represent eight island populations of *B. galapagoensis*. Island populations reading left to right are as follows: Santa Fe, Española, Pinzón, Marchena, Pinta, Isabela, Fernandina, Santiago; **(B)** Average agglutination titres (NAbs) ± SDM from 46 *B. galapagoensis* individuals vs. estimated host population genetic diversity (the relationship between NAb agglutination titres and genetic diversity was not linear, although significant

differences existed in average NAb agglutination titres among island-populations, one-way ANOVA: $F_{6,39}$, $p< 0.01$). Island populations reading left to right are as follows: Santa Fe, Española, Marchena, Pinta, Isabela, Fernandina, Santiago.

Figure 3. Linear relationship between **(A)** *Colpocephalum turbinatum* abundance and natural antibody (NAb) titres **(B)** *Degeeriella regalis* abundance and NAb titres from 43 individual *Buteo galapagoensis* hosts. Solid triangles = individuals from more inbred island populations (Española, Marchena, Santa Fe), solid circles = individuals from more outbred island populations (Fernandina, Isabela, Pinta, Santiago). Only *C. turbinatum* abundance was significantly and negatively related to agglutination titres after controlling for other host factors (the slope of this parameter estimate from the GLM was $\beta = -0.342$, $p = 0.05$).


Pinta
H = 0.251
Marchena
H = 0.106
Santiago
H =0.308
Swainson's Hawks
H = 0.646
Fernandina
H = 0.302
Pinzón
H = 0.101
X
Isabela
H = 0.287
Santa Fe
H = 0.04
X
X
Española
H = 0.095


A
Average louse abundance ln(x + 1)
0
1
2
3
4
5
6
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
B
Agglutination titre
5
6
7
8
9
10
11
12
0.2
0.3
0.4
0.5
0.6
0.7
0.8
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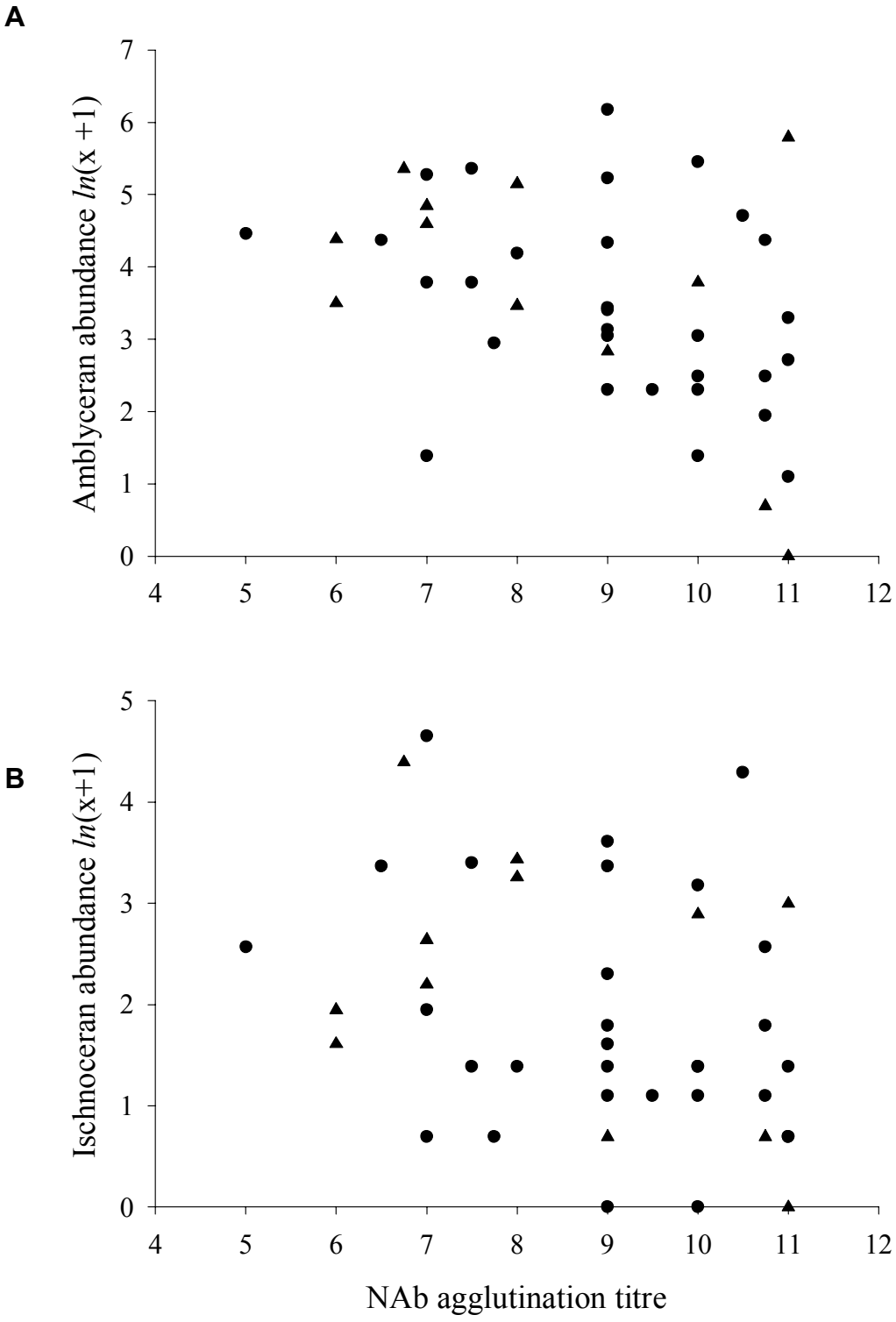
arcsin√Host heterozygosity


A
Amblyceran abundance ln(x +1)
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1
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6
7
8
9
10
11
12
B
Ischnoceran abundance ln(x+1)
0
1
2
3
4
5
4
5
6
7
8
9
10
11
12
NAb agglutination titre

Chapter VII.

Establishment of the avian disease vector *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador

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INTRODUCTION

Avian disease has been implicated as a major factor in decline of the endemic Hawaiian avifauna (Warner 1968; van Riper *et al.* 1986, 2002; Atkinson *et al.* 2000; Yorinks & Atkinson 2000). The introduction into Hawaii of avian pox (*Avipoxvirus* spp.), avian malaria (*Plasmodium relictum*) and a suitable vector, the Southern house mosquito (*Culex quinquefasciatus* Say 1823; Hardy 1960), are thought to be the mechanisms driving this decline (van Riper & Scott 2001, van Riper *et al.* 2002). *Culex quinquefasciatus* is a cyclopropagative vector (in which the pathogen undergoes further development and multiplication) for avian malaria, and a mechanical vector (in which the pathogen is carried on or in mouthparts, legs, etc., but does not undergo further development or multiplication), for avian pox in Hawaii. The endemic birds of Hawaii are more susceptible than are introduced birds, to both of these pathogens (van Riper *et al*. 2002, Atkinson *et al.* 2000, Yorinks & Atkinson 2000).

In contrast, the avifauna of the Galápagos Islands is largely intact (due to relatively recent human colonization; Snell *et al.* 2002), yet is highly endemic (84% of land birds are unique; Tye *et al.* 2002). Several endemic bird populations are in decline (Snell et al. 2002), although none are extinct archipelago-wide. For example, the Galápagos Hawk (*Buteo galapagoensis* Gould 1837) has been extirpated on three human-inhabited islands (de Vries 1975), while breeding populations still reside on eight islands. Invasive organisms and disease agents, including viruses such as West Nile Virus (WNV), now pose the greatest threat to the continued persistence of Galápagos' unique birds (Wikelski *et al.* 2004, Thiel *et al.* 2005). We report here the establishment in the Galápagos Islands of the avian disease vector *C. quinquefasciatus*, first reported from the archipelago in 1989 (Peck *et al.* 1998), and documented now as part of a larger survey of avian disease and their vectors in the archipelago begun in 2001. We also report the date 1985 as the first collection of this mosquito in the archipelago, earlier than was published previously (1989). The implications of the establishment of this insect in the Galápagos Islands, specifically the threat it poses to avian health, are discussed.

METHODS

Adult mosquitoes were sampled during a total of nine trapping attempts using U.S. Centers for Disease Control & Prevention miniature ultraviolet light traps on Isla Santa Cruz in the Galápagos Islands (Archipelago de Colón), Ecuador, in July and August, 2003 (purchased from BioQuip Products, Rancho Dominguez, CA, U.S.A). Light traps were turned on approximately one hour before dusk (~5 pm local time) and turned off from 1 to 5 hours after dawn (~7am-11am). Culicids were then separated from other insect taxa and stored in 95% ethanol for identification. Label information from

specimens collected prior to this study was obtained from vouchers housed at the Canadian National Collection of Insects in Ottawa, Canada. All 2003 collections were made in and around the coastal town of Puerto Ayora, Isla Santa Cruz, which lies within the Arid Zone (with focused sampling at the Charles Darwin Research Station; 0° 44' 20" S latitude, 90° 18' 25" W longitude; 6 m) and within the town of Bellavista, which lies within the upper Transition Zone (0° 42' S latitude, 90° 22' W longitude; 194 m). Bellavista, Isla Santa Cruz annually receives more rainfall and is cooler in temperature than Puerto Ayora, Isla Santa Cruz (Snell & Rea 1999).

Oviposition traps were made from 5 litre 'pitcher' style plastic water containers by cutting away the neck and front walls of the vessel to half height. The containers were filled with ~1.5 litres of fresh, potable water and a handful of dry straw and placed in partially shaded locations around the Galápagos National Park Service Headquarters in Puerto Ayora, Isla Santa Cruz. Two traps were set on consecutive days from 28 April -14 May 2004. Traps were checked daily and the number of eggs counted. Egg rafts were removed to separate hatching containers and allowed to complete the development cycle, after which a selection of adults was collected for identification. Identifications of culicid specimens were made using a species-diagnostic molecular analysis of the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal gene array (Crabtree *et al.* 1995), conducted at the Arbovirus Laboratories, Wadsworth Center, NY, U.S.A.

RESULTS

Eleven adult individuals of the Southern house mosquito (*C. quinquefasciatus*) were collected from two traps placed at two locations (one trap within the Arid Zone and one trap within the upper Transition Zone) on Isla Santa Cruz in August 2003 (Table 1). One

of the traps (placed in Bellavista) that produced two Southern house mosquitoes also produced 11 individuals of the black salt marsh mosquito (*Ochlerotatus taeniorhynchus* (Wiedemann 1821)). Seven traps placed in other areas, including near the Charles Darwin Research Station, produced 155 *O. taeniorhynchus* individuals and no *Culex* individuals. Thus, 11 Southern house mosquito and 166 black salt marsh mosquito individuals were collected from the nine trapping attempts. Voucher specimens of both species have been placed at the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany. Reexamination of museum label data from *C. quinquefasciatus* collected in the Galápagos Islands prior to this study indicate that the date of first record of occurrence in the Galápagos was not 1989 as reported by Peck *et al.* (1998), but rather 1985.

A total of 27 egg rafts were laid in oviposition traps between 28 April and 14 May 2004. Adults reared from these eggs rafts were subsequently confirmed as *C. quinquefasciatus* using the molecular analysis described above.

DISCUSSION

The establishment of *C. quinquefasciatus* on the Galápagos Islands after its first detection two decades ago, in 1985, is troubling from an avian conservation perspective. This species is capable of biting humans or migrating birds and transmitting exotic disease agents, such as WNV (Turell *et al.* 2001). West Nile Virus is present within other island systems in the New World tropics and it may be simply a matter of time before it enters the Galápagos ecosystem (Dupuis *et al.* 2003). This mosquito is also a mechanical vector for *Avipoxvirus*, now present in both domesticated and wild birds in the Galápagos (Thiel *et al.* 2005), and thus its presence may exacerbate the spread of pox within and between

islands. If *Plasmodium relictum* or another avian malaria species ever enters the Galápagos, *C. quinquefasciatus* can serve as a competent vector. This combination of events would likely be devastating to the local bird community.

Interestingly, the first 2003 *C. quinquefasciatus* collection locality on Isla Santa Cruz was in a small town (Bellavista), and only 5 km from the first collection locality (in 1985) on Isla Santa Cruz, at the Media Luna. However, these two sites, though geographically proximate, are separated by ~400 m in elevation. Bellavista is an agricultural settlement located ~8 km inland, situated in the more mesic highlands of the upper Transition Zone. The 1985 sampling locality (the Media Luna) remains uninhabited and is in the mesic Miconia Zone. The second 2003 collection location on Santa Cruz was located within the Arid Zone but a trap was intentionally placed near a laundry room of a private residence, where mosquitoes had been observed previously. *Culex quinquefasciatus* also readily oviposited in fresh water traps on Santa Cruz. Thus, *C. quinquefasciatus* has now been reported from three altitudinal zones within Isla Santa Cruz and from the Arid Zone within Isla San Cristóbal. Since breeding by *C. quinquefasciatus* could be limited by the presence of fresh water (it is a fresh water obligate; Patrick & Bradley 2000) its distribution in the Galápagos is probably most common near human habitations where fresh water can be found. However, *C. quinquefasciatus* is likely to increase its range within the Arid Zone during the wet season. Furthermore, the absence of *C. quinquefasciatus* from the majority of light traps may be due to the fact that we sampled during the dry season and not the wet season. Nonetheless, this species was present within both the Arid and Transition Zones during the dry season, which underscores the potential for *C. quinquefasciatus* to invade coastal

areas of other islands, particularly during the wet season and during El Niño Southern Oscillation events. Simple control measures, such as reducing the availability of human-made oviposition sites (e.g., used tires, open containers) may reduce the local abundance and the eventual spread of these obligate freshwater breeding mosquitoes in the archipelago. Other control measures, such as the use of the biological control agent *Bacillus sphaericus*, which is toxic to *C. quinquefasciatus* (Regis *et al.* 2000), could be implemented. However, resistance to the 'Bin toxin' has been observed (Oliveira *et al.* 2004). The toxin produced by *Bacillus thuringiensis israelensis* (Bti), the effects of which are also relatively specific to larval dipterans, would be preferable since mosquitoes do not develop resistance to it. However, non-target taxa, particularly other insects within the dipteran suborder Nematocera, such as chironomid midges, may be negatively affected by its application (Hershey *et al.* 1998).

Peck *et al.* (1998) speculated that *C. quinquefasciatus* arrived in the archipelago as larvae in water. However, local air travel now occurs among three islands within the archipelago (Islas Isabela, Santa Cruz, San Cristóbal) and between two islands and the mainland, including the city of Guayaquil, Ecuador, situated in the humid tropical lowlands. As Peck *et al.* (1998) noted, 11,448 insect specimens were collected from aircraft in Hawaii (Dethier 1948, see also Lounibos 2002). This route of dispersal is likely to ensure the presence of such invasive pests in Galápagos, and new mosquito-borne diseases are likely to be introduced unless control measures are implemented for aircraft flying into the archipelago (Kilpatrick et al. unpublished results). Tour operators, tourists, residents, and scientists on inter-island boat trips should be vigilant in ensuring that they are not transporting these mosquitoes. An educational campaign should be

instituted to alert communities on the Galápagos to eliminate standing water. Nonetheless, *C. quinquefasciatus* now appears to be established on Isla Santa Cruz and is quite likely still present on Isla San Cristóbal, where it was collected in 1989. It seems probable that this species is also present on Islas Isabela and Floreana, the only other islands inhabited by humans in the archipelago, but further sampling is needed to confirm this.

The black salt marsh mosquito (*O. taeniorhynchus*) is present on all main islands within the Galápagos and has been known since first record in the late 1890s (Linsley & Usinger 1966). This species breeds in brackish water and is regarded as less threatening as a vector of avian disease agents. However, it should not be ignored as a threat, because, although it may prefer feeding upon mammals, individuals also feed on birds (Edman 1971). *Ochlerotatus taeniorhynchus* individuals have been observed feeding on endemic birds within the Galápagos and locally high mosquito population densities have led to cases of nest desertion by endemic birds (Anderson & Fortner 1988). Moreover, individuals of *O. taeniorhynchus* have tested positive for WNV elsewhere (Hribar *et al.* 2003), and individuals are capable of transmitting WNV (Turell *et al.* 2001). This insect is also likely to serve as a mechanical vector of *Avipoxvirus* among birds in the Galápagos Islands (Thiel *et al.* 2005).

Data on host preferences (by genetically characterizing the identity of mosquito blood meals; Ngo & Kramer 2003), distribution, and intra- and inter-island movement of these mosquitoes (e.g., population genetics), and how each of these interacts with seasonality, are needed to more fully understand the threat posed by these vectors to the unique Galápagos avifauna.

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Table 1. Collection records of the Southern house mosquito (Diptera: Culicidae) *Culex quinquefasciatus* and the black salt marsh mosquito (*Ochlerotatus taeniorhynchus*)) from the Galápagos Islands, Ecuador.

Species	Date	Island	Location	Abundance
Culex quinquefasciatus	14.V. –13.VII.1985	Santa Cruz*	4 Km N Bellavista, Media Luna, 620 m	4
	10.II.1989*	San Cristóbal*	Puerto Baquerizo, hotel light, swarming	9
	01.VIII.2003	Santa Cruz	Town of Bellavista	2
	03.VIII.2003	Santa Cruz	Near laundry room of private residence in Puerto Ayora	9
				2003 Total: 11
Ochlerotatus taeniorhynchus	16-17.VII.2003	Santa Cruz	Charles Darwin Research Station, ~1 km E Puerto Ayora, 6 m (CDRS), near scientists' dormitories)	2
	17.VII.2003	Santa Cruz	Same data	1
	20.VII.2003	Santa Cruz	CDRS (near Iguana rearing pens)	4
	20.VII.2003	Santa Cruz	CDRS (near scientists' dormitories)	10
	23.VII.2003	Santa Cruz	CDRS (near Iguana rearing pens)	36
	28.VII.2003	Santa Cruz	CDRS (near scientists' dormitories)	91
	01.VIII.2003	Santa Cruz	Town of Bellavista, 194 m (collected in same light trap as *C. quinquefasciatus* collected on this date).	11
	03.VIII.2003	Santa Cruz	CDRS (outside of Ornithology Laboratory).	11
				2003 Total: 166

*Same collection data reported previously (Peck et al. 1998).

Chapter VIII.

Characterization of Canarypox-like Viruses Infecting Endemic Birds in the Galápagos Islands

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ABSTRACT

The presence of avian pox in endemic birds in the Galápagos Islands has led to concern that the health of these birds may be threatened by avian pox strains transmitted to them by domestic birds. We describe here a simple PCR-based method for identification and discrimination of pox strains similar to fowlpox or canarypox. This method, in conjunction with DNA sequencing of two PCR-amplified loci totaling about 800 bp, was used to identify two *Avipoxvirus* strains, Gal1 and Gal2, in pox lesions from warblers (*Dendroica petechia*), finches (*Geospiza* spp.) and mockingbirds (*Nesomimus parvulus*) from the inhabited islands of Santa Cruz and Isabela. Both strains were found in all three passerine taxa and both strains were <5% different from each other and from canarypox. In contrast, chickens in Galápagos were infected with a poxvirus that appears to be identical in sequence to the characterized fowlpox strain, and ~30% different from any member in the canarypox/Galápagos group in the regions sequenced. These results indicate a colonization of avipoxvirus infecting the endemic birds independent of the fowlpox virus infecting the chickens. Alignment of the sequence of a 5.9-kb region of

the genome revealed that sequence identities among Gal1, Gal2 and canarypox were clustered in discrete regions. This suggests that recombination between poxvirus strains combined with mutation led to the variants of canarypox that are now prevalent in the Galápagos.

Key Words: avian pox, Galápagos, poxvirus

INTRODUCTION

Pathogens can have especially severe effects when they are transmitted to novel environments where populations may lack natural resistance. Island populations may be particularly at risk, as they tend to have less pathogen diversity than their continental counterparts (Lewis, 1968a,b; Dobson, 1988; Fromont et al., 2001; Goüy de Bellocq et al., 2002). Founders likely carry only a subset of the parasites found in the donor population and virulent pathogens needing a large host population may be lost quickly (Dobson and May, 1986, Dobson, 1988). Paucity of parasites reduces selection for resistance and enhances host population densities, both of which facilitate the transmission of introduced pathogens (Dobson, 1988).

The Galápagos Islands are volcanic in origin (Christie et al., 1992, White et al., 1993) and located on the equator almost 1000 km west of mainland Ecuador in South America. Their isolation and relative desolation delayed permanent colonization by humans, and their biodiversity remains mostly intact, with only about five percent of species having been lost (Gibbs et al., 1999); this includes none of the 28 breeding land bird species, 26 of which are endemic. In 1959, 90% of the archipelago was set aside as a national park. However, the resident human population along with tourism has grown rapidly and exotics are continually being introduced despite increasing efforts to exclude

them. The Charles Darwin Research Station and the Galápagos National Park are concerned about the introduction of avian diseases that could result in extinctions of Galápagos avifauna similar to those in Hawaii (Wikelski et al., 2004). The appearance of avian pox-like lesions in domestic chickens and endemic birds heightened concerns regarding the possibility of disease transmission from introduced birds to endemics.

Avian pox is a mild to severe disease of birds diagnosed in approximately 60 species from 20 different avian families worldwide. A DNA virus of the family Poxviridae, genus *Avipoxvirus*, causes the disease; transmission occurs when a virion enters a break in the skin or, more commonly, when vectored by a biting insect. There are two primary manifestations of the disease: the most common cutaneous form consists of proliferative lesions that harden to thick scabs (Merck, 1993); the diptheritic or wet form results in mucosal lesions within the digestive and upper respiratory tracts (Merck, 1993). The cutaneous form is most commonly observed in passerine birds (Gerlach, 1999). In Galápagos, the order Passeriformes is represented by 8 families with 28 species (Castro and Phillips, 1996). Among these exist several severely threatened species populations, such as the mangrove finch (*Cactospiza heliobates*; total population approximately 100 individuals), the Floreana mockingbird (*Nesomimus trifasciatus*; approximately 200 individuals), the Española mockingbird (*Nesomimus macdonaldi*; approximately 2500 individuals), the medium tree finch (*Camarhynchus pauper*), and the large tree finch (*Camarhynchus psittacula*). The diptheritic form is observed most frequently in Psittaciformes, Phasianiformes, and several Columbiformes (Gerlach, 1999). In Galápagos, only Columbiformes occurs of these orders, represented by a single

endemic, the Galápagos dove (*Zenaida galapagoensis*) as well as introduced pigeons (*Columba livia*).

Thirteen strains of avipoxvirus have been identified worldwide. The strains vary in virulence and host specificity. Poxviruses from endemic forest birds in Hawaii (Apapane *H. sanguinea* and Hawaiian crow *Corvus hawaiiensis*) include two strains that differ significantly from fowlpox virus by RFLP genetic analysis (Tripathy et al., 2000); their pathogenicity was mild in chickens. Oral vaccination with fowlpox viruses provided immunity in chickens (Saini et al., 1990a, b; Sarma and Sharma, 1988), although protection may be of short duration (Saini et al., 1990a). However, quailpox virus vaccine provided no immunologic protection against pigeon- and fowlpox viruses, nor did psittacine poxvirus vaccine protect chickens and quail against quailpox challenge. Quail, psittacine, and fowl poxviruses induced protective immunologic response in chickens and quail when challenged with the matching virus, but no protection against challenge with a non-matching virus (Winterfield and Reed, 1985). The high specificity of some viruses and the taxonomically limited effectiveness of vaccines suggest significant antigenic differentiation among strains. This may result, in part, from rapid evolution of poxviruses by recombination between strains. Replication of the pox genome occurs through intermediates comprising many tandem repeats of the entire genome (Moyer and Graves, 1981). Recombination, which occurs at extraordinarily high frequencies in pox, is an essential part of replication during infection by a single type of virus (Ball, 1987); however, recombination may also contribute to the wide diversification of avian pox.

The dynamics of multihost pathogens in natural populations are key to understanding general patterns of rapid evolution of viruses and their impact on natural populations (Cleaveland et al., 2002, Woolhouse et al., 2001). In Galápagos, pox-like symptoms have been described in several species of endemic birds, including Galápagos mockingbirds (*N. parvulus parvulus*), Galápagos doves (*Z. galapagoensis*), yellow warblers (*D. petechia*), and some Galápagos finches (*Geospiza* spp.) (Jimenez 2003). Most data on the effects of avian pox are from the mockingbirds. During the 1982-1983 El Niño event, 56% of mockingbirds displaying lesions died on Genovesa, compared to 39% of asymptomatic individuals (Curry and Grant, 1989). In that study, significantly more adults were infected than juveniles, partly because the epizootic peaked before most of the juveniles hatched. Prevalence was higher in nestling and juvenile Galápagos mockingbirds than adults on the island of Santa Cruz, and much higher resighting rates for young birds without symptoms than those with lesions suggested higher mortality for infected birds (Vargas, 1987). Pox-like lesions were also observed during the 1982–83 El Niño among mockingbirds on Champion, an islet off Floreana (Grant et al., 2000).

There were two main objectives in this study. The first was to develop a simple, specific diagnostic test for avian pox that could be adapted for use in the Galápagos where propagation of the virus in vitro and subsequent testing is not economically feasible. The second was to determine the type of avianpox infecting the native birds. The availability of large published regions of sequence for fowlpox and canarypox provides the foundation for development of rapid PCR-based detection of these viruses. We describe here a simple PCR-based method for identification and discrimination of

pox strains similar to fowlpox or canarypox and the subsequent analysis of the avian pox strains identified in Galápagos wild bird populations.

METHODS

Field Methods

Between May and July of 2002 and 2003, birds from the wild populations were mistnetted near the Charles Darwin Research Station on the island of Santa Cruz, Galapagos, Ecuador. In January 2003 and July 2003, birds were mistnetted on the island of Isabela, Galapagos Ecuador. Samples were excised from cutaneous lesions that were dry and scab-like. Sections of lesions were removed using sterile scalpels, transferred to a plastic vial and frozen in liquid nitrogen for transport. In some instances samples were suspended in 95% ethanol and frozen. Any bleeding was stopped by applying mild pressure with sterile cotton; typically, there was little or no bleeding.

DNA extraction.

Samples of each lesion were frozen in liquid nitrogen, then pulverized to a powder and incubated at 65° C for at least 6 h in 250 µl Longmire's lysis buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M EDTA,10 mM NaCl, 0.5% SDS) with Proteinase-K (final concentration, 1.0 mg ml^{-1}). Samples were extracted with phenol/$CHCl_3$/isoamyl alcohol (25:24:1), and total DNA was precipitated with ethanol and then resuspended in 100 µl sterile TE (10 mM Tris-HCl, pH 8.0; 10 mM EDTA).

Primer design.

The sequence available for canarypox at the time this project began (6181 nucleotides; GenBank D86731) was aligned with the homologous region from fowlpox

(complete genome; AF198100) using ClustalW (Thompson et al., 1994). Overall, the sequence identity between the two avian pox strains in this region of the chromosome is about 70%. The alignment was inspected visually for regions of divergence, particularly insertions or deletions (indels) that would allow rapid screening of PCR products on the basis of size differences. The intergenic region between the canary genes CA.X and TK (thymidine kinase), designated CAX, was chosen because it provides an indel of 52 bp in a region of 426 bp. Highly conserved sequences within the coding regions of CA.X and TK provided the sequence for primers that could amplify DNA from fowlpox or canarypox (CAX'F AGATATAGTAGAATTTAGTG; CAX'R TTCTGCAAGATTTAATATC). The second locus, designated CA3-2, is a region spanning the CA.2 and the CA.3 genes of canarypox. A number of indels in this region led to a predicted total size difference between fowlpox and canarypox of 18 nucleotides. Highly conserved regions within CA.2 and CA.3 provided the sequence for primers that amplified DNA from fowlpox or canarypox (CA3-2F CTAATAGATACTAACGGAGAAG; CA3-2R TTAAATAAAGAAATGTAAAGAC).

PCR amplification and sequencing.

PCR amplification with primer set CA3-2 or CAX was performed in 50 µl volumes of 67 mM Tris-HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 1.5 mM $MgCl_2$, 2.0 mM each dNTP, 0.01mg bovine serum albumen, 0.6 µM for each primer, 1 unit *Taq* polymerase (Bioline) and 2 µl DNA (concentrations unknown) from field samples of avipox. A sample of DNA isolated from fowlpox from a chicken in the U.S. (kindly provided by D.N. Tripathy) was used as a positive control. A touch-down PCR program was used, beginning with an annealing temperature of 50° C and decreasing 0.5°

every cycle for 14 cycles to a final annealing temperature of 43° C for an additional 25 cycles. Denaturation was at 94°C and extension was 72°C, with a 45 second hold at each temperature in the cycle. Amplicon size was determined after electrophoresis in a 1.5% agarose gel by comparison with markers of known size. Amplification with the other primers (see section above for sequences) was performed as described for CA3-2 except that the initial annealing temperature varied with the T_m of each primer pair. For sequencing the 6-kb region of DNA from lesions of samples 502F (see Table 1) (named Gal1) and 100W (see Table 1) (named Gal2), 20 sets of primers were designed that produced 20 amplicons of 350-400 bp, with about 50 bp of overlap for adjacent amplicons, covering the 6-kb region. Amplicons were sequenced in both directions with the same primers used for amplification with the ABI Big Dye protocol on an ABI 377 sequencer. Sequencing of several amplicons of CA3-2 revealed the presence of *Spe*I or *Age*I restriction sites in amplicons from some strains, but not from others; therefore amplicons of CA3-2 were digested with *Spe*I or *Age*I in the buffer supplied with the enzyme. The accession number for the sequenced region of Gal1 is AY631870 and for Gal2 it is AY631871

Phylogenetic analysis.

Using ClustalW (Thompson et al., 1994), the sequenced regions of Gal1 and Gal2 were aligned with each other and with the sequences of the following strains: fowlpox (Afonso et al., 2000; GenBank AF19810), canarypox ATCC VR-111 (Tulman et al., 2004; GenBank AY318871), and canarypox (Amano et al., 1999; GenBank D86731). The sequence of the culture-adapted fowlpox strain, FP9 (Laidlaw and Skinner 2004;

GenBank AJ581527), was identical to the other fowlpox sequence (Afonso et al., 2000; GenBank AF19810) throughout this region; therefore it was not included in the analysis.

Given the limited nature of our sampling, we sought a simple genetic distance-based analysis to portray the gross phylogenetic relationships among the five pox sequences. To this end, the 5.9 kb alignment was converted to a distance matrix and analysed via the neighbor-joining method in PAUP*4.0 (Swofford 2002). However, the long branch leading to fowlpox relative to the canarypox and canarypox-like Gal1 and Gal2 sequences yielded a tree that was biologically untenable (fowlpox was joined sister to the Gal strains). Thus, we used a maximum-likelihood approach, which helps to minimize the problem of long branches in phylogenetic analysis (Felsenstein, 1978). A bootstrapped (10,000 replications) maximum-likelihood tree (with TBR branch-swapping) was produced for these aligned sequences using PAUP*4.0 (Swofford 2002), rooted with fowlpox. Maximum-likelihood evaluates trees using explicit evolutionary models. MODELTEST 3.06 (Posada and Crandall, 1998) selected the TrN+I+G evolutionary model as the most likely of the 56 possible evolutionary models. The log-likelihood score of the best tree was 14,404.83620.

Recombination analysis

Recombination plays an important role in the propagation of poxviruses (and thus their evolutionary history is not strictly one of association by descent) and has clear conservation implications should a bird become infected with two strains simultaneously. Thus, we conducted a preliminary recombination analysis. Specifically, we used a statistical analysis to detect the extent of historical recombination among ancestors of the five pox lineages. This was implemented using the GENECONV version 1.81 program

(an extension of Sawyer, 1989; Sawyer, 2004). This program is a substitution-based approach, which determines if segments of DNA between two taxa in the alignment are more similar to each other than would be expected given their overall level of similarity. After detecting recombination events, GENECONV then ranks them according to statistical significance, and reports where along the sequence the recombinatory segment begins and ends and its total length. This approach is widely used (e.g., Millman et al., 2001; Drouin, 2002), was more powerful than other recombination-detection methods in computer simulations, and did not over-estimate recombination events (Posada and Crandall, 2001). In our study, pairs of segments of sequences within the alignment that showed significant recombination are reported as global inner *P*-values. Significant recombination between a segment of a sequence from within the alignment and an unknown taxon outside the alignment, or a taxon within the alignment obscured by other evolutionary processes, are reported here as global outer *P*-values. Both are based on 10,000 permutations, and are corrected for multiple comparisons.

RESULTS

Identification of pox

Lesions were collected from a variety of endemic passerine birds on the inhabited islands of Santa Cruz and Isabela in the Galápagos Islands and from domestic chickens on Santa Cruz (Table 1). The CAX primers amplified a 374 bp fragment of DNA from lesions from Galápagos chickens (the size predicted from the genome of fowlpox) compared to a 426 bp amplicon from lesions from Galápagos finches, yellow warblers, and mockingbirds. The CA3-2 primers amplified a 374 bp fragment of DNA from lesions from Galápagos chickens (the size predicted from the genome of fowlpox)

compared to 392 or 381 bp (depending on the pox strain) from lesions from Galápagos finches, warblers, and mockingbirds. Each set of primers produced an amplicon of the expected size for fowlpox from DNA extracted from a control fowlpox strain from a U.S. chicken. The sizes of both the CAX and CA3-2 amplicons from the lesions from the Galápagos finches, warblers and mockingbirds were about the sizes predicted for those amplicons for canarypox and larger than the amplicons produced from the pox lesions from chickens.

The sequences of the CAX and CA3-2 amplicons from five pox strains from chickens from the Galápagos and one from the U.S. were identical to the published sequence for fowlpox at both loci. Thus, chickens in Galápagos were infected with a pox virus that is very similar, if not identical, to the strain that infects poultry in the U.S. In contrast, the sequences of several CA3-2 amplicons from the passerine birds indicated two distinct strains of avian pox, both very similar to canarypox. One of these amplicons, Gal1 (sequenced for 10 strains), contained restriction sites for *Spe*I and *Age*I, while the other, Gal2 (sequenced for 5 strains), did not. The Gal1 strain was more similar to canarypox than was Gal2 and was also the more prevalent of the two strains, particularly in the finches (Table 1). Both strains were found in finches, warblers and mockingbirds, indicating that both these canarypox variants can infect all of these species. We did not identify amplicons characteristic of fowlpox in any of the passerine bird samples. Thus, it appears that chickens on Santa Cruz were infected with fowlpox while the passerine birds were infected with two variants of canarypox. Because these primers were designed using the sequences of fowlpox and canarypox, it is likely that the primers would not

amplify DNA from avian pox strains that are not closely related to these two pox strains. Hence, other avian pox strains may be present in birds in the Galápagos.

Similarity of avian pox strains

In order to determine the similarities among the pox strains, we sequenced a 5.9-kb region of a Gal1 (strain 502F) and a Gal2 (strain 100W) representative strain corresponding to most of a sequenced canarypox region that contains the gene for thymidine kinase (Amano et al.1999; GenBank D86731). DNA from these two strains produced single amplicons with all primer pairs tested and the sequences of each amplicon were consistent with the presence of only a single pox strain in each specimen. Recently the complete genome sequence of a slightly different canarypox strain has been published (Tulman et al.2004; GenBank AY318871) providing another strain for comparison to Gal1 and Gal2. The 5.9-kb sequenced region spans from within gene CNPV117 to within gene CNPV109 (using the nomenclature of the genes for the complete canarypox genome [Tulman et al.2004]). Alignment of the Gal1 and Gal2 sequences with the two canarypox sequences and with fowlpox confirmed that Gal1 and Gal2 were most similar to both known canarypox sequences and not very similar to fowlpox. Within this region, Gal1 and Gal2 were 97.6% identical to each other, the two published canarypox strains were 98.7% identical to each other, Gal1 was 97-98% identical to the two canarypox strains, and Gal2 was 95-96% identical to the two canarypox. In contrast, fowlpox was only about 70% identical to the other strains. The aligned sequences were analyzed using maximum likelihood to produce a phylogenetic tree (Fig. 1). These results were fairly congruent with the pairwise comparisons. The

canarypox, Gal1 and Gal2 strains clustered together, and were separated by a long branch leading to fowlpox.

The quantitative analysis of recombination using the GENECONV program yielded 19 significant recombination events (global inner fragments) between ancestors of four of the five taxa included in the analysis (Table 2). Four instances, involving two taxa within the alignment, of significant recombination events with taxa outside the alignment or within, but obscured for some reason (global outer fragments), were also detected (Table 2). Notably, identical sequence segments or overlapping segments showing evidence of recombination occurred between more than one pair of sequences in many cases. More generally, there was statistical evidence of recombination at most nucleotide sites along the 5.9kb alignment between at least two lineages (Table 2).

A qualitative analysis of the alignment of the 5.9-kb region revealed that the identities among Gal1, Gal2 and canarypox were clustered in discrete regions further supporting recombination between strains. In the first 500 nucleotides, Gal1 matched canarypox perfectly, whereas Gal2 differed by about 5%. This was followed by a region of over 1500 nucleotides where Gal1 and Gal2 matched perfectly, while canarypox matched them in some regions but not in others. The next 350 nucleotides showed more variability, followed by a similar size region of identity among all three stains. For the remainder of the sequence Gal1 matched canarypox, while Gal2 matched in some regions but not in others. A region that showed many differences among all the strains was in the 5' end of the gene encoding thymidine kinase (CNPV113) (Fig.2). Many of these differences changed amino acids in the amino terminal region of the deduced proteins. Even the genes of the two canarypox strains showed differences in the amino acids

encoded in this region. The mosaic pattern of sequence identity combined with the many substitutions in the thymidine kinase gene suggests that recombination between poxvirus strains combined with mutation led to the variants of canarypox that are now prevalent on Santa Cruz and Isabela.

DISCUSSION

The introduction of domestic animals to archipelagos poses a threat to endemic species that are typically naïve hosts for foreign bacterial and viral infections and, hence, are highly susceptible. The loss of endemic birds in Hawaii has been attributed to the introduction of diseases by domestic birds, including avian pox and malaria (Warner, 1968; van Riper et al., 1986, 2002; Tripathy et al., 2000). To date there has been no report of *Plasmodium* blood parasites in Galápagos; however, avian pox has been prevalent on the islands for decades, infecting both domestic chickens and wild birds. Although many viruses are species specific, some, including pox (Reed et al., 2004) and influenza (Webby and Webster 2003), can cross species barriers, occasionally causing severe disease in the new host. An *Avipoxvirus* isolated from Amazon parrots was reported to infect chickens, suggesting that infection by avian pox across genera is possible (Boosinger et al., 1982). The presence of chickens on the inhabited islands of Galápagos has led to local concern that pox infection of these domestic birds could spread to the wild bird populations.

Restriction fragment length polymorphisms for the CA3-2 amplicon, as well as sequencing of a 5.9-kb region of the genome indicated that the endemic passerine birds, including finches, warblers and mockingbirds, were all infected with one of two closely

related variants of canarypox, but that none was infected with fowlpox. In contrast, chickens from Santa Cruz were infected with fowlpox but not the canarypox strains. These results indicated that on Santa Cruz and Isabela there was no evidence that the endemic bird species had been infected by pox viruses of chicken origin. However, the similarity of the Gal1 and Gal2 strains to canarypox suggested that the endemic birds might have been infected initially by a canarypox strain brought to the islands by either domestic or wild passerine bird(s). The canarypox-like strains that are now prevalent on these islands might have evolved from the initial *Avipoxvirus* strain by a combination of mutation and recombination. It will be interesting to determine the identity of pox strains from birds on the other islands of the Galápagos, particularly the uninhabited islands, where pox may be transmitted by migrant birds from other islands. Since the poxvirus is mechanically vectored, it can be transferred to a new host by any number of biting insects, as well as by shed virions in the substrate entering through any break in the skin. The community of vectors differs between the human-inhabited islands where biting insects requiring fresh water are found (e.g., the mosquito *Culex quinquefasciatus* and the blackfly *Simulium bipunctatum*). On islands without fresh water, the mosquito *Ochlerotatus taeniorhynchus* and a number of hippoboscid parasitic flies and ceratopogonid biting midges are common.

The genomes of all characterized poxviruses comprise a single chromosome of linear double-stranded DNA that has telomeric ends with covalently closed terminal hairpin loops. The virus encodes all the proteins required for DNA replication, which occurs in the cytoplasm of the host cell. Replication of the genome occurs through intermediates called concatamers, comprising many tandem repeats of the entire genome (Moyer and

Graves, 1981). Recombination, which occurs at extraordinarily high frequencies in pox, is an essential part of concatamer formation and replication (Ball, 1987). The virus-encoded DNA polymerase of vaccinia virus mediates recombination; mutants lacking this polymerase do not recombine DNA (Merchlinsky, 1989; Willer et al., 1999, 2001). Consistent with the role for recombination in replication of pox, the recombination pathway in vaccinia virus can very efficiently recombine pairs of linear molecules and requires only 12-20 bp of homology (Willer et al., 2000; Yao and Evans 2001).

Recombination is thought to serve a number of possible functions in pox: since there is no known primase, it may play a role in the priming of DNA replication it can function to repair double-stranded breaks in DNA; and it may provide a mechanism for acquisition of new genes from a coinfecting virus or from the host cell (Yao and Evans 2001). The similarity of many pox genes with mammalian genes provides strong evidence for the acquisition of host genes by pox, although the mechanism is not understood (Yao and Evans 2001). Intermolecular recombination between the genomes of different viruses has been implicated in the formation of new recombinant pox strains. Malignant rabbit fibroma virus, a lethal tumorigenic poxvirus of rabbits, resulted from recombination between Shope fibroma virus, which induces benign tumors in rabbits, and myxoma virus, which causes myxomatosis (Block et al., 1985; Upton et al., 1988). In another instance, the genome structures of one capripoxvirus isolate indicated that the progenitor of this strains resulted from recombination between the genomes of two other capripoxvirus strains (Gershon and Black, 1988; Gershon et al., 1989). Thus, recombination between different avipox strains may provide a powerful mechanism for rapid evolution of poxviruses in wild animals.

The gross phylogenic relationships among fowlpox, two canarypox strains, Gal1 and Gal2 portrayed here suggests that the two pox strains identified from endemic, wild, Galápagos passerines are close relatives of each other and canarypox lineages. However, since no strains from native, wild, mainland South American birds were included in this analysis, it is impossible to determine the nature and number of colonization events of *Avipoxvirus* into the Galápagos Archipelago. Nevertheless, the Gal1 and Gal2 strains are much more similar to other passerine poxviruses (e.g., canarypox) than they are to fowlpox. This phylogenetic reconstruction should be accepted with caution due to its narrow scope and since recombination can cause considerable error in tree estimation. There is clear evidence of recombination in the sequences of the 5.9-kb region. On Galápagos, the sympatry of fowlpox virus in the introduced chickens and the canarypoxvirus variants we describe here in endemic birds, presents opportunity for further recombinants of unknown effect.

The gene encoding thymidine kinase showed the greatest divergence of any gene in the 5.9-kb region even in the very closely related canarypox relatives. This viral enzyme is part of the salvage pathway that allows the pox virus to phosphorylate nucleotides for DNA synthesis. Although the gene is not essential, it is present in the genomes of all pox viruses sequenced to date except *Molluscum contagiosum* (Gubser et al., 2004). The difference in the amino acid sequences of the enzymes from very closely related strains suggests that it may evolve rapidly; hence, it may serve as a marker for identification of different avian pox strains and for measuring the rate of evolution of pox viruses from different islands in the Galápagos.

CONSERVATION IMPLICATIONS

Avipoxvirus infection can significantly increase mortality of Galapagos mockingbirds (Curry and Grant, 1989; Vargas, 1987). Presumably, it can have similar consequences for the other susceptible Galapagos endemics in which it occurs, although these have not been measured. Management plans for small populations further threatened by pathogens requires characterizing the pathogens: how many lineages are there, how are they related, how did they arrive, and can future impacts be predicted? This work suggests that the two lineages of avian pox described to date in endemic passerines in the Galapagos Islands did not arrive through the introduced chickens, which are infected with a very different poxvirus. The work does suggest, however, that significant recombination continues to occur among the strains in the endemic birds, which could continue to generate new forms of unknown pathogenicity. Three courses of action are suggested: (1) Work to understand the pathogenicity of the two extant strains, and any differences in their biology that would suggest avenues for control measures; (2) Focused studies of biting insect vectors of the two extant strains, to suggest avenues for vector control; and (3) Monitoring and sampling pox lesions from endemic birds proximate and distant to large chicken farms, and from the chickens themselves, for coinfections and recombination of fowlpox and the canarypox-like strains. .

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Table 1. Pox strains from birds in the Galápagos Islands

Strain	Species	Location	Pox type
300C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
301C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
302C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
303C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
304C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
305C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
306C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
307C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
308C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
309C	chicken (*Gallus gallus*)	Santa Cruz	fowlpox
100W	warbler (*Dendroica petechia)*	Santa Cruz	Gal2
101W	warbler (*Dendroica petechia)*	Santa Cruz	Gal1
102W	warbler (*Dendroica petechia)*	Santa Cruz	Gal2
500F	small ground finch (*Geospiza fuliginosa*)	Santa Cruz	Gal2
501F	small ground finch (*Geospiza fuliginosa*)	Santa Cruz	Gal1
502F	med. ground finch (*Geospiza fortis*)	Santa Cruz	Gal1
503F	med. ground finch (*Geospiza fortis*)	Santa Cruz	Gal1
504F	med. ground finch (*Geospiza fortis*)	Santa Cruz	Gal1
505F	finch (*Geospiza sp*.)	Santa Cruz	Gal1
508F	small ground finch (*Geospiza fuliginosa*)	Isabela	Gal1
509F	small ground finch (*Geospiza fuliginosa*)	Isabela	Gal1
510F	med. ground finch (*Geospiza fortis*)	Isabela	Gal1
512F	finch (*Geospiza sp*.)	unknown	Gal1
513F	cactus finch (*Geospiza scandens*)	Isabela	Gal1
514F	small ground finch (*Geospiza fuliginosa*)	Isabela	Gal2
515F	cactus finch (*Geospiza scandens*)	Santa Cruz	Gal2
516F	finch (*Geospiza sp*.)	Santa Cruz	Gal1
518F	finch (*Geospiza sp*.)	Santa Cruz	Gal1
519F	finch (*Geospiza sp*.)	Santa Cruz	Gal1
702M	mockingbird (*Nesomimus parvulus)*	Santa Cruz	Gal2
703M	mockingbird (*Nesomimus parvulus)*	Santa Cruz	Gal1

Table 2. Putative recombination events between ancestors of *Avipoxvirus* strains[a]

	Fragment	Sequence 1	Sequence 2	*P*[b]	Begin[c]	End[d]	Length[e]
1	GI	Canary	CanaryVR-111	0.0206	3605	4402	798
2	GI	Canary	Gal1	0.0007	464	897	434
3	GI	Canary	Gal1	0.0128	2591	2973	383
4	GI	Canary	Gal1	0.0000	3605	4402	798
5	GI	Canary	Gal2	0.0000	535	897	363
6	GI	Canary	Gal2	0.0047	2591	2838	248
7	GI	Canary	Gal2	0.0005	3326	3603	278
8	GI	Canary	Gal2	0.0002	3605	3940	336
9	GI	CanaryVR-111	Gal1	0.0001	1	927	927
10	GI	CanaryVR-111	Gal1	0.0000	2476	4431	1956
11	GI	CanaryVR-111	Gal1	0.0000	4433	5966	1534
12	GI	CanaryVR-111	Gal2	0.0001	535	927	393
13	GI	CanaryVR-111	Gal2	0.0126	2521	2838	318
14	GI	CanaryVR-111	Gal2	0.0000	3209	3940	732
15	GI	CanaryVR-111	Gal2	0.0000	4552	5258	707
16	GI	CanaryVR-111	Gal2	0.0020	5343	5684	342
17	GI	Gal1	Gal2	0.0000	535	2193	1659
18	GI	Gal1	Gal2	0.0001	3209	3940	732
19	GI	Gal1	Gal2	0.0013	4552	5258	707
20	GO	Fowlpox	N/A	0.0000	535	905	371
21	GO	Fowlpox	N/A	0.0160	2591	2838	248
22	GO	Fowlpox	N/A	0.0000	3209	3940	732
23	GO	Canary	N/A	0.0294	2152	2160	9

[a]Putative recombination events detected using GENECONV version 1.81 (Sawyer 1989). Nineteen significant ($P < 0.05$) recombination events were detected between members of the five-taxon sequence alignment (GI=Global inner fragments), and four significant recombination events were inferred between one taxon within and one unknown taxon outside the alignment or taxa within the alignment obscured by other evolutionary processes (GO=Global outer fragments).

[b]Based on global *P* value obtained by simulation via 10,000 permutations, corrected for multiple comparisons.

[c]Corresponds to the first nucleotide base of the recombinatory region.

[d]Corresponds to the last nucleotide base of the recombinatory region.

[e]Corresponds to the entire length of the recombinatory region.

Figure Legends

Fig. 1. A maximum likelihood phylogenetic tree (with TBR branch-swapping) of an alignment of a 5.9-kb region of DNA sequence from five *Avipoxvirus* strains rooted with fowlpox and bootstrapped (10,000 replications); support values are below the branches. Taxon labels are as follows: CanaryVR-111 (Genbank AY31887), Canary (GenBank D86731), Fowlpox (AF198100).

Fig. 2. Alignment of thymidine kinase. The deduced amino acids for the putative thymidine kinase genes of five strains of avian pox were aligned using ClustalW. Black highlights indicate residues that show some variability among all five strains. Grey highlights indicate residues that differ only in fowlpox. Canary1 from GenBank D86731; Canary2 from Genbank AY31887; Fowl from Genbank AF198100.

Fig. 1

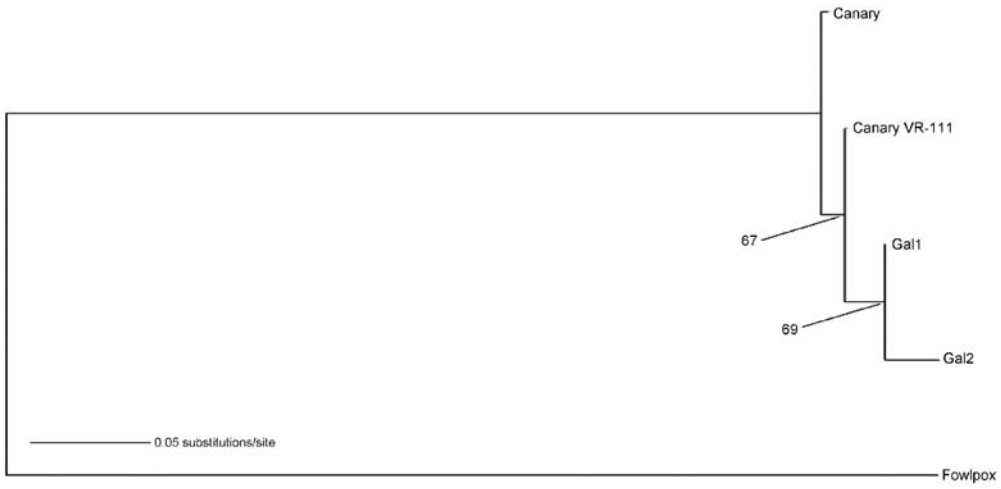
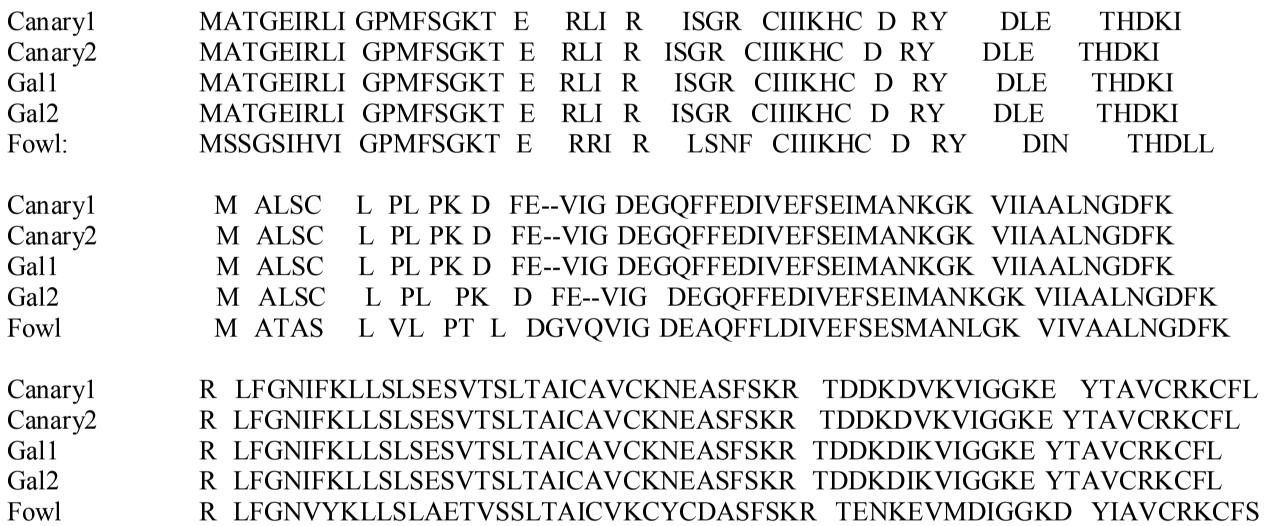

Canary
Canary VR-111
67
Gal1
69
Gal2
0.05 substitutions/site
Fowlpox

Fig. 2.


Canary1 MATGEIRLI GPMFSGKT E RLI R ISGR CIIIKHC D RY DLE THDKI
Canary2 MATGEIRLI GPMFSGKT E RLI R ISGR CIIIKHC D RY DLE THDKI
Gal1 MATGEIRLI GPMFSGKT E RLI R ISGR CIIIKHC D RY DLE THDKI
Gal2 MATGEIRLI GPMFSGKT E RLI R ISGR CIIIKHC D RY DLE THDKI
Fowl: MSSGSIHVI GPMFSGKT E RRI R LSNF CIIIKHC D RY DIN THDLL
Canary1 M ALSC L PL PK D FE--VIG DEGQFFEDIVEFSEIMANKGK VIIAALNGDFK
Canary2 M ALSC L PL PK D FE--VIG DEGQFFEDIVEFSEIMANKGK VIIAALNGDFK
Gal1 M ALSC L PL PK D FE--VIG DEGQFFEDIVEFSEIMANKGK VIIAALNGDFK
Gal2 M ALSC L PL PK D FE--VIG DEGQFFEDIVEFSEIMANKGK VIIAALNGDFK
Fowl M ATAS L VL PT L DGVQVIG DEAQFFLDIVEFSESMANLGK VIVAALNGDFK
Canary1 R LFGNIFKLLSLSESVTSLTAICAVCKNEASFSKR TDDKDVKVIGGKE YTAVCRKCFL
Canary2 R LFGNIFKLLSLSESVTSLTAICAVCKNEASFSKR TDDKDVKVIGGKE YTAVCRKCFL
Gal1 R LFGNIFKLLSLSESVTSLTAICAVCKNEASFSKR TDDKDIKVIGGKE YTAVCRKCFL
Gal2 R LFGNIFKLLSLSESVTSLTAICAVCKNEASFSKR TDDKDIKVIGGKE YTAVCRKCFL
Fowl R LFGNVYKLLSLAETVSSLTAICVKCYCDASFSKR TENKEVMDIGGKD YIAVCRKCFS

Chapter IX.

Population Genetics of The Galápagos Hawk (*Buteo galapagoensis*): Genetic Monomorphism Within Isolated Populations.

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ABSTRACT

Island populations tend to have less genetic variation and be more divergent than mainland populations due to their smaller size and isolation. We collected DNA samples from nine Galápagos hawk (*Buteo galapagoensis*) island populations, covering the entire species range. Neutral minisatellite DNA markers were used to calculate within-island genetic diversity and between-island genetic differentiation (F_{ST}). Typically, these markers mutate too quickly to be informative in such studies. However, in very small, isolated populations, concerns about high mutational rate are obviated by the relative force of genetic drift. Individuals within islands had the highest levels of reported genetic uniformity of any natural bird population, with mean within-population band-sharing similarity values ranging from 0.693 to 0.956, increasing with decreasing island size. Galápagos hawks exhibit cooperative polyandry to varying degrees across islands; however, we did not find an association between degree of polyandry and genetic

variability. Between-island F_{ST} values ranged from 0.017 to 0.896, with an overall archipelago value of 0.538; thus, most populations were genetically distinct. Also, we documented higher levels of genetic similarity between nearby populations. Our results indicated negligible gene flow among most Galápagos hawk populations, and genetic drift has played a strong role in determining structure at these minisatellite loci.

Population genetic structure reflects a number of processes, such as mutation rate, genetic drift, gene flow, natural selection, and phylogeographic history (Bohonak 1999; Ouborg et al. 1999). Genetic variability is lost via genetic drift and selection against some genotypes. Generally, genetic drift has a stronger effect in smaller populations; thus, a positive relationship between population size and genetic variation is expected (Nevo et al. 1984; Frankham 1996). Populations may diverge due to random fixation of different alleles, differences in selective pressures, or the addition of novel mutations. Gene flow, however, can have a homogenizing effect among populations and mitigate the loss of intra-population variation by adding new alleles or replacing alleles lost due to drift (Slatkin 1985).

Populations on islands often have lower levels of genetic variation than those on the mainland (Frankham 1997). Populations of birds on island archipelagos tend to be more strongly differentiated than geographically separate mainland populations as water acts as an effective barrier to gene flow for many species (Williamson 1981; Boag 1986; Baker et al. 1990). These patterns of decreased genetic variation and increased differentiation may result from founder events that occurred at the time of colonization (Mayr 1954). In many cases, though, founding flock sizes may be large enough that founder effects are negligible (e.g. Clegg et al. 2002). Even when the number of founders is known to be quite small, subsequent arrival of additional immigrants may prevent a measurable founder effect (Grant et al. 2001). Alternatively, lower variability and increased differentiation on islands may be due to sequential founder events (Clegg et al. 2002), long-term genetic drift working in small, isolated populations (Baker et al. 1990; Mundy et al. 1997), or a combination of the two.

The Galápagos hawk (Aves: Falconiformes: *Buteo galapagoensis*) is endemic to the Galápagos archipelago located almost 1000 km west of South America. The islands are volcanic in origin, having arisen from a mantle hotspot (Morgan 1971), and they have never been connected to the mainland. The oldest of the present islands is approximately four million years old (White et al. 1993). However, older, now submerged seamounts to the southeast of the archipelago indicate that islands have been present over the hotspot for at least seventeen million years and probably for much longer (Christie et al. 1992; Werner and Hoernle 2003).

Hawks are presently found on nine islands: Santa Fe, Española, Pinzón, Santiago, Santa Cruz, Isabela, Fernandina, Pinta, and Marchena (Fig. 1). Historically, humans have shot hawks, and the hawks are now extirpated on two human-inhabited islands, San Cristóbal and Floreana. The Santa Cruz population may also have been extirpated; no adults have been seen on the island in recent years, but juveniles are seen periodically. Islands with Galápagos hawk populations are separated by distances of less than 5 km up to around 240 km (Fig. 1). The level of hawk migration between islands is unknown but presumed to be low (de Vries 1975), as most *Buteos* are reluctant to cross large bodies of water (Kerlinger 1985). Swainson's hawks (*Buteo swainsoni*) are the Galápagos hawk's closest mainland relatives (Riesing et al. 2003), and they migrate long distances over land (from North America to Argentina) but avoid flying over water (Fuller et al. 1998). Galápagos hawk populations vary morphologically and behaviorally, also suggesting genetic isolation. They differ in overall body size, and in allometry to a lesser degree, across islands (de Vries 1973; Bollmer et al. 2003). Galápagos hawks exhibit cooperative polyandry, where territorial groups consist of one female and up to eight

(usually two or three) unrelated males (Faaborg and Patterson 1981; Faaborg et al. 1995). Paternity is shared within and among broods, though there are often more males in a group than the number of chicks produced per brood (1-2); all birds in the group defend the communal territory and care for the brood, including males that are not the genetic sires of the offspring (Faaborg et al. 1995; DeLay et al. 1996). One Galápagos hawk population appears to be monogamous (Española), while the rest exhibit cooperative polyandry to varying degrees, with mean group sizes ranging from 2.5 to 4.5 birds (de Vries 1975; Faaborg et al. 1980; Bollmer et al. 2003). The factors contributing to this variation in mating system (e.g. sex ratio, survivorship) are unstudied but are likely associated with differences in habitat structure and resource availability.

In this study, we described the genetic structure of all nine populations of Galápagos hawks (thus sampling the entire range of the species) using multilocus minisatellite DNA markers. Minisatellites are hypervariable regions of DNA consisting of tandem repeats of short units of nucleotides (Jeffreys et al. 1985), which have been used to characterize population structure (e.g. Freeman-Gallant 1996; Carneiro da Silva and Granadeiro 1999; Gullberg et al. 1999; Tarr and Fleischer 1999). We described the amount of genetic variation present in populations and measured the degree of differentiation among populations using Wright's F_{ST}, the standardized variance in allele frequencies among populations (Wright 1951, 1978). We tested the prediction that genetic variation increases with population size by using total island area and total area of appropriate habitat as indices of population size. In addition to population size, variation in mating system is predicted to partly determine genetic variability by impacting effective population size, mostly through biased sex ratios and variance in reproductive

success (Nunney 1993; Parker and Waite 1997). In the Galápagos hawk, there may be increased variance in reproductive success and more skewed sex ratios in the more polyandrous populations, which would lead to decreased effective population sizes relative to total population size and a more rapid loss in variation. We tested for an effect of mating system (degree of polyandry) on genetic variability after first controlling for island area. Finally, we asked whether populations closer in geographic proximity are more similar genetically due to increased gene flow or more recent separation (isolation by distance).

METHODS

Field methods. —We visited the Galápagos Islands for two to three months each year between May and August from 1998 to 2003. Hawks (n = 541) were captured on nine islands: 25 individuals from Santa Fe, 23 from three sites on Española (Gardner Bay, Punta Suarez, and Punta Cevallos), 287 from three sites on Santiago (James Bay, Sullivan Bay, and the highlands), 93 from Volcan Alcedo on Isabela, 41 from Pinta, 26 from Marchena, 10 from Pinzón, 32 from Fernandina, and 4 from Santa Cruz. The hawks were caught using two methods: a balchatri trap baited with a live prey animal such as a rat (Berger and Mueller 1959) or a rope noose on a stick to capture perched birds (Faaborg et al. 1980). We banded each hawk with an aluminum and/or anodized color band and took two 50 □l blood samples via venipuncture of the brachial vein. Samples were immediately put into 500 □l of lysis buffer (100 mM Tris, pH 8.0, 100

mM EDTA, 10 mM NaCl, 0.5% SDS; Longmire et al. 1988), shaken, and stored at ambient temperature.

Minisatellite DNA markers.—The use of hypervariable multi-locus minisatellite profiles (VNTRs) in studies of population genetic differentiation is typically problematic due to constraints imposed in part by a high mutational rate at these loci (Flint et al. 1999). Moreover, Flint et al. (1999) cautioned that calculating F_{ST} values between human populations using minisatellites yielded an underestimate of genetic differentiation when compared to the level found via other markers. Therefore, their use in characterizing population genetic differentiation, at least in light of this finding, is a statistically conservative methodology. However, in special cases, such as those involving isolated island vertebrate populations, "the fixation of restriction-fragment polymorphisms can outpace the generation of fragment-length variability through recombination" (Gilbert et al. 1990). This claim was buttressed by the finding that all bands were fixed within one population of the Channel Island fox, and that individual foxes within each island had diagnostic, island-specific bands. Clearly in this and analogous special cases, "differences among hypervariable restriction-fragment profiles can be used to estimate relative genetic variability and to reconstruct the evolutionary relationships of natural populations" (Gilbert et al. 1990) because concerns related to a high mutational rate are largely obviated by the relative force of genetic drift in small populations.

In this study, we extracted DNA and performed multilocus minisatellite DNA fingerprinting using the restriction endonuclease *Hae*III and Jeffreys' probe 33.15 (Jeffreys et al. 1985) following procedures described in Parker et al. (1995). After hybridization, we used a Storm 820 Phosphorimager to visualize fingerprints. For most

populations, we used only a subset of the samples (n = 163) for genetic analyses: 15 from Santa Fe, 15 from Española, 37 from Santiago, 22 from Isabela, 20 from Pinta, 20 from Marchena, and 20 from Fernandina. From Pinzón and Santa Cruz, we used all birds sampled (10 and 4, respectively), and they were all juveniles. For the other populations, we randomly selected individuals from the pool of sampled territorial adults (the class most likely to consist of non-relatives). We did not run all samples; however, fewer individuals are necessary to get a representative sample when populations (such as these) are lacking in genetic variability. We ran a total of nine gels, with 17 to 26 lanes each. We ran samples in alternating blocks of three to seven individuals from each island, so that multiple islands were represented on each gel. We chose four individuals from different islands as ladders and ran them on each of the gels. From the banding patterns, we created a presence-absence matrix of bands (alleles) encompassing all individuals. Due to high within-population genetic uniformity, the presence of a number of bands fixed across populations, and the ladders on each of the gels, we were able to reliably score across gels.

We assumed that bands were assorting independently and calculated within- and between-island similarity indices as $S = 2S_{AB} / (2S_{AB} + N_A + N_B)$, where S is the proportion of bands shared, S_{AB} is the number of bands shared by individuals A and B, N_A is the number of bands unique to individual A, and N_B is the number of bands unique to individual B (Wetton et al. 1987; Lynch 1988, 1990). We calculated these from our presence-absence matrix using the program GELSTATS v. 2.6 (Rogstad and Pelikan 1996).

In fingerprinting, individuals are often used in multiple pairwise comparisons, thus resulting in nonindependence of band-sharing values (Danforth and Freeman-Gallant 1996; Call et al. 1998; Leonard et al. 1999). We used the *p-dif* test (Bertorelle et al. 1999) in the program Watson (Bucchini et al. 1999), a test that permutes individuals, not band-sharing values, to ask if within-island band-sharing values significantly differed from between-island values. We calculated F_{ST} values for each pairwise comparison of islands, as well as an overall archipelago value, according to Lynch (1990, 1991). F_{ST} values attain a maximum value of one when two subpopulations are fixed for different alleles (complete differentiation) and fall to zero when alleles are distributed randomly among subpopulations (no differentiation).

We used a linear regression to test the prediction that population genetic uniformity (as measured by within-island similarity indices) decreases with increasing island area. We calculated total island area in the program ArcMap 9.1 using digitized vegetation coverage maps held by the Charles Darwin Research Station, and then we used the log of island area in the regression. Large portions of some of these islands (up to 75% of total island area) are barren of vegetation, making them less suitable for hawk territories. Total island area may therefore overestimate population size in some cases, so we did a second regression using the log of total vegetated area (excluding lava and beaches). We tested for an effect of mating system with a general linear model, using band-sharing values as the dependent variable, mean group size as a fixed factor, and log of total island area as a covariate. Due to the non-independence of minisatellite band-sharing values, we first randomly selected a subset of independent values (using each individual once) from each population. For mating system, we classified each island as

having a mean group size of less than two males or more than two males using published data from de Vries (1975) and Bollmer et al. (2003) and new data collected from Fernandina in 2003 (1.4 ± 0.5 males per group, n = 10 groups). So, we classified Española, Santa Fe, Pinzón, and Fernandina as less polyandrous (mean group sizes of 1-1.5 males) and Isabela, Santiago, Marchena, and Pinta as more polyandrous (mean group sizes of 2.3-3.5 males). We used a Mantel (1967) test to examine isolation by distance (Slatkin 1993), testing the prediction that genetic differentiation among populations (F_{ST}) should increase with increasing geographic distance between them. We log-transformed the distance between islands as measured between nearest points. We performed these analyses in SPSS v. 10.0.5 for Windows (SPSS Inc. 1999) and IBDWS v. 2.0 beta (Jensen et al. 2004). We excluded Santa Cruz from the above analyses due to its small sample size.

Because there does not appear to be a breeding population on Santa Cruz, we performed an assignment test to see whether the juveniles we captured on Santa Cruz closely matched any of the other populations, which would indicate they could be migrants. While there are no tests designed for codominant minisatellite data, the online program Doh (Brzustowski 2002) as first described in Paetkau et al. (1995) can accommodate data from dominant markers by treating each band as a separate locus. We performed a segregation analysis by tallying, within each population, the co-occurrences of each band with every other band in order to note cases of linkage (bands always appearing together within individuals) and allelism (individuals always having one or the other band but never both, indicating they belong to the same locus). We found no cases of linkage, and we eliminated all cases of allelism (most due to rare bands) by removing

the less frequent band from each allelic dyad. We entered the remaining 23 independent bands into the Doh program as presence/absence data for each individual. The program assigns each individual into the population in which its genotype has the highest probability of occurring.

RESULTS

Within-population similarity.—We scored an average (± SD) of 14.1 ± 1.42 bands for each individual. Within-island similarity indices were high, ranging from 0.693 for Isabela to 0.956 for Santa Fe (Table 1). The mean similarity index for Santa Cruz was slightly lower (0.657), but this is based on only six pairwise comparisons. Birds from Santa Fe were particularly lacking in genetic variation, having only a few variable bands. Specifically, 13 of the 16 Santa Fe bands scored were fixed in the population. All 15 Santa Fe birds were identical to two or three other birds, resulting in only four different genotypes in that population. In addition, four of the 10 birds on Pinzón were identical, while there were two sets of identical birds (two and three birds each) out of 15 individuals sampled on Española and four sets of identical birds (two or three birds each for nine total) on Marchena. The other populations (Isabela, Fernandina, Santiago, and Pinta) were more variable and had no identical individuals.

Regression analyses supported our prediction that genetic similarity among individuals in a population decreases with increasing total island area (r = -0.844, df = 7, P = 0.008; Fig. 2) and vegetated area (r = -0.846, df = 7, P = 0.008), though there was no substantial difference between the two measures. A general linear model showed there

was no effect of degree of polyandry on genetic variability after controlling for island area ($F = 0.537$, $P = 0.466$, $n = 78$), while there was still a strong island area effect after controlling for mating system ($F = 32.1$, $P < 0.0001$, $n = 78$).

Population differentiation.—Between-island F_{ST} values ranged from 0.017 to 0.896 (Table 2) with an overall archipelago value of 0.538. We performed pairwise permutation tests to test whether populations were significantly distinct from each other. There were 28 pairwise comparisons, so we used a Bonferroni correction to avoid Type I errors, which brought our alpha level down to 0.002. Twenty-three of the 28 comparisons still showed significant differences among populations ($P < 0.001$ for all). Four of the five nonsignificant values involved Pinzón compared to Isabela ($P = 0.058$), Fernandina ($P = 0.021$), Santiago ($P = 0.820$), and Pinta ($P = 0.006$). The remaining comparison, Isabela vs. Fernandina ($P = 0.203$), had the lowest F_{ST} value (0.017; Table 2). Three of the five nonsignificant values also represent the three smallest interisland distances.

We had predicted that populations would exhibit isolation by distance. A Mantel test confirmed this, showing a significant pattern of increasing genetic differentiation with increasing distance between islands ($r = 0.626$; $P \leq 0.003$; Fig. 3).

Between-island dispersal.—Over the past few decades, juveniles have occasionally been seen on islands where there was no resident hawk population, but no individual banded on one island had ever been observed on another island. In 2003, however, we observed two banded individuals on Fernandina, an island where hawks had not previously been studied. One individual, a territorial adult female, had been banded by us as a second-year juvenile on Volcan Alcedo, Isabela in 1998. The other bird was a territorial male

whose band could not be read. It is very likely he was also banded as a juvenile on Alcedo in 1998, since 70 birds were caught there in two days, 64 of which were juveniles. Also, it is unlikely he could have come from an island other than Isabela, because Isabela separates Fernandina from all the other islands (Fig. 1).

In Table 3 we present the results of the assignment test for each population. The program accurately assigned all the individuals from the more genetically monomorphic Española, Santa Fe, Pinzón, and Marchena populations to their home islands, while there were misassignments among the larger populations, likely due to their greater genetic variability. The assignment test placed the four Santa Cruz juveniles into the populations they most closely matched. One of the four individuals caught on Santa Cruz had a banding pattern identical to one of the Santa Fe genotypes, and the assignment test placed it within the Santa Fe population. Another of the Santa Cruz individuals had a banding pattern very similar to those on Pinzón (mean band-sharing between it and the Pinzón individuals was 0.911 ± 0.03), and the assignment test placed it within the Pinzón population. The last two Santa Cruz individuals matched Santiago best, though the chance for an assignment error is higher for the more variable populations.

DISCUSSION

Genetic variation within populations.—In this study, we were able to characterize population genetic structure of nine Galápagos hawk populations, covering their entire species range. The hawk populations exhibited very little genetic variation, having

within-population similarity indices ranging from 0.6 to over 0.9 at hypervariable minisatellite loci. To our knowledge, the smaller Galápagos hawk populations have the highest reported levels of monomorphism at minisatellite loci of any natural bird population, though some populations of New Zealand birds (reviewed in Miller et al. 2003) and other endangered island bird species (e.g. Rave 1995; Caparroz et al. 2001) are nearly as inbred. Gilbert et al. (1990) found even higher mean band-sharing values for populations of Channel Island foxes (*Urocyon littoralis*), another top predator, ranging from 0.75 up to 1.00. In contrast, unrelated birds in outbred mainland populations typically have band-sharing values around 0.2 and 0.3 (Parker Rabenold et al. 1991; Papangelou et al. 1998). Although there are no published studies using minisatellites in other Buteos, mean band-sharing within a small sample of migrating Swainson's hawks was 0.374 ± 0.10 (n = 8; unpubl. data). So, the Galápagos hawk's ancestral mainland polymorphism was likely much higher.

Extremely low genetic variability within this species is probably the result of a single founder event coupled with long-term genetic drift. The *Buteo* phylogeny by Riesing et al. (2003) shows a very recent divergence between Galápagos and Swainson's hawks, and mtDNA work underway on the Galápagos hawks indicates a single colonization event (Bollmer, Kimball et al., unpubl. data). Although there is evidence that island colonizations may not always result in a significant decrease in genetic diversity (Clegg et al. 2002; Grant 2002), in this case, the founding population of hawks may have been small enough that a severe bottleneck occurred. The high mean inter-island band-sharing (0.617) and the presence of bands that are fixed across all populations (even though most populations are currently genetically isolated) suggest that

hawks became inbred early on in their colonization of the islands. The close relationship between island area and genetic variation across populations indicates that long-term genetic drift has also been an important factor influencing the level of variability in the Galápagos hawk. The smallest populations have become fixed or nearly fixed for many of their bands, with different bands being common in different populations.

Within-island genetic uniformity decreased significantly with increasing population size, as approximated by total island area and vegetated area. While total island area explained a large portion of the variance in genetic similarity (r = -0.844), we had supposed that population size (and thus genetic variability) would correlate even more strongly with vegetated area due to the presence of large tracts of barren lava on some islands. Using only vegetated area, however, did not substantially improve the correlation (r = -0.846), even though five of the islands are less than 70% vegetated, two greatly so. We excluded Santa Cruz from this analysis because it differs from the rest of the islands in that it has an artificially small population on a large island due to the human impact there. Even though the Santa Cruz population is almost certainly the smallest in the archipelago, the four juvenile hawks sampled there exhibited the lowest mean similarity of any of the populations, probably due to inter-island movements of birds, which will be discussed below.

We found that there was no effect of mating system on genetic variability of Galápagos hawk populations. We had predicted that increased polyandry might result in lowered effective population sizes relative to total population size due to increased variance in male reproductive success or more strongly biased sex ratios. The lack of difference between low and high polyandry populations shows that mating system is not

a strong determinant of genetic variability in the Galápagos hawk; shared paternity may mitigate the effects of increased polyandry. Also, population size accounts for such a large portion of the variance in within-island genetic similarity that there is little remaining variability upon which other forces could act.

Genetic divergence among populations.—Overall, the high F_{ST} values indicate that Galápagos hawks are reluctant to cross large stretches of water, which is consistent with the migratory behavior of their closest mainland relatives (Fuller et al. 1998). Most hawk populations appear to be significantly genetically different from each other, with the exception of the interaction between Isabela and Fernandina and four comparisons involving Pinzón. The comparisons involving Pinzón are more suspect given that we sampled only 10 individuals on Pinzón, all of which were floater juveniles instead of territorial adults. Also, the use of the Bonferroni correction increased the probability of Type II errors, especially for the two comparisons with *P*-values of 0.006 (Pinzón vs. Pinta) and 0.021 (Pinzón vs. Fernandina). These two comparisons are also the most geographically distant of the nonsignificant values.

The hawk populations were divergent to varying degrees, as indicated by the pattern of isolation by distance. Lower F_{ST} values between nearby populations may be the result of ongoing (albeit relatively rare in most cases) gene flow between them, more recent population separation, or a combination of the two. Española and Santa Fe were the most divergent from the rest of the archipelago, with F_{ST} values between them and the other islands ranging from 0.5 to 0.9. Their relatively extreme divergence (especially from each other) is likely due to the random fixation of alleles in these populations that are not common on other islands.

Fernandina and Isabela were indistinguishable at these minisatellite loci. Of all island pairs, they are separated by the shortest distance (< 5 km), and we observed a bird banded on Isabela residing in a territory on Fernandina. The lack of differentiation between these two populations, therefore, could be due to ongoing gene flow. Alternatively, their similarity could be due to more recent separation or drift acting more slowly in larger populations. With the current data we are unable to distinguish among these scenarios.

The four juveniles we captured on Santa Cruz are likely migrants from neighboring islands. When fledglings leave their territories, they spend at least three or four years in a non-territorial floater population, roaming all over their native island and occupying areas not used by territorial birds (de Vries 1975). Because of this nomadic behavior, we suggest that juveniles are much more likely than adults to move between islands. Dispersal of juveniles to Santa Cruz could be more probable than movement to other islands, because Santa Cruz is mostly or entirely uninhabited by a territorial adult population, which means that suitable habitat is vacant, and juveniles are not likely to be harassed and driven away by adults. The assignment test placed two of the birds into the Santa Fe and Pinzón populations with high degrees of probability. The other two were most similar to Santiago, though there is more likely to be a misassignment when dealing with more variable populations. Santiago is a likely source population because it supports a large floater population and is an adjacent island. We cannot eliminate the possibility that one or more of these birds was born on Santa Cruz since we could not compare them to a sample of resident Santa Cruz territorial birds, because of the lack of known breeding adults there.

Island archipelagoes are well known as arenas for radiations of species (e.g. Darwin's finches, Hawaiian honeycreepers). Although we have described morphological and behavioral differences among populations of Galápagos hawks (Bollmer et al. 2003), and now the genetic differentiation shown here, these differences are on a microevolutionary scale. Presumably, hawks are one of the more recent arrivals to the archipelago, and have not been there long enough to diverge into subspecies or new species. Drift has had a strong influence on divergence at these neutral minisatellite markers, but the importance of drift in speciation is debatable (Barton 1998). Given the genetic isolation of many of these hawk populations, the Galápagos hawk may one day match the patterns seen in other sedentary species groups in the archipelago (e.g. the Galápagos tortoises [*Geochelone elephantopus* subspp.], lava lizards [*Microlophus* spp.]), with multiple subspecies or species restricted to one or a few islands.

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Table 1 Mean within-island Galápagos hawk minisatellite band-sharing value (± SD), total island area, and percent of each island that is vegetated (not lava or beach); islands are listed in order of increasing area as provided in Black (1973).

Island	Within-Island *S*	Area (ha)	% Vegetated
Pinzón	0.903 ± 0.067	1815	95.2
Santa Fe	0.956 ± 0.032	2413	100.0
Pinta	0.765 ± 0.083	5940	62.0
Española	0.900 ± 0.052	6048	98.2
Marchena	0.891 ± 0.047	12,996	25.4
Santiago	0.711 ± 0.086	58,465	68.6
Fernandina	0.719 ± 0.101	62,248	30.5
Santa Cruz	0.657 ± 0.157	95,555	~
Isabela	0.693 ± 0.086	458,812	66.5

Table 2 Pairwise comparisons of between-island differentiation. Mean between-island band-sharing values (± SD) are above the diagonal, with total number and number of independent pairwise comparisons scored in parentheses. F_{ST} values are reported below the diagonal.

	Española	Santa Fe	Pinzón	Isabela	Fernandina	Santiago	Marchena	Pinta
Española	~	0.306 ± 0.03 (225, 15)	0.656 ± 0.04 (150, 10)	0.546 ± 0.08 (330, 15)	0.534 ± 0.10 (300, 15)	0.593 ± 0.08 (555, 15)	0.579 ± 0.05 (300, 15)	0.563 ± 0.70 (300, 15)
Santa Fe	0.896	~	0.489 ± 0.04 (150, 10)	0.485 ± 0.08 (330, 15)	0.443 ± 0.08 (300, 15)	0.509 ± 0.07 (555, 15)	0.404 ± 0.05 (300, 15)	0.470 ± 0.07 (300, 15)
Pinzón	0.714	0.862	~	0.702 ± 0.08 (220, 10)	0.716 ± 0.09 (200, 10)	0.737 ± 0.07 (370, 10)	0.753 ± 0.05 (200, 10)	0.748 ± 0.07 (200, 10)
Isabela	0.551	0.659	0.322	~	0.701 ± 0.09 (440, 20)	0.669 ± 0.09 (814, 22)	0.641 ± 0.08 (440, 20)	0.632 ± 0.09 (440, 20)
Fernandina	0.591	0.708	0.335	0.017	~	0.675 ± 0.09 (740, 20)	0.631 ± 0.08 (400, 20)	0.636 ± 0.10 (400, 20)
Santiago	0.522	0.661	0.266	0.100	0.123	~	0.672 ± 0.07 (740, 20)	0.667 ± 0.08 (740, 20)
Marchena	0.752	0.872	0.583	0.421	0.472	0.393	~	0.753 ± 0.08 (400, 20)
Pinta	0.617	0.737	0.341	0.264	0.291	0.213	0.304	~

Table 3 Results of Galápagos hawk assignment test using minisatellite data. Rows represent the populations in which we sampled the individuals, while columns represent the populations to which Doh assigned the individuals. Santa Cruz is listed only as an island of capture, because there is no resident hawk population there with which possible migrants could be compared.

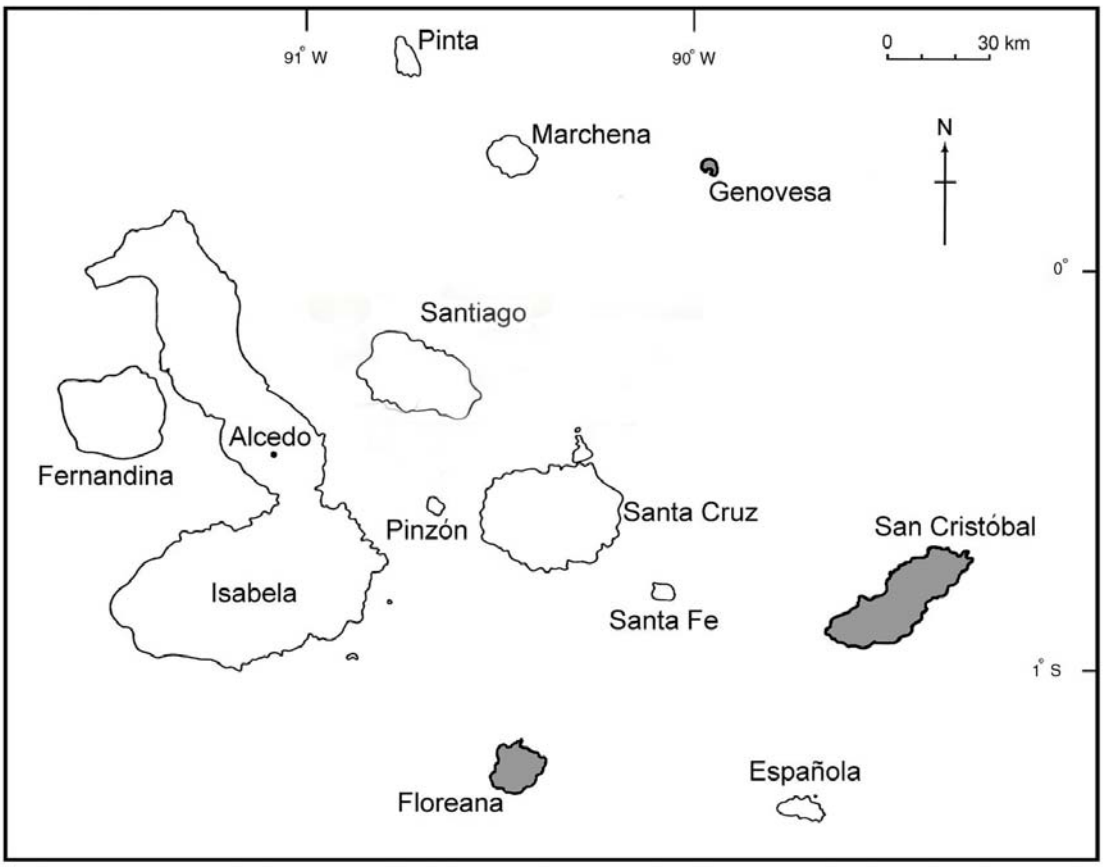
	Española	Santa Fe	Pinzón	Isabela	Fernandina	Santiago	Marchena	Pinta
Española	**15**							
Santa Fe		**15**						
Pinzón			**10**					
Isabela			**2**	**10**	**8**	**2**		
Fernandina				**5**	**13**	**2**		
Santiago			**1**	**5**	**5**	**23**		**3**
Marchena							**20**	
Pinta			**1**	**1**			**5**	**13**
Santa Cruz		**1**	**1**			**2**		

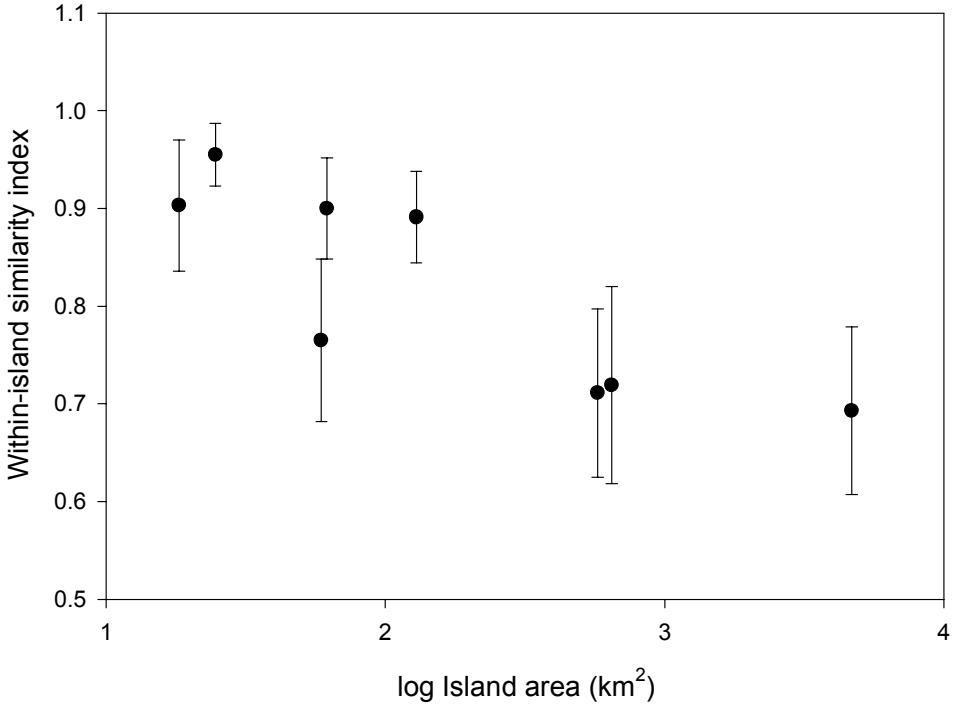
FIGURE LEGENDS

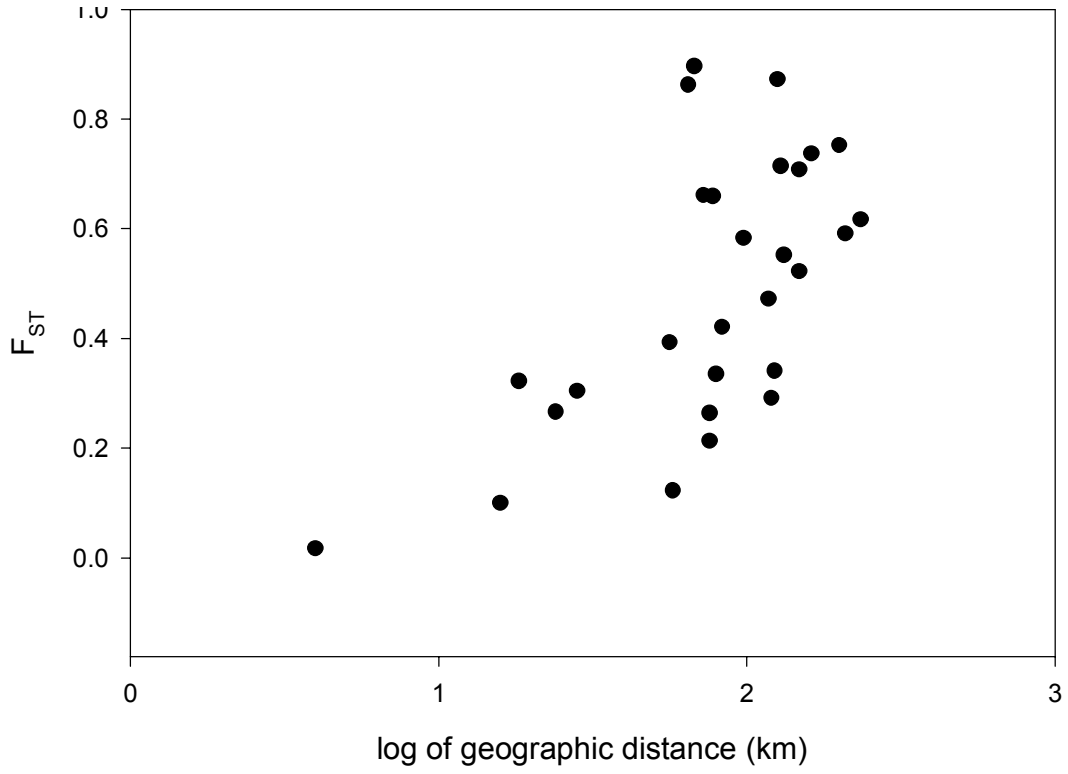
Fig. 1 Distribution of the Galápagos Hawk on the Galápagos Islands. All labeled islands currently have hawk populations except for three islands that are shaded. Genovesa has never supported a hawk population, and the populations on San Cristóbal and Floreana have been extirpated.

Fig. 2 Plot of mean genetic similarity (±SD) of individuals within islands against the log of island area (km^2). The data support our prediction that within-population genetic similarity should decrease with increasing island size.

Fig. 3 Plot of pairwise inter-island F_{ST} values against the log of geographic distances (km) between islands for Galápagos hawks. The degree of genetic differentiation between populations increases with increasing geographic distance.


91° W
Pinta
90° W
0 30 km
Marchena
N
Genovesa
0°
Santiago
Alcedo
Fernandina
Pinzón
Santa Cruz
San Cristóbal
Isabela
Santa Fe
1° S
Floreana
Española


1.1
1.0
0.9
0.8
0.7
0.6
0.5
Within-island similarity index
1
2
3
4
log Island area (km2)


1.0
0.8
0.6
0.4
0.2
0.0
F_{ST}
0
1
2
3
log of geographic distance (km)

Chapter X.

Phylogeography of The Galápagos Hawk (*Buteo galapagoensis*): A Recent Arrival to the Galápagos Islands

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ABSTRACT

Galápagos hawks (*Buteo galapagoensis*) are one of the most inbred bird species in the world, living in small, isolated island populations. We used mitochondrial sequence and nuclear minisatellite data to describe relationships among Galápagos hawk populations and their colonization history. We sampled ten populations (encompassing the entire current species range of nine islands and one extirpated population), as well as the Galápagos hawk's closest mainland relative, the Swainson's hawk (*B. swainsoni*). There was little sequence divergence between Galápagos and Swainson's hawks (only 0.42% over almost 3 kb of data), indicating that the hawks colonized Galápagos very recently, likely less than 300,000 years ago, making them the most recent arrivals of the studied taxa. There were only seven, closely related Galápagos hawk haplotypes, with most populations being monomorphic. The mitochondrial and minisatellite data together indicated a general pattern of rapid population expansion followed by genetic isolation of hawk breeding populations. The recent arrival, genetic isolation, and phenotypic

differentiation among populations suggest that the Galápagos hawk, a rather new species itself, is in the earliest stages of further divergence.

Key words: *Buteo galapagoensis*; Galápagos hawk; minisatellite DNA; mitochondrial DNA; phylogeography

1. Introduction

Island archipelagos have long been valuable for understanding evolutionary processes (Darwin, 1859; Grant, 1998; Whittaker, 1998). The relatively small size and isolation of populations on archipelagos often results in the occurrence of multiple, closely related yet distinct lineages on neighboring islands. There are numerous examples of radiations occurring in a variety of taxa on island systems around the world (e.g., Wagner and Funk, 1995). The refinement of phylogenetic techniques has opened up new avenues of investigation of these systems (Grant, 2001; Emerson, 2002), revealing mainland source populations and colonization patterns within archipelagos (e.g., Warren et al., 2003).

The Galápagos Islands, located on the equator 1000 km west of mainland Ecuador, are one of the most isolated archipelagos in the world and thus have a high degree of endemism. Almost a third of the plant species and half of the insect species are endemic (Tye et al., 2002). Fifty-nine percent of the vertebrates are endemic, including all of the native reptile and terrestrial mammal (rats) taxa (Tye et al., 2002). Endemism is high among the native terrestrial birds (84%) also, but it is much lower among the seabirds (26%) and shorebirds (23%; Tye et al., 2002). Though many taxa have speciated from their mainland ancestors, radiations within the Galápagos archipelago are relatively rare compared to other, older archipelagos where taxa have had more time to speciate (Tye et al., 2002).

The islands in the Galápagos archipelago form over a mantle hotspot and drift in a southeasterly direction with the movement of the Nazca plate. The current islands range from less than half a million years old in the west up to 4 million years old in the east

(White et al., 1993); however, older, now submerged islands indicate that islands have been present over the hotspot for at least 17 million years (Christie et al., 1992; Werner and Hoernle, 2003).

Radiations within Galápagos vertebrate lineages are skewed toward the reptiles and mammals, with few occurring among the birds (Table 1). There are about 40 recognized reptile taxa (including species and subspecies, depending on the latest taxonomic revisions). These 40 likely arose from only nine or ten original lineages from the mainland. The species and subspecies within taxa are generally isolated on different islands or volcanoes within an island. Within the mammals, the rice rats underwent a radiation, while neither of the two bat species have done so.

The pattern among the terrestrial birds is distinctly different from that of the reptiles. Only two of the founding bird lineages radiated into multiple species on the archipelago: the finches and the mockingbirds (Table 1). Two subspecies of Galápagos dove have been recognized (Swarth, 1931), but the rest of the taxa (even though they are all present on multiple islands) have not been subdivided. So, the 30 distinct lineages of terrestrial birds present now arose from only 14 colonizing lineages. This is a 2:1 ratio of current to colonizing lineages, whereas the reptiles are about 4:1. The 2:1 ratio is highly skewed by the finch radiation, the complexity of which is unique among Galápagos birds. Excluding the finches, the relationship drops to 1.4:1. None of the 32 lineages of seabird or aquatic/shorebird have radiated within the Galápagos Islands. This striking difference between birds and reptiles has two possible explanations. First, birds are obviously more mobile, and so gene flow among populations might be preventing further divergence. Second, most of the bird species might have colonized the archipelago more recently and

thus have not had time to diverge. Both explanations are supported by the lower degree of endemism seen among the birds, especially the waterbirds. It is possible that the lack of differentiation within bird lineages is due to their being not as well studied as the reptiles, but most Galápagos vertebrate lineages have been recognized for decades from extensive museum collections (long before genetic studies on particular taxa).

1.1. Galápagos hawk

Here, we characterize the population genetic structure and colonization history of one of these terrestrial bird species, the endemic Galápagos hawk (*Buteo galapagoensis*). The islands' only diurnal raptor, this hawk is widely distributed within the archipelago, currently inhabiting nine islands: Española, Santa Fe, Pinzón, Santiago, Isabela, Fernandina, Marchena, Pinta, and Santa Cruz. Once the "center of abundance" of the species distribution (Gifford, 1919), the Santa Cruz breeding population may now be extinct, though juveniles are occasionally seen there (Bollmer et al., 2005). To our knowledge, hawks have never existed on Genovesa, and their populations on Floreana (Steadman and DeLeon, 1999) and San Cristóbal were extirpated due to human activities. Morphological studies have been inconclusive as to the putative mainland sister species of the Galápagos hawk, focusing on several New World *Buteo* species (Brown and Amadon, 1968; Mayr and Short, 1970; Voous and de Vries, 1978). Molecular phylogenetic studies suggest that Galápagos hawks are most closely related to the Swainson's hawk (*B. swainsoni*; Fleischer and McIntosh, 2001; Riesing et al., 2003), a Neotropical migrant which breeds in North America but migrates annually to southern South America (Fuller et al., 1998). Swainson's hawks are generally smaller and more slender than Galápagos hawks, and Swainson's adults have three color morphs as

opposed to one dark morph in adult Galápagos hawks (Ferguson-Lees and Christie, 2001).

Island-populations of Galápagos hawks have extremely low levels of genetic variability as evidenced by mean similarity indices between 0.66 and 0.96 at hypervariable minisatellite loci, and genetic variation is positively correlated with island area, an index of population size (Bollmer et al., 2005). There is a significant amount of genetic differentiation among most populations; only two populations (Fernandina and Isabela) are statistically indistinguishable at minisatellite loci (Bollmer et al., 2005). Galápagos hawk populations vary behaviorally and morphologically (de Vries, 1973; Bollmer et al., 2003). The hawks breed in cooperatively polyandrous groups consisting of one female and up to eight males (Faaborg and Patterson, 1981; DeLay et al., 1996), and mean group size varies across islands (Bollmer et al., 2003). Galápagos hawks also vary in overall body size and shape across islands, with female mass in the smallest-bodied population averaging 22% less than in the largest-bodied population (26% in males; Bollmer et al., 2003).

In this study, we described the phylogeographic and population genetic structure of the Galápagos hawk, a species we know to be genetically monomorphic within populations but divergent between populations at nuclear loci. We collected mitochondrial sequence data from all nine extant populations of Galápagos hawk. We were also able to obtain sequence data from a San Cristóbal hawk (a population now extirpated) collected during the 1905-1906 California Academy of Sciences expedition. In addition, we sampled migratory Swainson's hawks and investigated the degree of divergence between the two species to determine when the Galápagos lineage likely

colonized the archipelago. Within Galápagos hawks, we examined relationships among different island populations at mitochondrial loci, using multilocus minisatellite data as a nuclear comparison, with the goal of elucidating the colonization history of the hawks in the archipelago.

2. Materials and methods

2.1. Field methods

We visited the Galápagos Islands for two to three months between May and August of each year from 1998 to 2003 and sampled 541 Galápagos hawk individuals from all nine extant populations (Table 2). We captured hawks using balchatri traps baited with rats (Berger and Mueller, 1959) and rope nooses on poles. We banded each hawk and took morphological measurements (see Bollmer et al., 2003) and two 50 µl blood samples via venipuncture. In addition, we captured and sampled thirty-four Swainson's hawks using balchatri traps placed in agricultural fields near the town of Las Varillas, in Córdoba province (Central Argentina) during January 2003.

The California Academy of Sciences in San Francisco, California has a single Galápagos hawk specimen collected in 1905 from the now extirpated San Cristóbal population. In order to obtain genetic data from this population, we visited the Academy in June 2004 and excised a toe pad from that specimen.

2.2. Laboratory methods

For most populations, we used a subset of the individuals in the genetic analyses (Table 2). When possible, we preferentially limited our pool of individuals to territorial, breeding adults, the class most likely to be genetically representative of the population and consist of nonrelatives (individuals within groups are unrelated [Faaborg et al., 1995]). On Pinzón and Santa Cruz, however, we captured only juveniles and used all of them in the analyses. Initially, we sequenced 26 hawks (Table 2) at four mitochondrial regions comprising 2860 bp. This included complete NADH dehydrogenase subunit 2 (ND2) sequences (1041 bp), 320 bases at the 3′ end of cytochrome *b* (CYB), 72 bp between CYB and the control region (CR), including tRNAthr, 415 bp of the 5′ end of CR (66 bp of the 5′ end of CR were problematic to sequence and are excluded from analyses), and 516 bp near the 5′ end and 496 bp near the 3′ end of cytochrome oxidase (COI). Among the Galápagos hawks sampled, most regions were invariant in this initial sample; therefore, we sampled 126 additional individuals (Table 2; 123 Galápagos and 29 Swainson's hawks) at only the variable 3′ end of COI and 415 bp of the CR.

The majority of sequences were single-stranded, though we obtained double-stranded sequences from those individuals where all gene regions were amplified, and for sequences where there were uncertainties. Table 3 lists the primers used to amplify and sequence the CYB-CR, COI, and ND2 regions. Unless noted, primers are named to indicate light (L) or heavy (H) strand and the 3′ position of the primer numbered according to the complete mitochondrial genome of *Gallus gallus* (Desjardins and Morais, 1990). The CYB-CR region was amplified with L15662 and H15414 (name indicates the 3′ end of the primer numbered according to the complete mitochondrion of *Buteo buteo*). To double-strand sequences, we used the internal primers H16065 and

L15004 (name indicates the 3′ end of the primer numbered according to the complete mitochondrion of *Buteo buteo*). COI was amplified in two reactions. The 5′ region was amplified with L6615 and H7539, and sequencing was done using L6615 or H7181. The 3′ region of COI was amplified with L7201 and H8214; sequencing was done using L7651 and H8214. ND2 sequences were obtained by amplifying and sequencing with primers L5216 and H6313. Sequences were double-stranded with internal primers L5716 and H5766.

PCR amplification followed standard protocols. We purified amplicons by precipitation using an equal volume of PEG:NaCl (20 %:2.5M) and washing with 70% ethanol. We sequenced purified amplicons using either ABI BigDye® Terminator v.1.0, BigDye® Terminator v.3.1, or Beckman DTCS Quickstart® chemistries. Manufacturers' recommendations were followed, except reaction volumes were cut to 1/2 - 1/6 of the recommended volume. Sequences were analyzed on an ABI Prism™ 310, ABI Prism™ 3100-Avant genetic analyzer (PE Applied Biosystems), or a CEQ™ 8000 (Beckman-Coulter™) genetic analysis system.

The 100-year-old San Cristóbal sample was processed in a lab dedicated to working with ancient DNA at the Florida Museum of Natural History located at the University of Florida. We extracted DNA from the toe pad and amplified the appropriate regions in the ancient DNA lab. Due to the poorer quality of the ancient DNA, we needed to sequence the regions in smaller segments using additional primers designed from Galápagos hawk sequences (primer sequences available from RTK upon request).

We performed multilocus minisatellite DNA fingerprinting using the restriction endonuclease *Hae*III and Jeffreys' probe 33.15 (Jeffreys et al., 1985) following

procedures described in general in Parker et al. (1995) and specifically for Galápagos hawks in Bollmer et al. (2005). We visualized hybridized fingerprints using a Storm 820 Phosphorimager. We fingerprinted a total of 119 of the 122 Galápagos hawks sequenced at the variable mitochondrial loci (Table 2). From the resulting banding patterns, we created a presence-absence matrix of bands (alleles) encompassing all individuals.

2.3. Data analysis

We examined and compared sequences using Sequencher™ 4.1 (Gene Codes Corp.). We used DnaSP v. 4.0.5 (Rozas et al., 2003) to calculate within-population genetic diversity indices: haplotype diversity (Nei, 1987) and nucleotide diversity (π; Nei, 1987). We generated a 95% statistical parsimony-based haplotype network using TCS v. 1.18 (Clement et al., 2000). Mean genetic distances (number of variable sites and uncorrected *p*-distances) within and between species were calculated using *MEGA* v. 2.1 (Kumar et al., 2001). Standard errors were calculated via bootstrapping (500 replicates). When the level of genetic differentiation between populations was ambiguous, we used pairwise differences to calculate F_{ST} values in Arlequin version 2.000 (Schneider et al., 2000).

To estimate divergence times, we assumed the mitochondrial protein-coding regions were diverging at 2% per million years (Shields and Wilson, 1987). There were six differences between Galápagos and Swainson's hawks (sites invariant within each species but variable between them) in the 2373 bp of protein-coding data used to determine divergence time: 3 in ND2, 1 in CYB, 1 in COI 5′, and 1 in COI 3′. There

were other variable sites where some individuals from both species shared the same nucleotide, but these were not used to calculate the divergence between the two species. We estimated a 95% confidence interval for the divergence time assuming a Poisson model of evolution (e.g., Braun and Kimball, 2001). While this method does not correct for ancestral polymorphism, we were primarily interested in setting an upper limit on divergence time, making a correction unnecessary.

For the nuclear minisatellite data, pairwise similarity values were calculated from the presence-absence matrix (based on 46 characters) using the program GELSTATS v. 2.6 (Rogstad and Pelikan, 1996). Similarity values, the proportion of bands shared between any two individuals (Lynch, 1990), were converted to distances (1 – similarity value). We used the distances to construct a neighbor-joining tree in *PAUP** v. 4.0b10 (Swofford, 2002), using midpoint rooting and constraining it to non-negative branch lengths.

3. Results

3.1. Haplotype variation within and between Galápagos and Swainson's hawks

Sequence data is available in GenBank, accession nos. **AY870866** to **AY870892**. For the 26 individuals sequenced at the four mitochondrial regions, polymorphic sites were present in only two of those regions, the CR and the 3′ end of COI (911 bp total), while the other regions (1949 bp total) were invariant within each species, differing by 5 bp between species. Among the 151 individuals (excluding the San Cristóbal hawk)

sequenced for the two variable regions, there were only 27 variable sites across all individuals: 6 found only within the 122 Galápagos hawks sampled, 16 only within the 29 Swainson's hawks, 3 in both species, and 2 monomorphic within species but variable between them (Table 4). There were a total of 19 haplotypes sequenced, 7 among the 122 Galápagos hawks and 12 among the 29 Swainson's hawks, indicating greater genetic variability in the Swainson's hawks (Tables 4, 5). The seven Galápagos hawk haplotypes differed from each other by an average of 3.14 ± 1.07 (SE) bases (mean uncorrected *p*-distance of 0.003 ± 0.001), while the 12 Swainson's hawk haplotypes differed by an average of 4.55 ± 1.10 bases (mean *p*-distance of 0.005 ± 0.001). The *p*-distances within Galápagos hawks ranged from 0 to 0.007, while they ranged from 0 to 0.011 in the Swainson's hawks. Including all the sampled individuals, the mean uncorrected *p*-distance was 0.002 ± 0.001 within Galápagos hawks and 0.003 ± 0.001 within Swainson's hawks. Galápagos and Swainson's hawk haplotypes differed from each other by an average of 10.43 ± 2.46 bases, with a mean *p*-distance of 0.011 ± 0.003, and *p*-distances ranged from 0.005 to 0.015. The smallest *p*-distance between Galápagos and Swainson's hawks (0.005) is less than the largest distance within either one of them (0.007 in Galápagos and 0.011 in Swainson's hawks). Including all the sampled individuals, Galápagos and Swainson's hawks differed by an average of 10.20 ± 2.75 bases, with a mean p-distance of 0.011 ± 0.003.

Using DnaSP, we inferred the amino acid sequences from 492 of the 496 bp at the 3′ end of COI, which resulted in 164 codons in an open reading frame. Interestingly, within the 122 Galápagos hawks, of the five nucleotide substitutions, four were

nonsynonymous and one was synonymous. Within the 29 Swainson's hawks, the only mutation in this region was synonymous.

Using a divergence rate of 2% per million years for the 2373 bp of coding DNA (Shields and Wilson, 1987), Galápagos and Swainson's hawks diverged approximately 126,000 years ago, with a 95% confidence interval between 51,000 and 254,000 years ago. While there is a large amount of error in molecular clock estimates (Arbogast et al., 2002; Lovette, 2004), our estimate still indicates that Galápagos hawks arrived in Galápagos very recently, likely less than 300,000 years ago.

3.2. Divergence among Galápagos hawk populations

There were only seven mitochondrial haplotypes present across the nine extant Galápagos hawk populations; multiple haplotypes were present in two populations (Isabela and Santa Cruz), while the other seven populations were fixed (Fig. 1). Three haplotypes were present on multiple islands. One (black circles in Fig. 1) was found in all individuals from the northern and central islands of Pinta, Marchena, Santiago, and Santa Fe, and in two of the four Santa Cruz birds. The second haplotype (black triangles) was shared among all Pinzón individuals, as well as five individuals from Isabela and one from Santa Cruz. The third haplotype (black squares) was found in all Fernandina individuals, the majority of the sampled individuals from Isabela, and the San Cristóbal individual (see below). The remaining four haplotypes were unique to individual islands: one present in all Española individuals, one in a single Santa Cruz individual, and two in two Isabela individuals. Interestingly, one Isabela haplotype was more similar to the

common haplotype present on the five central and northern islands than it was to other Isabela haplotypes. The genetic distances between populations were small, with the average number of base pair differences ranging from 0 to 4.25 (mean uncorrected *p*-distances ranging from 0 to 0.005).

Due to the degraded nature of the San Cristóbal sample, we sequenced a subset of the COI 3′ and CR regions. We were able to sequence 281 of the 496 bp of COI 3′ and 308 of the 415 bp of the CR, covering 65% of the 911 bp sequenced from the other individuals. These two fragments encompassed all but one of the sites that were variable in the other Galápagos hawks; the one missing site was a site that separated the Española haplotype from all the rest of the haplotypes, including the Swainson's haplotypes (site number 22 in Table 4). At the regions sequenced, the San Cristóbal haplotype was identical to the Fernandina/Isabela haplotype. While we cannot rule out possible variable sites in the 311 bp not sequenced for the San Cristóbal hawk, the rest of the Galápagos haplotypes were all monomorphic at those sites (except for site 22). It is likely that this individual is representative of the former population on San Cristóbal given that seven of the other nine populations were fixed for a single haplotype.

We calculated F_{ST} values between Isabela and Fernandina and Isabela and Pinzón, because Fernandina and Pinzón were each fixed for haplotypes present on Isabela, though Isabela had additional haplotypes. Both Fernandina ($F_{ST} = 0.216$, $P < 0.01$) and Pinzón ($F_{ST} = 0.451$, $P < 0.01$) were significantly differentiated from Isabela.

The minisatellite data indicated some differentiation among populations (Fig. 2). Española and Santa Fe individuals formed independent, distinct clusters. Most of the Pinzón individuals also clustered, though not as distinctly as those from Española and

Santa Fe. Marchena and Pinta individuals generally clustered together, with some differentiation between them. Only individuals from Santiago, Isabela, and Fernandina, the three largest and most variable populations, were indistinguishable from each other.

The four Santa Cruz birds were widely distributed in the tree. One individual fell within the Santa Fe cluster, having a banding pattern identical to four Santa Fe individuals. Another fell within the Pinzón cluster. These two birds also shared haplotypes with Santa Fe and Pinzón, respectively, suggesting these birds were born on those islands and subsequently dispersed to Santa Cruz. The other two Santa Cruz birds were not closely associated with any particular population.

The program TCS will estimate the root of a haplotype network based on the position of a haplotype in the tree and its frequency, which correlate with haplotype age (Castelloe and Templeton, 1994). When Swainson's hawk haplotypes were not included, TCS estimated that the most likely root of the Galápagos hawk haplotypes was the common one shared by Pinta, Marchena, Santiago, Santa Fe, and Santa Cruz. When Swainson's hawks were included, TCS still estimated that the most common Galápagos haplotype was the root, because the program does not take into consideration information about outgroups. The haplotype network (Fig. 1) created by TCS, though, identified the haplotype shared by the Fernandina, Isabela, and San Cristóbal populations as the one most closely related to Swainson's hawks, indicating it is the oldest of the Galápagos hawk haplotypes.

4. Discussion

4.1. Recent divergence between Galápagos and Swainson's hawks

The mitochondrial data indicated that Galápagos hawks form a monophyletic clade; thus, there was likely a single colonization event. They showed remarkably little divergence from their mainland sister species, the Swainson's hawk, differing by only 0.42% over almost 3 kb of data. The divergence between Swainson's and Galápagos hawks is on average greater than that within either of them. There is overlap, however, in the ranges of the genetic distances; the maximum divergence among Swainson's hawk lineages and among Galápagos hawk lineages is greater than the minimum divergence between the two species (Fig. 1). It may be that if we sampled Swainson's hawks more broadly and included additional outgroups, we would find that Swainson's hawks are paraphyletic.

Although the genetic divergence between Galápagos and Swainson's hawks is minimal, their morphological differences are great enough to have prevented their earlier identification as sister species (e.g., Brown and Amadon, 1968; de Vries, 1973). Many studies have found significant morphological differentiation between species that show little if any mitochondrial divergence (e.g., Seutin et al., 1995; Freeland and Boag, 1999; Piertney et al., 2001). In an analysis of Old World *Buteo* lineages, Kruckenhauser et al. (2004) also found little mitochondrial divergence among morphologically distinct species and subspecies. The life histories of Swainson's and Galápagos hawks (migratory vs. sedentary, prey base) differ greatly in ways that affect their morphology, especially their wings and talons. In addition to selection, the rapid morphological differentiation could be the result of genetic bottlenecks and ongoing drift in small island populations.

Swainson's and Galápagos hawks are not necessarily less divergent than other *Buteo* sister species. Using Riesing et al.'s (2003) sequence data for the mitochondrial gene nd6, we calculated a *p*-distance of 0.008 between Swainson's and Galápagos hawks and an average *p*-distance of 0.010 ± 0.002 (SD) within five other well-supported (based on bootstrap values) pairs of *Buteo* sister species. There are few other raptor mitochondrial studies; however, Groombridge et al. (2002) found similarly low levels of divergence between some kestrel species.

The extremely low level of divergence between the Galápagos and Swainson's hawks indicates that they separated only very recently (less than 300,000 years ago). Of the native Galápagos fauna studied to date, Galápagos hawks appear to be the most recently arrived lineage. Some taxa predate the current islands. The endemic land (*Conolophus*) and marine (*Amblyrhynchus*) iguanas are sister taxa, likely having diverged 10 to 20 million years ago (MYA) on the now sunken islands (Wyles & Sarich 1983; Rassmann 1997). Lava lizards (*Microlophus* spp.) likely colonized the islands multiple times between 6 and 20 MYA (Wright, 1983; Lopez et al., 1992; Kizirian et al., 2004), and *Galapaganus* weevils separated from their mainland relatives approximately 11 MYA (Sequeira et al., 2000). Other lineages arrived in Galápagos more recently, colonizing the current islands. The oldest divergence among the 11 extant Galápagos tortoise (*Geochelone nigra*) subspecies occurred 1.5 to 2 MYA (Caccone et al., 1999, 2002). Sato et al. (2001) estimated that Darwin's finches diverged from their closest mainland relative around 2.3 MYA, likely arriving in Galápagos from the Caribbean (Burns et al. 2002). The yellow warbler (*Dendroica petechia aureola*) diverged from the mainland form approximately 2.5 MYA (Collins, 2003).

4.2. Galápagos hawk phylogeography

Most Galápagos lineages underwent further differentiation as they colonized multiple islands, and, in many taxa, older lineages occur on the older eastern islands (San Cristóbal, Española, and Floreana) and younger lineages on the western islands (e.g., Rassmann et al., 1997; Sequeira et al., 2000; Beheregaray et al., 2004). For example, six of the 11 tortoise subspecies occur on different islands (the rest inhabiting the five volcanoes of Isabela), and mitochondrial and microsatellite data indicate significant genetic differentiation among them (Caccone et al., 2002; Ciofi et al., 2002). There should be greater genetic divergence among the older lineages due to a longer period of isolation. In the tortoises, differences among populations explain 97% of mitochondrial molecular variance for older islands and only 60% for younger islands (Beheregaray et al., 2004). Within geckos (*Phyllodactylus* spp.) and lava lizards, Wright (1983) found that the populations on the central and western islands tended to have higher allozyme similarities than the more divergent populations to the east.

The Galápagos hawk haplotype network shows a striking pattern of genetic monomorphism within populations and short genetic distances among populations at the mitochondrial loci. Four different populations (Santa Fe, Santiago, Marchena, and Pinta) comprising 58 sampled individuals were fixed for a single haplotype. Fernandina, Pinzón, and Española were also fixed but for different haplotypes. Only the populations on Isabela and Santa Cruz had any variability. Española hawks in the east have the highest mean genetic distance from the other populations; however, Española is not

necessarily the oldest population, but instead may have become the first population to be isolated from the rest. The paucity of different haplotypes and the small genetic distances among them suggests the hawks spread across the archipelago relatively quickly, with subsequent lineage sorting resulting in different haplotypes on different islands. The pattern on Isabela, with haplotypes that are not most closely related to each other, and the presence of the same haplotype on San Cristóbal as on Fernandina (at opposite ends of the archipelago) further supports this. It is difficult to say from which direction the initial hawk colonization of the archipelago occurred; the Swainson's hawks were most closely related to the Fernandina/Isabela/San Cristóbal haplotype that was located on the far eastern and western islands. Limitations due to lineage sorting and possible homoplasy prevent a more definitive determination of the colonization pattern. Our understanding is also hindered by the missing information from the extirpated Floreana population, and our four samples from Santa Cruz (the most central island) are likely not representative of the former population there (see next section).

The role of genetic drift in these island populations was also demonstrated by the finding that the majority of nucleotide substitutions in the 3′ end of COI within Galápagos hawks were nonsynonymous. This finding is unsurprising from a theoretical perspective, given that slightly deleterious mutations with respect to fitness are expected to drift to fixation at a higher rate within small populations relative to larger populations (reviewed in Johnson and Seger, 2001). This qualitative interpretation is supported further by Johnson and Seger's (2001) empirical study, which found elevated rates of nonsynonymous substitutions on lineages of island bird taxa compared to their mainland relatives. Finally, the fact that Galápagos hawks have very small island populations, the

majority of which are genetically isolated (Bollmer et al., 2005) also lends support for the role of drift in generating these patterns.

4.3. Mitochondrial vs. nuclear differentiation among populations

Mitochondrial and nuclear markers can often be used in conjunction to draw more accurate conclusions about genetic structure. The eastern population on Española was clearly genetically isolated at both mitochondrial and minisatellite loci. The central and northern populations (Santa Fe, Santiago, Marchena, and Pinta) share a common mitochondrial haplotype even though our pairwise F_{ST} estimates show significant differentiation among them at the more rapidly evolving minisatellite loci (Bollmer et al., 2005). The western populations of Fernandina and Isabela, less than 5 km apart, were statistically indistinguishable at minisatellite loci (Bollmer et al., 2005) and shared a mitochondrial haplotype; moreover, one female hawk banded as a juvenile on Isabela (Volcan Alcedo) in 1998 was observed in a territorial group on Fernandina in 2003, though we do not know which is its natal island (Bollmer et al., 2005). The presence of other haplotypes on Isabela, however, resulted in a significant F_{ST} value between them for the mitochondrial data. This discrepancy between the nuclear and mitochondrial data could be due to male-biased gene flow, though we have no other evidence that this occurs. Another explanation is that it is due to the differing natures of the two markers. Santiago, Isabela, and Fernandina are the largest of the hawk populations and have retained the most genetic variability. The fact that they are more distinguishable at mitochondrial loci than at minisatellite loci could be attributed to the shorter coalescent

time of the mitochondrial loci, thus allowing significant genetic structuring to arise more quickly.

The combined mitochondrial and nuclear data can also be used to determine the populations of origin of dispersers, which is of potential conservation importance, both from the perspective of disease transmission and population management. Given the apparent absence of a breeding population on Santa Cruz, both the mitochondrial and the minisatellite data suggest that the four Santa Cruz juveniles are likely dispersers from different islands. One was very likely born on Pinzón and one on Santa Fe; both their minisatellite and mitochondrial profiles are consistent with that. The origin of the other two individuals is less clear. Neither of them is closely associated with any of the more inbred populations at the minisatellite loci, leaving Fernandina, Isabela, and Santiago as possible source populations. One shares the same haplotype as Santiago; the other has a unique haplotype that is most closely related to the one shared by Isabela and Pinzón. Given the genetic monomorphism on Pinzón, the latter bird more likely originated on Isabela.

Taking both the nuclear and mitochondrial data into account, the overall pattern among Galápagos hawk populations is one of genetic isolation. The Santa Cruz population is certainly an exception in that juveniles appear to be dispersing there, and there may be gene flow between Fernandina and Isabela, since they are indistinguishable at the nuclear loci (though not at the mitochondrial loci). All the other populations show statistically significant divergence at nuclear or mitochondrial loci or both. This, combined with the morphological differentiation among populations and the recentness of its arrival, may mean that the Galápagos hawk is in the very early stages of speciation.

The much older finch colonization of the archipelago resulted in fourteen morphological species; however, mitochondrial data only distinguished four groups (Sato et al., 1999), and interspecific genetic distances at microsatellite loci were generally lower among sympatric populations than among allopatric populations, likely due to introgressive hybridization (Grant et al., 2005). Galápagos hawks are less vagile, and most of their populations, like those of other sedentary species in the archipelago (e.g., tortoises, lava lizards), appear to be on separate evolutionary trajectories. Although the colonization history of the Galápagos hawk remains unclear, reconstructing the genealogies of its parasites (de Vries, 1975; Whiteman and Parker, 2005) may yield insight into the hosts' movements within the archipelago.

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Figure Legends

Fig. 1. Mitochondrial haplotype network of Galápagos and Swainson's hawks. Within the Galápagos hawks, each haplotype is represented by a different symbol (corresponding to symbols in Table 4 and Figure 2), and the Swainson's hawks haplotypes are represented by different letters (corresponding to those in Table 4). Only one haplotype was found in each Galápagos hawk population except for Isabela (four haplotypes) and Santa Cruz (three haplotypes). The number of individuals with each haplotype is listed next to the corresponding symbol. It should be noted that while the Swainson's hawk haplotypes are drawn connecting to the Fernandina/Isabela haplotype, that same haplotype is also present on San Cristóbal, though it is based on fewer sequenced sites.

Fig. 2. A midpoint rooted neighbor-joining tree of Galápagos hawk populations based on minisatellite distances (1–similarity). Populations are identified with abbreviations: E = Española, F = Fernandina, I = Isabela, M = Marchena, PT = Pinta, PZ = Pinzón, SA = Santiago, SC = Santa Cruz, and SF = Santa Fe. The symbols following the population abbreviations represent mitochondrial haplotypes and correspond to those on the haplotype network (Fig. 1). The four Santa Cruz individuals are in boxes.

Table 1

Summary of terrestrial vertebrate taxa of Galápagos, including the number of lineages that evolved on the archipelago, the number of colonizing species from which they evolved, and whether they are endemic. Only native, resident taxa are listed (i.e., no introduced species or seasonal migrants), and lineages that arose in Galápagos but have since gone extinct are included. There are references listed where genetic studies have determined the likely number of founding events; otherwise, the numbers reflect what is believed based on morphological characters.

Class	Taxa	Number of lineages	Number of founding taxa	Endemic
Reptilia	giant tortoises (*Geochelone nigra*)	11 subspecies	1 (Caccone et al., 1999)	yes
	marine (*Amblyrhynchus cristatus*) and land (*Conolophus subcristatus, C. pallidus*) iguanas	7 subspecies (marine), 2 species (land)	1 (Rassmann, 1997)	yes
	lava lizards (*Microlophus* spp.)	7 species	2 (Kizirian et al., 2004)	yes
	geckos (*Phyllodactylus* spp.)	6 species	2 (Wright, 1983)	yes
	snakes (*Philodryas hoodensis*, *Antillophis slevini*, *A. steindachmeri*, *Alsophis biseralis* subspp.)	3 species, 3 subspecies	at most 4	yes
	Total	**40**	**10**	
Mammalia	rice rats (*Oryzomys* spp., *Nesoryzomys* spp., *Megaoryzomys curiori*)	at least 8 species	3	yes
	bats (*Lasiurus brachyotis*, *L. cinerius*)	2 species	2	yes (*L. brachyotis*)
	Total	**10**	**5**	
Aves	Darwin's finches (*Geospiza* spp., *Camarhynchus* spp., *Cactospiza* spp., *Platyspiza crassirostris*, *Certhidea olivacea*	13 species	1 (Sato et al., 1999; Burns et al., 2002)	yes
	Galápagos mockingbirds (*Nesomimus* spp.)	4 species	1	yes
	Galápagos dove (*Zenaida galapagoensis*)	2 subspecies	1	yes
	Galápagos hawk (*Buteo galapagoensis*)	1 species	1 (this study)	yes
	Barn owl (*Tyto alba punctatissima*)	1 subspecies	1	subspecies
	Short-eared owl (*Asio flammeus galapagoensis*)	1 subspecies	1	subspecies
	Galápagos martin (*Progne modesta*)	1 species	1	yes
	Yellow warbler (*Dendroica petechia aureola*)	1 subspecies	1 (Collins, 2003)	subspecies
	Galápagos flycatcher (*Myiarchus magnirostris*)	1 species	1	yes
	Vermilion flycatcher (*Pyrocephalus rubinus*)	1 species	1	no
	Dark-billed cuckoo (*Coccyzus melacoryphus*)	1 species	1	no
	Galápagos rail (*Laterallus spilonotus*)	1 species	1	yes
	Paint-billed crake (*Neocrex erythrops*)	1 species	1	no
	Common gallinule (*Gallinula chloropus*)	1 species	1	no
	Total	**30**	**14**	

Table 2

Sample sizes of Galápagos and Swainson's hawks sequenced at mitochondrial loci and fingerprinted at minisatellite loci

Species	Population	No. sequenced at all regions	No. sequenced at variable regions	No. fingerprinted at minisatellite loci
Galápagos hawk	Española	2	10	10
	Santa Fe	2	9	9
	Santa Cruz	4	4	4
	Santiago	2	21	20
	Pinzón	2	10	10
	Marchena	2	15	15
	Pinta	2	13	12
	Isabela	4	20	19
	Fernandina	2	20	20
	San Cristóbal	0	1	0
Swainson's hawk		4	29	0
Total		26	152	119

A total of 26 hawks were sequenced at all four mitochondrial regions (CYB, CR, COI, and ND2). An additional 126 hawks were then sequenced at the two variable regions (COI 3′ and CR) for a total of 152 hawks sequenced at those regions, though the San Cristóbal hawk sequence is incomplete.

Table 3

Primers used in this study to amplify and sequence three hawk mitochondrial regions

Region	Primer	Source	Sequence (5′ to 3′)	T_M (°C)
CYB-CR	L15662	Kimball et al., 1999	CTAGGCGACCCAGAAAACTT	54°,
	H15414	this study	CAAGTAGTGCTAGGGGTTTAGG	30 sec
	L15004	this study	CACATATCATGAACTATTATGGG	Seq. only
	H16065	Kimball et al., 1999	TTCAGTTTTTGGTTTACAAGAC	Seq. only
COI	L6615	modified from Sorenson et al., 1999	TCTGTAAAAAGGACTACAGCC	52°, 30 sec
	H7539	Sorenson et al., 1999	GATGTAAAGTAGGCCGGGTGTCTAC	
	H7181	this study	TACGAATAGGGGTGTTTGG	Seq. only
	L7201	this study	ACCAAACACCCCTATTCGTATG	54°,
	H8214	this study	ATGCRGYTGGCTTGAAACC	30 sec
	L7651	this study	GGAACTATCAAATGAGACCC	Seq. only
ND2	L5216	Sorenson et al., 1999	GCCCATACCCCRAAAATG	52°,
	H6313	Sorenson et al., 1999	CCTTATTTAAGGCTTTGAAGGC	30 sec
	L5716	this study	CCCTACTYACCYTCCTAGCAAT	Seq. only
	H5766	modified from Sorenson et al., 1999	GATGARAAGGCTAGGATYTTTCG	Seq. only

Table 4

The polymorphic sites within the variable COI 3′ and CR regions of the Galápagos and Swainson's hawk mitochondrial DNA

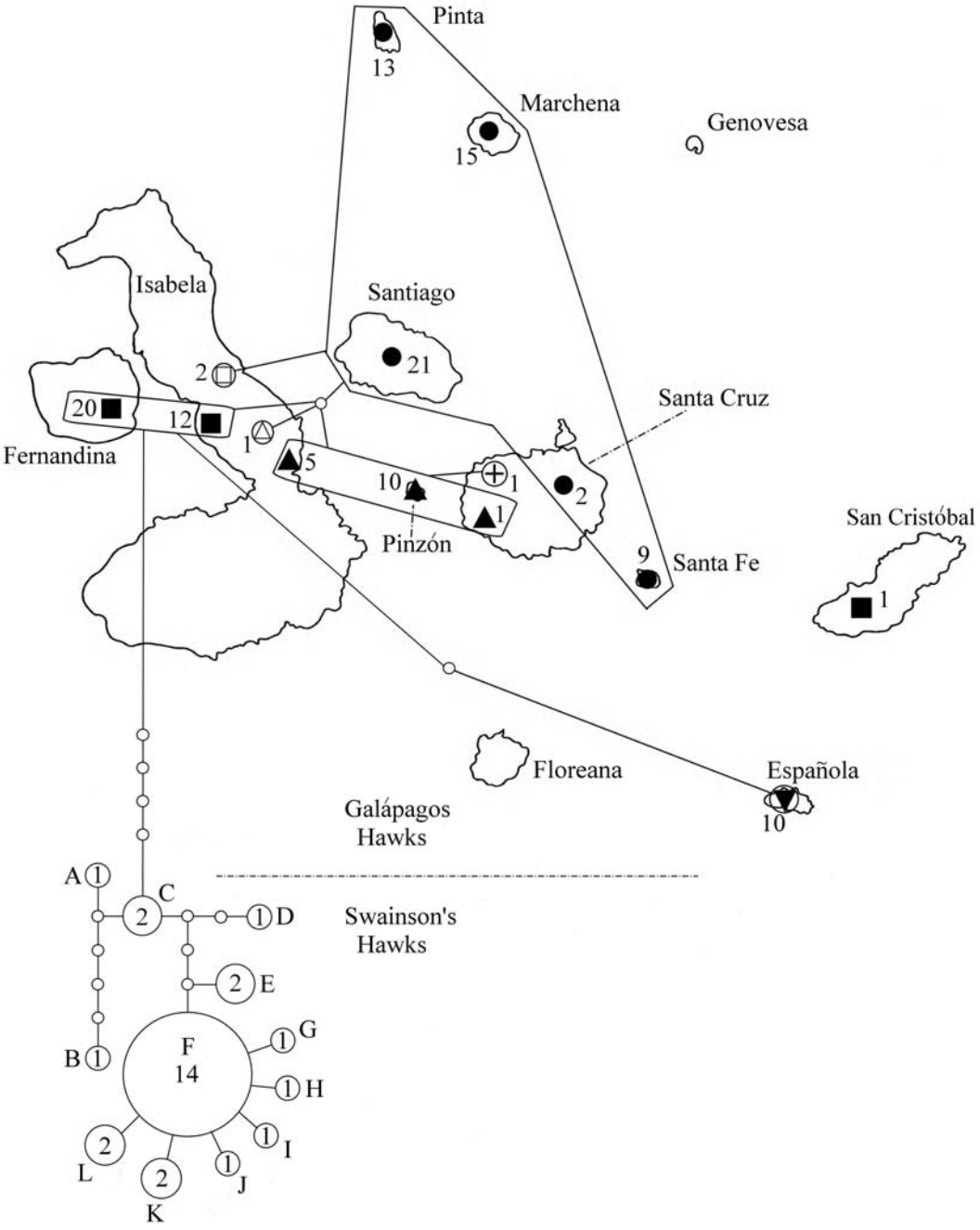
		1 2 2 4 5 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7
		2 7 0 0 4 7 1 1 1 1 5 6 7 0 0 0 1 1 1 2 2 2 2 3 4 6 7
		2 1 1 7 3 3 0 2 6 8 6 8 7 7 8 9 2 4 9 0 1 4 7 1 4 4 0
Galápagos hawks	▼	CTGAT CACCA TGTCT TGAGA CGTTTAC
	■	TTGGT CACCA TGTCT TGAGA CGTTTAC
	Δ	TTGGT CGTCA TGTCT TGAGA CGTTTAC
	□	TTAGT CGCCA TGTCT TGAGT TGTTTAC
	●	TTAGT CGCCA TGTCT TGAGA CGTTTAC
	▲	TTGGT TGCCA TGTCT TGAGA CGTTTAC
	+	TTGGC TGCCA TGTCT TGAGA CGTTTAC
Swainson's hawks	A	TTGGC CACCA TGTCT TAGGA CATCTGT
	B	TTGGC CACTG TGTCT TGGGA TATTTGT
	C	TTGGC CACCA TGTCT TAAGA CATTTGT
	D	TCGGC CACCA TGTTT CAAGA CATTTGT
	E	TTGGC CACCA TATTC TAAGA CATTCGT
	F	TTGGC CACCA TGCTC TAAGA CATTCGT
	G	TTGGC CACCA TGCTC TAAGA CACTCGT
	H	TTGGC CACCA CGCTC TAAGA CATTCGT
	I	TTGGC CACCA TGCTC TAAGT CATTCGT
	J	TTGGC CACCA TGCTC TAAAA CATTCGT
	K	TTGGC TACCA TGCTC TAAGA CATTCGT
	L	TTGGC CACCA TGCTC TAAGA CGTTCGT

Of the 911 bp sequenced at the COI 3′ and CR regions, there were 27 variable sites. The sites are numbered according to their position within our combined COI and CR dataset; positions 1-496 are COI sites and positions 497-911 are CR sites. Each Galápagos hawk haplotype is labeled with a symbol corresponding to the symbols in Figures 2 and 3. Each Swainson's hawk haplotype is labeled with a letter corresponding to the letters in Figure 2.

Table 5

Genetic variability at five mitochondrial regions within Galápagos ($N = 122$; excluding the San Cristóbal hawk) and Swainson's ($N = 29$) hawks

		CYB, ND2, COI 5′ (1949 bp)	COI 3′ (496 bp)	CR (415 bp)	COI 3′/CR combined (911 bp)
B. galapagoensis	No. of polymorphic sites	0	4	5	9
	Nucleotide diversity	0	0.0017	0.0019	0.0018
	No. of haplotypes	1	4	5	7
	Haplotype diversity (±SD)	0	0.578 ± 0.023	0.625 ± 0.025	0.671 ± 0.030
B. swainsoni	No. of polymorphic sites	0	1	18	19
	Nucleotide diversity	0	0.0001	0.0059	0.0028
	No. of haplotypes	1	2	12	12
	Haplotype diversity (±SD)	0	0.069 ± 0.063	0.766 ± 0.081	0.766 ± 0.081


Pinta
13
Marchena
15
Genovesa
Isabela
Santiago
21
2
20
12
1
5
10
1
1
2
Santa Cruz
Fernandina
Pinzón
9
Santa Fe
San Cristóbal
1
Floreana
Española
10
Galápagos
Hawks
A 1
C
2
1 D
Swainson's
Hawks
2 E
B 1
F
14
1 G
1 H
1 I
2
L
2
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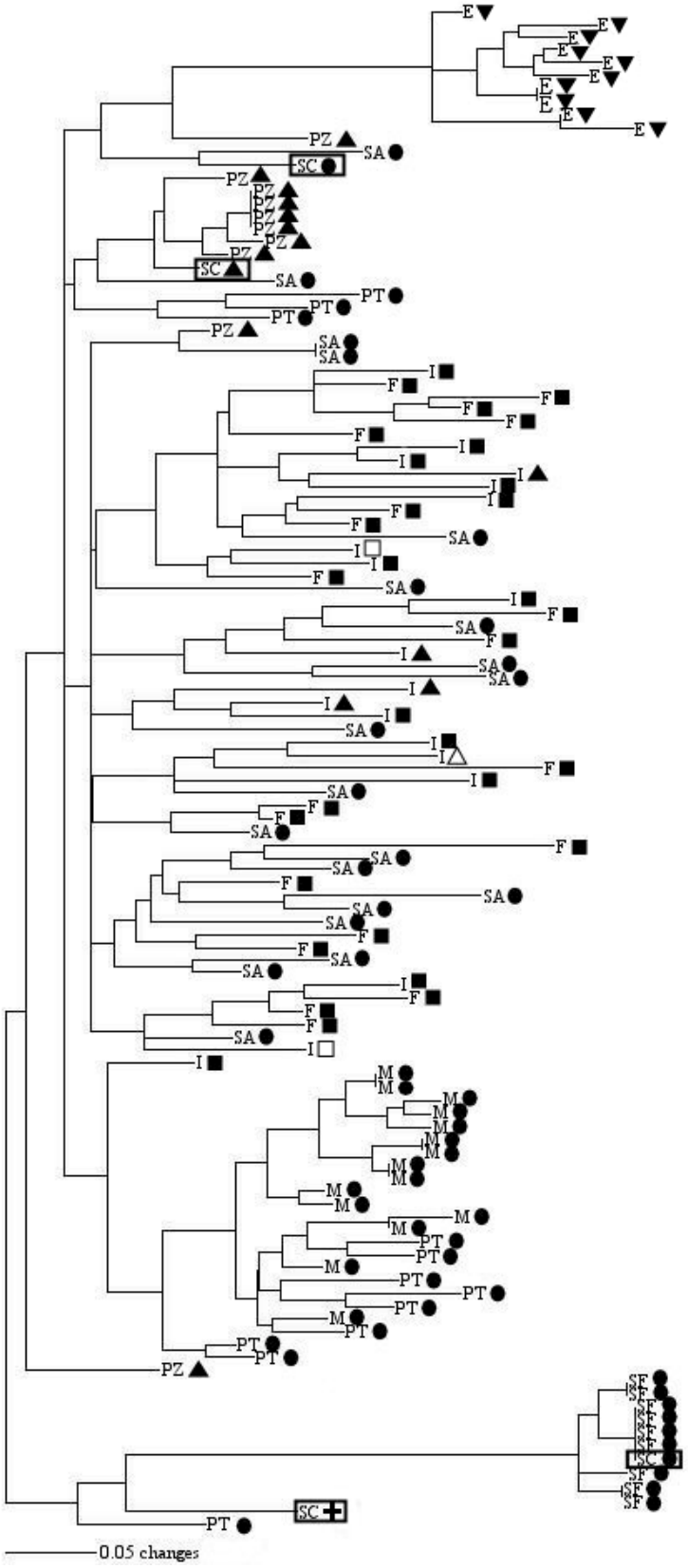
1
J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Chapter XI.

Health Assessment of Seabirds on Isla Genovesa, Galápagos Islands

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ABSTRACT

A multi-species colony of seabirds was studied on the island of Genovesa, in the northern part of the Galápagos archipelago, Ecuador, in 2003 to establish baseline health parameters, and to test specifically for *Chlamydophila psittaci,* known to exist elsewhere in the archipelago. Ninety-three individual Red-Footed Boobies (*Sula sula*), Great Frigatebirds (*Fregata minor*), Nazca Boobies (*Sula granti*) and Swallow-Tailed Gulls (*Creagrus furcatus*) were hand restrained for venipuncture and collection of lacrimo-choanal-cloacal combination swabs. White blood cell counts, differentials and packed erythrocyte volumes were obtained and plasma chemistry analyses performed on the blood samples. Presence/absence and parasitemias of circulating hemoparasites were determined by microscopic evaluation of peripheral blood smears. *Haemoproteus*-like hemoparasites were found in three of the seabird species sampled. Prevalences were 29.2% (7/24) in Great Frigatebirds, followed by 15.8% (3/19) in Swallow-Tailed Gulls,

8.7% (2/23) in Red-Footed Boobies and none of 25 Nazca Boobies were infected. Parasitemias were relatively low within each of the infected species. Individual Great Frigatebirds birds with *Haemoproteus* infections also exhibited significantly higher heterophil to lymphocyte concentration ratios than birds not infected with *Haemoproteus*, an indication that birds infected with *Haemoproteus* were also physiologically stressed or alternatively that they were actively fighting the malaria infection. *Haemoproteus* prevalences within Great Frigatebirds on Genovesa were not significantly different from those previously reported from conspecific hosts in the Hawaiian Islands. To compare seabird hemoparasite data with a sympatric terrestrial species, Galápagos Doves (*Zenaida galapagoensis*) were sampled on Genovesa in 2004 and screened for *Haemoproteus* previously reported in Galápagos Doves on other islands. Prevalence in this terrestrial endemic was high (42.3%; 11/26), and several birds exhibited relatively high parasitemias. *Chlamydophila psittaci* was not found in any birds by either serology or antigen detection methods.

Key Words (4 to 8 words): *Haemoproteus*, Galápagos Islands, seabirds, Red-Footed Booby (*Sula sula*), Great Frigatebird (*Fregata minor*), Nazca Booby (*Sula granti*), Swallow-Tailed Gull (*Creagrus furcatus*), Galápagos Dove (*Zenaida galapagoensis*).

INTRODUCTION

The Galápagos Islands are located on the equator in the Pacific Ocean, almost 1000 km west of mainland Ecuador in South America. They have been inhabited by humans for less than two centuries, and their biodiversity remains largely intact, with only about five percent of species having been lost (Gibbs et al. 1999); all of the 28 breeding land bird species, 26 of which are endemic, remain. High endemism extends to seabirds, despite the huge individual ranges of some members of this group (Dearborn et al. 2003). Nineteen seabird species nest in the Galápagos Islands, including four endemic species and six endemic subspecies (Harris 1984).

In 1959, 90% of the area of the archipelago was set aside as a national park. However, the resident human population has grown rapidly and exotics are continually being introduced despite increasing efforts to exclude them. The Charles Darwin Research Station and the Galápagos National Park are concerned about the introduction of avian diseases that could result in extinctions of Galápagos avifauna, similar to those in Hawaii (Wikelski et al. 2004). Disease has been implicated as a major factor in the population declines and extinctions in the endemic Hawaiian avifauna (Warner 1968; van Riper et al. 1986, 2002), which now comprises less than half of the species present at Polynesian settlement. Avian pox (*Avipoxvirus* spp.) and avian malaria (*Plasmodium* spp.) have been implicated as the major pathogens contributing to this wave of Hawaiian extinctions beginning in the mid-1800s (van Riper and Scott 2001, van Riper et al. 2002). Island populations may be particularly susceptible to new pathogens, as they have been exposed to fewer pathogens than mainland populations (Lewis 1968a,b; Dobson 1988; Fromont et al. 2001; Goüy de Bellocq et al. 2002). Colonizers from the mainland will

not represent all of the parasites found in their population of origin (Dobson and May 1986). Populations may grow quickly in the absence of pathogens and selection for resistance is relaxed, facilitating the invasion of pathogens arriving later (Dobson 1988). To illustrate using the example of Hawaiian songbirds, some Hawaiian endemic birds had higher *Plasmodium* parasitemia levels and higher pox prevalence and heavier infections than introduced birds (van Riper et al. 1986, 2002).

Concerns over the status of native avian populations in archipelagos and the effects of introduced diseases have been the impetus for an active health surveillance project in the Galápagos Islands (Miller et al. 2002; Wikelski et al. 2004). To date, health surveys of Galápagos birds have found a number of pathogens in domestic chickens (Gottdenker et al. 2005). Tests have shown clearly that the *Avipoxvirus* in the chickens is a different virus than the two canarypox-like *Avipoxvirus* strains infecting the endemic landbirds (Thiel et al. 2005). Studies aimed at seeing whether other pathogens are transferring from chickens to endemics are underway (Padilla, Parker, Soos, et al. in prep.). General health surveys conducted on the Waved Albatross (*Phoebastria irrorata*) established baseline values for a generally healthy population (Padilla et al. 2003). A four-island comparison of the introduced Rock Pigeon (*Columba livia*) and the only endemic dove, the Galápagos Dove (*Zenaida galapagoensis*), revealed no evidence of transmission of pathogens in either direction, despite the occurrence of *Trichomonas gallinae* in the Rock Pigeons on San Cristobal, *Chlamydophila psittaci* in the Galápagos Dove on Espanola, and the near-ubiquitous presence of a *Haemoproteus*-like blood parasite in the Galápagos Doves on every island sampled. Interestingly, the two single cases of inter-specific parasite transmission found thus far involved prey to predator

transmission of relatively benign chewing lice (Phthiraptera: Ischnocera). Louse species typically found only on Galápagos Doves (*Columbicola*, *Physconelloides*) and introduced goats (*Bovicola*) were found as 'stragglers' on Galápagos hawks (*Buteo galapagoensis*), to which they dispersed during predation events (Whiteman et al. 2004). However, it was the presence of *Chlamydophila psittaci* on Espanola that prompted this survey of seabirds on Genovesa. *Chlamydophila psittaci* can spread rapidly as an epizootic agent in dense bird colonies (Franson 1999), and the colonial breeding habit is associated with higher rates of parasitism (Tella 2002). Few islands rival the number and density of seabirds nesting on the island of Genovesa, and we designed this survey specifically to test for the presence of *Chamydophila*, while gathering general health baseline data.

The colonial breeding habits and limited geographic distribution of most seabirds are thought to make them vulnerable to mass mortality events (Warham 1996), including infectious disease epizootics and environmental disasters. Health studies on free-ranging island-nesting populations of seabirds are limited, and few species-specific reference ranges are available (Work 1996; Work 1999). Establishing reference parameters from free-ranging animals is essential to conservation efforts, setting a baseline for recognition of health-related threats to a population (Spalding and Forrester, 1993). Monitoring pelagic seabirds has also been suggested as a way of assessing the overall health of marine ecosystems (Uhart et al. 2003). The purpose of this study was to establish species-specific baseline health parameters for a multi-species colony of seabirds from Genovesa, an island in the northeastern part of the Galápagos archipelago, and to test them specifically for *Chamydophila* and blood parasites, known to exist on the archipelago, and in seabirds elsewhere.

MATERIALS AND METHODS

Between 11 and 15 July 2003, 93 seabirds from a multi-species colony were sampled on Darwin Bay, on the southern part of Genovesa (00º19' N, 89º57' W), Galápagos Islands, Ecuador. Procedures were conducted in accordance with Saint Louis Zoo institutional animal care and use committee standards. Individuals included 24 Great Frigatebirds (*Fregata minor*), 23 Red-Footed Boobies (*Sula sula*), 25 Nazca Boobies *(Sula granti*) and 20 Swallow-Tailed Gulls (*Creagrus fulcatus*). In addition, we sampled one Yellow-Crowned Night Heron (*Nyctanassa violacea*), two Short-Eared Owls (*Asio flammeus galapagoensis*) and three Galápagos Doves (*Zenaida galapagoensis*) all of which frequently observed foraging within the colony. Lava Gulls (*Larus fuliginosus*) were also seen occasionally in this colony, but were not sampled. The colony was chosen on the basis of size, species diversity and accessibility, and deemed representative of other multi-species colonies on this island. This area is frequently monitored by Galápagos National Park personnel, and no historical mass mortalities have been documented. Seabirds were hand captured while resting or standing on the ground. Owls and doves were captured with hand-nets. Each bird was marked with a single permanent marker dot on either the right leg or the ventral aspect of the beak to avoid repeated sampling. In 2004, 30 additional Galápagos Doves (*Zenaida galapagoensis*) were sampled on the same site.

Blood was collected from the ulnar vein and birds were inspected for hemostasis before being returned to the capture site. Microhematocrit tubes were filled and fresh blood smears were prepared. The remaining blood was preserved in lithium heparin blood collection tubes. Blood smears were fixed in ethanol. Blood was also preserved in

in a lysis buffer (Longmire et al. 1988) for molecular sexing (as described by Fridolfsson and Ellegren 1999) and future genetic and population studies. Microhematocrit tubes were centrifuged (using Mobilespin Model 128, Vulcon Technologies, Grandview, Missouri, USA), and packed erythrocyte volumes (PCV) were measured. Heparinized plasma was separated into nalgene cryotubes (Nalge Nunc International, Rochester, New York, USA) and stored in liquid nitrogen until further analysis. Blood smears were stained with a modified Wright-Giemsa stain (JorVet Dip-Quick, Jorgensen Laboratories, Loveland, Colorado, USA) prior to being individually examined for presence of hemoparasites. Blood smears were assessed for hemoparasite presence by searching 200 oil immersion fields at 100X magnification. Parasitemias (infection intensities) were recorded as the total number of infected erythrocytes observed during the search of 200 oil immersion fields. An estimated leukocyte count was obtained as described by Fudge (2000). Differential white blood cell counts were performed by counting 100 leukocytes under oil immersion. Within Great Frigatebirds, differential white blood cell counts were transformed into concentrations by multiplying the estimated total white blood cell values (white blood cells (* 1000/μL) with the differential value, the product of which was then divided by 100. These values (in units of 1000/ μL for heterophils and lymphocytes) were then used to calculate heterophil to lymphocyte ratios for each Great Frigatebird from which both estimated white blood cell concentrations and differential data were available (n = 16). Since heterophil to lymphocyte concentration ratios were not normally distributed (based on a one-sample Kolmogorov-Smirnov test), a Mann-Whitney U test was then used to compare concentration ratios between birds infected and uninfected with *Haemoproteus*. Plasma remained frozen until shipped for

Chlamydophila psittaci serology testing by elementary body agglutination at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA) and for plasma chemistry testing at a commercial veterinary laboratory (AVL Veterinary Clinical Laboratory, Saint Louis, Missouri, USA), using the ACE clinical chemistry analyzer system (Alfa Wasserman, Inc., West Caldwell, New Jersey, USA).

Sterile Dacron® tip applicators were used to collect combination swabs of the conjunctiva, choana and cloaca, and were subsequently frozen in nalgene cryotubes with no preservatives. Swabs were submitted to the Infectious Diseases Laboratory, University of Georgia - College of Veterinary Medicine, Athens, Georgia, USA, for *C. psittaci* antigen detection and by polymerase chain reaction (PCR).

Results were analyzed using commercial statistical software packages (NCSS, Kaysville, Utah, USA; SPSS v. 13.0, 2004). Data were tested for normality using a Shapiro-Wilk W test or a one-sample Kolmogorov-Smirnov test, and Mann-Whitney U tests were used to compare groups if the data were not normally distributed. Quantitative Parasitology 2.0 (Reiczigel, J. & Rózsa, L. 2001), which utilized distribution-free statistical tests specifically designed for comparative parasitology, was used to compare *Haemoproteus* prevalences and intensities among seabirds and within species, between sexes and age classes. This program was also used to calculate parasite distributions within species. Since 13 plasma chemistry parameters and the white blood cell count were compared for each subset of the data, a Bonferroni-corrected p-value (0.05 / 14 = 0.004) was used to establish statistical significance. No Bonferroni-correction was performed on the one dataset (Great Frigatebird) used to compare heterophil to lymphocyte concentration ratios between birds infected and uninfected with

Haemoproteus given that the relationship between the variables was hypothesized *a priori*.

RESULTS

Screening of blood smears revealed circulating hemoparasites in three of the four seabirds examined, with Great Frigatebirds exhibiting the highest prevalence (29.2%; 7/24), followed by Swallow-Tailed Gulls (15.8%; 3/19), and Red-Footed Boobies (8.7% 2/23); none were seen in Nazca Boobies (0/25; Table 1 summarizes the hematology values and hemoparasite distribution data). All circulating hemoparasites were morphologically consistent with *Haemoproteus*-like organisms (Figure 1). *Haemoproteus* parasites were confirmed in one of the three Galápagos Doves tested in 2003 and in 11/26 (42.3%) of those examined in 2004, but not in the single yellow-crowned night heron, nor in the short-eared owls. *Haemoproteus* prevalence was not significantly different between sexes within species or among individual species. Mean parasitemias (infection intensities) were low in all three positive seabirds, but relatively high in Galápagos Doves in both 2003 and 2004. We then compared prevalences within Great Frigatebirds sampled in this study (29.2%; 7/24) to those published by Work and Rameyer (1996) from conspecific hosts sampled within another Pacific Island oceanic archipelago, Laysan and Tern Islands (35.6%; 32/90) in Hawaii, and found no significant differences in *Haemoproteus* prevalence between the two populations ($p>0.05$).

Table 2 summarizes plasma chemistry values for the four seabird species sampled. No evidence of *C. psittaci* was found by either plasma serology or PCR of swabs in any of the birds tested. Swallow-Tailed Gulls had consistently lower total white blood cell (WBC) counts than the other species sampled. We compared the plasma

chemistry values between hemoparasitized and non-parasitized birds and found no differences between them. Across seabird species, hemoparasitized birds had slightly lower WBC counts than non-hemoparasitized birds (WBC = 9.0 ± 5.3 x 10^3 / μL vs. 8.2 ± 4.1 x 10^3 / μL; n=91, U = 531, p=0.01), but this difference was not significant when Bonferroni correction was applied. Male and female birds did not differ in occurrence of hemoparasitism, total white blood cell counts or PCV values. However, heterophil to lymphocyte concentration ratios were significantly higher for haemoparasite-infected than for non-infected Great Frigatebirds (Fig. 2; n =16 U = 9, p = 0.036). Low prevalence in the other two infected seabird species precluded using this test to compare the heterophil to lymphocyte concentration ratios between infected and uninfected birds.

Plasma chemistry values showed no sex-related differences, with the exception of plasma phosphorous (Table 3). A trend seen across species was that females had higher phosphorous levels (n=81, U= 1135, p = 0.04), and slightly higher total plasma calcium levels (n=81, U=958.5, p =0.01). Within species, phosphorous values were higher in female than male Nazca (n=23, U=119.5, p=0.0002), Red-Footed Boobies (n=20, U =30, p=0.03), and Swallow-Tailed Gulls (n=14, U=22, p=0.9), but not in Frigatebirds (n=24, U=71.5, p=0.9). This difference was considered significant in Nazca Boobies.

DISCUSSION

We observed *Haemoproteu*s parasites in peripheral blood smears from three of four species of Genovesa island seabirds, as well as in the sympatric and endemic Galápagos Dove. Hemoparasites in the *Haemoproteus* genus have traditionally been considered incidental and relatively non-pathogenic parasites of birds and reptiles,

although effects on host fitness components have been demonstrated (Earlé et al. 1993; Merino et al. 2000; Marzal et al. 2005) and pathogenicity has been shown for certain hosts of certain hemoparasite species (Valkiunas et al. 2003; Garvin et al. 2003). However, molecular phylogenetic studies of malarial parasites has revealed considerable convergence in the morphological and life-history traits used to traditionally classify lineages (Perkins and Schall 2002). Furthermore, *Haemoproteus* parasites may not be monophyletic (Perkins and Schall 2002). Galápagos Doves exhibited the highest hemoparasite prevalence and parasitemias, although most individuals were sampled one year after the seabirds. Thus, these differences within and among host species may be due to differences in exposure to vectors (Sol et al. 2000), host physiology (constrained by phylogeny or life history) or genetics, parasite factors (strain type or within-host evolutionary dynamics), environmental conditions or a combination of these and other factors (Goater and Holmes, 1997).

The pathogenicity of these parasites in the seabirds and doves sampled, or the effects on host fitness or reproductive success are unknown. However, within Great Frigatebirds, birds infected with *Haemoproteus* parasites exhibited significantly higher heterophil to lymphocyte concentration ratios than uninfected birds. In chickens, this ratio increased when birds were exposed to social stress or corticosterone in feed and it is thus considered to be a reliable indication of environmental stress (Gross and Siegel, 1983). Alternatively, this finding could simply be a consequence of a direct immune response to malarial pathogens. Although only correlational, our finding of significantly higher heterophil to lymphocyte concentration ratios in infected Great Frigatebirds is notable and the direction of causality should be investigated. Work and Rameyer (1996)

did not find significant differences in blood chemistries between infected and uninfected Great Frigatebirds in Hawaii, although there were no significant differences in *Haemoproteus* infection prevalences between Hawaii and Galápagos, and parasitemias were relatively low in both studies.

In general, hemoparasites have been considered rare in wild populations of seabirds (Greiner et al. 1975; Bishop and Bennett 1992; Bennett et al. 1994; Peirce 1981). More recent reports, however, show that prevalences of hemoparasites can be quite high in some wild populations of seabirds (e.g., gulls: Esparza et al. 2004; Ruiz et al. 1995; Martinez-Abrain et al. 2004 and frigatebirds: Work and Rameyer 1996; Work and Rameyer 1997) while they are still rare in other groups (e.g., penguins: Jones and Shellam 1999a, 1999b). A previous study of another Galápagos seabird, the waved albatross, *Phoebastria irrorata*, on the island of Española, showed no evidence of hemoparasites (Padilla et al. 2003), although *Haemoproteus*-like organisms are extremely common in Galápagos doves (*Zenaida galapagoensis*) on the same island (Padilla et al. 2004). Relatively long embryonic development periods of seabirds (Ricklefs 1992) and the relative paucity of competent vectors in marine environments were proposed as hypotheses explaining the general absence of blood parasites from seabirds. Interestingly, Great Frigatebirds have an extremely long 57-day incubation period and provide parental care for one year after hatching (Dearborn et al. 2001), yet *Haemoproteus* parasites have been reported from at least three populations of Great Frigatebirds, although infection intensities were relatively low in Hawaii and Galápagos (Aldabra Atoll by Lowry 1971; Hawaiian Islands by Work and Rameyer, 1996; Galápagos Islands, present study)

Haemoproteus parasites can be vectored by hippoboscid flies and ceratopogonid midges (Atkinson 1991). While no ceratopogonids were reported from Hawaii, several species occur within the Galápagos Islands (B. J. Sinclair, personal communication). Given the absence of ceratopogonids in Hawaii, Work and Rameyer (1996) speculated that the *Haemoproteus* present within Great Frigatebirds was likely vectored by *Olfersia* hippoboscid species associated with Pelicaniform birds, and which they observed on Great Frigatebirds. *Olfersia* species are also present within Galápagos, and *O. aenescens*, which is associated with Pelicaniformes, was collected in light traps on Genovesa during the 2004 sampling. Galápagos Doves also harbor a species of hippoboscid (*Microlynchia galapagoensis*) and hippoboscids are often restricted to a few host families or orders (Maa 1963). Thus, presently it is impossible to implicate either hippoboscids or ceratopogonids as vectors of *Haemoproteus* among the four bird species infected on Genovesa. Further characterization and differentiation of the Galápagos seabird hemoparasites through molecular techniques (e.g., Bensch et al. 2000; Schrenzel et al. 2003), and long-term studies will help understand the biology of these hemoparasites, and their ecological implications at the population level and may reveal whether these birds share hemoparasite lineages and vectors. Frigatebirds exhibit high philopatry to nesting sites, but travel thousands of kilometers from their nesting islands (Dearborn et al. 2003), creating opportunities for transmission of vectors and *Haemoproteus* between individuals during these long-distance movements. Therefore, placing *Haemoproteus* lineages from Galápagos and Hawaiian populations of Great Frigatebirds and the *Haemoproteus* lineages from the two other Genovesa seabirds in a broader phylogenetic context is warranted.

When compared to hematologic parameters published for other adult free-ranging Red-Footed Boobies and Great Frigatebirds, the range of total white blood cell counts are comparable (Work 1999, Work 1995). We observed slightly higher PCV values, and slightly lower total white cell counts in Great Frigatebirds, but our values were comparable to previously published biological ranges for that species in the wild (Work 1996). Female gulls and the two sulid species tested had higher phosphorous and calcium levels than males, but this was only significant for phosphorous. Work (1999) observed higher phosphorous values in breeding female Brown Boobies (*Sula leucogaster*) than in males of that species, which is consistent with our findings, although we did not differentiate breeding or non-breeding females at time of sampling. Reproductive status, dietary preferences, or different activity levels may be speculated as explanations for these differences. This relationship was absent in Great Frigatebirds in both studies, and has not been observed in other Pelicaniformes. In general, plasma chemistry values were comparable to those published in the literature for other species of the same taxa.

The absence of *Chlamydophila psittaci* antigen in all the birds tested is notable, and the absence of *C. psittaci* antibodies by EBA suggests that none of these birds had active clinical infections. In a previous study done on Galápagos Doves, *C. psittaci* was only present in doves from Isla Española (Padilla et al. 2004), which, like Genovesa, also contains large congregations of colonial seabirds. Charadriiforms (gulls and terns) have been reported as commonly infecting free-living birds (Brand, 1989), but few studies have documented *Chlamydophila* infections in wild seabirds (Franson and Pearson, 1995).

This study presents baseline health parameters for a free-ranging colony of several species of seabirds in the Galápagos Islands. The most notable finding was the presence of hemoparasites in three of the seabird species surveyed and in a sympatric terrestrial endemic bird, along with associated signs of physiological stress in *Haemoproteus*-infected Great Frigatebirds. Further characterization of these hemoparasites, as well as long term population studies, are suggested to understand the implications of these findings for the protection and conservation of avifauna of the Galápagos Islands.

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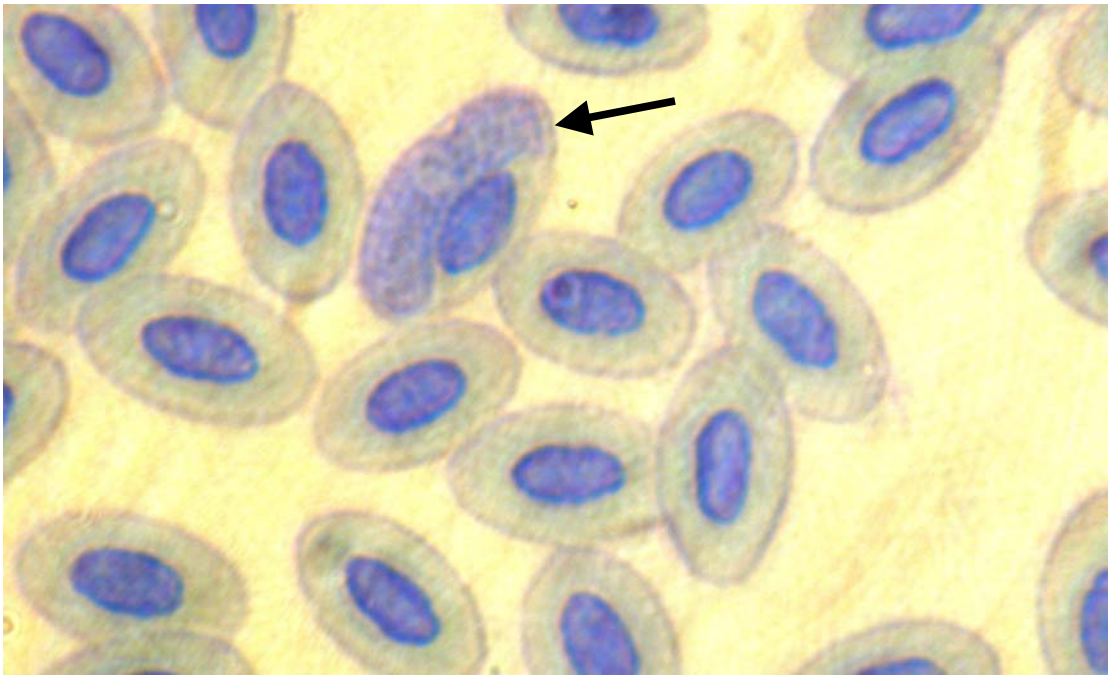
FIGURE LEGENDS

Fig. 1. Photomicrographs of haemoparasites in Galapagos birds.

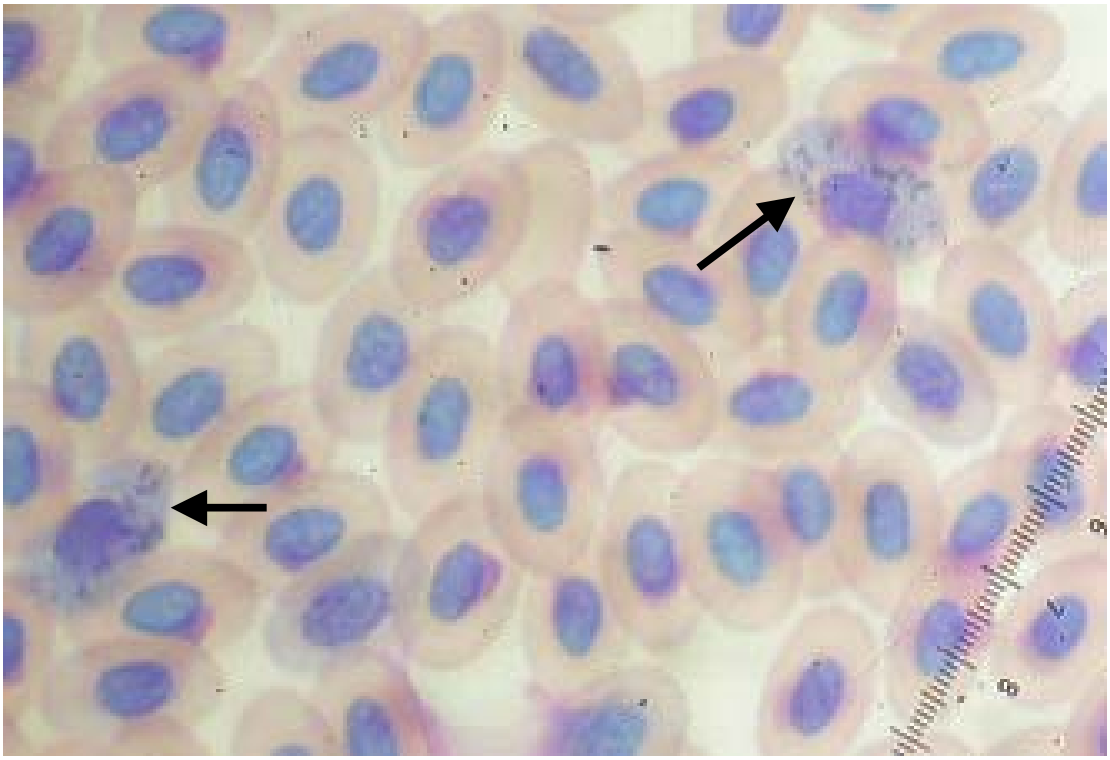
A. Erythrocytes from a thin blood smear from Galapagos Dove (*Zenaida galapagoensis*). Haemoproteus-like parasite indicated by arrow.

B. Erythrocytes from a thin blood smear from Great Frigatebird (*Fregata minor*). Haemoproteus-like parasites indicated by arrows.

Fig. 2 Boxplot showing mean (dotted line within box), median (solid line within box), 25^{th} and 75^{th} percentiles (lower and upper box limits), and 5^{th} and 95^{th} percentiles (whiskers) for the ratio of heterophil to lymphocyte concentration from *Fregata minor* on Isla Genovesa, Galápagos (standard differentials from thin smears were transformed into concentrations by multiplying the total white blood cell values (WBC (* 1000/µL) with the differential value, divided by 100). The mean ratios of heterophils to lymphocytes were significantly higher in birds positive for *Haemoproteus* infection compared to birds negative for *Haemoproteus* infection ($n = 16$, $U = 9$, two-tailed $P = 0.036$).



A. Photomicrograph of erythrocytes from Galapagos Dove (*Zenaida galapagoensis*) containing a Haemoproteus-like parasite indicated by an arrow.



B. Photomicrograph of erythrocytes from a Great Frigatebird (*Fregata minor*) containing Haemoproteus-like parasites indicated by arrows.

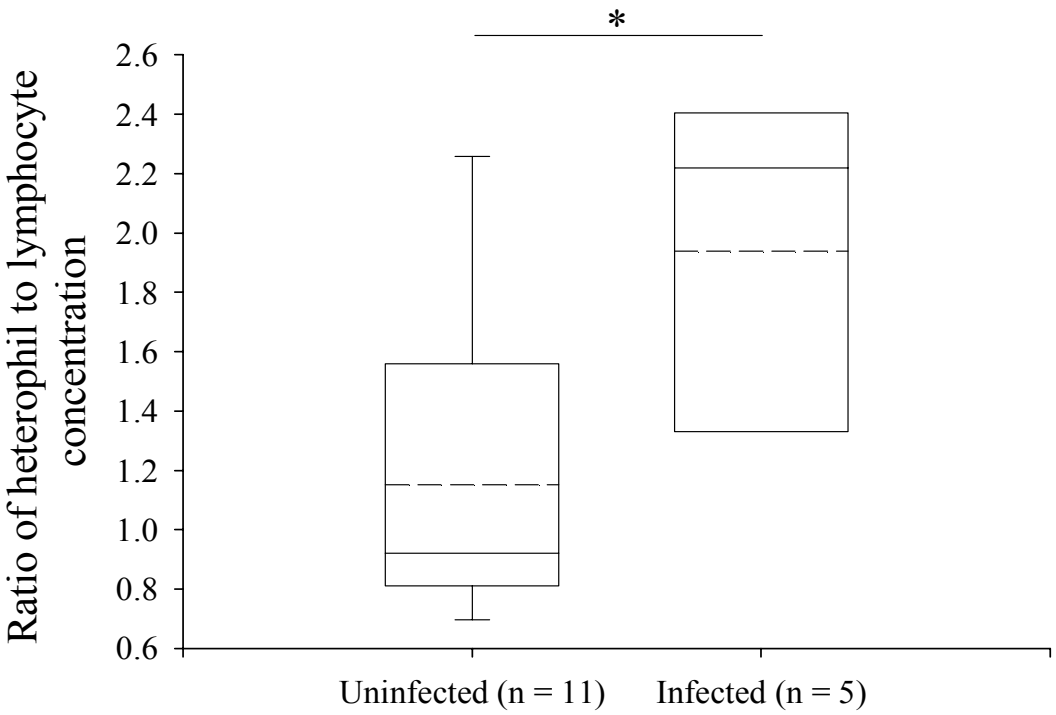

*
Ratio of heterophil to lymphocyte concentration
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
Uninfected (n = 11)
Infected (n = 5)
Haemoproteus infection status for Fregata minor

Table 1: Hematological parameters and prevalence, intensity and aggregation level (Var/mean ratio) of *Haemoproteus* spp. from four seabirds sampled in 2003 and one terrestrial species (Galápagos Dove) sampled in 2004 inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands.

Species	**PCV (%)**	**WBC (x 10^3/ uL)**	**Heterophils (%)**	**Lymphocytes (%)**	**Basophils (%)**	**Eosinophils (%)**	**Monocytes (%)**	***Haemoproteus* prevalence[1]**	***Haemoproteus* mean intensity (range if >1)**	***Haemoproteus* Var/mean ratio**
Great Frigatebird *(Fregata minor)*	55 ± 8 (n=23)	7.5 ± 2.7 (n=24)	39.0 ± 8.5 (n=16)	40.0 ± 12.0 (n=16)	0.6 ± 0.9 (n=16)	17.9 ± 2.7 (n=16)	2.5 ± 1.6 (n=16)	7/24 (29.2%)	1.29 (1-2)	1.12
Red Footed Booby *(Sula sula)*	50 ± 7 (n=20)	10.3 ± 4.7 (n=23)	36.1 ± 16.7 (n=18)	54.8 ± 17.5 (n=18)	0.2 ± 0.4 (n=18)	5.2 ± 3.6 (n=18)	3.7 ± 2.6 (n=18)	2/23 (8.7%)	1.00	0.95
Nazca Booby *(Sula granti)*	51 ± 3 (n=23)	9.4 ± 3.5 (n=25)	46.7 ± 14.3 (n=24)	34.4 ± 14.2 (n=24)	0.5 ± 0.7 (n=24)	15.4 ± 7.9 (n=24)	3.8 ± 2.2 (n=24)	0/25 (0%)	0.00	0
Swallow-Tailed Gull *(Creagrus furcatus)*	54 ± 5 (n=19)	4.8 ± 2.7 (n=20)	54.0 ± 11.1 (n=14)	36.2 ± 10.9 (n=14)	0.5 ± 0.8 (n=14)	4.4 ± 1.8 (n=14)	4.9 ± 4.2 (n=14)	3/19 (15.8%)	1.00	0.89
Galápagos Dove[2] (***Zenaida galapagoensis***)	N/A	N/A	18 ± 5.7 (n =18)	69.06 ± 10.17 (n =18)	0.06 ± 0.24 (n =18)	8.4 ± 8.1 (n =18)	4.41 ± 2.28 (n =18)	11/26 (42.3%)	29.36 (1-270)	225.76

[1] Prevalence established by detection of circulating *Haemoproteus*-like organisms on smear examination.
[2] Three doves were sampled in 2003, one of which was positive for *Haemoproteus* with an infection intensity of 8.

Table 2: Plasma biochemistry values of four seabird species sampled in 2003 inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands

Species	Uric Acid (mg/dl)	CK (U/L)	AST (U/L)	Total Bilirubin (mg/dl)	Glucose (mg/dl)	Ca^{++} (mg/dl)	Phos (mg/dl)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cl- (mmol/L)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Great Frigatebird (*Fregata minor*) (n=24)	7.7± 7.7	556 ± 421	248.1 ± 95.1	0.6 ± .5	212.1 ± 45.7	9.1 ± 0.66	4.7 ± 1.5	145.4 ± 8.2	3.0 ± 1.6	114.6 ± 4.5	3.58 ± 0.5	0.94 ± 0.1	2.6 ± 0.4
Red Footed Booby (*Sula sula*) (n=21)	10.9 ± 8.2	940.1 ± 371.4	465.8 ± 181.1	0.6 ± 0.2	180.5 ± 64.0	9.3 ± 0.7	10.8 ± 4.6	151.4 ± 3.7	6.6 ± 2.1	116.0 ± 4.7	3.56 ± 0.3	1.17 ± 0.1	2.0 ± 0.9
Nazca Booby (*Sula granti*) (n=24)	12.1 ± 7.2	871.8 ± 271.1	310.3 ± 141.7	0.47 ± 0.3	252.0 ± 34.6	9.5 ± 0.64	5.6 ± 2.6	154.5 ± 3.6	3.0 ± 1.2	117.9 ± 3.2	3.76 ± 0.4	1.22 ± 0.1	2.4 ± 0.6
Swallow-Tailed Gull (*Creagrus furcatus*) (n=14)	7.5 ± 5.9	263.3 ± 323.1	361.6 ± 172.2	1.2 ± 1.6	280.6 ± 31.8	11.0 ± 2.9	4.6 ± 4.9	155.0 ± 9.2	3.0 ± 0.7	122.2 ± 7.5	4.13 ± 0.8	1.57 ± 0.4	1.8 ± 1.3

Table 3: Plasma phosphorous (mg/dl) values reported by sex of four seabird species sampled in 2003 inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands

	Male	Female	p
Great Frigatebird (*Fregata minor*)	4.9 ± 1.69 (14)	4.5 ± 1.21 (10)	0.9
Red Footed Booby (*Sula sula*)	8.6 ± 4.12 (10)	13.3 ± 3.93 (10)	0.03
Nazca Booby (*Sula granti*)	4.6 ± 0.66 (13)	6.8 ± 3.56 (10)	0.0002
Swallow-Tailed Gull (*Creagrus furcatus*)	1.7 ± 1.10 (8)	8.4 ± 5.40 (6)	0.9

Chapter XII

Cryptic Host Specificity and Genetic Epidemiology of a Generalist Avian Skin Mite (Acari: Astigmata) from Two Sympatric and Threatened Galápagos Endemic Birds

Unpublished manuscript:

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ABSTRACT

Hitchhiking by one parasite on another (phoresis) may be an important force shaping parasite evolution and host-parasite dynamics, because the vector may allow the hitchhiker to invade new host individuals and species. In species where phoresis is common, the hitchhiker's host specificity is predicted to mirror its vector's, but this has not been tested. The 'generalist' avian skin mite *Myialges caulotoon* Speiser (Acari: Epidermoptidae) is unusual because female mites require an insect vector to complete their life cycle. This species was previously reported in phoretic associations with two lousefly species (Diptera: Hippoboscidae) parasitizing Flightless Cormorants (*Phalacrocorax harrisi*) and Galápagos Hawks (*Buteo galapagoensis*) within the Galápagos Islands, a surprising distribution given that the lousefly species are specific to pelicaniform or falconiform birds. We tested for cryptic host specificity in *Myialges* mites from the same islands and hosts where the two endemic avian and fly species were previously sampled in allopatry, and additionally from hosts on an island where host

species were sympatric. Mitochondrial DNA sequence data revealed two reciprocally monophyletic *Myialges* clades that sorted out perfectly with respect to host species. One clade was restricted to hawk flies and the other to cormorant flies, each with divergent demographic histories and distributions within the respective fly populations. The large genetic distance between the clades (17% uncorrected *p*-distance), indicates that these mite lineages separated from a common ancestor prior to the colonization of the Galápagos Islands by the Galápagos Hawk. Within the hawk *Myialges* clade, genetic differentiation between two island populations mirrored that of its host's. Wildlife health implications are discussed in light of these findings.

Key words: Cryptic species; DNA barcoding; Epidermoptidae; Host specificity; Galápagos Islands; Phoresy

1. Introduction

Parasite systematics has been troubled by a tendency for parasites to be morphologically conservative relative to their hosts, which has resulted in the use of host relationships to define parasite species limits (Eichler, 1948). The application of molecular phylogenetics has revealed this practice to be highly problematic (e.g., Johnson et al., 2002a). On the other hand, the morphologically conservative tendency of parasites, combined with a faster rate of molecular evolution relative to their hosts, has led to the artificial grouping of cryptic parasite species, including protozoans (Perkins, 2000), helminths (Hung et al., 1999; Criscione and Blouin, 2004), lycaenid butterflies (Als et al., 2002), chewing lice (Johnson et al. 2002b; Whiteman et al., 2004) and ticks (McCoy et al., 2005). Thus, our understanding of parasite distribution, evolutionary history, ecology, and transmission dynamics are of questionable quality (Miura et al., 2005). To

facilitate species identifications and test species delimitations, DNA-based species identification systems have been advocated generally for all species (Hebert et al., 2003), and specifically for parasites (McManus and Bowles, 1996; Besansky et al., 2003; Whiteman et al., 2004; Whiteman and Parker, 2005), although 'molecular taxonomy' remains highly controversial (e.g., Ebach and Holdrege, 2005).

In addition to its utility in differentiating among parasite species, molecular-genetic characterization of parasite lineages and populations also reveals information about the processes driving parasite diversification (Hebert et al. 2004; Criscione and Blouin 2005; Whiteman and Parker 2005). Many parasite species have complex life-cycles involving intermediate hosts or free-living stages; these dynamics influence their population genetic structure and phylogenetic history (Nadler 1995).

Phoresis is an adaptive explanation for the occurrence of one parasite individual attached to a more vagile parasite individual (Keirans, 1975). Phoresis allows the hitchhiker access to a new host individual, which may be particularly important when the parasite's host is dying or dead (Clayton et al., 2003). Theresa Clay (1949; Clay and Meinertzhagen, 1943) was among the first to speculate that phoretic involvement of lice (Phthiraptera) on volant louseflies (Hippoboscidae) of birds has played an important role in speciation within lice. Several recent studies have revisited the importance of phoresy as an ecological force underpinning ectoparasite diversification (Clayton et al., 2003; Johnson and Clayton, 2003; Johnson et al., 2002a, b; Weckstein, 2004). However, ecological studies of phoresis are largely absent from the literature.

The genera *Myialges* and *Microlichus* within the mite family Epidermoptidae (Acari: Astigmata), are, unlike lice, obligately phoretic on ectoparasitic insects and are

therefore ideal systems in which to investigate how phoresy has shaped ectoparasite transmission dynamics and evolution. Accordingly, the phoretic relationship between these mites and their insect vectors has been invoked to explain the relatively low degree of host specificity of these mites among birds (Fain, 1965). The insect vectors are generally thought to have a low degree of host specificity and other, non-phoretic mite genera in the Epidermoptidae are generally highly host specific (Fain, 1965). Gravid females of *Myialges* and *Microlichus* are exclusively found attached to louseflies or, more infrequently, to lice, of birds (Fain, 1965). Inseminated adult female mites move from the bird and onto the insect, to which they become permanently attached. Female mites oviposit while attached to the insect and eggs are anchored to the host insect's cuticle. The eclosed immature mites disperse from the insect vector onto an avian host to complete development where they feed on bird epidermal tissue and body fluids (Evans et al., 1963). Immatures, adult males and non-gravid female mites live in the avian host's skin and have not been found on insects (Fain, 1965). Members of the subfamily Epidermoptinae, which includes *Myialges*, burrow into the upper skin layers of their avian hosts and causes pityriasis, dermatitis and mange, leading to host mortality and morbidity, including in endangered seabird species (Evans et al., 1963; Fain, 1965; Greve, 1984; Gilardi, 2001). The entire life-cycle is depicted in Figure 1.

The "low grade of specificity" of phoretic and hyperparasitic epidermoptid species (Fain, 1965) is typified by *Myialges caulotoon* Speiser, which has been reported from at least nine hippoboscid species collected in association with at least eight avian host species, comprising three avian orders and five families (Table 1). Remarkably, Fain (1965) reported that: (1) no illustrations accompanied the original descriptions of

M. caulotoon by Speiser (1907) rendering species recognition, in relation to the original description, impossible; (2) notwithstanding this, Ferris (1928), without reexamining the type specimens, reported *M. caulotoon* from two hippoboscids collected in the Philippines; and (3) Several authors (e. g., Thompson, 1936; Dubinin, 1953) accepted Ferris' (1928) designation even though Dubinin (1953) "confused the tarsus of *M. anchora* with that of *M. caulotoon*" according to Fain (1965). Fain's (1965) attempts to Speiser's types (1907) did not succeed, but he did examine *Myialges* specimens from the type-fly host. Fain (1965) designated lectotypes from these after listing 11 characters (10 of which are continuous morphological characters, e.g., "Legs I longer.") by which *M. caulotoon* could be distinguished from other *Myialges* species. Only females of *M. caulotoon* are known, yet males are typically needed to confirm species limits in these mites (Phillips and Fain, 1991). Thus, the taxonomy of this genus, and our understanding of host specificity, is of highly questionable value and requires further study and testing using molecular genetic tools. This is especially important since previously defined "generalist" parasites have been shown to actually be comprised of host races or cryptic species (McManus and Bowles, 1996; McCoy et al., 2005; Miura et al. 2005). Ecologically and morphologically similar skin mites of mammals in the genera *Psoroptes* and *Sarcoptes* are also notoriously difficult to differentiate between species (Sweatman, 1958). Molecular genetic data have shed a great deal of light on the degree of host specificity within and between putative mange mite species and have therefore informed wildlife and public health management strategies (Ochs et al., 1999; Walton et al., 1999, 2004; Zaher et al., 1999; Ramey et al., 2000). No genetic studies on epidermoptid mites have been published previously.

1.1. Myialges caulotoon in the Galápagos Islands

Females of *Myialges caulotoon* were previously reported from the Galápagos Islands (Table 1; Madden and Harmon, 1998). Hippoboscid flies were collected from Galápagos hawks (Falconiformes: Accipitridae: *Buteo galapagoensis*) on Isla Santiago and Flightless cormorants (Pelecaniformes: Phalacrocoracidae: *Phalacrocorax harrisi*) and Brown pelicans (Pelecaniformes: Pelecanidae: *Pelecanus occidentalis*) on Isla Fernandina during a survey of parasitic mites from the Galápagos Islands (Harmon et al., 1990). Mites found on flies from hawks (*Icosta nigra*) and the two pelicanform birds (*Olfersia sordida*) were identified as *Myialges caulotoon* by A. Fain (Institut Royal des Sciences Naturelles de Beglique), the world authority on Epidermoptidae. However, Harmon and Madden (1998) noted that the Galápagos specimens differed morphologically from the type specimens (Fain 1965) and that "other less detectable morphological differences exist between this population and those previously described. The Galápagos population may thus be a clinal or ecotypic population of *M. caulotoon*." Harmon and Madden further suggested that "*M. caulotoon* might not be a very good indicator of host phylogeny or ecology because it occurs on at least 2 hippoboscid species and 3 unrelated bird species with at least 2 very different niches in Galapagos." However, only one *M. caulotoon* individual was collected and examined from the *O. sordida* flies associated with Flightless Cormorants, raising further doubts as to the validity of this identification, which is understandably difficult since 10 of the 11 characters used to differentiate some congeners are continuously variable (Fain, 1965).

Given the broad geographic and host range of *M. caulotoon*, Fain's (1965) and Madden and Harmon's (1998) general characterization that members of the 'phoretic'

epidermoptid genera exhibit low host specificity, seems straightforward. However, several points suggest that cryptic host specificity may be present and detectable within this *Myialges* species. First, dispersal of *Myialges* mites among avian hosts is likely largely restricted to phoresy via hippoboscid flies. Thus, it follows that host specificity of *Myialges* mites may mirror that of the lousefly vectors, as suggested by Phillips and Fain (1991). Although host specificity of hippoboscids is highly variable across fly species, most are restricted to one avian order or family, and many are highly host-specific (Maa, 1963; Marshall, 1981). Moreover, Phillips and Fain (1991) suggested that *M. caulotoon* may, in fact, be a complex of species. The lousefly *Icosta nigra* is typically restricted to members of the Falconiformes and *Olfersia sordida* to two families within the Pelecaniformes (Phalacrocoracidae and Pelicanidae; Maa, 1963). The Galápagos Hawk, Flightless Cormorant and Brown Pelican are the only resident birds from these avian lineages within the archipelago. Thus, *I. nigra* is likely restricted to Galápagos Hawks and *O. sordida* to Flightless Cormorants and Brown Pelicans within the archipelago, a distribution that may prevent dispersal of *Myialges* mites between avian host orders. Finally, if host specialization has occurred, genetic differentiation should be apparent between sympatric hosts (Jaenike, 1981; McCoy et al., 2001), although morphological differentiation may not be apparent.

2. Materials and Methods

2.1. Field methods

Since *Myialges* mites occur under their avian host's skin, sampling from birds requires invasive procedures not feasible for threatened Galápagos Hawks and Flightless Cormorants. Thus, we collected female *Myialges* mites attached to the cuticle of

louseflies (Fig. 2) associated with each avian host species from the entire breeding range of Galápagos Hawks (2001-2003) and Flightless Cormorants (2004-2005). Sampling was associated with an ongoing avian disease survey involving the University of Missouri-St. Louis, the Saint Louis Zoo, Charles Darwin Research Station and Galápagos National Park. In all cases, avian subjects were live-captured, sampled for subsequent disease testing, and released unharmed. Louseflies were collected from each host species on separate sampling trips. Eight Galápagos Hawk populations on Española, Fernandina, Isabela, Marchena, Pinta, Pinzón, Santa Fe, and Santiago Islands were sampled and *I. nigra* fly specimens were collected from birds by hand (Bollmer et al. 2005). *Olfersia sordida* flies were collected by hand from Flightless Cormorants from Islas Fernandina and Isabela. On Fernandina, sampling sites for cormorants included areas from which hawks were also sampled (e.g., coastal areas within hawk territories). All flies were placed in labeled vials of 95% ethanol, transported to the United States and placed in a freezer at -20º C to maximize DNA preservation. Dr. B. J. Sinclair, (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany) identified representative specimens of flies from both hosts and retained voucher specimens, which were deposited at the Museum Alexander Koenig. *Olfersia sordida* specimens were not collected from Brown Pelicans, since this taxon was not included in the collaborative avian disease survey. All field sampling procedures were approved by the University of Missouri-St. Louis Institutional Animal Care and Use Committee and the Galápagos National Park. Dr. Heather Proctor, University of Alberta, provided specimens of *Neodermation* sp. (Dermationidae) from a Pileated Woodpecker (*Dryocopus pileatus*) for inclusion as an outgroup in the phylogenetic analyses.

2.2. Specimen examination

Flies were thoroughly examined under a binocular dissecting microscope at UM-St. Louis for the presence of *Myialges* mites. If present, their attachment sites were recorded (see Fig. 2A and B for examples of mite attachment sites). Basic metrics describing parasite load (e.g., prevalence, abundance, intensity, index *k*) were calculated for mites from each fly host species (from each population sampled) and compared between host species using the program Quantitative Parasitology 2.1 (QP; Reiczigel and Rózsa., 2001). For mites from *I. nigra* only (which had reasonably high *Myialges* prevalences), comparisons of these measures among main body segments and among appendages of the thorax (legs I, II III, wings and main thorax) were carried out in QP. *Myialges* prevalence data from Madden and Harmon's (1998) study were also included and analyzed for comparative purposes. Finally, one *I. nigra* specimen (with several *Myialges* females attached) was dehydrated in an ethanol series, critical point dried (SPI Jumbo critical point drier; Structure Probe, West Chester, Pennsylvania, USA) and then gold sputter-coated in a Polaron E5000 sputter coater (Quorum Technologies, Hailsham, UK). Images were taken on an Hitachi S450 scanning electron microscope (SEM; Tokyo, Japan) at 20 kV at the University of Missouri-St. Louis.

2.3. DNA extraction

The Cruickshank et al. (2001) voucher method was used to extract DNA from individual *Myiagles* mites collected from both *Icosta nigra* flies (from Galápagos Hawks) and *Olfersia sordida* flies (from Flightless Cormorants) at the University of Missouri-St. Louis. Specifically, individual mites and their brood (if present) were removed from the alcohol-preserved hippoboscid fly hosts (e.g,. Fig. 2) using sterile syringe needles. Since

mitochondrial DNA (mtDNA) is typically maternally inherited in animals, mitochondrial sequences from mother mites and their brood should be identical (including brood increased the quantity of DNA and therefore the quality of subsequent PCR amplicons). Mites were dried on the benchtop in clean watchglasses for five minutes and the abdomens sliced into two approximately equal pieces with the beveled edge of a sterile needle tip. The mites were then individually transferred to 1.5 µL Eppdendorf tubes and the animal tissue extraction instructions for the DNAeasy Tissue Extraction Kit (Qiagen, Inc., Valencia, California, USA) were followed with these modifications: (1) mites were left in incubation at 55º C for two nights and (2) the final elution step consisted of only one 40 µL volume of warmed elution buffer (EB). Mite exoskeletons (which were 'cleared' by the extraction process) were retrieved from the 1.5 Eppdendorf tubes, further cleared, slide mounted using standard protocols and deposited in the Ohio State University Acarology Collection. Some mite exoskeletons or portions thereof were lost during the retrieval process owing to their small size. One female *Myiagles* mite from *O. sordida* collected in association with a Flightless cormorant was slide mounted intact, and thus its DNA was not extracted. However, DNA from that mite's brood was extracted following the protocol above. The *Neodermation* sp. mite specimens were extracted following the above procedure except that three batches of mites (4-5 individuals each) rather than individuals were extracted due their extremely small size.

2.4. PCR, cleanup and DNA sequencing

The primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAACTTCAGGGTGACCAAAAAATCA-3') was used to PCR amplify a 658 bp fragment of the mitochondrial gene cytochrome oxidase *c* subunit I (COI; near the

5' end; Folmer et al. 1994). This particular locus has been used successfully as a DNA barcode to identify arthropod species (Hebert et al. 2003). Each PCR tube contained 47 µL of a PCR master mix comprised of the following components: 2.5 µL of PCR buffer (provided with DNA polymerase), 1.5 µL of BIOLASE Red DNA polymerase (Midwest Scientific, St. Louis, Missouri, USA), 3.9 µL of 25 mM $MgCl_2$ (provided with DNA polymerase), 3 µL of each primer (diluted to 100 µM), 1.6 µL of 100 µM DNTPs, 31.5 µL of sterile dH_2O. Three microlitres of template mite DNA was added from each individually extracted mite, yielding a final PCR volume of 50 µL. Negative controls (tubes with no template DNA) were included in each set of reactions. The PCR amplifications for COI were performed using the following conditions: An initial denaturing step at 94°C for 4 minutes, followed by 35 cycles beginning with a denaturation step at 94°C for 1 minute, an annealing step at 40°C for 1 minute, an extension step at 70°C for 1 minute, followed by a final extension step at 72°C for 7 minutes after the completion of the 35 cycles. Amplicon size was verified on 1-2% TBE agarose gels stained with ethidium bromide and visualized under UV light and then purified with QIAQuick PCR columns or agarose gel purified and then extracted using QIAQuick Gel Extraction Kit following the manufacturer's instructions (Qiagen Inc., Valencia, California, USA). Purified amplicons were then verified on an agarose gel following the above. Direct sequencing was performed on both strands of each amplicon by Macrogen, Inc. (Seoul, Korea) using the primers above with ABI PRISM® BigDye Terminator PCR cycling conditions and sequenced on Applied Biosystems 3730xl DNA Analyzers (Applied Biosystems Division, Foster City, CA). We also amplified a 330-334 bp (the fragment size variation results from the presence of in-dels) fragment of the 12S

mitochondrial ribosomal RNA gene from the same samples using the primer pair 12SAI (5'-CTAGGATTAGA-TACCCTATT-3' and 12SBI (5'-AAGAGCGACGGGCGATG-3') published previously (Simon et al. 1994). Volumes of reagents and template DNA in PCRs as well as purification (using QIAQuick PCR columns only) were identical to the above. The PCR amplifications for 12S amplifications were performed using the following conditions: An initial denaturing at 94°C for 2 minutes, followed by 35 cycles beginning with a denaturation step at 94°C for 30 seconds, annealing at 46°C for 30 seconds, extension at 70°C for 30 seconds, followed by a final extension step at 72°C for 7 minutes after the completion of the 35 cycles. Sequencing was carried out by Macrogen as described above (using 12SAI and 12SBI primers for sequencing).

2.5. DNA sequence analyses

Raw sequence chromatograms of forward and reverse strands were assembled for each amplicon in Seqman II (DNASTAR, Inc., Madison, WI, USA). The entire length of each strand was evaluated by eye. Poor quality data and primer sequences were trimmed from both strands. Seqman II was used to assemble the contigs (consensus sequences) resulting from the double stranded sequences for each gene, which were aligned in Se-Al (Rambaut, 1996) or ClustalX (Thompson et al., 1997). We then returned to the original chromatograms to ensure that variable sites were unambiguously assigned. Sequences have been deposited in Genbank under the accession numbers XXXXXX-XXXXXX. Alignments of both loci have been deposited in TreeBASE (http://www.treebase.org/treebase/index.html).

Neighbor joining analyses were performed on each dataset (COI, 12S and combined) in Paup*. To roughly test the monophyly of each *Myialges* clade identified,

heuristic parsimony and neighbor-joining searches were performed in Paup* on an alignment of the COI *Myialges* sequences and two outgroup COI sequences: *Neodermation* sp. and *Varroa destructor*, (12S sequences, which are highly variable in length between invertebrate species, were not used for the analyses including distant related outgroups). To examine cryptic genetic diversity within each putative *Myialges* lineage (those from hawks and cormorants), a statistical parsimony haplotype network was constructed using the TCS program (Clement et al., 2000) for each locus.

We preformed analyses of mismatch distributions (Slatkin and Hudson, 1991; Rogers and Harpending, 1992) to provide a visual and statistical framework for investigating historical demography of populations. With this method, histograms show the pairwise frequencies of individuals that differ by *i* nucleotide sites. These patterns correlate with trends in historical population size in predictable ways. For example, populations that have undergone a single, ancient bottleneck, a sudden expansion or continued exponential growth exhibit a unimodal distribution (a single wave). Mismatch distributions of populations that have very recently undergone a large reduction in size follow a pattern of exponential decay (convergence to the new equilibrium is established rapidly; Rogers and Harpending, 1992). Repeated bottlenecks followed by population expansion produce multimodal distributions (multiple waves; Jolly et al., 2005), although, interestingly, these patterns can also be generated by population stability over long periods of time. Mismatch analyses for the cormorant *Myialges* lineage (combined Islas Fernandina and Isabela due to small overall sample size and lack of island-specific haplotypes) and for the two hawk *Myialges* populations for which reasonable sample sizes were obtained (Islas Fernandina and Santiago), were performed. Tajima's D

(Tajima 1989) was also used to test for recent population expansion within each population. The pairwise F_{ST} value was calculated for *Myialges* sequences obtained from hawk flies (*I. nigra*) from Islas Fernandina and Santiago to test for population differentiation. To compare the level of genetic differentiation between the hawk mite and its avian host, we also calculated pairwise F_{ST} values between these islands for the Galápagos Hawk using mtDNA sequences obtained previously (Bollmer et al., in press). The preceding analyses were conducted in Arlequin (Schneider et al., 2000). Pairwise F_{ST} values from multilocus minisatellites were also obtained (Bollmer et al. 2005) from the same hawk populations for an estimate of differentiation of the host at nuclear loci.

3. Results

3.1. Fly collections

A total of 296 *I. nigra* specimens was collected from eight island populations of *B. galapagoensis* hosts, which comprises the entire breeding range of these endemic birds. *Icosta nigra* sample sizes by island were as follows: Española $N = 14$, Fernandina $N = 46$, Isabela $N = 20$, Pinta $N = 46$, Pinzon $N = 18$, Santa Fe $N = 5$ and Santiago $N = 147$. No *I. nigra* specimens were collected or observed to be associated with hawks from Isla Marchena, despite capturing and examining 26 hawk individuals on Marchena during the same season as the other island populations. A total of 105 *O. sordida* specimens were collected from Islas Isabela and Fernandina (and several small islets associated with Isabela), which comprise the entire species range of *P. harrisi* hosts. *Olfersia sordida* sample sizes by island were as follows: Fernandina $N = 59$ and Isabela $N = 46$.

3.2. Mite collections and distributions

A total of 127 *Myialges* individuals was found attached to 53 of the 296 *I. nigra* specimens from four of the eight islands on which hawk hosts were sampled (Islas Fernandina, Isabela, Santiago and Pinzon). Seven *Myialges* individuals were found attached to 7 of the 105 *O. sordida* specimens (from both Isabela and Fernandina) and one *Myialges* individual was found free floating in the collection vial associated with three of the 105 *O. sordida* specimens and is assumed to have been present on one of those fly individuals prior to immersion in alcohol. Prevalence of mites on *I. nigra* (hawk louseflies) differed significantly among islands (Table 2), whereas prevalence of mites on *O. sordida* (cormorant louseflies) did not differ significantly among islands (Table 2).

In both the 1998 study and the present study (using a combined dataset among islands), mites were more significantly prevalent and abundant on *I. nigra* (from hawks) than on *O. sordida* (from coromorant) fly hosts (Table 3). Distributions of mites among flies approximated a negative binomial distribution (Fig. 3). Within *I. nigra* flies infected with mites, female *Myialges* were most prevalent on the thorax, followed by the thorax and head (Table 4). Considering only the thorax for flies infected with *Myialges*, mites were most prevalent on the hind legs, followed by attachment to the wings, main thorax, middle legs and fore legs (Table 3).

3.3. DNA sequence, population genetic and phylogenetic analyses

We obtained double-stranded sequences of 658 bp of COI and the 330-334 bp of 12S mtDNA from 28 *Myialges* individuals (sample size by island: N = 2 Isabela; N = 1 Pinzon; and N = 25 Santiago) associated with 28 *I. nigra* louseflies from 24 different Galápagos Hawks and 8 *Myialges* individuals (N = 3 Isabela; N = 5 Fernandina)

associated with 8 *O. sordida* louseflies from 8 different Flightless cormorants. In the case of the *I. nigra* samples, one mite each was sampled from two different louseflies collected from the same hawk in four cases (hawk bands G44, G16 and N40 from Isla Santiago and hawk band R5E from Fernandina). Alignments of both loci were unambiguous within *Myialges* sampled from Galápagos hosts. The inferred amino acid residues translated (using the arthropod mtDNA code) from 658 bp of the protein-coding COI sequences resulted in an open reading frame comprising 219 codons (in the second frame). The non-protein coding 12S fragments amplified from *Myialges* collected from cormorant flies were 4 bp shorter (330 bp) than the fragment amplified from *Myialges* collected from hawk flies (334 bp). The concatenated alignment (COI + 12S) was 988 bp in length (gaps were inserted in the cormorant *Myialges* sequences). A neighbor joining analysis using the combined 988 bp alignment and an analysis of the 12S data set only from the 35 *Myialges* individuals revealed trees essentially identical to the NJ tree resulting from an analysis of only COI data shown in Fig. 4. We chose to display the COI tree (Fig. 4) because we were able to align outgroup sequences unambiguously with the ingroup sequences. In all of the trees (COI, 12S and combined), one main lineage was comprised only of *Myialges* collected from cormorant flies (*O. sordida)* and these haplotypes were separated from the other main lineage, comprised only of *Myialges* collected from hawk flies (*I. nigra*), by 16.05-16.55% uncorrected *p*-distance. For the COI phylogeny, the monophyly of the two host-specific *Myialges* was highly supported in both NJ and parsimony bootstrap analyses (100% in each case; Fig. 4). The cormorant *Myialges* lineage was further comprised of two clades, separated by ~1% uncorrected *p*-distance at COI (7 mutations) and ~0.3% uncorrected *p*-distance at 12S (one mutation).

Mite sequences collected from cormorant flies on both Fernandina and Isabela were recovered in each clade (no island-specific lineages). Table 5 lists the polymorphic sites in the cormorant *Myialges* samples. Sequences of *Myialges* mites from hawk louseflies sampled from the same hawk host individuals (but from two different hippoboscid individuals within each bird) were identical at both loci. A Fernandina-specific hawk *Myialges* clade comprised of five *Myialges* individuals (see haplotype network below) was also recovered. Eight sites varied across the 988 bp alignment within the hawk *Myialges* lineage (Table 6).

Haplotype networks for each lineage (cormorant *Myialges* and hawk *Myialges*) showed very different patterns (Fig. 5). While the cormorant *Myialges* clade only harbored two haplotypes separated by eight mutational steps, the hawk *Myialges* clade was star-like and individual haplotypes were all closely related, with one central, and common haplotype that likely comprises the ancestral haplotype. All six COI mutations within the hawk *Myialges* clade were synonymous. Within the cormorant *Myiagles* clade, six COI mutations were synonymous and one was nonsynonymous.

The mismatch distributions of mite haplotypes from the hawk *Myialges* clade were unimodal and did not differ significantly from the theoretical model of rapid population expansion (Rogers and Harpending, 1992), for either island population analyzed (Fernandina or Santiago; Fig. 6a,b). However, the mismatch analysis for mite haplotypes from the cormorant *Myialges* clade did differ significantly from the theoretical model of rapid population expansion and exhibited a bimodal distribution (Fig. 6c). Tajima's D values for hawk *Myialges* were negative (indicative of a recent population expansion; Tajima, 1989) for both Fernandina (Tajima's D = –0.43; N = 8; *P*

> 0.05) and Santiago (Tajima's D = –1.94; N = 17; $P < 0.05$), and positive for cormorant *Myialges* (Tajima's D = 1.88; N = 8; $P < 0.05$).

Myialges populations collected from hawks on Islas Fernandina and Santiago were differentiated from each other ($F_{ST} = 0.33$, $P < 0.00001$) based on the combined (COI + 12S) mtDNA dataset. Hawk populations from these two islands were completely differentiated (though were invariant at the homologous 5' end of COI used here) from each other based on nearly 3kb of mtDNA ($F_{ST} = 1$, $P < 0.00001$) obtained for hawk individuals sampled previously (N = 23 Santiago; N = 22 Fernandina; Bollmer et al. in press. Bollmer et al. (2005) also showed that hawks from Santiago (N =37) and Fernandina (N =20) were significantly differentiated at multilocus minisatellite (nuclear) loci ($F_{ST} = 0.123$).

4. Discussion

We genetically characterized and quantified the distributions of epidermoptid mite populations associated with endemic Flightless Cormorants and Galápagos Hawks in the Galápagos Islands. To determine if these mites exhibited cryptic host specificity or instead were being transmitted between host species, we sampled mites from locations where the two avian hosts were sympatric and shared habitat (Isla Fernandina) and from locations where only the Galápagos hawk was present, including Isla Santiago. Islas Fernandina (the sympatric setting) and Santiago were also the island populations from which *Myialges* mites were collected from Flightless Cormorant and Galápagos Hawk louseflies, respectively, by Harmon & Madden (1998). Despite the opportunity for horizontal transfer between fly or avian host species, significant genetic divergence and reciprocal monophyly was revealed between mite populations collected from each host

species. These *Myialges* populations thus exhibit cryptic host specificity. We found further cryptic genetic diversity within each major host-specific *Myialges* clade, including an island-specific clade of *Myialges* associated with hawks on Isla Fernandina and two relatively divergent non-island specific clades among *Myialges* sequences from cormorants. A DNA-barcoding approach of mite eggs laid on a cormorant fly revealed the identity of the *Myialges* mother (which was identical to the most abundant cormorant *Myiagles* haplotype), which had been vouchered without being genotyped.

The haplotype network indicates that the most widespread haplotype within hawk associated *Myialges* dataset is most likely the ancestral DNA sequence. Interestingly, this haplotype is most abundant on Isla Santiago, and rare on Isla Fernandina. Together with the F_{ST} data (discussed below), this suggests that these island populations of *Myialges* are genetically isolated from one another just as their host populations are differentiated between these two islands.

The inferred demographic history of *Myialges* populations from Galápagos hawks is consistent with one of a very recent severe population bottleneck (Rogers and Harpending, 1992). This is consistent with the interpretation that these *Myialges* populations were derived from a few, closely related individuals, a pattern also observed in the demographic history of its hawk host (Bollmer et al., in press). Conversely, the bimodal mismatch distribution of cormorant *Myialges* sequences is explained to the presence of two relatively divergent haplotypes, which were both present in each of the island populations of cormorant louseflies. This, along with a negative Tajima's D value, is consistent with a model of population expansion followed by introduction of new haplotypes, possibly by Brown Pelicans, which may share *Myialges* mite lineages with

Flightless Cormorants considering that they both harbor *O. sordida* . Alternatively, the bimodal distribution may be explained by repeated historical fragmentation of the cormorant *Myialges* populations due to bottlenecking (Rogers and Harpending, 1992), followed by secondary contact of the genetically differentiated and once isolated populations (Jolly et al., 2005). Lastly, this trend may also indicate long-term stability in the cormorant *Myialges* population size (Rogers and Harpending, 1992). Valle's (1995) analysis indicates that its host species has an extremely small, but demographically stable, population size of ~1,000 individuals. However, a population crash in 1982 was preceded by a severe El Niño Southern Oscillation Event, from which it rapidly recovered. Future sampling and sequencing of *Myialges* mites from Brown Pelicans will help distinguish between these scenarios.

Myialges mites within *Icosta nigra* fly populations collected from Galápagos Hawks were genetically differentiated between Islas Fernandina and Santiago, consistent with their host's pattern of restricted nuclear and mitochondrial gene flow between these two islands (Bollmer et al., 2005; Bollmer et al., in press). However, the pairwise F_{ST} values for Fernandina and Santiago were lower for the mites than the hawk hosts, a possible indication that parasite gene flow has occurred in the absence of host gene flow (Dybdahl and Lively, 1996). Although we have inferred that Galápagos hawks exhibit high natal philopatry (most island populations are highly differentiated genetically), we have previously observed movement of marked juvenile hawks from Isla Isabela to Isla Fernandina and inferred occasional inter-island movements of juvenile hawks using genetic markers (Bollmer et al. 2005; Bollmer et al., in press). Thus, parasite gene flow without host gene flow is plausible. However, the population size of *Myialges* within the

hawk population is likely much larger than the size of the hawk population (Bollmer et al., 2005). Thus the time to coalescence may be longer in the mites (despite their faster generation time and likely faster DNA substitution rate), which may also underlie their pattern of lower genetic differentiation (Nadler 1995). Conversely, the level of differentiation between hawk populations on Santiago and Fernandina is much lower at variable nuclear loci (minisatellites; $F_{ST} = 0.122$), which could either be due to recent separation of these populations combined with the longer coalescence time relative to mtDNA or recurrent gene flow between them.

Mites were significantly more prevalent among hawk flies than cormorant flies, and exhibited a pattern of distribution typical for that of most parasites (most hosts have no parasite individuals and few have many parasite individuals). Within hawk flies, prevalence of female mites varied across island populations, mirroring the isolation of most hawk populations. Among infected hawk flies (all island populations combined) prevalence also varied across the major fly body segments and within the main thoracic structures. Nearly 80% of infected hawk flies harbored mites on the thorax and within the thorax; the most common attachment size (based on prevalence) was the rear legs. Several possible explanations for the nonrandom *Myialges* distributions exist, including competition among mites for attachment sites (Hayashi and Ichiyangi, 2005) and differences in the host's integument among sites (McAloon and Durden, 2000). However, we are unable to determine the cause of the patterns found here.

The genetic and distributional data of *Myialges* within and between host species suggest that hippoboscid flies, which in this case are typically limited to Falconiformes (*I. nigra*) and Pelicaniformes (*O. sordida*) hosts, also limit maternal dispersal of *Myialges*

mites. Moreover, inter-island dispersal of hawk-associated *Myialges* is also limited, and concordant with that of their hawk hosts. Phoresis and other modes of inter-host dispersal over evolutionary time has been invoked to explain evolutionary patterns of many ectoparasites, including mites and lice (Clay 1949; Fain 1965; Johnson et al. 2001; Johnson et al. 2002; Weckstein 2004; Whiteman et al. 2004). Accordingly, Harmon and Madden (1998) observed that *Myiagles caulotoon*, which is phoretic or hyperparasitic on the louseflies *I. nigra* and *O. sordida* in the Galápagos Islands, do not serve as useful indicators of host phylogeny or ecology. In contrast, our results reveal cryptic host specificity in a *Myialges* mite between the two sympatric hippoboscid species associated with two sympatric and often syntopic avian host species from different avian orders. Vercammen-Grandjean (1966) first suggested that studies of mite host specificity may be particularly helpful in informing our understanding of host ecological and evolutionary history within taxa inhabiting the Galápagos Islands. Without Harmon and Madden's (1998) study, we would not have extensively collected flies from both of these hosts, which enabled, given the low prevalence on *Myialges* on cormorants, the genetic characterization of the mite populations from each host species. Their survey of parasitic mites in Galápagos hosts served as a springboard for further research, and together, these studies show how little is known generally with regard to parasite biodiversity (Whiteman and Parker, 2005). The DNA sequences presented in this paper are the first to have been published or analyzed from any member of the mite family Epidermoptidae, which further underscores this point.

When did these lineages of *Myialges* colonize the Galápagos Islands? One reasonable scenario is that the two cryptic *Myialges* lineages revealed in this study

diverged before the arrival of one or both of these hosts' ancestors in the Galápagos Islands. The Galápagos hawk's ancestors are likely to have only recently colonized the islands based on the lineage's relatively recent split (estimated to be <300,000 ybp, Bollmer et al. in press), from its common ancestor with the Swainson's hawk (*B. swainsoni*; Reising et al. 2003). Assuming that arthropod COI sequences diverge at a rate of roughly 2-3% per million years (Hebert et al., 2003), the hawk and cormorant *Myialges* lineages (which differ by ~16% COI sequence divergence) have clearly been separated for much longer than 300,000 years. However, divergence of these mites within the Galápagos Islands prior to the colonization of one or both hosts followed by transfer to these hosts cannot be excluded. Hebert et al. (2003) found that within Chelicerata (which includes Acari), the percent sequence divergence between congeneric species pairs for COI was 14.4% (±3.6 SDM) and most congeneric species within Chelicerata were 8-16% divergent followed by nearly half of congeneric species pairs with COI divergences of 16-32%. Given a relatively ancient separation from a common ancestor, it seems reasonable to predict that in addition to dispersal barriers, secondary isolating mechanisms via a coevolutionary arms race, are present between these *Myialges* lineages and their hosts (McCoy et al. 2002). *Myialges* mites feed on the host's (both avian and fly) body fluids and are thus subjected to two layers of host immune responses (Fain, 1965). Therefore, dispersal barriers and local adaptation to hosts may maintain the genetic isolation of these cryptic *Myialges* species.

Although the endemic avifauna of the Galápagos Islands remains relatively intact, the human population is rapidly expanding, causing concern that exotic wildlife diseases will be introduced into the archipelago, precipitating a Hawaii-like extinction

among the native vertebrates (Warner, 1968; Wikelski et al., 2004). Therefore, understanding parasite transmission dynamics within these island populations is essential to the overall goal of reducing the threat posed by invasive disease agents, but little information is presently available on dynamics of either invasive or native disease agents (Wikelski et al., 2004; Whiteman et al., 2004; Gottdenker et al., 2005; Thiel et al., 2005), despite the important role transmission mode plays in the evolution of virulence (Clayton and Tompkins, 1994). Understanding the transmission dynamics of *Myialges* mites is of wildlife health importance in this case, since epidermoptid mange causes significant mortality in island birds (Gilardi et al., 2001). Gilardi et al. (2001) speculated that *Myialges* mites were introduced to Laysan Albatrosses on Midway Atoll via hippoboscid flies or introduced birds. Similarly, Harmon and Madden (1998) suggested that *M. caulotoon* was transmitted between Flightless Cormorants and Galápagos Hawks, both species of conservation concern. We have shown that hawk and cormorants are epidemiologically isolated with respect to these skin mites, even though both birds are often syntopic. Similar results were obtained by Ochs et al. (2000) and Walton et al. (1999; 2004), who found that sympatric mammalian host species harbored putatively conspecific skin mite populations that were genetically distinct from each other. Clearly, basic information on parasite identity, host specificity and transmission among is essential for informed conservation and public policy management decisions, in addition to informing our understanding of parasite diversification

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Figure 1. Life cycle of *Myialges* mites (after Evans et al. 1963), which alternates between hippoboscid fly or louse hosts and avian hosts.

Figure 2. Scanning electron micrographs of a hippoboscid fly specimen (*Icosta nigra*) with female *Myialges* mites (with brood) attached to **(A)** the left wing (black arrow) and **(B)** the hind leg (white arrow indicating a developing mite in an egg laid by this female mite). The fly was collected from a Galápagos hawk (*Buteo galapagoensis*).

Figure 3. Frequency histogram of *Myialges* mite distribution among (**A**) 296 *Icosta nigra* lousefly individuals from seven island populations of Galápagos hawk hosts and (**B**) 105 *Olfersia sordida* lousefly individuals from two island populations of Flightless cormorant hosts. Mites were significantly more prevalent on hawk flies than on cormorant flies.

Figure 4. Tree from neighbor-joining (NJ) analysis of 658 bp of mitochondrial cytochrome oxidase *c* subunit I (COI) sequences from 8 female *Myialges* female mite individuals removed from *Olfersia sordida* louseflies associated with Flightless Cormorants (*Phalacrocorax harrisi*) and 28 female *Myialges* individuals removed from *Icosta nigra* louseflies associated with Galápagos hawks (*Buteo galapagoensis*). Homologous COI sequences from *Varroa destructor* (Accession Number X) a mesostigmatid mite and a *Neodermation* sp. (Neodermatdionidae) individual, which is a close relative of the Epidermoptidae, were included as outgroups in the analyses. A heuristic parsimony bootstrap analysis yielded a tree with a topology nearly identical to the NJ tree (all nodes common to both trees are indicated by black dots). Numbers on the tree represent bootstap support values for nodes, estimated using 10,000 replications for

the NJ tree and 1000 replications for the parsimony tree implemented in Paup* v.4.0b (values left to right: NJ values/parsimony values).

Figure 5. 95% statistical parsimony haplotype networks estimated in TCS for 988 bp of combined cytochrome oxidase *c* subunit I and 12S data from *Myialges* mites collected from **(A)** *Olfersia sordida* louseflies associated with Flightless Cormorants and **(B)** *Myialges* mites collected from *Icosta nigra* louseflies associated with Galápagos Hawks. Haplotype frequencies are listed by island population within each haplotype. Haplotypes drawn as squares were estimated as the most ancestral (most likely root) haplotype in TCS.

Figure 6. Mismatch distributions of the observed mtDNA haplotype variation in *Myialges* mites compared to the theoretical distribution representing rapid population expansion as implemented in Arlequin 2.0 for: (**A**) specimens collected from Galápagos hawk louseflies (*Icosta nigra*) on Isla Santiago; (**B**) specimens collected from Galápagos hawk louseflies (*Icosta nigra*) on Isla Fernandina; (**C**) specimens collected from Flightless cormorants collected from louseflies (*Olfersia sordida*) on Islas Fernandina and Isabela (data were combined to increase sample size and because there were no island-specific haplotypes in cormorant *Myialges*).

Table 1. Published reports of lousefly species host to the avian skin mite (Epidermoptidae) *Myialges caulotoon* Speiser (*sensu* Fain 1965) and the avian hosts from which the flies were collected.

Lousefly host (Hippoboscidae)	**Avian host**	**Locality**	**Reference**
Icosta ardeae (Macquart)	*Ardea purpurea* (Ciconiiformes, Ardeidae)	Tanganyika Territory (Kibonoto on Kilimandjaro and Tanga)	Bequaert 1953; Speiser 1907; Fain 1965
	Botaurus lentiginosus (Ciconiiformes, Ardeidae)	St. Paul, Minnesota, USA	Phillips and Fain 1991
Icosta chalcolampra (Speiser)	Unknown	Solomon Islands	Phillips and Fain 1991
Icosta duckei (Austen)	*Urotriorchis macrourus* (Falconiformes, Accipitridae)	Bantanga, Congo	Phillips and Fain 1991
Icosta nigra (Perty)	Unknown	Isla Santa Cruz, Galápagos Islands, Ecuador	Hill et al. 1967; Phillips and Fain 1991
	Buteo galapagoensis (Falconiformes, Accipitridae)	Isla Santiago (San Salvador), Galápagos Islands, Ecuador	Madden and Harmon 1998
	Unknown	Tuscon, Arizona, USA	Phillips and Fain 1991
Olfersia fossulata Macquart	Unknown	Peru	Phillips and Fain 1991
Olfersia fumipennis (Sahlberg)	*Pandion haliaetus* (Falconiformes. Accipitridae)	British Honduras	Phillips and Fain 1991
Olfersia sordida Bigot	*Phalacrocorax harrisi* (Pelicaniformes, Phalacrocoracidae) *Pelicanus occidentalis* (Pelicaniformes, Pelicanidae)	Isla Fernandina, Galápagos Islands, Ecuador	Madden and Harmon 1998
Olfersia spinifera (Leach)	*Fregata magnificens* (Pelicaniformes, Fregatidae)	Mathews, Jamaica	Phillips and Fain 1991
Ornithomyia remota Walcher	Unknown	Chile	Bequaert 1953

Table. 2. Comparisons of *Myialges* (Acari: Epidermoptidae) prevalences between island populations of lousefly hosts (louseflies were parasitizing Flightless Cormorants and Galápagos Hawk hosts and each species was analyzed separately) within the Galápagos Islands. Raw prevalence data (number of flies infected/total number sample) are followed by the percentage infected (with bootstrap 95% confidence intervals in parentheses). Mite prevalence differed significantly across hawk lousefly (*Icosta nigra*) island populations ($\chi^2 = 26.32$, df = 6, $P < 0.001$), but not between the two island populations of cormorant louseflies (*Olfersia sordida*; $\chi^2 = 0.14$, df = 1, $P > 0.05$).

Island Population	Prevalence of *Myiagles* ex. *Icosta nigra* from Galápagos hawks	Prevalence of *Myialges* ex. *Olfersia sordida* from Flightless cormorants
Fernandina	8/46 flies infected 17.4% (7.8–31.4%)	5/59 flies infected 8.5% (2.8–18.7%)
Isabela	2/20 flies infected 10% (1.2–31.7%)	2/20 flies infected 10% (1.2–31.7%)
Española	0/14 flies infected 0%	
Santa Fe	0/5 flies infected 0%	
Pinzon	2/18 flies infected 11.1% (1.4–34.7%)	
Pinta	0/46 flies infected 0 %	
Santiago	41/144 flies infected 28.5% (21.3–33.6%)	

Table 3. Comparisons of *Myiagles* (Acari: Epidermoptidae) loads between two lousefly (Diptera: Hippoboscidae) host species collected in association with two endemic Galápagos bird species. Data are from Madden and Harmon (1998) and the present study. Using the software package Quantitative Parasitology 2.1, Fisher's exact tests were used to compare prevalences, bootstrap t-tests were used to compare intensities and abundances, and Mood's test of medians was used to compare typical levels of mite infestation. Raw prevalence data (number of flies infected/total number sample) are followed by the percentage infected. Numbers in parenthesis are 95% bootstrap confidence intervals for each metric.

Metric	**Study**	***Myialges* ex. *Olfersia sordida* from Flightless cormorants**	***Myiagles* ex. *Icosta nigra* from Galápagos hawks**	***P***	***t***
Number of flies sampled	Present study	105	296	---	---
	Madden and Harmon	15	13	---	---
Mite prevalence	Present study	8/105 flies infected 7.6% (3.33–14.57%)	53/296 flies infected 17.9% (13.7–22.76%)	0.011*	---
	Madden and Harmon	1/15 flies infected 6.7% (0.16–31.95%)	7/13 flies infected 53.8% (25.13–80.78%)	0.011*	---
Mean mite abundance	Present study	0.08 (0.02–0.11%)	0.43 (0.24–0.61%)	0.025*	3.39
Mean mite intensity	Present study	1	2.396 (1.53–3.30%)	0.078	2.91
Median mite intensity	Present study	1	1 (1–2%)	0.018*	---

Table 4. Attachment site data of 127 female *Myialges* mite individuals attached to 53 *Icosta nigra* louseflies associated with Galápagos hawks (*Buteo galapagoensis*).

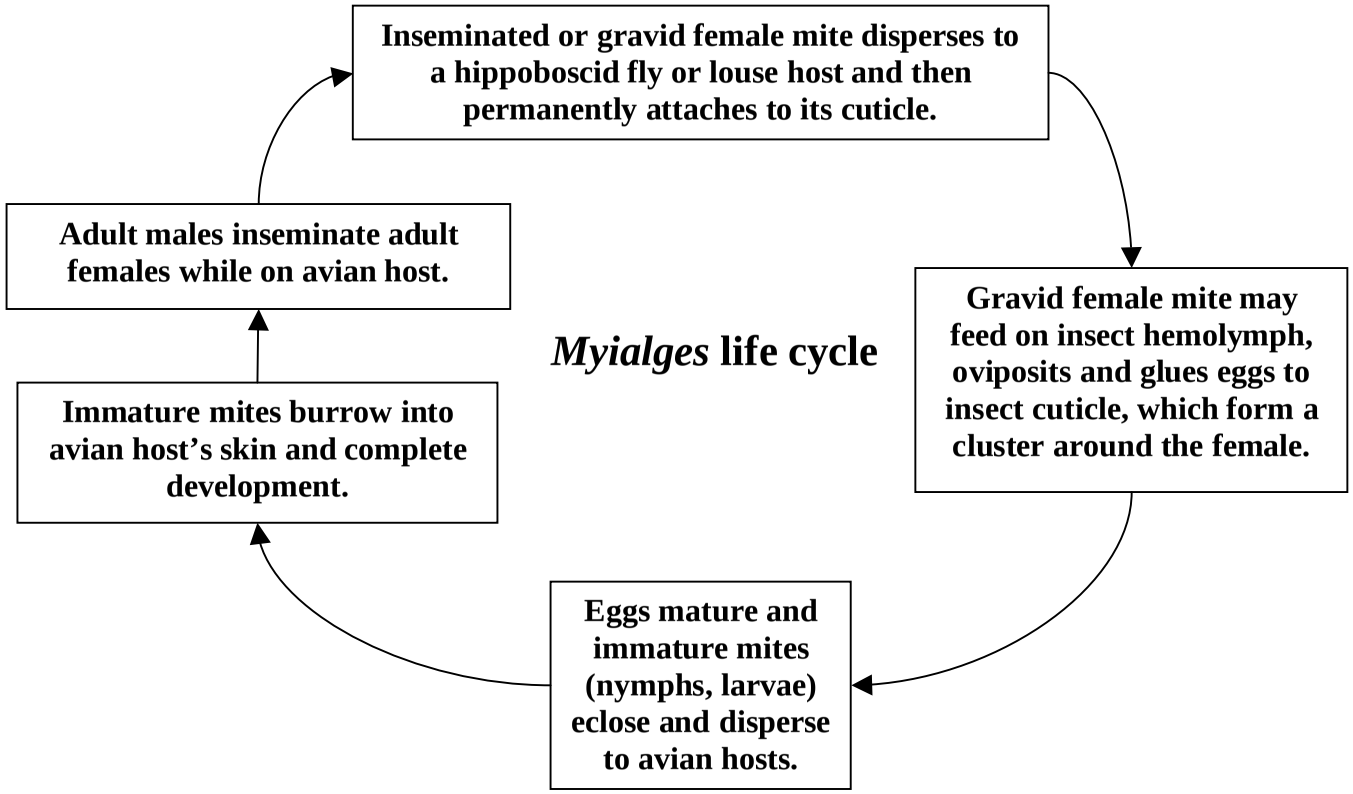
Mite attachment site	**Prevalence**	**Mean abundance**	**Mean Intensity**	**Median Intensity**
Head	9.4%	0.09	1	1
Thorax	79.2%	1.08	1.36	1
Abdomen	47.2%	1.23	2.6	1
P	< 0.001			
Within thorax only				
Main thorax	21.4%	0.24	1.11	1
wing	28.3%	0.28	1	1
legs I	7.5%	0.08	1	1
legs II	13.2%	0.17	1.29	1
legs III	32.1%	0.36	1.12	1
P	< 0.01			

Table 5 List of samples and polymorphic sites along the 988 bp combined COI and 12S mtDNA alignment within *Myialges* collected from *Icosta nigra* louseflies associated with Galápagos Hawks only. Sites are read vertically (e.g., the first variable site is at position 238).

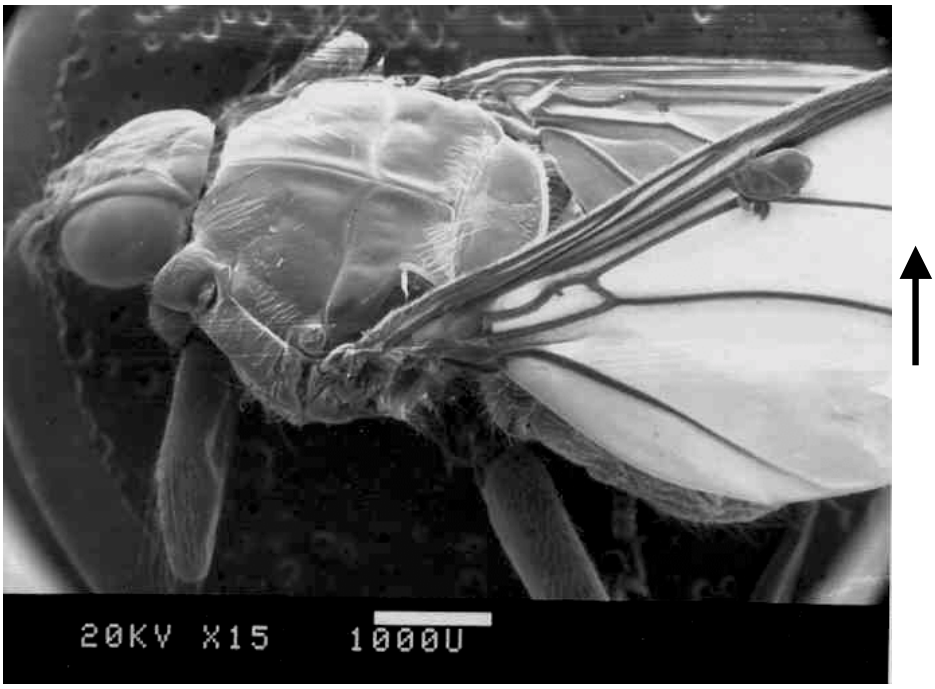
Accession (*Myialges* from *Icosta nigra* flies)	**238**	**304**	**338**	**482**	**556**	**631**	**737**	**834**
***Myialges* Hawk N85 Fernandina**	**A**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R5E A Fernandina**	**A**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R5E B Fernandina**	**A**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G3S Fernandina**	**A**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk N88 Fernandina**	**C**	**T**	**T**	**T**	**C**	**A**	**A**	**A**
***Myialges* Hawk NHI Fernandina**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**G**
***Myialges* Hawk NIE Fernandina**	**A**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R3U Fernandina**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk N93 Pinzon**	**C**	**C**	**C**	**T**	**C**	**G**	**A**	**A**
***Myialges* Hawk 170 Isabela**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk B4E A Isabela**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk B3Y B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk B4R Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R90 Santiago**	**C**	**T**	**C**	**T**	**C**	**G**	**G**	**A**
***Myialges* Hawk G16 A Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G16 B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G44 A Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G44 B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G84 Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk O15 A Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk OOM B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R86 A Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk B4K B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk N40 A Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk N40 B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk R3R B Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**
***Myialges* Hawk G49 Santiago**	**C**	**T**	**T**	**C**	**T**	**A**	**A**	**A**
***Myialges* Hawk G50 Santiago**	**C**	**T**	**T**	**C**	**C**	**A**	**A**	**A**

Table 6. List of samples and polymorphic sites along the 984 bp combined COI and 12S mtDNA alignment within *Myialges* collected from *Olfersia sordida* louseflies associated with Flightless Cormorants only. Sites are read vertically (e.g., the first variable site is at position 107).

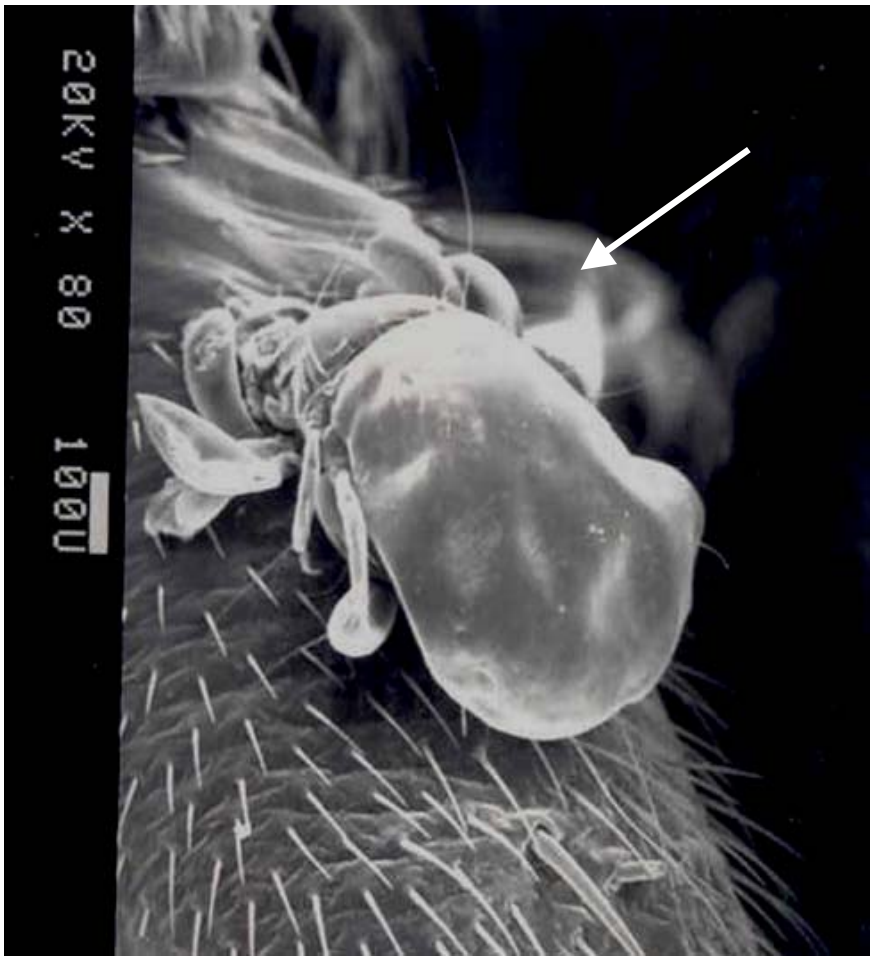
Accession (*Myialges* from *Olfersia sordida* flies)	**1 0 7**	**2 1 1**	**3 4 6**	**4 3 3**	**4 3 6**	**4 9 6**	**5 2 0**	**9 7 6**
***Myialges* Cormorant 922 Isabela**	**G**	**A**	**C**	**A**	**G**	**T**	**A**	**C**
***Myialges* Cormorant 836 Fernandina**	**G**	**A**	**C**	**A**	**G**	**T**	**A**	**C**
***Myialges* Cormorant 868 Fernandina**	**G**	**A**	**C**	**A**	**G**	**T**	**A**	**C**
***Myialges* Cormorant 884 Fernandina**	**A**	**G**	**T**	**G**	**A**	**C**	**G**	**T**
***Myialges* Cormorant 889 Fernandina**	**A**	**G**	**T**	**G**	**A**	**C**	**G**	**T**
***Myialges* Cormorant 890 Fernandina**	**A**	**G**	**T**	**G**	**A**	**C**	**G**	**T**
***Myialges* Cormorant 2094 Isabela**	**A**	**G**	**T**	**G**	**A**	**C**	**G**	**T**
***Myialges* Cormorant 655 Isabela**	**A**	**G**	**T**	**G**	**A**	**C**	**G**	**T**

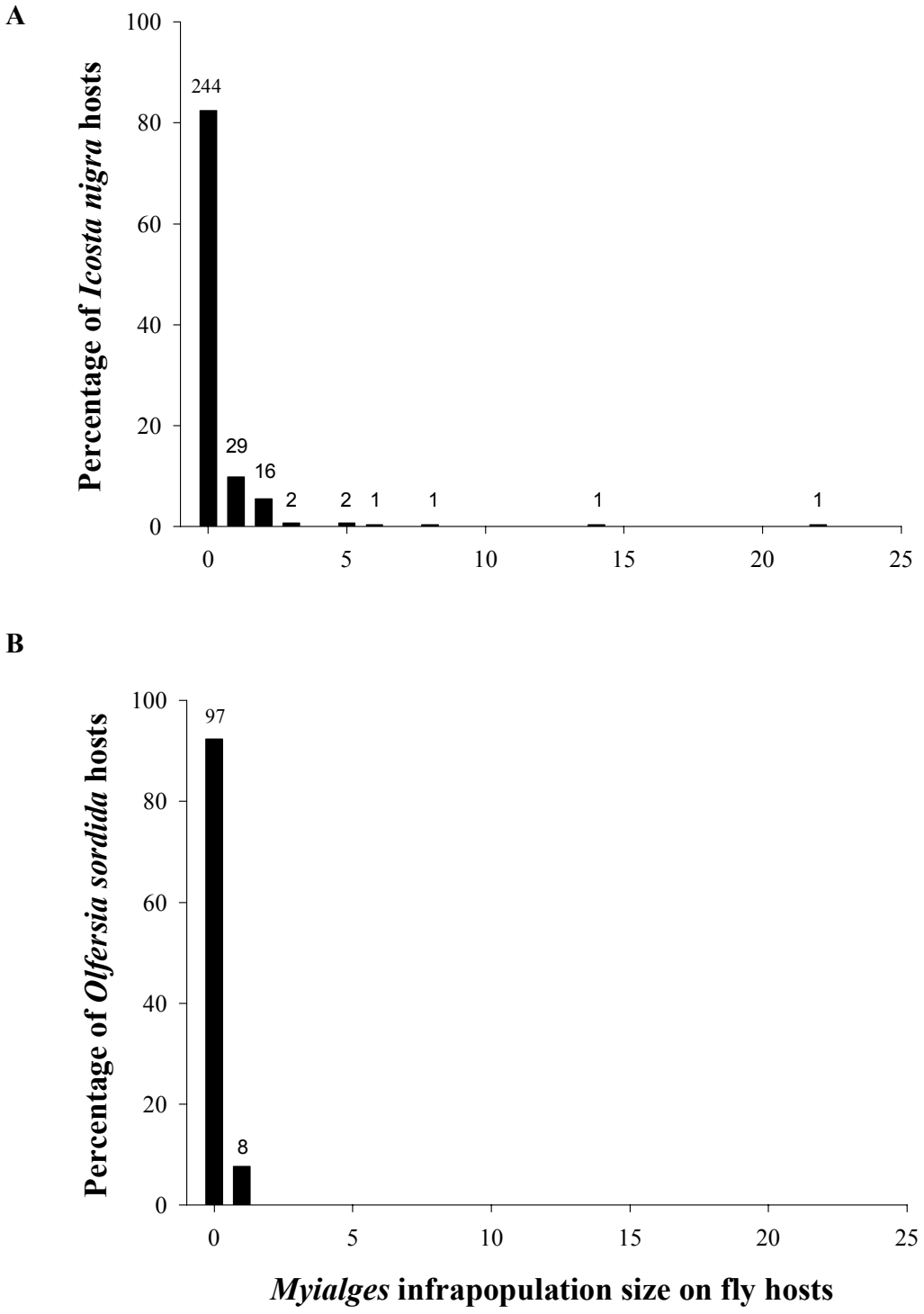

Inseminated or gravid female mite disperses to a hippoboscid fly or louse host and then permanently attaches to its cuticle.
Gravid female mite may feed on insect hemolymph, oviposits and glues eggs to insect cuticle, which form a cluster around the female.
Eggs mature and immature mites (nymphs, larvae) eclose and disperse to avian hosts.
Immature mites burrow into avian host's skin and complete development.
Adult males inseminate adult females while on avian host.
Myialges life cycle

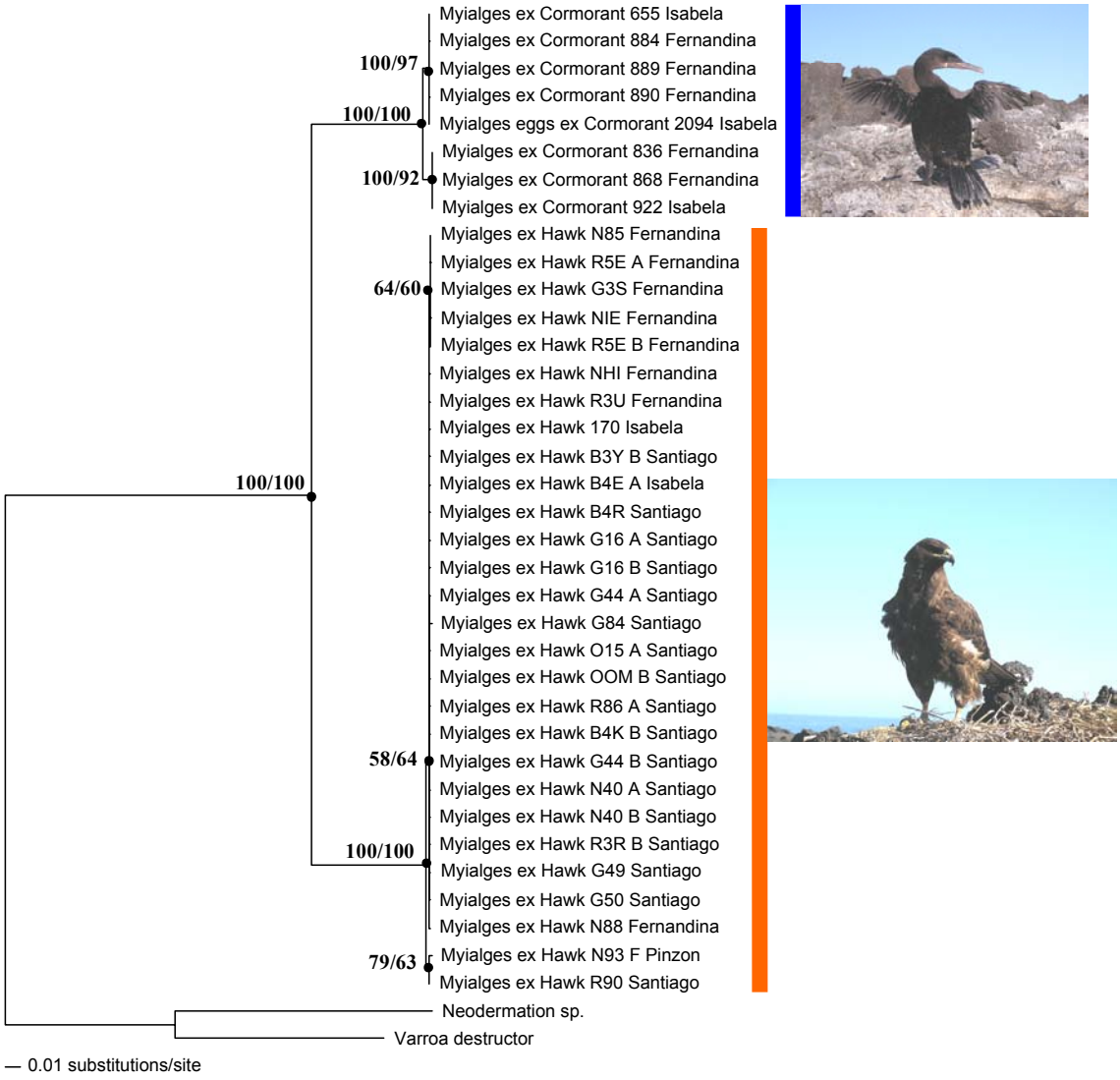
A



B




A
Percentage of *Icosta nigra* hosts
100
80
60
40
20
0
244
29
16
2
2
1
1
1
1
0
5
10
15
20
25
B
Percentage of *Olfersia sordida* hosts
100
80
60
40
20
0
97
8
0
5
10
15
20
25
Myialges infrapopulation size on fly hosts


Myialges ex Cormorant 655 Isabela
Myialges ex Cormorant 884 Fernandina
100/97
Myialges ex Cormorant 889 Fernandina
Myialges ex Cormorant 890 Fernandina
100/100
Myialges eggs ex Cormorant 2094 Isabela
Myialges ex Cormorant 836 Fernandina
100/92
Myialges ex Cormorant 868 Fernandina
Myialges ex Cormorant 922 Isabela
Myialges ex Hawk N85 Fernandina
Myialges ex Hawk R5E A Fernandina
64/60
Myialges ex Hawk G3S Fernandina
Myialges ex Hawk NIE Fernandina
Myialges ex Hawk R5E B Fernandina
Myialges ex Hawk NHI Fernandina
Myialges ex Hawk R3U Fernandina
Myialges ex Hawk 170 Isabela
Myialges ex Hawk B3Y B Santiago
100/100
Myialges ex Hawk B4E A Isabela
Myialges ex Hawk B4R Santiago
Myialges ex Hawk G16 A Santiago
Myialges ex Hawk G16 B Santiago
Myialges ex Hawk G44 A Santiago
Myialges ex Hawk G84 Santiago
Myialges ex Hawk O15 A Santiago
Myialges ex Hawk OOM B Santiago
Myialges ex Hawk R86 A Santiago
Myialges ex Hawk B4K B Santiago
58/64
Myialges ex Hawk G44 B Santiago
Myialges ex Hawk N40 A Santiago
Myialges ex Hawk N40 B Santiago
100/100
Myialges ex Hawk R3R B Santiago
Myialges ex Hawk G49 Santiago
Myialges ex Hawk G50 Santiago
Myialges ex Hawk N88 Fernandina
79/63
Myialges ex Hawk N93 F Pinzon
Myialges ex Hawk R90 Santiago
Neodermation sp.
Varroa destructor
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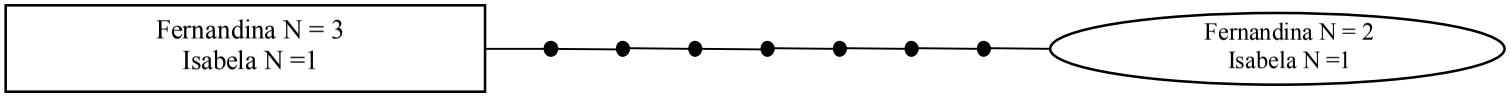
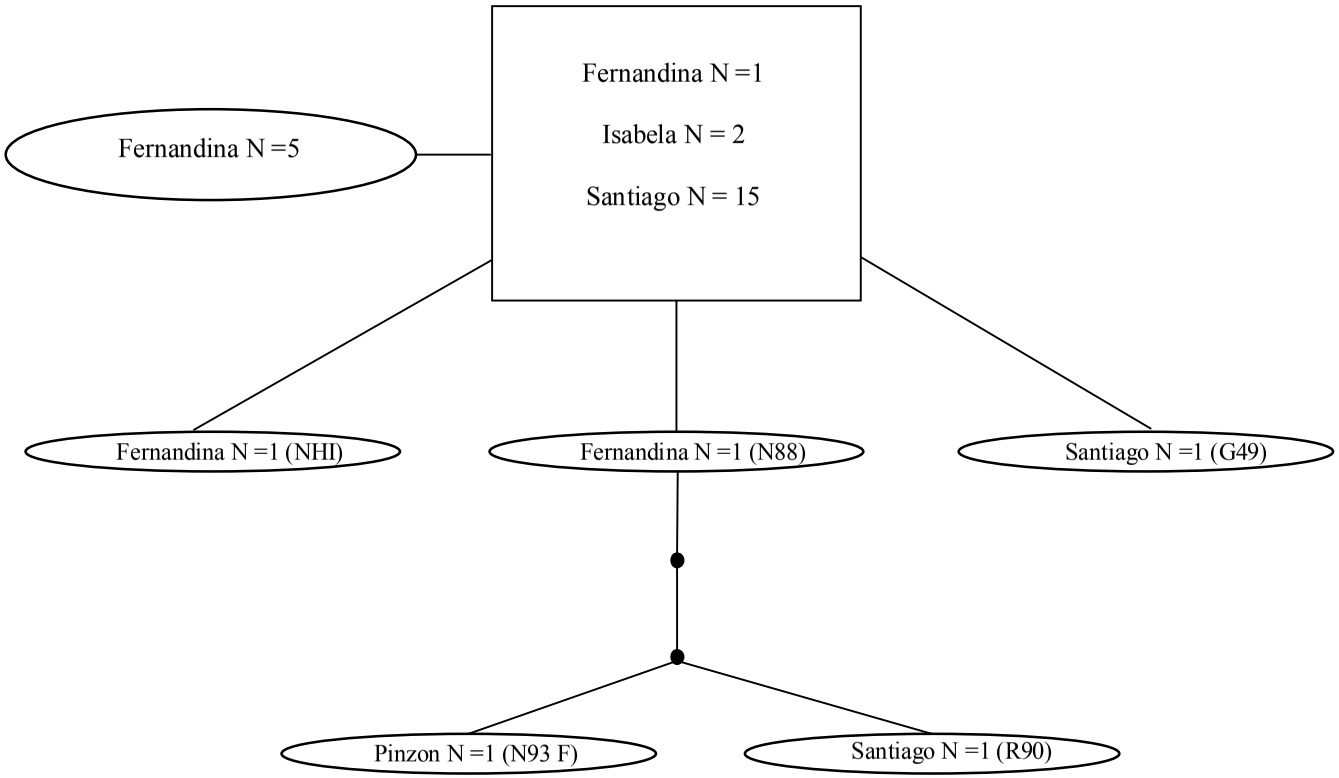
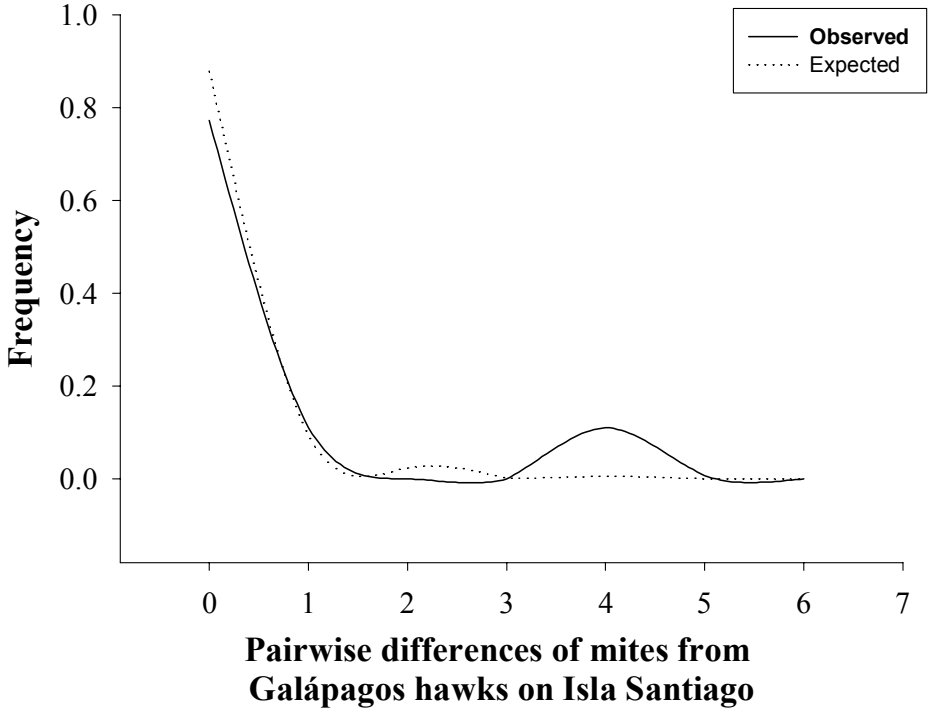


***Myialges* from Flightless Cormorants**

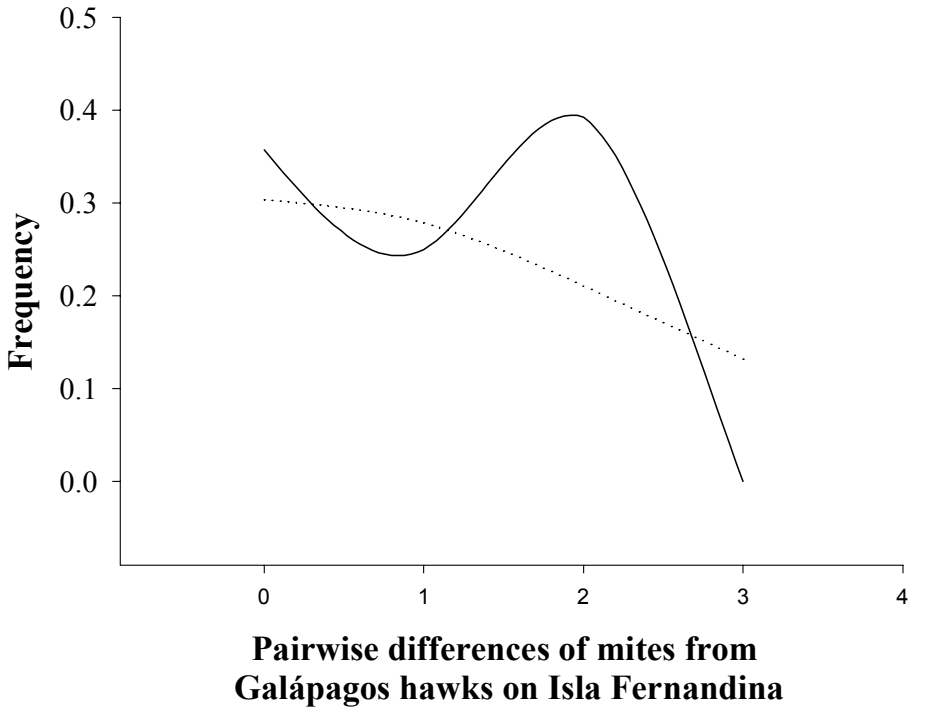
***Myialges* from Galápagos Hawks**



A


Observed
Expected
Frequency
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
Pairwise differences of mites from
Galápagos hawks on Isla Santiago

B


Frequency
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3
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Pairwise differences of mites from
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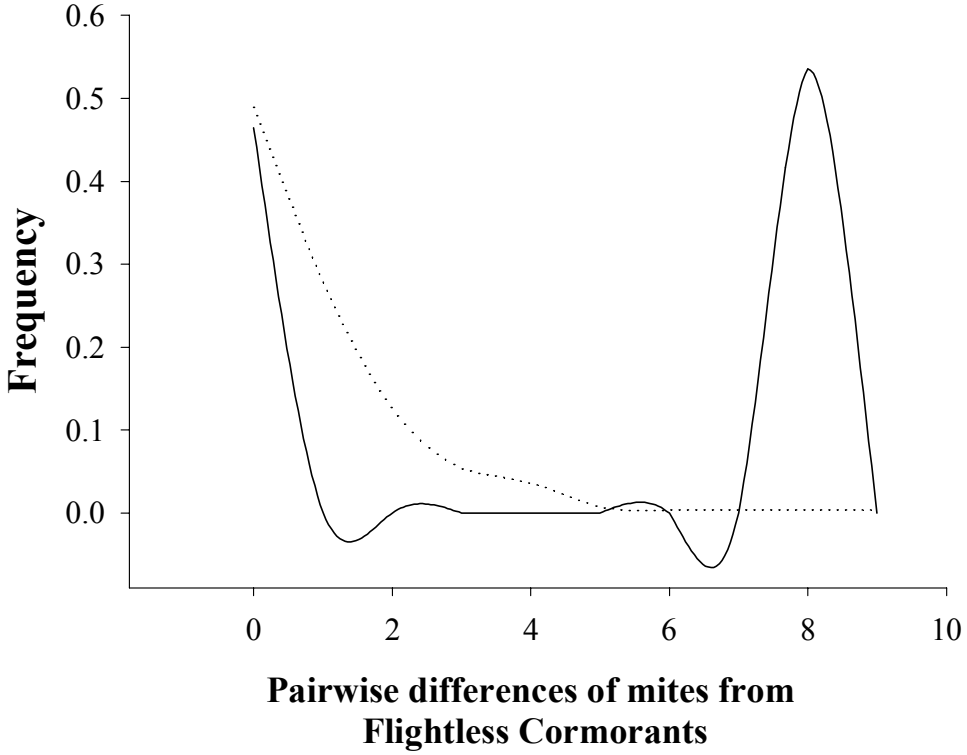
C


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Frequency
0
2
4
6
8
10
Pairwise differences of mites from
Flightless Cormorants

Chapter XIII.

Comparative Population Genetics and Phylogeography of a Model Host-Parasite System from the Galápagos Islands

Unpublished:

Whiteman, N.K. & P. G. Parker

ABSTRACT

Despite its importance as a natural evolutionary laboratory, no host-parasite microevolutionary studies have been reported from the ecologically simplified Galápagos Islands. More generally, very little is known regarding the microevolutionary processes underlying parasite diversification, despite their ecological importance and evolutionary success. We studied the comparative population genetic structure and phylogeography of three evolutionarily independent ectoparasite species with varying natural histories (ecological replicates) and that of their host, the threatened and endemic Galápagos Hawk (*Buteo galapagoensis*). Island populations of the host and each of the three parasite species were highly genetically differentiated. The host was invariant at one mitochondrial (mtDNA) locus that was variable in the three parasite species, to varying degrees, and was in turn consistent with differences in parasite natural history. Overall, ~1kb of parasite mtDNA was richer in haplotype diversity than ~3kb of host mtDNA. However, patterns of differentiation among the three parasite species differed considerably. One parasite (an ischnoceran feather louse) in which vertical transmission is likely the primary mode of dispersal, tracked host nuclear gene flow, and its mtDNA

was diversifying at rate >1.5 times that of the host's under an island model of speciation, while two more vagile and less host-specific ectoparasites (an amblyceran louse and hippoboscid fly) did not track the host's gene flow and therefore comparisons of evolutionary rate were not informative. The amblyceran and ischnoceran lice, however, both showed patterns of host race formation among islands (island-specific haplotypes fixed in most or all individuals), but the amblyceran's genealogy was not correlated with the host's genealogy, indicating that this species may undergo periodic local turnover due to tracking of host dispersal rather than gene flow *per se*. Overall level of population genetic diversity was directly related to population sizes of each species. These findings have implications for our understanding of local adaptation of parasites, antagonistic coevolution and disease ecology. Estimated genealogies revealed that the parasites' mtDNA was a more informative marker of host colonization history, recurrent gene flow and dispersal than that of the hosts. The high degree of cryptic diversity recovered in these parasites species underscores the utility of using parasites to infer host history and how little is known with respect to their evolutionary history and ecology.

Key words: Aves; Coevolution; Galápagos Islands; Hippoboscidae: Island Biogeography; Minisatellites; Mitochondrial DNA; parasite; Phthiraptera; phylogeography

INTRODUCTION

> The student of Mallophaga, in this aspect of his work, can be compared to the palaeontologist. He delves into the past, not by quarrying in the rocks for fragments of bones, but by studying the morphology and distribution of these living fossils. As he pieces together the story of their evolution, he likewise unfolds the story of the evolution of the birds (Rothschild and Clay 1952).

Although most lineages of life on Earth are parasitic (Price 1980), very little is known regarding the microevolutionary processes underlying their diversification (reviewed in Nadler 1995; Jarne and Théron 2001; Criscione and Blouin 2005a, b; Huyse et al. 2005; Whiteman and Parker 2005). General principles hypothesized to underpin parasite speciation, such as the importance of parasite and host natural history, geography, and population dynamics in mediating coevolutionary outcomes, remain largely untested (Clay 1949; Thompson 1994, 1999; Nadler 1995; Huyse et al. 2005; Morgan et al. 2005). This is surprising, considering the large number of studies that have examined host-parasite systems at macroevolutionary scales (Brooks 1979; Brooks and McLennan 1993; Page 2003) and the effects parasite have on human, wildlife and agricultural health. Many studies have approached the problem of parasite diversification by testing for congruence between host and parasite phylogenies (Page 2003). Although there is evidence to indicate that cospeciation has occurred between a large number of diverse taxa (e.g., Hafner et al. 1994; Clark et al. 2000), these patterns do not reveal the processes that led to them (Funk et al. 2000; Page 2003).

Uncovering the contemporary and historical factors driving parasite diversification also requires an understanding of host ecology through space and time (Rannala and Michalakis 2003; Huyse et al. 2005). Accordingly, findings from studies simultaneously examining microevolutionary patterns of parasites suggest that parasite

and host natural history, geographic distribution and population dynamics are key in mediating parasite microevolutionary processes, and therefore the coevolutionary process (e.g., reviewed in Criscione and Blouin 2005 a; Whiteman and Parker 2005; Nieberding et al. 2004; Kaliszewska et al. 2005; McCoy et al. 2005; Noël et al. 2005; Prugnolle et al. 2005; Wirth et al. 2005; Whiteman et al. in prep.).

Taxonomically and geographically limited microevolutionary studies of parasites are of the greatest utility for parasite microevolutionary investigations (Hafner et al. 2003; Whiteman et al. 2004). Similarly, Nadler (1995) suggested "studies of genetic structure in parasites may benefit from a comparative approach in which several species of parasites that co-occur in populations of a single host species are investigated simultaneously." Nadler (1995), Clayton et al. (2004), Huyse et al. (2005) proposed that parasite natural history factors should, in principle, shape parasite microevolution and, ultimately, macroevolution. However, few studies at the microevolutionary level exist that have examined multiple, phylogenetically independent parasite lineages on a single host species (Criscione and Blouin 2004, 2005 a). Thus, one goal of the present study was to examine how between-species variance in parasite natural history traits (e.g., population size, vagility, generation time) influences population genetic structure.

Study System, Conceptual Framework and Predictions

Simplified ecosystems hold great promise in informing the study of parasite evolution (Perkins 2001; Fallon et al. 2003). In particular, the Galápagos Islands have served as exceptional natural laboratories from which key evolutionary and ecological insights have emerged (e.g., Darwin 1859, 1909; Grant et al. 1976; Grant and Grant 2002) and is an ideal location in which to investigate host-parasite dynamics (Whiteman

et al. 2004, in press, in preparation). This volcanic island system, which has never been connected to a continental land mass, provides geographical opportunity for allopatric populations to evolve independently, eventually resulting in speciation (e.g., Grant 1986; Finston and Peck 1997). Although anthropogenic disturbance has devastated the ecological communities of many similar systems (e.g., Polynesia), the relatively intact Galápagos biota is the most undisturbed of any oceanic archipelago in the world (Tye et al. 2002). Due to its relatively young geological age, many organisms inhabiting the Galápagos remain in the process of speciation (Caccone et al. 2002; Bollmer et al. 2005, in press). Thus, it is a superb setting in which to dissect microevolutionary processes (Tye et al. 2002). However, anthropogenic threats jeopardize the evolutionary potential of the unique Galápagos populations (Wikelski et al. 2004), and basic studies of its flora and fauna should be prioritized in light of these (Grehan 2001). Surprisingly, no studies of parasite population genetics or phylogeography have been conducted in the Galápagos Islands.

Before the use of DNA sequence data were widely used to reconstruct evolutionary history, others have argued that parasites of Galápagos vertebrates could help inform the evolutionary histories of their hosts (Vercammen-Grandjean 1966; Ayala and Hutchings 1974). We revisited this idea in the context of the more rapid evolutionary rate exhibited by parasite DNA (Whiteman and Parker 2005). In addition to revealing information about parasite diversification, microevolutionary studies of parasites may reveal host historical ecology and genealogy (Reed et al. 2004; Wirth et al. 2005; Nieberding et al. 2005; Whiteman and Parker 2005). In particular, chewing lice (Phthiraptera) of vertebrates are particularly informative of host history at relatively deep

and shallow evolutionary timescales (Hafner and Nadler 1988, 1990; Barker et al. 1991a, b; Nadler et al. 1990; Hafner et al. 1994; Weckstein 2004; Whiteman et al. 2004). Thus, a second goal of the present study was to use parasite population genetics and phylogeographical patterns (Avise et al. 1987) to infer host movements and colonization history within the Galápagos Islands in a case where the host harbored extremely low genetic diversity, rendering recovery of its genealogy difficult (Bollmer et al. in press).

The study host was the Galápagos Hawk (*Buteo galapagoensis*), the only resident falconiform and top diurnal predator in the Galápagos terrestrial ecosystem (de Vries 1975). The Galápagos Hawk was first studied to characterize its unusual mating system, which varies from monogamy to cooperative polyandry, within and among island populations (de Vries 1975; Faaborg et al. 1995; Bollmer et al. 2003). All breeding groups on Isla Espanola are monogamous pairs, whereas all breeding groups from Isla Marchena and Pinta are polyandrous, while other islands, including Islas Fernandina, Santa Fe, and Santiago, have breeding groups of both types (Bollmer et al. 2003). Thus, a continuing goal of our research is to place the variable mating system of *B. galapagoensis* in a phylogenetic context, which has been difficult due to the extremely low level of DNA sequence variation in this species. The eight breeding populations of the Galápagos Hawk (*Buteo galapagoensis*) are genetically and morphologically distinct (Bollmer et al. 2003, 2005, in press) and the overall level of genetic structure within the archipelago is relatively high (F_{st} = 0.538) based on rapidly evolving nuclear multilocus minisatellites (VNTRs; Bollmer et al. 2005). The smallest island populations of hawks are the most genetically uniform of any wild bird species at these most rapidly evolving loci (Bollmer et al. 2005), but this uniformity is population-specific; these populations are

also highly differentiated from each other (nearly all pairwise inter-island F_{st} values were significantly greater than zero and island-specific alleles are fixed or nearly fixed among individuals within island populations; Bollmer et al. 2005). Genetic diversity of VNTRs is directly related to island area, which is directly related to hawk population size, and the degree of genetic differentiation between island populations increases with increasing geographic distance (isolation by distance). These VNTR data, however, are not phylogenetically informative and cannot be used with confidence to estimate genealogy. However, overall variation within these same populations of ~3 kb of mitochondrial DNA (mtDNA) is extremely low (Bollmer et al. in press), and only one haplotype is typically fixed in each population, resulting in either no genetic variance between island populations ($F_{st} = 0$) or complete differentiation ($F_{st} = 1$ and haplotypes typically differ between islands by one or two mutations), in nearly every inter-island comparison. Moreover, *B. galapagoensis* and its sister species *B. swainsoni* (Riesing et al. 2003) differ by only 0.42% sequence divergence across ~3 kb of mtDNA sequence. Island populations that share the same mtDNA haplotype are highly differentiated at VNTR loci, indicating that that genealogy of the hawks based on mtDNA may never be resolved due to an extreme bottleneck during the founder event, even though island populations are genetically isolated. Finally, hawks maintain all-purpose territories year-round, and group membership is highly stable over time (e.g., breeding adult birds banded in 1989 were observed in the same territories on Isla Santiago in 2004; de Vries 1975; Faaborg et al. 1995; Bollmer et al. 2003). All of these data suggest that: (1) gene flow between most island populations of *B. galapagoensis* is extremely low, and (2) the hawk is a

relatively recent arrival to the archipelago (likely separated from a common ancestor with *B. swainsoni* < 300,000 ybp; Bollmer et al. in press).

Interestingly, although *B. galapagoensis* gene flow is highly restricted among islands, we have directly (by population censuses of color-banded individuals) and indirectly (by assignment of multilocus VNTR and mitochondrial genotypes) observed rare inter-island movements of non-breeding hawks (Bollmer et al. 2005, in press). Thus, although most hawk populations are genetically isolated from each other, there exists a low rate of inter-island hawk dispersal potentially irrespective of gene flow. In this context, differences in the natural histories of multiple lineages of hawk parasites may be used to predict which parasites will track host gene flow and colonization history and which parasites will track host dispersal irrespective of host gene flow.

We examined three phylogenetically independent ectoparasite lineages of the Galápagos Hawk. This included two species from the paraphyletic chewing lice (Phthiraptera), *Colpocephalum turbinatum* (Amblycera: Menoponidae) and *Degeeriella regalis* (Ischnocera: Philopteridae), and a species of lousefly (Diptera: Pupipara: Hippoboscidae), *Icosta nigra*, all reported previously from *B. galagoensis* (Clay 1958; Price and Beer 1963; de Vries 1975).

Lice are soft-bodied, hemimetabolous, wingless, permanent ectoparasites of birds and mammals, and comprise the largest number of ectoparasitic insect species. As permanent ectoparasites, their entire life-cycle is completed on the host, and dispersal usually occurs via direct host-host bodily contact (Marshall 1981). The two louse suborders (Amblycera and Ischnocera) comprising the chewing lice are derived from different free-living ancestors within the Psocodea (Johnson et al. 2004). Accordingly,

species from each clade are typically highly divergent with respect to natural history (Marshall 1981; Whiteman and Parker 2004a). Amblycerans feed on most epidermal tissues and blood, and are generally less host-specific, less restricted to a particular region of the host's body, are influenced by host sociality and more vagile than feather- and dead skin-feeding ischnoceran lice (Marshall, 1981). Horizontal transmission may be a more important dispersal route in amblyceran lice than ischnoceran lice (Whiteman and Parker 2004a; cf. Keirans 1975). In light of these differences in their natural histories, Whiteman and Parker (2004a, b) showed that within Galápagos Hawks, the distribution of *C. turbinatum* and *D. regalis* corresponded to these basic differences in natural history (summarized in Table 1). These two louse species are at opposite ends of the host-range spectrum. The typical hosts of *D. regalis* are limited to *B. galapagoensis* and *B. swainsoni* in the New World (Clay 1958). This is in contrast to the typical hosts of *C. turbinatum* (although it likely comprises a species complex, Price and Beer, 1963), which include at least 35 host species within the Falconiformes and the rock dove (*Columba livia*) according to Price and Beer (1963), and 47 species in total according to Price et al. (2003). Although development time varies among louse species, typically about one month is required from egg to reproductive maturity (Marshall 1981).

The natural history of the lousefly *I. nigra* is less well known, but its host range is restricted to falconiform birds (Maa 1963). In an important distinction from lice in the context of this study, hippoboscid females have very low fecundity (estimated at 7 larvae/female *Ornithomyia fringillinia* over the entire reproductive life; Corbet 1956). The pupariation period of *O. fringillia* in the UK was an average of 303 days. In *I. nigra*, both sexes are winged, pairs of flies have been observed with regularity on individual

hawks and horizontal transfer has been observed to occur with regularity (nearly every time in which multiple *B. galapagoensis* individuals were handled at the same time) between hawk hosts (and to humans holding those hosts; N.K. Whiteman pers. obs.). This species is not a permanent parasite of the host, although adult flies may be highly specific to particular bird individuals (Corbet 1956).

Given the restricted taxonomic affinities of these three parasites to falconiform birds, and the absence of collection records of these species from other host species in the Galapagos (Bequaert 1933a,b; R. Palma personal communication), it is likely that all three parasites are typically restricted to *B. galapagoensis* in the Galapagos, although straggling may occur with enough regularity to be important from an evolutionary perspective in this system (Whiteman et al. 2004). Following the logic of Johnson and Clayton (2003), we therefore postulate that upon colonization of the islands, the ancestor of *B. galapagoensis* brought with it the ancestral forms of the extant parasite fauna (conspecific parasites of *B. galapagoensis* are also found on other *Buteo* species). Thus, given the significant degree of population genetic structure among island populations of the host, it is reasonable to predict that any shared ″biogeographic event (*sensu* Johnson and Clayton 2003),″ such as colonization of a novel island by the host, should cause co-cladogenesis amongst all parasite lineages accompanying the host.

We assumed that host range correlated positively with parasite dispersal abilities, consistent with other authors (e.g., Clayton and Johnson 2003; Johnson et al. 2003; Clayton et al. 2004) and that population size was directly related to overall degree of genetic variation. This and other general parasite life-history differences (Table 1) may

generate predictable differences in parasite population genetic parameters (Nadler 1995; Clayton et al. 2004; Huyse et al. 2005), which we have summarized in Table 2.

MATERIALS AND METHODS

Field methods

We quantitatively sampled lice from 200 Galápagos Hawk individuals across the entire eight island breeding range (Islas Española, Fernandina, Isabela, Marchena, Pinta, Pinzón, Santa Fe, and Santiago) from 2001-2003, within the Parque Nacional Galápagos, Ecuador. We also sampled lice from a juvenile hawk in captivity (rehabilitating from an injury) on Isla Santa Cruz, Galápagos, using the dust-ruffling technique (Walther and Clayton 1997) as is described specifically for these birds elsewhere (Whiteman and Parker 2004a, b; Whiteman et al. 2004). Hippoboscid flies were captured from birds being sampled for lice (some were immobilized by the insecticide used in the dust-ruffling) on nearby objects or humans during hawk handling (all flies captured were in contact with a hawk individual preceding capture). In an attempt to obtain parasites for outgroup analyses, we also collected ectoparasites (using the same methods) from Swainson's Hawks (*B. swainsoni*) in New Mexico (2002) and from an overwintering population in Córdoba, Argentina (2003). Within the Galápagos, sampling was associated with an ongoing avian disease survey involving the University of Missouri-St. Louis, the Saint Louis Zoo, Charles Darwin Research Station and Parque Nacional Galápagos. In all cases, avian subjects were live-captured, sampled and released unharmed.

DNA extraction

The Cruickshank et al. (2001) voucher method was used to extract DNA from individual lice and flies at the University of Missouri-St. Louis. For each extraction, an individual louse or fly was removed from a preservation vial containing 95% etOH (stored at -20 º C). In the case of lice, it was then dried on the benchtop in clean watchglasses for five minutes and the head was either removed with sterile jeweler's forceps or sliced laterally through the thorax with the beveled edge of a sterile needle tip. The two pieces were then individually transferred to 1.5 µL Eppdendorf tubes and extracted using the protocol below. In the case of the flies, two legs were removed from each individual fly, which were then dried in clean watchglasses as above and a sterile needle tip was used to slice the tibia and femur along the long axes in order to expose the muscle (rich in mtDNA). These legs were then placed in the same 1.5 µL Eppdendorf tube and then crushed with the end of a sterile pipette tip. For both lice and flies, the animal tissue extraction instructions for the DNAeasy Tissue Extraction Kit (Qiagen, Inc., Valencia, California, USA) were followed with these modifications: (1) samples were left in incubation at 55º C for two nights and (2) the final elution step consisted of only one 40 µL volume of warmed elution buffer (EB). Louse exoskeletons were retrieved from the 1.5 Eppdendorf tubes and are stored in the Parker Laboratory of Animal Molecular Ecology at the University of Missouri-St. Louis. Voucher specimens of unextracted lice have been deposited at the Illinois Natural History Survey Phthiraptera Collection, Urbana, Illinois (K. P. Johnson) and the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand (R. L. Palma). Voucher specimens of flies have been deposited in the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (B. J. Sinclair).

PCR, cleanup and DNA sequencing

The primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAACTTCAGGGTGACCAAAAAATCA-3') was used to PCR amplify a 658 bp fragment of the mitochondrial gene cytochrome oxidase *c* subunit I (COI; near the 5' end; Folmer et al. 1994). This particular locus has been used successfully as a DNA barcode to identify arthropod species (Hebert et al. 2003). Each PCR tube contained 47 µL of a PCR master mix comprised of the following components: 2.5 µL of PCR buffer (provided with DNA polymerase), 1.5 µL of BIOLASE Red DNA polymerase (Midwest Scientific, St. Louis, Missouri, USA), 3.9 µL of 25 mM $MgCl_2$ (provided with DNA polymerase), 3 µL of each primer (diluted to 100 µM), 1.6 µL of 100 µM DNTPs, 31.5 µL of sterile dH_2O. Three microlitres of template mite DNA was added from each individually extracted mite, yielding a final PCR volume of 50 µL. Negative controls (tubes with no template DNA) were included in each set of reactions. The PCR amplifications for COI were performed using the following conditions: An initial denaturing step at 94°C for 4 minutes, followed by 35 cycles beginning with a denaturation step at 94°C for 1 minute, an annealing step at 40°C for 1 minute, an extension step at 70°C for 1 minute, followed by a final extension step at 72°C for 7 minutes after the completion of the 35 cycles. Amplicon size was verified on 1-2% TBE agarose gels stained with ethidium bromide and visualized under UV light and then purified with QIAQuick PCR columns or agarose gel purified and then extracted using QIAQuick Gel Extraction Kit following the manufacturer's instructions (Qiagen Inc., Valencia, California, USA). Purified amplicons were then verified on an agarose gel following the above. Direct sequencing was performed on both strands of each amplicon

using the primers above with ABI PRISM® BigDye Terminator PCR cycling conditions (followed by an appropriate clean-up step according the manufacturer's instructions) and sequenced on Applied Biosystems 3730xl DNA Analyzers (Applied Biosystems Division, Foster City, CA) by Macrogen, Inc. (Seoul, Korea) or on an Applied Biosystems 377 DNA Analyzer at the University of Missouri-St. Louis. We also amplified a fragment of the 12S mitochondrial ribosomal RNA gene from the same samples using the primer pair 12SAI (5'-CTAGGATTAGA-TACCCTATT-3' and 12SBI (5'-AAGAGCGACGGGCGATG-3') published previously (Simon et al. 1994). Volumes of reagents and template DNA in PCRs as well as purification (using QIAQuick PCR columns only) were identical to the above. The PCR amplifications for 12S amplifications were performed using the following conditions: An initial denaturing at 94°C for 2 minutes, followed by 35 cycles beginning with a denaturation step at 94°C for 30 seconds, annealing at 46°C for 30 seconds, extension at 70°C for 30 seconds, followed by a final extension step at 72°C for 7 minutes after the completion of the 35 cycles. Sequencing was carried out as described above (using 12SAI and 12SBI primers for sequencing).

DNA sequence analyses

Raw sequence chromatograms of forward and reverse strands were assembled for each amplicon in Seqman II (DNASTAR, Inc., Madison, WI, USA), for each species (three separate alignments per locus). The entire length of each strand was evaluated by eye. Poor quality data and primer sequences were trimmed from both strands. Seqman II was used to assemble the contigs (consensus sequences) resulting from the double stranded sequences for each gene, which were aligned in Se-Al (Rambaut, 1996) or

ClustalX (Thompson et al., 1997). We then returned to the original chromatograms to ensure that variable sites were unambiguously assigned in each case; if any ambiguity existed within a sequence, that sequence was discarded from the alignment. Sequences have been deposited in Genbank under the accession numbers XXXXXX-XXXXXX. Alignments of both loci for each species have been deposited in TreeBASE (http://www.treebase.org/treebase/index.html).

The alignments (COI, 12S and combined) for each parasite species were analyzed in DNAsp (Rozsas and Rozsas 1999) in order to calculate standard population genetic parameters (Table 7), to deduce the amino acid sequences from the COI sequences, for calculation of overall (combined across islands) F_{st} values for each parasite species (using the method of Hudson et al. 1992) and *Nm* derived from Wright's island model (1951) and derived from $F_{st} = 1/(1+2Nm)$. Arlequin 2.01 (Schneider et al., 2000) was used to calculate pairwise inter-island F_{st} values from haplotype frequencies for each parasite species (using the combined 12S+COI alignment) and to determine if these values were significantly greater than zero. We also obtained population genetic data from the same island populations of the host as those sampled for the three parasite species, to compare parasite and host inter-island differentiation and rates of evolution. Importantly, the same 5' locus of COI was sequenced from the Galápagos hawks to estimate maternal host gene flow, along with an additional 2.5 kb of mtDNA data (Bollmer et al. in press). Host nuclear gene flow was estimated using multilocus minisatellites (VNTRs) described in detail elsewhere (Bollmer et al. 2005). We created a scatterplot of parasite (mtDNA) vs. host (mtDNA and nrVNTR) F_{st} values to show the relationship between host and parasite differentiation across the archipelago. To control for multiple comparisons, we used a

Mantel (1967) procedure in Arlequin to determine if there was a significant correlation between a matrix of pairwise inter-island F_{st} values from the host nuclear VNTR data and matrices of the mtDNA derived inter-island F_{st} values for each parasite species. Since host inter-island F_{st} values were typically either 0 or 1, we did not attempt to correlate these with the parasite F_{st} values. However, following Hafner and Nadler (1990), we regressed inter-island parasite mtDNA Kimura 2-parameter (K_2P) corrected genetic distance values (calculated in Arelquin) against the same inter-island pairwise comparisons of host mtDNA K_2P genetic distances, to compare relative rates of mtDNA evolution between the hosts and parasites (first in a bivariate fashion using SPSS and then to control for multiple comparisons of island populations, correlations between matrices were tested using Mantel tests in Arlequin). This also shows the overall level of mtDNA differentiation for parasites relative to the host. We chose to relate host and parasite inter-island mtDNA genetic distances rather than by haplotype divergence (e.g., Nieberding et al. 2004) even though some of the host island populations were identical and fixed for one common haplotype, because the more rapidly evolving nuclear minisatellite data suggest that nearly all hawk populations are genetically isolated from one another (Bollmer et al. 2005). We also used Arelquin to test for isolation by distance (Rousset 1997) in each parasite species by comparing a matrix of inter-island mtDNA F_{st} values to a matrix of inter-island distances (ln transformed).

Three statistical parsimony haplotype networks were constructed using the TCS 1.8 program (Clement et al., 2000) for the combined 12S+COI dataset from each parasite species to determine the extent of geographic structuring among island populations of each parasite species. We also reconstructed the intraspecific phylogenies of the two

louse species using neighbor-joining in Paup*. Sequences from putative parasite outgroups (*Colpocephalum* and *Degeeriella*) were included for each locus and the sequences were then re-aligned in Clustal X as described above. Specifically, outgroup sequences from *Degeeriella* specimens collected from *B. swainsoni* and, in the likelihood analysis, from *Accipiter cirrocephalus*. This alignment (due to the large number of in-dels with the inclusion of *A. cirrocephalus*) consisted of 1145 characters, 731 of which were constant, 390 were parsimony uninformative, 24 were parsimony informative and gaps were treated as fifth bases. For *Colpocephalum*, specimens collected from *B. swainsoni* were included in the neighborjoining analyses. Since little variation was found within the *I. nigra* sequences, we did not subject the alignments to phylogenetic analysis and we were not able to obtain any *Icosta* specimens for inclusion as outgroups (none were observed on *B. swainsoni* in New Mexico or South America, and numerous attempts at obtaining museum specimens were not successful due to the rarity of louseflies in entomology collections).

RESULTS

Parasite collections and distributions within the host population

We collected a total of 14,843 individuals of the louse *C. turbinatum* and 2,858 individuals of the louse *D. regalis* from 199 Galápagos hawks sampled for lice across its 8 breeding populations (the single Santa Cruz bird sampled for lice was not included in the total number sampled or in the distributional analyses because it was sampled while in captivity. We collected 296 *I. nigra* individuals from 7 hawk populations (no flies were recovered on hosts from Isla Marchena despite sampling from 26 birds). We also found lice of both species from two nestling (near fledging-age) hawks on Isla

Fernandina, confirming that both louse species are likely vertically transmitted (both individuals were still being provisioned by their parents and were in a nest in the middle of an aa lava field). Only *C. turbinatum* was only found on a smaller nestling sampled on Isla Santiago, suggesting that *D. regalis* may require more mature plumage before it colonizes a host. Lice from Galápagos doves (*Zenaida galapagoensis*) including *Columbicola macrourae* and *Physconelloides galapagensis* and a louse from a goat (*Bovicola* sp.), which do not typically occur on hawks, were also collected from hawks (presumed to be stragglers from prey to hawks during predation events; Whiteman et al. 2004), but at such a low prevalence and abundance that they were not considered in this analysis. The infection intensities from each island population are shown Fig. 1 and for the entire sampling effort (all island populations pooled) in Table 1. Notably, *C. turbinatum* was significantly more prevalent, abundant, had higher infection intensities and was more evenly distributed within the hawks than *D. regalis* ($P < 0.001$ in every case; tests performed in Quantitative Parasitology 2.0). *C. turbinatum* was > five times more abundant on hawks than *D. regalis*. Since *I. nigra* specimens routinely were observed on more than one host prior to capture, we only present abundance data (sum of flies collected/sum of hawks sampled across all 7 populations). Nonetheless, the fly was highly prevalent within nearly all hawk populations (with the exception of Marchena) and infection intensities were low relative to the lice; most hosts harbored only one or two fly individuals.

Population Genetic Data

Sequences from both regions of the mitochondrion (COI and 12S) were obtained from each of 111 *D. regalis* individuals collected from 111 different *B. galapagoensis*

individuals (8 island populations + 1 accession from the juvenile Santa Cruz bird), 127 *C. turbinatum* individuals collected from 127 *B. galapagoensis* individuals (8 populations) and 117 *I. nigra* individuals (7 populations) (see haplotype networks for population-specific sample sizes). Sequences from 118 *B. galapagoensis* (analyzed in Bollmer et al. 2005) and haplotypes are available under GenBank accession numbers XXXXXXXX-XXXXXXXXX.

Populations of each of the three parasite species were significantly genetically differentiated across island populations of the Galápagos Hawk (Fig. 2), which was also significantly differentiated at both nuclear (Bollmer et al. 2005) and mitochondrial loci (Fig. 2). Using the 911 bp of COI and CR that varied among hawk populations, the differentiation among 9 island population was significant ($F_{st} = 0.73$; $\chi^2 = 360.15$, $P < 0.0001$), and 21/28 inter-island F_{st} comparisons were significant (Table 3). For the parasites, , *D. regalis* was most differentiated ($F_{st} = 0.895$; $\chi^2 = 513.59$, $P < 0.0001$), followed by *C. turbinatum* ($F_{st} = 0.70$; $\chi^2 = 476.04$, $P < 0.0001$), and *I. nigra* ($F_{st} = 0.65$; $\chi^2 = 109.60$, $P < 0.0001$). Of 28 inter-island pairwise comparisons of *D. regalis* F_{st} values, 25 were significantly differentiated (89.3%), while 22/28 comparisons of *C. turbinatum* F_{st} values (78.6%) and 12/21 comparisons (57.1%) of *I. nigra* F_{st} values were significantly greater than zero (Tables 4-6). Similarly, average pair-wise inter-island K_2P genetic distances of mtDNA were highest in *D. regalis*, followed by *C. turbinatum* and *D. regalis* (Table 7).

A significant and positive correlation was found only between *D. regalis* inter-island pairwise mtDNA F_{st} values and the host's nuclear multilocus minisatellite F_{st} values ($R = 0.64$; $P < 0.01$; 1000 Mantel permutations). No significant relationship was

found for *C. turbinatum* ($R = 0.115$; $P = 0.354$; Mantel 1000 permutations) or *I. nigra* ($R = -0.093$; $P = 0.675$), and host nuclear differentiation, although there was a slight positive relationship between the two variables in each (Figs. 3 A, B, C). We then determined if there was a pattern of isolation by distance (Figs. 4 A, B, C), which was the case for *D. regalis* ($R = 0.19$; $P < 0.01$; 1000 Mantel permutations) and less strong, but nonetheless significant, for *I. nigra* ($R = 0.11$; $P < 0.05$; 1000 permutations). No significant pattern of isolation by distance was found for *C. turbinatum* ($R = 0.19$; $P = 0.195$).

At the homologous 5' COI locus sequenced in all three parasites and the host, the host was invariant across the archipelago and only the host's variable mtDNA regions (3'COI and CR) were used in the analysis comparing rates of mtDNA evolution between hosts and parasites (although the invariance at this locus in the host, but not the parasites, is itself illustrative). Only *D. regalis* inter-island population K_2P genetic distances were positively related to those of the host's mtDNA genetic distances (Fig. 5A). The slope of the regression line was significantly positive and greater than one (bivariate: $\beta = 1.50$, $P < 0.00001$; Mantel: $\beta = 1.54$, $P < 0.05$) while the intercept was not significantly different from zero (bivariate: $\beta = 0.424$, $P > 0.05$). There was no relationship between the relative rates of mtDNA between the other two parasite species and that of the host (Fig. 5 B, C; $P > 0.05$ for bivariate and Mantel test).

Table 7 summarizes the basic population genetic parameters elucidated from the DNA sequences. The pattern of genetic variation follows the overall F_{st} patterns. *Degeeriella regalis* sequences were the most variable at both 12S and COI, followed by *C. turbinatum* and *I. nigra*. Interestingly, however, the total number of polymorphic

(segregating) sites (in the combined 12S + COI dataset) was very similar in the two louse species, and very low overall in the lousefly species. Also notable is the fact that there was a smaller number of haplotypes at 12S than at COI in *D. regalis*, but this pattern was reversed for the other two parasite species (only slightly so in the case of *C. turbinatum*). The host did not harbor any genetic variability within or across island population at the COI locus sequenced in the parasites. Although only 22 Galápagos Hawk host individuals were sequenced at this locus (from nine island populations), an additional ~2.5 kb of mtDNA sequences from an additional 129 individuals from the same populations (Bollmer et al. 2005) revealed low variability in the control region and the 3' end of COI, which resulted in a high level of differentiation between some populations even though overall variation was extremely low ($F_{st} = 0.80$; $\chi^2 = 360.15$, $P < 0.0001$). Overall, ~3kb of mtDNA from the host yielded fewer polymorphic sites, haplotypes and haplotype diversity than from ~1 kb of two of the three parasite species.

A high degree of population subdivision correlated with geography is apparent in *D. regalis* and *C. turbinatum* mtDNA networks (Fig. 6 B, C) and phylogenetic trees (Fig. 7 A, B) relative to the host (Fig. 6A) and *I. nigra* (Fig. 6 D). Variation within *I. nigra* sequences was very low, but nonetheless indicates that many island populations are genetically isolated from each other, a pattern similar to host's. Notably, both *D. regalis* and *C. turbinatum* populations from Marchena and Santa Fe hawk populations were each completely genetically differentiated or nearly so (see below) from the other populations. The Española population was the most differentiated from the rest of the *D. regalis* island populations, which is also the case for the host's haplotype network (Bollmer et al. in press). Moreover, the five most inbred and smallest island populations of hawks that

were significantly differentiated at minisatellite loci also harbored highly differentiated and unique *D. regalis* mtDNA haplogroups, which, in the context of the significant *D. regalis* mtDNA vs. *B. galapagoensis* nrDNA F_{st} inter-island comparison, supports the conclusion that *D. regalis'* genealogy is directly tied to *B. galapagoensis* genealogy. Nonetheless, all three parasite species harbored within-island private alleles, with *D. regalis* harboring the most (16 alleles in all 8 island populations), followed by *C. turbinatum* (12 alleles in 5 island populations), *I. nigra* (3 in 3 island populations) and the host harbored 4 private alleles in 3 island populations.

The phylogenetic reconstructions of *D. regalis* and *C. turbinatum* (Fig. 7 A, B) are typified by the presence of short branches, typical of most Galápagoan taxa (Tye et al. 2002). However, the inclusion of the outgroups in these analyses reinforces the interpretation that for *D. regalis*, the Española population is sister to the rest and perhaps first to have branched off from the common Galápagoan *D. regalis* ancestor (if indeed this is a monophyletic lineage). The other major clusterings include a Pinta+(Marchena+Santa Fe) clade, a Pinzón-predominating clade derived from the F+I+S lineage. Within *C. turbinatum*, the Marchena population is the most divergent from the other island populations, and is sister to the rest of the Galápagoan haplotypes. The Santa Fe haplogroup, is differentiated from the other populations. There is also a *C. turbinatum* clade within Fernandina that appears to be unique to that island.

DISCUSSION

In this study, we recovered a significant amount of population genetic structure and phylogeographic signal in three unrelated ectoparasites collected from the entire breeding range of the Galápagos Hawk. As predicted, the degree of population genetic

structure and relative rate of co-divergence with the host varied for each parasite species in ways that were predictable in the context of each parasite's natural history (Marshall 1981; Nadler 1995; Huyse et al. 2005). Seventeen mtDNA lineages of *D. regalis*, 16 of *C. turbinatum*, 5 of *I. nigra* and 7 of *B. galapagoensis* were recovered, many of which where private with respect to an island population. *Degeeriella regalis* diversification was significantly correlated with the diversification of its host, while that of *C. turbinatum* and *I. nigra* was not. Although all species were highly differentiated among islands in the archipelago (in particular *D. regalis* and C. *turbinatum*), the pattern of diversification was different among the lineages. This illuminates the importance of both association by descent (Brooks 1979; Page 2003) in the case of *D. regalis*, and association by colonization, in *C. turbinatum*, and perhaps *I. nigra*, in underpinning macroevolutionary patterns of parasite evolution (Hoberg et al. 1997).

The *D. regalis* haplotype network indicates that the smallest and most inbred hawk populations also harbored highly differentiated *D. regalis* populations. The Española population is the most differentiated from the rest of the *D. regalis* island populations, which is also the case for the host's haplotype network (Bollmer et al. in press). In the *D. regalis* neigborjoining phylogeny, the Española population was sister to a clade that contained the rest of the Galapágoan sequences and two sequences of *D. regalis* from *B. swainsoni*. Thus, its sister relationship with the rest of the Galápagos *D. regalis* sequences (and several *B. swainsoni*-derived *D. regalis* sequences) suggests that this population diverged first from the rest of the haplotypes, during the hawk's colonization history, and has remained isolated. There appears to have been a rapid radiation among the rest of the *D. regalis* clades from a common ancestor, followed by

some secondary contact of once isolated haplotypes (in light of the above population genetic data). This is in accord with a pattern across Galápagoan endemics, of older lineages inhabiting geologically older islands (Rassmann et al. 1997; Sequeira et al. 2000; Beheregaray et al. 2004; Kizirian et al. 2004). Interestingly, breeding groups of hawks inhabiting Española are entirely monogamous, which is also the case for *B. swainsoni*, the sister species. In addition to the Española population, *D. regalis* inhabiting hawks on Santa Fe are completely differentiated from other *D. regalis* island populations. The Santa Fe haplogroup is derived from the haplotype fixed in the Marchena *D. regalis* population (both islands are situated in the eastern part of the archipelago). Interestingly, one *D. regalis* individual from a territorial male hawk on Isla Santiago matched exactly the Marchena haplotype, indicating either an ancestral polymorphism remains in the Santiago *D. regalis* population, or that a hawk dispersed from Marchena to Santiago, resulting in *D. regalis* gene flow in the very recent past. Similarly, the *D. regalis* population from Pinta formed a unique haplogroup, but one *D. regalis* individual was an intermediate haplotype between the Pinta and the most common Fernandina, Isabela, and Santiago (F+I+S) haplotype. The Pinta and Marchena+Santa Fe haplogroups are very closely related to each other (all hawks from these islands share the same haplotype; Bollmer et al. in press). The *D. regalis* population on Pinzón was fixed for a haplotype that was rare on Santiago hawks, and also present in one juvenile bird from Santa Cruz. That Santa Cruz bird's haplotype was one mutational step away from the haplotype fixed in all of the Pinzón birds and rare in the Isabela hawk population (Bollmer et al. in press). Together this evidence may indicate that the *D. regalis* (and thus the hawk) on the Santa Cruz bird was derived from the Pinzón population given that breeding hawks are unlikely

to occur on Santa Cruz. Nonetheless, this *D. regalis* haplotype is very closely related (one mutational step) from the common F+I+S *D. regalis* haplotype indicating that this haplotype, as a tip, is derived from the F+I+S haplotype. The common F+I+S haplotype was present among the three largest hawk island populations within the archipelago, which are also relatively close to each other geographically. The pairwise inter-island *D. regalis* F_{st} values were not significantly greater than zero among these islands. The fact that the Santiago *D. regalis* population was not differentiated from Fernandina and Isabela is surprising considering that hawks from Santiago are completely genetically differentiated at mtDNA. However, Fernandina and Isabela hawk populations were not significantly differentiated based on the nuclear markers and F_{st} values between those two populations and the Santiago hawk population were the next lowest of all inter-island pairwise comparisons (Bollmer et al. 2005), suggesting that nuclear (male) gene flow may occur irrespective of mitochondrial (female) gene flow. Alternatively, because all three hawk populations are large and contain the largest VNTR variation, the coalescence process is incomplete even though gene flow is negligible among these islands. The *Degeeriella* data suggest that gene flow may be restricted between all three islands (though not significantly so). Isabela is sandwiched between Santiago and Fernandina. Thus, if gene flow of *D. regalis* is restricted in the context of these three islands, an expectation is that given the overall pattern of isolation by distance exhibited by this species, pairwise F_{st} values between Isabela and Fernandina and between Isabela and Santiago would each be lower (each is ~0.01 for *D. regalis*) than that between Santiago and Fernandina (~0.06), which is the case for both *D. regalis* mtDNA and *B. galapagoensis* nuclear DNA.

The *C. turbinatum* network and phylogeny indicates that the *B. swainsoni*-derived *C. turbinatum* and *B. galapagoensis*-derived *C. turbinatum* are each monophyletic although our sampling of this species (often used as an example of the higher limits of host range in lice; Marshall 1981) is very limited. Within the *B. galapagoensis*-derived *C. turbinatum*, the Marchena haplotype is sister to the rest of the Galápagoan haplotypes. Two main clades are nested within this clade, including a clade of *C. turbinatum* found only from Pinta+Fernandina, which also contained the monophyletic Santa Fe clade. The other main clade consisted of haplotypes from Española, Fernandina Isabela, Pinzón, and Santiago. Barker et al. (1991a, b) also found significant population subdivision in the wallaby louse *Heterodoxus* which occurs on rock wallabies whose populations are geographically isolated. Thus, despite their very different natural histories from ischnocerans in many cases, there appears to be an effect of geography on the population genetic structure of amblyceran lice as well, although the present study suggests that this is not significantly related to host gene flow or a simple model of isolation by distance. The larger population size of *C. turbinatum* relative to *D. regalis* may significantly increase the amount of time to coalescence even though the latter may be tracking host gene flow (Rannala and Michalakis 2003). The low host specificity of many amblycerans (Clay, 1949) is one aspect of their natural history correlating with their low species diversity relative to ischnoceran lice. However, this and Barker et al.'s (1991a, b) studies suggest that cryptic diversity is present within the Amblycera defined using morphological characters. Moreover, Page et al. (1998) found significant cospeciation between *Dennyus* lice (within the same family as *C. turbinatum*) and their swiftlet hosts,

underscoring that generalizations regarding the factors underlying parasite diversification should be made with caution.

The *I. nigra* population within Galápagos appears to have undergone a bottleneck on the same order as that of the host, based on low mtDNA diversities in both species. Despite this low variation in *I. nigra*, there existed significant differentiation among islands, with a pattern of isolation by distance (though weak). Although this is the first study of hippoboscid population genetics, an almost identical pattern of low overall diversity and high differentiation among populations was observed in the well-studied tsetse fly (*Glossina pallidipes*) in Africa, which is phylogenetically closely related (in the monophyletic Hippoboscoidea; Nirmala et al. 2001) and ecologically similar to hippoboscid flies (e.g., hematophagous, winged flies with low fecundity and adenotrophic viviparity; females average ~2 offspring over the entire lifespan within wild populations of *Glossina*; Gooding and Krafsur 2005). This species underwent a severe population bottleneck, based on mtDNA and microsatellite data, exhibited pattern of isolation by distance and, surprisingly, significant differentiation among populations (Krafsur 2002, 2003). Thus, despite being highly potentially volant, low abundances may correlate with low effective population sizes to a point where genetic drift becomes a major microevolutionary force. Indeed, a population crash in the natural hosts of *Glossina* associated with a Rinderpest outbreak beginning in 1887 is consistent with the pattern of low genetic diversity in the southern African population of *G. morsitans centralis* and *G. pallipides* (Gooding and Krafsur 2005). Moreover, tsetse flies, despite being fully winged, typically take short flights (~5 minutes in length) and fly for ~20 minutes per day, and are thus capable range expansion in the African savannah at a rate

of 7 km/ year (Gooding and Krafsur 2005). A similar scenario may have taken place in the *I. nigra* populations studied here, given that a very small number of host individuals were likely founded the ancestral *B. galapagoensis* population (based on nr and mtDNA genetic variance).

Understanding parasite diversification at microevoultionary scales is of practical importance given the threat posed by emerging infectious diseases in the context of an increasingly anthropogenically disturbed biosphere. Understanding the processes underlying host switching or the maintenance of fidelity to particular host lineages may allow predictions of which parasites of wildlife pose the greatest threat to humans and wildlife populations of conservation concern (Brooks and Ferrao 2005; Whiteman et al. 2004). Given that the debate over parasite macroevolution is largely centered on the nature of the processes that lead to parasite speciation and distribution, considerable insight into this problem would be gained by accumulating data from comparative studies of parasite microevolution (Nadler 1995; Hafner et al. 2003; Huyse et al. 2005). Our findings support's Clay's (1949), Clay's and Rothschild (1952) and Huyse et al.'s (2005) hypothesis that allopatric speciation may underpin much of the initial diversification of parasite lineages, in one case where the parasites tacked host gene flow (*D. regalis*) and two other cases where populations were significantly structured, but not in relation to the pattern of host gene flow. The less host specific, less aggregated, more vagile, prevalent and abundant amblyceran *C. turbinatum* harbored a similar degree of population genetic diversity and genetic differentiation to that of *D. regalis*, but did not appear to track the host's gene flow or any signal of host colonization history was likely lost as a result of recurrent inter-island gene flow and frequent turnover of island populations. However,

several island populations of *C. turbinatum* were highly differentiated from all other populations sampled. This combination of relatively high genetic diversity overall, recurrent gene flow between and apparent local differentiation within populations may facilitate local adaptation to hosts and host race formation (Gandon et al. 1996; Lively 1999; Morgan et al. 2005; Whiteman et al. 2005). Notably, host races appear to have formed in parasites on island populations where the hawk hosts exhibit extremely invariant minisatellite profiles and innate immune responses (Whiteman et al. 2005). Moreover, *C. turbinatum* louse abundance was related in a negative fashion to host natural antibody titres (Whiteman et al. 2005) in this system and across deep evolutionary time-scales, amblyceran species richness across hosts is explained by T-cell mediated host immune response (Møller and Rózsa 2005). The level of parasite gene flow among genetically structured parasite populations is directly related to the ability of the parasites to adapt locally to hosts (Lively 1999; Thompson 1999; Morgan et al. 2005). The hawk-ectoparasite system is one in which host inbreeding, parasite load and immunity are linked, and one in which the parasites appear to be taking advantage of genetically depauperate hosts populations. Thus, placing this system in a metapopulation context may be highly informative (e.g., modeling local population turnover, particularly in *C. turbinatum*) with respect to our understanding of disease ecology and evolution in this host-parasite system (Fallon et al., 2004). It may be similarly important for understanding how avian parasites and pathogens move among the islands of the Galápagos given that invasive diseases may pose the greatest risk to the continued persistence of the world's most intact oceanic avifaunas (Wikelski et al. 2004).

The rate of mtDNA evolution across pairwise inter-island comparisons was ~1.6% higher in *D. regalis* (5' COI+12S mtDNA) than in the host in the context of an island model of diversification. Although uncorrected (see Hafner et al. 2003) for potential biases, this is consistent with a rate of 2-3 times that of the host in other studies comparing rates of DNA substitution in other louse-vertebrate systems (Page 1998). However, at the homologous 5' COI locus sequenced in the host and parasites, both *C. turbinatum* and *D. regalis* harbored significant inter-island pairwise differences, while the host was invariant. Thus, this ~1.6-fold higher relative rate of evolution is a conservative estimate and the actual rate is clearly higher, but a graphical depiction of this relationship at the 5' end of COI for hosts and parasites is not helpful due to the fact that each pairwise comparison of the host was zero genetic distance at this locus. Lice in general have an elevated rate of mtDNA substitution (Hafner et al. 1994; Page et al. 1998, 2002; Johnson et al. 2003; Yoshizawa and Johnson 2003). Interestingly, *C. turbinatum* and *I. nigra* did not have elevated rates of diversification in the context of this island model relative to the host's divergence, which indicates that cospeciation *sensu stricto* (Page 2003) is not occurring based on these markers between these two parasites and the host.

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Working on it.

Figure captions

Figure 1. Infection intensities of three ectoparasite species quantitatively sampled from Galápagos Hawks (±SE). No hippoboscids were found on hosts from Isla Marchena. Note that *C. turbinatum* is the most abundant species, followed by *D. regalis* and *I. nigra.*

Figure 2. Plot of overall F_{st} values for mtDNA from three ectoparasites of the Galápagos hawk (*B. galapagoensis*): *D. regalis*, *B. galapagoensis*, *C. turbinatum*, and *I. nigra*. Also note the overall F_{st} values for *B. galapagoensis* based on minisatellites. The effective number of migrants/generation is given below each for mtDNA data (using Wright's 1952 island model as implemented in DNAsp). Note that the highest population

genetics structure and is found in the ischnoceran *D. regalis* and the lowest in the lousefly *I. nigra*.

Figure 3. Scatterplot of Galápagos inter-island pairwise F_{st} values from *B. galapagoensis* (x-axis) vs. inter-island pairwise F_{st} values from mtDNA from each of three ectoparasite species (A) *D. regalis*, (B) *C. turbinatum* and (C) *I. nigra*. The relationship was significant only in *D. regalis*.

Figure 4. Scatterplot of Galápagos inter-island pairwise geographic distances (ln transformed) vs. inter-island pairwise F_{st} values from mtDNA from each of three ectoparasite species of the Galápagos hawk (*B. galapagoensis*): (A) *D. regalis*, (B) *C. turbinatum* and (C) *I. nigra*. The relationship was significant and positive in *D. regalis* and *I. nigra*.

Figure 5. Relationship between inter-island pairwise Kimura 2-parameter mtDNA distances from *B. galapagoensis* vs. inter-island pairwise Kimura 2-parameter mtDNA distances from each of three ectoparasite species (A) *D. regalis*, (B) *C. turbinatum* and (C) *I. nigra*. The relationship was significant only in *D. regalis*.

Figure 6. 95% statistical parsimony haplotype networks of combined mtDNA sequence data (3' COI and CR mtDNA) for (A) the Galápagos hawk (*Buteo galapagoensis*) and combined mtDNA sequence data (12S+COI) from each of three ectoparasites species of the Galápagos hawk (*B. galapagoensis*): (B) *D. regalis*, (C) *C. turbinatum* and (D) *I. nigra*. Geographical locations are color-coded in the accompanying map. Each connection (dash) between haplotypes represents one mutational step and small black circles are inferred (unsampled or extinct) haplotypes. Sampled haplotypes are represented by circles or rectangles (squares represent the putative ancestral or oldest

halpotype based on the TCS algorithm and Castelloe and Templeton's 1994 method). If > 1 island populations harbored a haplotype, its frequency in each is indicated by the pie diagrams or the proportionally divided rectangles. The highest amount of geographic structuring was observed in *D. regalis* (A), followed by *C. turbinatum* and *I. nigra*. The latter two species are more vagile than *D. regalis*. Notice also the low amount of variation in *I. nigra* sequences, which has relatively small population sizes relative to the two lice (and is highly vagile).

Figure 7. Phylogenetic trees estimated in Paup* based on combined 12S+COI sequences representing (A) Neigborjoining analysis of *D. regalis* from nine island populations of *B. galapagoensis* and several sequences from *Degeeriella* collected from *B. swainsoni*, the sister species of *B. galapagoensis* using neighborjoining; (B) Neigborjoining analysis of *C. turbinatum* from nine island populations of *B. galapagoensis* and several sequences from *Colpocephalum* collected from *B. swainsoni*, the sister species of *B. galapagoensis* using neighborjoining.

Table 1. Natural history factors of three phylogenetically independent Galápagos hawks likely mediating population genetic parameters. Data from all islands were pooled to illustrate inter-specific differences in these factors.

Parasite species	Taxonomic position	Relative vagility	Relative host specificity	Prevalence	Mean abundance	Mean intensity	*k*	Var/mean ratio	Total
Colpocephalum turbinatum	Insecta: Phthiraptera: Amblycera: Menoponidae	Moderate (vertical and horizontal transmission)	Low (47 diurnal raptors + rock dove)	97.5% (94.23-99.18%)	74.59 (58-89.98)	76.51 (60.79-93.16)	0.64	194.77	14,843
Degeeriella regalis	Insecta: Phthiraptera: Ischnocera: Philopteridae	Low (primarily vertical transmission)	Low (Two New World *Buteo* spp.)	85.4% (79.74-90.02%)	14.36 (11.05-17.51)	16.81 (13.02-20.07)	0.48	40.43	2,858
Icosta nigra	Insecta: Diptera: Pupipara: Hippoboscidae	High (volant)	High (Diurnal raptors)	High*	1.49	N/A	N/A	N/A	296

*On Isla Marchena, no *I. nigra* individuals were collected (despite sampling from 26 hosts, or ~1/4 of the total hawk population), thus its prevalence was zero in this population. Moreover, since individual flies were often collected from multiples hosts (flies moved between hosts while we searched for flies) no data on prevalence is given. For abundance, the total number of flies captured was simply divided by the total number of hawks searched for flies across the archipelago.

Table 2. Conceptual framework for this study. Each parasite species has been qualitatively scored in light of natural history factors *sensu* Nadler (1995), Clayton et al. (2004), Huyse et al. (2005) predicted to influence population genetics parameters.

Parasite Species	**Predicted Relative Population Genetic Diversity**	**Predicted Relative Population Genetic Structure Among Islands**	**Predicted Utility in Tracing Host Inter-Island Gene Flow**	**Predicted Utility in Tracing Host Inter-Island Dispersal**
Colpocephalum turbinatum	High	Low	Low	High
Degeeriella regalis	High	High	High	Low
Icosta nigra	Low	Low	Low	High

Table 3. Pairwise comparisons of inter-island genetic differentiation in the *Buteo galapgoensis*, the focal host in this host-parasite system. Data are based on 911 bp of mtDNA sequences (parital COI and CR) collected from 118 individual hawks 8 Galápagos hawk island populations. Pairwise F_{st} values estimated from haplotype frequencies are below the diagonal and the *P* values of those comparisons are above the diagonal.

	Fernandina	**Pinta**	**Marchena**	**Isabela**	**Santiago**	**Pinzón**	**Española**	**Santa Fe**
Fernandina	–	<0.00001	<0.00001	<0.01	<0.00001	<0.00001	<0.00001	<0.00001
Pinta	1	–	1	<0.00001	1	<0.00001	<0.00001	1
Marchena	1	0	–	<0.00001	1	<0.00001	<0.00001	1
Isabela	0.257	0.656	0.671	–	0.273	<0.001	<0.00001	<0.00001
Santiago	1	0	0	0.708	–	<0.00001	<0.00001	1
Pinzón	1	1	1	0.520	1	–	<0.00001	<0.001
Española	1	1	1	0.630	1	1	–	<0.00001
Santa Fe	1	0	0	0.620	0	1	1	–

Table 4. Pairwise comparisons of inter-island genetic differentiation in the louse *Degeeriella regalis*. Data are based on 1099 bp of mtDNA sequences (parital COI and 12S) collected from 110 individual lice from 8 Galápagos hawk island populations. Pairwise F_{st} values estimated from haplotype frequencies are below the diagonal and the *P* values of those comparisons are above the diagonal.

	Fernandina	**Pinta**	**Marchena**	**Isabela**	**Santiago**	**Pinzón**	**Española**	**Santa Fe**
Fernandina	–	<0.00001	<0.00001	0.348	0.129	<0.00001	<0.00001	<0.00001
Pinta	0.747	–	<0.00001	<0.00001	<0.00001	<0.001	<0.001	<0.001
Marchena	0.846	0.747	–	<0.00001	<0.00001	<0.00001	<0.001	<0.00001
Isabela	0.010	0.610	0.708	–	0.273	<0.00001	<0.00001	<0.00001
Santiago	0.048	0.519	0.591	0.009	–	<0.00001	<0.00001	<0.00001
Pinzón	0.899	0.780	0.899	0.726	0.562	–	<0.001	<0.00001
Española	0.899	0.780	0.899	0.726	0.611	1.0	–	<0.00001
Santa Fe	0.757	0.639	0.757	0.623	0.531	0.791	0.791	–

Table 5. Pairwise comparisons of inter-island genetic differentiation in the louse *Colpocephalum turbinatum*. Data are based on 950 bp of mtDNA sequences (parital COI and 12S) collected from 127 individual lice from 8 Galápagos hawk island populations. Pairwise F_{st} values estimated from haplotype frequencies are below the diagonal and the *P* values of those comparisons are above the diagonal.

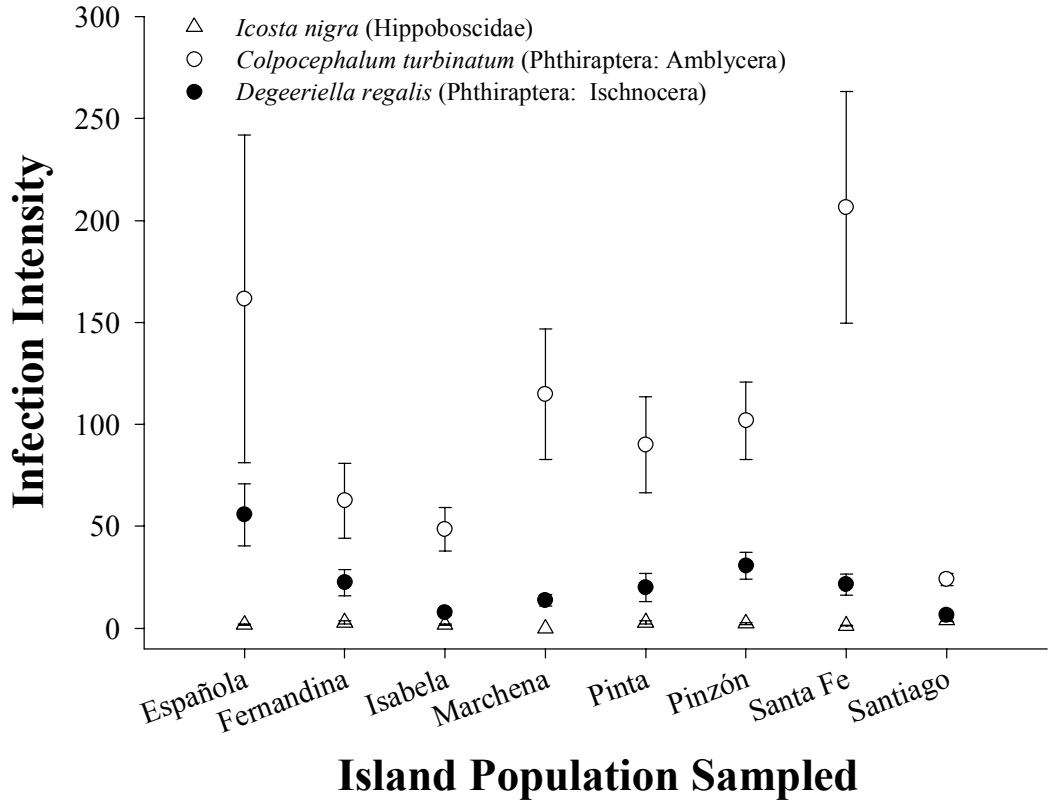
	Fernandina	**Pinta**	**Marchena**	**Isabela**	**Santiago**	**Pinzón**	**Española**	**Santa Fe**
Fernandina	–	<0.00001	<0.00001	0.122	<0.00001	<0.05	0.226	<0.00001
Pinta	0.584	–	<0.00001	<0.01	<0.00001	<0.00001	<0.00001	<0.00001
Marchena	0.348	0.759	–	<0.001	<0.00001	<0.001	<0.01	<0.00001
Isabela	0.103	0.485	0.348	–	<0.00001	0.067	0.110	<0.00001
Santiago	0.204	0.802	0.561	0.431	–	<0.05	0.101	<0.00001
Pinzón	0.141	0.822	0.328	0.213	0.225	–	.0352	<0.001
Española	0.052	0.872	0.337	0.178	0.068	0.047	–	<0.01
Santa Fe	0.450	0.886	0.480	0.495	0.624	0.474	0.501	–

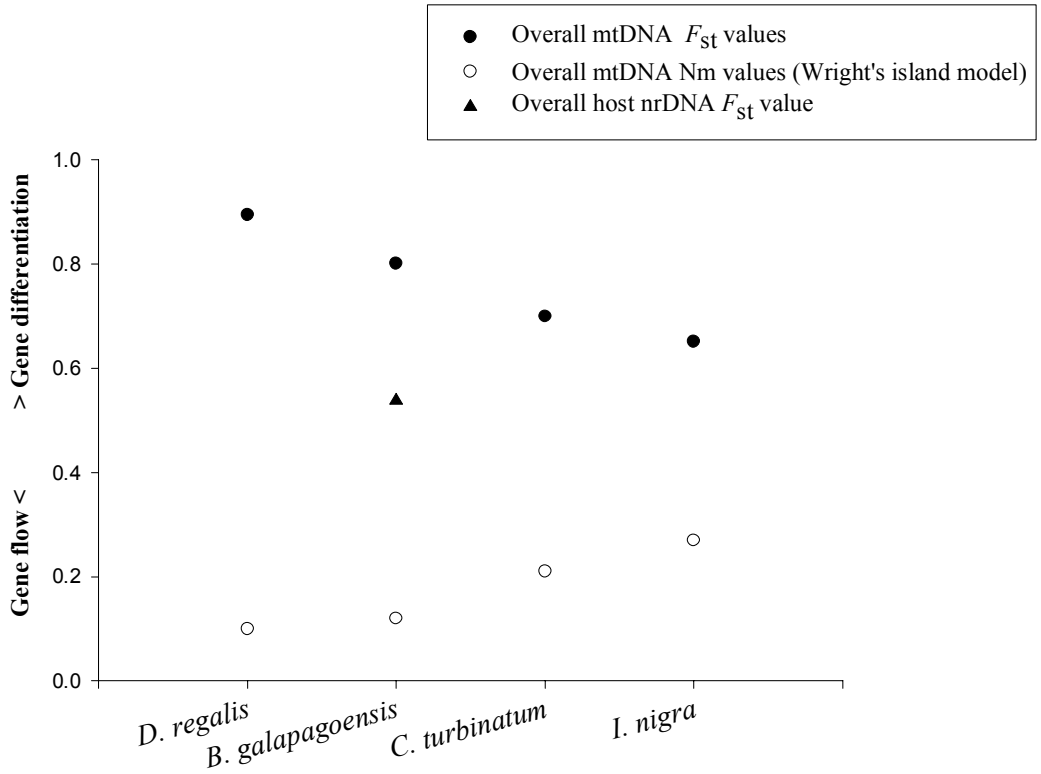
Table 6. Pairwise comparisons of inter-island genetic differentiation in the lousefly *Icosta nigra*. Data are based on 937 bp of mtDNA sequences (partial COI and 12S) collected from 117 individual flies from 7 Galápagos hawk island populations. Pairwise F_{st} values estimated from haplotype frequencies are below the diagonal and the *P* values of those comparisons are above the diagonal.

	Fernandina	**Pinta**	**Isabela**	**Santiago**	**Pinzón**	**Española**	**Santa Fe**
Fernandina	–	<0.00001	0.497	0.170	<0.00001	<0.00001	<0.00001
Pinta	1.0	–	<0.00001	<0.00001	0.100	0.221	0.248
Isabela	0.008	0.880	–	0.291	<0.00001	<0.00001	<0.00001
Santiago	0.095	0.740	0.011	–	<0.00001	<0.00001	<0.001
Pinzón	0.769	0.162	0.642	0.509	–	0.602	0.637
Española	0.852	0.045	0.736	0.614	–0.024	–	0.100
Santa Fe	0.898	0.241	0.736	0.606	–0.020	–0.050	–

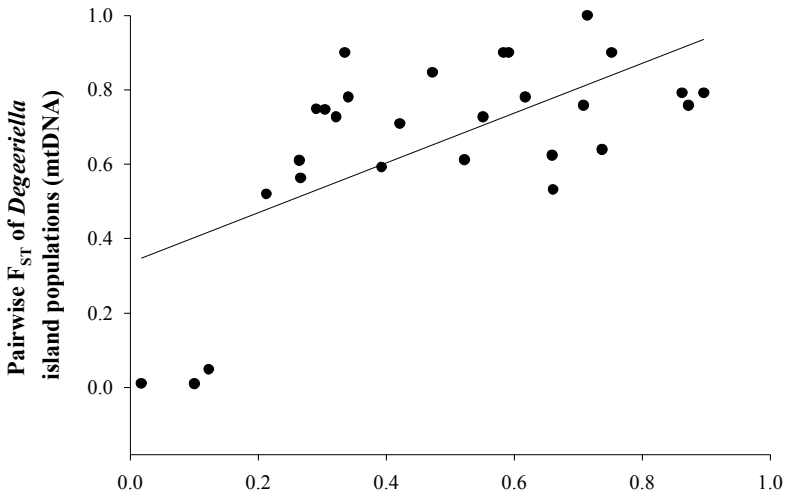
Table 7. Genetic variability mitochondrial DNA loci sequenced from three ectoparasites collected from the entire breeding range of the Galápagos Hawk (*Buteo galapagoensis*), which was also sequenced. The hawk was invariant at the homologous 5' end of COI across island populations (Bollmer et al. in press); two other mitochondrial fragments (the 3' end of COI and the control region or CR) were variable in the host and are listed below in bold. The 12S fragment was not sequenced from the host.

Species	Population genetic parameters	COI 5'	12S	COI 5' + 12S
D. regalis (N = 111 louse individuals)	Aligned length	603 bp	496 bp	1099 bp
	No. of polymorphic sites	13	8	21
	Nucleotide diversity	0.00222	0.00114	0.00207
	No. of haplotypes	10	8	17
	Haplotype diversity (±SD)	0.627±0.047	0.400±0.056	0.768±.0.35
	Number of synonymous/nonsynonymous mutations	9/4 (200 codons)	–	–
	Average inter-island pairwise genetic distance (K_2P)	2.37 ±1.03 (SD)	1.51 ±1.42 (SD)	3.12 ± 2.62 (SD)
C. turbinatum (N = 127 louse individuals)	Aligned length	601 bp	349 bp	950 bp
	No. of polymorphic sites	8	9	17
	Nucleotide diversity	0.00168	0.00178	0.00171
	No. of haplotypes	8	10	16
	Haplotype diversity (±SD)	0.635±0.025	0.496 ±0.052	0.769±0.028
	Number of synonymous/nonsynonymous mutations	6/2 (199 codons)	–	–
	Average inter-island pairwise genetic distance (K_2P)	0.91 ±1 (SD)	0.55 ±0.56 (SD)	1.47 ±1.45 (SD)
I. nigra (N = 117 fly individuals)	Aligned length	612 bp	325	937 bp
	No. of polymorphic sites	1	3	4
	Nucleotide diversity	0.00003	0.00163	0.00058
	No. of haplotypes	2	4	5
	Haplotype diversity (±SD)	0.00028±0.017	0.00035±0.019	0.520±0.020
	Number of synonymous/nonsynonymous mutations	1/0 (203 codons)	–	–
	Average inter-island pairwise genetic distance (K_2P)	0	0.42 ± 0.39 (SD)	0.42 ± 0.39 (SD)
B. galapagoensis (N = 22 individuals from 9 island populations; 122 additional hawks sequenced at variable regions).	Aligned length	516 bp	–	**911 bp 3' COI+CR**
	No. of polymorphic sites	0	–	**9**
	Nucleotide diversity	0	–	**0.0018**
	No. of haplotypes	1	–	**7**
	Haplotype diversity (±SD)	0	–	**0.671 ± 0.030**
	Number of synonymous/nonsynonymous mutations	0	–	**1/4 (164 codons from 3' COI)**
	Average inter-island pairwise genetic distance (K_2P)	0	–	**1.80 ± 1.31 (SD)**

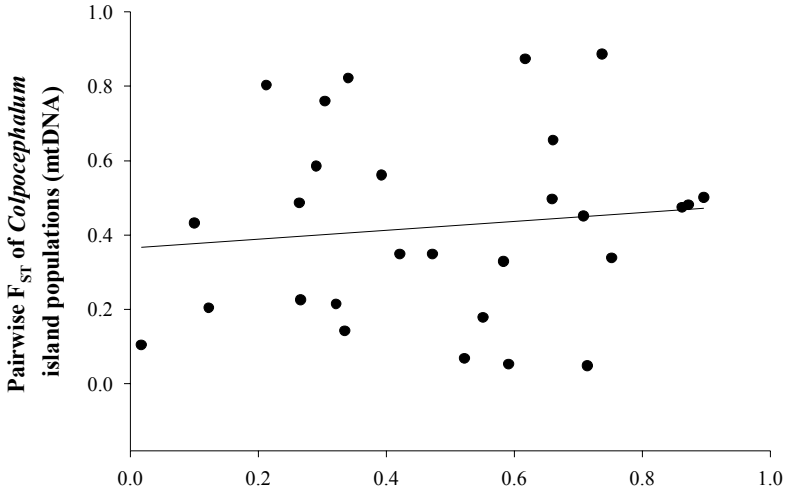

300
250
200
150
100
50
0
Infection Intensity
Icosta nigra (Hippoboscidae)
Colpocephalum turbinatum (Phthiraptera: Amblycera)
Degeeriella regalis (Phthiraptera: Ischnocera)
Española
Fernandina
Isabela
Marchena
Pinta
Pinzón
Santa Fe
Santiago
Island Population Sampled


Overall mtDNA F_{st} values
Overall mtDNA Nm values (Wright's island model)
Overall host nrDNA F_{st} value
Gene flow < > Gene differentiation
1.0
0.8
0.6
0.4
0.2
0.0
D. regalis
B. galapagoensis
C. turbinatum
I. nigra

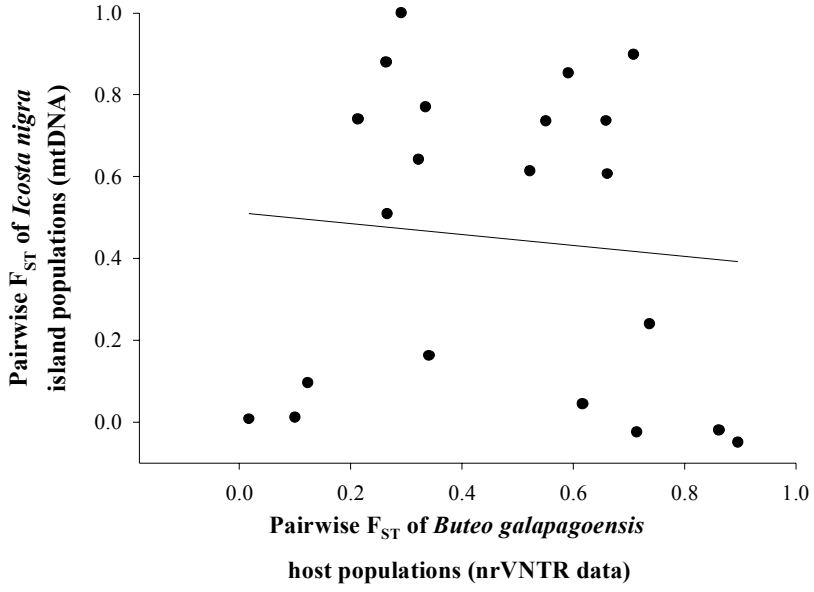
A

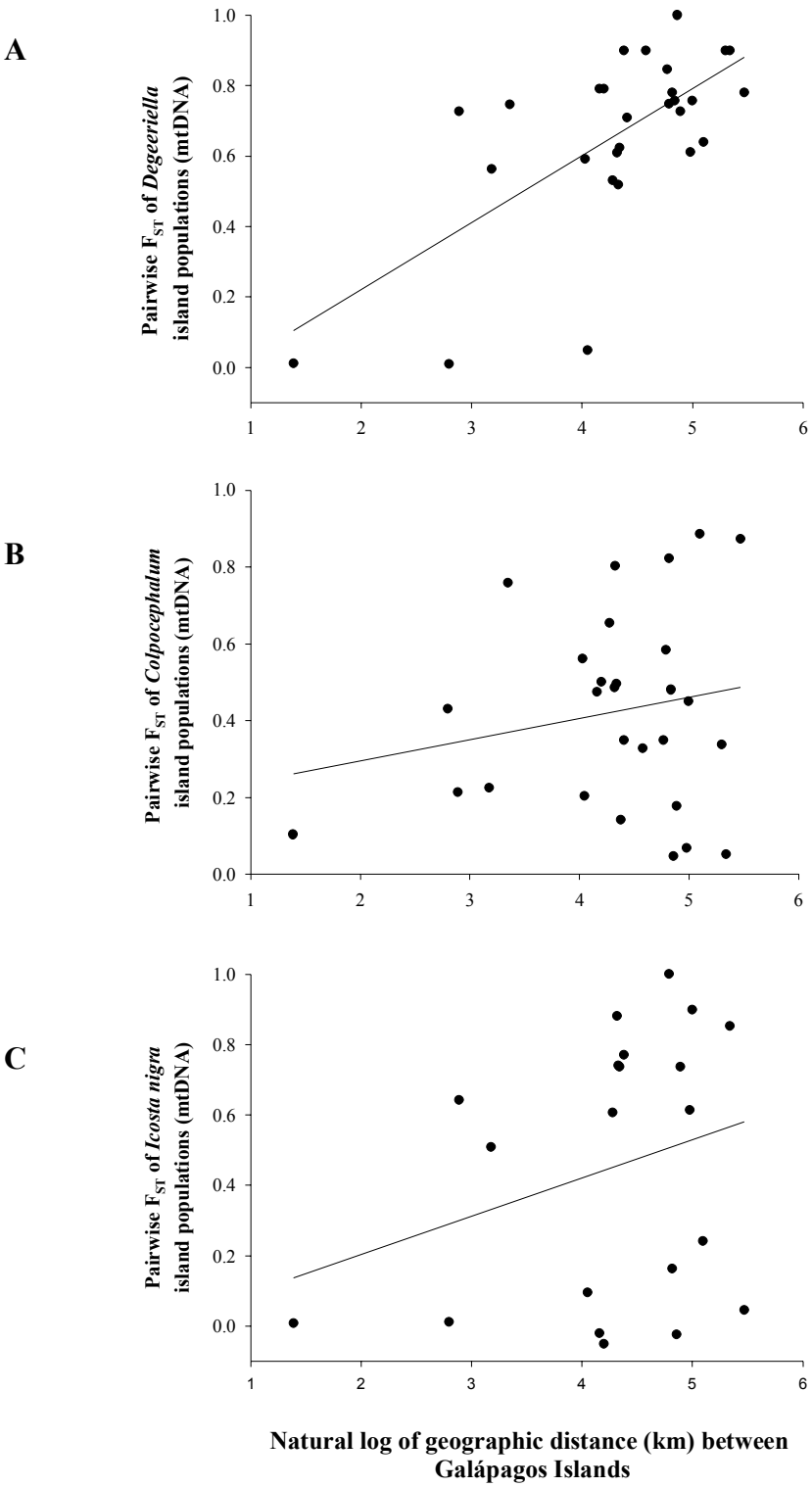

Pairwise F_{ST} of *Degeeriella* island populations (mtDNA)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0

B

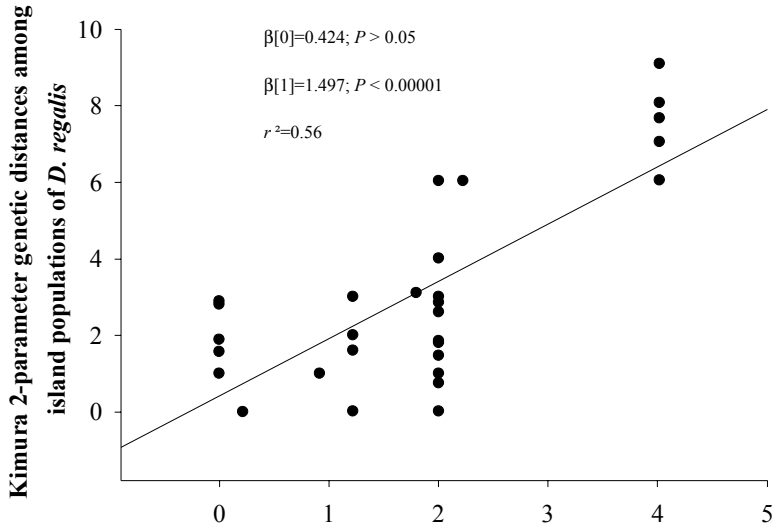

Pairwise F_{ST} of *Colpocephalum* island populations (mtDNA)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0

C

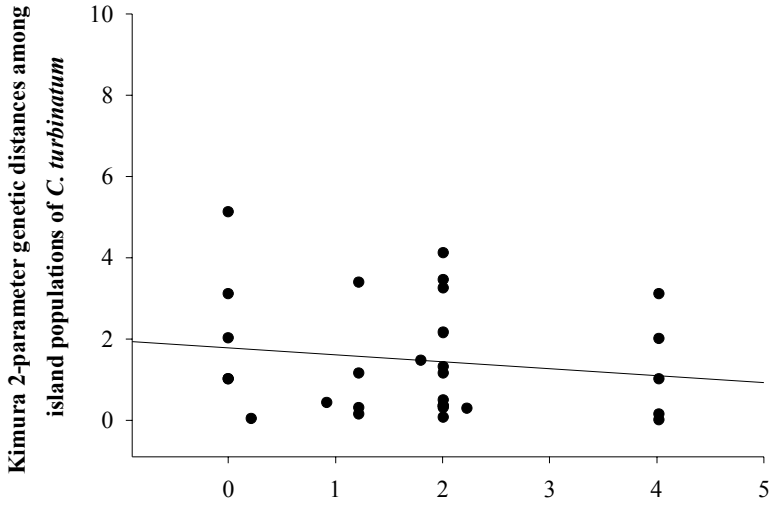

Pairwise F_{ST} of *Icosta nigra* island populations (mtDNA)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Pairwise F_{ST} of *Buteo galapagoensis* host populations (nrVNTR data)


A
B
C
Pairwise F_{ST} of *Degeeriella* island populations (mtDNA)
Pairwise F_{ST} of *Colpocephalum* island populations (mtDNA)
Pairwise F_{ST} of *Icosta nigra* island populations (mtDNA)
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
Natural log of geographic distance (km) between Galápagos Islands

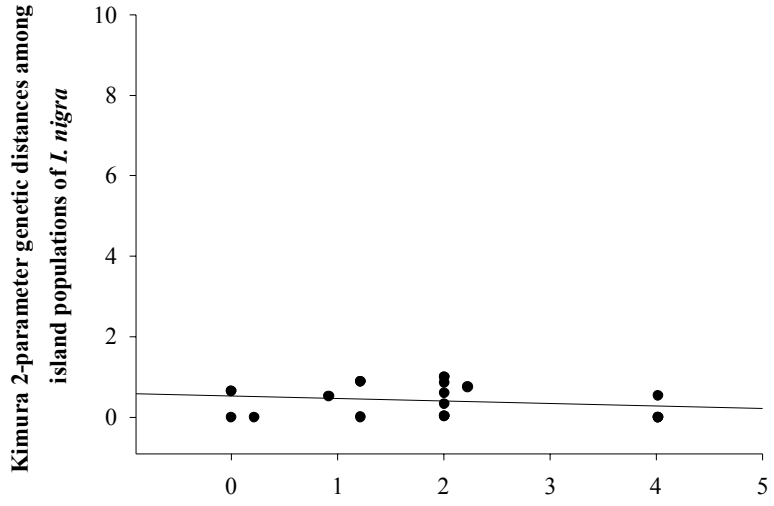
A



B



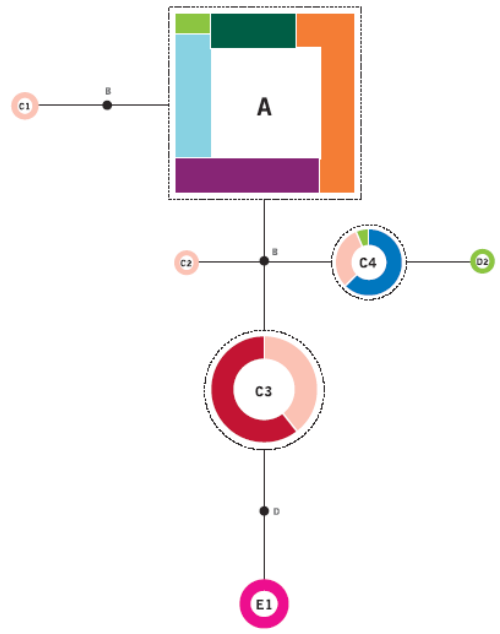
C



A

Kimura 2-parameter genetic distances among island populations of *B. galapagoensis* (mtDNA)

FIGURE 6a



Buteo galapagoensis
CO1 + CR + CYTB + ND2

KEY

Buteo galapagoensis
CO1 + CR + CYTB + ND2

GALÁPAGOS ISLANDS MAP

ISLAND	HAPLOTYPE FREQUENCY
PINTA	**A** N=13
FERNANDINA	**C3** N=20
ISABELA	**C1** N=2 **C2** N=1 **C3** N=13 **C4** N=5
SANTA FE	**A** N=9
SANTIAGO	**A** N=21
ESPAÑOLA	**E1** N=10
PINZÓN	**C4** N=10
MARCHENA	**A** N=15
SANTA CRUZ	**A** N=2 **C4** N=1 **D2** N=1
	Extirpated populations

NETWORK COMPONENTS

———	*Mutational step*
- - - - - -	*Multi-island haplotype*
●	*Unobserved haplotype*
A	*Inferred root haplotype*
B–E	*Letter sequence corresponds to step from root haplotype*
1–4	*Numbers identify haplotypes within a step*

Note: *Haplotype size depicted in network is proportional to frequency; See above for haplotype frequencies by island*

B

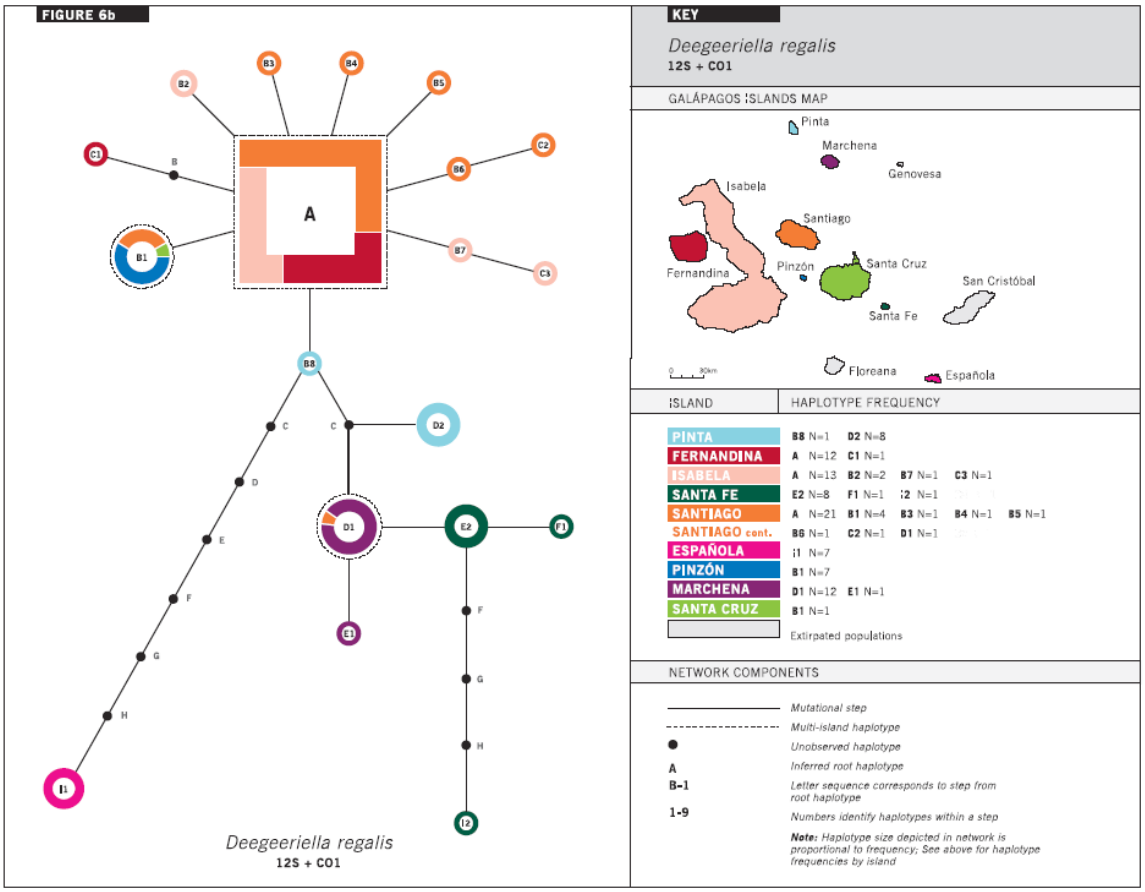


FIGURE 6b
A
B1
B2
B3
B4
B5
B6
B7
B8
C1
C2
C3
D1
D2
E1
E2
F1
I1
I2
Deegeeriella regalis
12S + CO1
KEY
Deegeeriella regalis
12S + CO1
GALÁPAGOS ISLANDS MAP
Pinta
Marchena
Genovesa
Isabela
Santiago
Pinzón
Santa Cruz
Fernandina
San Cristóbal
Santa Fe
Floreana
Española
ISLAND
HAPLOTYPE FREQUENCY
PINTA B8 N=1 D2 N=8
FERNANDINA A N=12 C1 N=1
ISABELA A N=13 B2 N=2 B7 N=1 C3 N=1
SANTA FE E2 N=8 F1 N=1 I2 N=1
SANTIAGO A N=21 B1 N=4 B3 N=1 B4 N=1 B5 N=1
SANTIAGO cont. B6 N=1 C2 N=1 D1 N=1
ESPAÑOLA I1 N=7
PINZÓN B1 N=7
MARCHENA D1 N=12 E1 N=1
SANTA CRUZ B1 N=1
Extirpated populations
NETWORK COMPONENTS
Mutational step
Multi-island haplotype
Unobserved haplotype
A
Inferred root haplotype
B-1
Letter sequence corresponds to step from root haplotype
1-9
Numbers identify haplotypes within a step
Note: Haplotype size depicted in network is proportional to frequency; See above for haplotype frequencies by island

C

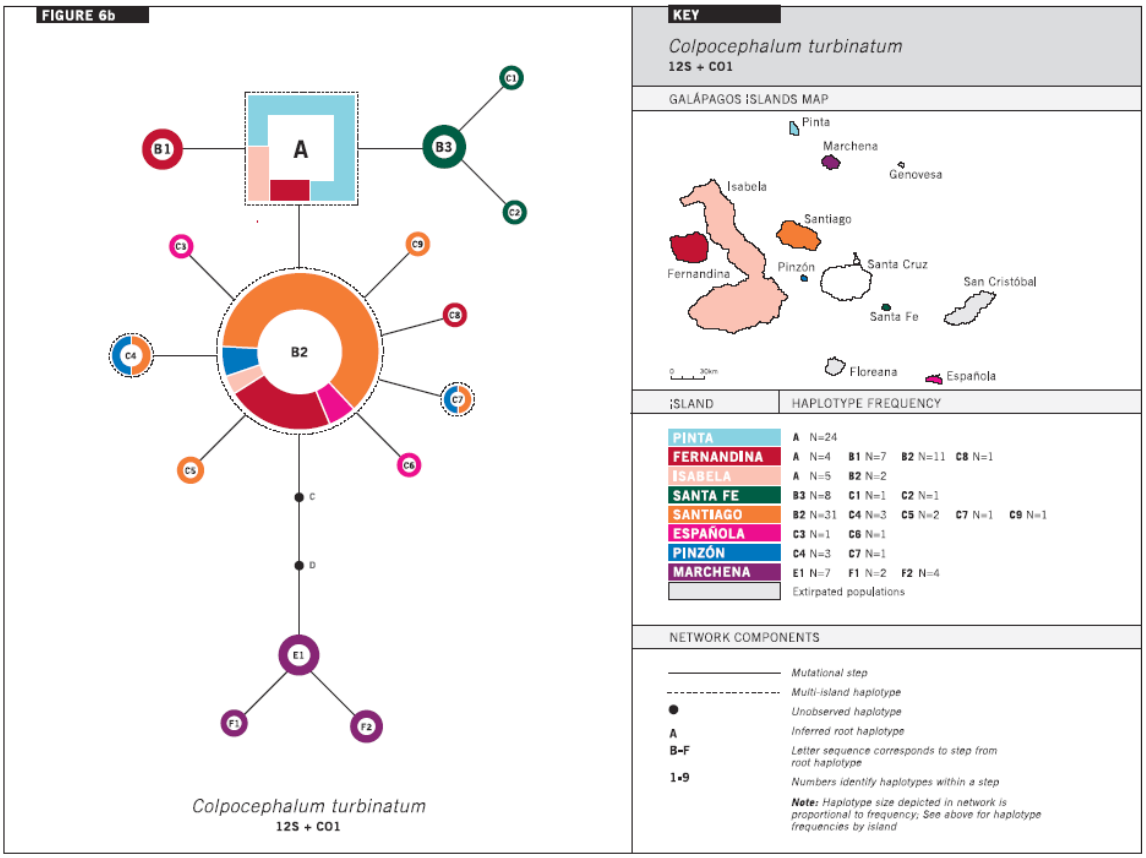


FIGURE 6b
A
B1
B3
C1
C2
B2
C3
C9
C8
C4
C7
C5
C6
C
D
E1
F1
F2
Colpocephalum turbinatum
12S + CO1
KEY
Colpocephalum turbinatum
12S + CO1
GALÁPAGOS ISLANDS MAP
Pinta
Marchena
Genovesa
Isabela
Santiago
Pinzón
Santa Cruz
Fernandina
San Cristóbal
Santa Fe
Floreana
Española
ISLAND
HAPLOTYPE FREQUENCY
PINTA
A N=24
FERNANDINA
A N=4 B1 N=7 B2 N=11 C8 N=1
ISABELA
A N=5 B2 N=2
SANTA FE
B3 N=8 C1 N=1 C2 N=1
SANTIAGO
B2 N=31 C4 N=3 C5 N=2 C7 N=1 C9 N=1
ESPAÑOLA
C3 N=1 C6 N=1
PINZÓN
C4 N=3 C7 N=1
MARCHENA
E1 N=7 F1 N=2 F2 N=4
Extirpated populations
NETWORK COMPONENTS
Mutational step
Multi-island haplotype
Unobserved haplotype
A
Inferred root haplotype
B–F
Letter sequence corresponds to step from root haplotype
1–9
Numbers identify haplotypes within a step
Note: Haplotype size depicted in network is proportional to frequency; See above for haplotype frequencies by island

D

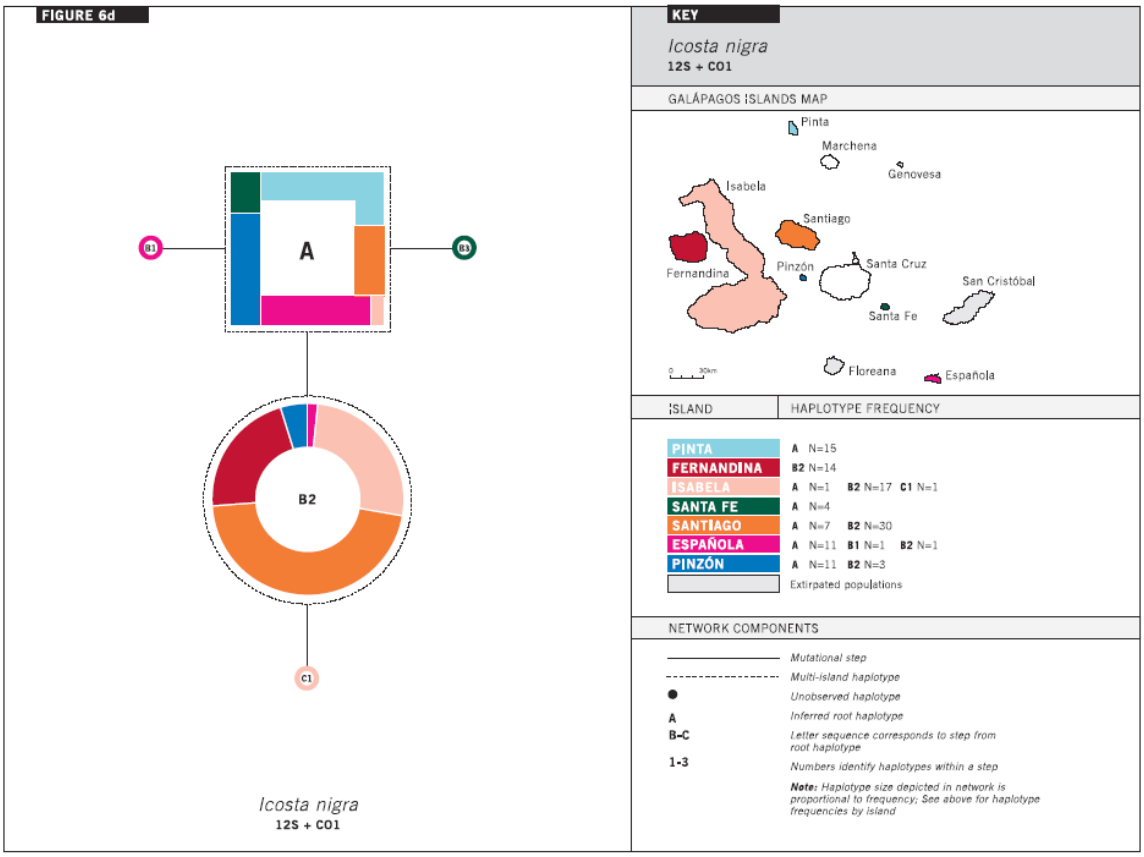
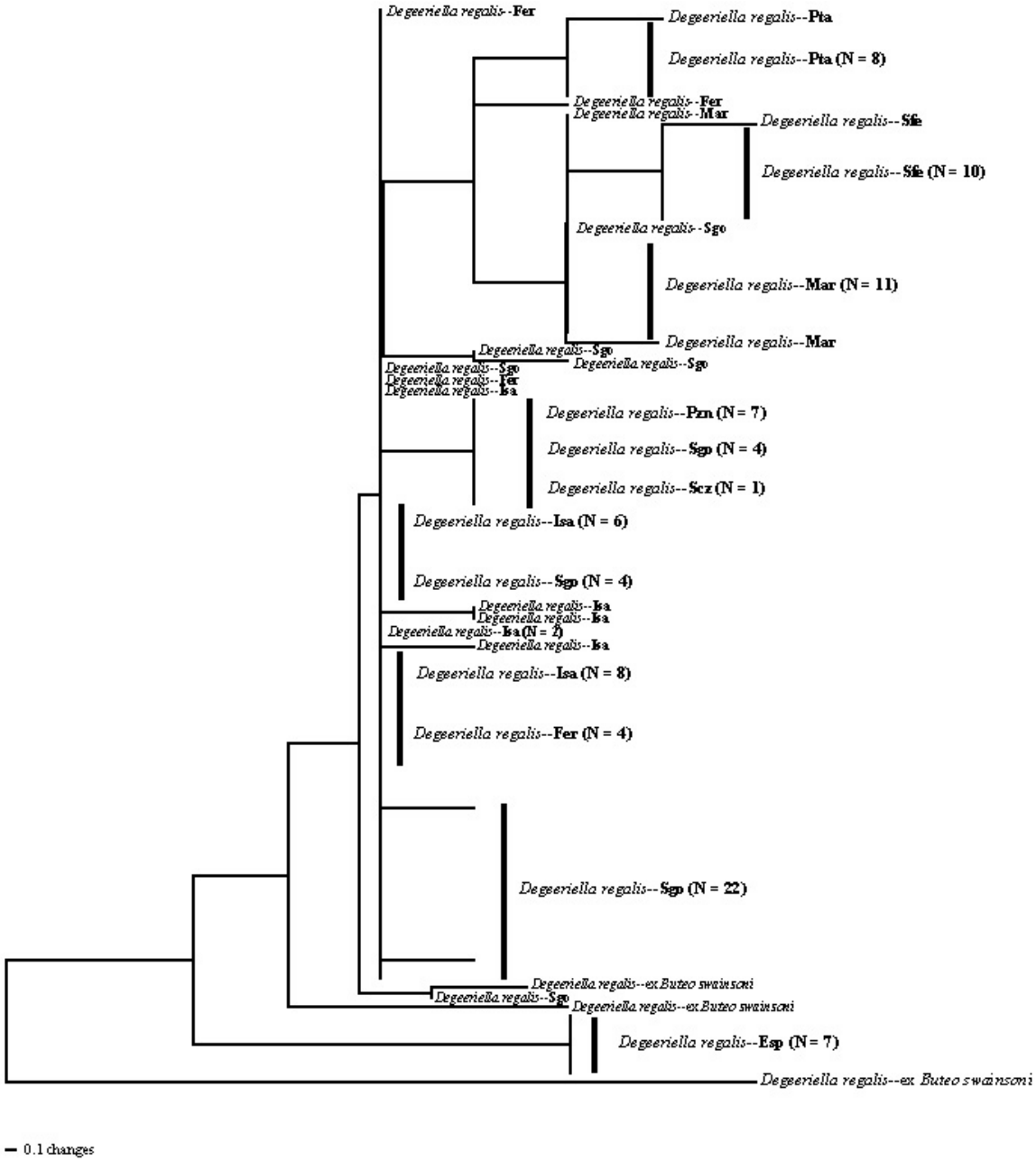


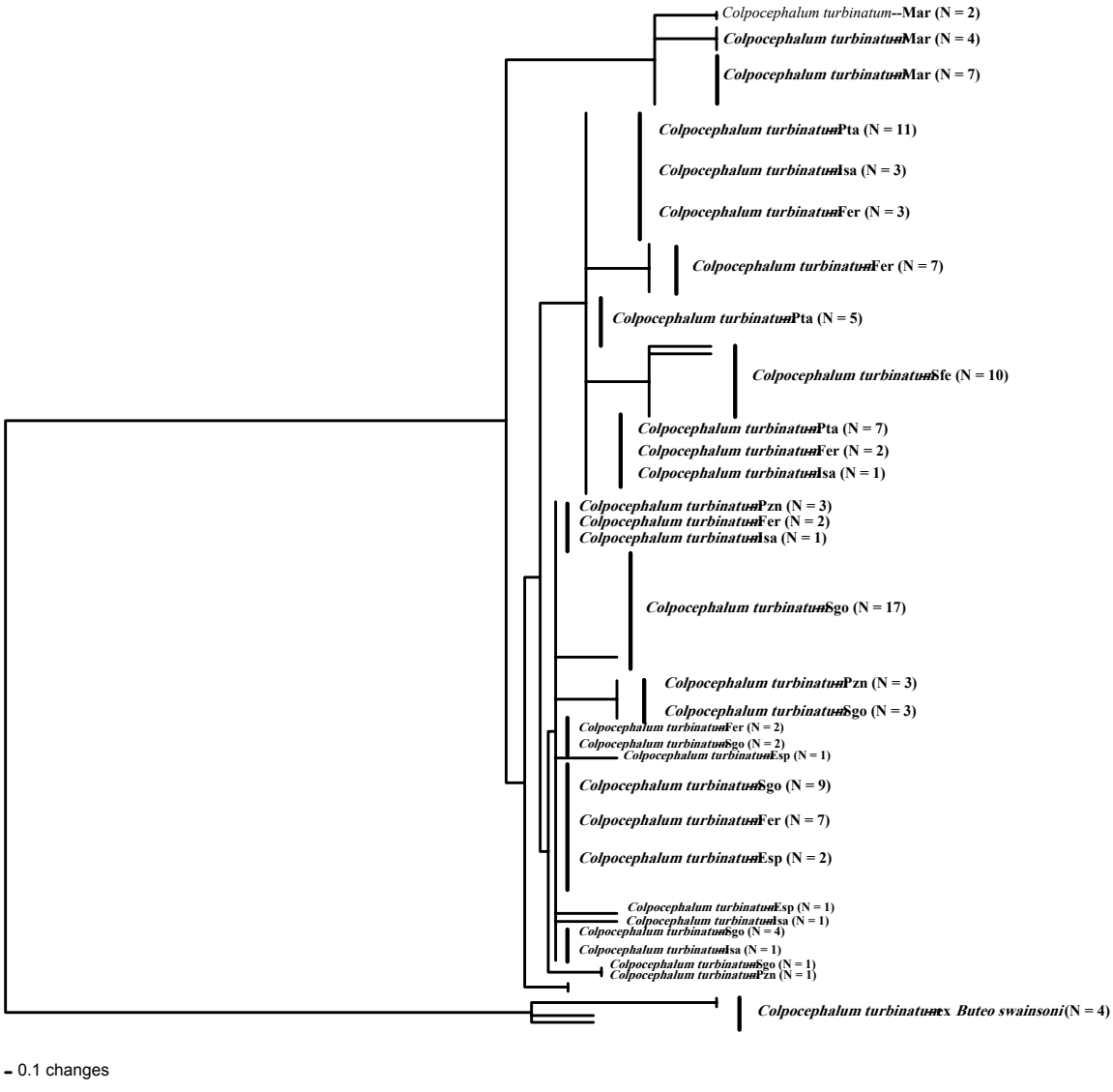
FIGURE 6d
A
B1
B2
C1
Icosta nigra
12S + CO1
KEY
Icosta nigra
12S + CO1
GALÁPAGOS ISLANDS MAP
Pinta
Marchena
Genovesa
Isabela
Santiago
Fernandina
Pinzón
Santa Cruz
San Cristóbal
Santa Fe
Floreana
Española
ISLAND
HAPLOTYPE FREQUENCY
PINTA A N=15
FERNANDINA B2 N=14
ISABELA A N=1 B2 N=17 C1 N=1
SANTA FE A N=4
SANTIAGO A N=7 B2 N=30
ESPAÑOLA A N=11 B1 N=1 B2 N=1
PINZÓN A N=11 B2 N=3
Extirpated populations
NETWORK COMPONENTS
Mutational step
Multi-island haplotype
Unobserved haplotype
A
Inferred root haplotype
B-C
Letter sequence corresponds to step from root haplotype
1-3
Numbers identify haplotypes within a step
Note: Haplotype size depicted in network is proportional to frequency; See above for haplotype frequencies by island

A



Degeeriella regalis 12S + COI
Neighbor-joining phylogeny

B



Colpocephalum turbinatum 12S + COI Neighborjoining