THE ECOLOGICAL BASIS OF GENETIC VARIATION

IN PARASITE POPULATIONS

by

Emily Ann DiBlasi

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STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Emily Ann DiBlasi**

has been approved by the following supervisory committee members:

Sarah E. Bush	, Chair	**5/12/17** Date Approved
Dale H. Clayton	, Member	**5/12/17** Date Approved
Kevin P. Johnson	, Member	**5/12/17** Date Approved
Jon Seger	, Member	**5/12/17** Date Approved
Alan Rogers	, Member	**5/12/17** Date Approved
Jennifer Koop	, Member	**5/12/17** Date Approved

and by **M. Denise Dearing** , Chair of the Department of **Biology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Dispersal is the main process leading to gene flow in populations. Gene flow influences the spatial distribution of populations, individuals and genes. Natural populations are typically subdivided, or structured, in space and genetic composition in part due to limited dispersal. Understanding how genetic variation is partitioned and the factors that govern this process is a major goal of evolutionary biologists because the degree to which populations are structured affects rates of local adaptation, speciation and extinction. In my dissertation I examine how one life history trait, phoretic dispersal, influences gene flow and genetic differentiation in populations. Here I compare related and sympatric species that are ecologically very similar, but explicitly differ in dispersal behavior. Wing lice (*Columbicola columbae*) frequently engage in phoresis or "hitchhike" on the pigeon lousefly (*Pseudolynchia canariensis*), while body lice (*Campanulotes compar*) do not. In the first part of my dissertation I characterize the genetic makeup of wing and body lice populations on a local scale. I found that in a geographic area where flies occur, wing lice have significantly less population genetic structure than body lice. Next I identified geographic areas where flies are absent from pigeon populations. Finally, I compared patterns of genetic differentiation on a global scale for wing and body lice populations. I found that in areas without flies, wing lice and body lice have similar patterns of

genetic differentiation. These findings strongly suggest that ongoing phoretic dispersal erodes population genetic structure and enhances population connectivity for wing lice.

“Nothing in evolution makes sense except in the light of population genetics”

—Michael Lynch

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CHAPTER 1

INTRODUCTION

Evolution is a change in genotype frequencies in a population over time. At its core, evolution is a population genetic process that is influenced by four main forces: selection, drift, mutation and gene flow (Lynch 2007). Many different characteristics of organisms and their environments influence the strength of these forces and often leave behind imprints as genetic signatures. In my dissertation, I focus on one life-history trait: dispersal. Dispersal is defined as the movement of individuals away from their birthplace and is the main process leading to gene flow in populations (Clobert *et al.* 2012). Gene flow influences the spatial distribution of populations, individuals and genes (Bohonak 1999), and can be measured indirectly with genetic data (Broquet & Petit 2009).

Natural populations are typically subdivided, or structured, in space and genetic composition (Nielsen & Slatkin 2013). This non-uniform distribution occurs because geographic or ecological factors limit dispersal and keep organisms from randomly mating. Understanding how genetic variation is partitioned in populations and the factors that shape this structure is a major goal of evolutionary biologists because the degree to which populations are structured affects rates of local adaptation, speciation and extinction.

For many free-living species, the spatial distribution of individuals is correlated with habitat quality, where optimal habitat patches are occupied and suboptimal parts of the landscape are not. Dispersal of free-living organisms often occurs between these landscape fragments (Clobert *et al.* 2012). In parasites this is also the case, but with host individuals often representing the habitat patches. Permanent, obligate external parasites (ectoparasites) with direct life cycles are found *only* on hosts (Poulin 2006). Many ectoparasites are small and relatively immobile (Blasco-Costa and Poulin, 2013); for these parasites dispersal between hosts is often a major challenge. The Koop *et al.* (2014) study of ectoparasitic lice on hawks, and the Harper *et al.* (2015) study of ectoparasitic lice on pocket gophers demonstrated that hosts represent "islands" with parasite gene flow restricted between host individuals. However this is not the case for all parasites. Many parasites can easily move between hosts and may even be able to disperse more than hosts (Maze-Guilmo *et al.* 2016).

Most birds and mammals host multiple species of parasites (Dobson *et al.* 2008). In some host-parasite systems it is possible to compare parasite species that have very similar ecological niches on the same host, but which differ in key life history traits. Comparison of related and sympatric species that are ecologically similar, yet differ in dispersal ability, allows for examination of how dispersal influences population genetic structure and other microevolutionary patterns. For my thesis, I used a highly tractable, host-parasite system for which previous ecological studies and experiments have laid a solid foundation on which to generate and test *a priori* hypotheses about how particular modes of

dispersal affect gene flow and shape population genetic structure.

I used a host-parasite system that consisted of birds and their feather lice (Suborder: Ischnocera). Feather lice are permanent, obligate parasites that complete their entire life-cycle on bird hosts. Feather lice disperse in three main ways: (1) Vertical transmission – where lice move from parent to offspring during contact in the nest; (2) Direct horizontal transmission – where lice move between hosts that are in direct, physical contact, such as that between mates, or contact between other host individuals at shared foraging, watering sites, or communal roosting sites; and (3) Indirect horizontal transmission – where lice move between hosts that do not come into physical contact (Lee & Clayton 1995; Harbison *et al.* 2008; Harbison *et al.* 2009; Harbison & Clayton 2011; Clayton *et al.* 2016). Louse movement is referred to as "transmission" by parasitologists. Transmission and dispersal are interchangeable terms (Clayton *et al.* 2016).

An intriguing example of indirect horizontal transmission in lice is phoresis. "Phoresy" is a behavior where one organism disperses by “hitching a ride” on another more mobile organism (Farish & Axtell 1971). Phoresis often occurs among relatively immobile species (e.g., wingless species) that use highly mobile organisms (e.g., flies) to disperse among isolated resource patches. Phoresy has been documented in 19 genera of lice (Bartlow *et al.* 2016). These lice use hippoboscid flies, which are common blood-feeding parasites of birds, to disperse. Lice are not the only dispersal-limited organisms to use hippoboscid flies; 17 genera of mites have also been documented engaging in phoresis on flies (Philips & Fain 1991). Furthermore, phoresy is a common dispersal strategy

for animals in a diverse range of taxonomic groups. In addition to mites and lice, many species of nematodes, beetles, pseudoscorpions, echinoderms and worms regularly engage in phoresis (Treat 1956; Roubik & Wheeler 1983; Athias-Binche & Morand 1993; Ohtsuka *et al.* 2009). Despite the pervasiveness of this dispersal strategy, the effect that phoresis has on the population connectivity and structure of these organisms is unknown.

Rock pigeons are commonly infested with “wing lice” (*Columbicola columbae*), which frequently engage in phoresis with the pigeon lousefly (*Pseudolynchia canariensis*), a hippoboscid fly that is a common blood-feeding parasite of pigeons. Experimental and field studies in the Clayton-Bush Lab have shown that wing lice can disperse on *P. canariensis* to novel host pigeons and novel host species in sufficient numbers to establish new populations (Harbison *et al.* 2008; Harbison *et al.* 2009; Harbison & Clayton 2011). Importantly for my study, rock pigeons are commonly co-infested with wing lice and “body lice” (*Campanulotes compar*). Like all feather lice, wing and body lice are small wingless insects that live, feed and reproduce on the feathers of their host. Both species of lice eat the downy portions of the host's abdominal feathers. Females of both species lay one large egg at a time, which they glue to the host's feathers with glandular cement. When the eggs hatch, immature stages molt through three nymphal stages before molting into the adult stage. Both species reach reproductive maturity within ten days of hatching (Nelson & Murray 1971; Marshall 1981). In short, wing and body lice are ecologically very similar. Despite these similarities, however, wing and body lice differ in their ability to disperse

among hosts. Wing lice are phoretic on *P. canariensis* while body lice are not (Harbison *et al.* 2009; Harbison & Clayton 2011).

I hypothesized that this dispersal difference between wing and body lice would create distinct genetic patterns in each species. I assessed wing and body lice populations on free ranging feral rock pigeon populations on both small and large geographic scales. In Chapter 2, I compare the population genetic patterns of wing and body lice at a local scale among 3 sites in Salt Lake City, Utah. To do this, I developed molecular tools to genotype lice at eight, highly variable nuclear microsatellite markers unique to each of the two species. I used a hierarchical strategy to sample rock pigeons and their feather lice from three sites in Salt Lake City, Utah. Each site has a pigeon flock where flies occur, and all sites are within a six-mile radius. Ten pigeons from each flock that were found co-infested with at least 10 wing lice and 10 body lice were used in this study. This even sampling allowed me to assess the genetic variation of wing and body lice at several levels. Here I use the term "infrapopulation" to refer to all the “metapopulation” to refer to all the conspecific lice on birds in a single flock and all the conspecific lice among flocks in the study. I found that body lice exhibited significantly higher genetic differentiation than wing lice at all levels assessed, among infrapopulations, among flocks and within the metapopulation. The genetic patterns observed are consistent with key differences in dispersal ability between wing lice and body lice. This pattern is consistent with phoretic dispersal of wing lice as a potential driver of this genetic pattern.

If phoretic dispersal has a recurring influence on patterns of gene flow in

many wing lice populations, phoresis may have a large influence on microevolutionary patterns of diversification. However, to thoroughly test the hypothesis that fly-mediated phoretic dispersal erodes population structure, I compared wing and body louse populations in areas where flies are present and areas where flies are absent. In Chapter 3, I inferred the distribution of the phoretic vector of wing lice, *P. canariensis*. I did this by sampling pigeon populations for parasites at a global scale and surveying literature that included geographic records of the fly. Indeed, I found that pigeon parasite community composition varies geographically. *P. canariensis* was restricted to areas where the average low temperature is greater than 5˚C. The known geographic range of *P. canariensis* is between 45.5 °N and 33 °S. Additionally, I examined genetic differentiation among several fly populations from North America and one fly population from South America. I found little divergence in four mitochondrial genes, suggesting that rates of gene flow and population connectivity are high for *P. canariensis*.

Importantly, wing lice and body lice also co-occur on pigeons in areas where flies are absent and phoretic dispersal on flies is impossible. In the absence of the flies, horizontal dispersal of both wing lice and body lice should be limited to periods of direct contact between pigeons. Therefore I predicted that in areas without flies, wing lice populations would have a similar amount of genetic differentiation as body lice populations. This is because wing and body lice should have nearly the same rates of dispersal. In Chapter 4 I tested this hypothesis. To do this, I collected wing and body lice from pigeon populations in

areas where flies were present and in three pigeon populations where flies are known to be absent. I found that wing lice infrapopulations in both sites with flies exhibited less population genetic structure than body lice infrapopulations. At two of the three sites without flies, I found that wing lice infrapopulations have a similar amount of genetic structure compared to body lice infrapopulations. I also found that wing lice have higher genetic effective population sizes than body lice in areas with flies but have the same effective population size as body lice in areas without flies. These findings are consistent with the hypothesis that phoretic dispersal enhances gene flow for wing lice.

In summary, the results of my thesis strongly suggest that ongoing phoretic dispersal erodes population genetic structure and enhances population connectivity for wing lice. Collectively with other studies, these results provide a bridge between micro- and macroevolutionary patterns observed in wing and body lice. Other genera of pigeons and doves also have different species of wing lice, body lice and louseflies that co-occur on hosts. Phoresis likely explains patterns of host specificity in wing and body lice (Johnson *et al.* 2002; Clayton & Johnson 2003; Johnson & Clayton 2003). Body lice occur on fewer host species than wing lice and cospeciate with their hosts more often than wing lice (Clayton *et al.* 2003; Clayton & Johnson 2003). More broadly, my dissertation work provides evidence that dispersal patterns influence mircoevolutionary patterns of diversification. These microevolutionary patterns are parallel to patterns over macroevolutionary time.

References

Athias-Binche, F. & Morand, S. (1993). From phoresy to parasitism: the example of mites and nematodes. *Res. Rev. Parasit.*, 53, 73-79.

Bartlow, A.W., Villa, S.M., Thompson, M.W. & Bush, S.E. (2016). Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. *Int. J. Parasitol.*, 46, 221-227.

Bohonak, A.J. (1999). Dispersal, gene flow, and population structure. *Q. Rev. Biol.*, 74, 21-45.

Broquet, T. & Petit, E.J. (2009). Molecular estimation of dispersal for ecology and population genetics. *Annu. Rev. Ecol. Evol. Syst.*, 40, 193-216.

Clayton, D.H., Bush, S.E., Goates, B.M. & Johnson, K.P. (2003). Host defense reinforces host-parasite cospeciation. *Proc. Natl. Acad. Sci.*, 100, 15694-15699.

Clayton, D.H., Bush, S.E. & Johnson, K.P. (2016). *Coevolution of Life on Hosts: Integrating Ecology and History*. Univ. of Chicago Press.

Clayton, D.H. & Johnson, K.P. (2003). Linking coevolutionary history to ecological process: doves and lice. *Evolution*, 57, 2335-2341.

Clobert, J., Baguette, M., Benton, T.G. & Bullock, J.M. (2012). *Dispersal Ecology and Evolution*. Oxford Univ. Press, Oxford.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F. & Jetz, W. (2008). Homage to Linnaeus: how many parasites? how many hosts? *Proc. Natl. Acad. Sci.*, 105, 11482-11489.

Farish, D.J. & Axtell, R.C. (1971). Phoresy redefined and examined in Macrocheles muscaedomesticae (Acarina: Macrochelidae). *Acarologia*, 13, 16-29.

Harbison, C.W., Bush, S.E., Malenke, J.R. & Clayton, D.H. (2008). Comparative transmission dynamics of competing parasite species. *Ecology*, 89, 3186-3194.

Harbison, C.W. & Clayton, D.H. (2011). Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proc. Natl. Acad. Sci.*, 108, 9525-9529.

Harbison, C.W., Jacobsen, M.V. & Clayton, D.H. (2009). A hitchhiker's guide to parasite transmission: the phoretic behaviour of feather lice. *Int. J. Parasitol.*, 39, 569-575.

Harper, S.E., Spradling, T.A., Demastes, J.W. & Calhoun, C.S. (2015). Host behaviour drives parasite genetics at multiple geographic scales: population genetics of the chewing louse, *Thomomydoecus minor*. *Mol. Ecol.*, 24, 4129-4144.

Johnson, K.P. & Clayton, D.H. (2003). Coevolutionary hisory of ecological replicates: comparing phylogenies of wing and body lice to Columbiform hosts. In: *Tangled Trees: Phylogenies, Cospeciation and Coevolution* (ed. Page, RDM). The Univ. Chicago Press, Chicago, pp. 262 - 285.

Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J. & Clayton, D.H. (2002). The population genetics of host specificity: genetic differentiation in dove lice (Insecta : Phthiraptera). *Mol. Ecol.*, 11, 25-38.

Koop, J.A.H., DeMatteo, K.E., Parker, P.G. & Whiteman, N.K. (2014). Birds are islands for parasites. *Biol. Lett.*, 10. http://dx.doi.org/10.98/rsbl.2014.0255

Lee, P.L.M. & Clayton, D.H. (1995). Population biology of swift (apus-apus) ectoparasites in relation to host reproductive success. *Ecol. Entomol.*, 20, 43-50.

Lynch, M. (2007). *The Origins of Genome Architecture*. Sinauer Associates, Inc., Sunderland, MA.

Marshall, A.G. (1981). *The Ecology of Ectoparasitic Insects*. Academic Press, London.

Maze-Guilmo, E., Blanchet, S., McCoy, K.D. & Loot, G. (2016). Host dispersal as the driver of parasite genetic structure: a paradigm lost? *Ecol. Lett.*, 19, 336-347.

Nelson, B. & Murray, M. (1971). The distribution of Mallophaga on the domestic pigeon (Columba livia). *Int. J. Parasitol.*, 1, 21-29.

Nielsen, R. & Slatkin, M. (2013). *An Introduction to Population Genetics: Theory and Applications*. Sinauer Associates, Inc., Sunderland, MA.

Ohtsuka, S., Koike, K., Lindsay, D., Nishikawa, J., Miyake, H. & Kawahara, M. (2009). Symbionts of marine medusa and ctenophores. *Plankton Benthos Res.*, 4, 1-13.

Philips, J.R. & Fain, A. (1991). Acarine symbionts of louseflies (Diptera, Hippoboscidae). *Acarologia*, 32, 377-384.

Poulin, R. (2006). *Evolutionary Ecology of Parasites*. Second Edition. Princeton Univ. Press.

Roubik, D.W. & Wheeler, Q.D. (1983). Flightless beetles and stingless bees:

Phoresy of scotocryptine beetles (Leiodidae) on their meliponine hosts (Apidae). *J. Kans. Entomol.*, 125-135.

Treat, A.E. (1956). A pseudoscorpion on moths. *Lepid. News*, 87-89.

CHAPTER 2

DISPERSAL ECOLOGY PREDICTS POPULATION GENETIC STRUCTURE IN PARASITES

Abstract

Understanding how and why populations are structured is important because structure affects rates of local adaptation, speciation and extinction. Dispersal is one factor that influences population structure, but few empirical studies have directly assessed its impact. Here we use a tractable host-parasite system to test how differences in parasite dispersal predict differences in genetic structure. Our study system consists of rock pigeons that are parasitized by two species of feather lice that are ecologically similar, but which differ in dispersal ecology: "wing lice" disperse phoretically on hippoboscid flies, while "body lice" do not. We hypothesized that this difference in dispersal will result in wing louse populations that are less genetically differentiated than body louse populations. We found lower levels of genetic differentiation in wing lice than body lice at three spatial scales. Our results confirm that dispersal plays a fundamental role in the population genetic structure of parasites.

Introduction

Speciation, extinction, and local adaptation are processes influenced by the partitioning of genetic variation within and between populations. Studies assessing population subdivision provide a bridge between ecological and macroevolutionary time scales because ecological processes influence selection, genetic drift, and gene flow that act to structure populations. One major ecological process that influences population genetic structure is dispersal, which is the movement of organisms from one place to another (Clobert *et al.* 2001; Clobert *et al.* 2012). Dispersal influences the spatial distribution of populations, individuals and genes (Bohonak 1999; Broquet & Petit 2009).

Studies assessing how dispersal shapes the genetic structure of natural populations should investigate systems where differences in dispersal have been rigorously quantified, and should generate *a priori* hypotheses about expected patterns of population genetic structure (Bohonak 1999; Marko & Hart 2011). Such a study could be done by comparing the population genetic structure of two related and sympatric species that are ecologically very similar, but differ in patterns of dispersal (Nadler 1995; Bohonak 1999). Host-parasite systems lend themselves to this type of approach because a single host species, and even an individual host, can harbor two or more species of parasites that are ecologically similar yet phylogenetically independent (Clayton *et al.* 2016). By examining parasite species that have very similar ecological niches on the same host, yet differ in key dispersal characteristics, it is possible to use host-parasite systems to test specific hypotheses about how differences in dispersal influence

population genetic structure (Nadler 1995; Johnson *et al.* 2002; Whiteman *et al.* 2007; Criscione 2008).

Much of the work on parasite population genetic structure compares parasite structure to host structure. Traditionally, it has been argued that the genetic structure of host-specific parasites should mirror that of their hosts. Yet a recent meta-analysis of 38 host-parasite co-structure studies revealed that variables related to host dispersal are often not the main predictors of population genetic structure in parasites (Maze-Guilmo *et al.* 2016). Most parasites do not exclusively mirror their hosts; their own life history is also important in shaping the distribution of genetic variation (Criscione *et al.* 2005; Barrett *et al.* 2008; van Schaik *et al.* 2014). Interestingly, Maze-Guilmo *et al.* (2016) found that molecular estimates of gene flow are frequently higher for parasites than for their respective hosts. This suggests that rates of dispersal are often greater for parasites than for their hosts. However, this meta-analysis was not able to directly assess how parasite dispersal influences patterns of population genetic structure because dispersal has not been directly measured in these systems.

Here, we take advantage of a highly tractable host-parasite system consisting of two species of ectoparasitic feather lice (Insecta: Phthiraptera: Ischnocera) that co-infest rock pigeons (*Columba livia*). Feather lice are small wingless insects that live, feed and reproduce on feathers. Rock pigeons are infested with "wing lice" (*Columbicola columbae*) and "body lice" (*Campanulotes compar*). Wing and body lice are considered “ecological replicates” because they are distantly related yet have very similar life histories (Johnson & Clayton 2003;

Clayton *et al.* 2016). Both species of lice eat the downy portions of the host's feathers. Both species glue their eggs to the host's feathers with glandular cement. When the eggs hatch, immature lice molt through three nymphal stages before becoming adults, and both species reach reproductive maturity approximately one month after hatching (Nelson 1971).

In some respects, wing and body lice have similar dispersal constraints and mechanisms. Both species are largely immobile off host feathers (Bartlow *et al.* 2016). Both species disperse primarily between hosts that are in direct physical contact (Clayton *et al.* 2016), such as that between parents and offspring, or between mates. Harbison *et al.* (2008) documented that both wing lice and body lice disperse vertically from parents to offspring in the nest. Harbison *et al.* (2008) also showed that direct horizontal transmission rates of wing and body lice are similar.

Despite these similarities, wing and body lice differ in *indirect* horizontal transmission; wing lice disperse phoretically on winged parasitic flies. Phoresis is a relatively common form of indirect horizontal transmission in which immobile organisms hitchhike on other more mobile organisms (Bartlow *et al.* 2016). Some species of lice are phoretic, as are many species of nematodes, mites, beetles and pseudoscorpions (Treat 1956; Keirans 1975; Roubik & Wheeler 1983; Houck & OConnor 1991; Zeh & Zeh 1992; Athias-Binche & Morand 1993). Wing lice from rock pigeons are often recorded hitching rides on hippoboscid flies (*Pseudolynchia canariensis*), which are highly mobile parasites of pigeons (Figure 2.1). In contrast, body lice do not disperse phoretically (Bartlow *et al.*

2016). Harbison *et al.* (2009) and Harbison and Clayton (2011) showed that, even when wing and body lice are given equal access to flies, body lice do not engage in phoresis. Given this difference in dispersal, we predict that body louse populations will be more genetically structured than wing louse populations. We tested this hypothesis by comparing the population genetic structure of pigeon wing and body lice at several spatial scales. We used a hierarchical study design that compares the genetic variation of wing lice and body lice populations (1) on a single host individual (2) among host individuals within a single pigeon flock and (3) among host flocks. We also compared the genetic differentiation between each species of louse and their pigeon hosts.

Materials and Methods

Field sampling

Population genetic analyses are strongly influenced by sampling design (Meirmans 2015; Papadopoulou & Knowles 2016). We used a hierarchical strategy to sample rock pigeons and their feather lice from three sites in Salt Lake City, Utah. Each site was within 10.5 kilometers of the others (Figure 2.2). Each site contained a pigeon flock containing several hundred birds (pers. obs.). Pigeons were trapped in June of 2014 and 2015 and examined for ectoparasites at each site until we captured 10 pigeons from each flock that were co-infested with at least 10 wing lice and 10 body lice. This even sampling allowed us to assess the genetic variation of wing and body lice at multiple levels. We use "infrapopulation" to refer to all the conspecific lice living on a single host pigeon

(Bush *et al.* 1997). We use “metapopulation” to refer to all the conspecific lice on birds in a single flock and all the lice in every flock assessed in the study. Infrapopulation size was obtained for each species by counting the number of lice removed from each bird.

A total of 225 birds were captured and blood was collected from each host (~10 µL) on filter paper for DNA extraction. Ectoparasites were collected by fumigating each bird using the live-fumigation method developed by Clayton and Drown (2001). Samples were preserved in 95% ethanol for identification and DNA extraction. Lice were identified to species under an Olympus SZCTV stereoscope.

Microsatellite development and genotyping

We developed 17 microsatellite primer sets specific to wing lice (*Columbicola columbae*) and 13 primer sets specific to body lice (*Campanulotes compar*). Variable nuclear microsatellite loci were identified by searching for STR motifs (di, tri, tetra) with msatcommander (Rozen & Skaletsky 2000; Faircloth 2008) in sequences generated by Illumina sequencing from 30 pooled individuals. Sequences used to search for microsatellite motifs had BLAST alignment scores ≥200 compared with the human body louse (*Pediculus humanus corporis*) genome, which is the only published louse genome (Kirkness *et al.* 2010). Each microsatellite locus was evaluated with a multistep screening process to ensure quality data as suggested by Selkoe and Toonen (2006) and Fernandez-Silva *et al.* (2013). This filtering yielded 8 microsatellites specific to

wing lice and 8 microsatellites specific to body lice that were appropriate for analyses. The 17 pigeon microsatellite loci used to genotype the host birds were developed by Chun-lee *et al.* (2007), Stringham *et al.* (2012), and Traxler *et al.* (2000). DNA extractions of wing lice, body lice, and pigeons were performed using the DNeasy Blood and Tissue kit (Qiagen). DNA was extracted from louse individuals as described by Johnson *et al.* (2001).

Multiplex PCRs with a universal primer and fluorophore were used to genotype the samples (Schuelke 2000; Blacket *et al.* 2012). The universal primer tail M13 (5' CAC GAC GTT GTA AAA CGA C 3') was added to the 5' end of the locus-specific forward primer. M13 labeled primers were tagged with FAM, PET, NED, or VIC (Applied Biosystems). The two forward primers and the appropriate locus-specific reverse primer were used in PCR reactions. An ABI 3100 Genetic Analyzer (Applied Biosystems) was used to resolve PCR products and was run with the 500 LIZ size standard. Genemapper v 3.7 (Applied Biosystems) was used to determine allele sizes. A total of 10 wing lice and 10 body lice were genotyped from each host individual at 8 different microsatellite loci. Lice were genotyped from 10 host individuals at each of the three flocks for a total of 300 wing lice and 300 body lice from 30 birds. The 30 birds were also genotyped at 17 microsatellite loci.

Population genetic analyses

Genotyping error and null allele frequencies were estimated with Micro-checker (Van Oosterhout *et al.* 2004). Linkage disequilibrium and deviations from

Hardy-Weinberg equilibrium for each marker within the two species were assessed with Genepop (Raymond & Rousset 1995). Descriptive statistics, including mean number of alleles observed (N_A), observed heterozygosity (H_O), heterozygosity within populations (H_S), total heterozygosity (H_T), the inbreeding coefficient (G_{IS}) and standard errors were calculated using GenoDive (v 2.0) (Meirmans & Van Tienderen 2004). For each louse species we compared infrapopulation H_O to the infrapopulation size of lice on the bird using Kruskal–Wallis rank sum tests in R (v 3.1.0) (R Core Team 2014).

To compare genetic differentiation between louse infrapopulations, pairwise F_{ST} values were calculated in Arlequin (v 3.5) and significance was tested with 10,000 permutations (Excoffier *et al.* 2005). Critical significance levels were computed with corrections for false discovery rates to control for multiple comparisons (Benjamini & Hochberg 1995). Global F_{ST} values and 95% confidence intervals (CIs) were calculated using a bootstrapping approach (10,000 iterations) calculated in the R package “diveRsity” (Keenan *et al.* 2013). For polymorphic microsatellite loci empirical maximum values of F_{ST} are often lower than the theoretical maximum of 1 (Hedrick 2005; Jost 2008). Therefore, multiple differentiation statistics and estimators were calculated and compared.

Louse genetic variation was also partitioned into three biologically relevant levels: (1) on a single host individual, (2) among host individuals within a single pigeon flock and (3) between host flocks. All of the lice genotyped from a single bird (10 wing lice and 10 body lice) were treated as an *a priori* defined population. To assess if there was significant population structure of wing and

body lice at each of the three levels, we performed an Analysis of Molecular Variance (AMOVA) in Arlequin (v 3.5) (Excoffier *et al.* 2005).

To test for an association between genetic and geographic distance matrices for each louse species, Mantel tests with 10,000 permutations were used (Mantel 1967). The pairwise geographic and genetic distance matrices used in the Mantel tests were calculated in GenoDive. Geographic distances were taken from coordinates at the center of each of the three distinct flock sites. Genetic distance matrices of pairwise F_{ST} values were transformed to F_{ST} /(1 - F_{ST}). Additionally, partial Mantel tests corrected for geographic distance were implemented in GenoDive with 10,000 permutations to compare genetic distance matrices of pairwise F_{ST} values of each species of louse to genetic distance matrices of pairwise F_{ST} values of the pigeon host from which they were collected.

Multivariate analyses

To identify the optimal number of genetic clusters in the data without predefining populations, we used the *find.clusters* function implemented in the R package “adegenet” (Jombart & Ahmed 2011).The optimal number of genetic clusters was chosen for each species by selecting the lowest Bayesian Information Criterion (BIC) values. For wing and body lice, we tested values of *k* = 1–30 corresponding to the 30 louse infrapopulations and for pigeons we tested values of *k* = 1–3 corresponding to the three flock sites, with multiple runs at each value of k. Using the groupings from k-means clustering, we used the *dapc*

function to describe the genetic clusters with the Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.* 2010). Ordination plots were used to visualize the DAPC analysis; the axes represent the first two principal components of the DAPC. For each analysis, principal components were retained to account for at least 84% of the total variance in the data. For each louse species, populations were also predefined by the flock site from which they were collected. When louse genotypes were grouped by flock, two discriminant functions were retained. The results of the DAPC analyses are shown in several ordination plots.

Modeling private vs. shared alleles

We compared allele frequencies of two different louse species using microsatellite markers. In order to directly compare the degree of genetic differentiation between wing and body lice, we used generalized linear mixed-effects models (GLMM) with a binomial distribution and logit link. We predicted private alleles within each species across sampling site by modeling the fixed effects of the ratio of private to shared alleles in each louse species, with sampling locality included as a random effect. The model had 48 observations from three sites and the model intercept was set as the ratio of private to shared alleles for body lice. We also predicted private alleles within each species across host sampled by modeling the fixed effects of the ratio of private to shared alleles in each louse species and, with host included as a random effect. The model had 480 observations from 30 hosts and the model intercept was set as the ratio of

private to shared alleles for body lice. The ‘lme4’ package in R was used to fit each GLMM (Bates *et al.* 2015).

Results

Louse population genetic analyses

Wing lice had a higher prevalence and mean abundance than body lice at all three sites. Three of the 300 individual body lice did not yield enough DNA for amplification of all microsatellites, so they were excluded from the analyses. None of the microsatellites for either louse species showed evidence of allelic dropout. Nor was linkage disequilibrium significant among loci for either louse species. Populations of wing and body lice on birds did not show departures from Hardy-Weinberg equilibrium. The mean number of alleles in body lice was 12.6 while in wing lice the mean number of alleles was 9.1. The mean observed heterozygostiy (H_o) was 0.449 for body lice and 0.557 for wing lice. Overall, the inbreeding coefficent (G_{IS}) was 0.061 for body lice and 0.036 for wing lice. Infrapopulation size (H_o) did not correlate with population size for either species (body lice $r = 0.0816$, $p = 0.6681$; wing lice $r = 0.1980$, $p = 0.2942$). A weak yet significant pattern of isolation by distance was found for body lice (Mantel $r = 0.061$, $p = 0.015$), while no significant correlation was found between genetic and geographic distances for wing lice (Mantel $r = 0.062$, $p = 0.060$).

Host population genetic analyses

In total, 30 pigeons were genotyped. The mean number of alleles per locus was 4.9. The 30 birds from which lice were sampled were the most heavily co-infested birds in each flock. The hierarchical AMOVA (Table 2.1) indicates that pigeons have low, yet significant, genetic differentiation among the three flocks (F_{ST} 0.01206, $p < 0.0001$). Almost all of the genetic variance (98%) among pigeons was accounted for by sampling within pigeon flocks. The ordination plot showing the first two principal components of the DAPC for the host genotypes shows overlapping clusters. Pigeon population genetic structure did not correlate with louse population genetic structure either for body lice (partial-Mantel controlled for geographic distance, $r = 1.000$, $p = 0.584$; Mantel $r = -0.492$, $p = 0.492$) or wing lice (partial-Mantel controlled for geographic distance, $r = 1.000$, $p = 0.581$; Mantel $r = 0.979$, $p = 0.328$).

Wing and body louse genetic structure

Both species of lice were significantly structured between birds in the same flock and between birds in different flocks (Table 2.1). Genetic differentiation between body louse infrapopulations was larger than that seen in wing louse infrapopulations in 83% (360/435) of all possible comparisons (Figure 2.3). Most body louse infrapopulations were significantly structured. After correcting for false discovery rates, 92% (401/435) of pairwise F_{ST} values were significantly different from zero. In contrast, only 61% (265/435) of pairwise F_{ST} values in wing lice were significantly different from zero. For both wing and body

lice, bird 3 sampled from flock 3 had the largest pairwise F_{ST} values (F_{ST} = 0.28 for wing lice and F_{ST} = 0.73 for body lice). G"$_{ST}$ values followed the same pattern as F_{ST} values for both wing and body lice, yet the degree of differentiation was larger for G"$_{ST}$ values. Additionally, 23% of the total genetic variation in body lice was distributed among body louse infrapopulations (F_{SC} 0.21478, $p < 0.0001$; Table 2.1) compared with 8% among wing louse infrapopulations (F_{SC} 0.06880, $p < 0.0001$).

The Global F_{ST} value from a hierarchical AMOVA indicates that body lice are highly genetically differentiated among different flocks (F_{ST} 0.22542, $p < 0.0001$; Table 2.2), while wing lice are moderately genetically differentiated (F_{ST} 0.07484, $p < 0.0001$). The 95% CIs of F_{ST} values did not overlap when compared within or between flocks (Figure 2.4). When body louse infrapopulations were grouped by flock, the first two principal component axes of the DAPC separated the distributions of genetic clusters, indicating a high degree of genetic differentation of body lice between the three flocks (Figure 2.5). In contrast, the first two principal component axes of the DAPC for wing lice revealed overlapping distributions of genetic clusters, indicating a lower degree of genetic differentation between flocks (Figure 2.5).

The k-means clustering algorithm displayed the lowest BIC values at 14 clusters for body lice and 10 clusters for wing lice. Body louse clusters 2, 3, and 13 show a large degree of separation from the other clusters. Louse individuals assigned membership to clusters 2, 3, and 13, corresponded to body louse infrapopulations from Bird 3, Bird 8 and Bird 6 in flock 3. Wing lice clusters 2 and

6 show a small degree of separation from the other clusters. Wing louse individuals assigned membership to clusters 2 and 6 parasitized birds at all three flock sites, and thus do not correspond to individual birds.

Modeling private vs. shared alleles

The ratio of private to shared alleles for wing lice was lower than the ratio of private to shared alleles for body lice analyzed with respect to site (GLMM; Z = -6.012; p <0.001) and host bird (GLMM; Z = -8.808; p <0.001). Specifically, the probability of finding a private allele in a flock is 1.9 times higher for body lice than for wing lice. The probability of finding a private allele on a bird is 3.4 times higher for body lice than for wing lice.

Discussion

Wing lice of rock pigeons sometimes engage in indirect horizontal dispersal by attaching to hippboscid flies to move between hosts (Harbison *et al.* 2009; Harbison & Clayton 2011). However, body lice of rock pigeons do not disperse in this manner (Keirans 1975; Harbison & Clayton 2011). Based on these ecological differences, we hypothesized that body louse populations would be more genetically differentiated than wing louse populations on individual birds (infrapopulation), among birds in a single flock, and among flocks. Using microsatellite markers for these two parasite species, we found that body lice are indeed more genetically structured than wing lice at all of these spatial scales.

Overall, both wing and body louse populations exhibited a significant

amount of structure at all spatial scales examined. Most wing louse infrapopulations were genetically differentiated. More than 60% of pairwise F_{ST} values for wing louse infrapopulations were significantly different from zero. However, body louse infrapopulations had an even higher degree of genetic differentiation, with more than 90% of pairwise F_{ST} values genetically differentiated.

We compared the degree of genetic structure observed between wing and body lice on single host individuals and found that body lice had higher pairwise F_{ST} values than wing lice in 83% of comparisons. Furthermore, when we directly compared the genetic differentiation of the two species by modeling the ratio of private to shared alleles within each species across hosts sampled we found that it was 2.9 times more likely for body lice on a given bird to have a private allele than it is for wing lice to have a private allele.

We compared genetic differentiation of wing and body lice among pigeon flocks and found that both species were significantly structured. However, genetic clusters of body lice exhibited a larger degree of separation than wing lice, indicating a more genetic differentiation between sites. Moreover, when we modeled the ratio of private to shared alleles within each louse species across flocks we found that it was 1.9 times more likely for body lice than wing lice in a given flock to have a private allele, suggesting that gene flow is more restricted in body lice.

In this study, only hosts with at least 10 wing lice and 10 body lice were included. It is conceivable that birds with a higher parasite load are more social,

and possibly receive lice from other birds more often through direct contact. If this were the case, we would expect the measures in this study to be conservative estimates of genetic differentiation for each louse species. We would expect the degree of structure to be even greater between louse infrapopulations on pigeons with lower parasite loads.

Like feather lice, many other parasites are small and relatively immobile (Blasco-Costa & Poulin 2013). For these parasites, dispersal between hosts is often a major challenge. Koop *et al.* (2014) and Harper *et al.* (2015) demonstrated that Galapagos hawks and pocket gophers are "islands" for their host-specific lice, where the movement of parasites between hosts occurs only during direct physical contact. Our study further demonstrates the small spatial scale at which genetic differentiation can occur, even when parasites disperse in ways that are independent of host contact, such as by phoresis.

We found that the pigeon flocks in this study had a low, yet significant amount of genetic structure among sites less than 10.5 kilometers apart (Table 2.2). This differentiation in the bird flocks themselves is surprising considering the mobility of pigeons, which often fly > 25 km per day in search of food and water (Johnston & Janiga 1995). These results suggest that pigeons exhibit site fidelity. However, pigeons are known to also move between sites (Johnston & Janiga 1995). In our study, both the wing lice and body lice from Bird 3 at site 3 were the most genetically differentiated from other louse infrapopulations. These data suggest that Bird 3 was a recent immigrant.

If population structure were shaped mainly by vertical transmission of

parasites from parents to offspring we would expect that genetic distance matrices of the parasite and host would be correlated (van Schaik *et al.* 2014). Host and parasite genetic distances were not correlated with the host for either wing or body lice. Thus, there is no support from the genetic data that vertical dispersal is a major factor driving the structure of pigeon louse populations.

In addition to direct vertical transmission, experimental evidence shows that direct horizontal transmission occurs frequently and at equal rates among wing lice and body lice (Harbison *et al.* 2008). Thus, neither vertical nor direct horizontal transmission can account for the observed differences in genetic structure between the two parasite species. Differences in indirect horizontal transmission (i.e., phoresis) between wing and body lice, are consistent with the observed differences in population genetic structure detected at every scale in our study. Further comparisons of the genetic structure of wing and body lice in areas with and without hippoboscid flies could enhance our understanding of how this mode of dispersal influences population genetic structure.

It is likely that different evolutionary forces (drift, mutation, and gene flow), interact to influence the observed patterns of genetic differentiation (Marko & Hart 2011). For example, population size influences the strength of genetic drift in structuring populations. Thus, we examined the census population sizes of wing lice and body lice at each site in our study. We found that the size of the wing and body louse populations were not correlated with the genetic diversity of the lice. However, this snap-shot measure of louse population size does not reflect the demographic history or historical patterns of gene flow that may have

played roles in structuring louse populations. In our study wing lice were more prevalent and abundant than body lice. Consequently, body lice may experience less connectivity among metapopulations than wing lice simply because their populations are smaller. Moreover, body lice may also be more likely than wing lice to experience local extinctions because body lice have smaller infrapopulation sizes. Body lice are also unable to escape from a dying host by phoresis. It is therefore plausible that frequent bottlenecks in body louse populations, as well as limited dispersal opportunities, may have interacted to structure populations.

We assessed microevolutionary patterns of population genetic structure on a local scale in wing and body lice that co-infest rock pigeons. We found that wing lice are less genetically differentiated than body lice across all spatial scales. This pattern is consistent with earlier work that measured genetic structure of different species of wing and body lice on a larger macroevolutionary scale. Using mitochondrial data (COI) Johnson *et al.* (2002) compared genetic differentiation of wing lice species (*Columbicola*) and body lice species (*Physconelloides*) that co-occur on sympatric species of doves. The wing lice species in the study were less host-specific than the body lice species. Hippoboscid flies, the phoretic vectors of wing lice, are also less host-specific and can transport wing lice between host individuals of the same or different species (Harbison & Clayton 2011). Our study suggests that patterns of population genetic structure for wing and body lice also hold on a microevolutionary scale. Genetic differentiation and local adaptation, in turn, can

ultimately influence phylogenetic diversification. Differences in dispersal by wing and body lice in ecological time predict differences at both microevolutionary and macroevolutionary scales.

When individuals disperse they contribute to the gene pool of the new population, and population genetic structure is reduced (Bohonak 1999; Broquet & Petit 2009). However, few empirical studies have directly assessed how dispersal affects the magnitude of this structure. Moreover, little is known about how particular modes of dispersal effect population genetic structure. By examining the population differentiation of two parasite species that occupy the same host and differ primarily in a single mode of dispersal, we show that increased horizontal transmission likely erodes population genetic structure in wing lice, compared to body lice. In the future, studies that compare the genetic structure of wing and body louse populations in different geographic regions that either have, or do not have, phoretic vectors will improve our understanding of how phoresis influences population genetic structure at even greater spatial scales.

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References

Athias-Binche, F. & Morand, S. (1993). From phoresy to parasitism: the example of mites and nematodes. *Res. Rev. Parasit.*, 73-79.

Barrett, L.G., Thrall, P.H., Burdon, J.J. & Linde, C.C. (2008). Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends Ecol. Evol.*, 23, 678-685.

Bartlow, A.W., Villa, S.M., Thompson, M.W. & Bush, S.E. (2016). Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. *Int. J. Parasitol.*, 46, 221-227.

Bates, D., Machler, M., Bolker, B.M. & Walker, S.C. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, 67, 1-48.

Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Methodol.*, 57, 289-300.

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F. & Miller, A.D. (2012). Universal primers for fluorescent labelling of PCR fragments-an efficient and cost-effective approach to genotyping by fluorescence. *Mol. Ecol. Res.*, 12, 456-463.

Blasco-Costa, I. & Poulin, R. (2013). Host traits explain the genetic structure of parasites: a meta-analysis. *Parasitology*, 140, 1316-1322.

Bohonak, A.J. (1999). Dispersal, gene flow, and population structure. *Q. Rev. Biol.*, 74, 21-45.

Broquet, T. & Petit, E.J. (2009). Molecular estimation of dispersal for ecology and population genetics. *Annu. Rev. Ecol. Evol. Syst.*, 40, 193-216.

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, 83, 575-583.

Chun-Lee, J., Tsai, L.-C., Kuan, Y.-Y., Chien, W.-H., Chang, K.-T., Wu, C.-H. *et al.* (2007). Racing pigeon identification using STR and chromo-helicase DNA binding gene markers. *Electrophoresis*, 28, 4274-4281.

Clayton, D.H., Bush, S.E. & Johnson, K.P. (2016). *Coevolution of Life on Hosts: Integrating Ecology and History*. Univ. of Chicago Press.

Clayton, D.H. & Drown, D.M. (2001). Critical evaluation of five methods for quantifying chewing lice (Insecta : Phthiraptera). *J. Parasitol.*, 87, 1291-1300.

Clobert, J., Danchin, E., Dhondt, A.A. & Nichols, J.D. (2001). *Dispersal*. Oxford Univ. Press, Oxford.

Clobert, J., M. Baguette, Benton, T.G. & J. M. Bullock (2012). *Dispersal Ecology and Evolution*. Oxford Univ. Press, Oxford

Criscione, C.D. (2008). Parasite co-structure: Broad and local scale approaches. *Parasite Paris Fr.*, 15, 439-443.

Criscione, C.D., Poulin, R. & Blouin, M.S. (2005). Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Mol. Ecol.*, 14, 2247-2257.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinform.*, 1, 47-50.

Faircloth, B.C. (2008). Msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol. Ecol. Res.*, 8, 92-94.

Fernandez-Silva, I., Whitney, J., Wainwright, B., Andrews, K.R., Ylitalo-Ward, H., Bowen, B.W. *et al.* (2013). Microsatellites for next-generation ecologists: a post-sequencing bioinformatics pipeline. *Plos One*, 8. https://doi.org/10.1371/journal.pone.0055990

Harbison, C.W., Bush, S.E., Malenke, J.R. & Clayton, D.H. (2008). Comparative transmission dynamics of competing parasite species. *Ecology*, 89, 3186-3194.

Harbison, C.W. & Clayton, D.H. (2011). Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proc. Natl Acad. Sci.*, 108, 9525-9529.

Harbison, C.W., Jacobsen, M.V. & Clayton, D.H. (2009). A hitchhiker's guide to parasite transmission: The phoretic behaviour of feather lice. *Int. J. Parasitol.*, 39, 569-575.

Harper, S.E., Spradling, T.A., Demastes, J.W. & Calhoun, C.S. (2015). Host behaviour drives parasite genetics at multiple geographic scales: population genetics of the chewing louse, *Thomomydoecus minor*. *Mol. Ecol.*, 24, 4129-4144.

Hedrick, P.W. (2005). A standardized genetic differentiation measure. *Evolution*, 59, 1633-1638.

Houck, M.A. & OConnor, B.M. (1991). Ecological and evolutionary significance of phoresy in the Astigmata. *Annu. Rev. Entomol.*, 36, 611-636.

Johnson, K.P., Adams, R.J. & Clayton, D.H. (2001). Molecular systematics of Goniodidae (Insecta : Phthiraptera). *J. Parasitol.*, 87, 862-869.

Johnson, K.P. & Clayton, D.H. (2003). Coevolutionary hisory of ecological replicates: Comparing phylogenies of wing and body lice to Columbiform hosts. In: *Tangled Trees: Phylogenies, Cospeciation and Coevolution* (ed. Page, RDM). The Univ. of Chicago Press, Chicago, pp. 262 - 285.

Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J. & Clayton, D.H. (2002). The population genetics of host specificity: genetic differentiation in dove lice (Insecta : Phthiraptera). *Mol. Ecol.*, 11, 25-38.

Johnston, R.F. & Janiga, M. (1995). *Feral Pigeons*. Oxford Univ. Press, New York.

Jombart, T. & Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070-3071.

Jombart, T., Devillard, S. & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.*, 11.

Jost, L. (2008). G(ST) and its relatives do not measure differentiation. *Mol. Ecol.*, 17, 4015-4026.

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W. & Prodohl, P.A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods Ecol. Evol.*, 4, 782-788.

Keirans, J.E. (1975). A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). *J. Med. Entomol.*, 12, 71-76.

Kirkness, E.F., Haas, B.J., Sun, W.L., Braig, H.R., Perotti, M.A., Clark, J.M. *et al.* (2010). Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proc. Natl Acad. Sci.*, 107, 12168-12173.

Koop, J.A.H., DeMatteo, K.E., Parker, P.G. & Whiteman, N.K. (2014). Birds are islands for parasites. *Biol. Lett.*, 10. http://dx.doi.org/10.1098/rsbl.2014.0255

Mantel, N. (1967). Detection of disease clustering and a generalized regression approach. *Cancer Res.*, 27, 209-&.

Marko, P.B. & Hart, M.W. (2011). The complex analytical landscape of gene flow

inference. *Trends Ecol. Evol.*, 26, 448-456.

Maze-Guilmo, E., Blanchet, S., McCoy, K.D. & Loot, G. (2016). Host dispersal as the driver of parasite genetic structure: a paradigm lost? *Ecol. Lett.*, 19, 336-347.

Meirmans, P.G. (2015). Seven common mistakes in population genetics and how to avoid them. *Mol. Ecol.*, 24, 3223-3231.

Meirmans, P.G. & Van Tienderen, P.H. (2004). GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes*, 4, 792-794.

Nadler, S.A. (1995). Microevolution and the genetic-structure of parasite populations. *J. Parasitol.*, 81, 395-403.

Nelson, B., Murray, M. (1971). The distribution of Mallophaga on the domestic pigeon (Columba livia). *Int. J. Parasitol.*, 1, 21-29.

Papadopoulou, A. & Knowles, L.L. (2016). Toward a paradigm shift in comparative phylogeography driven by trait-based hypotheses. *Proc. Natl Acad. Sci.*, 113, 8018-8024.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.Rproject.org/.

Raymond, M. & Rousset, F. (1995). Genepop (version-1.2) - population-genetics software for exact tests and ecumenicism. *J. Hered.*, 86, 248-249.

Roubik, D.W. & Wheeler, Q.D. (1983). Flightless beetles and stingless bees: Phoresy of scotocryptine beetles (Leiodidae) on their meliponine hosts (Apidae). *J. Kans. Entomol.*, 55, 125-135.

Rozen, S. & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* (Clifton, N.J.), 132, 365-386.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotech.*, 18, 233-234.

Selkoe, K.A. & Toonen, R.J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.*, 9, 615-629.

Stringham, S.A., Mulroy, E.E., Xing, J., Record, D., Guernsey, M.W., Aldenhoven, J.T. *et al.* (2012). Divergence, convergence, and the ancestry of feral populations in the domestic rock pigeon. *Curr. Biol.*, 22, 302-308.

Traxler, B., Brem, G., Muller, M. & Achmann, R. (2000). Polymorphic DNA microsatellites in the domestic pigeon, Columba livia var. domestica. *Mol. Ecol.*, 9, 366-368.

Treat, A.E. (1956). A pseudoscorpion on moths. *Lepid. News*, 10, 87-89.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*, 4, 535-538.

Van Schaik, J., Kerth, G., Bruyndonckx, N. & Christe, P. (2014). The effect of host social system on parasite population genetic structure: comparative population genetics of two ectoparasitic mites and their bat hosts. *BMC Evol. Biol.*, 14.

Whiteman, N.K., Kimball, R.T. & Parker, P.G. (2007). Co-phylogeography and comparative population genetics of the threatened Galapagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Mol. Ecol.*, 16, 4759-4773.

Zeh, D.W. & Zeh, J.A. (1992). On the function of harlequin beetle-riding in the pseudoscorpion, Cordylochernes scorpioides. *J. Arachnol.*, 20, 47-51

Table 2.1. Analysis of molecular variance for wing lice, body lice and pigeons.

Source of Variation	% Variation	Fixation Indices	P value
Wing lice			
Among infrapopulations between flocks	0.6	F_{ST} = 0.07484	p<0.001
Among infrapopulations within flocks	6.8	F_{SC} = 0.06880	p<0.001
Infrapopulations on individual birds	92.5	F_{CT} = 0.00649	p<0.05
Body lice			
Among infrapopulations between flocks	1.4	F_{ST} = 0.22542	p<0.001
Among infrapopulations within flocks	21.2	F_{SC} = 0.21478	p<0.001
Infrapopulations on individual birds	77.5	F_{CT} = 0.01355	p<0.05
Pigeon			
Among Flocks	1.2	F_{ST} = 0.01206	p<0.001
Within Flocks	98.8		

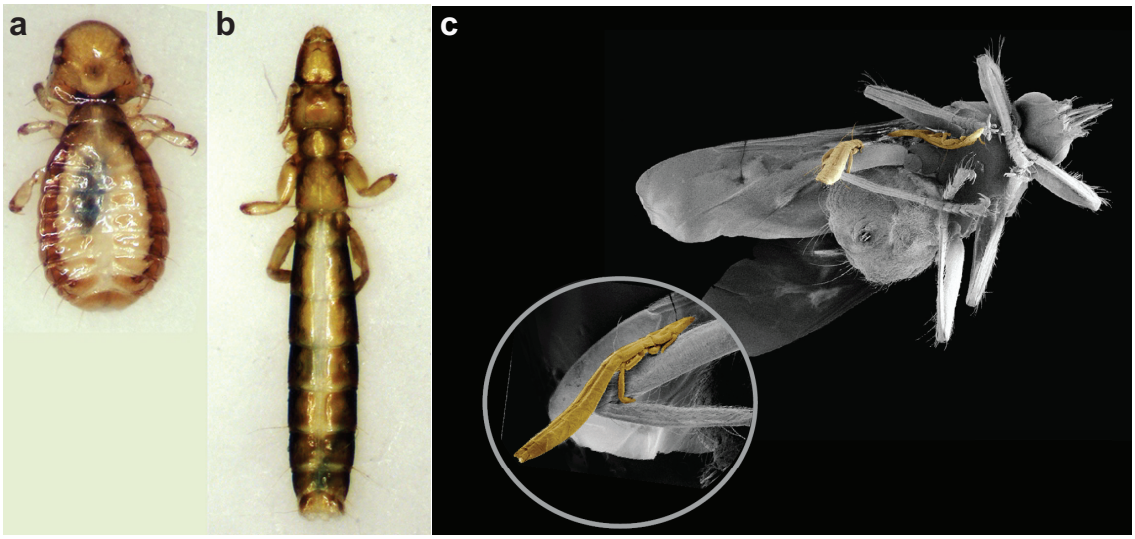

Figure 2.1. The pigeon louse study system. (a) The body louse, *Campanulotes compar*. (b) The wing louse, *Columbicola columbae*. (c) Wing lice engage in phoresis or “hitchhiking” on the Hippoboscid fly (*Pseudolynchia canariensis*) SEM (Harbison & Clayton 2011).

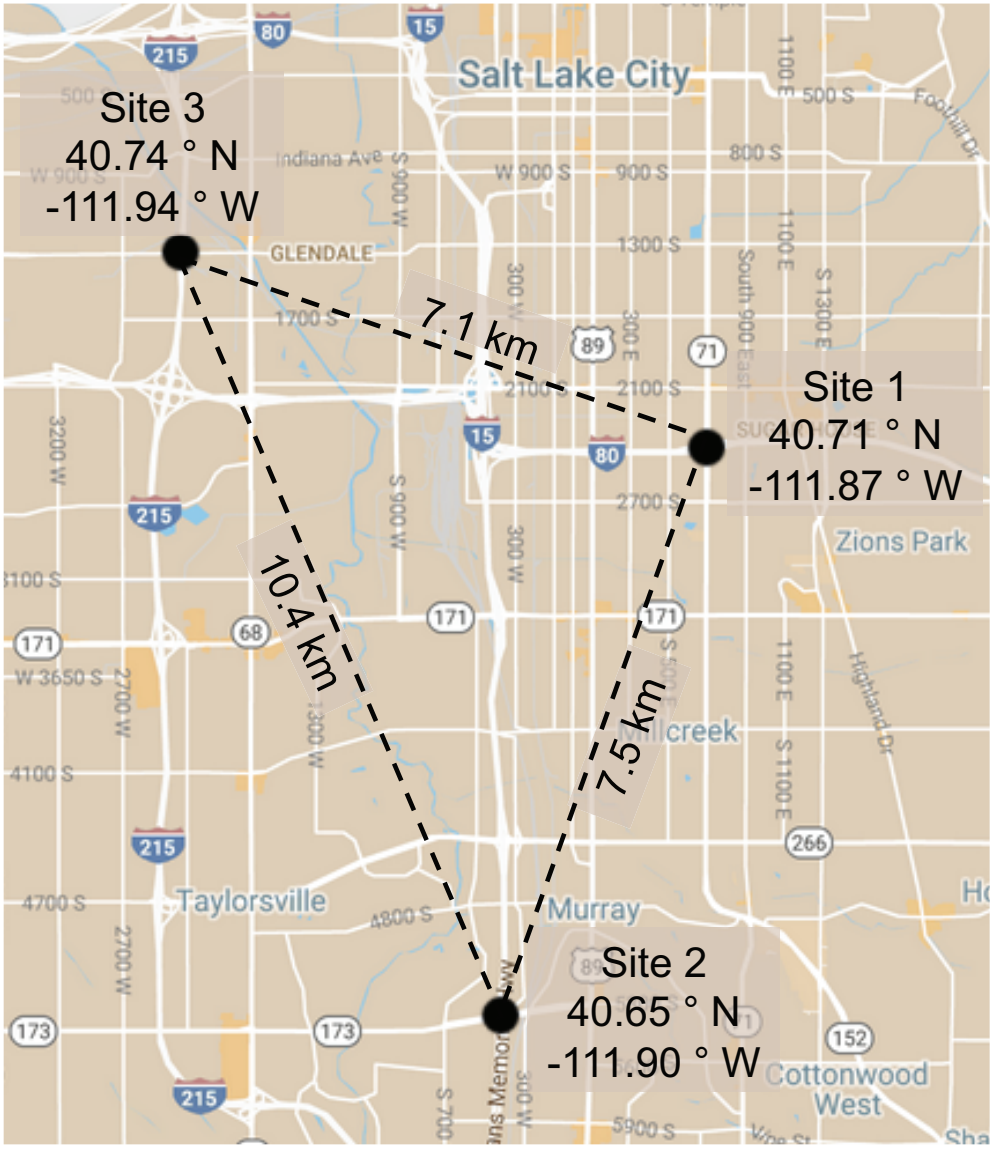

Figure 2.2. Map of sampling sites in Salt Lake City, Utah.

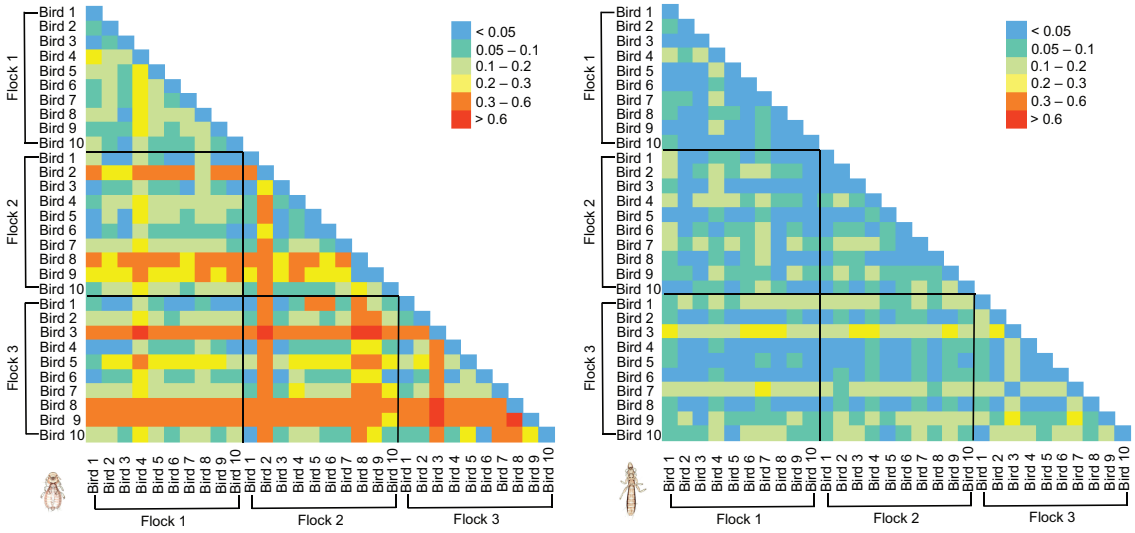

Figure 2.3. Matrix of pairwise F_{ST} values between genotypes of body louse infrapopulations (left) and wing lice infrapopulations (right). Warmer colors indicate a larger amount of genetic differentiation between comparison.

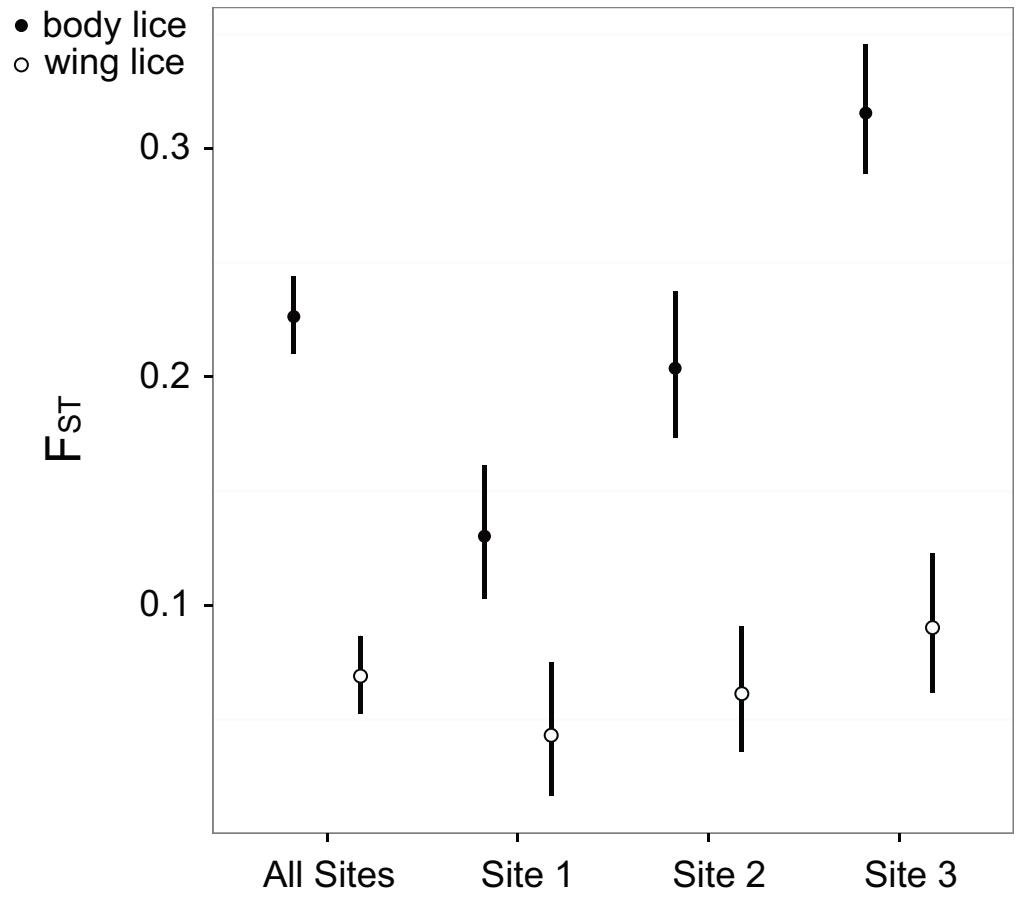

Figure 2.4. Global F_{ST} values for wing and body lice infrapopulations at all sites and within each site. Bars on the graphs represent 95% confidence intervals obtained from bootstrapping.

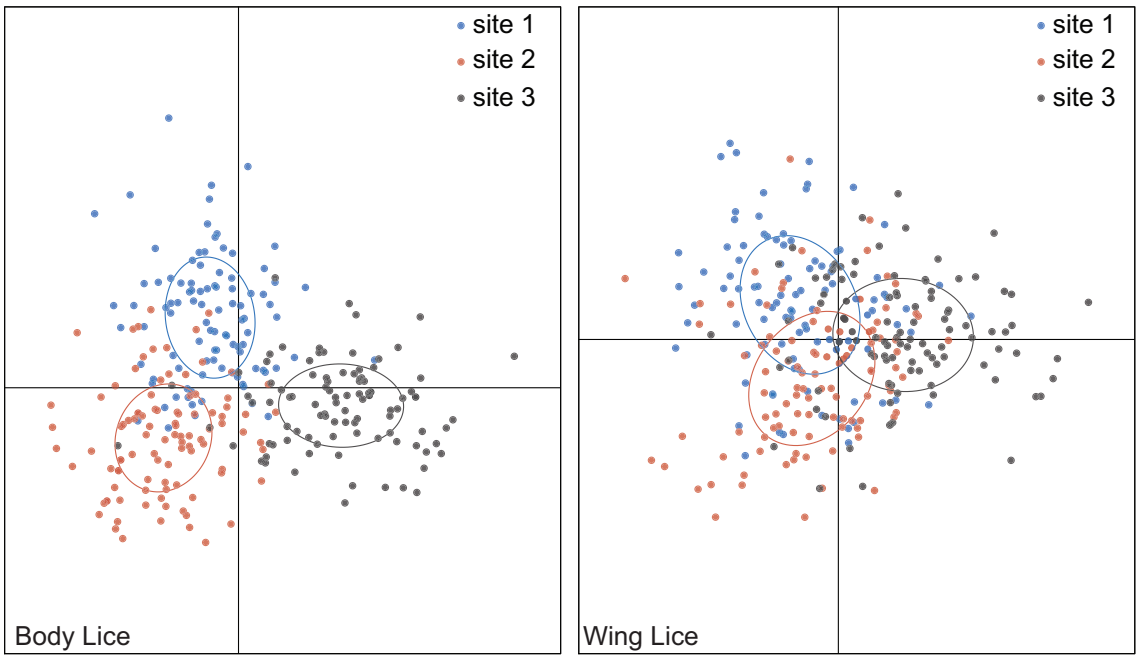

Figure 2.5. Ordination plots showing the first two principal components of the DAPC for body lice (left) and wing lice (right). Colors indicate which flock of birds each louse was collected from (Site 1 is blue, Site 2 is red, Site 3 is gray) and dots represent genotypes of individual lice. Circles represent confidence intervals of the DAPC.

CHAPTER 3

TEMPERATURE DEPENDENT DISTRIBUTION PATTERNS AND LOW POPULATION GENETIC STRUCTURE IN THE DIPTERAN VECTOR, *PSEUDOLYNCHIA CANARIENSIS*

Abstract

Invasive species may be successful in part because they escape their native parasites and pathogens. This phenomenon, termed “enemy release,” can occur either because parasites are not introduced along with their hosts, or because parasites fail to establish in the invasive range. Although the spread and distribution of invasive hosts has received much attention, relatively little is known about the distributions of their parasites. Tracking distributions of parasites and pathogens is complicated, in part, because they are often difficult to census and identify, and because they can be patchily distributed, both in space and time. In this study we assess the spatial distribution of the pigeon lousefly (*Pseudolynchia canariensis*). The distribution and dispersal of *P. canariensis* has implications for the distributions of other parasites because it is an important vector of the malarial blood parasite, *Haemoproteus columbae*. It is also a phoretic vector of five species of mites and one species of feather louse. We assessed spatial genetic structure of *P. canariensis* across its geographic

range to examine population connectivity. We found no flies or published geographic records of the fly in extreme latitudes (north of 45.5 °N, or south of 33 °S), even though rock pigeons occur in those areas. Extended periods of cold temperature (<5°C) in these latitudes may limit the fly's distributions, given that we found a positive correlation between fly prevalence and temperature. We found little divergence in fly mitochondrial genes, suggesting high rates of gene flow between flies on different host populations. Also, nine flies were found in phoretic association with Epidermoptid mites (Acari: Astigmata). Pigeon populations in extreme latitudes appear to represent areas where *P. canariensis* is absent. This absence may impact pigeons and the community of organisms they host in various ways.

Introduction

Birds are host to communities of parasites, pathogens, commensal and mutualist organisms (Poulin & Morand 2000; Dobson *et al.* 2008). The geographic distributions of these symbionts does not always mirror that of their hosts; their ranges can vary over large and small spatial scales (Poulin 2006; Hoberg & Brooks 2008). Studies assessing spatial host-parasite ecology consider movement patterns of both the host and the parasite because these patterns are critical for examining eco-evolutionary dynamics of host-parasite interactions (Kubisch *et al.* 2014; Harper *et al.* 2015; Boulinier *et al.* 2016; Engelbrecht *et al.* 2016; Maze-Guilmo *et al.* 2016; Bonte & Dahirel 2017). An indirect way to measure movement and dispersal is through studies of population

genetic differentiation (Broquet & Petit 2009; Kim & Sappington 2013).

Host-parasite genetic co-structure has been measured in a variety of bird and mammal systems. One of the best known systems is that of colonial seabirds and their ticks. *Ixodes uriae* is a globally distributed parasite of many seabird species. Patterns of genetic structure on a global scale correspond to biogeographic colonization patterns beginning in the early Miocene (22 Million years ago) as well as host life history traits (McCoy *et al.* 2005; Dietrich *et al.* 2014). Examining *I. uriae's* global distribution patterns also allows for the inference of disease spread, as this hard tick vectors the bacterium (*Borrelia burgdorferi)*, which is responsible for Lyme disease in animals (Duron *et al.* 2016). The genetic structure of vector populations provides other clues to how parasites and pathogens may spread. For example, Streicker *et al.* (2016) used large datasets on vampire bats and rabies to connect sex-biased dispersal of bats to the spread of rabies across Latin America (Streicker *et al.* 2016).

Introduced species may or may not bring their parasites with them to their new range (Clay 2003; Colautti *et al.* 2004; Liu & Stiling 2006; MacLeod *et al.* 2010; Marzal *et al.* 2011). Parasites may "miss the boat," meaning that by chance they are not introduced along with their hosts. They may also "drown on arrival," meaning that they fail to establish viable populations once they arrive in the new range (Marzal *et al.* 2011). Low levels of genetic variation do not limit invasion success in many invasive species (Barrett & Schluter 2008; Prentis *et al.* 2008; Cheng *et al.* 2016). However, less is known about how life history traits and environmental gradients affect invasion success (Bock *et al.* 2015). In this

study we assess the spatial scale of an ectoparasite's range on an invasive and globally distributed host. We do this by surveying published records, collecting samples from host populations and by assessing spatial genetic structure.

For ectoparasites, geographic distribution may be limited by abiotic factors because they are exposed to the external environment. For example in the Eurasian otter (*Lutra lutra*), tick (*Ixodes hexagonus*) abundance is correlated with climate patterns (North Atlantic Oscillation). More ticks are associated with warmer and wetter weather (Sherrard-Smith *et al.* 2012). Temperature also limits the distribution of mites (*Ornithonyssus sylviarum*) on chickens (De La Riva *et al.* 2015). Additionally, relative humidity influences louse abundance on birds; birds in arid areas have fewer lice than in humid areas (Moyer *et al.* 2002). Here we examine if two abiotic factors (temperature and humidity) influence the distribution of a parasite on an invasive host.

Study System

In this study we focus on a parasite of feral pigeons (*Columba livia*), which are globally distributed birds. Their wild ancestors likely originated in Northern Africa and the Mediterranean and were domesticated about 3,000–5,000 years ago (Johnston & Janiga 1995; Driscoll *et al.* 2009). Escaped domestic pigeons then established feral populations in the Old World and were introduced to North America around 400 years ago (Johnston & Janiga 1995). Pigeons are commonly infested with various species of arthropods, bacteria, protozoa and fungi (Johnston & Janiga 1995) but the geographical distribution of many of these

organisms is unknown.

The hippoboscid fly, *Pseudolynchia canariensis* (Macquart), is a common blood-feeding parasite of pigeons (*Columba livia*) and other birds (Maa 1966). This fly, commonly called the “pigeon fly,” is thought to have originated in the Old World with pigeons and was first reported in in North America in 1896 (Bishopp 1929). Currently, it is unclear if the fly’s range mirrors that of pigeons or if it is absent in areas where feral pigeon populations are found. Determining the known range of *P. canariensis* may help to elucidate how parasites of invasive species track, or do not track, their hosts.

Pigeon flies are dorso-ventrally flattened and move swiftly through feathers. Although pigeons are the preferred hosts for these flies, *P. canariensis* is not host specific; it has been recorded from 33 bird genera that span 13 families and eight orders of birds (Maa 1966). Most studies assessing fly ecology do so using pigeon hosts. Flies spend about 70% of their time on pigeons (Waite *et al.* 2014) and the rest of their time off the host. Blood meals are taken several times a day, during which flies can feed for up to 80 minutes (Arcoverde *et al.* 2009). Adult flies found off pigeons are typically mating or depositing pupae. Female flies deposit pupae every 2-3 days in or around pigeon nests (Bishopp 1929). Female flies are often followed by mate guarding males when depositing pupae (Coatney 1931; Yuval 2006). Off of the host, flies can easily fly between individual pigeons, between populations of pigeons, and between host species (Harbison *et al.* 2009; Harbison & Clayton 2011; Clayton *et al.* 2016).

Bishop (1929) reported that *P. canariensis* likely inhabits the tropics and warm temperate regions of the world. Other researchers have speculated that cold temperatures at extreme latitudes prevent over-winter survival of the pupal stages of these flies. In an experimental study, Klei & Degiusti (1975a) observed that temperatures of 13°C and 37 °C were lethal to *P. canariensis* pupae in a laboratory setting. They also described the optimum temperature range for maintaining adult flies in the laboratory to be between 26.6°C and 30°C. These data suggest that abiotic factors, such as temperature, may constrain fly distribution.

The range and dispersal of *P. canariensis* is also important for several members of the pigeon community. The pigeon lousefly is the only known vector of the malarial blood parasite, *Haemoproteus columbae* (Valkiunas 2005), as well as a phoretic vector of five mite species and one louse species (Philips & Fain 1991; Sol *et al.* 2000; Bartlow *et al.* 2016). *H. columbae* is an intracellular parasite that infects the red blood cells of pigeons. Asexual reproduction of *H. columbae* takes place in the pigeon while sexual reproduction takes place in *P. canariensis* (Valkiunas 2005). Both male and female flies ingest infected pigeon blood and transmit *H. columbae* between hosts. *H. columbae* is not transmitted transovarially (Valkiunas 2005; Santiago-Alarcon *et al.* 2012). Thus, we predict that prevalence of *H. columbae* will be closely tied to that of its vector.

Four avian skin mites in the family Epidermoptidae (*Myialges anchora*, *M. falconis*, *M. lophortyx*, *M. macdonaldi)* and one mite in the family Cheyletiellidae (*Ornithocheyletia hallae*) are frequently found in association with *P. canariensis*

(Feres & Flechtmann 1991; Philips & Fain 1991; Macchioni *et al.* 2005). Phoresy is a behavior in which one organism disperses by “hitching a ride” on another more mobile organism (Farish & Axtell 1971). In *Myialges spp.* gravid female mites often attach to louseflies to oviposit and fasten eggs to the fly’s cuticle (Fain, 1965). Eclosed mites then disperse from the fly to the skin of host birds (Evans *et al.* 1963). It is thought that some skin mites require flies to complete their life cycle (Evans *et al.* 1963).

Wing lice (*Columbicola columbae*) that parasitize pigeons are regularly found engaging in phoresis with *P. canariensis*. Wing lice are permanent, obligate parasites that complete their entire life cycle on pigeons. Wing lice are highly immobile off of the host (Bartlow *et al.* 2016) and are typically limited to dispersal between pigeons that are in direct physical contact (Harbison *et al.* 2008). Lab and field studies have shown that wing lice can disperse on *P. canariensis* to novel host pigeons and novel host species in sufficient numbers to establish new populations (Harbison *et al.* 2008; Harbison & Clayton 2011). Therefore determining the geographic range of *P. canariensis* may also have important implications for pigeon associated community interactions.

Finally, since *P. canariensis* is highly mobile and not host specific we expect the fly to have a large degree of population connectivity across its range. Since the fly is not host specific we expect that it will be able to follow pigeons throughout their invasive range. We test this hypothesis by assessing population genetic structure for flies sampled from pigeon populations across a large spatial scale.

Materials and Methods

Literature search

To determine the known geographic distribution of *P. canariensis* we queried the Scopus database, the ISI Web of Science database and Google Scholar during September of 2015 and April of 2016. We used the following search terms: "*Pseudolynchia canariensis,"* "*Pseudolynchia"* and "pigeon lousefly." The following taxonomic synonyms for *P. canariensis* were also used as search terms: *P. maura*, *Olfersia testacea*, *O. rufipes*, *O. falcinelli*, *O. maura*, *O. lividicolor*, *O. capensis*, *O. exornata*, *Lynchia simillima* (Maa, 1966). In total, 271 relevant studies were examined dating from 1839 to 2016 that included *P. canariensis* (or taxonomic synonyms).

Sample collection

Samples were collected from pigeons by fumigating live birds with ethyl acetate, or washing euthanized birds (Clayton and Drown 2001). Overall, 256 pigeons from 18 locations across the world were examined (Table 3.1). Fly samples were preserved in 95% ethanol and identified to species under an Olympus SZCTV stereoscope.

Using blood parasites as a proxy for louseflies

H. columbae prevalence has been shown to be correlated with fly prevalence (Sol et al. 2000). Flies are mobile parasites and are patchily distributed in time and space. Among discrete pigeon flocks in Salt lake City,

Utah, flies are present at some but not all flock sites (Harbison *et al.* 2008). Among pigeon flocks in Detroit, Michigan, fly populations increase during late summer and reach a peak in the fall, and continue to be present in low numbers during the winter (Klei & Degiusti 1975b). In contrast, pigeons can have chronic *H. columbae* infections that persist even when flies are not apparent. For example, 754 pigeons were examined for parasites over a period of 27 months, throughout 1966 – 1968, in Detroit, Michigan (Klei & Degiusti 1975b). Over 197 flies were collected from 16% of pigeons, while 78% of the pigeons were infected with *H. columbae* (Klei & Degiusti 1975b). More than 20 studies have documented *H. columbae* in feral pigeon populations (Waite 2012). To confirm that *P. canariensis* occurred in pigeon flocks we screened birds for *H. columbae*. Since *P. canariensis* is the only known vector of *H. columbae,* the blood parasite served as a “smoking gun” indicating that the birds in question had been parasitized by the fly at some point.

A total of 149 pigeons at nine sites were screened for *H. columbae*. For each pigeon caught, 10μL of blood was sampled by brachial venipuncture with a heparinized capillary tube. Blood smears were made on microscope slides with two blood dots from each tube. Slides were fixed with methanol, then stained with Giemsa. Slides were examined under oil immersion at 1000x with a light microscope. One slide per host was screened for malaria by examining all red blood cells in 1 microscopic field, and counting infected cells per 100 non-overlapping fields (Valkiunas 2005).

DNA sequencing and population analyses

Subsamples of *P. canariensis* specimens collected at each sampling locality were used in molecular analyses. For 85 flies, DNA was extracted from a single hind leg using the Qiagen DNeasy Blood and Tissue Kit. Fragments of the mitochondrial 12S ribosomal RNA (12S), 16S ribosomal RNA (16S), cytochrome b (Cytb) and cytochrome c oxidase subunit I (COI) genes were sequenced for a subset of specimens. A 1224 bp COI fragment was PCR amplified with the primer pairs LCO1490 and HCO2198 (Folmer *et al.* 1994) and COI-2F and COI-2R (Simon *et al.* 1994). A 218 bp 12S fragment was PCR amplified with the primer pair 12SAI and 12SBI (Simon *et al.* 1994). A 383 bp16S fragment was PCR amplified with the primer pair 16sf and 16sr (Simon *et al.* 1994). A 425 bp Cytb fragment was PCR amplified with the primer pair L11122 and H11823 (Page *et al.* 1998). Sequences were assembled, edited and aligned in Geneious v7.1.6 (Kearse *et al.* 2012).

All genes were analyzed separately as well as in a concatenated 2525 bp alignment. Minimum-spanning haplotype networks were constructed with statistical parsimony software, TCS (Clement *et al.* 2000), implemented in the package PopART (Leigh & Bryant 2015). Haplotype and nucleotide diversity were calculated in DnaSP 5.10.1 (Librado & Rozas 2009).

Environmental variables

We tested whether mean annual humidity, or annual minimum temperature was correlated with the presence of flies. Climate data were

obtained from climatemps.com. Statistical analyses were conducted in JMP v.12.1.

Results

Lousefly distribution

Fifty publications included geographic records for the pigeon louse fly (Appendix A). All published geographic records of the fly occurred between the latitudes of 45.5 °N and 33 °S (Figure 3.1). All published geographic records of *H. columbae* also occurred within this range.

For this paper 184 new *P. canariensis* specimens were collected in 2014-15 from 29 pigeons across seven sites in North America, one site in India, and one site in Brazil (Table 3.1). Fly prevalence ranged from 0 – 100%. In areas where the fly was present, mean abundance ranged from 0.1 – 17.6 (Table 3.1). Most pigeons (82%) infested with flies had 1 – 4 flies per host. Two pigeons from Guarujá, Brazil harbored the most flies, with 40 and 80 *P. canariensis* individuals. No flies were collected from pigeons in Ružomberok, Slovakia; Regina, Saskatchewan; Winnipeg, Manitoba; Grand Forks, North Dakota; Seattle, Washington; Laramie, Wyoming or Albuquerque, New Mexico, USA.

No flies or *H. columbae* were found in Laramie, Wyoming; Regina, Saskatchewan; Seattle, Washington, or Winnipeg, Manitoba. At two sites flies were not collected, but *H. columbae* infected red blood cells were found (Albuquerque, New Mexico and Salt Lake City, Utah A). Epidermoptid Mites (Acari: Astigmata: Epidermoptidae) were found riding on flies in Guarujá, São

Paulo, Brazil and New Orleans, Louisiana (Table 3.2). Most mites were attached to the abdomens of the flies. Two flies vectored mites with egg sacs.

Population genetic analysis

Haplotype networks showed limited population subdivision (Figure 3.2) as variation in *P. canariensis* mtDNA sequences was low (Table 3.3). The mtDNA network from the concatenated data set of all genes (Figure 3.2e) revealed divergence between North and South American fly haplotypes. Flies collected in São Paulo exhibited the most within population subdivision, followed by flies collected in Louisiana.

Environmental variables

Fly prevalence was positively correlated with average annual minimum temperature (Spearman rank correlation: $r_s = 0.68$, $P = 0.0015$; Figure 3.3) but not with average annual relative humidity (Spearman rank correlation: $r_s = 0.41$, $P = 0.0820$). No flies or *H. columbae* infected red blood cells were found in areas where the average annual minimum temperature was below 5˚C.

Discussion

P. canariensis is not found throughout the entire invasive range of its host, the feral pigeon. No flies, *H. columbae* infected red blood cells, or published records of the fly or *H. columbae,* were found in areas north of 45.5 °N, or south of 33 °S. Although the pigeon host is present outside this zone, flies are likely

constrained to areas without prolonged cold spells below 5˚C. Future studies could track spatial distributions of other pigeon parasites to identify areas where host-parasite pressures differ.

Fly populations showed low population differentiation across this broad geographic scale. In temperate areas of the globe the patchy distribution and seasonality of the fly may increase the chance of local extinctions and generate population structure at small spatial scale. However genetic differentiation is likely eroded at this large scale given the fly's high mobility and low host specificity. It is not likely that pigeon louseflies "missed the boat" upon introductions to temperate and tropical regions of the New world given their low genetic differentiation across a large spatial scale. However, since *P. canariensis* has been reported from over 30 bird species in addition to feral pigeons and likely has high gene flow between populations it is surprising that these flies "drown on arrival," in extreme latitudes.

The genetic variation observed in this study is similar to previous studies of genetic variation in hippoboscid flies. Relatively low mitochondrial variation was found in three lousefly species parasitizing birds in the Galápagos Islands. Gene flow was found to be very high in *Olfersia spinifera* which parasitizes great frigatebirds (*Fregata minor*), *Olfersia aenescens* which parasitizes Nazca boobies (*Sula granti*), and *Icosta nigra* which parasitizes the Galápagos hawk (*Buteo galapagoensis*) (Whiteman *et al.* 2007; Levin & Parker 2013).

Furthermore, two species of wingless Nycteribiid bat flies also exhibited low levels of genetic differentiation among populations. Both the host specific bat

fly, *Nycteribia schmidlii,* which parasitizes the bent-winged bat (*Miniopterus schreibersii)* and the bat fly, *Cyclopodia horsfieldi*, which parasitizes multiple *Pteropus* bat species exhibited extremely low levels of population genetic structure across a wide geographic range (Olival *et al.* 2013; Witsenburg *et al.* 2015).

For at least two kinds of pigeon parasites, lice and flies, abundance is higher in warm, humid regions. It would be interesting to test if increased parasite pressure in the tropics differentially impacts community interactions. For example in Guarujá, Brazil one pigeon hosted over 80 flies and three species of lice. It is likely phoresis of lice on flies occurs more in areas where flies are more abundant. It is also possible that flies infected with *H. columbae* may take blood meals on flies more frequently, increasing opportunities for phoresis of mites and lice. Waite *et al*. (2012) showed that *P. canariensis* females infected with *H. columbae* had lower fitness than male flies. The authors suggested that female flies might feed more to compensate for the costs associated with vectoring *H. columbae* as other blood parasites increase feeding habits of dipteran vectors.

P. canariensis may influence the evolutionary trajectories of the organisms it disperses by enhancing gene flow and population connectivity. In several areas in northern latitudes where flies were absent we found *Myialges spp.* and *Columbicola columbae* infesting pigeons. Future studies could examine the genetic structure of these mites and lice in areas with and without flies to examine the role vector mediated phoresis plays in shaping the spatial distribution of species (Chapter 4).

Pigeons established feral populations in North America around 400 years ago (Johnston & Janiga 1995). Several species of parasites were introduced along with the pigeons, including feather lice, flies and blood parasites. However, pigeon parasites may be constrained by abiotic factors that don't necessarily affect pigeons in invasive ranges. Thus, through introduction to ranges where their parasites cannot survive, invasive species can experience “enemy release” from their native parasites and pathogens. The success of some invasive species is attributed to this phenomenon (Torchin et al. 2003). It is unclear if release from flies and the associated *Hemoproteus* blood parasites has contributed to the success of pigeons in colder regions, in part because the cost of these parasites to the pigeon host is unclear (Waite 2012). Future studies should investigate this hypothesis. Additionally the absence of the pigeon lousefly in the host’s rage may influence host associated community interactions.

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References

Arcoverde, A.R., Soares Ferreira Rodrigues, A.F. & Daemon, E. (2009). Feeding and breeding aspects of Pseudolynchia canariensis (Macquart, 1839) (Diptera, Hippoboscidae) under laboratory conditions. *Parasitol. Res.*, 104, 277-280.

Barrett, R.D.H. & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends Ecol. Evol.*, 23, 38-44.

Bartlow, A.W., Villa, S.M., Thompson, M.W. & Bush, S.E. (2016). Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. *Int. J. Parasitol.*, 46, 221-227.

Bishopp, F.C. (1929). The pigeon fly - An important pest of pigeons in the United States. *J. Econ. Entomol.*, 22, 974-980.

Bock, D.G., Caseys, C., Cousens, R.D., Hahn, M.A., Heredia, S.M., Hubner, S. *et al.* (2015). What we still don't know about invasion genetics. *Mol. Ecol.*, 24, 2277-2297.

Bonte, D. & Dahirel, M. (2017). Dispersal: a central and independent trait in life history. *Oikos*, 126, 472-479.

Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A. *et al.* (2016). Migration, prospecting, dispersal? What host movement matters for infectious agent circulation? *Intgr. Comp. Biol.*, 56, 330-342.

Broquet, T. & Petit, E.J. (2009). Molecular Estimation of Dispersal for Ecology and Population Genetics. *Annu. Rev. Ecol. Evol. Syst.*, 40, 193-216.

Cheng, D.F., Liang, G.W. & Xu, Y.J. (2016). Meta-analysis reveals asymmetric reduction in the genetic diversity of introduced populations of exotic insects. *Biol. Invas.*, 18, 1163-1175.

Clay, K. (2003). Conservation biology: Parasites lost. *Nature*, 421, 585-586.

Clayton, D.H., Bush, S.E. & Johnson, K.P. (2016). *Coevolution of Life on Hosts: Integrating Ecology and History*. Univ. of Chicago Press.

Clayton, D.H. & Drown, D.M. (2001). Critical evaluation of five methods for quantifying chewing lice (Insecta : Phthiraptera). *J. Parasitol.*, 87, 1291-1300.

Clement, M., Posada, D. & Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies. *Mol. Ecol.*, 9, 1657-1659.

Coatney, G. (1931). On the biology of the pigeon fly, Pseudolynchia maura Bigot

(Diptera, Hippoboscidae). *Parasitology*, 23, 525–532.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecol. Lett.*, 7, 721-733.

De La Riva, D.G., Soto, D. & Mullens, B.A. (2015). Temperature governs on-host distribution of the northern fowl mite, Ornithonyssus sylviarum (acari: macronyssidae). *J. Parasitol.*, 101, 18-23.

Dietrich, M., Kempf, F., Boulinier, T. & McCoy, K.D. (2014). Tracing the colonization and diversification of the worldwide seabird ectoparasite Ixodes uriae. *Mol. Ecol.*, 23, 3292-3305.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F. & Jetz, W. (2008). Homage to Linnaeus: How many parasites? How many hosts? *Proc. Natl Acad. Sci. USA*, 105, 11482-11489.

Driscoll, C.A., Macdonald, D.W. & O'Brien, S.J. (2009). From wild animals to domestic pets, an evolutionary view of domestication. *Proc. Natl Acad. Sci. USA*, 106, 9971-9978.

Duron, O., Cremaschi, J. & McCoy, K.D. (2016). The high diversity and global distribution of the intracellular bacterium *Rickettsiella* in the polar seabird tick *Ixodes uriae*. *Microb. Ecol.*, 71, 761-770.

Engelbrecht, A., Matthee, S., du Toit, N. & Matthee, C.A. (2016). Limited dispersal in an ectoparasitic mite, Laelaps giganteus, contributes to significant phylogeographic congruence with the rodent host, Rhabdomys. *Mol. Ecol.*, 25, 1006-1021.

Evans, G.O., Fain, A. & Bafort, J. (1963). De´couverte du cyclee´volutif du genre Myialges avec description d'une espe`ce nouvelle (Myialgidae: Sarcoptiformes). *Bulletin et Annales de la Socie´te´ Entomologique de Belgique* 99, 486–500.

Farish, D.J. & Axtell, R.C. (1971). Phoresy redefined and examined in Macrocheles muscaedomesticae (Acarina: Macrochelidae). *Acarologia*, 13, 16-29.

Feres, R.J.F. & Flechtmann, C.H.W. (1991). Ocorrencia de acaros "parasitos-foréticos" (Acari: Epidermoptidae, Cheyletiellidae) sobre moscas hipoboscideas de pombo, em Sao José do Rio Preto. *Naturalia*, 16, 155-160.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotech.*, 3, 294–299.

Harbison, C.W., Bush, S.E., Malenke, J.R. & Clayton, D.H. (2008). Comparative

transmission dynamics of competing parasite species. *Ecology*, 89, 3186-3194.

Harbison, C.W. & Clayton, D.H. (2011). Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proc. Natl Acad. Sci. USA*, 108, 9525-9529.

Harbison, C.W., Jacobsen, M.V. & Clayton, D.H. (2009). A hitchhiker's guide to parasite transmission: The phoretic behaviour of feather lice. *Int. J. Parasitol.* 39, 569-575.

Harper, S.E., Spradling, T.A., Demastes, J.W. & Calhoun, C.S. (2015). Host behaviour drives parasite genetics at multiple geographic scales: population genetics of the chewing louse, *Thomomydoecus minor*. *Mol. Ecol.*, 24, 4129-4144.

Hoberg, E.P. & Brooks, D.R. (2008). A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host-parasite systems. *J. Biogeo.*, 35, 1533-1550.

Johnston, R.F. & Janiga, M. (1995). *Feral Pigeons*. Oxford Univ. Press, New York.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S. *et al.* (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647-1649.

Kim, K.S. & Sappington, T.W. (2013). Population genetics strategies to characterize long-distance dispersal of insects. *J. Asia-Pacific Entomol.*, 16, 87-97.

Klei, T.R. & Degiusti, D.L. (1975a). Observations on bionomics of Pseudolynchia-canariensis (diptera-hippoboscidae). *Parasitology*, 70, 195-202.

Klei, T.R. & Degiusti, D.L. (1975b). Seasonal occurrence of haemoproteus-columbae and its vector Pseudolynchia-canariensis. *J. Wildlife Dis.*, 11, 130-135.

Kubisch, A., Holt, R.D., Poethke, H.-J. & Fronhofer, E.A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, 123, 5-22.

Leigh, J.W. & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.*, 6, 1110-1116.

Levin, I.I. & Parker, P.G. (2013). Comparative host-parasite population genetic structures: obligate fly ectoparasites on Galapagos seabirds. *Parasitology*, 140, 1061-1069.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451-1452.

Liu, H. & Stiling, P. (2006). Testing the enemy release hypothesis: a review and meta-analysis. *Biol. Invasions*, 8, 1535-1545.

Maa, T.C. (1966). On the genus Pseudolynchia bequaert. *Pacific Insects Monograph*, 10, 125 - 138.

Macchioni, F., Magi, M., Mancianti, F. & Perrucci, S. (2005). Phoretic association of mites and mallophaga with the pigeon fly Pseudolynchia canariensis. *Parasite Paris Fr.*, 12, 277-279.

MacLeod, C.J., Paterson, A.M., Tompkins, D.M. & Duncan, R.P. (2010). Parasites lost - do invaders miss the boat or drown on arrival? *Ecol. Lett.*, 13, 516-527.

Marzal, A., Ricklefs, R.E., Valkiunas, G., Albayrak, T., Arriero, E., Bonneaud, C. *et al.* (2011). Diversity, loss, and gain of malaria parasites in a globally invasive bird. *Plos One*, 6. https://doi.org/10.1371/journal.pone.0021905

Maze-Guilmo, E., Blanchet, S., McCoy, K.D. & Loot, G. (2016). Host dispersal as the driver of parasite genetic structure: a paradigm lost? *Ecol. Lett.*, 19, 336-347.

McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Le Bohec, C. *et al.* (2005). Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. Lond B Biol. Sci.*, 272, 2389-2395.

Moyer, B.R., Drown, D.M. & Clayton, D.H. (2002). Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos*, 97, 223-228.

Olival, K.J., Dick, C.W., Simmons, N.B., Morales, J.C., Melnick, D.J., Dittmar, K. *et al.* (2013). Lack of population genetic structure and host specificity in the bat fly, Cyclopodia horsfieldi, across species of Pteropus bats in Southeast Asia. *Parasit. Vectors*, 6. https://doi.org/10.1186/1756-3305-6-231

Page, R.D.M., Lee, P.L.M., Becher, S.A., Griffiths, R. & Clayton, D.H. (1998). A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Mol. Phylogenet. Evol.*, 9, 276-293.

Philips, J.R. & Fain, A. (1991). Acarine symbionts of louseflies (Diptera, Hippoboscidae). *Acarologia*, 32, 377-384.

Poulin, R. (2006). *Evolutionary Ecology of Parasites*. Second Edition. Princeton Univ. Press.

Poulin, R. & Morand, S. (2000). The diversity of parasites. *Q. Rev. Biol.*, 75, 277-293.

Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. & Lowe, A.J. (2008). Adaptive evolution in invasive species. *Trends Plant Sci.*, 13, 288-294.

Santiago-Alarcon, D., Palinauskas, V. & Schaefer, H.M. (2012). Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biol. Rev.*, 87, 928-964.

Sherrard-Smith, E., Chadwick, E. & Cable, J. (2012). Abiotic and biotic factors associated with tick population dynamics on a mammalian host: *Ixodes hexagonus* infesting otters, *Lutra lutra*. *Plos One*, 7. https://doi.org/10.1371/journal.pone.0047131.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Ann. Entomol. Soc. Am.*, 87, 651-701.

Sol, D., Jovani, R. & Torres, J. (2000). Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography*, 23, 307-314.

Streicker, D.G., Winternitz, J.C., Satterfield, D.A., Condori-Condori, R.E., Broos, A., Tello, C. *et al.* (2016). Host-pathogen evolutionary signatures reveal dynamics and future invasions of vampire bat rabies. *Proc. Natl. Acad. Sci.,* 113, 10926-10931.

Valkiunas, G. (2005). *Avian Malaria Parasites and Other Haemosporidia*. CRC Press, Boca Raton.

Waite, J.L. (2012). Quantifying fitness costs in a vector-borne parasite system: experiments with pigeons, hippoboscid flies, and an avian malaria parasite. In: *Department of Biology*. Univ. of Utah, Salt Lake City.

Waite, J.L., Henry, A.R., Adler, F.R. & Clayton, D.H. (2012). Sex-specific effects of an avian malaria parasite on an insect vector: support for the resource limitation hypothesis. *Ecology*, 93, 2448-2455.

Waite, J.L., Henry, A.R., Owen, J.P. & Clayton, D.H. (2014). An experimental test of the effects of behavioral and immunological defenses against vectors: do they interact to protect birds from blood parasites? *Parasit. Vectors*, 7. https://doi.org/10.1186/1756-3305-7-104

Whiteman, N.K., Kimball, R.T. & Parker, P.G. (2007). Co-phylogeography and

comparative population genetics of the threatened Galapagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Mol. Ecol.*, 16, 4759-4773.

Witsenburg, F., Clement, L., Lopez-Baucells, A., Palmeirim, J., Pavlinic, I., Scaravelli, D. *et al.* (2015). How a haemosporidian parasite of bats gets around: the genetic structure of a parasite, vector and host compared. *Mol. Ecol.*, 24, 926-940.

Yuval, B. (2006). Mating systems of blood-feeding flies. *Annu.l Rev. Entomol.*, 51, 413-440.

Table 3.1. Locations where pigeon populations were examined for parasites. Sites are ordered by average annual minimum temperature. We found no flies and no H. columbae at six sites. “NA” refers to areas where no blood samples were screened for H. columbae infected cells. Abbreviations: CA = Canada, US = United States, SK = Slovakia, BR = Brazil, IN = India; Temp = mean annual minimum temperature; Hum. = mean annual relative humidity; Prev.= prevalence (percentage of infected hosts); Abun. = abundance (mean number of parasites in sampled hosts, including uninfected hosts).

Inferred fly range	Location (City, State, Country)	Temp. (°C)	Hum. (%)	# Hosts	*Pseudolynchia canariensis*		*Haemoproteus Columbae*	
					Prev.	Abun.	Prev.	Abun.
	Regina, Saskatchewan, CA	-3.3	65	20	0	0	0	0
	Winnipeg, Manitoba, CA	-2.8	67	9	0	0	0	0
	Laramie, Wyoming, US	-2.8	29	10	0	0	0	0
	Ružomberok, SK	-1.1	60	10	0	0	NA	NA
	Grand Forks, North Dakota, US	-0.5	66	11	0	0	NA	NA
	Bruneau, Idaho, US	3.3	55	31	0	0	NA	NA
	Salt Lake City A, Utah, US	5.6	55	23	0	0	48	21.4
	Salt Lake City B, Utah, US	5.6	55	14	50	0.7	NA	NA
	Apollo, Pennsylvania, US	6.1	68	22	0	0	NA	NA
	Albuquerque, New Mexico, US	7.2	36	21	0	0	86	111
	Seattle, Washington, US	7.8	73	7	0	0	0	0
	Guarujá, São Paulo, BR	13.9	86	8	88	17.6	NA	NA
	Tucson, Arizona, US	14.4	38	8	25	0.25	100	479.6
	San Antonio Texas, US	15	67	7	40	0.7	71	11.7
	New Orleans, Louisiana, US	16.1	76	13	30	0.7	100	144.3
	Jacksonville, Florida, US	16.7	76	6	67	2.3	100	102.5
	Phoenix, Arizona, US	17.2	37	21	5	0.1	9.5	0.19
	Rampur, Uttar Pradesh, IN	18.3	56	11	9	0.1	NA	NA
	Ft. Lauderdale, Florida, US	20	73	4	35	0.25	100	103.8

Table 3.2. Avian skin mites in the family Epidermoptidae (Myialges spp.) were phoretic on 5% of the *P. canariensis* collected. Gravid female Epidermoptinae mites often attach to louseflies to oviposit and fasten eggs to the cuticle. Eclosed mites then disperse from the fly to the skin of host birds. It is thought that these mites require flies to complete their life cycle. Most mites were attached to fly abdomens near the hind legs.

Host #	Fly #	Location	# Mites	Eggs	Mite Attachment Sites
9	23	Louisiana, US	1	absent	Abdomen
22	49	São Paulo, BR	1	present	Abdomen
	52	São Paulo, BR	1	absent	Abdomen
23	55	São Paulo, BR	2	absent	Abdomen
	58	São Paulo, BR	2	absent	Abdomen
25	59	São Paulo, BR	1	present	Thorax
	58	São Paulo, BR	2	absent	Abdomen
26	72	São Paulo, BR	2	absent	Abdomen
28	85	São Paulo, BR	1	absent	Abdomen

Table 3.3. Haplotype and genetic diversity estimates for mtDNA sequences from P. canariensis. Sequenced flies were collected from five sites throughout North America, and one site in South America. Overall, mitochondrial variation is relatively low.

Gene	Population	# Seq.	# Variable Sites	# Haplotypes	Haplotype Diversity ± standard deviation	Nucleotide Diversity ± standard deviation
Cytb	All	34	18	10	0.765 ± 0.061	0.00732 ± 0.00136
425 bp	Arizona	1	NA	NA	NA	NA
	São Paulo	11	9	3	0.564 ± 0.134	0.00705 ± 0.00224
	Florida	6	0	1	0 ± 0	0 ± 0
	Louisiana	5	10	4	0.900 ± 0.161	0.01368 ± 0.00365
	Texas	3	0	1	0 ± 0	0 ± 0
	Utah	6	0	1	0 ± 0	0 ± 0
COI	All	19	11	6	0.468 ± 0.140	0.00140 ± 0.00055
1224 bp	Arizona	2	0	1	0 ± 0	0 ± 0
	São Paulo	3	8	3	1.000 ± 0.272	0.00436 ± 0.00150
	Florida	5	1	2	0.400 ± 0.237	0.00033 ± 0.00019
	Louisiana	3	0	1	0 ± 0	0 ± 0
	Texas	3	1	2	0.667 ± 0.314	0.00054 ± 0.00026
	Utah	2	0	1	0 ± 0	0 ± 0
12S rDNA	All	76	1	2	0.191 ± 0.056	0.00088 ± 0.00026
218 bp	Arizona	2	0	1	0 ± 0	0 ± 0
	São Paulo	36	1	2	0.356 ± 0.078	0.00163 ± 0.00036
	Florida	14	0	1	0 ± 0	0 ± 0
	Louisiana	10	0	1	0 ± 0	0 ± 0
	Texas	3	0	1	0 ± 0	0 ± 0
	Utah	8	0	1	0 ± 0	0 ± 0
16S rDNA	All	67	6	7	0.614 ± 0.035	0.00216 ± 0.00030
383 bp	Arizona	2	1	2	1.000 ± 0.500	0.00261 ± 0.00131
	São Paulo	27	2	2	0.262 ± 0.097	0.00137 ± 0.00051
	Florida	13	4	3	0.410 ± 0.154	0.00221 ± 0.00337
	Louisiana	9	3	4	0.750 ± 0.112	0.00305 ± 0.00079
	Texas	4	0	1	0 ± 0	0 ± 0
	Utah	12	1	2	0.485 ± 0.106	0.00127 ± 0.00028

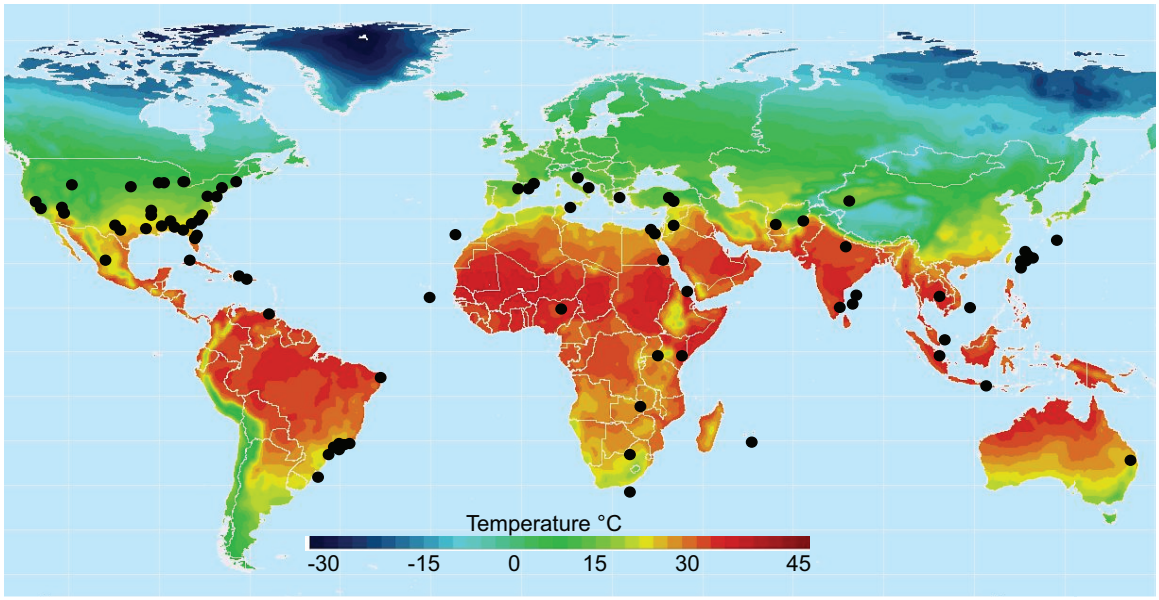

Figure 3.1. Global distribution of *Pseudolynchia canariensis* in relation to the mean annual temperature. Black dots show areas where the fly was collected or reported from the literature. Fly distribution is limited to areas between 45.5 °N and 33 °S.

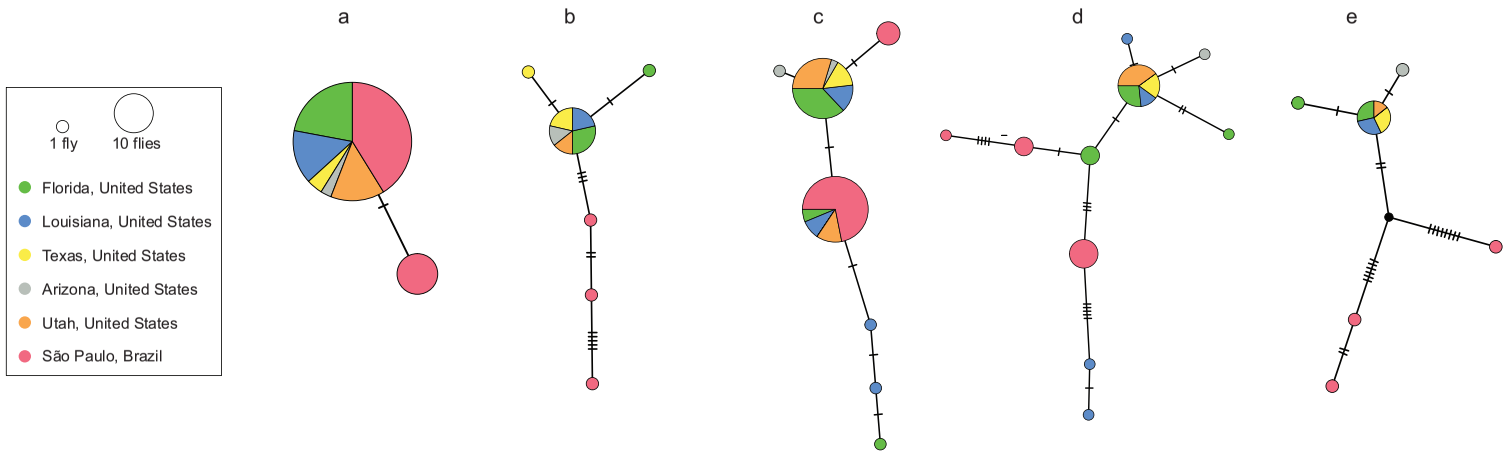

Figure 3.2. Pigeon fly population structure estimated with statistical parsimony haplotype networks. Haplotype networks were constructed from four mitochondrial genes: (a) 12S (b) COI (c) 16S (d) Cytb and (e) a combined dataset of all genes. The size of circles is proportional to the number of flies that share that same haplotype. Colors correspond to sampling sites. Small black dashes are inferred (undetected) haplotypes. Tick-marks on connections represent the amount of genetic divergence.

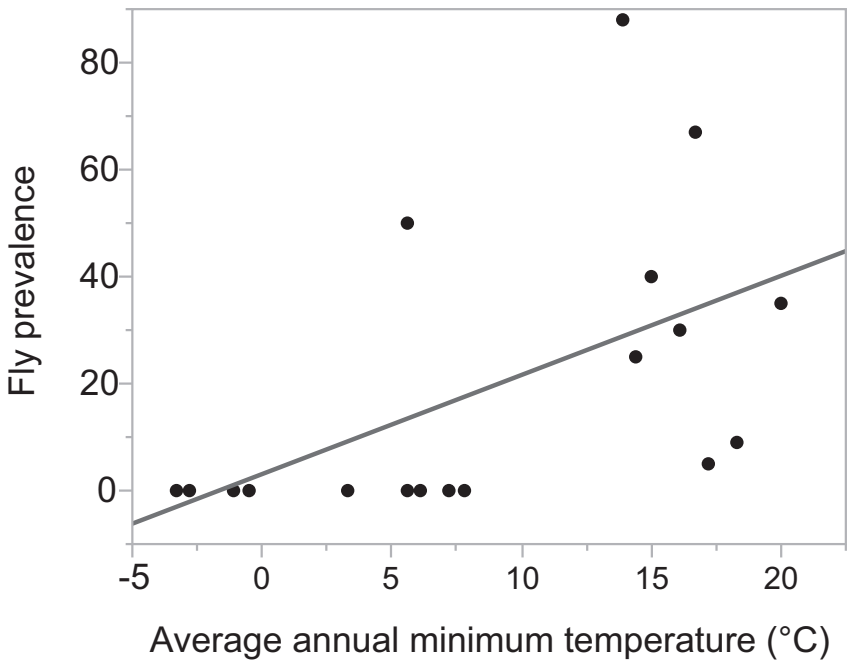

Figure 3.3. Fly prevalence is correlated with average annual minimum temperature (Spearman rank correlation: r_s = 0.68, P = 0.0015). Flies were only found in areas with an annual minimum temperature above 5°C.

CHAPTER 4

COMMUNITY INTERACTIONS GOVERN INTRASPECIFIC GENETIC VARIATION IN PARASITE POPULATIONS

Abstract

Genetic variation is influenced by many biotic and abiotic factors. However, relatively little is known about how biotic interactions shape microevolutionary patterns. We examined two sympatric species of feather lice (Phthiraptera: Ischnocera) that parasitize pigeons. The two species, “wing lice” (*Columbicola columbae*) and “body lice” (*Campanulotes compar*), are ecologically very similar. However, wing lice differ from body lice in one key trait. Wing lice disperse phoretically, or “hitchhike,” on parasitic flies (Diptera: Hippoboscidae). Body lice do not disperse phoretically. To quantify how phoretic dispersal influences patterns of genetic variation, we compared genotypes of wing and body lice in geographic regions with and without flies. We found that wing lice populations in areas with flies show less population genetic structure than body lice populations. In two out of three areas where flies are absent, wing lice and body lice populations have similar genetic differentiation. We also found that wing lice have a larger genetic effective population size than body lice in areas with flies, but the same effective population size in areas without flies. Our

findings strongly suggest that phoretic dispersal erodes population genetic structure and enhances population connectivity for wing lice.

Introduction

Dispersal is a central life history trait that shapes eco-evolutionary dynamics in organisms (Clobert *et al.* 2012; Kubisch *et al.* 2014; Bonte & Dahirel 2017). The movement of individuals away from their birthplace affects the spatial distribution of populations, which in turn affects demographic and microevolutionary patterns. Examining how dispersal behavior shapes metapopulation connectivity across landscapes is of critical importance when predicting how species distributions will respond to environmental change (Hanski & Mononen 2011; Alberti 2015; Massol & Debarre 2015; Cote *et al.* 2017; Legrand *et al.* 2017; Thompson & Gonzalez 2017).

Many population genetic studies have investigated factors that impede or enhance dispersal by inferring gene flow from genetic data. Studies comparing different ecologically similar species within a shared landscape are particularly effective at identifying factors that affect gene flow and shape population genetic structure (Waples 1998; Bohonak 1999; Criscione 2008). For example, landscape features differentially affect patterns of gene flow in co-occurring wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*) in the northeastern United States. The presence of roads is associated with genetic structure for the wood frog, while the occurrence of rivers explains genetic differentiation better for the spotted salamander (Richardson 2012). Contrasting

patterns of genetic structure, attributed to the occurrence of rocky or sandy habitat types, have also been found for three sympatric species of cichlid fish in Eastern Africa with contrasting habitat preferences (Wagner & McCune 2009).

While abiotic factors can shape patterns of gene flow, the impact of biotic factors on dispersal and population structure remains poorly understood (Hand *et al.* 2015). Evaluation of the biotic factors that shape dispersal is critical to understanding eco-evolutionary dynamics (Kubisch *et al.* 2014). Comparison of related and sympatric species that are ecologically very similar, but explicitly differ in dispersal, allows for examination of how these differences influence population genetic structure. Host-parasite systems lend themselves to this type of approach and these systems are thought to be a natural starting point for thoroughly assessing eco-evolutionary dynamics (Weber *et al.* 2017). In host-parasite systems it is possible to compare parasite species that have very similar ecological niches on the same host, yet differ in life history traits. Here we use a highly-tractable, host-parasite system where previous ecological studies and experiments have laid a solid foundation on which to generate and test *a priori* hypotheses about how particular species interactions affect dispersal patterns and shape population genetic structure.

Background

Rock pigeons (*Columba livia*) have successfully colonized most metropolitan areas of the world. These ubiquitous birds are commonly co-infested with feather lice (Order: Phthiraptera), which are permanent, obligate

parasites that complete their entire life cycle on their host. Two co-occurring species of feather lice are “wing lice” (*Columbicola columbae*) and “body lice” (*Campanulotes compar*). Despite the fact that wing and body lice are distantly related, they are ecologically very similar. Both species live, feed and reproduce on pigeon feathers. Both species disperse at similar rates vertically from parent to offspring in the nest, as well as horizontally when hosts are in direct contact (Harbison *et al.* 2008). However, wing lice differ from body lice in one key dispersal trait: their ability to disperse phoretically, or “hitchhike,” on blood-feeding Hippoboscid flies (Diptera: Hippoboscidae) (Harbison *et al.* 2009; Harbison & Clayton 2011).

Dispersal between hosts is a major challenge for many immobile parasites like lice (Harper et al. 2015). Wing and body lice are highly immobile off the host (Bartlow *et al.* 2016) and are typically limited to dispersal between pigeons that are in direct physical contact (Harbison *et al.* 2008). However, for wing lice, fly-mediated phoretic dispersal may allow these parasites to escape an isolated host or to avoid intraspecific or interspecific competition on hosts (Clayton *et al.* 2016). Hippoboscid flies are much more mobile than lice. Experiments with captive birds have shown that wing lice can disperse on *P. canariensis* to novel host individuals, as well as novel host species, in sufficient numbers to establish new populations (Harbison *et al.* 2008; Harbison & Clayton 2011).

If phoretic dispersal has a recurring influence on patterns of gene flow in wing lice populations, then phoresis may have a large influence on microevolutionary patterns of diversification. Consistent with this hypothesis,

wing lice should have more gene flow among populations, compared to body lice, which are not phoretic. Among pigeon flocks and between individual birds in Salt Lake City, Utah, wing lice have significantly less population genetic structure than body lice (Chapter 2). The genetic patterns observed are consistent with key differences in dispersal ability between wing lice and body lice. However, as an additional test of the influence of phoretic dispersal on population structure, we here compare wing and body louse population structure in geographic regions where flies are present, versus regions where they are absent.

Pigeon parasite community composition varies geographically. The distribution of *P. canariensis* is restricted to regions where the average low temperature is greater than 6˚C (Chapter 3). In these regions they often co-occur with both wing and body lice on individual hosts. However, wing and body lice also co-occur on pigeons in areas where flies are absent and phoretic dispersal is impossible (Chapter 3). In the absence of the flies, dispersal of both wing lice and body lice should be limited to periods of direct contact between pigeons. We predicted that in areas without flies, wing lice populations will have similar genetic differentiation as body lice. In areas with flies, where phoresis occurs, wing lice should have less genetic differentiation than body lice.

We compared the population genetic structure of wing lice and body lice in geographic regions with and without *P. canariensis*, the phoretic vector of wing lice. Since wing and body lice are significantly structured between birds in pigeon flocks (Chapter 2), we compared wing and body lice populations living on the same individual birds. We use the term "infrapopulation" (Bush *et al.* 1997) to

refer to all the conspecific adult lice living on a single host bird. Infrapopulations can be considered local populations, or demes, that are connected by dispersal and gene flow to the larger metapopulation (Criscione & Blouin 2005; Clayton *et al.* 2016). We genotyped and compared 36 wing and body lice infrapopulations on pigeons in two areas where phoresis occurs and 21 wing and body lice infrapopulations on pigeons in three areas where fly mediated phoresis is impossible. We also estimated the genetic effective population size for both species at each sampling site with the prediction that phoretic dispersal of wing lice should enhance population connectivity and effective population size compared with body lice.

Materials and Methods

Field Sampling

Wing and body lice were collected from feral pigeons in five cities across four countries (Table 4.1). Samples were collected in June and July of 2014 – 2016. In four of the cities (Salt Lake City, Utah, USA; Guarujá, São Paulo, Brazil; Grand Forks, North Dakota, USA and Ružomberok, Slovakia), lice were collected from wild birds. In Winnipeg, Manitoba, Canada, lice were collected from wild pigeons brought to two rehabilitation hospitals throughout 2012 - 2013. Lice were collected by fumigating or washing individual pigeons as in Clayton and Drown (2001). Samples were preserved in 95% ethanol for identification and DNA extraction. Lice were identified to species under an Olympus SZCTV stereoscope. In total, we compared wing and body lice infrapopulations from 55

pigeons (Table 4.1).

Genotyping

DNA was extracted from lice using the DNeasy Blood and Tissue kit (Qiagen) as described by Johnson *et al.* (2001). To genotype lice, we identified microsatellites specific to each species (Chapter 2). A total of 540 wing lice and 534 body lice were genotyped at 8 different microsatellite loci. Multiplex PCRs with a universal primer and fluorophor were used to genotype the samples (Schuelke 2000; Blacket *et al.* 2012). The universal primer tail M13 (5' CAC GAC GTT GTA AAA CGA C 3') was added to the 5' end of the locus-specific forward primer. M13 labeled primers were tagged with FAM, PET, NED, or VIC (Applied Biosystems). The two forward primers and the appropriate locus-specific reverse primer were used in PCR reactions. An ABI 3100 Genetic Analyzer (Applied Biosystems) was used to resolve PCR products and was run with the 500 LIZ size standard. Genemapper v 3.7 (Applied Biosystems) was used to determine allele sizes.

Population genetic analyses

Currently there is no consensus as to which index of genetic differentiation is best suited for assessing population structure (Neigel 2002; Meirmans & Hedrick 2011; Whitlock 2011). Meirmans and Hedrick (2011) recommend reporting F_{ST} along with the unbiased F_{ST} estimator that is best suited for the objectives of the study. Here we report F_{ST} and the unbiased F_{ST} estimater

Hedrick's G'_{ST}. G'_{ST} is best suited for comparisons between organisms with different effective population sizes (Hedrick 2005).

Global F_{ST} and G'_{ST} values with 95% confidence intervals (CIs) were calculated using a bootstrapping approach (10,000 iterations) in the R (v 3.1.0) (R Core Team 2014) package "diveRsity" (Keenan *et al.* 2013). To compare genetic differentiation between louse infrapopulations, pairwise F_{ST} and G'_{ST} values were also calculated in diveRsity. The difference in infrapopulation pairwise G'_{ST} values between body lice and wing lice was plotted by sampling site.

To test if lice were significantly structured between infrapopulations at a sampling site, we performed an Analysis of Molecular Variance (AMOVA) in Arlequin (v 3.5) (Excoffier *et al.* 2005). Louse genetic variation was partitioned among and within host birds at each site for both species.

Census population size analyses

We counted the number of conspecific adult lice on a pigeon to quantify infrapopulation size, which is used here as a proxy for the population size. The infapopulation size of wing and body lice was compared on the same host. Mean infrapopulation sizes between wing and body lice were compared using a matched-pair t-test. Allelic richness (the mean number of alleles per microsatellite locus) was calculated using diveRsity. Linear regressions were used to model the relationships between: (a) body louse infrapopulation size and wing louse infrapopulaton size, (b) body louse infrapopulation allelic richness and

wing louse infrapopulation allelic richness and (c) allelic richness and infrapopulation size for both body lice and wing lice. To test whether slopes of the regression lines comparing allelic richness and infrapopulation size were different between louse species we performed an ANCOVA. Statistical analyses were conducted in JMP v.12.1.

Additionally, we examined how lice were spatially distributed among pigeons. Most ectoparasites have aggregated distributions, in which they are not distributed evenly on individuals within host populations (Poulin & Morand 2000). A highly aggregated distribution is thought to increase genetic structure of parasites (Huyse *et al.* 2005). To assess if wing or body lice have aggregated distributions, we calculated the index of dispersion from variance to mean ratios for each species (Poulin 2006).

Effective population size analyses

The effective population size (N_e) is defined as the number of breeding individuals in an idealized population whose allele frequencies would show the same signatures of evolutionary forces as the population under consideration (Wright 1931). Factors that affect N_e in sexual organisms include inbreeding, changes in census population size, and spatial structure (Charlesworth 2009). For parasites, increased dispersal distances are thought to increase N_e whereas frequent local extinctions are thought to decrease N_e (Criscione & Blouin 2005; Barrett *et al.* 2008). Changes in N_e that result from fluctuations in population size may play a major role in parasite evolution (Papkou *et al.* 2016). For example,

example, theory predicts that drift should have a larger impact in populations with a lower N_e. Here we used a linkage disequilibrium method to approximate the contemporary effective population size from neutral genetic variation in NeEstimator v2 (Do *et al.* 2014). N_e and parametric 95% CIs were estimated with the random mating model and the lowest allele frequency used was 0.05.

Isolation by distance and environment

Isolation by distance (IBD) results from limited dispersal across space (Wright 1943). IBD is the null hypothesis for patterns of population genetic differentiation in organisms. To test for an association between genetic and geographic distance we used Mantel tests (Mantel 1967) with 10,000 permutations calculated in GenoDive (v 2.0) (Meirmans & Van Tienderen 2004). Coordinates taken from the center of each sampling site were transformed into Euclidean distances. For intraspecific comparisons, genetic Chord distances were calculated from microsatellite data. In interspecific comparisons between wing and body lice standardized genetic distances (F'_{ST}) were calculated from microsatellite data.

Partial Mantel tests implemented in GenoDive with 10,000 permutations were used to test the association between distance matrices while controlling for a third variable. We tested if infrapopulation size was correlated with genetic distance while controlling for geographic distance. Environmental variables also varied across our sampling sites. Therefore we tested for a correlation between genetic distance and environmental distance while controlling for geographic

distance. Previous studies with lice have found that infrapopulation sizes are higher in areas with high relative humidity (Moyer *et al.* 2002). Therefore, louse population genetic differentiation may also be influenced by abiotic factors such as humidity and temperature. For each sampling site, mean estimates of annual temperature and relative humidity were taken from climatemps.com (Table 4.2). An environmental distance matrix was made from a combined data set of mean temperature and relative humidity values transformed into Euclidean distances.

Results

Population genetic analyses

Global F_{ST} and G'_{ST} values had nonoverlapping 95% CIs for wing and body lice (Table 4.3) at four sites (Salt Lake City, USA 1-3 and Ružomberok, Slovakia). In contrast, global F_{ST} and G'_{ST} CIs were overlapping for wing and body lice (Table 4.3) at three sites (Guarujá, Brazil; Winnipeg, Canada and Grand Forks, North Dakota). Wing lice had higher infrapopulation pairwise G'_{ST} values than body lice in more than half of the comparisons in Grand Forks, North Dakota (52%, 11/21 comparisons) and Winnipeg, Canada (67%, 24/36 comparisons) (Figure 4.3). Wing lice had higher infrapopulation pairwise G'_{ST} values in less than half of the comparisons at Salt Lake City 1 (2%, 1/45 comparisons), Salt Lake City 2 (20%, 9/45 comparisons), Salt Lake City 3 (18%, 9/45 comparisons), Guarujá, Brazil (27%, 4/15 comparisons) and Ružomberok, Slovakia (0%, 0/6 comparisons) (Figure 4.1).

The hierarchical AMOVA (Table 4.4) indicates that both wing and body

lice are significantly structured between birds at all sites. More of the genetic variance was accounted for by sampling between pigeon hosts for body lice than for wing lice at all sites except Grand Forks, North Dakota, USA and Winnipeg, Manitoba, Canada. The Global F_{ST} values indicate that body lice are highly genetically differentiated between hosts (F_{ST} 0.12 – 0.34) at all sites except Canada where body lice populations are moderately genetically differentiated (0.08). Wing lice are moderately genetically differentiated (F_{ST} 0.02 – 0.09) at all sites except Grand Forks, North Dakota, USA where they are highly genetically differentiated (F_{ST} 0.24).

Census population size analyses

Mean infrapopulation sizes were significantly different between louse species (Matched-pair t-test, $t = 5.39$, $df = 54$, $p < 0.0001$). Body lice had a median population size of 23 and wing lice had a median population size of 50. For the 55 infrapopulations assessed in this study, 45% of body lice infrapopulations were made up of 2 – 10 lice, compared with the 13% of wing lice populations that were that size. Whereas 67% of wing louse infrapopulations consisted of 20 – 90 lice in comparison, 36% of body lice populations were that size. Frequencies of infrapopulations above 90 lice were similar for body lice and wing lice at 18% and 20%, respectively.

Body louse infrapopulation size was correlated with wing louse infrapopulation size ($r = 0.72$, $p < 0.0001$). Moreover, allelic richness in body lice infrapopulations was correlated with allelic richness in wing louse

infrapopulations (r = 0.50, p <0.0001). We also found significant relationships between infrapopulation allelic richness and infrapopulation size for body lice (r = 0.52, p <0.0001; Figure 4.2a), as well as wing lice (r = 0.38, p = 0.005; Figure 4.2b). The slopes of the regressions between infrapopulation allelic richness and infrapopulation size for wing and body lice were not significantly different (ANCOVA, $F_{1, 54}$ = 0.79, p = 0.38). The variance to mean ratio was 211 for body lice and 99 for wing lice, indicating that both species have aggregated distributions.

Effective population size analyses

Values of N_e were undefined for wing lice genotypes in Ružomberok, Slovakia likely due to the small sample size. Estimates of N_e had nonoverlapping 95% CIs for wing and body lice at four sites: Salt Lake City, USA 1-3 and Guarujá, Brazil (Figure 4.3). Estimates of N_e had overlapping 95% CIs for wing and body lice in Winnipeg, Canada and Grand Forks, North Dakota (Figure 4.3).

Isolation by distance and environment

We found support for isolation by distance among body lice populations (Mantel test, r = 0.31, p = 0.001; Table 4.5) but not among wing lice populations (Mantel test, r = 0.02; p = 0.383; Table 4.5). After correcting for infrapopulation size, the pattern of isolation by distance in body lice was no longer significant (partial-Mantel, r = 0.31, p = 0.501; Table 4.5). Genetic distance and infrapopulation size were negatively correlated for body lice (Mantel test, r = -

0.19; p = 0.010; Table 4.5) but not for wing lice (Mantel test, r = 0.001; p = 0.46; Table 5). Genetic distance and ecological distance were negatively correlated for body lice (Mantel test, r = -0.18; p = 0.013; Table 4.5) although this association was not statistically significant after correcting for geographic distance (partial-Mantel, r = -0.18, p = 0.511; Table 4.5). In wing lice, we found no association between genetic distance and ecological distance (Mantel test, r = -0.01; p = 0.711; Table 4.5). We found that genetic distance matrices of wing and body lice were correlated (Mantel test, r = 0.20; p = 0.032; Table 4.5) but not after correcting for geographic distance (partial-Mantel, r = 0.21, p = 0.517; Table 4.5).

Discussion

Wing lice of pigeons frequently disperse phoretically on hippboscid flies; which can result in movement between individual birds (Harbison *et al.* 2009; Harbison & Clayton 2011). Body lice do not disperse in this manner (Keirans 1975; Harbison & Clayton 2011; Barlow et al. 2016). A previous study (Chapter 2) showed that wing lice have significantly less population genetic structure than body lice at sites where flies are present (Chapter 2). However, this study was confined to populations within Salt Lake City, Utah. Here we expand upon this study to include multiple geographic regions with and without flies. We found that wing lice infrapopulations in areas with flies have less population genetic structure than body lice infrapopulations. At two of the three sites without flies, we found that wing lice and body lice infrapopulations have similar population genetic structure. We also found that wing lice have higher genetic effective

population sizes than body lice in areas with flies, but that they have similar effective population sizes in areas without flies. These findings strongly suggest that ongoing phoretic dispersal erodes population genetic structure and enhances infrapopulation connectivity for wing lice.

Body lice infrapopulations were significantly more differentiated than wing lice infrapopulations in all pairwise comparisons in Ružomberok, Slovakia. This result was unexpected, as Ružomberok is in a geographic region where flies are absent (Chapter 3). This result may be due to our small sample size in that region. At the Ružomberok site we were only able to compare wing and body lice populations from 4 pigeons because only 4 of the 10 birds captured had body lice (all 10 had wing lice). In contrast, at all other sites we compared wing and body lice populations on 6 or more birds. Ecological factors other than phoresis may have influenced genetic structure at the Ružomberok site. Differences in host sociality and host demography are also thought to influence population genetic structure (Barrett *et al.* 2008). Lice were collected from newly fledged young birds in the attic of a building at this site. It is possible that some of these birds were siblings and acquired lice by vertical transmission from their parents. Since wing lice have slight, yet significantly higher rates of vertical transmission, compared to body lice (Harbison *et al.* 2008), the low population differentiation in wing lice may reflect this.

Three of the fly sites used in this study were in Salt Lake City, Utah. Although lice were sampled from spatially isolated pigeon flocks, it is not likely that these sites are completely independent of each other owing to some level of

pigeon dispersal. Therefore, it may be more accurate to consider Salt Lake City a single site. Future studies assessing the effect of phoretic dispersal on parasite populations should compare the genetic differentiation of wing and body lice across a more even spatial distribution of sites.

Despite these sampling issues, we found contrasting spatial distribution patterns of wing and body lice on pigeons. Body lice were more aggregated than wing lice, suggesting that dispersal of lice among individual hosts is more limited for body lice than wing lice. Nearly half of the pigeons sampled had small body lice infrapopulations (2-10 lice) but intermediate wing lice populations (>20 lice). Since body lice populations are typically smaller in size than wing lice populations, we expected drift to have played a greater role in shaping population genetic differentiation in body lice than wing lice. Consistent with this hypothesis, genetic distance and infrapopulation size were significantly negatively correlated for body lice, but not wing lice. However, allelic richness was correlated with infrapopulation size in both species of lice, suggesting that drift likely plays a role in structuring both wing and body lice populations. A positive IBD pattern was found for body lice but not wing lice, indicating that body lice are likely in the gene flow-drift equilibrium (Hutchison & Templeton 1999). Wing lice may not be in the gene flow-drift equilibrium because of increased gene flow due to phoretic dispersal.

One reason that immobile parasites, like lice, may use arthropod vectors for dispersal is if vector dispersal is greater than host dispersal. For example, the bat fly, *Nycteribia schmidlii*, exhibits less population genetic structure than its

host, the bent-winged bat (*Miniopterus schreibersii*) across its European range, suggesting that the fly is more mobile than its host (Witsenburg *et al.* 2015). Since hippobsocid flies are more mobile than lice, future studies could investigate this hypothesis by comparing fly population genetic structure and host population genetic structure at a local scale.

There are many costs and trade-offs associated with dispersal (Bonte *et al.* 2012), which could be further investigated with this system. Phoresis in wing lice may have evolved as a competition-colonization tradeoff (Harbison *et al.* 2008; Clayton *et al.* 2016). Wing lice, which are superior disperses, are inferior competitors with body lice on the same host (Bush & Malenke 2008). Competition-colonization tradeoffs are known from other parasites as well as free-living animals (Mordecai *et al.* 2016). In Glanville butterflies (*Melitaea cinxia*), females heterozygous at a phosphoglucose isomerase (*Pgi*) SNP are superior disperses among local populations compared to females homozygous at the *Pgi* SNP (Niitepold *et al.* 2009; Zheng *et al.* 2009; Niitepold *et al.* 2011). Butterflies with the “high dispersal genotype” can reproduce at a younger age but are inferior competitors compared to females with the “low dispersal genotype” when resource availability is limited (Saastamoinen 2008; Saastamoinen *et al.* 2009). It would be fascinating to see if there is intraspecific variation in phoretic dispersal and if phoretic dispersal is associated with a particular genotype in wing lice.

Our study further demonstrates the importance of considering species ecology and life history when assessing patterns of genetic differentiation. For

many species, genome wide genetic diversity is correlated with life history (Romiguier *et al.* 2014; Ellegren & Galtier 2016); therefore, thorough knowledge of species life history is likely critical to accurately interpret patterns of population genetic differentiation in many other organisms (Rodriguez-Verdugo *et al.* 2017; Weber *et al.* 2017). Currently, few empirical studies directly link life history traits of organisms to micro- and macroevolutionary patterns of diversification. One notable exception is a study by Riginos *et al*. (2014) that links dispersal ecology with population genetic structure and species richness in reef fishes. Across these fish species, genetic differentiation and species richness are correlated with parental investment in larval dispersal. Members of fish families that guard eggs (low larval dispersal) have significantly more population structure and greater species richness than those that release eggs into the water column (high larval dispersal) (Riginos *et al.* 2014). Given that dispersal is integral in shaping the abundance and distribution of species, examining dispersal-related life history traits is critical in linking micro and macroevolutionary patterns or organisms.

Different genera of pigeons and doves are host to different species of wing lice, body lice and louse flies. Phoresis may explain micro and macroevolutionary patterns of host specificity in wing and body lice (Johnson *et al.* 2002; Clayton & Johnson 2003; Johnson & Clayton 2003). Body lice are more host specific than wing lice and cospeciate with hosts to a greater extent than wing lice (Clayton *et al.* 2003; Clayton & Johnson 2003). Our study is consistent with the hypothesis that phoretic dispersal plays a major role in shaping the eco-evolutionary

dynamics of feather lice.

Furthermore, demonstrating how dispersal related life history traits affect community interactions is critical for a comprehensive understanding of eco-evolutionary dynamics, yet empirical work in this area is limited (Baguette *et al.* 2013; Kubisch *et al.* 2014; Cote *et al.* 2017; Massol *et al.* 2017). A recent modeling study showed that dispersal rates are fundamental in altering species interactions and retaining community composition. Interestingly, the model also showed that interspecific differences in dispersal ability can alter community interactions (Thompson & Gonzalez 2017). Empirically testing how species differences in dispersal shape community composition is of critical importance as habitat fragmentation and global change continues to threaten ecosystem connectivity and patterns of biodiversity (Alberti 2015; Harrisson *et al.* 2016; Cote *et al.* 2017; Legrand *et al.* 2017).

Future studies could use host-parasites systems like this one to empirically test how parasite dispersal influences host community composition. For example, many species of birds and mammals host hippoboscid flies that interact with other members of the host community. Phoresy on hippoboscid flies has been documented in 17 genera of mites and in 19 genera of lice (Philips & Fain 1991; Bartlow *et al.* 2016). Moreover, many hippoboscid flies vector blood parasites that cause diseases in humans and wildlife and are of medical significance (Santiago-Alarcon *et al.* 2012). Fly-mediated dispersal may play a role in host associated community dynamics.

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References

Alberti, M. (2015). Eco-evolutionary dynamics in an urbanizing planet. *Trends Ecol. Evol.*, 30, 114-126.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V.M. & Turlure, C. (2013). Individual dispersal, landscape connectivity and ecological networks. *Biol. Rev.*, 88, 310-326.

Barrett, L.G., Thrall, P.H., Burdon, J.J. & Linde, C.C. (2008). Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends Ecol. Evol.*, 23, 678-685.

Bartlow, A.W., Villa, S.M., Thompson, M.W. & Bush, S.E. (2016). Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. *Int. J. Parasitol.*, 46, 221-227.

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F. & Miller, A.D. (2012). Universal primers for fluorescent labelling of PCR fragments-an efficient and cost-effective approach to genotyping by fluorescence. *Mol. Ecol. Resour.*, 12, 456-463.

Bohonak, A.J. (1999). Dispersal, gene flow, and population structure. *Q. Rev. of Biol.*, 74, 21-45.

Bonte, D. & Dahirel, M. (2017). Dispersal: a central and independent trait in life history. *Oikos*, 126, 472-479.

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M. *et al.*

(2012). Costs of dispersal. *Biol. Rev.*, 87, 290-312.

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, 83, 575-583.

Bush, S.E. & Malenke, J.R. (2008). Host defence mediates interspecific competition in ectoparasites. *J. Anim. Ecol.*, 77, 558-564.

Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Rev. Genet.*, 10, 195-205.

Clayton, D.H., Bush, S.E., Goates, B.M. & Johnson, K.P. (2003). Host defense reinforces host-parasite cospeciation. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 15694-15699.

Clayton, D.H., Bush, S.E. & Johnson, K.P. (2016). *Coevolution of Life on Hosts: Integrating Ecology and History*. Univ. Chicago Press.

Clayton, D.H. & Drown, D.M. (2001). Critical evaluation of five methods for quantifying chewing lice (Insecta : Phthiraptera). *J. Parasitol.*, 87, 1291-1300.

Clayton, D.H. & Johnson, K.P. (2003). Linking coevolutionary history to ecological process: Doves and lice. *Evolution*, 57, 2335-2341.

Clobert, J., M. Baguette, Benton, T.G. & J. M. Bullock (2012). *Dispersal Ecology and Evolution*. Oxford Univ. Press, Oxford.

Cote, J., Bestion, E., Jacob, S., Travis, J., Legrand, D. & Baguette, M. (2017). Evolution of dispersal strategies and dispersal syndromes in fragmented landscapes. *Ecography*, 40, 56-73.

Criscione, C.D. (2008). Parasite co-structure: Broad and local scale approaches. *Parasite Paris Fr.*, 15, 439-443.

Criscione, C.D. & Blouin, M.S. (2005). Effective sizes of macroparasite populations: a conceptual model. *Trends Parasitol.*, 21, 212-217.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J. & Ovenden, J.R. (2014). Neestimator v2: re-implementation of software for the estimation of contemporary effective population size (N-e) from genetic data. *Mol. Ecol. Resour.*, 14, 209-214.

Ellegren, H. & Galtier, N. (2016). Determinants of genetic diversity. *Nature Rev. Genet.*, 17, 422-433.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin (version 3.0): An

integrated software package for population genetics data analysis. *Evol. Bioinform.*, 1, 47-50.

Hand, B.K., Lowe, W.H., Kovach, R.P., Muhlfeld, C.C. & Luikare, G. (2015). Landscape community genomics: understanding eco-evolutionary processes in complex environments. *Trends Ecol. Evol.*, 30, 161-168.

Hanski, I. & Mononen, T. (2011). Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. *Ecol. Lett.*, 14, 1025-1034.

Harbison, C.W., Bush, S.E., Malenke, J.R. & Clayton, D.H. (2008). Comparative transmission dynamics of competing parasite species. *Ecology*, 89, 3186-3194.

Harbison, C.W. & Clayton, D.H. (2011). Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proc. Natl Acad. Sci. U.S.A.*, 108, 9525-9529.

Harbison, C.W., Jacobsen, M.V. & Clayton, D.H. (2009). A hitchhiker's guide to parasite transmission: The phoretic behaviour of feather lice. *Int. J. Parasitol.*, 39, 569-575.

Harper, S.E., Spradling, T.A., Demastes, J.W. & Calhoun, C.S. (2015). Host behaviour drives parasite genetics at multiple geographic scales: population genetics of the chewing louse, Thomomydoecus minor. *Mol. Ecol.*, 24, 4129-4144.

Harrisson, K.A., Yen, J.D.L., Pavlova, A., Rourke, M.L., Gilligan, D., Ingram, B.A. *et al.* (2016). Identifying environmental correlates of intraspecific genetic variation. *Heredity*, 117, 155-164.

Hedrick, P.W. (2005). A standardized genetic differentiation measure. *Evolution*, 59, 1633-1638.

Hutchison, D.W. & Templeton, A.R. (1999). Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, 53, 1898-1914.

Huyse, T., Poulin, R. & Theron, A. (2005). Speciation in parasites: a population genetics approach. *Trends Parasitol.*, 21, 469-475.

Johnson, K.P., Adams, R.J. & Clayton, D.H. (2001). Molecular systematics of Goniodidae (Insecta : Phthiraptera). *J. Parasitol.*, 87, 862-869.

Johnson, K.P. & Clayton, D.H. (2003). Coevolutionary hisory of ecological replicates: Comparing phylogenies of wing and body lice to Columbiform hosts. In: *Tangled Trees: Phylogenies, Cospeciation and Coevolution* (ed. Page, RDM).

Univ. Chicago Press, Chicago, pp. 262 - 285.

Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J. & Clayton, D.H. (2002). The population genetics of host specificity: genetic differentiation in dove lice (Insecta : Phthiraptera). *Mol. Ecol.*, 11, 25-38.

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W. & Prodohl, P.A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods Ecol. Evol.*, 4, 782-788.

Kubisch, A., Holt, R.D., Poethke, H.-J. & Fronhofer, E.A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, 123, 5-22.

Legrand, D., Cote, J., Fronhofer, E.A., Holt, R.D., Ronce, O., Schtickzelle, N. *et al.* (2017). Eco-evolutionary dynamics in fragmented landscapes. *Ecography*, 40, 9-25.

Mantel, N. (1967). Detection of disease clustering and a generalized regression approach. *Cancer Res.*, 27, 209.

Massol, F., Altermatt, F., Gounand, I., Gravel, D., Leibold, M.A. & Mouquet, N. (2017). How life-history traits affect ecosystem properties: effects of dispersal in meta-ecosystems. *Oikos*, 126, 532-546.

Massol, F. & Debarre, F. (2015). Evolution of dispersal in spatially and temporally variable environments: The importance of life cycles. *Evolution*, 69, 1925-1937.

Meirmans, P.G. & Hedrick, P.W. (2011). Assessing population structure: F-ST and related measures. *Mol. Ecol. Res.*, 11, 5-18.

Meirmans, P.G. & Van Tienderen, P.H. (2004). GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes*, 4, 792-794.

Mordecai, E.A., Jaramillo, A.G., Ashford, J.E., Hechinger, R.F. & Lafferty, K.D. (2016). The role of competition - colonization tradeoffs and spatial heterogeneity in promoting trematode coexistence. *Ecology*, 97, 1484-1496.

Moyer, B.R., Drown, D.M. & Clayton, D.H. (2002). Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos*, 97, 223-228.

Neigel, J.E. (2002). Is F-ST obsolete? *Conserv. Genet.*, 3, 167-173.

Niitepold, K., Mattila, A.L.K., Harrison, P.J. & Hanski, I. (2011). Flight metabolic

rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. *Oecologia*, 165, 847-854.

Niitepold, K., Smith, A.D., Osborne, J.L., Reynolds, D.R., Carreck, N.L., Martin, A.P. *et al.* (2009). Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. *Ecology*, 90, 2223-2232.

Papkou, A., Gokhale, C.S., Traulsen, A. & Schulenburg, H. (2016). Host-parasite coevolution: why changing population size matters. *Zoology*, 119, 330-338.

Philips, J.R. & Fain, A. (1991). Acarine symbionts of louseflies (diptera, hippoboscidae). *Acarologia*, 32, 377-384.

Poulin, R. (2006). *Evolutionary Ecology of Parasites*. Second Edition. Princeton Univ. Press.

Poulin, R. & Morand, S. (2000). The diversity of parasites. *Q. Rev. Biol.*, 75, 277-293.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Richardson, J.L. (2012). Divergent landscape effects on population connectivity in two co-occurring amphibian species. *Mol. Ecol.*, 21, 4437-4451.

Riginos, C., Buckley, Y.M., Blomberg, S.P. & Treml, E.A. (2014). Dispersal capacity predicts both population genetic structure and species richness in reef fishes. *Am. Nat.*, 184, 52-64.

Rodriguez-Verdugo, A., Buckley, J. & Stapley, J. (2017). The genomic basis of eco-evolutionary dynamics. *Mol. Ecol.*, 26, 1456-1464.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A. *et al.* (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature*, 515, 261-U243.

Saastamoinen, M. (2008). Heritability of dispersal rate and other life history traits in the Glanville fritillary butterfly. *Heredity*, 100, 39-46.

Saastamoinen, M., Ikonen, S. & Hanski, I. (2009). Significant effects of Pgi genotype and body reserves on lifespan in the Glanville fritillary butterfly. *Proc. R. Soc. Lond.*, 276, 1313-1322.

Santiago-Alarcon, D., Palinauskas, V. & Schaefer, H.M. (2012). Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biol. Rev.*, 87, 928-964.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotech.*, 18, 233-234.

Thompson, P.L. & Gonzalez, A. (2017). Dispersal governs the reorganization of ecological networks under environmental change. *Nature Ecol. Evol.*, 1, 0162.

Wagner, C.E. & McCune, A.R. (2009). Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. *Evolution*, 63, 1312-1326.

Waples, R.S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *J. Hered.*, 89, 438-450.

Weber, M.G., Wagner, C.E., Best, R.J., Harmon, L.J. & Matthews, B. (2017). Evolution in a community context: on integrating ecological interactions and macroevolution. *Trends Ecol. Evol.*, 32, 291-304.

Whitlock, M.C. (2011). G '(ST) and D do not replace F-ST. *Mol. Ecol.*, 20, 1083-1091.

Witsenburg, F., Clement, L., Lopez-Baucells, A., Palmeirim, J., Pavlinic, I., Scaravelli, D. *et al.* (2015). How a haemosporidian parasite of bats gets around: the genetic structure of a parasite, vector and host compared. *Mol. Ecol.*, 24, 926-940.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 16, 0097-0159.

Wright, S. (1943). Isolation by distance. *Genetics*, 28, 114-138.

Zheng, C.Z., Ovaskainen, O. & Hanski, I. (2009). Modelling single nucleotide effects in phosphoglucose isomerase on dispersal in the Glanville fritillary butterfly: coupling of ecological and evolutionary dynamics. *Philos. Trans. Royal Soc. B.*, 364, 1519-1532.

Table 4.1. Sampling design for the 55 infrapopulations at seven sampling sites across five cities. “Infrapopulation” refers to the conspecific adult lice on a single bird host.

Site Description	Latitude	Longitude	# Louse Infrapopulations
1 Salt Lake City, Utah, USA	40.71883	-111.87129	10
2 Salt Lake City, Utah, USA	40.65505	-111.90167	10
3 Salt Lake City, Utah, USA	40.74088	-111.94931	10
Guarujá, Brazil	-23.99005	-46.2408	6
Winnipeg, Manitoba, Canada	49.80592	-97.13787	10
Grand Forks, North Dakota, USA	47.93141	-97.07051	7
Ružomberok, Slovakia	49.08168	19.30459	4

Table 4.2. Demographic information and ecological variables specific to each sampling site.

	# hosts	Wing louse abun.	Wing louse prev.	Body louse abun.	Body louse prev.	Flies	Hum.	Temp. (°C)
Salt Lake City 1	83	31	100	6	71	present	55	6
Salt Lake City 2	70	34	99	6	73	present	55	6
Salt Lake City 3	72	22	73	9	71	present	55	6
Guarujá	8	52	100	41	75	present	86	14
Winnipeg	>250	110	64	95	87	absent	67	-2.7
Grand Forks	12	10	58	5	92	absent	66	-1
Ružomberok	10	34	100	5	100	absent	60[B]	-1

Table 4.3. Genetic information relevant to each sampling site.

	# Wing lice	# Hosts	# Body lice	# Hosts	F_{ST} wing lice	95% CIs	F_{ST} body lice	95% CIs	Wing Lice N_e	95% CIs	Body Lice N_e	95% CIs
Salt Lake City 1	100	10	100	10	0.0432	[0.0167, 0.0751]	0.1303	[0.1031, 0.1616]	91.5	[56.6, 181.1]	28.9	[22.4, 37.6]
Salt Lake City 2	100	10	100	10	0.0614	[0.0359, 0.0907]	0.2038	[0.1733, 0.2372]	172.1	[83.6, 1054.6]	10.5	[7.7,13.9]
Salt Lake City 3	100	10	97	10	0.0903	[0.062, 0.1229]	0.3155	[0.2889, 0.3454]	37.1	[27.1, 52.3]	5.3	[3.7, 7.3]
Guarujá	60	6	60	6	0.0695	[0.0314, 0.1164]	0.1212	[0.0762, 0.1744]	62.9	[31.1, 242.6]	17.4	[11.6, 26.4]
Winnipeg	100	10	100	10	0.0776	[0.0499, 0.1098]	0.0732	[0.0451, 0.1064]	9.6	[6.2, 14.8]	12.3	[8.1, 18.7]
Grand Forks	36	7	41	7	0.2273	[0.1586, 0.3083]	0.1651	[0.088, 0.2614]	0	[0, 0]	23.6	[12.5, 61.1]
Ružomberok	40	4	36	7	0.0237	[-0.0112, 0.0677]	0.1192	[0.0372, 0.2191]	49.5	[33.7, 78.3]	44.8	[31.4, 67.4]

Table 4.4. Results of AMOVA used to evaluate the amount of population genetic structure both among infrapopoulations and on individual birds for body lice and wing lice. F_{ST} values were calculated from microsatellite data. F_{ST} values range from zero to one. Populations are considered structured if F_{ST} values are significantly different from zero. P values are associated with F_{ST} values. In panmictic (unstructured) louse populations, we would expect to see nearly 100% of the variation arise from within infrapopulations. The AMOVA indicates that wing and body lice populations at all geographic locations are structured between birds.

Site	Source of Variation	% Variation	F_{ST}	P value
Salt Lake City 1 USA	Wing lice			
	Among infrapopulations within site	4.6	0.04688	<0.001
	Infrapopulations on individual birds	95.3		
	Body lice			
	Among infrapopulations within site	12.9	0.12976	<0.001
	Infrapopulations on individual birds	87.0		
Salt Lake City 2 USA	Wing lice			
	Among infrapopulations within site	6.2	0.06226	<0.001
	Infrapopulations on individual birds	93.7		
	Body lice			
	Among infrapopulations within site	18.8	0.18834	<0.001
	Infrapopulations on individual birds	81.1		
Salt Lake City 3 USA	Wing lice			
	Among infrapopulations within site	9.1	0.09153	<0.001
	Infrapopulations on individual birds	90.8		
	Body lice			
	Among infrapopulations within site	34.2	0.34264	<0.001
	Infrapopulations on individual birds	65.7		
Guarujá Brazil	Wing lice			
	Among infrapopulations within site	7.8	0.07821	<0.001
	Infrapopulations on individual birds	92.2		
	Body lice			
	Among infrapopulations within site	12.3	0.12337	<0.001
	Infrapopulations on individual birds	86.7		
Winnipeg Canada	Wing lice			
	Among infrapopulations within site	8.3	0.08249	<0.001
	Infrapopulations on individual birds	91.7		
	Body lice			
	Among infrapopulations within site	7.6	0.07622	<0.001
	Infrapopulations on individual birds	92.4		

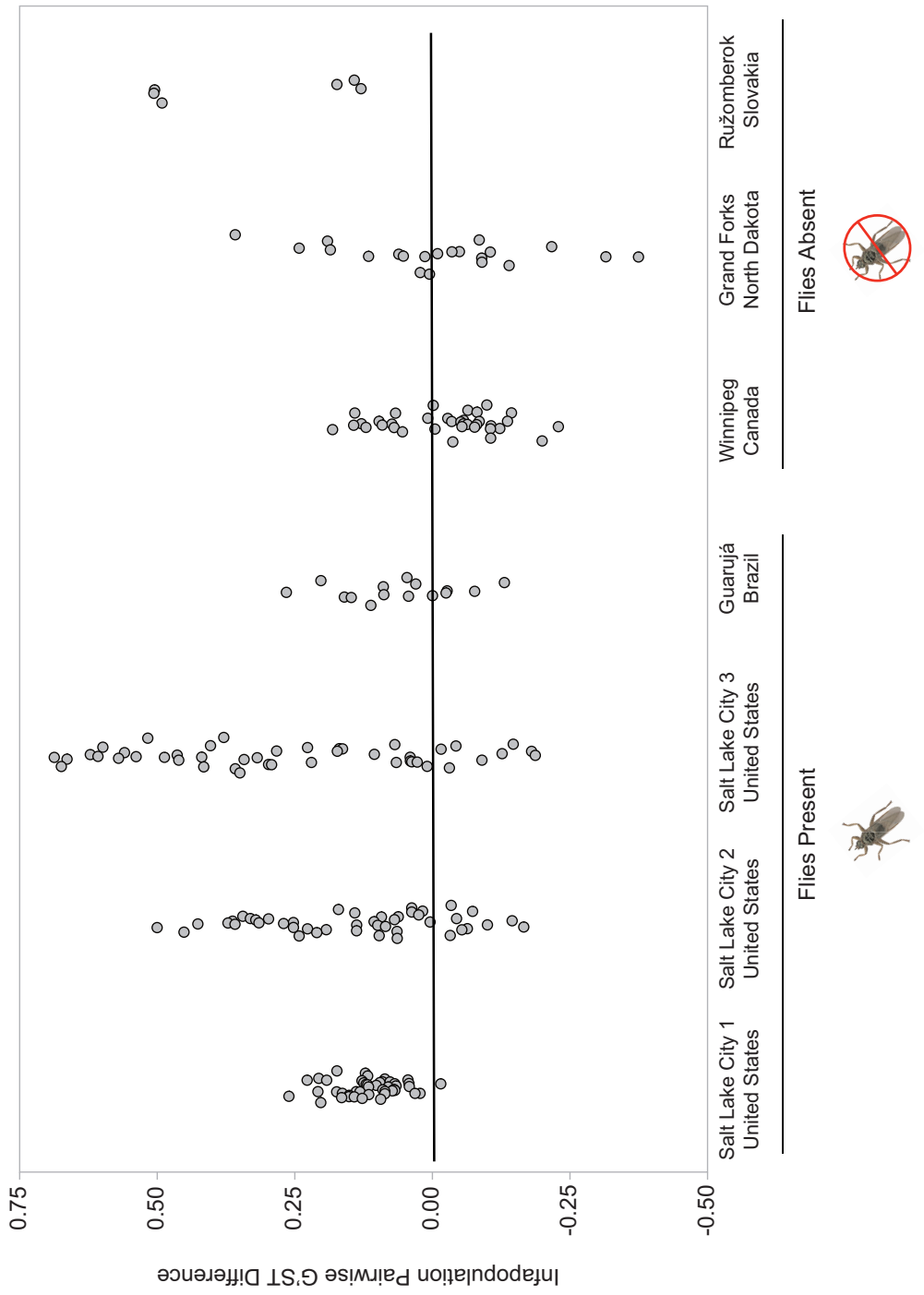
Table 4.4. continued

Site	Source of Variation	% Variation	F_{ST}	P value
Grand Forks USA	Wing lice			
	Among infrapopulations within site	23.8	0.23766	<0.001
	Infrapopulations on individual birds	76.2		
	Body lice			
	Among infrapopulations within site	18.5	0.18450	<0.001
	Infrapopulations on individual birds	81.5		
Ružomberok Slovakia	Wing lice			
	Among infrapopulations within site	2.6	0.02622	<0.05
	Infrapopulations on individual birds	97.4		
	Body lice			
	Among infrapopulations within site	16.1	0.16085	<0.001
	Infrapopulations on individual birds	83.9		

Table 4.5. Factors other than life history traits may influence patterns of genetic differentiation. To assess if allele frequencies change with geographic location due to limited dispersal across space (isolation by distance; IBD) or if allele frequencies change with ecological variables, independent of geographic location due to limited dispersal across ecological gradients (Isolation by environment; IBE) we used Mantel and partial Mantel tests. We tested for a significant relationship between genetic distances and geographic distances or ecological distance between sampling sites. For partial Mantel tests, parentheses indicate the controlled variable in the analysis. Genetic distances were calculated from microsatellites. Genetic Chord distances were used in all comparisons except in intraspecific comparisons where F'_{ST} distances were used to control for comparisons between species. Geographic distances were calculated from coordinates taken at the center of each sampling site and transformed into Euclidian distances. Ecological distance was calculated from a combined data set of average annual low temperature and average annual relative humidity at each sampling site and transformed into Euclidian distances. Asterisks indicate significant results of Mantel or partial Mantel tests.

Comparisons	Mantel's r	p-value
Body Lice		
genetic distance – geographic distance	0.307	0.001 *
genetic distance – geographic distance (infrapopulation size)	0.311	0.501
genetic distance – infrapopulation size	-0.194	0.010 *
genetic distance – infrapopulation size (geographic distance)	-0.178	0.512
genetic distance – ecological distance	-0.184	0.013 *
genetic distance – ecological distance (geographic distance)	-0.178	0.511
Wing Lice		
genetic distance – geographic distance	0.022	0.383
genetic distance – geographic distance (infrapopulation size)	0.059	0.631
genetic distance – infrapopulation size	0.001	0.455
genetic distance – infrapopulation size (geographic distance)	0.006	0.719
genetic distance – ecological distance	0.009	0.429
genetic distance – ecological distance (geographic distance)	0.011	0.711
Interspecific comparison		
body lice genetic distance – wing lice genetic distance	0.201	0.032 *
body lice genetic distance – wing louse genetic distance (geographic distance)	0.209	0.517

Figure 4.1. Each point represents the difference between body lice infrapopulation pairwise G'_{ST} values and wing lice infrapopulation pairwise G'_{ST} values on a single bird. The points are jittered for clarity. The horizontal line at zero signifies the area where there is no difference between the genetic structure of body lice and wing lice. Points above the line indicate that body lice are more structured than wing lice. Points below the line indicate that wing lice are more structured than body lice. In areas with flies most points are above the line indicating that wing lice have less genetic differentiation than body lice, likely due to phoresis. In Winnipeg and Grand Forks points are nearly equally distributed above and below the line indicating that wing and body lice exhibit a similar amount of genetic differentiation at two sites without flies. All points at the Ružomberok site fall above the line. This may indicate that in some geographic areas without flies factors other than phoretic dispersal erode population genetic structure in wing lice when compared with body lice. G'_{ST} values were calculated from microsatellite data. G'_{ST} values range from zero to one like F_{ST}. G'_{ST} was used instead of F_{ST} because it is considered an unbiased estimator of population genetic differentiation and is comparable between species with different effective population sizes.


Infrapopulation Pairwise G'ST Difference
0.75
0.50
0.25
0.00
-0.25
-0.50
Salt Lake City 1 United States
Salt Lake City 2 United States
Salt Lake City 3 United States
Guarujá Brazil
Winnipeg Canada
Grand Forks North Dakota
Ružomberok Slovakia
Flies Present
Flies Absent

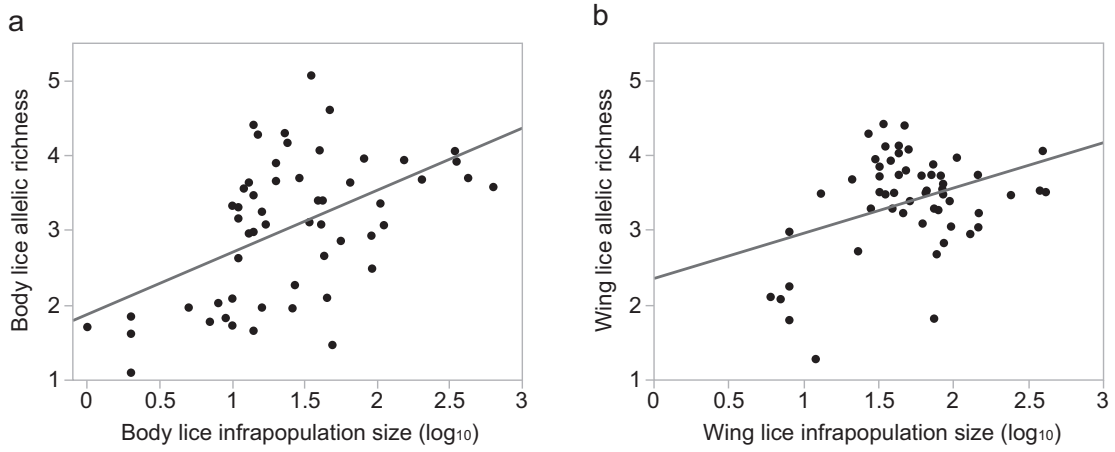


Figure 4.2. The relationship between louse infapopulation size and allelic richness (mean number of alleles per microsatellite locus). (a) Body louse infrapopulation size covaries significantly with allelic richness (see text for details). (b) Wing louse infrapopulation size also covaries significantly with allelic richness (see text for details). Since smaller infrapopulations harbor less genetic diversity than large infrapopulations for both wing and body lice, random genetic drift should play a stronger role in smaller populations of both species.

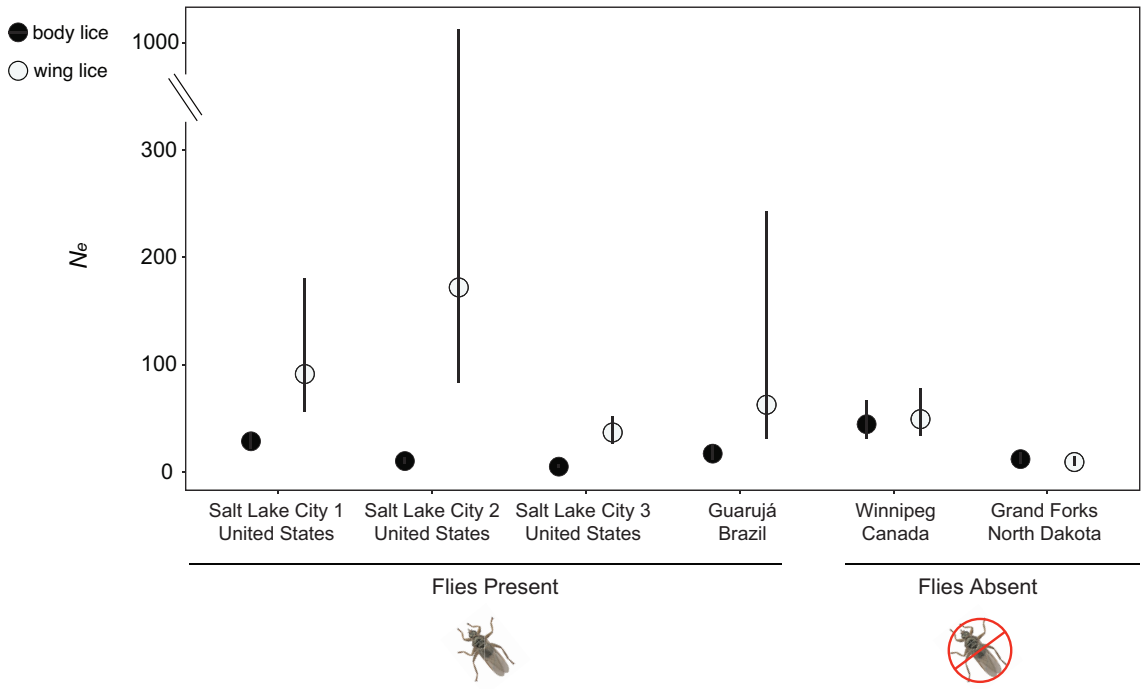


Figure 4.3. Estimates of contemporary effective population size (Ne) with 95% CIs for wing and body lice estimated from microsatellite data. Wing louse estimates of Ne at the Slovakian site were undefined and therefore this site was excluded. The greater Ne of wing lice compared to body lice at sites where flies are present is consistent with enhanced population connectivity due to phoretic dispersal. In the two areas without flies the Ne of wing and body lice have overlapping CIs (not significantly different).

APPENDIX A

SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Table A.1. Microsatellite loci developed for this study. We developed 17 primer sets specific to wing lice (Columbicola columbae) and 13 primer sets specific to body lice (Campanulotes compar). Variable nuclear microsatellite loci were identified by searching for STR motifs (di, tri, tetra) with msatcommander (Rozen & Skaletsky 2000; Faircloth 2008) in sequences generated by Illumina sequencing from 30 pooled individuals. Sequences used to search for microsatellite motifs had BLAST alignment scores ≥200, compared with the human body louse (*Pediculus humanus corporis*) genome, which is the only published louse genome (Kirkness *et al.* 2010). Each microsatellite locus was evaluated with a multistep screening process to ensure quality data as suggested by Selkoe and Toonen (2006) and Fernandez-Silva *et al.* (2013). This filtering yielded 8 microsatellites specific to wing lice, and 8 microsatellites specific to body lice, that were appropriate for analyses. The 17 microsatellite loci used to genotype host pigeons were developed by Chun-lee *et al.* (2007), Stringham *et al.* (2012), and Traxler *et al.* (2000). DNA extractions of wing lice, body lice, and pigeons were performed using the DNeasy Blood and Tissue kit (Qiagen). DNA was extracted from lice as described by Johnson *et al.* (2001). Multiplex PCRs with a universal primer and fluorophore were used to genotype the samples (Schuelke 2000; Blacket *et al.* 2012). The universal primer tail M13 (5’ CAC GAC GTT GTA AAA CGA C 3’) was added to the 5’ end of the locus-specific forward primer. M13 labeled primers were tagged with FAM, PET, NED, or VIC (Applied Biosystems). The two forward primers and the appropriate locus-specific reverse primer were used in PCR reactions. An ABI 3100 Genetic Analyzer (Applied Biosystems) was used to resolve PCR products and was run with the 500 LIZ size standard. Genemapper v 3.7 (Applied Biosystems) was used to determine allele sizes.

	#	Repeat	Forward Primer	Reverse Primer	Length
Body Lice *Campanulotes compar*	2B	(AG) * 19	M13_GACAAGTGACCGTCTCTATCGCG	AGTATGGCCGGTGACTTTCG	351
	5B	(ACT) * 23	M13_GACCGGCTCGAAGTCAAGAAACC	GCACTCTGACAGGCGTTC	423
	6B	(ACT) * 16	M13_GACAAGTCAGTTCGCTCCCTTTG	TCCACCTCCACAACTTTCCG	373
	9B	(AC) * 8	M13_GACTCTCGAAATTTCCACGTGCC	TTGTGTGGCCAAACTCTTCG	318
	10B	(AAG) * 7	M13_GACTTCCTCTCAGAACGCTCAGG	AAGCTTCCAGTTCCCTCTCG	167
	11B	(AG) * 18	M13_GACGAACCGGTTCGAGGAATGTC	AGGAGCGATTAATGCCAAGC	416
	12B	(AGC) * 6	M13_GACAAAGGGAGTGGAGGGTACGG	CGCGATCCAGGTCATTTCAC	277
	13B	(AT) * 7	M13_GACAGTAAGCAGTGGTCAGCAGG	CGTCTTTCCGCGTTAAGTTTG	414
Wing Lice *Columbicola columbae*	3W	(AG) * 14	M13_GACGGAGAGATAGGACGGGCTTG	AAACAAGGACCTCGGACCC	417
	6W	(AAG) * 7	M13_GACGGCGGTGGATGATTGAACAG	TCACGTATGGATCCGGACAG	376
	9W	(AG) * 9	M13_GACTCACACTGGAGGCAAGGAAG	GCACATTTGGACGAGTGGAG	327
	10W	(ATC) * 6	M13_GACTTGAAACTGAGAGGAGTGCC	TCAATTTGACGATCCTGCTGG	443
	12W	(AG) * 9	M13_GACAAAGTCTGGGAGAGGTCAGC	GTTGGTCGAGCAGGAATGAC	300
	13W	(AG) * 13	M13_GACGGTAACTTGTGCGTCCGTAAG	ATCTTCATTTCCGGTTGCGG	376
	15W	(ACAG) * 6	M13_GACGTCTTCTCTTGAGTGTTGCAAC	AATCGTGTGTAGCGTGCAAG	406
	16W	(AG) * 12	M13_GACTTATTCACAGCCTCCCTCCG	ACGGGTCATAAGTGGCAAAG	422

Table A.2. Comparison of body and wing lice prevalence and abundance on pigeons at each site. Prevalence is the percent of pigeons sampled with at least one parasite. Abundance is the mean (± standard error) number of lice removed from each fumigated pigeon by ruffling (including parasite free birds).

Flock #	**Site #**	**# pigeons sampled**	**Parasite prevalence**		**Parasite abundance**	
			Body lice	Wing lice	Body lice	Wing lice
Flock 1	Site 1	83	71.1	100.0	6.0 ± 0.9	31.5 ± 3.4
Flock 2	Site 2	70	72.9	98.6	6.4 ± 1.3	33.8 ± 4.8
Flock 3	Site 3	72	70.8	83.3	8.7 ± 3.2	21.9 ± 3.5

Table A.3. Indices of genetic diversity for body and wing lice subpopulations on each host individual. The mean number of alleles observed (N_A), Observed heterozygosity (H_O), heterozygosity within populations (H_S), and total heterozygosity (H_T) are reported.

			Body Lice - *Camanulotes compar*				Wing Lice - *Columbicola columbae*			
Sampling Site	Host Bird	Host ID	N_A	H_O	H_S	H_T	N_A	H_O	H_S	H_T
Flock 1	Bird1	1SLC4235	4.714	0.470	0.514	0.514	4.500	0.707	0.685	0.685
	Bird2	1SLC42	4.500	0.475	0.560	0.560	4.875	0.675	0.668	0.668
	Bird3	1SLC67	4.500	0.549	0.616	0.616	3.875	0.556	0.608	0.608
	Bird4	1SLC72	2.750	0.500	0.459	0.459	3.750	0.537	0.545	0.545
	Bird5	1SLC50	3.750	0.475	0.489	0.489	4.125	0.483	0.678	0.678
	Bird6	1SLC78	4.625	0.562	0.565	0.565	5.000	0.575	0.683	0.683
	Bird7	1SLC82	5.375	0.565	0.568	0.568	4.250	0.556	0.604	0.604
	Bird8	1SLC56	4.375	0.415	0.546	0.546	4.000	0.532	0.569	0.569
	Bird9	1SLC77	4.000	0.615	0.550	0.550	4.000	0.584	0.678	0.678
	Bird10	1SLC4229	4.000	0.519	0.566	0.566	4.250	0.507	0.620	0.620
Flock 2	Bird1	2SLC4290	5.000	0.581	0.570	0.570	4.375	0.534	0.647	0.647
	Bird2	2SLC50	2.500	0.264	0.320	0.320	3.500	0.488	0.539	0.539
	Bird3	2SLC51	5.125	0.575	0.597	0.597	4.625	0.583	0.641	0.641
	Bird4	2SLC56	4.250	0.519	0.522	0.522	3.875	0.494	0.561	0.561
	Bird5	2SLC69	4.625	0.450	0.527	0.527	4.875	0.562	0.626	0.626
	Bird6	2SLC68	5.125	0.465	0.535	0.535	4.500	0.513	0.589	0.589
	Bird7	2SLC63	3.375	0.513	0.481	0.481	3.750	0.460	0.532	0.532
	Bird8	2SLC53	2.125	0.339	0.271	0.271	4.500	0.632	0.651	0.651
	Bird9	2SLC57	2.375	0.263	0.318	0.318	3.750	0.625	0.569	0.569
	Bird10	2SLC54	3.750	0.356	0.457	0.457	4.625	0.643	0.648	0.648
Flock 3	Bird1	3SLC4316	5.143	0.481	0.574	0.574	3.250	0.529	0.558	0.558
	Bird2	3SLC46	3.250	0.444	0.516	0.516	4.250	0.625	0.611	0.611
	Bird3	3SLC39	1.500	0.158	0.179	0.179	3.000	0.422	0.423	0.423
	Bird4	3SLC45	5.000	0.562	0.587	0.587	4.250	0.569	0.626	0.626
	Bird5	3SLC38	3.000	0.354	0.467	0.467	4.500	0.475	0.619	0.619
	Bird6	3SLC54	5.000	0.519	0.537	0.537	5.250	0.604	0.672	0.672
	Bird7	3SLC65	3.875	0.532	0.570	0.570	3.875	0.425	0.492	0.492
	Bird8	3SLC53	1.750	0.425	0.273	0.273	4.375	0.713	0.653	0.653
	Bird9	3SLC66	1.875	0.222	0.230	0.230	3.750	0.550	0.538	0.538
	Bird10	3SLC4315	4.375	0.390	0.493	0.493	4.000	0.550	0.576	0.576

Table A.4. Pairwise FST estimates for body lice (Campanulotes compar) populations estimated from 8 microsatellite markers. Population comparisons that are statistically significant after correcting for false discovery rates are shaded in gray.

		Flock 1										Flock 2										Flock 3									
	Body Lice	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10
Flock 1	Bird1	0.00																													
	Bird2	0.07	0.00																												
	Bird3	0.04	0.07	0.00																											
	Bird4	0.23	0.18	0.19	0.00																										
	Bird5	0.14	0.10	0.09	0.22	0.00																									
	Bird6	0.08	0.11	0.08	0.20	0.12	0.00																								
	Bird7	0.05	0.10	0.07	0.24	0.15	0.05	0.00																							
	Bird8	0.14	0.11	0.04	0.24	0.10	0.12	0.16	0.00																						
	Bird9	0.08	0.08	0.07	0.20	0.10	0.09	0.14	0.10	0.00																					
	Bird10	0.12	0.09	0.03	0.16	0.09	0.05	0.07	0.11	0.08	0.00																				
Flock 2	Bird1	0.11	0.00	0.04	0.10	0.07	0.02	0.01	0.12	0.02	0.03	0.00																			
	Bird2	0.34	0.27	0.28	0.44	0.34	0.31	0.30	0.28	0.36	0.37	0.36	0.00																		
	Bird3	0.00	0.05	0.05	0.19	0.08	0.05	0.04	0.10	0.06	0.02	-0.02	0.29	0.00																	
	Bird4	0.06	0.11	0.10	0.24	0.16	0.15	0.12	0.18	0.12	0.12	0.05	0.35	0.09	0.00																
	Bird5	0.04	0.10	0.04	0.24	0.13	0.10	0.08	0.12	0.10	0.09	0.05	0.31	0.04	0.09	0.00															
	Bird6	0.04	0.07	0.03	0.18	0.07	0.05	0.07	0.09	0.07	0.04	0.00	0.28	0.04	0.08	0.04	0.00														
	Bird7	0.11	0.13	0.11	0.21	0.13	0.10	0.12	0.15	0.11	0.05	0.05	0.32	0.08	0.18	0.13	0.09	0.00													
	Bird8	0.35	0.26	0.30	0.46	0.32	0.30	0.28	0.37	0.33	0.38	0.32	0.42	0.26	0.38	0.30	0.29	0.31	0.00												
	Bird9	0.26	0.20	0.25	0.41	0.26	0.25	0.23	0.33	0.28	0.32	0.25	0.39	0.21	0.32	0.25	0.22	0.25	0.00	0.00											
	Bird10	0.09	0.11	0.10	0.28	0.12	0.10	0.09	0.18	0.13	0.14	0.07	0.32	0.07	0.09	0.08	0.07	0.11	0.23	0.14	0.00										
Flock 3	Bird1	0.09	-0.01	-0.02	0.15	0.05	0.04	0.00	0.07	0.04	0.02	0.04	0.30	-0.03	0.06	0.00	0.00	0.08	0.31	0.26	0.09	0.00									
	Bird2	0.11	0.14	0.11	0.22	0.15	0.08	0.13	0.15	0.14	0.10	0.06	0.36	0.09	0.19	0.14	0.09	0.12	0.38	0.32	0.15	0.07	0.00								
	Bird3	0.46	0.55	0.47	0.61	0.56	0.52	0.50	0.52	0.51	0.58	0.50	0.66	0.49	0.53	0.51	0.52	0.55	0.71	0.68	0.59	0.51	0.56	0.00							
	Bird4	0.02	0.03	0.04	0.15	0.07	0.08	0.07	0.10	0.05	0.03	-0.05	0.32	0.03	0.09	0.05	0.05	0.09	0.28	0.22	0.07	-0.08	0.10	0.52	0.00						
	Bird5	0.09	0.24	0.21	0.35	0.25	0.21	0.27	0.25	0.22	0.19	0.11	0.45	0.17	0.23	0.21	0.21	0.24	0.48	0.42	0.28	0.14	0.22	0.54	0.19	0.00					
	Bird6	0.04	0.08	0.06	0.21	0.11	0.07	0.09	0.14	0.07	0.09	0.00	0.33	0.04	0.10	0.05	0.07	0.07	0.29	0.22	0.03	0.02	0.06	0.53	0.01	0.18	0.00				
	Bird7	0.12	0.11	0.12	0.23	0.13	0.12	0.18	0.12	0.15	0.11	0.07	0.31	0.09	0.21	0.17	0.12	0.16	0.33	0.30	0.23	0.06	0.14	0.51	0.11	0.16	0.15	0.00			
	Bird8	0.32	0.38	0.34	0.42	0.44	0.39	0.35	0.40	0.42	0.39	0.38	0.53	0.35	0.39	0.34	0.39	0.41	0.55	0.52	0.42	0.32	0.45	0.71	0.33	0.46	0.38	0.41	0.00		
	Bird9	0.36	0.35	0.32	0.48	0.40	0.34	0.31	0.41	0.36	0.41	0.33	0.51	0.31	0.36	0.34	0.33	0.34	0.50	0.43	0.27	0.34	0.34	0.73	0.30	0.47	0.22	0.44	0.62	0.00	
	Bird10	0.11	0.10	0.08	0.23	0.10	0.06	0.10	0.12	0.09	0.10	0.03	0.34	0.07	0.14	0.10	0.06	0.07	0.31	0.25	0.08	0.05	0.05	0.59	0.05	0.22	0.02	0.15	0.43	0.28	0.00

Table A.5. Pairwise FST estimates for wing lice (Columbicola columbae) populations estimated from 8 microsatellite markers. Population comparisons that are statistically significant after correcting for false discovery rates are shaded in gray.

		Flock 1										Flock 2										Flock 3									
	Wing Lice	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10	Bird1	Bird2	Bird3	Bird4	Bird5	Bird6	Bird7	Bird8	Bird9	Bird10
Flock 1	Bird1	0.00																													
	Bird2	0.07	0.00																												
	Bird3	0.02	0.03	0.00																											
	Bird4	0.16	0.07	0.13	0.00																										
	Bird5	-0.02	0.01	0.04	0.12	0.00																									
	Bird6	0.04	0.01	0.01	0.09	0.00	0.00																								
	Bird7	0.05	0.06	0.02	0.16	0.02	-0.02	0.00																							
	Bird8	0.05	0.02	0.00	0.08	0.07	0.00	0.05	0.00																						
	Bird9	0.01	0.03	0.02	0.11	0.03	0.03	0.07	0.03	0.00																					
	Bird10	0.05	-0.01	0.04	0.07	0.02	0.01	0.05	0.00	0.03	0.00																				
Flock 2	Bird1	0.09	-0.01	0.02	0.05	0.06	0.01	0.07	0.03	0.04	0.00	0.00																			
	Bird2	0.11	0.02	0.06	0.13	0.07	0.10	0.13	0.07	0.06	0.04	0.04	0.00																		
	Bird3	0.06	0.00	0.01	0.11	0.01	0.01	0.04	0.03	0.03	0.01	0.00	0.04	0.00																	
	Bird4	0.11	0.04	0.10	0.10	0.11	0.07	0.14	0.05	0.05	0.02	0.05	0.10	0.05	0.00																
	Bird5	0.03	0.04	-0.01	0.09	0.01	0.03	0.05	0.00	0.02	0.03	0.02	0.07	0.03	0.06	0.00															
	Bird6	0.11	0.03	0.08	0.04	0.06	0.05	0.10	0.02	0.06	0.02	0.02	0.08	0.07	0.02	0.06	0.00														
	Bird7	0.13	0.09	0.10	0.09	0.10	0.08	0.13	0.03	0.11	0.08	0.09	0.14	0.14	0.11	0.09	0.01	0.00													
	Bird8	0.06	0.03	0.03	0.05	0.04	0.05	0.10	0.01	0.03	0.02	0.01	0.06	0.05	0.04	0.04	0.01	0.03	0.00												
	Bird9	0.09	0.06	0.04	0.13	0.06	0.05	0.06	0.02	0.11	0.06	0.08	0.12	0.09	0.15	0.06	0.09	0.05	0.08	0.00											
	Bird10	0.04	0.03	0.05	0.06	-0.01	0.07	0.10	0.07	0.02	0.03	0.04	0.06	0.04	0.08	0.02	0.06	0.13	0.06	0.10	0.00										
Flock 3	Bird1	0.09	0.10	0.09	0.16	0.05	0.11	0.15	0.12	0.11	0.11	0.10	0.11	0.14	0.14	0.09	0.07	0.10	0.09	0.12	0.10	0.00									
	Bird2	0.06	0.02	0.01	0.08	0.01	0.01	0.00	0.00	0.05	0.00	0.03	0.07	0.03	0.07	0.02	0.05	0.07	0.04	0.04	0.06	0.11	0.00								
	Bird3	0.21	0.16	0.19	0.15	0.17	0.22	0.26	0.21	0.16	0.19	0.15	0.11	0.20	0.24	0.13	0.13	0.19	0.14	0.23	0.10	0.17	0.20	0.00							
	Bird4	0.06	0.01	0.01	0.09	0.03	0.03	0.04	0.02	0.03	0.01	0.00	0.02	0.02	0.07	0.03	0.04	0.09	0.02	0.07	0.06	0.08	0.01	0.12	0.00						
	Bird5	0.08	-0.01	0.02	0.03	0.03	0.02	0.05	0.00	0.05	0.00	-0.01	0.04	0.03	0.06	0.02	0.00	0.05	0.02	0.05	0.04	0.07	0.00	0.12	0.00	0.00					
	Bird6	0.04	0.03	0.02	0.07	0.02	0.00	0.02	0.01	0.02	0.01	0.02	0.09	0.04	0.05	0.02	0.02	0.06	0.02	0.08	0.06	0.09	0.00	0.19	0.02	0.00	0.00				
	Bird7	0.15	0.12	0.14	0.11	0.15	0.18	0.24	0.14	0.12	0.14	0.11	0.09	0.16	0.18	0.10	0.13	0.16	0.09	0.18	0.10	0.15	0.16	0.04	0.10	0.11	0.17	0.00			
	Bird8	0.06	0.01	0.02	0.05	0.02	0.03	0.06	0.02	0.02	0.02	0.00	0.05	0.03	0.06	0.02	0.02	0.07	0.03	0.09	0.03	0.09	0.01	0.12	0.02	-0.01	0.00	0.11	0.00		
	Bird9	0.09	0.10	0.04	0.20	0.09	0.06	0.09	0.09	0.10	0.09	0.07	0.17	0.08	0.12	0.06	0.12	0.18	0.11	0.15	0.13	0.10	0.07	0.28	0.08	0.06	0.05	0.24	0.07	0.00	
	Bird10	0.08	0.09	0.09	0.15	0.04	0.12	0.17	0.10	0.09	0.08	0.09	0.10	0.11	0.09	0.03	0.09	0.12	0.09	0.12	0.05	0.03	0.11	0.16	0.11	0.09	0.10	0.13	0.09	0.14	0.00

Table A.6. Measures of genetic differentiation. (a) Global estimates of FST values with lower and upper 95% CIs in brackets for body and wing lice. (b) Global estimates of G''_{ST} values with lower and upper 95% CIs in brackets for body and wing lice.

a

F_{ST}	Body Lice	Wing Lice
All sites	0.226 [0.210, 0.244]	0.069 [0.053, 0.087]
Site 1	0.130 [0.103, 0.162]	0.043 [0.017, 0.075]
Site 2	0.204 [0.173, 0.237]	0.061 [0.036, 0.091]
Site 3	0.316 [0.289, 0.345]	0.090 [0.062, 0.123]

b

G''_{ST}	Body Lice	Wing Lice
All sites	0.444 [0.421, 0.468]	0.181 [0.150, 0.214]
Site 1	0.295 [0.248, 0.347]	0.130 [0.071, 0.198]
Site 2	0.376 [0.331, 0.423]	0.161 [0.110, 0.216]
Site 3	0.576 [0.546, 0.607]	0.220 [0.169, 0.274]

Table A.7. The optimal number of genetic clusters chosen from wing and body lice genotypes. We tested values of k = 1–30 corresponding to the 30 subpopulations of each louse species. Body lice grouped into more clusters than wing lice. Half of the body lice clusters contained genotypes sampled from ≤ 5 pigeons. In contrast for wing lice, the smallest number of hosts in a cluster was 11. This suggests that dispersal between birds is more restricted in body lice than wing lice.

Species	Cluster	# Lice in cluster	# Hosts in cluster
Body Lice	1	24	5
	2	10	1
	3	10	1
	4	39	16
	5	16	6
	6	27	15
	7	27	13
	8	15	5
	9	31	13
	10	15	4
	11	11	2
	12	31	13
	13	10	1
	14	31	15
Wing Lice	1	29	15
	2	27	12
	3	27	11
	4	37	20
	5	25	11
	6	26	16
	7	33	19
	8	36	17
	9	25	14
	10	35	16

Table A.8. GLMM summary testing the probability that a given allele is (a) unique to the subpopulation (private allele) compared to all other lice at each collection site respective of species or (b) is a private allele for that louse subpopulation on its individual host bird. Asterisks indicate statistical significance in probability tests.

a	Random effects	Variance	Standard deviation		
	Site	1.15E-16	1.07E-08		
	Fixed effects	Estimate	Standard error	Z-value	Pr(>|z|)
	Intercept (body lice)	-0.488	0.086	-5.65	<0.001*
	wing lice	-0.920	0.153	-6.01	<0.001*
b	Random effects	Variance	Standard deviation		
	Host	0.013	0.114		
	Fixed effects	Estimate	Standard error	Z-value	Pr(>|z|)
	Intercept (body lice)	-1.599	0.089	-17.91	<0.001*
	wing lice	-1.361	0.155	-8.81	<0.001*

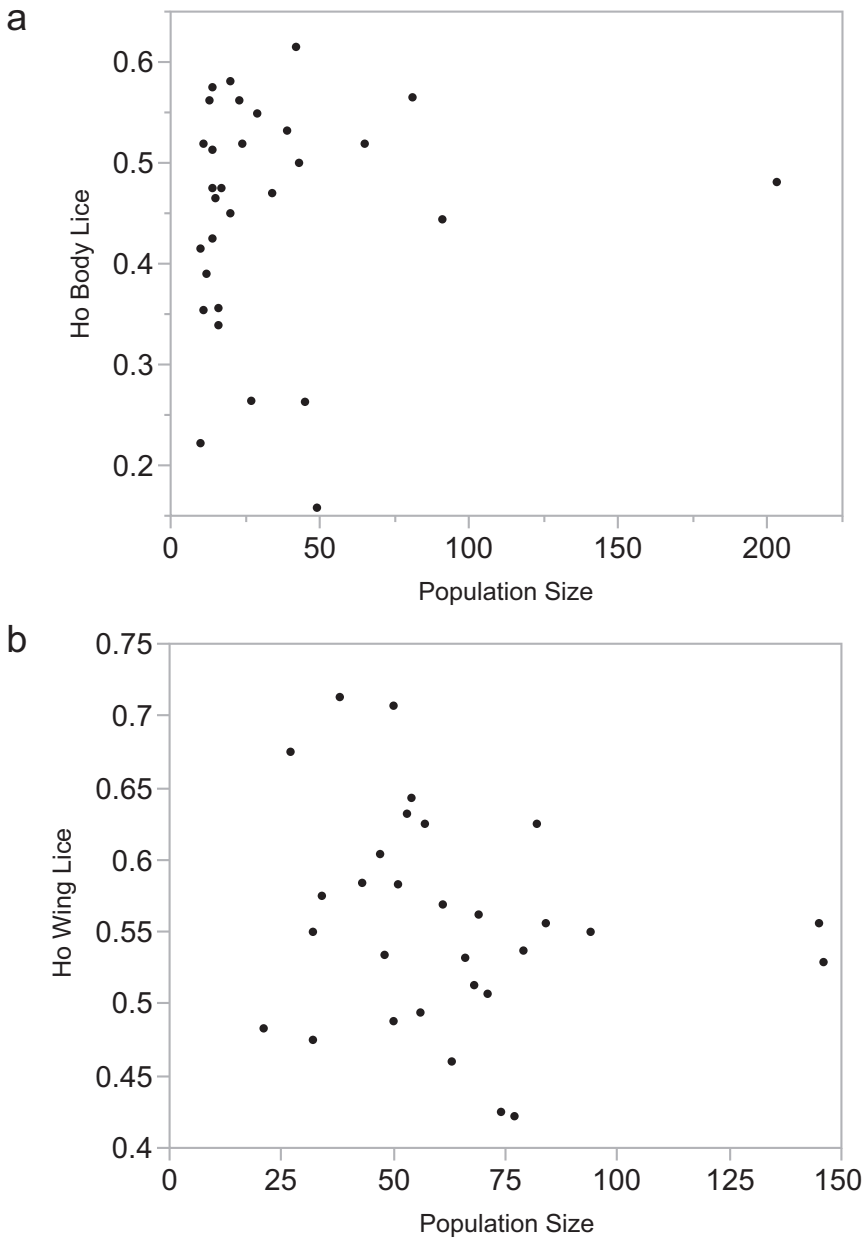


Figure A.1. Observed heterozygosity in relation to subpopulation size for (a) body lice and (b) wing lice.

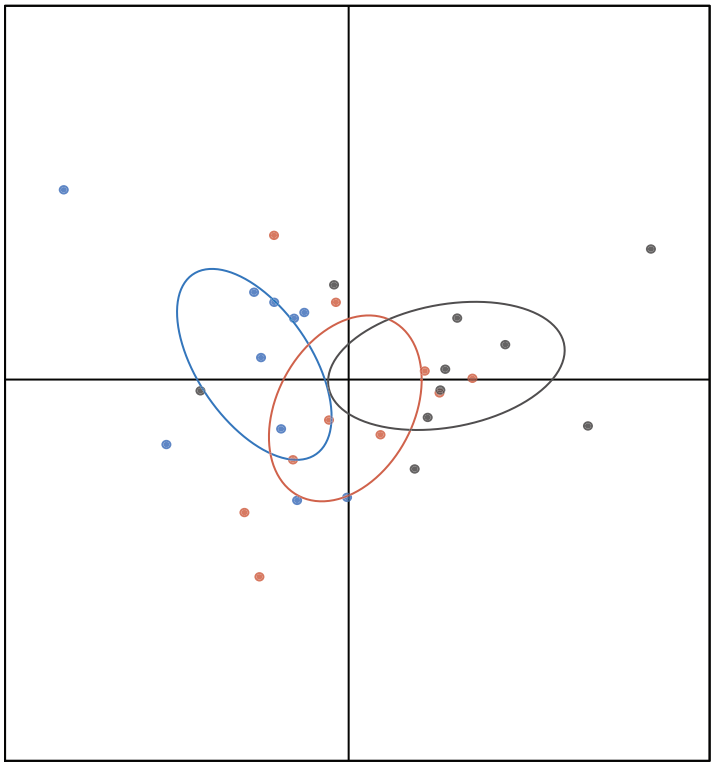


Figure A.2. Ordination plot showing the first two principal components of the DAPC for pigeons. Colors show the sites/flock from which birds were collected (Site 1: blue; Site 2: red; Site 3: gray). Dots represent individual pigeon genotypes. Circles represent confidence intervals of the DAPC.

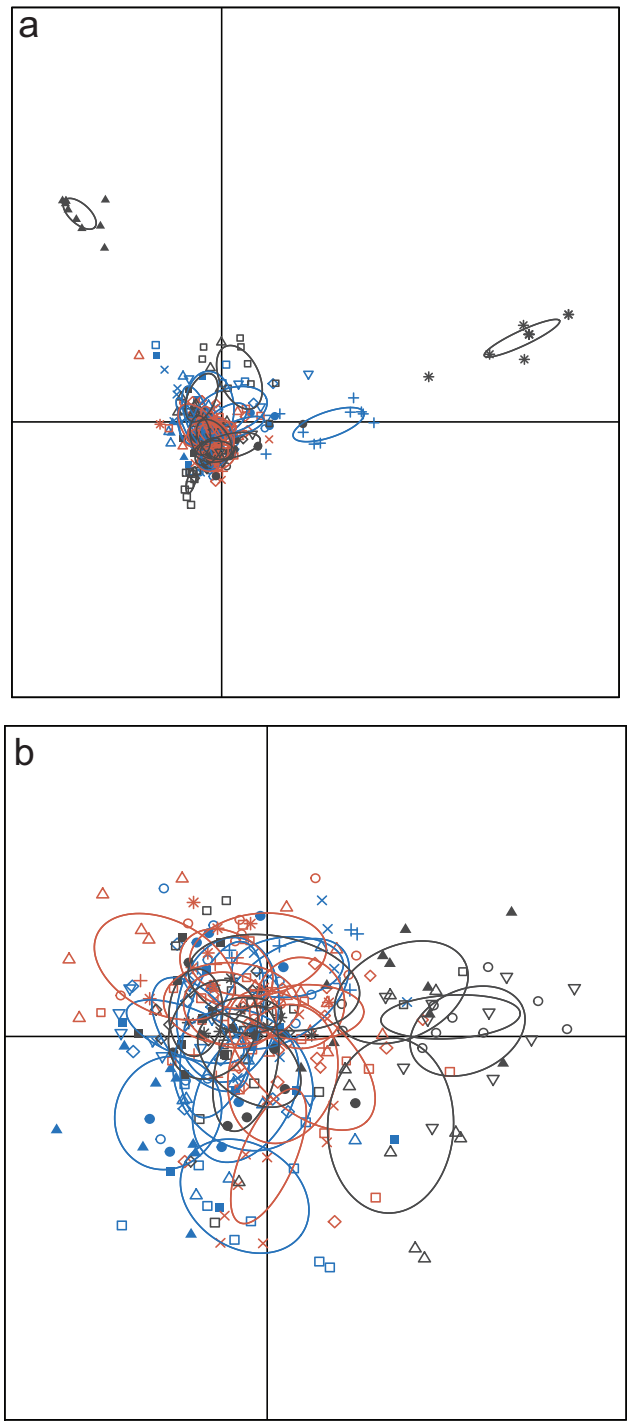


Figure A.3. Ordination plots showing the first two principal components of the DAPC for (a) body lice and (b) wing lice, grouped by bird. Colors show the sites from which lice were collected (Site 1: blue; Site 2: red; Site 3: gray). Different symbols represent different subpopulations of lice. Circles represent confidence intervals of the DAPC.

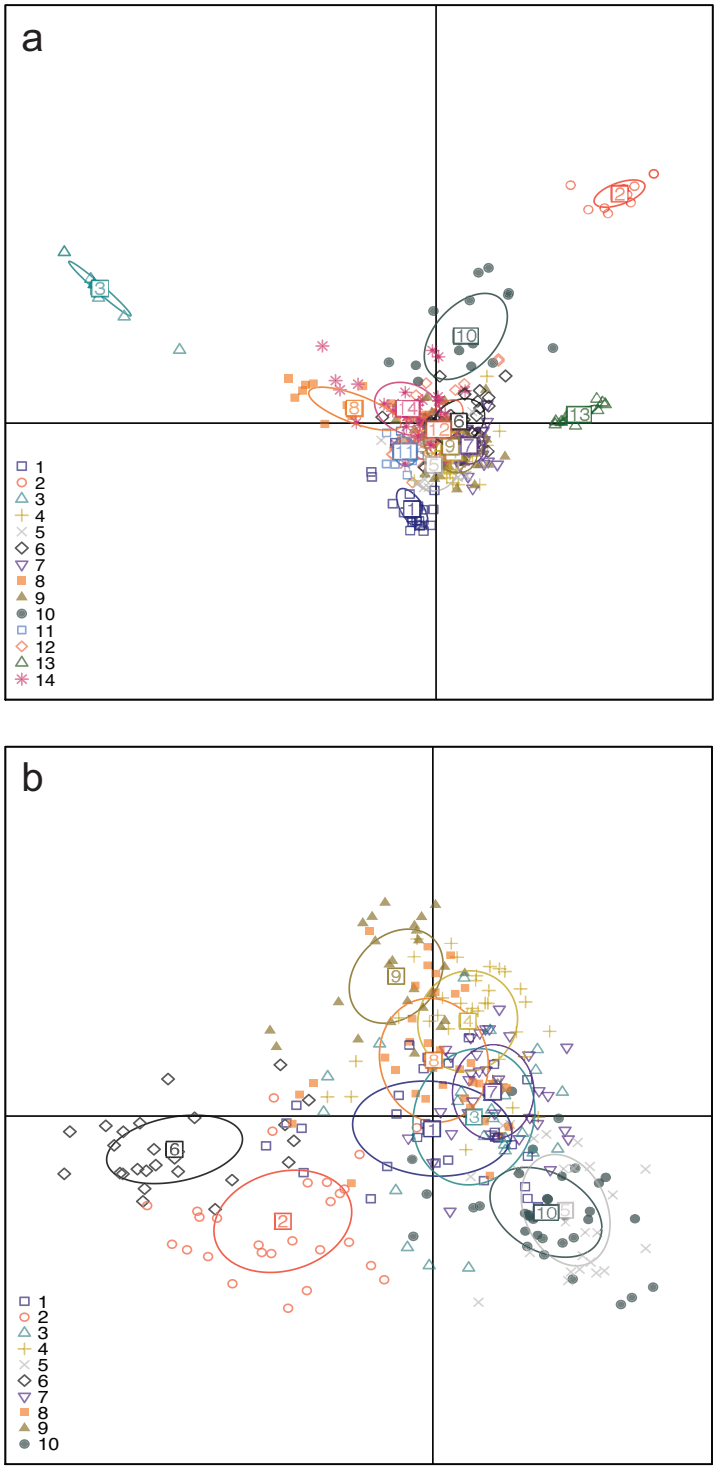


Figure A.4. Ordination plots showing the first two principal components of the DAPC for (a) body lice and (b) wing lice grouped by k means clustering. Different colors indicate different genetic clusters and different symbols represent different louse subpopulations. Circles represent confidence intervals of the DAPC.

References

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F. & Miller, A.D. (2012). Universal primers for fluorescent labelling of PCR fragments-an efficient and cost-effective approach to genotyping by fluorescence. *Mol. Ecol. Res.*, 12, 456-463.

Chun-Lee, J., Tsai, L.-C., Kuan, Y.-Y., Chien, W.-H., Chang, K.-T., Wu, C.-H. et al. (2007). Racing pigeon identification using STR and chromo-helicase DNA binding gene markers. *Electrophoresis*, 28, 4274-4281.

Faircloth, B.C. (2008). MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol. Ecol. Res.*, 8, 92-94.

Fernandez-Silva, I., Whitney, J., Wainwright, B., Andrews, K.R., Ylitalo-Ward, H., Bowen, B.W. et al. (2013). Microsatellites for next-generation ecologists: a post-sequencing bioinformatics pipeline. *Plos One*, 8. https://doi.org/10.1371/journal.pone.0055990

Kirkness, E.F., Haas, B.J., Sun, W.L., Braig, H.R., Perotti, M.A., Clark, J.M. et al. (2010). Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proc. Natl Acad. Sci. USA*, 107, 12168-12173.

Rozen, S. & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* (Clifton, N.J.), 132, 365-386.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnol.*, 18, 233-234.

Selkoe, K.A. & Toonen, R.J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.*, 9, 615-629.

Stringham, S.A., Mulroy, E.E., Xing, J., Record, D., Guernsey, M.W., Aldenhoven, J.T. et al. (2012). Divergence, convergence, and the ancestry of feral populations in the domestic rock pigeon. *Curr. Biol.*, 22, 302-308.

Traxler, B., Brem, G., Muller, M. & Achmann, R. (2000). Polymorphic DNA microsatellites in the domestic pigeon, *Columba livia* var. *domestica*. *Mol. Ecol.*, 9, 366-368.

APPENDIX B

GEOGRAPHIC RECORDS OF *PSEUDOLYNCHIA CANARIENSIS*

Reference #	City/ Region	Country	Reference
1	Campania Region	Italy	(Dipineto *et al.* 2013)
2	Porto de Pelotas	Brazil	(da Cunha Amaral *et al.* 2013)
3		Turkey	(Dik 2012)
4	South Khorasan	Iran	(Radfar *et al.* 2012)
5	Yaeyama Islands	Japan	(Yamauchi *et al.* 2011)
6	Pisa	Italy	(Macchioni *et al.* 2005)
7	Santa Crus de Tenerife	Canary Archipelago	(Foronda *et al.* 2004)
8	Zaria	Nigeria	(George *et al.* 2004)
9	Tel Aviv	Singapore Israel	(Paperna & Smallridge 2002)
10		India	(Mandal 1991)
11	Barcelona	Spain	(Jovani *et al.* 2001)
12	Catalonia	Spain	(Sol *et al.* 2000)
13	Kampala	Uganda	(Dranzoa *et al.* 1999)
14	Thrissur	India	(Vel & Pillai 1998)
15		Israel	(Bear & Freidberg 1995)
16	Fort Dix, New Jersey	United States	(Harlan & Kramer 1979)
17	Zaria	Nigeria	(Shotter 1978)
18	Amami-Oshima	Japan	(Mogi 1977)
19	Detroit	Michigan	(Klei & Degiusti 1975b)
20	South Bend	United States	(Sanders & Petersen 1975)
21	Canungra, Queensland	Australia	(Roberts 1945)
22	Chicago	United States	(Ward 1953)
23	New York Massachusetts Washington D.C. South Carolina Georgia Florida	United States	(Bequaert 1943b)

	Alabama Mississippi Louisiana Texas Arkansas Iowa California		
24	Detroit, Michigan	United States	(Klei & Degiusti 1975a)
25		England United States	(Santiago-Alarcon *et al.* 2014)
26	Washington D.C. South Carolina Georgia Florida Alabama Mississippi Louisiana Texas Arkansas California	United States	(Bequaert 1939)
27	Agra, Borinquen, Natal	India, Puerto Rico, Trinidad, Brazil	Flies on an aircraft Bequaert, J. Hippoboscidae (Diptera) Transported by Aircraft
28	Sao Paulo	Brazil Mexico Guyana	(Bequaert 1943a)
29	Madras	India	(Subbiah & Joseph 1986)
30	California	United States	(Kadner 1941)
31	Cairo	Egypt	(Rashdan 1998)
32	Shah Alam, Selangor	Malaysia	(Amin-Babjee *et al.* 1993)
33	Boston	United States	(Brown 1971)
34	Sebele, Gaborone	Botswana	(Mushi *et al.* 2000)
35	Rio de Janeiro	Brazil	(Valim & Gazeta 2007)
36	Morogoro Municipality	Tanzania	(Msoffe *et al.* 2010)

37	Juiz de For a	Brazil	(Arcoverde *et al.* 2009)
38	Rio de Janeiro	Brazil	(Gredilha *et al.* 2008)
39	Cairo	Egypt	(Ahmed & Mohammed 1977)
40	Juiz de For a	Brazil	(Arcoverde *et al.* 2007)
41	Captive Racing Pigeons	Brazil	(Resende *et al.* 2001)
42	Morrum	Sweden	(Andersson 1985)
43	Captive Pigeons in Juiz de For a	Brazil	(Marcelino *et al.* 2009)
44	Sumter, South Carolina	United States	(Owiny & French 2000)
45	Lages, Santa Catarina	Brazil	(Marques *et al.* 2007)
46	Havana	Cuba	(Gregor *et al.* 1973)
47	Barcelona, Zaragoza	Spain	(Carles-Tolrá 1998)
48	Morrum, Attica, Tunis, Aswan, Fogo, Shyock Valley between Karghalik and Saser Brangsa, Asmara, Kuisip, St. Denis, Tharangambadi, Phan Rang, Jefferson County Florida, St. Croix, Rio de Janeiro, Taipei, Isinchu city, Nantou County, Kaohsiung County, Taitung County, Taipei County	Greece, Tunis, Egypt, Cape Verde Island, Afghanistan, NW China, Eritrea, Congo, Kenya, South Africa, Reunion Island, India, Sumatra, Java, Thailand, Vietnam, Taiwan, U.S.A., West Indies, Brazil	(Maa 1966)
49	Shahrekord	Iran	(Pirali-Kheirabadi *et al.* 2016)

50	Atlantico (Baranquilla) Bolivar (Cartagena) Cundinamarca (Bogotá) Vale del Cauca (Cali, Palmira)	Columbia	(Graciolli & Barros de Carvalho 2003; Graciolli 2016) [LB1]
		U.S.A (including Hawaii Islands) Mexico Honduras Costa Rica Panama Cuba Jamaica Haiti Puerto Rico Virgins Islands St. Croix, St. Kitts Antigua and Barbados Trinidad and Tobago Venezuela Guiana Brazil Argentina Uruguay	

References

Ahmed, F.E. & Mohammed, A.H. (1977). Schizogony in Haemoproteus columbae Kruse. *J. Protozool.*, 24, 389–393.

Amin-Babjee, S.M., Lee, C.C. & Mohna, S.S. (1993). Parasites of the house crow (corvus splendens). *J. Vet. Malaysia*, 5, 59-60.

Andersson, H. (1985). The Swedish louse-flies (Diptera: Hippohoscidae). *Entomologisk tidskrift* 106, 15-25.

Arcoverde, A.R., Rodrigues, A.F.F. & Daemon, E. (2007). A new technique for feeding Pseudolynchia canariensis (Macquart, 1839) (Diptera, Hippoboscidae) under laboratory conditions. *Parasitol. Res.*, 102, 135-137.

Arcoverde, A.R., Soares Ferreira Rodrigues, A.F. & Daemon, E. (2009). Feeding and breeding aspects of Pseudolynchia canariensis (Macquart, 1839) (Diptera, Hippoboscidae) under laboratory conditions. *Parasitol. Res.*, 104, 277-280.

Bear, A. & Freidberg, A. (1995). Contribution to the knowledge of the Ornithomyini of israel (Diptera, Hippoboscidae). *Israel J. Zool.*, 41, 109-124.

Bequaert, J. (1939). Hippoboscid flies from North American doves. *Science*, 89, 267-268.

Bequaert, J. (1943a). Notes on Hippoboscidae. 16. Hippoboscidae from Southern Brazil. With the Description of a New Species of Lynchia. *J. Parasitol.*, 29, 131-135.

Bequaert, J. (1943b). The Pigeon-fly, Pseudolynchia canariensis (Macquart), in New England and New York (Hippoboscidae, Diptera). *Bull. Brooklyn Entomol. Soc.*, 37, 185-186.

Brown, N.S. (1971). A survey of the arthropod parasites of pigeons (Columba livia) in Boston. *J. Parasitol.*, 57, 1379-1380.

Carles-Tolrá, M. (1998). New data on the Diptera from Spain (Díptera: Scenopinidae, Xylomyidae, Lonchopteridae, Hippoboscidae, Nycteribiidae). *Boletín de la Asociación española de Entomología*, 22, 75-79.

da Cunha Amaral, H.L., Bergmann, F.B., Silveira, T., Silveira dos Santos, P.R. & Krueger, R.F. (2013). Pseudolynchia canariensis (Diptera: Hippoboscidae): distribution pattern and phoretic association with skin mites and chewing lice of Columba livia (Aves: Columbidae). *J. Nat. Hist.*, 47, 2927-2936.

Dik, B. (2012). The First Record of *Ornithocheyletia hallae* Smiley, 1970 (Acariformes: Actinedida Cheyletidae) and Its Prevalance on Pseudolynchia canariensis (Mcquart, 1840) (Diptera: Pupipara: Hippoboscidae) in Turkey. *Turkiye Parazitol. Derg.*, 36, 169-173.

Dipineto, L., Borrelli, L., Pepe, P., Fioretti, A., Caputo, V., Cringoli, G. *et al.* (2013). Synanthropic birds and parasites. *Avian Dis.*, 57, 756-758.

Dranzoa, C., Ocaido, M. & Katete, P. (1999). The ecto-, gastro-intestinal and haemo-parasites of live pigeons (Columba livia) in Kampala, Uganda. *Avian Pathol.*, 28, 119-124.

Foronda, P., Valladares, B., Rivera-Medina, J.A., Figueruelo, E., Abreu, N. & Casanova, J.C. (2004). Parasites of Columba livia (Aves: Columbiformes) in Tenerife (Canary Islands) and their role in the conservation biology of the Laurel pigeons. *Parasite*, 11, 311-316.

George, B.D.J., Sa'idu, L. & Abdu, P.A. (2004). A review and case reports of parasitic arthropods infesting local poultry and their roles in the transmission of haemoparasites in Nigeria. *Tropical Veterinarian*, 22, 61-71.

Graciolli, G. (2016). Family Hippoboscidae. *Zootaxa*, 4122, 771-779.

Graciolli, G. & Barros de Carvalho, C.J. (2003). Hippoboscidae (Diptera, Hippoboscoidea) in the State of Paraná, Brazil: keys, hosts and geographic distribution. *Revista Brasileira de Zoologia*, 20, 667-674.

Gredilha, R., Balthazar, D.D., Spadetti, A.L., Fedullo, L.P.L. & Mello, R.P. (2008). Pseudolynchia canariensis (Diptera:Hippoboscidae) on Buteogallus aequinoctialis (Ciconiiformes: Accipitridae) in the State of Rio de Janeiro, Brasil. *Revista Brasileira De Parasitologia Veterinaria*, 17, 110-112.

Gregor, F., Cerny, V. & De La Cruz, J. (1973). On the avian Hippoboscidae (Diptera) of Cuba. *Folia Parasitologica*, 20, 183-187.

Harlan, H.J. & Kramer, R.D. (1979). Ectoparasites of mammals and birds from Fort Dix, New Jersey. *Entomol. News*, 90, 79-87.

Jovani, R., Tella, J.L., Sol, D. & Ventura, D. (2001). Are hippoboscid files a major mode of transmission of feather mites? *J. Parasitol.*, 87, 1187-1189.

Kadner, C.G. (1941). Pigeon malaria in California. *Science*, 93, 281.

Klei, T.R. & Degiusti, D.L. (1975a). Observations on bionomics of pseudolynchia-canariensis (diptera-hippoboscidae). *Parasitology*, 70, 195-202.

Klei, T.R. & Degiusti, D.L. (1975b). Seasonal occurrence of haemoproteus-

columbae and its vector Pseudolynchia-canariensis. *J. Wildlife Dis.*, 11, 130-135.

Maa, T.C. (1966). On the genus pseudolynchia bequaert. *Pac. Insects Monograph*, 10, 125 - 138.

Macchioni, F., Magi, M., Mancianti, F. & Perrucci, S. (2005). Phoretic association of mites and mallophaga with the pigeon fly Pseudolynchia canariensis. *Parasite-Journal De La Societe Francaise De Parasitologie*, 12, 277-279.

Mandal, F.B. (1991). A preliminary report on the incidence of blood parasites in pigeon columba-livia and pigeon fly Pseudolynchia-canariensis Macquart. *Indian J. Animal Health*, 30, 29-32.

Marcelino, V., Arcoverde, A.R. & Daemon, E. (2009). Phoretic Association of the Mites Myialges spp. (Astigmata: Epidermoptidae) and Ornitocheyletia hallae Volgin (Prostigmata: Cheyletidae) with the Fly Pseudolynchia canariensis (Macquart) (Diptera: Hippoboscidae). *Neotrp. Entomol.*, 38, 578-581.

Marques, S.M.T., Quadros, R.M., Silva, C.J. & Baldo, M. (2007). Parasites of pigeons (Columba livia) in urban areas of lages, Southern Brazil. *Parasitología latinoamericana*, 62, 183 -187.

Mogi, M. (1977). Notes on hippoboscidae diptera from the Ryukyu Islands, Japan with description of a new Icosta sp. *Kontyu*, 45, 395-398.

Msoffe, P.L.M., Muhairwa, A.P., Chiwanga, G.H. & Kassuku, A.A. (2010). A study of ecto- and endo-parasites of domestic pigeons in Morogoro Municipality, Tanzania. *African J. Agricultural Res.*, 5, 264-267.

Mushi, E.Z., Binta, M.G., Chabo, R.G., Ndebele, R. & Panzirah, R. (2000). Parasites of domestic pigeons (Columba livia domestica) in Sebele, Gaborone, Botswana. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging*, 71, 249-250.

Owiny, J.R. & French, E.D. (2000). Ectoparasites in a pigeon colony. *Comparative Medicine*, 50, 229-230.

Paperna, I. & Smallridge, C. (2002). Haemoproteus columbae infection of feral pigeons in Singapore and Israel. *Raffles Bulletin of Zoology*, 50, 281-286.

Pirali-Kheirabadi, K., Dehghani-Samani, A., Ahmadi-Baberi, N. & Najafzadeh, V. (2016). A first report of infestation by *Pseudolynchia canariensis* in a herd of pigeons in Shahrekord (Southwest of Iran). *J. Arthropod-Borne Dis.*, 10, 426-430.

Radfar, M.H., Asl, E.N., Seghinsara, H.R., Dehaghi, M.M. & Fathi, S. (2012). Biodiversity and prevalence of parasites of domestic pigeons (Columba livia

domestica) in a selected semiarid zone of South Khorasan, Iran. *Trop. Animal Health Prod.*, 44, 225-229.

Rashdan, N.A. (1998). Bionomics and laboratory transmission of Haemoproteus columbae Kruse by Pseudolynchia canariensis Maquart (Diptera: Hippoboscidae) to two bird hosts in Egypt. *J. Egyptian Soc. Parasitol.*, 28, 59-65.

Resende, J.S., Martins, N.R.S. & Jorge, M.A. (2001). An outbreak of malaria by Haemoproteus columbae in pigeons. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 53, 361-363.

Roberts, F.H. (1945). The occurrence of the pigeon fly (Pseudolynchia canariensis Macq.) in Australia, with a note on Australian Hippoboscidae. *Aust. Vet. J.*, 21, 150-151.

Sanders, D.P. & Petersen, J.L. (1975). The occurrence of the pigeon fly Pseudolynchia-canariensis new-record in Indiana. *Proc. Indiana Acad. Sci.*, 84, 287-288.

Santiago-Alarcon, D., Rodriguez-Ferraro, A., Parker, P.G. & Ricklefs, R.E. (2014). Different meal, same flavor: cospeciation and host switching of haemosporidian parasites in some non-passerine birds. *Parasites & Vectors*, 7, 286.

Shotter, R.A. (1978). Aspects of the biology and parasitology of the speckled pigeon Columba guinea L. from Ahmadu Bello University Campus, Zaria, North Central State, Nigeria. *Zoological Journal of the Linnean Society*, 62, 193-203.

Sol, D., Jovani, R. & Torres, J. (2000). Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography*, 23, 307-314.

Subbiah, T.V. & Joseph, S.A. (1986). Studies on the bionomics and vector potential of the pigeon fly pseudolynchia-canariensis in the transmission of haemoproteus-columbae in pigeons. *Cheiron*, 15, 113-118.

Valim, M.P. & Gazeta, G.S. (2007). Phoretic association of the mites Myialges anchora Sergent & Trouessart (Acaridida, Epidermoptidae) and Ornithocheyletia hallae smiley (Actinedida, Cheyletiellidae) with Pseudolynchia canariensis (Macquart) (Diptera, Hippoboscidae). *Revista Brasileira De Entomologia*, 51, 518-519.

Vel, K.S. & Pillai, K.M. (1998). Ectoparasites of pigeons in Thrissur, Kerala. *J. Vet. Anim. Sci.*, 27, 64-65.

Ward, R.A. (1953). Additional record of phoresy of Mallophaga on Hippoboscidae. *Bull. Brooklyn Entomol. Soc.*, 48, 128.

Yamauchi, T., Tsuda, Y., Sato, Y. & Murata, K. (2011). Pigeon louse fly, pseudolynchia canariensis (Diptera: Hippoboscidae), collected by dry-ice trap. *J. Am. Mosq. Cont. Assoc.*, 27, 441-443.

APPENDIX C

UNLOCKING THE BLACK BOX OF FEATHER LOUSE DIVERSITY: A MOLECULAR PHYLOGENY OF THE HYPER-DIVERSE GENUS *BRUEELIA*

Bush S.E., Weckstein J.D., Gustaffson D.R., Allen J., DiBlasi E., Shreve S.M., Boldt R., Skeen H.R., Johnson K.P.(2016). Unlocking the black box of feather louse diversity: A molecular phylogeny of the hyper-diverse genus *Brueelia*. Molecular Phylogenetics and Evolution, 94, 737–751. Reprinted with permission from *Molecular Phylogenetics and Evolution*.

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Unlocking the black box of feather louse diversity: A molecular phylogeny of the hyper-diverse genus *Brueelia* ☆

Sarah E. Bush [a,*], Jason D. Weckstein [b,1], Daniel R. Gustafsson [a], Julie Allen [c], Emily DiBlasi [a], Scott M. Shreve [c,2], Rachel Boldt [c], Heather R. Skeen [b,3], Kevin P. Johnson [c]

[a] Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
[b] Field Museum of Natural History, Science and Education, Integrative Research Center, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA
[c] Illinois Natural History Survey, University of Illinois, 1816 South Oak Street, Champaign, IL 61820, USA

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ABSTRACT

Songbirds host one of the largest, and most poorly understood, groups of lice: the *Brueelia*-complex. The *Brueelia*-complex contains nearly one-tenth of all known louse species (Phthiraptera), and the genus *Brueelia* has over 300 species. To date, revisions have been confounded by extreme morphological variation, convergent evolution, and periodic movement of lice between unrelated hosts. Here we use Bayesian inference based on mitochondrial (COI) and nuclear (EF-1α) gene fragments to analyze the phylogenetic relationships among 333 individuals within the *Brueelia*-complex. We show that the genus *Brueelia*, as it is currently recognized, is paraphyletic. Many well-supported and morphologically unified clades within our phylogenetic reconstruction of *Brueelia* were previously described as genera. These genera should be recognized, and the erection of several new genera should be explored. We show that four distinct ecomorphs have evolved repeatedly within the *Brueelia*-complex, mirroring the evolutionary history of feather-lice across the entire order. We show that lice in the *Brueelia*-complex, with some notable exceptions, are extremely host specific and that the host family associations and geographic distributions of these lice are significantly correlated with our understanding of their phylogenetic history. Several ecological phenomena, including phoresis, may be responsible for the macroevolutionary patterns in this diverse group.

> "Taxonomist's nightmare... evolutionist's delight"
> [*MacIntyre (1967), after A.J. Cain*]

1. Introduction

In 2012 a British birder was the first person to see 9000 different species of birds (McCarthy, 2012). This impressive tally is roughly 85–90% of all known bird species. Although a few new species of birds are being discovered and described each year (Sangster and Luksenburg, 2015), it is estimated that over 95% of all bird species have already been described (Mayr, 1982). In short, birds are among the best known groups of organisms on the planet. Despite this knowledge, however, birds represent many additional layers of undiscovered diversity. Each bird species harbors a complex community of parasites and other symbionts, many of which are undescribed and understudied.

Songbirds (Passeriformes), the largest order of birds, are host to one of the largest, and most poorly understood groups of feather lice. The genus *Brueelia* Kéler 1936 has over 300 described species (Price et al., 2003; Cicchino, 2004; Rékási and Saxena, 2005; Valim and Palma, 2006, 2015; Cicchino and González-Acuña, 2008, 2009; Sychra et al., 2009, 2010a, 2010b; Valim and Weckstein, 2011; Najer et al., 2012a, 2012b, 2012c; Mey and Barker, 2014; Najer et al., 2014; Valim and Silveira, 2014), and thousands of slides with specimens of unidentified and undescribed species of *Brueelia* line the drawers of museum collections around the world.

Lice in the genus *Brueelia* are incredibly diverse. They vary enormously in body shape: from short, round, "head" louse ecomorphs, to long, thin, "wing" louse ecomorphs (Johnson et al., 2012). They

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* Corresponding author.
E-mail address: bush@biology.utah.edu (S.E. Bush).
[1] Current address: Department of Ornithology and Department of Biodiversity, Earth, and Environmental Sciences, Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, USA.
[2] Current address: Division of Mathematics and Natural Sciences, Brescia University, Owensboro, KY 42301, USA.
[3] Current address: Biology Department, Loyola University Chicago, Chicago, IL 60660, USA.

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vary in color from light to dark (Bush et al., 2010), and in pigmentation patterns from simple to complex. Indeed, the morphological diversity within *Brueelia* echoes the diversity among all feather lice in the order Phthiraptera. A thorough understanding of the macroevolutionary patterns within *Brueelia* promises to illuminate the ecological and evolutionary forces influencing diversity among lice in general. However, this tantalizing diversity is a quintessential example of a "taxonomist's nightmare... evolutionist's delight" (MacIntyre, 1967). Convergent evolution of similar morphological characteristics is known to occur among lice (Johnson et al., 2012), which suggests that taxonomy based solely on morphological characters may obscure our understanding of the phylogenetic relationships within this group.

Lice in the genus *Brueelia* are also perplexing from another perspective. Among the groups of lice studied thus far, host specificity tends to correlate with cospeciation (Clayton et al., 2004). Lice on gophers are extremely host specific, and show among the strongest patterns of cospeciation in any system. Similarly, body lice on doves are quite host specific, and show a significant degree of cospeciation with their hosts, whereas wing lice on the same hosts are less host specific and show significantly less cospeciation than body lice (Clayton and Johnson, 2003; Clayton et al., 2004). *Brueelia* are considered to be highly host specific, with over 85% of described species recorded from just a single host species (Price et al., 2003). Despite this apparent high degree of host specificity, however, a preliminary cophylogentic analysis did not support a hypothesis of cospeciation (Johnson et al., 2002a).

There are at least two plausible explanations for this pattern. First, while specificity is a necessary condition for cospeciation, it is not a sufficient condition. For example, herbivorous beetles in the genus *Belpharida* are specific to particular host plants (*Bursera*), yet the beetle phylogeny is not congruent with the phylogeny of the host plants (Becerra, 1997). This is, in part, because these insects are relatively mobile organisms and can move between different host plants. In contrast, most lice are relatively immobile, only moving between hosts during periods of direct contact (Clayton et al., in press). *Brueelia* species, however, are known to hitch rides on hippoboscid flies (Fig. 1). This phoretic behavior may provide these lice with opportunities to switch to and adapt to new host species. If phoresis between host species is rare, and gene flow is limited, then lice may specialize and become quite specific on the "new" host species. Thus, rare phoretic events over macroevolutionary time could simultaneously support high levels of host specificity while disrupting patterns of cospeciation at a coarse macroevolutionary time-scale.

Alternatively, the apparent host specificity of lice in the *Brueelia* complex may be a taxonomic artifact. Early louse taxonomists tended to describe new species on the basis of host associations, rather than on the basis of the lice themselves. This unfortunate practice has required synonymization of nearly 2000 species and subspecies of chewing lice in comparison to only 4464 valid species and subspecies (Price et al., 2003). Indeed, initial molecular studies of lice in this genus indicate that a single species of *Brueelia* can infest multiple host species across several distantly related host families (Johnson et al., 2002a). A comprehensive taxonomic revision, independent of louse morphology, and host associations, is needed to identify the ecological and evolutionary drivers of diversity in this group.

Here we provide a molecular based phylogenic reconstruction for lice in the genus *Brueelia* and related lice in the genera *Bizarrifrons*, *Buerelius*, *Meropoecus*, *Motmotnirmus*, and *Sturnidoecus*, which are core members of the "*Brueelia* complex" (Clay and Tandan, 1967; Ledger, 1980; Valim and Palma, 2012, 2015). These genera are primarily found on songbirds (Passeriformes), although a few species are known to occur on Coraciiformes (bee-eaters), Piciformes (woodpeckers, barbets, and toucans), Trogoniformes (trogons), and Cuculiformes (couas). Our sampling includes lice from all of these host groups. We use DNA sequences of nuclear (EF-1α) and mitochondrial (COI) genes to provide a phylogenetic reconstruction of a worldwide sampling of over 300 specimens of lice from the *Brueelia*-complex and related genera (Johnson et al., 2002a). This is the largest phylogenetic reconstruction for any group in the order Phthiraptera. We discuss these results in the context of prior generic classifications and recommend that several previously recognized genera be considered valid. We also discuss emerging patterns of host specificity, biogeography, morphology, and behavior that are intimated by our new understanding of the phylogenetic relationships of these feather lice.

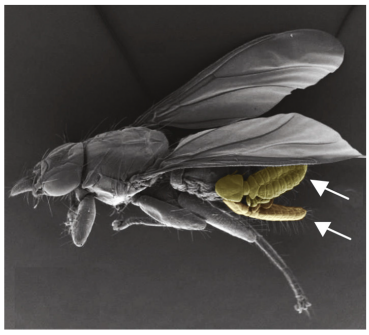


Fig. 1. Phoretic *Brueelia* sp. (arrows) hitching a ride on a hippoboscid fly. Fly collected from a blackbird *Turdus merula*. False-color SEM (SEM by V.S. Smith).

2. Materials and methods

2.1. Sampling

We sampled a total of 333 louse specimens belonging to the *Brueelia*-complex (see Table 1 in Bush et al., in press). These lice were sampled from 250 bird species belonging to 66 bird families and five orders (Passeriformes, Coraciiformes, Cuculiformes, Piciformes, and Trogoniformes). Sampled lice include 38 known species and 211 lice that represent either new species of lice or new host associations. These samples were collected from 23 countries and all continents except Antarctica. An additional 30 outgroup taxa for rooting the phylogeny were selected to represent nested sister taxonomic relationships within the family Philopteridae (Cruickshank et al., 2001; Johnson et al., 2001a; Smith et al., 2011). These 30 louse outgroup species were from 27 host species, in 17 host families, collected from 9 countries.

Lice were collected either from euthanized bird specimens using ethyl acetate fumigation or from live birds dusted with pyrethrum powder (Clayton and Drown, 2001; Bueter et al., 2009). Care was taken to make sure that individual hosts were kept separate at all times and to clean all working surfaces between fumigation. Lice were collected by the authors and colleagues during field-work conducted over several decades and were stored in vials of 95% ethanol, usually in ultracold (−80 °C) freezers.

2.2. DNA extraction, amplification and alignment

DNA was extracted from lice using either the Qiagen DNeasy micro-kit (Valencia, California, USA) following the manufacturer's protocol as described by Valim and Weckstein (2011), or the Qiagen DNeasy tissue kit (Valencia, California, USA) following the manufacture's protocol as described by Johnson et al. (2001b).

After DNA was extracted from individual lice, the exoskeletons were retained and mounted on microscope slides (Palma, 1978). These voucher slides were used to identify each specimen to genus using the keys in Price et al. (2003). Specific-level identifications were based on original descriptions, specific keys if possible, and comparison with identified slide mounted material. Voucher slides are deposited in the Illinois Natural History Survey Insect Collection (INHS), Price Institute for Parasite Research at the University of Utah (PIPeR), and Field Museum of Natural History (FMNH) (Table 1 in Bush et al., in press).

Portions of one mitochondrial (COI) and one nuclear gene (EF-1α) were selected because these genes have successfully resolved phylogenies of closely related groups of parasitic lice and more distantly related "bark lice" (Johnson et al., 2002c, 2003, 2004; Smith et al., 2004, 2011). We used PCR to amplify and sequence portions of the mitochondrial cytochrome oxidase I (COI; 379 bp) and the nuclear gene elongation factor 1a (EF-1α; 347 bp) using published amplification and sequencing protocols (Johnson et al., 2004; Smith et al., 2004). Purified PCR products were cycle sequenced using ABI Big Dye (Applied Biosystems, Foster City, California) and run on an ABI Prism 3730 DNA sequencer (Applied Biosystems). Raw sequence data was trimmed, edited, and reconciled using Sequencher 5.0.1 (Genecodes CO., Ann Arbor, Michigan) or Geneious (version 7.0.3, Biomatters LTD). Both genes are protein coding and therefore we were able to easily align them by eye according to codons. These aligned gene sequences were then concatenated for phylogenetic analysis (GenBank Accession Numbers original to this study KT892064–KT892646, for all other GenBank Accession Numbers see Table 1 of Bush et al., in press).

2.3. Phylogenetic analyses

The final sequence alignment was analyzed using PartitionFinder (v1.1.1; Lanfear et al., 2012, 2014), an open source python script that selects the best-fit partitioning schemes and models of molecular evolution for phylogenetic analysis. We tested whether the two genes (COI, EF-1α) should be analyzed together under the same model and parameters or as two separate partitions. We tested only these two partitions because separating each of these genes by codon would only provide 100 bps for each partition, a very small amount of sequence for estimating parameters and would likely result in over-parameterization. The PartitionFinder analysis found that a single partition and GTR+I+G model of molecular evolution best fit the data, using both AICc and BIC criterion. Using these parameters, which were estimated as part of the analysis, and a flat Dirichlet prior for state frequencies, we ran a Bayesian analysis in MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012) for 10,000,000 generations. Each Bayesian analysis included two parallel runs, each with four Markov chains, to ensure that our analyses were not stuck at local optima (Huelsenbeck and Bollback, 2001). Markov chains were sampled every 500 generations, yielding 20,000 parameter point-estimates. We used these 20,000 point-estimates minus the burn-in generations (500 point-estimates, 250,000 generations) to create a 50% majority-rule consensus tree and calculated Bayesian posterior probabilities to assess nodal support. We rooted the Bayesian tree using a nested set of sister taxa within the family Philopteridae (Cruickshank et al., 2001; Johnson et al., 2004, 2012; Smith et al., 2004).

2.4. Operational Taxonomic Units (OTUs)

The number of OTUs was calculated using a single locus, the COI dataset, with two methods. First, OTUs were estimated using Mothur (Schloss et al., 2009), which takes into account sequence divergence. We used a cutoff of 5% for each OTU, and in lice values over this are generally associated with different species, whereas values under 5% generally carry little additional biological information (such as distinct patterns of host association) (Johnson et al., 2002b, 2007; Weckstein, 2004; Price et al., 2008; Price and Johnson, 2009). Second, OTUs were estimated with bGMYC, a coalescent method (Reid and Carstens, 2012) that takes into account phylogenetic uncertainty and determines a threshold for which the branching in the phylogenetic tree switches from interspecific to intraspecific. We used BEAST (Drummond and

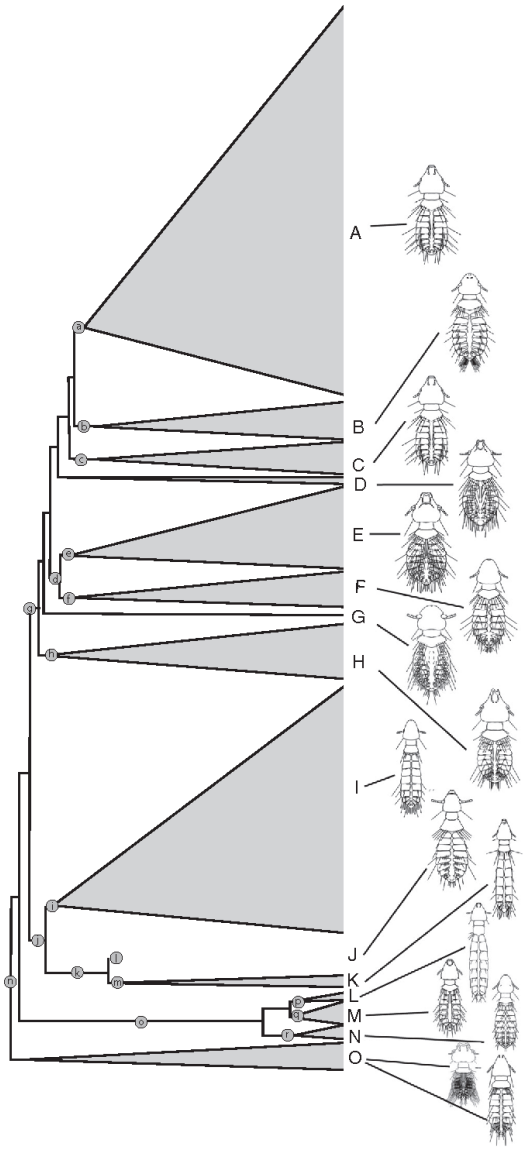


Fig. 2. Phylogenetic and graphical overview of clades in the *Brueelia* complex. Bullets indicate the presence of morphological characters unique to designated clades. These characters are defined in Table 1.

Rambaut, 2007) with the original COI alignment to obtain ultrametric trees using a GTR+G model of molecular evolution for 50 million generations, the burn-in was set at 20% and 100 trees were randomly selected from the post burn-in distribution for use in the bGMYC analysis. The threshold between intra and interspecific branching was calculated using the R package bGMYC (Reid and Carstens, 2012) running each tree for 5000 generations removing 2000 as burn-in and results calculated across the trees and the number of OTUs estimated based on the intra-interspecific threshold.

2.5. Test for phylogenetic signal with respect to host family and geographic distribution

We recorded the associated host family (Clements et al., 2014; Dickinson et al., 2014) and bioregion (see map, Olson et al., 2001; Fig. 3a) for each louse OTU. Several OTUs were associated with more than one host family or bioregion. To account for these polymorphic characters we randomly sampled 100 trees from the post-burnin Mr. Bayes analysis, and pruned each tree to represent only one taxon per OTU selected at random. Thus, our trees only contained one character per OTU, as each individual taxon was associated with only a single character. However, polymorphic characters were incorporated in the analyses because a single taxon was selected at random from each OTU for each tree; therefore, it is likely that all possible characters associated with each OTU are represented in the analyses. For each character (host family and bioregion) we ran a Maddison and Slatkin (1991) randomization procedure as a test of phylogenetic signal with Perl and R (Perl scripts and R code is available at www.github.com/juliema/publications). Specifically, we randomly assigned character states to taxa for each OTU tree 999 times and calculated the parsimony scores for each random assignment. We calculated the parsimony score for the true character states and determined whether the empirical parsimony score was significantly different than the random distribution. Significance indicates that the character in question is significantly conserved with respect to the tree topology as compared to random assignment of character states. This was done for all 100 randomly sampled OTU trees with: (1) host family as the character, (2) with bioregion as a character and (3) with host family and bioregion as a combined character. In total these analyses included 300 Maddison–Slatkin tests.

3. Results

The phylogenetic tree resulting from Bayesian analysis of the two gene regions identified several major clades, many of which are unified by gross morphology (Fig. 2, Table 1). Within clades, there was often strong phylogenetic resolution and support (Fig. 3a–f). However, the relationships among many of these clades were not very highly supported (see clade by clade results below). The OTU analyses of the 333 ingroup taxa indicated that there are between 114 and 166 operational taxonomic units in this dataset (Table 1 in Bush et al., in press). Based on the 5% species delimitation cutoff, Mothur calculated 166 OTUs and bGMYC calculated 114 OTUs. The majority of the OTUs identified in each analysis correspond well with the clades identified in the tree.

Tests that explored patterns of host association and geographic distribution among bioregions indicate a significant amount of phylogenetic signal in these characters, suggesting that these characters are more conserved with respect to the phylogenetic tree than expected by chance. Maddison and Slatkin tests for phylogenetic signal with respect to louse associations with host family, bioregion, and host family × bioregion were all significant ($p < 0.05$ in all cases), indicating that host associations and geographical distributions are significantly conserved.

Table 1
Key morphological characters that apply to clades of lice from the *Brueelia* complex. Letters refer to labeled bullets in Fig. 2.

	Morphological characters unique to designated clades
a	Marginal carina interrupted laterally and submedianly, connected by dorsal pre-antennal suture that may be transversally continuous posterior to dorsal anterior plate. Mesomere small compared to other genital elements, typically more or less triangular. Parameral heads axe-shaped or rectangular. Abdomen not very setose
b	Sternites modified antero-laterally into thickened bars.[a] Pleurites with complex anterior ends. Terminal abdominal segment very setose in male. Marginal carina uninterrupted. Male subgenital plate with subsidiary lateral plates. Mesomere either minute, triangular, or large and shield-shaped. Parameres elongated distally
c	As Clade A, but head elongately trapezoidal, and mesomere generally larger proportional to other genital elements. Not easily separable from Clade A on morphological grounds
d	Mesomere thickened distally, either transversally continuous, convergent along lateral margins of mesomeral lobes, divergent along median margins of lobes. Or along both margins of the lobes, but not transversally continuous
e	Head broadly triangular. Tergites generally with transversally continuous rows of setae. Marginal carina interrupted laterally and submedianly, connected by dorsal pre-antennal suture that is transversally continuous posterior to dorsal anterior plate; this plate has a flat or slightly concave posterior margin. Coni very prominent. Male genitalia very variable between species groups
f	Marginal carina not interrupted, but displaced medianly. Mesomeral lobes generally fused distally. Prominent rugose nodi on distal mesomere
g	AS3 absent. Extrusor muscles as parallel or convergent lines on mesomere extending onto basal plate,[a] male with *spa* on at least some tergites,[a] parameres with folded heads overlapping with mesomere, mesomere overlapping with basal plate
h	Mesomere much extended anteriorly
i	Marginal carina un-interrupted[a]; no dorsal pre-antennal suture[a]; *spa* present on at least some male tergites
j	Parameral heads cup-shaped, blunt or digitate; mesomere with scaly, hairy, or brushlike distal margin; parameres triangular (but may be extended distally); tergal setae missing on at least segments II–III; *stp* on at least some male tergites[a]
k	Marginal carina interrupted at least laterally, dorsal pre-antennal suture present
l	Dorsal anterior suture transversal; marginal carina interrupted only laterally, but modified into wide interior plate at frons; abdomen teardrop-shaped. Female subgenital plate may be divided transversally
m	Marginal carina interrupted laterally and submarginally; dorsal pre-antennal suture continuous with both interruptions and may entirely encircle a dorsal anterior plate
n	No setae on anterior end of tergite II, male tergites II–IX divided medianly,[a] female tergites II–VIII divided medianly[a]; at most one *mts* macroseta[a]
o	Parameral heads small, blunt; proximal mesomere flat or rounded; *as3* present; parameral heads folded into V- or heart-shapes[a]
p	Parameres lobe-like, typically with large pore in distal half; *ss* absent on anterior tergites[a]; anterior end of head typically with distinct nail-shaped thickening; *pns* microseta
q	Marginal carina interrupted laterally and submedianly; all Post-Nodal Setae microsetae; parameral heads large, heart-shaped; *ss* on all tergites; *pns* microseta
r	Splayed mesomeres with serrated distal margins; marginal carina uninterrupted or interrupted only submedianly; parameral heads small, pointed; *pns* long[a]; proximal mesomere fishtail-shaped; *ss* on all tergites

[a] A few taxa do not exhibit this character. Terminology of head characters and setae follows Clay (1951), Mey and Barker (2014); abdominal chaetotaxy follows Cicchino and Castro (1996).

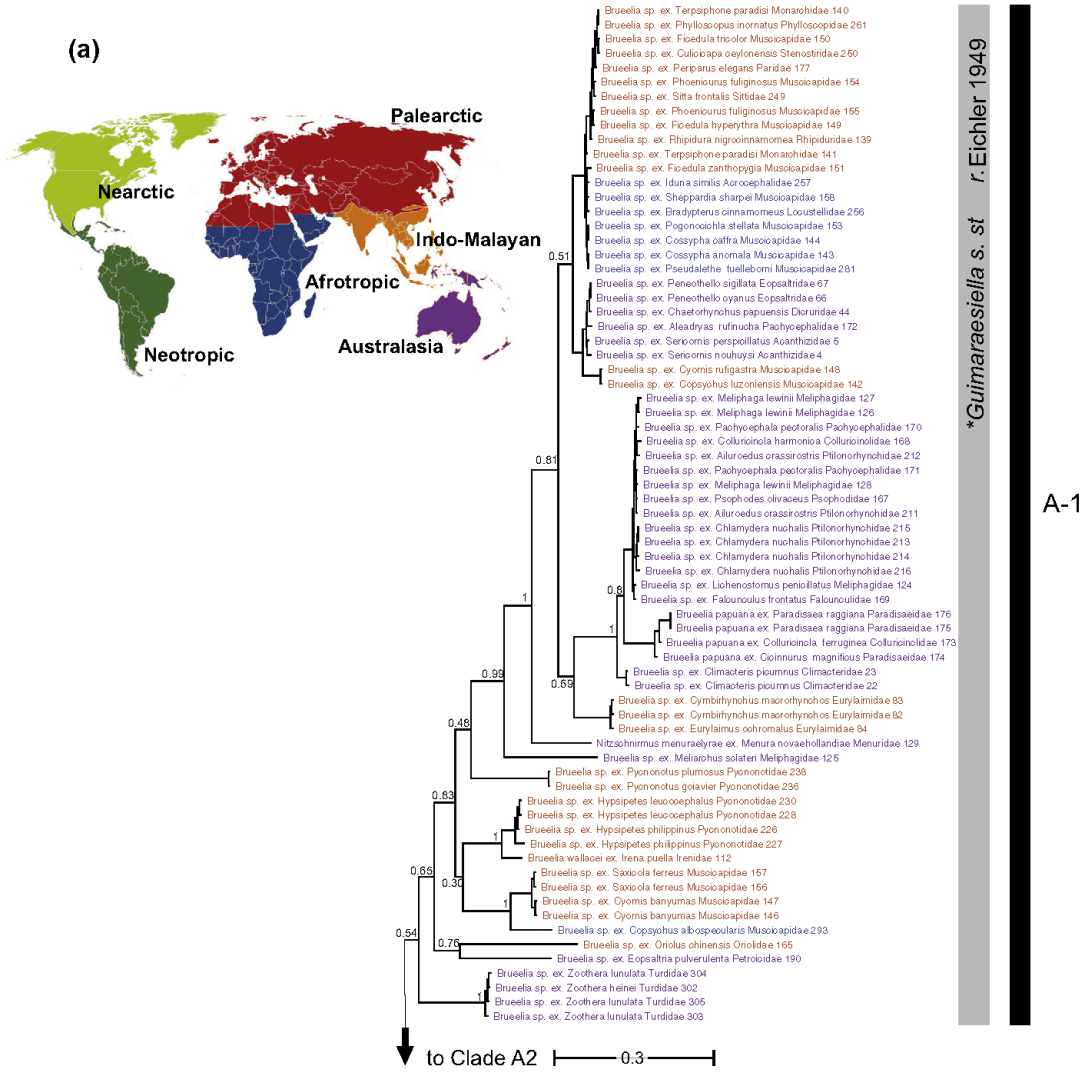


Fig. 3. Consensus tree from Bayesian analysis of combined COI and EF-1α sequences for *Brueelia*-complex species and outgroup taxa. Branches proportional to substitutions per site for the consensus tree (scale indicated). Numbers associated with nodes are posterior probabilities for the clade from a 10 million generation MCMC analysis, sampled every 1000 generations and excluding the first 1 million generations as burn-in (values <0.5, and values associated with short terminal branches not shown here; all support values >0.5 are shown in Fig. 2, Bush et al., in press). Text color refers to the geographic bioregion (map) where the specimen was collected. Numbers after taxonomic names refer to Table 1 in Bush et al., (in press). Louse taxonomy follows the classification of Price et al. (2003) and subsequent publications. Taxonomy indicated in the vertical gray bars indicate generic classifications; * indicates alternate, historical treatments of genera that are considered junior synonyms of *Brueelia* by Price et al. (2003); † indicates genera that have been recognized as valid in taxonomic studies published after Price et al. (2003), see results for details. Host taxonomy follows Clements et al. (2014) and Dickinson et al. (2014): host genus, species, and family are all indicated. Tree partitioned into six portions (a–f). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1. Clade A (Fig. 3a and b)

This strongly supported clade includes taxa that are morphologically similar to the type species of *Guimaraesiella* Eichler 1949. Although Price et al. (2003) considered this genus synonymous with *Brueelia*, species within Clade A form a strongly supported monophyletic group based on both genetic and morphological characters. The lice in this genus exhibit variable head characters, yet, they are unified by some pre-antennal head characters, abdominal chaetotaxy, and the shape and structure of the male genitalia (Table 1a). This clade also includes a specimen recently placed in a new genus, *Nitzschnirmus menuraelyrae* (Mey and Barker, 2014) (#129, Table 1 in Bush et al., in press). Given the molecular and morphological distinctions of this clade, the resurrection of the genus *Guimaraesiella* is warranted, which would make *Nitzschnirmus* a junior synonym of *Guimaraesiella*.

Clade A is distributed worldwide; however, within Clade A there are monophyletic units that are geographically restricted.

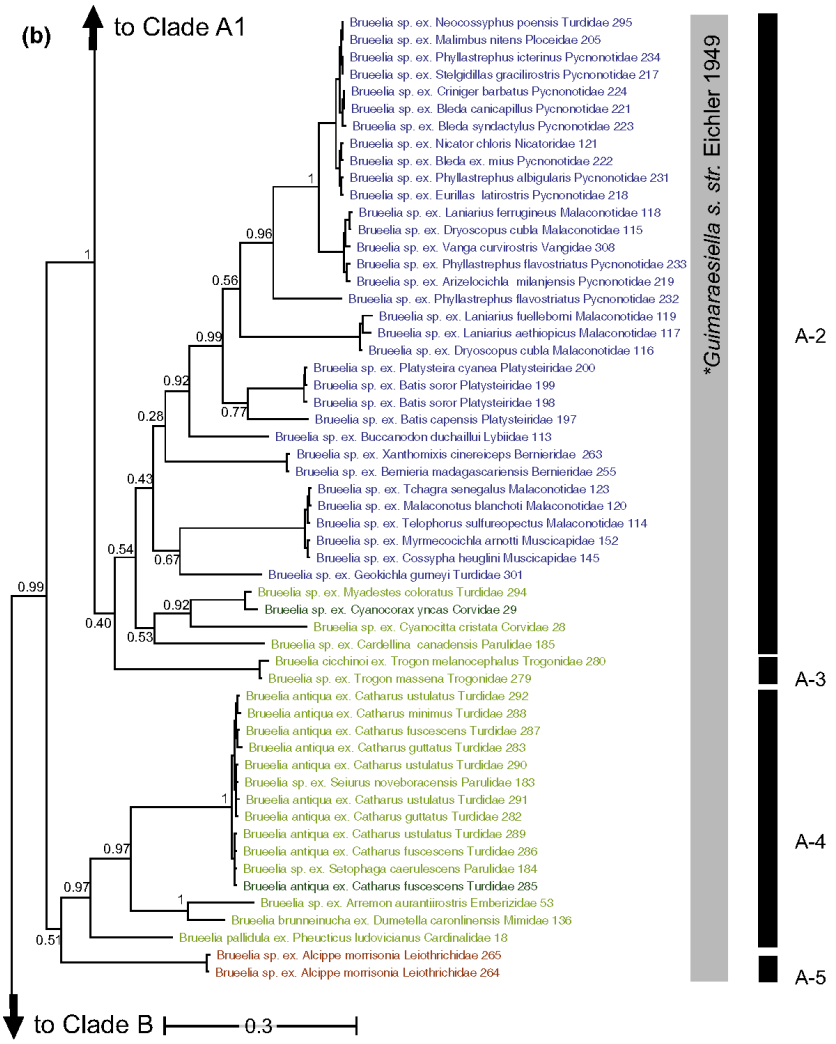


Fig. 3 (*continued*)

Clade A-1 is restricted to the Old World. Most of the taxa in this clade are from Indo-Malaya and Australasia, but a few taxa are from the Afrotropics. Samples from mainland Africa generally cluster together; however, they are genetically extremely close (<2% COI divergence) to samples from the Indo-Malayan region. The only remaining African taxon in Clade A-1 is a louse found on *Copsychus albospecularis*, a flycatcher from Madagascar, and this louse appears in a strongly supported clade with lice from Indo-Malayan Bulbuls and Old World Flycatchers.

Clade A-2+3 (Fig. 3b) contains a large monophyletic clade of lice from Africa, and smaller monophyletic clades from the New World, although neither of these have strong support. The separation and distinction of Clade A-1 and Clade A-2+3 is strongly supported by molecular data (100% posterior probability); yet, an initial morphological examination did not reveal any characteristics that clearly discriminate taxa in A-1 relative to A-2+3. There are, however, several strongly supported groups within in Clade A that have distinct morphological features. Although morphologically similar to *Guimaraesiella* s. str., the lice on trogons (Clade A-3; Fig. 3b) have unique pre-antennal head characteristics, (see Valim and Weckstein, 2011), and the lice on fulvettas (Clade A-5; Fig. 3b) have unique male genitalic characteristics. In each of these clades, very

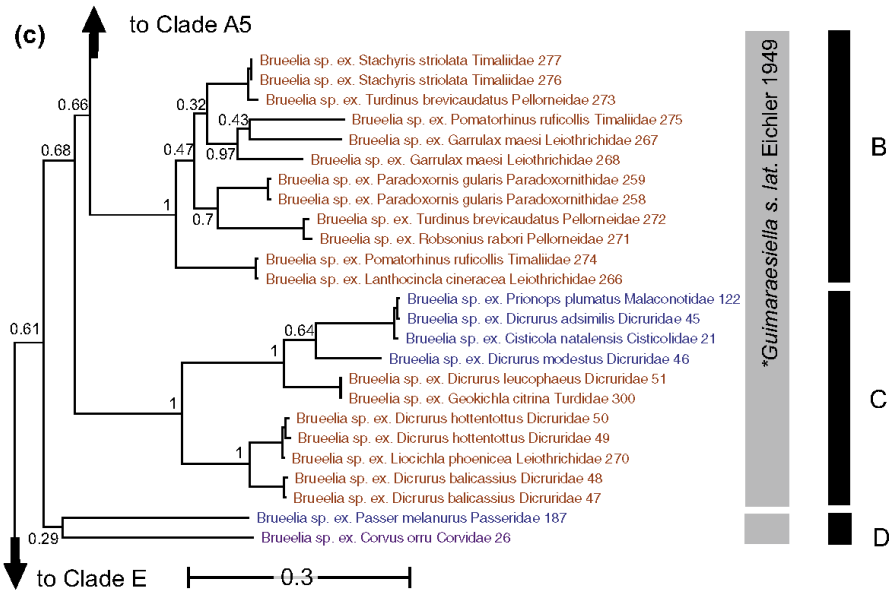


Fig. 3 (*continued*)

few lice were available for molecular analyses and their exact placement within Clade A is not well supported.

The lice on Nearctic songbirds (Clade A-4; Fig. 3b) form a strongly supported clade (97% posterior probability) based on molecular data, and these lice all share a dorsal pre-antennal head suture that completely separates the dorsal anterior plate from the main head plate. This character is ubiquitous in the New World, but is only rarely found in Old World taxa [e.g. in *Brueelia myiophoneae* (Clay, 1936)]. Most (10 out of 15) of the lice in this clade are found on thrushes (Turdidae); however, lice in this group are also found on four other passerine families: Mimidae, and more distantly related Parulidae, Cardinalidae, and Emberizidae (Cracraft and Barker, 2009).

3.2. Clades B–D (Fig. 3c)

Clade B is a strongly supported clade of lice geographically restricted to Indo-Malaya, and found only on Old World babblers (Timaliidae). Lice in Clade B share some chaetotaxy characters with lice in Clade A, but these chaetotaxy characters are somewhat variable within Clade A. However, striking differences in preantennal head structure and male genitalia separate lice in Clade B from other lice in the *Brueelia*-complex (Table 1b). This clade is placed as the sister group to Clade A (although this is weakly supported).

Morphologically, lice in Clade C are very similar to lice in Clade A. Subtle characteristics of the head unite this group (Table 1c), but these taxa have thoracic and genitalic characters that are extremely similar to those of *Guimaraesiella* s. str. (Clade A). Lice in this clade are restricted to the Old World. They are found largely on drongos (Dicuridae), but they also occur on other passerine families (Turdidae, Cisticolidae, and Malaconotidae).

Clade D is poorly supported. Both of the sequenced specimens were nymphs, which makes morphological characterization difficult. However, the sample from *Passer melanurus* appears to represent the genus *Rostrinirmus* Zlotorzycka, 1964, whereas the sample from *Corvus orru* likely represents *Corvonirmus* Eichler, 1944. Morphological differences in the preantennal head and abdominal chaetotaxy suggests that these two genera are not closely related. Additional samples for morphological and molecular work are sorely needed to sort out the phylogenetic placement and host specificity of these taxa.

Clades B, C, and D most likely represent distinct genera. However, additional taxonomic work is needed to formally address the taxonomic status of Clades B and C, and more specimens are needed to resolve the taxonomic status of Clade D.

3.3. Clades E–H (Fig. 3d)

Clade E is a strongly supported clade that contains lice in the genus *Sturnidoecus* Eichler 1944. These lice are unified by several morphological characters (Table 1e), including a distinctly round body with a broad triangular head, a phenotype typical of "head" lice (Johnson et al., 2012). Within Clade E several strongly supported monophyletic clades appear to be restricted to particular host families and/or geographic regions. For example, one clade includes lice from most Indo-Malayan thrushes (Turdidae), and another clade includes most lice from Nearctic thrushes. Lice from African weavers also group together into two distinct clades. As a whole, *Sturnidoecus* spp. in our study parasitize hosts from four different avian families: Sturnidae, Turdidae, Ploceidae, and Malaconotidae that diverged roughly 50 mya (Cracraft and Barker, 2009). Described species of *Sturnidoecus* are known from an additional 12 passeriform families, for which we did not have specimens for molecular work (Price et al., 2003). Thus, additional sampling of *Sturnidoecus* spp. is required before rigorous conclusions about radiations of lice in this genus across host families can be made.

Clade F is a strongly supported clade as is its position as a sister to *Sturnidoecus* (Clade E; 93% posterior probability). Although no described species from the previously recognized genus *Olivinirmus* Zlotorzycka, 1964 were sequenced in this study,

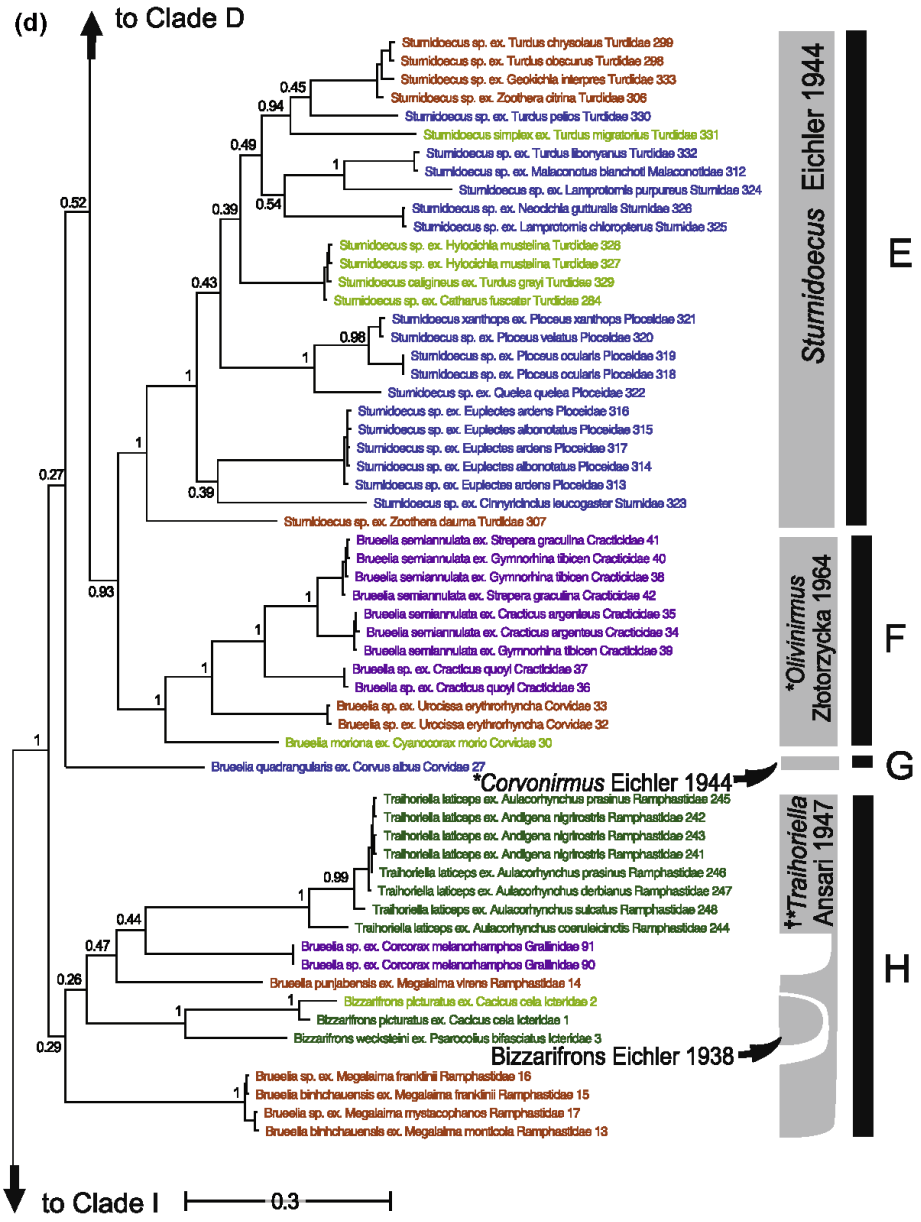
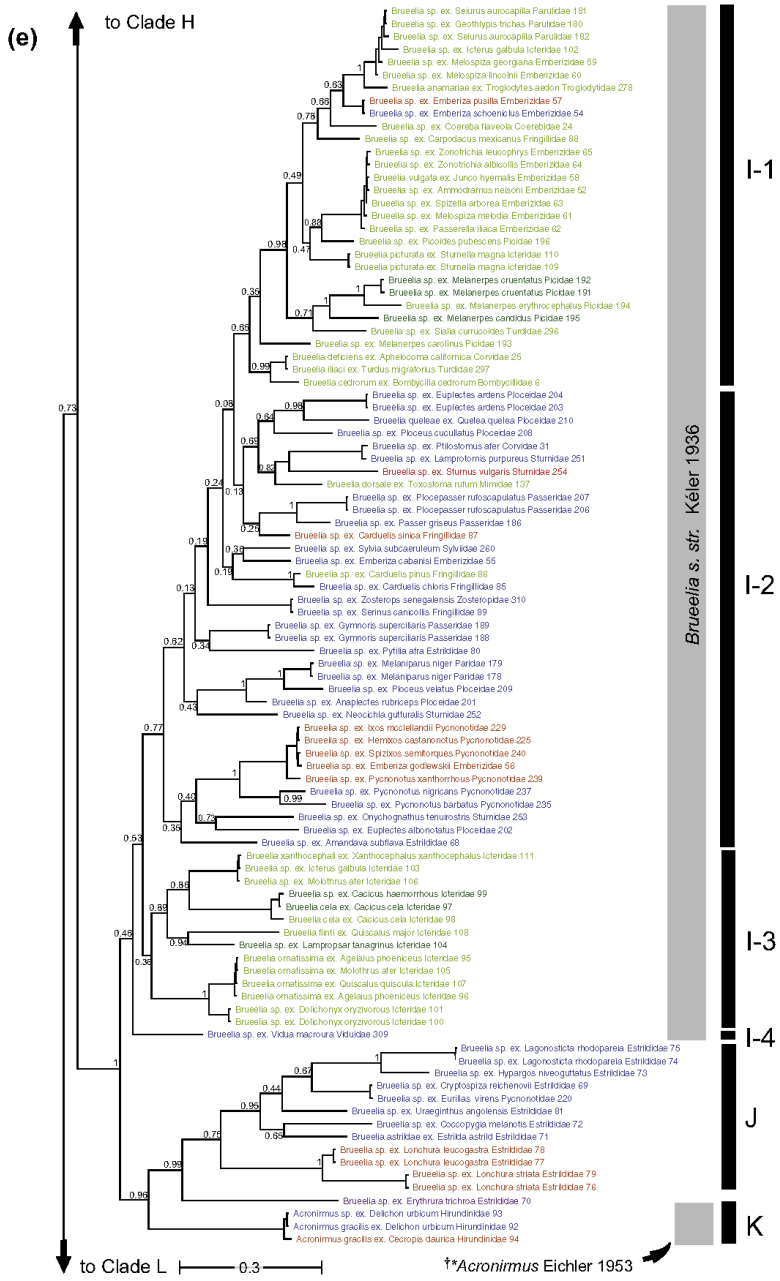


Fig. 3 (*continued*)

the shape of the male genitalic mesomere and pre-antennal head of the taxa in Clade F are unique (Table 1f) and are most similar to the generic description of *Olivinirmus*, which was considered a synonym of *Brueelia* by Ledger (1980). Given the observed molecular support, the resurrection of the genus *Olivinirmus* should be considered pending sequencing of described *Olivinirmus* spp. or formal taxonomic comparisons of the taxa in this study with *Olivinirmus glandarii* (Denny, 1842), the type species of *Olivinirmus*.

"Clade" G is a single louse from *Corvus albus* (Corvidae) that fits the description of the genus *Corvonirmus* erected by Eichler (1944). This genus was considered synonymous with *Brueelia* by Hopkins and Clay (1952). The position of this sample is sister to Clades A–F; however, this relationship is poorly supported. Future



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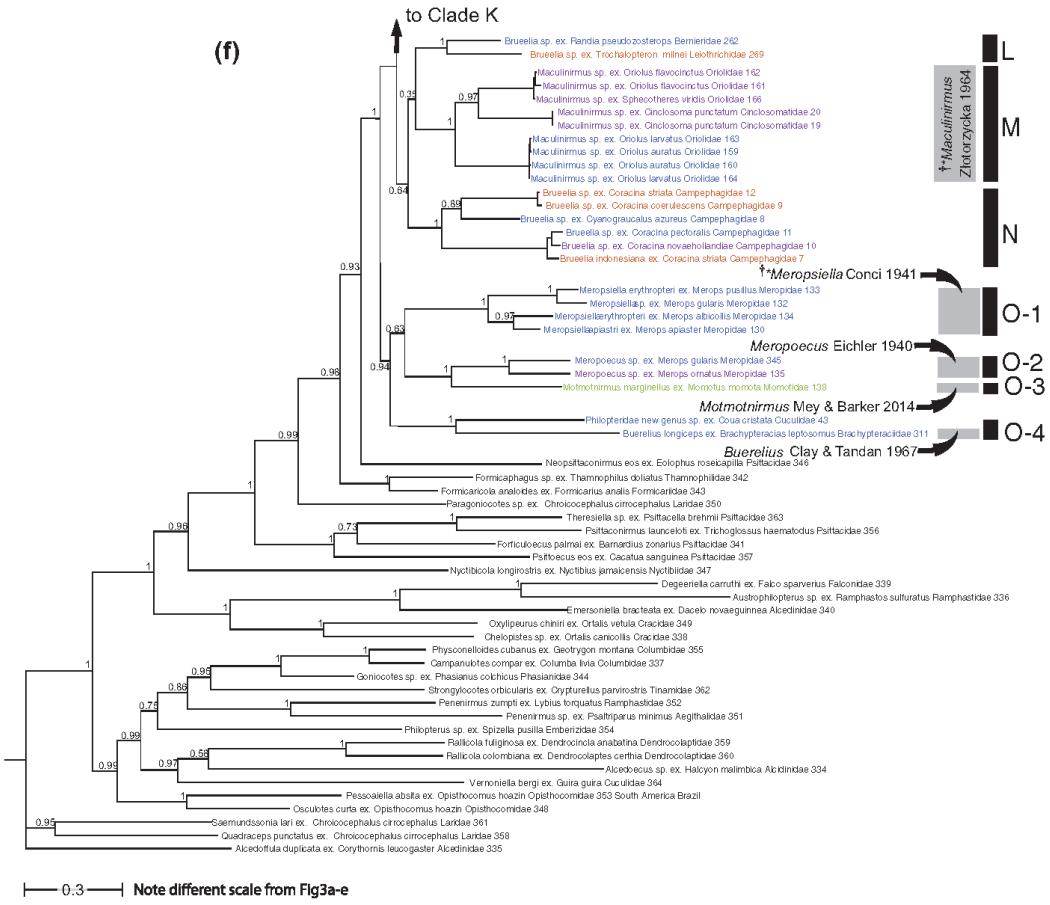

Fig. 3 (*continued*)

collections of fresh, sequenceable, material of described *Corvonirmus* spp. will greatly improve our understanding of how *Corvonirmus* is related to other genera within the *Brueelia* complex, and whether the resurrection of this genus is warranted.

Clade H is a poorly supported clade that contains lice from hosts in the orders Passeriformes and Piciformes. Ansari (1947) erected the genus *Traihoriella*, which included lice on toucans and barbets (Ramphastidae). This genus was later considered a synonym of *Brueelia* by Hopkins and Clay (1952). More recently, however, *Traihoriella* was considered a valid genus by Mey and Barker (2014) and Valim and Palma (2015). Lice from toucans form a strongly supported clade. Similarly, lice from most (4 out of 5) of the barbets (*Megalaima*) form a strongly supported clade. Morphologically, lice from toucans and barbets are quite similar, but they can be separated from each other by head shape and abdominal chaetotaxy: lice on toucans have broad, bell-shaped heads, whereas those on barbets are more roundly triangular. Within Clade H, there is also a strongly supported clade of three taxa found on New World Icteridae; these are all lice in the currently recognized genus *Bizarrifrons* Eichler, 1939. Lice in this genus all have an asymmetrical pre-antennal area, which easily separates them from all other taxa included in this study. Other songbird lice in Clade H are from Australian white-winged choughs (*Corcorax melanorhamphos*). Morphologically, these lice are no more similar to *Traihoriella* and *Bizarrifrons* than they are to other lice in Clades A–H. Indeed, lice within Clade H represent two distinct ecomorphs. Unfortunately, there is little support for the basal nodes within Clade H. Greater sampling is needed to resolve the molecular relationships of these morphologically disparate taxa.

3.4. Clade I (Fig. 3e)

Clade I contains 81 samples that belong to *Brueelia sensu stricto*. This clade is found world-wide, with the exception of Australasia, and it is found on 18 families of songbirds (Passeriformes: Bombycillidae, Coerebidae, Corvidae, Emberizidae, Estrildidae, Fringillidae, Icteridae, Mimidae, Paridae, Parulidae, Passeridae, Ploceidae, Pycnonotidae, Sturnidae, Sylviidae, Troglodytidae, Turdidae, Zosteropidae) and on woodpeckers (Piciformes: Picidae). Morphologically, lice in this genus are separated from other lice in the *Brueelia* complex by distinct pre-antennal and chaetotaxy characteristics (Table 1i).

Clade I-1, although not strongly supported by molecular data, is morphologically unified by the presence of a ventral anterior plate located in the feeding canal. This group is largely restricted to the New World, but two taxa were collected in the Old World (#54 and #57), from host species that are known to occur, albeit rarely, in the New World (*Emberiza schoeniclus* and *Emberiza pusilla*) (Lepage, 2015). Lice in this clade occur mostly on related passerine families in the parvorder Passerida (Cracraft and Barker, 2009), but they also occur on the distantly related passerine (*Aphelocoma*: Corvidae) and on woodpeckers in the distantly related order Piciformes.

I-2 is a paraphyletic grade of lice that are largely restricted to Old World hosts in the parvorder Passerida. Two geographic exceptions are lice from Nearactic hosts within Passerida (#86 and #137). Notably, associations of lice with particular host families are not phylogenetically conserved along this part of the tree. The morphology and relationships of these species are generally poorly known, and the majority of the species are undescribed. Poor molecular support of basal nodes with this grade prevents rigorous interpretation of the relationships between these taxa.

Clade I-3 contains lice that form the species group *B. ornatissima* (Cicchino and Castro, 1996). This group is restricted to New World blackbirds (Icteridae). Species in this group are morphologically similar to other *Brueelia* s. str., but are easily recognized by strikingly complex pigmentation patterns. Cicchino and Castro (1996) further divided the *B. ornatissima* species group into "*cela*" and "*amazonae*" subgroups, separated by different pigmentation characteristics. The two well-supported clades with within *B. ornatissima* may reflect this morphological split, but additional morphological work on the undescribed species in this clade is needed to confirm the monophyly of the group and subgroups proposed by Cicchino and Castro (1996).

3.5. Clades J–K

Clade J is a strongly supported clade of lice that are largely restricted to Old World finches (Estrildidae). Clade K is a small, but well supported clade of lice on swallows and martins (Hirundinidae). The sister relationships of Clade I, and Clades J + K, are supported morphologically as well as from the molecular data (Table 1i). Gross differences in morphology are apparent; lice in Clade J generally have a much rounder, almost tear-drop shaped body, whereas lice in Clade K have long, slender forms such as the "wing" lice in Johnson et al. (2012). These two clades most likely represent distinct genera, but additional taxonomic work is needed to formally address the taxonomic status of Clade J. Lice in Clade K belong to the genus *Acronirmus* (Eichler, 1953), which was considered synonymous with *Brueelia* by Price et al. (2003). Lice in this clade were placed in the genus *Hirundiniella* Carriker 1963, (a taxonomic treatment recently cited by Mey (2009), Mey and Barker (2014) and Valim and Palma (2015)) but this name is a junior synonym of *Acronirmus* Eichler 1953. Based on both molecular and morphological data the resurrection of the genus *Acronirmus* is warranted for Clade K.

3.6. Clades L–N

Clades L–N form a monophyletic group that is poorly supported with molecular data, but the taxa are united by similar male genitalic structures (Table 1o). Clade L is a small but strongly supported clade that is further unified by similarities in male genitalia (Table 1p). The relative phylogenetic arrangement of Clades L, M, and N is not well resolved, but morphological similarities suggest that Clades M and N are more closely related. Lice in Clade L have long, slender abdomens (Fig. 2), whereas lice in clades M and N have more ovate body forms reminiscent of the "head" and "generalist" ecomorphs (Fig. 4).

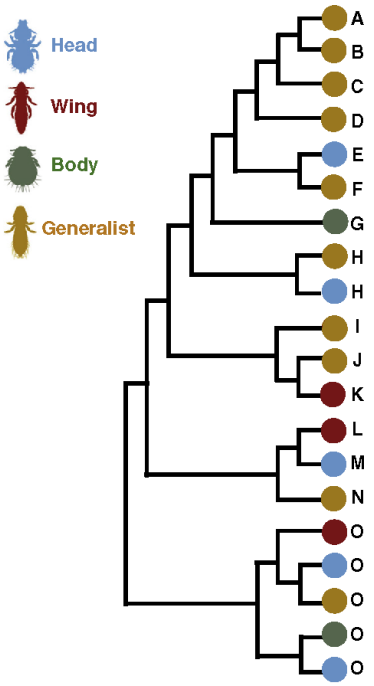

Fig. 4. Distribution of four louse ecomorphs across the *Brueelia*-complex. Head lice have oval bodies with large, triangular heads. The broad temples of head lice support large muscles attached to the mandibles, which enhance their grip, and presumably help these lice to avoid getting dislodged by a scratching host (Clay, 1949). Scratching is the principle host defense against head lice. Other ecomorphs are susceptible to being removed by the bird's bill during preening. Wing lice have long slender bodies, and long legs. They spend most of their time on the large flight feathers of the host's wings or tail, where they insert themselves between adjacent feather barbs to avoid preening (Clayton, 1991; Bush et al., 2006). Body lice have oval bodies and round heads. They live in the abdominal contour feathers, where they avoid preening by dropping between adjacent feathers, or by burrowing into the downy portions of feathers (Clayton, 1991). Generalist lice have intermediate body shapes. They can be found on most regions of the host's body, where they escape from preening by running quickly.

Clade M is a strongly supported clade of lice restricted to Old World orioles (Oriolidae) and quail-thrushes (Cinclosomatidae). These lice have morphologically similar heads and male genitalia (Table 1q). Lice in Clade M are morphologically most similar to the genus *Maculinirmus* Zlotorzycka 1964, which was considered a synonym of *Brueelia* by Ledger (1980). More recently, however, *Maculinirmus* is considered a valid genus by and Mey and Barker (2014) and Valim and Palma (2015). Molecular support for this monophyletic clade suggests that the resurrection of *Maculinirmus* may be warranted.

Clade N is a strongly supported clade of Old World lice restricted to cuckoo-shrikes (Campephagidae). Although lice in this clade are similar in many respects to Clade M, they are morphologically distinct in both head and male genitalic characters (Table 1r) and may represent a new genus.

3.7. Clade O

Clade O is a strongly supported clade of lice that occur on non-passerine hosts. There are no obvious morphological characters that unite the extremely variable taxa in this clade. Four taxa from

bee-eaters form the strongly supported Clade O-1; these lice are representatives of the genus *Meropsiella* Conci 1941a, which was later considered synonymous with *Brueelia* by Hopkins and Clay (1952) and Price et al. (2003). More recently, however, *Meropsiella* has been treated as a valid genus by Mey and Barker (2014) and Valim and Palma (2015). Taxa belonging to the currently recognized genus *Meropoecus* Eichler 1940 form Clade O-2. The single sequenced specimen of *Motmotnirmus* Mey and Barker 2014 (Clade O-3) is sister to *Meropoecus*. Clade O-4 includes the single sequenced specimen from *Buerelius* Clay and Tandan 1967, and an undescribed species from *Coua cristata* (#43), which shares some genitalic similarities with *Buerelius* spp. but it is dissimilar with respect to head characters, abdominal chaetotaxy, and other somatic characters. Although many of the nodes in this clade are strongly supported, additional sampling of lice from of these distinct morphological groups would increase the confidence for interpretations of the evolutionary history among these disparate taxa.

4. Discussion

Our phylogeny supports the monophyly of the *Brueelia*-complex (100% posterior probability, including the currently recognized genera *Brueelia*, *Buerelius*, *Bizarrifrons*, *Sturnidoecus*, and *Meropoecus*) (Clay and Tandan, 1967; Ledger, 1980; Price et al., 2003; Valim and Palma, 2012). Together members of this complex form a larger clade (100% posterior probability) with the genera *Forficuloecus* Conci 1941b, *Formicaricola* Carriker 1957, *Formicaphagus* Carriker 1957, *Neopsittaconirmus* Conci 1942, *Paragoniocotes* Cummings 1916, *Psittaconirmus* Harrison 1915, *Psittoecus* Conci 1942, and *Theresiella* Guimarães 1971. Previous taxonomists have variably considered the genera *Formicaricola* and *Formicaphagus* to be part of the *Brueelia*-complex (Clay and Tandan, 1967; Valim and Palma, 2012, 2015; Mey and Barker, 2014). However, based on our tree it appears that these genera may be nested within a clade of lice associated with parrots and thus are not strongly allied with the remainder of the *Brueelia*-complex. A more thorough molecular and morphological study of the species in these and related genera is necessary to determine the taxonomic limits of the *Brueelia*-complex.

Our phylogeny also indicates that the genus *Brueelia* as recognized by Price et al., 2003 is not monophyletic; the currently recognized genera *Bizzarrifrons*, *Buerelius*, *Meropoecus*, *Motmotnirmus*, and *Sturnidoecus* are firmly nested within *Brueelia* s. lat. (Price et al., 2003). Thus, a monophyletic definition of *Brueelia* would either need to be expanded to include the very distinct morphologies of these genera, or be limited to one or a few of the clades named here. We advocate the latter, as shared-derived morphological characters can be identified for well-supported clades in the molecular phylogeny (Fig. 2, Table 1). Indeed, many of these morphological characters were identified and used by previous taxonomists as generic level characters. Our molecular data suggests that re-elevation of the following genera would be warranted: *Guimaraesiella* Eichler 1949, *Olivinirmus* Zlotorzycka 1964, *Acronirmus* Eichler 1953, *Maculinirmus* Zlotorzycka 1964, *Meropsiella* Conci 1941a. Three undescribed, yet well-supported, clades are also apparent: Clades J, L, and N. In the future, formal alpha taxonomic work will likely lead to the description of specimens in these clades as new genera. For the previously recognized genera *Corvonirmus* Eichler 1944, *Rostrinirmus* Zlotorzycka 1964, and *Traihoriella* Ansari 1947, the molecular data is inconclusive, mainly because of limited taxon sampling of these groups. The sequencing of additional material, as well as a more thorough morphological examination of preserved specimens may clarify our understanding of the phylogenetic relationships of these groups within the *Brueelia*-complex.

The *Brueelia*-complex includes many clades with very different gross morphologies, and many of these clades align with previously recognized genera (Figs. 2 and 3). These lice can be divided into four common "ecomorphs" that specialize on different microhabitats of the host: head lice, wing lice, body lice, and generalist lice (Clay, 1949). These ecomorphs have evolved repeatedly across the order Phthiraptera (Johnson et al., 2012), and our phylogenetic reconstruction indicates that these forms have also evolved repeatedly within the *Brueelia*-complex (Fig. 4). The variation in morphology among these ecomorphs is thought to be associated with the different ways in which these lice escape from host defense (preening) on different microhabitats of the host. Note, however, that the characterization of lice in the *Brueelia*-complex as different ecomorphs is based primarily on gross morphology. More research is needed to confirm whether similar "ecomorphs" do, in fact, behave similarly and use similar microhabitats on the surface of the host. Indeed, studies that examine the microhabitat preferences and behavior of these lice would considerably further our understanding of the evolution of these divergent forms within the *Brueelia*-complex and across the order Phthiraptera.

With 333 ingroup samples and 31 outgroup taxa, this is the largest phylogenetic reconstruction for any group in the order Phthiraptera. Only 73 (22%) of these samples could be assigned to formally described species (37 of them), which is less than 15% of all described species in the *Brueelia*-complex. The remaining 78% are either undescribed species of lice or new host associations for which a formal species assignment could not be conducted in the absence of a complete species level revision of the group. The paucity of described species in this large data set indicates that this group of lice is vastly under-sampled even with the large number of individuals ($n = 333$) included in this study. Alpha-taxonomic work on the thousands of unidentified specimens in museum collections as well as future collecting efforts are likely to reveal many more species in this group of lice.

The genus *Brueelia*, as recognized by Price et al. (2003), is so speciose that there has been no comprehensive revision of this genus. Instead taxonomists have attempted to revise groups of *Brueelia* from related hosts, e.g. *Brueelia* from Picidae (Dalgleish, 1971) and Corvidae (Ansari, 1956, 1957). This practice has been questioned because it assumes lice are specific to a particular host family (Johnson et al., 2002). While this assumption may be true among many genera of lice (Hafner and Nadler, 1990), Johnson et al.'s (2002) study of a small group of lice in the genus *Brueelia* found that some lice within *Brueelia* are shared among distantly related hosts. Indeed, their study showed that "*Brueelia* sp. 1" (Clade A-1) was found on birds in four distantly related passerine families: Muscicapidae, Paridae, Rhipiduridae, and Sittidae. This material was included in our study (specimens #149, 177, 139, and 249 respectively), and we confirm that these specimens are closely related to each other as well as to specimens from several other host species. Our 5% OTU analysis lumps these specimens along with 14 others into one OTU (5% OTU #1, Table 1 in Bush et al., in press) that parasitizes 11 different host families within the order Passeriformes. The bGMYC OTU analysis considers this OTU even less specific and groups lice from 27 host species and 17 host families in a single OTU (bGMYC OTU#114, Table 1 in Bush et al., in press). This species is clearly one of the most extreme cases, as most species in the *Brueelia*-complex are only found on a single host species. Indeed, the percentage of species in the *Brueelia*-complex with only one recorded host species is over 85% in Price et al. (2003). In comparison, the OTU analyses of our data set indicate that 72.3% and 55.3% of the OTUs are found on only one host species for 5% OTU and bGMYC OTU analyses respectively.

Taxonomic revisions of lice that are circumscribed by a host family are also erroneous if lice on related hosts are not closely related. This pattern frequently occurs in the *Brueelia*-complex. For example, lice on jays and crows (Corvidae) occur in four distantly related clades and lice on thrushes (Turdidae) occur in at least eight different clades (Fig. 3). In short, our data indicates that revisionary work on lice in the *Brueelia*-complex cannot be accurately divided into groups based on the host family with which they are associated. Thus, revisionary taxonomy would benefit from a more holistic approach including both molecular and morphological data.

Why is the *Brueelia*-complex so diverse? One argument that has been suggested is that species richness of this group is a taxonomic artifact, generated by a tendency of taxonomist to over-describe species of lice in this group (Johnson et al., 2002). This hypothesis can be addressed by comparing the taxonomic treatments of Price et al. (2003) and the results of the two species delimitation methods (bGMYC and 5% OTU for the 73 taxa that are shared by Price et al. (2003) and our molecular data set. Price et al. (2003) treats these taxa as 37 species with a mean host specificity of 1.49 (1 sd ± 1.07) hosts per louse species. Our bGMYC OTU analysis indicates that these are best delimited as 34 OTUs with a mean host specificity of 1.53 (1 sd ± 1.02) hosts per OTU, and the 5% OTU analysis indicated that these taxa are best delimited as 43 OTUs with a mean host specificity of 1.35 (1 sd ± 0.78) hosts per OTU. Host specificity does not differ significantly among these three taxonomic treatments (Kruskal Wallis, df = 2, $\chi^2 = 0.45$, $P = 0.80$); moreover, all of these taxonomic treatments reflect very high levels of host specificity, all with ⩽1.53 hosts per louse species. Thus, the patterns of diversity and high host specificity without cospeciation, as noted by Johnson et al. (2002), cannot be attributed to over-described species; instead ecological factors should be considered to explain these phenomenon.

What ecological processes are likely to influence the macroevolutionary patterns in the *Brueelia*-complex? Although we provide evidence that alpha-taxonomic work relying on host-family associations can lead to erroneous groupings among taxa in the *Brueelia*-complex (as suggested by Johnson et al. (2002)), we did find a significant correlation between host-family associations and the phylogenetic tree. This is, in part, a matter of scale. Smaller clades within the complex are often restricted to one or a few host families. At this scale, limited dispersal of lice among different host species may lead to cospeciation via shared vicariance events and/or louse adaptations to particular types of hosts may prevent them from establishing successfully on more distantly related hosts. Experiments testing whether lice that are naturally restricted to one host family can establish on "novel" hosts are needed to understand patterns of diversification in this system.

We also found that biogeographic region had significant phylogenetic signal. This pattern is not entirely independent of host-family, as many avian host families are restricted to particular biogeographic regions. It is, however, interesting to note that in most cases where louse OTUs are found on multiple host families, the families are from the same geographic region. This pattern in particular suggests that ecological factors such as variation in dispersal could be very important to some groups within the *Brueelia*-complex. Unlike most other groups of lice, members of the *Brueelia*-complex commonly use phoretic dispersal by attaching to hippoboscid flies (Fig. 1). In fact, 88% of all recorded phoretic events are lice currently recognized as *Brueelia* spp. or *Sturnidoecus* spp. (reviewed by Harbison (2008) and Harbison and Clayton (2011)). Indeed, records of phoresis are known from species of lice associated with clades: A, E, F, G, and I.

Phoresis is a particularly interesting form of horizontal dispersal because hippoboscid flies are very mobile and may visit multiple host species. This provides an opportunity for phoretic lice to move between different host species. In a survey of documented cases, Harbison (2008) found that, within the genus *Brueelia*, species that are phoretic are significantly less host specific than species that are not phoretic (38% of phoretic spp. occurred on more than on host species whereas only 11% of non-phoretic spp. occurred on more than one host species). It is possible that phoretic dispersal is a key innovation that allows lice in the *Brueelia*-complex to disperse to, and then radiate on new host families. This pattern of "escape and radiate co-evolution" (Ehrlich and Raven, 1964) has been described for herbivorous insects that specialize on host plants (Becerra, 1997), but it has not been suggested for ectoparasites. In the future, cophylogenetic comparisons of lice and their hosts are needed to test hypotheses about the nature of coevolution in this system.

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References

Ansari, M.A.R., 1947. Mallophaga (Ischnocera) infesting birds in the Punjab (India). Proc. Nat. Inst. Sci. India 13, 255–303.

Ansari, M.A.R., 1956. A revision of *Brueelia* (Mallophaga) species infesting the Corvidae, Part I. Bull. Brit. Mus. Nat. Hist., London 4, 371–406.

Ansari, M.A.R., 1957. A revision of the *Brueelia* (Mallophaga) species infesting the Corvidae. Part II. Brit. Mus. Nat. Hist., London 5, 145–182.

Becerra, J.X., 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276, 253–256.

Bueter, C., Weckstein, J.D., Johnson, K.P., Bates, J.M., Gordon, C.E., 2009. Comparative phylogenetic histories of two louse genera found on *Catharus* thrushes and other birds. J. Parasitol. 95, 295–307.

Bush, S.E., Sohn, E., Clayton, D.H., 2006. Ecomorphology of parasite attachment: experiments with feather lice. J. Parasitol. 92, 25–31.

Bush, S.E., Kim, D., Reed, M., Clayton, D.H., 2010. Evolution of cryptic coloration in ectoparasites. Am. Nat. 176, 529–535.

Bush, S.E., Weckstein, J.D., Gustafsson, D.R., Allen, J., DiBlasi, E., Shreve, S.M., Boldt, R., Skeen, H.R., Johnson, K.P., in press. Data supporting a molecular phylogeny of the hyper-diverse genus *Brueelia*. Data in Brief.

Carriker Jr., M.A., 1957. Studies in neotropical Mallophaga. XVI: bird lice of the suborder Ischnocera. Proc. US Nat. Mus. 106, 409–439.

Carriker Jr., M.A., 1963. Neotropical Mallophaga (Insecta) miscellany, No. 13. Rev. Bras. Biol. 23, 293–316.

Cicchino, A.C., 2004. Adición a la revisión de las especies del género *Brueelia* Kéler, 1936 (Phthiraptera: Philopteridae) parásitas de Icterinae. Las especies parásitas del género *Agelaius* Vieillot, 1816 (Aves: Passeriformes: Fringillidae). Bol. Mus. Nac. Hist. Nat. Parag. 15, 66–81.

Cicchino, A.C., Castro, D.C., 1996. Revisión preliminar de las especies del género *Brueelia* Kéler, 1936 (Phthirapter, Philopteridae) parásitas de Icterinae (Aves, Passeriformes, Fringillidae). Graellsia 52, 3–30.

Cicchino, A.C., González-Acuña, D., 2008. Two new species of *Brueelia* (Phthiraptera: Philopteridas s. l.) parasitic on two species of *Phrygilus* (Aves: Emberizidae) from Chile. Neotrop. Entomol. 37, 301–304.

Cicchino, A.C., González-Acuña, D., 2009. A new species of *Brueelia* Kéler (Phthiraptera: Philopteridae) parasitic on the Common Diuca-Finch, *Diuca diuca diuca* (Aves: Emberizidae) in Chile. Neotrop. Entomol. 38, 504–507.

Clay, T., 1936. New species of Mallophaga recorded from Asiatic birds. Proc. Zool. Soc. Lond. 4, 905–914.
Clay, T., 1949. Some problems in the evolution of a group of ectoparasites. Evolution 3, 279–299.
Clay, T., 1951. An introduction to a classification of the avian Ischnocera (Mallophaga): Part I. Trans. Roy. Entomol. Soc. Lond. 102, 171–194.
Clay, T., Tandan, B.K., 1967. A new genus and species of Philopteridae (Mallophaga). Proc. Roy. Entomol. Soc. Lond. B 36, 34–40.
Clayton, D.H., 1991. Coevolution of avian grooming and ectoparasites avoidance. In: Loye, J.E., Zuk, M. (Eds.), Bird Parasite Interactions: Ecology, Evolution and Behavior. Oxford Ornithology Series 2, pp. 259–287.
Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). J. Parasit. 87, 1291–1300.
Clayton, D.H., Johnson, K.P., 2003. Linking coevolutionary history to ecological process: doves and lice. Evolution 57, 2335–2341.
Clayton, D.H., Bush, S.E., Johnson, K.P., 2004. Ecology of congruence: past meets present. Syst. Biol. 53, 165–173.
Clayton, D.H., Bush, S.E., Johnson, K.P., 2015. Coevolution of Life on Hosts: Integrating Ecology and History. University of Chicago Press. Chicago and London (in press).
Clements, J.F., Schulenberg, T.S., Iliff, M.J., Roberson, D., Fredericks, T.A., Sullivan, B. L., Wood, C.L., 2014. The eBird/Clements Checklist of Birds of the World: Version 6.8. Downloaded from http://www.birds.cornell.edu/clementschecklist/download/.
Conci, C., 1941a. Due nuovi generi e tre nuove specie di Mallofagi dei *Meropes*. Boll. Soc. Entomol. Ital. 73, 99–107.
Conci, C., 1941b. Nuovi generi di Mallofagi (nota preliminare). Boll. Soc. Entomol. Ital. 73, 126–127.
Conci, C., 1942. I Mallofagi deglie Psittaciformes. Boll. Soc. Entomol. Ital. 74, 33–41.
Cracraft, J., Barker, K., 2009. Passerine birds (Passeriformes). In: Hedges, S.B., Kumar, S. (Eds.), The Timetree of Life. Oxford Press, pp. 1–10.
Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H., Page, R.D.M., 2001. Phylogenetic analysis of partial sequences of Elongation Factor 1*a* identifies major groups of lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 19, 202–215.
Cummings, B.F., 1916. New species of lice. Ann. Mag. Nat. Hist. 8, 90–107.
Dalgleish, R.C., 1971. The *Brueelia* (Mallophaga: Ischnocera) of the Picidae (Aves: Piciformes). J. NY Entomol. Soc. 79, 139–146.
Denny, H., 1842. Monographia Anoplurorum Britannie; or, an Essay on the British Species of Parasitic Insects. Henry G. Bohn, London.
Dickinson, E.C., Remsen Jr., J.V., Christidis, L. (Eds.), 2014. The Howard and Moore Complete Checklist of the Birds of the World, fourth ed., vols. 1–2. Aves Press Ltd.
Drummond, A.J., Rambaut, A., 2007. Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. http://dx.doi.org/10.1186/1471-2148-7-214.
Ehrlich, P.R., Raven, P.H., 1964. Butterflies and plants: a study in coevolution. Evolution 18, 586–608.
Eichler, W., 1940. Notulae Mallophagologicae. IV. Neue Gattungen und höhere Einheiten von Kletterfederlingen. Zool. Anz. 130, 97–130.
Eichler, W., 1944. Notulae Mallophagologicae. XI. Acht neue Gattungen der Nirmi und Docophori. Stett. Entomol. Zeit. 105, 80–82.
Eichler, W., 1949. Phthirapterorum nova genera. Boll. Soc. Entomol. Ital. 79, 11–13.
Eichler, W., 1953. Von Alexander Koenig gesammelte Federlinge. I. Von Singvögeln und Spechten. Bonn. Zool. Beitr. 4, 333–343.
Guimarães, L.R., 1971. A new genus and two new species of parrot Mallophaga (Philopteridae) from New Guinea. Pap. Avol. Zool., São Paolo 24, 213–219.
Hafner, M.S., Nadler, S.A., 1990. Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. Syst. Biol. 39, 192–204.
Harbison, C.W., 2008. Ecology and Evolution of Transmission in Feather-feeding Lice (Phthiraptera: Ischnocera). University of Utah, Dissertation, Department of Biology.
Harbison, C.W., Clayton, D.H., 2011. Community interactions govern host-switching with implications for host–parasite coevolutionary history. Proceed. Nat. Acad. Sci. 108, 9525–9529.
Harrison, L., 1915. On a new family and five new genera of Mallophaga. Parasitology 7, 383–407.
Hopkins, G.H.E., Clay, T., 1952. A check list of the genera and species of mallophaga. Brit. Mus. Nat. Hist., London.
Huelsenbeck, J.P., Bollback, J.P., 2001. Empirical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. 50, 351–366.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Johnson, K.P., Moyle, R.G., Witt, C.C., Faucett, R.C., Weckstein, J.D., 2001a. Phylogenetic relationships in the louse genus *Penenirmus* based on nuclear (EF-1α) and mitochondrial (COI) DNA segments. Syst. Entomol. 26, 491–497.
Johnson, K.P., Adams, R.J., Clayton, D.H., 2001b. Molecular systematics of Goniodidae (Insecta: Phthiraptera). J. Parasitol. 87, 862–869.
Johnson, K.P., Adams, R.J., Clayton, D.H., 2002a. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. Biol. J. Linn. Soc. 77, 233–247. http://dx.doi.org/10.1046/j.1095-8312.2002.00107.x.
Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J., Clayton, D.H., 2002b. The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). Mol. Ecol. 11, 25–38.
Johnson, K.P., Weckstein, J.D., Witt, C.C., Faucett, R.C., Moyle, R.G., 2002c. The perils of using host relationships in parasite taxonomy: phylogeny of the *Degeeriella* complex. Mol. Phylogenet. Evol. 23, 150–157.
Johnson, K.P., Cruickshank, R.H., Adams, R.J., Smith, V.S., Page, R.D.M., Clayton, D.H., 2003. Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 26, 231–242.
Johnson, K.P., Yoshizawa, K., Smith, V.S., 2004. Multiple origins of parasitism in lice. Proc. R. Soc. Lond. B 271, 1771–1776. http://dx.doi.org/10.1098/rspb.2004.2798.
Johnson, K.P., Reed, D.L., Hammond-Parker, S.L., Kim, D., Clayton, D.H., 2007. Phylogenetic analysis of nuclear and mitochondrial genes supports species groups for *Columbicola* (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 45, 506–518.
Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. BMC Biol. 10, 52. http://dx.doi.org/10.1186/1741-7007-10-52.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Lanfear, R., Calcott, B., Kainer, D., Mayer, C., Stamatakis, A., 2014. Selecting optimal partitioning schemes for phylogenomic datasets. BMC Evol. Biol. 14, 82.
Ledger, J. 1980. The Arthropod Parasites of Vertebrates in Africa South of the Sahara, vol. IV. Phthiraptera (Insecta).
Lepage, D., 2015. Avibase: The World Bird Database. Bird Studies Canada.
MacIntyre, G.T., 1967. Foramen pseudovale and quasi-mammals. Evolution 21, 834–841.
Maddison, W.P., Slatkin, M., 1991. Null models for the number of evolutionary steps in a character on a phylogenetic tree. Evolution 45, 1184–1197.
Mayr, E., 1982. The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Harvard University Press.
McCarthy, M., 2012. World's greatest birdwatcher sets a new record – then hangs up his binoculars. The Independent [London] October 15, 2012.
Mey, E., 2009. Zweiter europäischer Nachweis von Coloceras tovornikae Tendeiro (Phthiraptera, Ischnocera) und weitere Federlingsbefunde bei Pskow (Russland). Entomologische Nachrichten und Berichte 53, 169–180.
Mey, E., Barker, S.C., 2014. Eine neue auf den Feenvögeln (Irenidae) lebende Brueelia-Art (Insecta, Phthiraptera, Ischnocera, Philopteridae), nebst Anmerkungen zur Gattung Brueelia Kéler, 1936 sensu lato. Rudolstädter Nat. Hist. Schr. 19, 73–114.
Najer, T., Sychra, O., Literák, I., Procházka, P., Capek, M., Koubek, P., 2012a. Chewing lice (Phthiraptera) from wild birds in Senegal, with descriptions of three new species of the genera *Brueelia* and *Philopteroides*. Acta Parasitol. 57, 90–98.
Najer, T., Sychra, O., Hung, N.M., Capek, M., Podzemny, P., Literak, I., 2012b. New species and new records of chewing lice (Phthiraptera: Amblycera and Ischnocera) from bulbuls (Passeriformes: Pycnonotidae) in Vietnam. Zootaxa 3357, 37–48.
Najer, T., Sychra, O., Hung, N.M., Capek, M., Podzemny, P., Literak, I., 2012c. Chewing lice (Phthiraptera: Amblycera, Ischnocera) from wild passerines (Aves: Passeriformes) in northern Vietnam, with descriptions of three new species. Zootaxa 3530, 59–73.
Najer, T., Sychra, O., Kounek, F., Papousek, I., Hung, N.M., 2014. Chewing lice (Phthiraptera: Amblycera and Ischnocera) from wild birds in southern Vietnam, with descriptions of two new species. Zootaxa 3755, 419–433.
Olson, D.M. et al., 2001. Terrestrial ecoregions of the world: a new map of life on earth a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. Bioscience 51 (11), 933–938.
Palma, R.L., 1978. Slide-mounting of lice: a detailed description of the Canada balsam technique. N. Zeal. Entomol. 6, 432–436.
Price, R.D., Johnson, K.P., 2009. Five new species of *Myrsidea* Waterston (Phthiraptera: Menoponidae) from tanagers (Passeriformes: Thraupidae) in Panama. Zootaxa 2200, 61–68.
Price, R.D., Hellenthal, R.A. Palma, R.L., Johnson, K.P., Clayton, D.H., 2003. The Chewing Lice. World Checklist and Biological Overview. Illinois Natural History Survey.
Price, R.D., Johnson, K.P., Palma, R.L., 2008. A review of the genus *Forficuloecus* Conci (Phthiraptera: Philopteridae) from parrots (Psittaciformes: Psittacidae), with descriptions of four new species. Zootaxa 1859, 49–62.
Reid, N.M., Carstens, B.C., 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12, 196. http://dx.doi.org/10.1186/1471-2148-12-196.
Rékási, J., Saxena, A.K., 2005. A new Phthiraptera species (Philopteridae) from the Red Avadavat (*Amandava amandava*). Aquila 112, 87–93.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Ronquist, F., Teslenko, M., Mark, P.V.D., Ayres, D.L., Darling, A., Hohana, S.H., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. Syst. Biol. 61, 1–4.
Sangster, G., Luksenburg, J.A., 2015. Declining rates of species described per taxonomist: slowdown of progress or a side-effect of improved quality in taxonomy? Syst. Biol. 64, 144–151.
Schloss, P.D., Westcott, S.L., Rayabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakely, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing

and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Smith, V.S., Page, R.D.M., Johnson, K.P., 2004. 2004 Data incongruence and the problem of avian louse phylogeny. Zool. Scr. 30, 239–259. http://dx.doi.org/10.1111/j.0300-3256..00149.x.

Smith, V.S., Ford, T., Johnson, K.P., Johnson, P.C.D., Yoshizawa, K., Light, J.E., 2011. Multiple lineages of lice pass through the K-Pg boundary. Biol. Lett. 7, 782–785.

Sychra, O., Literák, I., Hung, N.M., Podzemny, P., 2009. Chewing lice from wild passerines (Aves, Passeriformes) from Vietnam, with description of a new species of the genus *Brueelia* (Phthiraptera, Ischnocera, Philopteridae). Acta Parasitol. 54, 154–157.

Sychra, O., Literák, I., Najer, T., Capek, M., Koubek, P., Procházka, P., 2010a. Chewing lice (Insecta: Phthiraptera) from estrildid finches (Aves: Passeriformes: Estrildidae) and louse flies (Insecta: Diptera: Hippoboscidae) from birds in Senegal, with descriptions of three new species of the genus *Brueelia*. Zootaxa 2714, 59–68.

Sychra, O., Barlev, E., Literák, I., Capek, M., Koubek, P., Procházka, P., 2010b. The chewing lice (Phthiraptera) of Red-billed Quelea (*Quelea quelea*) in Senegal, with a description of a new species. Afr. Entomol. 18, 17–22.

Valim, M.P., Palma, R.L., 2006. A new species of *Brueelia* Kéler, 1936 (Phthiraptera: ischnocera: Philopteridae) from the blue-back grassquit (Aves: Passeriformes: Emberizidae) in Brazil. Zootaxa 1153, 27–32.

Valim, M.P., Palma, R.L., 2012. Redescriptions of two species and descriptions of three new species of the louse genus *Bizarrifrons* Eichler, 1938 (Phthiraptera: Ischnocera: Philopteridae). Zootaxa 3273, 28–50.

Valim, M.P., Palma, R.L., 2015. A new genus and two new species of feather lice (Phthiraptera: Ischnocera: Philopteridae) from New Zealand endemic passerines (Aves: Passeriformes). Zootaxa 3926, 480–498.

Valim, M.P., Silveira, L.F., 2014. A new species and five new records of chewing lice (Insecta: Phthiraptera: Ischnocera) from an isolated population of the solitary tinamou *Tinamus solitarius* (Aves: Tinamiformes). Zootaxa 3838 (1), 127–142.

Valim, M.P., Weckstein, J.D., 2011. Two new species of *Brueelia* Kéler, 1936 (Ischnocera, Philopteridae) parasitic on Neotropical trogons (Aves, Trogoniformes). ZooKeys 128, 1–13.

Weckstein, J.D., 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. Syst. Biol. 53, 154–164.

Zlotorzycka, J., 1964. Mallophaga parasitizing Passeriformes and Pici. II. Brueeliinae. Acta Parasitologica Polonica 12, 239–282.

APPENDIX D

DATA SUPPORTING A MOLECULAR PHYLOGENY OF THE HYPER-DIVERSE GENUS *BRUEELIA*

Bush S.E., Weckstein J.D., Gustaffson D.R., Allen J., DiBlasi E., Shreve S.M., Boldt R., Skeen H.R., Johnson K.P.(2015). Data supporting a molecular phylogeny of the hyper-diverse genus *Brueelia*. Data in Brief, 5, 1078–1091.

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Data Article

Data supporting a molecular phylogeny of the hyper-diverse genus *Brueelia*

Sarah E. Bush [a,*], Jason D. Weckstein [b,1], Daniel R. Gustafsson [a], Julie Allen [c], Emily DiBlasi [a], Scott M. Shreve [c,2], Rachel Boldt [c], Heather R. Skeen [b,3], Kevin P. Johnson [c]

[a] Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
[b] Field Museum of Natural History, Science and Education, Integrative Research Center, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA
[c] Illinois Natural History Survey, University of Illinois, 1816 South Oak Street, Champaign, IL 61820, USA

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ABSTRACT

Data is presented in support of a phylogenetic reconstruction of one of the largest, and most poorly understood, groups of lice: the *Brueelia*-complex (Bush et al., 2015 [1]). Presented data include the voucher information and molecular data (GenBank accession numbers) of 333 ingroup taxa within the *Brueelia*-complex and 30 outgroup taxa selected from across the order Phthiraptera. Also included are phylogenetic reconstructions based on Bayesian inference analyses of combined COI and EF-1α sequences for *Brueelia*-complex species and outgroup taxa.

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* Corresponding author.
E-mail address: bush@biology.utah.edu (S.E. Bush).
[1] Current address: Department of Ornithology and Department of Biodiversity, Earth, and Environmental Sciences, Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, USA.
[2] Current address: Department of Biology, Centre College, Danville, KY 40422, USA.
[3] Current address: Biology Department, Loyola University Chicago, Chicago IL 60660, USA.

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Specifications table

Subject area	Biology, genetics and genomics
More specific subject area	Phylogenetics
Type of data	Specimen matrix, phylogenetic reconstruction
How data was acquired	Phylogenetic reconstruction using Bayesian inference methods
Data format	Raw, analyzed
Experimental factors	n/a
Experimental features	n/a
Data source location	worldwide
Data accessibility	Within this article, sequences available in GenBank

Value of the data

- Evolutionary history of feather lice in the Brueelia-complex was reconstructed.
- Data support re-recognition of historic genera, and erection of several new genera.
- Associations of lice with geography and host-family are correlated with phylogeny.
- Host association and geographic origin of each sequenced specimen are provided.

1. Data, materials and methods

The data presented herein supports a phylogenetic reconstruction of the *Brueelia*-complex; these data complement the companion article by Bush et al. [1].

1.1. Sampling

We sampled a total of 333 louse specimens belonging to the *Brueelia*-complex (Supplemental Table 1). These lice were sampled from 250 bird species belonging to 66 bird families and five orders (Passeriformes, Coraciiformes, Cuculiformes, Piciformes, and Trogoniformes). Sampled lice include 38 known species and 211 lice that represent either new species of lice or new host associations. These samples were collected from 23 countries and all continents except Antarctica. An additional 30 outgroup taxa for rooting the phylogeny were selected to represent nested sister taxonomic relationships within the family Philopteridae [2,3]. These 30 louse outgroup species were from 27 host species, in 17 host families, collected from 9 countries.

Lice were collected either from euthanized bird specimens using ethyl acetate fumigation or from live birds dusted with pyrethrum powder [4,5]. Care was taken to make sure that individual hosts were kept separate at all times and to clean all working surfaces between fumigation. Lice were collected by the authors and colleagues during field-work conducted over several decades and were stored in vials of 95% ethanol, usually in ultracold (−80 °C) freezers.

1.2. DNA extraction, amplification and alignment

DNA was extracted from lice using either the Qiagen DNeasy micro-kit (Valencia, California, USA) following the manufacturer's protocol as described by Valim and Weckstein [6], or the Qiagen DNeasy tissue kit (Valencia, California, USA) following the manufacture's protocol as described by Johnson et al. [7]. After DNA was extracted from individual lice, the exoskeletons were retained and mounted on microscope slides [8]. These voucher slides were used to identify each specimen to genus using the keys in Price et al. [9]. Specific-level identifications were based on original descriptions, specific keys if possible, and comparison with identified slide mounted material. Voucher slides are deposited in

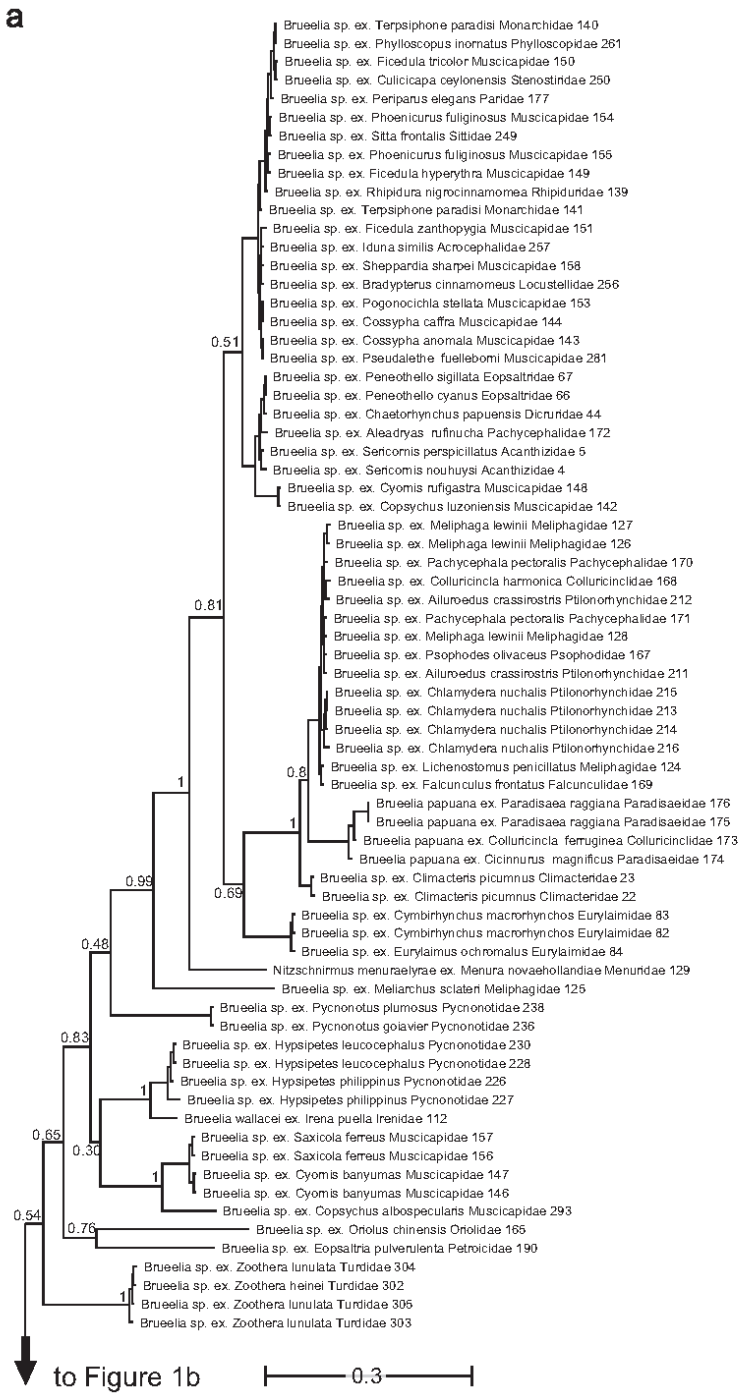
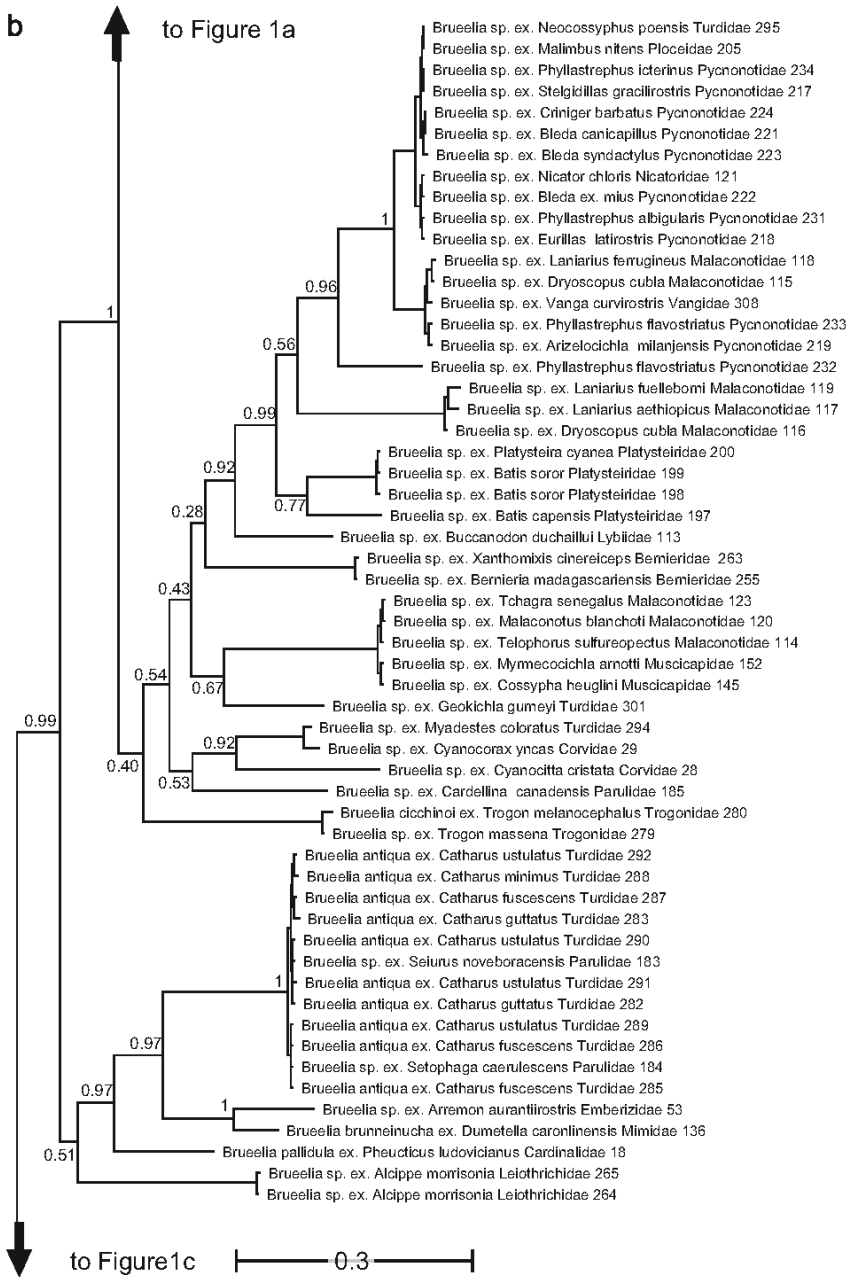


Fig. 1. Consensus tree from Bayesian analysis of combined COI and EF-1α sequences for *Brueelia*-complex species and outgroup taxa. Branches proportional to substitutions per site for the consensus tree (scale indicated). Numbers associated with nodes are posterior probabilities for the clade from a 10 million generation MCMC analysis, sampled every 1000 generations and excluding the first 1 million generations as burn-in (values < 0.5, and values associated with short terminal branches not shown here; all support values > 0.5 are shown on Fig. 2). Numbers after taxonomic names refer to Supplemental Table 1. Louse taxonomy follows the classification of Price et al. [9] and subsequent publications. Host taxonomy follows Clements et al. [21] and Dickinson et al. [22]: host genus, species, and family are all indicated. Tree partitioned into six portions (a-f).



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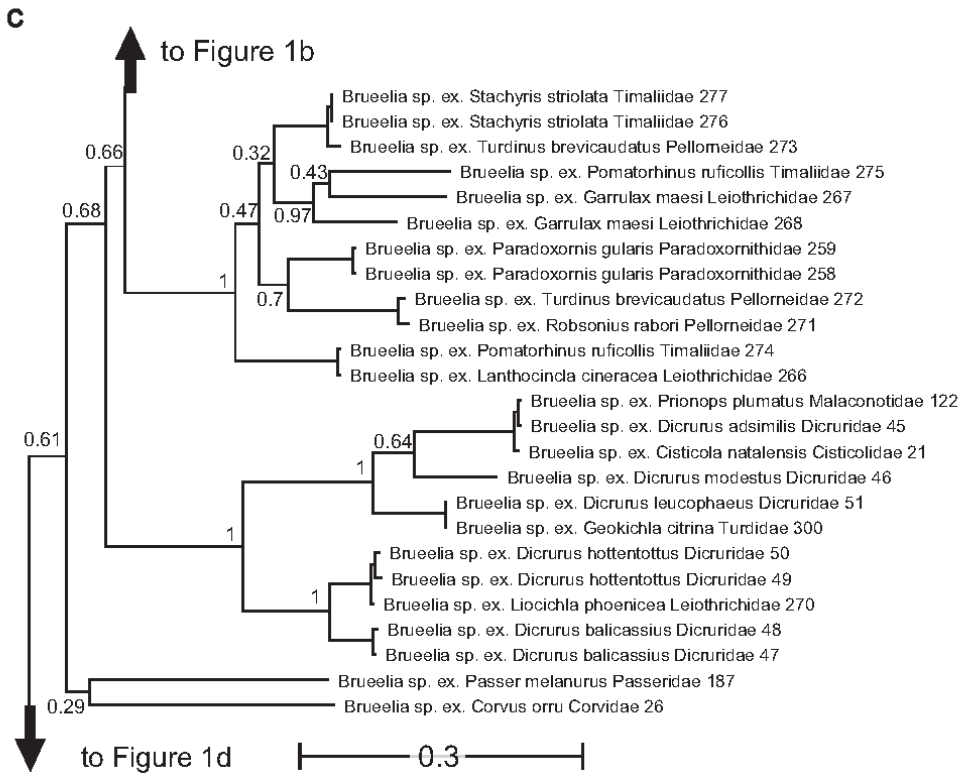

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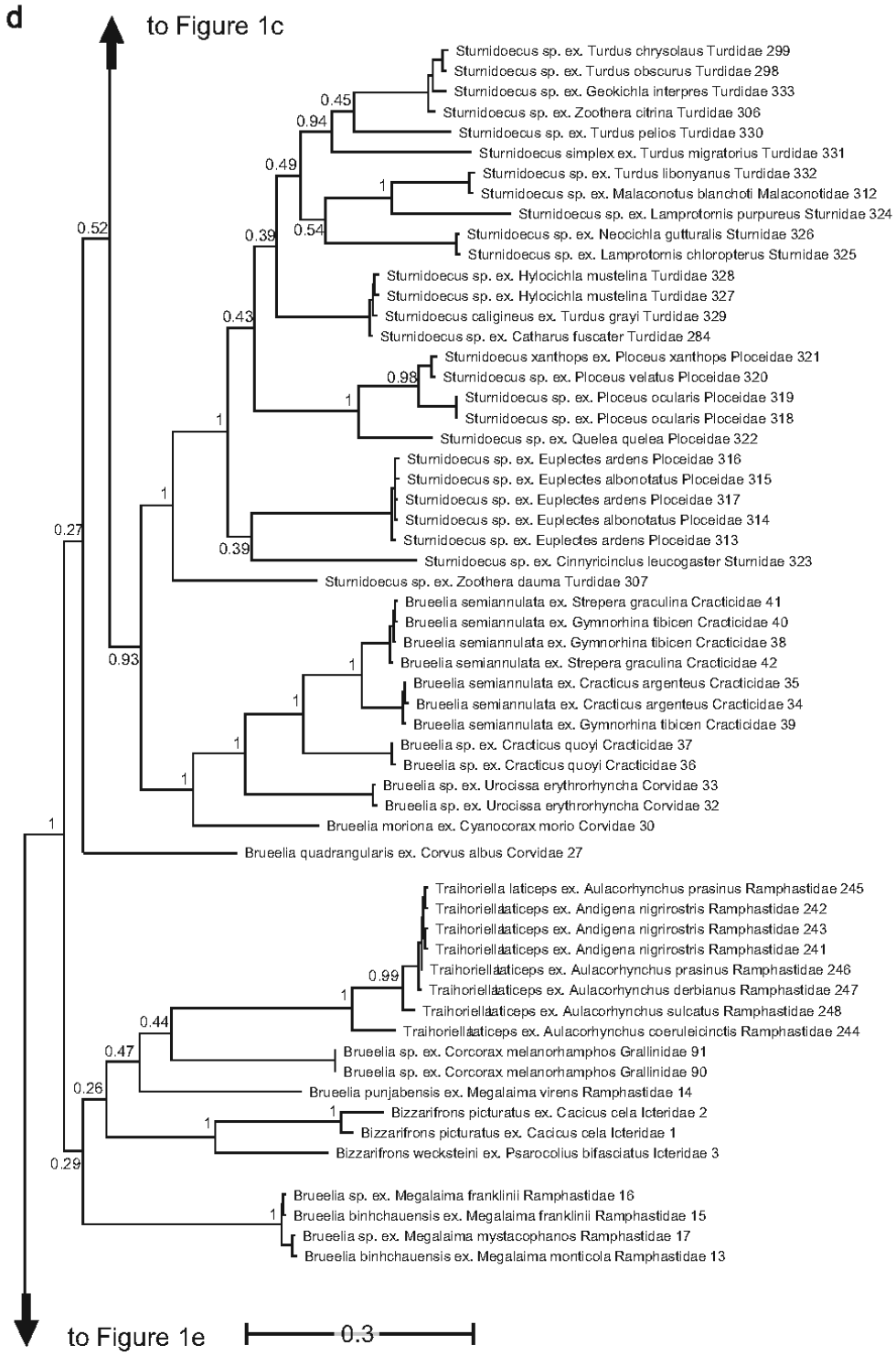
the Illinois Natural History Survey Insect Collection (INHS), Price Institute for Parasite Research at the University of Utah (PIPeR), and Field Museum of Natural History (FMNH) (Supplemental Table 1).

Portions of one mitochondrial (COI) and one nuclear gene (EF-1α) were selected because these genes have successfully resolved phylogenies of closely related groups of parasitic lice and more distantly related "bark lice" [3,10–13]. We used PCR to amplify and sequence portions of the mitochondrial cytochrome oxidase I (COI; 379 bp) and the nuclear gene elongation factor 1a (EF1 α; 347 bp) using published amplification and sequencing protocols [12,13]. Purified PCR products were cycle sequenced using ABI Big Dye (Applied Biosystems, Foster City, California) and run on an ABI Prism 3730 DNA sequencer (Applied Biosystems). Raw sequence data were trimmed, edited, and reconciled using Sequencher 5.0.1 (Genecodes CO., Ann Arbor, Michigan) or Geneious (version 7.0.3, Biomatters LTD). Both genes are protein coding and therefore we were able to easily align them by eye according to codons. These aligned gene sequences were then concatenated for phylogenetic analysis.

1.3. *Phylogenetic analyses*

The final sequence alignment was analyzed using PartitionFinder (v1.1.1; [14,15]), an open source python script that selects the best-fit partitioning schemes and models of molecular evolution for phylogenetic analysis. We tested whether the two genes (COI, EF1 α) should be analyzed together under the same model and parameters or as two separate partitions. We tested only these two partitions because separating each of these genes by codon would only provide 100 bps for each partition, a very small amount of sequence for estimating parameters and would likely result in over-parameterization. The PartitionFinder analysis found that a single partition and GTR+I+G model of molecular evolution best fit the data, using both AICc and BIC criterion. Using these parameters,



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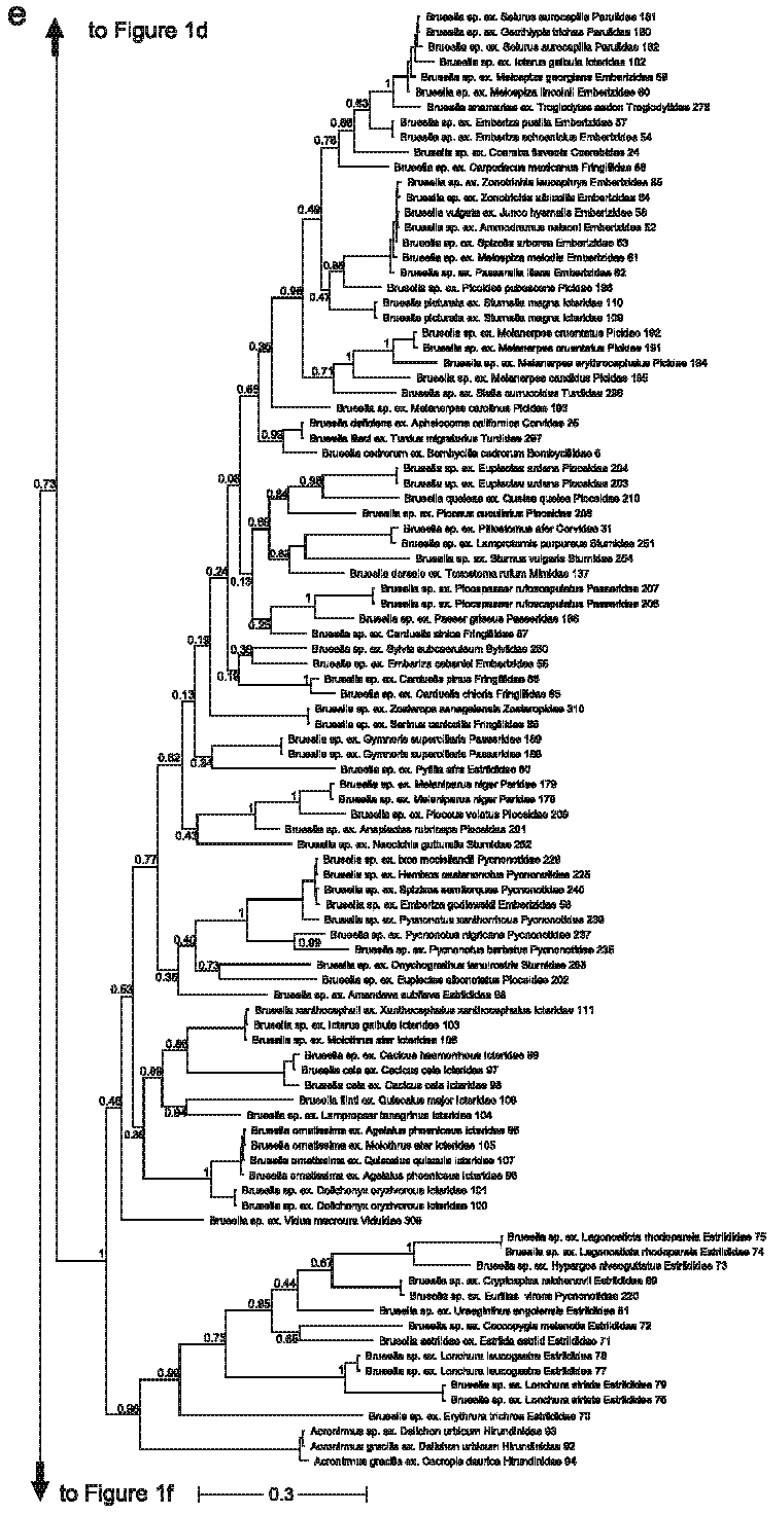

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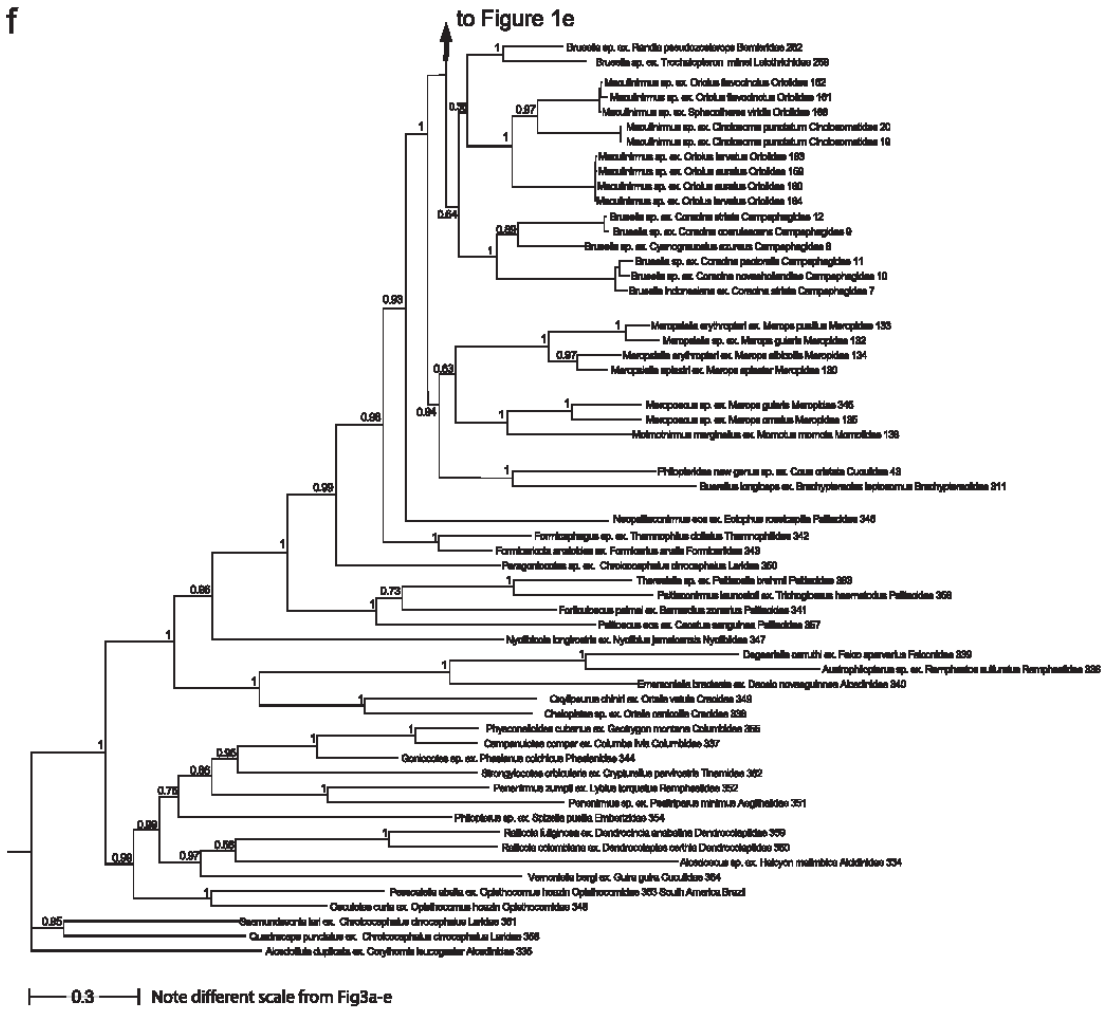

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which were estimated as part of the analysis, and a flat Dirichlet prior for state frequencies, we ran a Bayesian analysis in MrBayes 3.2.2 [16–18] for 10,000,000 generations. Each Bayesian analysis included two parallel runs, each with four Markov chains, to ensure that our analyses were not stuck at local optima [19]. Markov chains were sampled every 500 generations, yielding 20,000 parameter point-estimates. We used these 20,000 point-estimates minus the burn-in generations (500 point-estimates, 250,000 generations) to create a 50% majority-rule consensus tree and calculated Bayesian posterior probabilities to assess nodal support. We rooted the Bayesian tree using a nested set of sister taxa within the family Philopteridae [2,12,13,20].

A consensus tree from the Bayesian analysis of combined COI and EF-1α sequences for *Brueelia*-complex is shown in Fig. 1. A cladogram of the consensus tree from the Bayesian analysis is shown in Fig. 2.

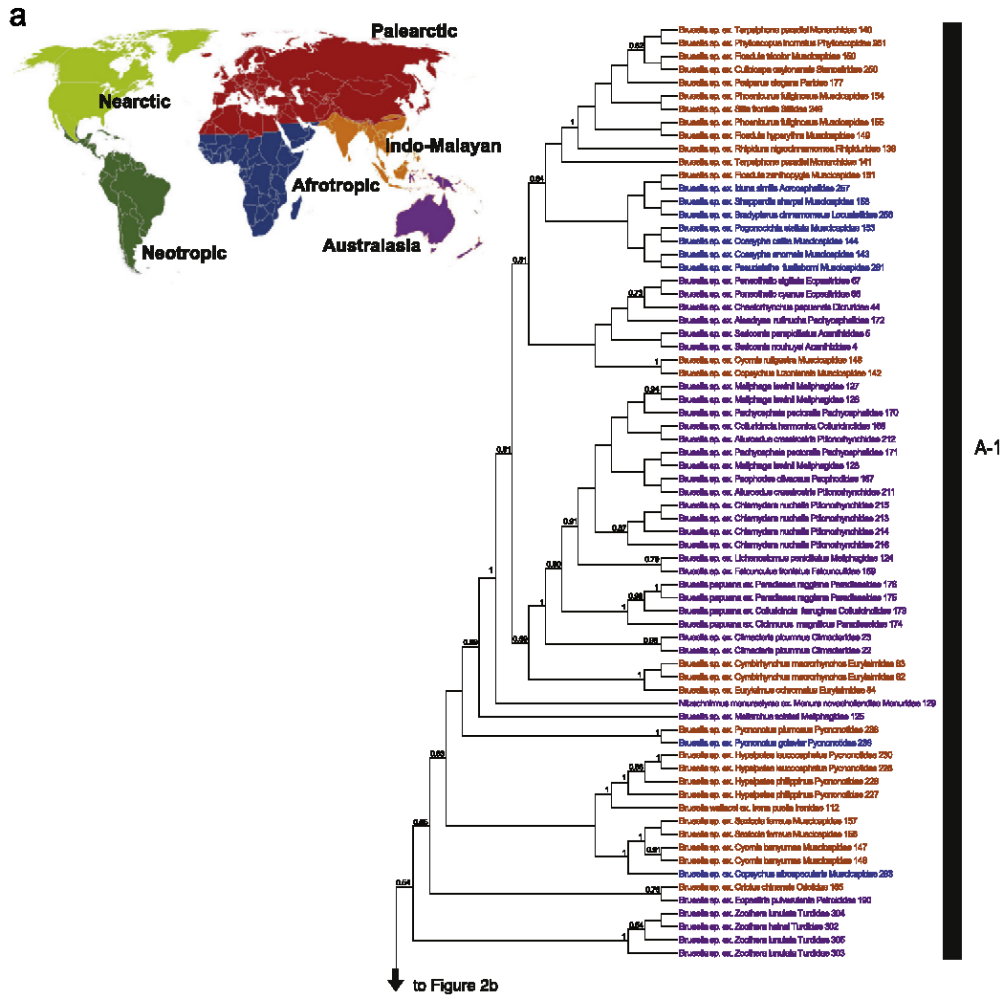

Fig. 2. Cladogram of the consensus tree from a Bayesian analysis of combined COI and EF-1α sequences for *Brueelia*-complex species and outgroup taxa. Numbers associated with nodes are posterior probabilities calculated from 10 million MCMC generations sampled every 1000 and excluding the first 1 million generations as burn-in (values < 0.5 not shown). Taxa colored to indicate geographic origin as indicated in map in 2a. Conventions as in Fig. 1. Tree partitioned into five portions (a–e).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.10.022. Specifically, Supplemental Table 1, which is a list of studied specimens, their voucher numbers, host associations, geographic origin, and GenBank accession numbers.

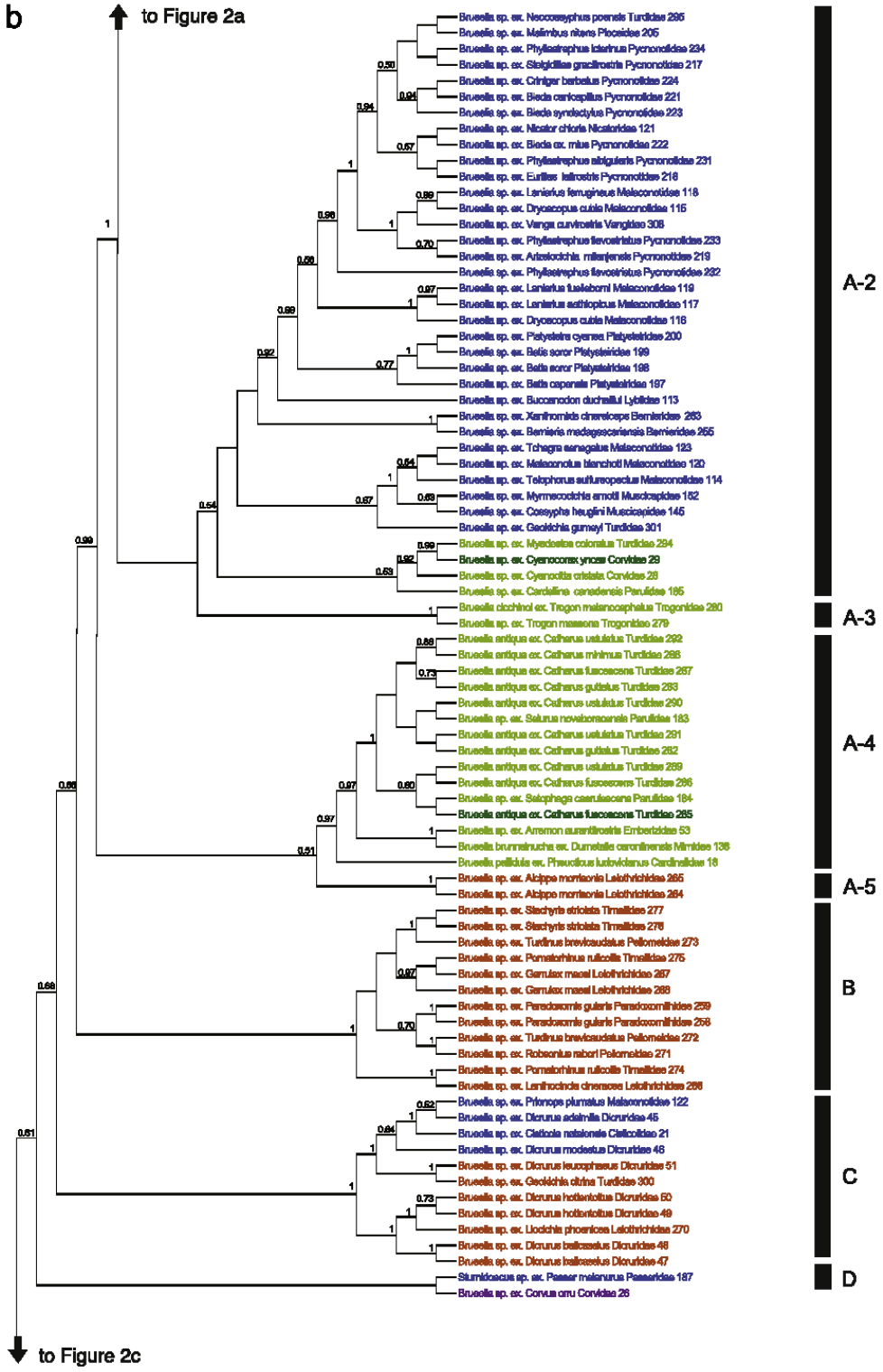

Fig. 2. (*continued*)

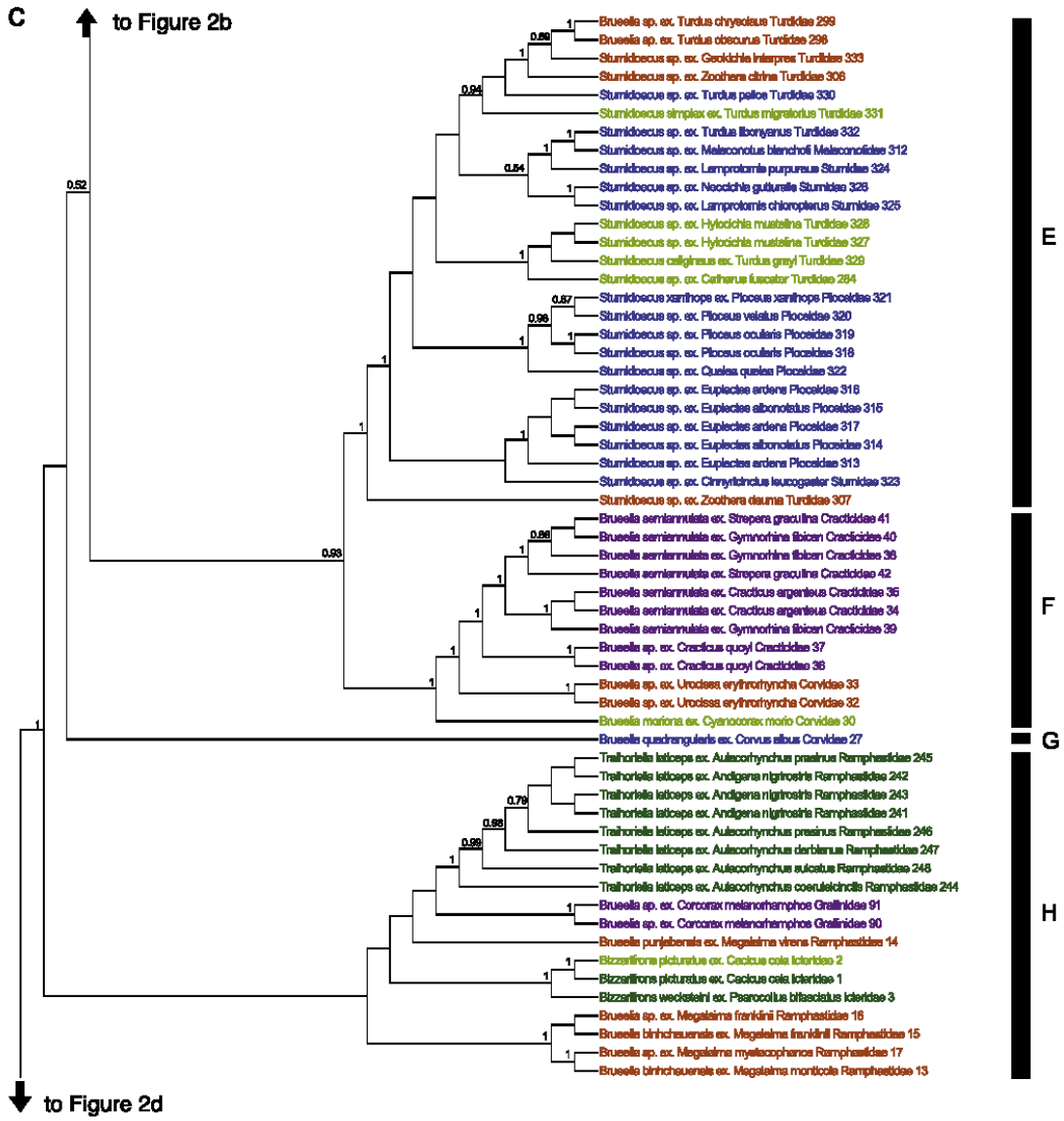

Fig. 2. (*continued*)

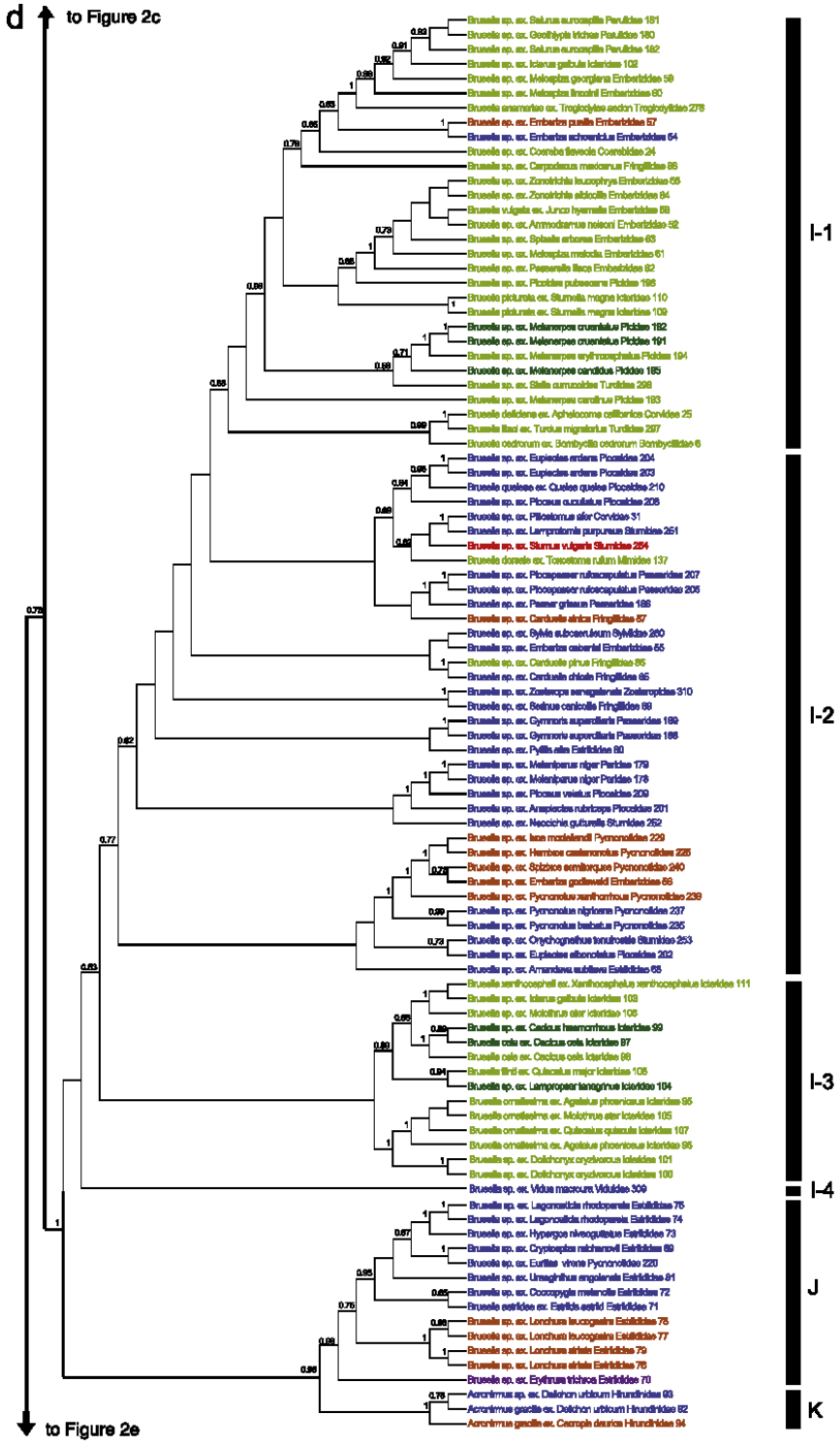

Fig. 2. (*continued*)

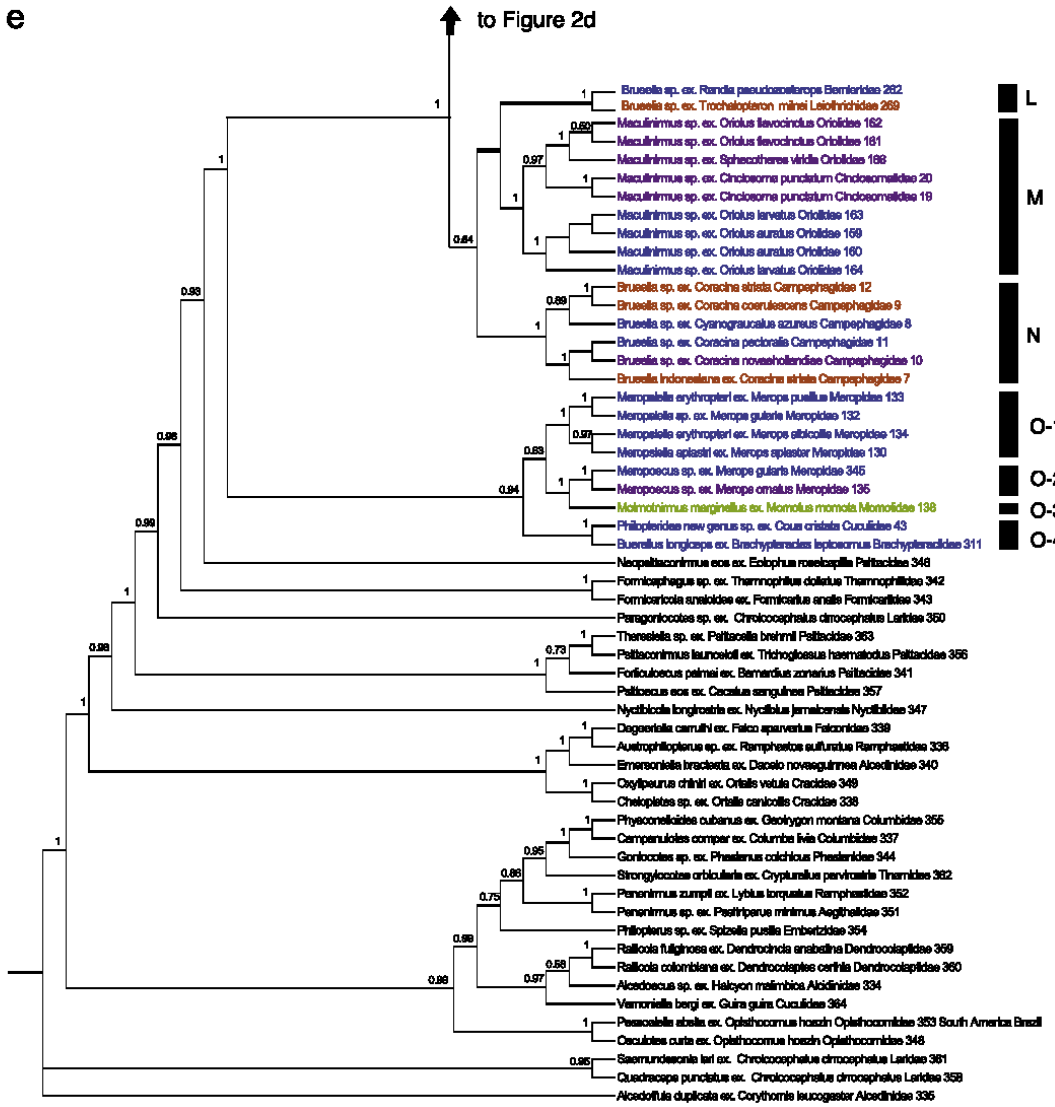

Fig. 2. (*continued*)

References

[1] S.E. Bush, J.D. Weckstein, D.R. Gustafsson, J. Allen, E. DiBlasi, S.M. Shreve, R. Boldt, H.R. Skeen, K.P. Johnson, Unlocking the black box of feather louse diversity: a molecular phylogeny of the hyper-diverse genus *Brueelia*, Mol. Phylogenet. Evol. (2015), in press.

[2] R.H. Cruickshank, K.P. Johnson, V.S. Smith, R.J. Adams, D.H. Clayton, R.D.M. Page, Phylogenetic analysis of partial sequences of elongation factor 1*a* identifies major groups of lice (Insecta: Phthiraptera), Mol. Phylogenet. Evol. 19 (2001) 202–215.

[3] V.S. Smith, T. Ford, K.P. Johnson, P.C.D. Johnson, K. Yoshizawa, J.E. Light, Multiple lineages of lice pass through the K–Pg boundary, Biol. Lett. 7 (2011) 782–785.

[4] D.H. Clayton, D.M. Drown, Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera), J. Parasitol. 87 (2001) 1291–1300.

[5] C. Bueter, J.D. Weckstein, K.P. Johnson, J.M. Bates, C.E. Gordon, Comparative phylogenetic histories of two louse genera found on *Catharus* thrushes and other birds, J. Parasitol. 95 (2009) 295–307.
[6] M.P. Valim, J.D. Weckstein, Two new species of *Brueelia* Kéler, 1936 (Ischnocera, Philopteridae) parasitic on Neotropical trogons (Aves, Trogoniformes), ZooKeys 128 (2011) 1–13.
[7] K.P. Johnson, R.J. Adams, D.H. Clayton, Molecular systematics of Goniodidae (Insecta: Phthiraptera), J. Parasitol. 87 (2001) 862–869.
[8] R.L. Palma, Slide-mounting of lice: a detailed description of the Canada balsam technique, N. Z. Entomol. 6 (1978) 432–436.
[9] R.D. Price, R.A. Hellenthal, R.L. Palma, K.P. Johnson, D.H. Clayton, The Chewing Lice: World Checklist and Biological Overview, Illinois Natural History Survey, 2003.
[10] K.P. Johnson, J.D. Weckstein, C.C. Witt, R.C. Faucett, R.G. Moyle, The perils of using host relationships in parasite taxonomy: phylogeny of the *Degeeriella* complex, Mol. Phylogenet. Evol. 23 (2002) 150–157.
[11] K.P. Johnson, R.H. Cruickshank, R.J. Adams, V.S. Smith, R.D.M. Page, D.H. Clayton, Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera), Mol. Phylogenet. Evol. 26 (2003) 231–242.
[12] K.P. Johnson, K. Yoshizawa, V.S. Smith, Multiple origins of parasitism in lice, Proc. R. Soc. Lond. B 271 (2004) 1771–1776. http://dx.doi.org/10.1098/rspb.2004.2798.
[13] V.S. Smith, R.D.M. Page, K.P. Johnson, Data incongruence and the problem of avian louse phylogeny, Zool. Scr. 30 (2004) 239–259. http://dx.doi.org/10.1111/j.0300-3256. 2004.00149.x.
[14] R. Lanfear, B. Calcott, S.Y.W. Ho, S. Guindon, PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses, Mol. Biol. Evol. 29 (2012) 1695–1701.
[15] R. Lanfear, B. Calcott, D. Kainer, C. Mayer, A. Stamatakis, Selecting optimal partitioning schemes for phylogenomic datasets, BMC Evolut. Biol. 14 (2014) 82.
[16] J.P. Huelsenbeck, F. Ronquist, MrBayes: Bayesian inference of phylogeny, Bioinformatics 17 (2001) 754–755.
[17] F. Ronquist, J.P. Huelsenbeck, MRBAYES 3: Bayesian phylogenetic inference under mixed models, Bioinformatics 19 (2003) 1572–1574.
[18] F. Ronquist, M. Teslenko, P.V.D. Mark, D.L. Ayres, A. Darling, S.H. Hohana, B. Larget, L. Liu, M.A. Suchard, J.P. Huelsenbeck, MrBayes 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space, Syst. Biol. 61 (2012) 1–4.
[19] J.P. Huelsenbeck, J.P. Bollback, Empirical and hierarchical Bayesian estimation of ancestral states, Sys. Biol. 50 (2001) 351–366.
[20] K.P. Johnson, S.M. Shreve, V.S. Smith, Repeated adaptive divergence of microhabitat specialization in avian feather lice, BMC Biol. 10 (2012) 52. http://dx.doi.org/10.1186/1741-7007-10-52.
[21] J.F. Clements, T.S. Schulenberg, M.J. Iliff, D. Roberson, T.A. Fredericks, B.L. Sullivan, C.L. Wood, The eBird/Clements checklist of birds of the world: Version 6.8. Downloaded from ⟨http://www.birds.cornell.edu/clementschecklist/download/⟩, 2014.

APPENDIX E

GALÁPAGOS MOCKINGBIRDS ARE TOLERANT HOSTS OF INTRODUCED PARASITES THAT THREATEN DARWIN'S FINCHES

Knutie S.A., J.P. Owen, S.M., McNew, A.W. Bartlow, E. Arriero, J.M. Herman, E. DiBlasi, M.Thompson, J.A.H. Koop, and D.H. Clayton. 2016. Galapagos mockingbirds are tolerant hosts of introduced parasites that threaten Darwin's finches. Ecology 97(4): 940-950. Reprinted with permission from *Ecology.*

Ecology, 97(4), 2016, pp. 940–950

Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches

Sarah A. Knutie,[1,4,6] Jeb P. Owen,[2] Sabrina M. McNew,[1] Andrew W. Bartlow,[1] Elena Arriero,[3] Jordan M. Herman,[1] Emily DiBlasi,[1] Michael Thompson,[1] Jennifer A. H. Koop,[1,5] and Dale H. Clayton[1]

[1]*Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA*
[2]*Department of Entomology, Washington State University, Pullman, Washington 99164, USA*
[3]*Department of Zoology and Physical Anthropology, Complutense University of Madrid, E-28040, Madrid, Spain*

Abstract. Introduced parasites threaten native host species that lack effective defenses. Such parasites increase the risk of extinction, particularly in small host populations like those on islands. If some host species are tolerant to introduced parasites, this could amplify the risk of the parasite to vulnerable host species. Recently, the introduced parasitic nest fly *Philornis downsi* has been implicated in the decline of Darwin's finch populations in the Galápagos Islands. In some years, 100% of finch nests fail due to *P. downsi*; however, other common host species nesting near Darwin's finches, such as the endemic Galápagos mockingbird (*Mimus parvulus*), appear to be less affected by *P. downsi*. We compared effects of *P. downsi* on mockingbirds and medium ground finches (*Geospiza fortis*) on Santa Cruz Island in the Galápagos. We experimentally manipulated the abundance of *P. downsi* in nests of mockingbirds and finches to measure the direct effect of the parasite on the reproductive success of each species of host. We also compared immunological and behavioral responses by each species of host to the fly. Although nests of the two host species had similar parasite densities, flies decreased the fitness of finches but not mockingbirds. Neither host species had a significant antibody-mediated immune response to *P. downsi*. Moreover, finches showed no significant increase in begging, parental provisioning, or plasma glucose levels in response to the flies. In contrast, parasitized mockingbird nestlings begged more than nonparasitized mockingbird nestlings. Greater begging was correlated with increased parental provisioning behavior, which appeared to compensate for parasite damage. The results of our study suggest that finches are negatively affected by *P. downsi* because they do not have such behavioral mechanisms for energy compensation. In contrast, mockingbirds are capable of compensation, making them tolerant hosts, and a possible indirect threat to Darwin's finches.

Key words: *Galápagos Islands:* Geospiza fortis; *host defense:* Mimus parvulus; *nest parasite:* Philornis downsi; *tolerance.*

Introduction

Introduced parasites can threaten native host populations that lack effective defenses (Daszak et al. 2000, Keesing et al. 2010). Not all hosts are vulnerable to introduced parasites, however. The fitness of some host species is clearly reduced, while the fitness of other hosts is relatively unaffected. "Unaffected" hosts may alleviate parasite damage with defense mechanisms that can include both resistance and tolerance. These two forms of defense are important to distinguish, because resistant hosts lower parasite populations, whereas tolerant hosts do not negatively affect parasite populations. Therefore, tolerant hosts provide a more stable resource for the introduced parasite (Schmid-Hempel 2011). By supporting the parasite population, tolerant hosts sustain, or increase, the "force of infection" for vulnerable host populations, defined as the fraction of the susceptible host population that the infected hosts can infect per unit of time (Anderson and May 1991, Hudson et al. 2002). For example, the introduction of parapoxvirus to Great Britain by tolerant, nonnative grey squirrel hosts has been implicated in the decline of native red squirrels (Tompkins et al. 2003). Tolerant hosts, such as the grey squirrel, maintain high levels of the parasite in the environment, while more vulnerable host species decline (Nokes 1992). For this reason, tolerant hosts can represent an indirect threat to populations of more vulnerable host species (Daszak et al. 2001, McCallum 2012).

Small island populations are particularly vulnerable to the effects of introduced parasites (Wikelski et al. 2004, Atkinson and Lapointe 2009). A classic example involves the historical introduction of avian malarial parasites and their mosquito vectors to the Hawaiian Islands. This

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[4]Present address: Department of Integrative Biology, University of South Florida, Tampa, Florida 33620, USA.

[5]Present address: Biology Department, University of Massachusetts-Dartmouth, Dartmouth, Massachusetts 02747, USA.

[6]E-mail: saknutie@gmail.com

introduction is thought to be partly responsible for the extinction of 17 endemic honeycreeper species (Atkinson and Lapointe 2009). Some species of honeycreepers have been relatively unaffected by introduced malarial parasites, however. Experiments with captive birds suggest that the amakihi honeycreeper (*Hemignathus virens virens*) is tolerant of the malarial parasite, and that this species of honeycreeper may therefore be a reservoir host that helps maintain the parasite in the local environment (Atkinson et al. 2000). In other words, the amakihi may be a host that essentially amplifies the negative effect of the malarial parasite on more vulnerable and declining honeycreeper species (Atkinson and Lapointe 2009).

The concept of host tolerance has seldom been tested directly under natural conditions (Read et al. 2008, Råberg et al. 2009, Svensson and Råberg 2010, Medzhitov et al. 2012). Testing for tolerant hosts requires comparing the fitness of different host genotypes or species under similar environmental conditions. Such studies are challenging because the most rigorous method for assessing the relative effects of parasites is to experimentally manipulate parasite abundance, which can be very difficult under natural conditions (McCallum and Dobson 1995).

Introduced parasites have colonized the Galápagos Islands of Ecuador in recent decades, threatening endemic birds as well as other groups of animals and plants (Wikelski et al. 2004). A notorious example of an introduced parasite is the nest fly *Philornis downsi*, which has been implicated in the decline of critically endangered species of Darwin's finches, such as the mangrove finch (*Camarhynchus heliobates*) (O'Connor et al. 2009, Fessl et al. 2010). Adult flies, which are not parasitic, lay their eggs in the nests of finches and other land birds in the Galápagos. Once the eggs hatch, fly larvae feed on the blood of nestlings and adult females as they brood the nestlings. Several studies have shown that *P. downsi* reduces the reproductive success of the medium ground finch (*Geospiza fortis*) and other species of Darwin's finches (reviewed in Koop et al. 2011). In some years, 100% of finch nests fail to produce fledglings due to *P. downsi* (Koop et al. 2011, 2013*a*, O'Connor et al. 2013). Moreover, Kleindorfer et al. (2014) recently suggested that *Philornis*-related mortality in finches has increased over the past decade, with nestling age at mortality decreasing due to *P. downsi* infestations earlier in the nestling developmental period.

Other host species nesting near Darwin's finches, such as the endemic Galápagos mockingbird (*Mimus parvulus*), may be less affected by *P. downsi* infestation. Anecdotal observations suggest that mockingbird nestlings often do not die when parasitized by *P. downsi* (personal observation). If so, then mockingbirds could be tolerant hosts that effectively amplify the force of infection for vulnerable hosts, such as Darwin's finches. The goal of the current study was to test this hypothesis by comparing the effects of *P. downsi* on the fitness of mockingbirds and medium ground finches at the same time and location. We measured the effects of parasites on nestling mockingbirds and finches over two field seasons, then compared the reaction norms of host nestling survival and parasite density between the two host species.

During the first season, we compared the effect of *P. downsi* on the size and fledging success of nestling mockingbirds and finches. We predicted a significant negative effect of *P. downsi* on the size and fledging success of finches, but not mockingbirds. We also tested for evidence of nestling immune responses that combat *P. downsi* in mockingbirds and finches.

During the second field season, we repeated these comparisons, and also explored possible mechanisms of tolerance, such as the rapid replacement of blood lost to the parasite. To test this possibility, we compared the effect of *P. downsi* on the hemoglobin of finch and mockingbird nestlings. Another possible mechanism of tolerance is increased parental care of parasitized nestlings (Tripet and Richner 1997, Hurtrez-Bousses et al. 1998, Tripet et al. 2002). Parents of such nestlings might increase their feeding rates to compensate for energy lost to parasites. One cue known to lead to increased feeding rates is increased begging by parasitized nestlings (Bengtsson and Rydén 1983, Christe et al. 1996). In some cases, however, parasitized nestlings appear to be too weak to solicit more food by begging. We compared parental and nestling behavior, as well as energy levels (via glucose), of finches and mockingbirds with and without parasites in the nest. We predicted that mockingbird nestlings in parasitized nests would beg more than nestlings in unparasitized nests, and that parents in parasitized nests would provision nestlings more than parents in nonparasitized nests. We further predicted that increased begging would lead to increased glucose levels in parasitized nestlings. In contrast, finch parental provisioning does not differ in response to parasitism (Koop et al. 2013*a*). Thus, we predicted that parasitized and nonparasitized finch nestling begging and glucose levels would decrease or not differ because parasitized nestlings are too weak to increase begging.

Methods

Study system

The study was conducted January–April in both 2012 and 2013 on the island of Santa Cruz in the Galápagos Archipelago. Our 3 × 4 km field site, known as El Garrapatero, is located in the arid coastal zone; it is located approximately 10 km east of the main town of Puerto Ayora. Galápagos mockingbirds and medium ground finches are abundant at the site. Mockingbirds build open, cup-shaped nests, which are made of *Acacia* thorns (bottom layer), moss (middle layer), and coarse grasses (top layer/nest liner) and primarily found in giant prickly pear cacti (*Opuntia echios gigantea*) or *Acacia* trees. Mockingbird clutch size ranges from 1 to 5 eggs, and females incubate the eggs for about 15 d. Nestlings

spend an average of 15 d in the nest, and both the adult females and males feed them. Mockingbirds feed their nestlings by placing food items in the nestling's open mouth rather than by regurgitating food, as in the case of finches (see below). Mockingbirds usually lay a single clutch of eggs per breeding season; however, they have been reported to re-nest in a new location when the first clutch fails, sometimes due to delayed rains. Mockingbirds normally do not reuse the same nest.

Finches build dome-shaped nests, which are made of coarse grasses (exterior layer) and fine grasses (nest liner that contacts the nestlings in the cup of the nest) and primarily found in giant prickly pear cacti or *Acacia* trees (Grant 1999). Their clutch sizes range from 2 to 5 eggs, with females incubating the eggs for 10–14 d. Nestlings spend an average of 12 d in the nest, and adult females and males both feed the nestlings by regurgitating food into the nestling's throat. In years of favorable weather and food resources, medium ground finches may lay additional clutches of eggs over the course of a single breeding season; however, like mockingbirds, they do not reuse the same nest (Grant 1999).

Experimental manipulation of parasites

To quantify the effect of *P. downsi* on host fitness, experimental nests were fumigated with a 1% aqueous permethrin solution (Permectrin™ II). Control nests were sham-fumigated with water. Permethrin, which has been used in previous studies (Fessl et al. 2006, Koop et al. 2013*a*,*b*, O'Connor et al. 2013), is harmless to birds, including newly hatched nestlings. Nests were sprayed soon after the first nestling hatched, then again 4–6 d later. Nest contents (nestlings, unhatched eggs, and the nest liner) were removed during the spraying process. Nest contents were then returned to the nest once it had dried (<10 min). Parents quickly returned to the nest following treatment, with no cases of nest abandonment due to treatment observed for either bird species.

Nestling size and fledging success

In 2012, each nestling was weighed twice: within 24 h of hatching, then again at 9–10 d of age. In 2013, each nestling was weighed three times: within 24 h of hatching, then at one-third and again at two-thirds of the nestling developmental period. Thus, the second weighing occurred when finch nestlings were 4–5 d old, and mockingbird nestlings were 5–6 d old. The third weighing occurred when finch nestlings were 8–9 d old, and mockingbird nestlings were 10–11 d old.

Each nestling was banded with a unique darvic color band combination. Successful fledging was confirmed by identifying birds once they left the nest, as in previous studies (Koop et al. 2011, 2013*a*,*b*). After the birds in a nest had all fledged or died, the nest was collected and placed in a sealed plastic bag. The number of *P. downsi* in the nest was then quantified, as described below. Fledging success for finches from 2013 was first reported in Knutie et al. (2014).

Quantifying P. downsi

Each nest was carefully dissected within 8 h of collection and *P. downsi* larvae, pupae, and eclosed pupal cases were counted (Koop et al. 2011, 2013*a*,*b*). First instar larvae can live subcutaneously in nestlings, making them impossible to quantify reliably. Therefore, parasite abundance was the sum of counts of second and third instar larvae, pupae, and eclosed pupal cases (for both infested and uninfested nests). Parasite abundance was used to calculate parasite density, which is the number of parasites per unit of host (Bush et al. 1997). For mockingbirds and finches, density was calculated by dividing the number of parasites per nest by the total mass of nestlings for a given nest at 2/3 of the mean nestling developmental period.

Larvae and pupae were reared to the adult stage to confirm that they were *P. downsi* (Dodge and Aitken 1968). Most larvae were third instars when the nests were collected; these larvae usually pupated within 24 h. Younger larvae, which require a blood meal, died soon after they were collected from the nest and were therefore not reared to adulthood. The length and width of pupae were measured with digital calipers in mm. These measurements were then used to estimate pupal volume as a measure of individual parasite size, which is related to lifetime fitness in other Muscid flies (Schmidt and Blume 1973, Moon 1980).

Nestling hemoglobin

In 2012, blood was sampled from 9 to 10 d old nestlings. In 2013, blood was sampled from nestlings when they were at one-third and two-thirds of the nestling period. A small blood sample (<30 μL) was collected in a microcapillary tube via brachial venipuncture. Using a portion of this blood, hemoglobin concentration was quantified immediately in the field (2013 only). Hemoglobin concentration can provide an accurate estimate of ectoparasite-induced anemia (O'Brien et al. 2001, Dudaniec et al. 2006, Carleton 2008). Hemoglobin was measured with a HemoCue® HB 201+ portable analyzer, using ten microliters of whole blood per disposable microcuvette. Hemoglobin was measured in g/dL.

The remainder of each blood sample was stored on wet ice in the field. Within 6 h of collection, samples were spun at 8000 rpm for 10 min in a centrifuge. Plasma and red blood cells were stored separately in 0.5 mL vials in a −20°C freezer at the Charles Darwin Research Station. Samples were later frozen at −80°C after being transported in liquid nitrogen to the University of Utah. The samples were ultimately used for the immunological and glucose assays described below.

Nestling immunology

Enzyme-linked immunosorbent assays (ELISA) were used to detect the presence of *P. downsi*-binding antibodies in the plasma of finches and mockingbirds, with a modification of the protocol in Koop et al. (2013*a*). Ninety-six well plates were coated with 100 μL/well of *P. downsi* protein extract (capture antigen) diluted in carbonate coating buffer (0.05 mol/L, pH 9.6). Plates were incubated overnight at 4°C, then washed and coated with 200 μL/well of bovine serum albumin (BSA) blocking buffer and incubated for 30 min at room temperature on an orbital table. Between each of the following steps, plates were washed five times with a Tris-buffered saline wash solution, loaded as described, and incubated for 1 h on an orbital table at room temperature. Triplicate wells were loaded with 100 μL/well of individual host plasma (diluted 1:100 in sample buffer). Plates were then loaded with 100 μL/well of Goat-αBird-IgG (diluted 1:50,000) (Antibodies Online, Atlanta, GA, USA; ABIN351982). Finally, plates were loaded with 100 μL/well of peroxidase substrate (tetramethylbenzidine, TMB; Bethyl Laboratories, Montgomery, TX, USA) and incubated for exactly 30 min. The reaction was halted using 100 μL/well of stop solution (Bethyl Laboratories). Optical density (OD) was measured with a spectrophotometer (BioTek, Winooski, VT, USA; PowerWave HT, 450-nanometer filter).

On each plate, a positive control of pooled plasma from naturally *P. downsi* parasitized adult female finches from the 2013 field season was used in triplicate to correct for inter-plate variation (Koop et al. 2013*a*). In addition, each plate contained a non-specific binding (NSB) sample in which capture antigen and detection antibody were added, but plasma was excluded. Finally, each plate included a blank sample in which only the detection antibody was added, but plasma and capture antigen were excluded. NSB absorbance values were subtracted from the mean OD value of each sample to account for background binding of the detection antibody to the capture antigen.

Nestling glucose

Plasma glucose was measured using blood samples taken from mockingbird and finch nestlings at about the same time their behavior was quantified; see below. An Endpoint Autokit (Wako, Diagnostics, Mountain View, CA, USA) was used to measure plasma glucose for mockingbirds and finches with a modified protocol based on Guglielmo et al. (2013). The kit provided 500 mg/dL and 200 mg/dL standards. Following the manufacturer's protocol, the buffer solution and color reagent were mixed together, then refrigerated at 4°C until they were used in the assay. Three microliters of sample or standard were run primarily in duplicate, assuming sufficient sample was available, on Nunc® MicroPlate™ 96-well polystyrene plates (Sigma-Aldrich, St. Louis, MO, USA). The buffer solution was pre-warmed to 37°C, then 300 μL were added to each well. The plate was incubated at 37°C on a microplate incubator shaker (Stat Fax® 2200) for 10 min, then shaken for 10 s on low speed. Optical density (OD) was measured using a spectrophotometer (BioTek; PowerWave HT, 505-nanometer filter). Samples were corrected for intraplate variation based on the 500 mg/dL standard. From the standards, a standard curve was created ranging from 50 to 500 mg/dL. Glucose concentration (mg/dL) for each sample was calculated using the OD value of the sample (x), and the slope and intercept of the line from the standard curve ($y = 0.003x + 0.0352$).

Nestling and adult behavior

Mockingbird behavior was recorded during the 2013 field season. Because we had a limited number of nest cameras and recording devices, and because we collected behavioral data from fumigated and sham-fumigated finch nests from the same field site in 2010, we chose to concentrate on recording mockingbird data in 2013; see Koop et al. (2013*a*) for details on finch behavior. Behavior was monitored with battery-powered Sony® video camera systems. Small nest cameras (31 mm in diameter, 36 mm in length) were suspended above nests; seven-meter long cables connected the cameras to small recording devices (PV700 Hi-res DVR; StuntCams, Grand Rapids, MI, USA) hidden near the base of the tree supporting the nest. Behavior was recorded between 0600 and 1000 using haphazard subsamples of fumigated and sham-fumigated nests.

Mockingbird behavior was quantified from videos by one of the authors (M.T.) who was blind to nest identity or treatment. A similar "blind" approach was used for finch behavior (Koop et al. 2013*a*). Videos were analyzed with the software VLC media player (VideoLAN, Paris, France), except in the case of begging, which was analyzed using CowLog v.2.1 (Hänninen and Pastell 2009). A single day of video from each of two nests was paired between treatments to control for hatch date, brood size, and nestling age. There was no significant difference in brood size or nestling age between treatments.

Nestling begging was defined as one or more nestlings tilting their head back, with the neck extended and the open mouth showing (Christe et al. 1996). Begging time was calculated as the proportion of total video time. The proportion of video time with nestling agitation behavior, defined as shaking, repositioning, or jumping in the nest, was also quantified.

Adult behaviors included the proportion of time each adult spent at the nest. We were unable to distinguish female and male mockingbirds because they are not sexually dimorphic. The following adult behaviors were quantified: brooding nestlings, standing erect in the nest, standing motionless on the rim of the nest, nest sanitation, self-preening, allopreening nestlings, and provisioning (feeding) nestlings. Brooding was defined as the adult sitting on the nest in direct contact with nestlings. Nest sanitation was defined as the adult contacting or

manipulating nest material with its bill. Provisioning of nestlings was defined as the insertion of the bill into the mouths of nestlings by adults; note, however, that we were unable to determine how much food was actually delivered. Because adults often preen themselves while brooding nestlings, self-preening was analyzed separately from the other behaviors. All other behaviors were analyzed as the proportion of total time that adults were observed.

Mockingbird behaviors were quantified from a total of 41 h of video, with an average of 2.5 h of video for each of the 16 mockingbird nests (eight fumigated, eight sham-fumigated). Mockingbird nestlings in the videos ranged in age from 3 to 6 d, and brood size ranged from 1 to 5 nestlings. Finch behaviors were quantified from a total of 54 h of video, with an average of 3 h of video for each of the 18 finch nests (nine fumigated, nine sham-fumigated; Koop et al. 2013*a*). Finch nestlings in the videos ranged in age from 2 to 6 d, and brood size ranged from 1 to 5 nestlings. The data for adult finch behavior were reported separately by sex in Koop et al. (2013*a*). For our analyses these data were pooled.

Statistical analyses

Parasite abundance, density, and volume were analyzed using generalized linear models (GLM) with a negative binomial family and logit link function for abundance and a Gaussian family and identity link function for density and volume; year (2012 or 2013) and host species (mockingbird or finch) were fixed effects for all three variables and treatment (fumigated or sham-fumigated) was a fixed effect for parasite abundance.

Data for individual nestlings were analyzed with generalized linear mixed models (GLMM) using nest as a random effect and year, host species, age, and treatment as fixed effects. Fledging success was modeled with a binomial family and logit link function; year, host species, and treatment were fixed effects. Mass, immune response, hemoglobin, and glucose were modeled with a Gaussian family and identity link function; year, host species, age, and treatment were fixed effects for mass and immune response, host species, age, and treatment were fixed effects for hemoglobin, and host species and treatment were fixed effects for glucose.

For each of the GLM and GLMM analyses, we developed a set of *a priori* models that included single, additive, and interactive effects of variables that we hypothesized had biologically meaningful effects of the response variables of interest. For example, year, host species, and treatment were predicted to affect parasite abundance; therefore, we analyzed the effect of year, species, and treatment alone, and all two and three-way interactions (Appendix S1). We ranked models using Akaike's Information Criterion with adjustment for small sample size (AICc). We report AICc differences (ΔAICc) and Akaike weights (ω) to determine the strength of evidence for each model, relative to the set of candidate models (Burnham and Anderson 2002). To account for model selection uncertainty, we averaged across all models to calculate model-averaged parameter estimates ($\tilde{\beta}$) with shrinkage, as well as *z*-values and *P*-values, for each variable and their interaction(s).

Host nestling and adult behavior were compared between treatments using a chi-square test to match previously reported analyses of finch behavior from Koop et al. (2013*a*); specific behaviors were compared between treatments using Fisher's exact tests. GLMM and GLM analyses were performed in the program RStudio, version 3.1.1 (R Core Team 2014) using the lme4, MuMIn, nlme, and MASS packages. Prism® v.5.0b (GraphPad Software, Inc., La Jolla, CA, USA) was used for all other analyses and to create figures.

Results

Quantifying P. downsi

Top ranked models included the effect of treatment and host species on parasite abundance and both variables were in every model with an Akaike weight of >0.10, indicating their importance (Appendix S1: Table S2). The experimental treatment of nests with permethrin was effective at reducing parasite abundance, compared to sham-fumigated control nests for both host species in both years of the study (GLM, Treatment, $\tilde{\beta} \pm SE = -5.06 \pm 0.57$, $P < 0.0001$; Appendix S1: Table S3). Parasite abundance was significantly higher in mockingbird nests than finch nests in both years (Species, $\tilde{\beta} \pm SE = 1.11 \pm 0.39$, $P < 0.001$; Appendix S1: Table S3). However, variation in parasite density (parasites per gram of nestling) and parasite size (pupal volume) was not explained by any of the predictors that we measured in our study (Density: Species, $\tilde{\beta} \pm SE = -0.06 \pm 0.21$, $P = 0.80$; Table 1; Appendix S2: Tables S2 and S3 Size: Species, $\tilde{\beta} \pm SE = 0.01 \pm 0.04$, $P = 0.67$; Table 1; Appendix S3: Tables S2 and S3).

Nestling mass

For nestling mass, all top models included the effect of age (ω > 0.10; Appendix S4: Table S3). Nestling mass increased significantly with increasing age in both species (GLMM, Age, $\tilde{\beta} \pm SE = 1.43 \pm 0.05$, $P < 0.0001$; Appendix S4: Table S4). Top models (ω > 0.10) also included the interaction between age and species and age and treatment. As expected, mockingbirds weighed significantly more than finches (Age × Species, $\tilde{\beta} \pm SE = 1.96 \pm 0.05$, $P < 0.0001$; Appendix S4: Table S4). *P. downsi* had a significant effect on the mass of both mockingbird and finch nestlings, but only in older nestlings (Age × Treatment, $\tilde{\beta} \pm SE = 0.13 \pm 0.05$, $P = 0.01$; Appendix S4: Table S4). The body mass of older nestlings in fumigated nests was significantly greater than the body mass of older nestlings in sham fumigated nests (Appendix S4: Table S1).

TABLE 1. Comparison of *Philornis downsi* number and size, and host fledging success in mockingbirds and finches in fumigated (F) and sham-fumigated (SF) nests. Parasite density is the number of parasites per gram of host.

	Galápagos mockingbird				Medium ground finch			
	2012		2013		2012		2013	
	F	SF	F	SF	F	SF	F	SF
Mean ± SE parasite density (Number of nests)	—	1.00 ± 0.29 (14)	—	1.06 ± 0.47 (13)	—	1.49 ± 0.52 (8)	—	1.06 ± 0.42 (12)
Mean ± SE pupal volume, mm^3 (Number of nests)	—	115.20 ± 6.57 (13)	—	120.10 ± 8.52 (14)	—	108.30 ± 6.93 (9)	—	117.50 ± 5.70 (9)
Fledglings, %	76.5	77.8	70.0	66.7	86.0	34.2	83.3	53.7
(Number of nestlings)	(51)	(54)	(47)	(54)	(43)	(38)	(60)	(54)
Nests with at least one fledgling, %	87.5	87.5	76.5	76.5	91.7	50.0	95.0	64.7
(Number of nests)	(16)	(16)	(17)	(17)	(12)	(12)	(20)	(17)

Fledging success

For fledging success, all top models ($\omega > 0.10$) included the effect of an interaction between species and treatment (Appendix S5: Table S1). Treatment had a significant effect on the fledging success of finches, but not mockingbirds (GLMM, Species × Treatment, $\tilde{\beta} \pm SE = -4.33 \pm 1.14$, $P < 0.001$; Tables 1; Fig. 1; Appendix S5: Table S2). That is, *P. downsi* reduced fledging success of finches but had no effect on mockingbirds. Parasite density was a significant predictor of fledging success in finches, but not mockingbirds (GLM, Density × Species, $\chi^2 = 16.24$, df = 1, $P < 0.0001$; Fig. 1).

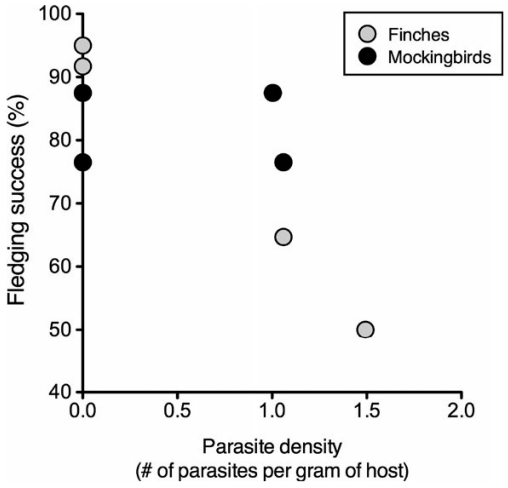

FIG. 1. Reaction norms for fledging success in finches and mockingbirds across different *Philornis downsi* densities. Each point represents percentage of fledging success, or the percentage of hatchlings that successfully left the nest, plotted against mean parasite density within a treatment and year. Mockingbirds are more tolerant to *P. downsi*; parasite density is not a significant predictor of fledging success. In contrast, parasite density is a significant predictor of fledging success in finches.

Nestling immunology

Philornis downsi was not a significant predictor of antibody levels because treatment was not included in any of the top models ($\omega > 0.10$; Appendix S6: Table S3). Antibody levels within each species were low (Appendix S6: Table S1). However, the top models included an effect of year, species, and their interaction, on antibody levels (Appendix S6: Table S3). For finches, antibody levels were significantly higher in 2012 than 2013 (GLMM, Species × Year, $\tilde{\beta} \pm SE = 0.16 \pm 0.04$, $P < 0.001$; Appendix S6: Table S4). Finches also had significantly higher antibody levels than mockingbirds (Species, $\tilde{\beta} \pm SE = -320.70 \pm 88.40$, $P < 0.001$; Appendix S6: Table S4).

Nestling hemoglobin

Top models included the effect of age and an age × treatment interaction on nestling hemoglobin levels ($\omega > 0.10$; Appendix S7: Table S1). Mockingbird and finch nestling hemoglobin increased significantly with age (GLMM, Age, $\tilde{\beta} \pm SE = 0.33 \pm 0.04$, $P < 0.0001$; Appendix S7: Table S3). There was a significant effect of *P. downsi* on the hemoglobin of older nestlings in both species of hosts (Age × Treatment, $\tilde{\beta} \pm SE = 0.30 \pm 0.06$, $P < 0.0001$; Appendix S7: Table S3). Older mockingbird nestlings in fumigated nests had 40% more hemoglobin than similar aged nestlings in sham-fumigated nests (Fig. 2; Appendix S7: Table S1). Older finch nestlings in fumigated nests had 14% more hemoglobin than similar aged nestlings in sham-fumigated nests (Fig. 2; Appendix S7: Table S1).

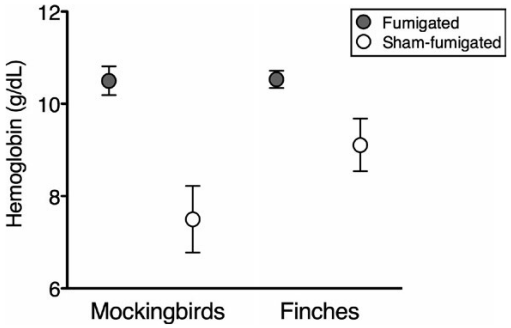

FIG. 2. Mean (±SE) hemoglobin in nestlings from fumigated and sham-fumigated nests. Nestlings in fumigated nests had significantly higher hemoglobin levels than nestlings in sham-fumigated nests for both species of birds.

Nestling glucose

The top model included the effect of a species by treatment interaction on nestling glucose levels and carried nearly all of the Akaike weight ($\omega = 0.99$; Appendix S8: Table S2). Mockingbird nestlings in fumigated nests had significantly lower plasma glucose levels than mockingbird nestlings in sham-fumigated nests (GLMM, Species × Treatment, $\hat{\beta} \pm SE = -31.10 \pm 15.79$, $P = 0.05$; Fig. 3; Appendix S8: Table S3). In contrast, parasite abundance was not a significant predictor of glucose concentration in finch nestlings (Fig. 3).

Nestling and adult behavior

Agitation behavior did not differ significantly between mockingbird nestlings from fumigated and sham-fumigated nests (Table 2). However, mockingbird nestlings from sham-fumigated nests spent significantly more time begging than nestlings from fumigated nests (Table 2; Fig. 4A).

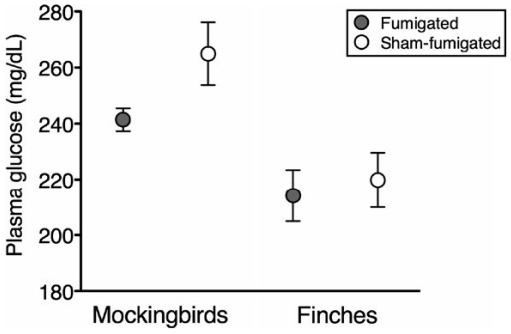

FIG. 3. Mean (±SE) plasma glucose levels in mockingbird and finch nestlings from fumigated and sham-fumigated nests. Mockingbird nestlings in sham-fumigated nests had higher glucose levels than nestlings in fumigated nests. In contrast, glucose levels did not differ significantly between treatments for finches.

The amount of time adult mockingbirds spent at fumigated and sham-fumigated nests did not differ significantly (Table 2). There was no significant effect of treatment on self-preening by adult mockingbirds ($W = -3.00$, $P = 0.81$); note, however, that the birds spent <0.01% of their time engaged in self-preening at the nest. We did not collect data on self-preening or other behaviors in birds away from the nest.

Adult mockingbirds differed significantly in the time they devoted to other (mutually exclusive) behaviors at fumigated vs. sham-fumigated nests (Chi-square test: $\chi^2 = 18.90$, df = 5, $P < 0.001$). The largest difference was in the time adult mockingbirds spent brooding nestlings, with adults at fumigated nests spending significantly more time brooding than adults at sham-fumigated nests (Table 2). When mockingbirds from sham-fumigated nests were not brooding, they were either standing erect in the nest, or standing erect on the rim of the nest (Table 2); however, these behaviors did not differ significantly between treatments. Adults on the rim of nests occasionally probed nest material (nest sanitation behavior), allopreened nestlings, or provisioned nestlings.

Adult mockingbirds spent very little time in nest sanitation behavior, and there was no significant effect of treatment on this behavior (Table 2). When adult mockingbirds from sham-fumigated nests were not brooding, but were still present at the nest, they spent most of their time allopreening nestlings while standing on the rim of the nest; however, there was no significant difference in allopreening between treatments (Table 2).

Adults from fumigated nests spent significantly less time in provisioning behavior, compared to adults from sham-fumigated nests (Table 2; Fig. 4A). The amount of time parents spent in provisioning behavior was positively correlated with the amount of time nestlings spent begging (Spearman rank correlation: $r_s = 0.52$, $P = 0.04$).

In contrast to mockingbirds, nestlings in fumigated finch nests did not beg more than nestlings in sham-fumigated finch nests (Table 2; Fig. 4B). The time adult finches spent at fumigated and sham-fumigated nests did not differ significantly (Table 2). The time parents spent in provisioning behavior was correlated with nestling begging time (Spearman rank correlation: $r_s = 0.81$, $P < 0.0001$). However, adult finches did not differ significantly in the amount of time they spent in provisioning behavior at fumigated and sham-fumigated nests (Table 2; Fig. 4B). See Koop et al. (2013*a*) for further details of finch behavior.

DISCUSSION

The effect of *P. downsi* varied considerably between finches and mockingbirds within the same years and location. *P. downsi* reduced the fledging success of Darwin's finch nestlings; however, despite a similar density of flies in mockingbird nests, *P. downsi* had no significant effect on mockingbird fledging success. Thus,

TABLE 2. Behavior of nestling and adult mockingbirds and finches in fumigated and sham-fumigated nests. For mockingbirds, each treatment contained eight nests; for finches, each treatment contained nine nests. For nestlings, values are the mean ± SE percent time out of total video time. For adults, most values are the mean ± SE percent time out of total attendance time at nest. Attendance time is the total time at the nest out of total video time. Wilcoxon signed rank tests were used to compare treatments within each behavior. See Koop et al. (2013*a*) further details of finch behavior.

	Fumigated (%)	Sham-fumigated (%)	*W* statistic	*P*-value
Galápagos mockingbird				
Nestlings				
Begging	3.12 ± 0.74	5.78 ± 0.98	−32.00	0.02
Agitation	10.38 ± 2.16	12.86 ± 3.07	−6.00	0.74
Adults				
Attendance at nest	54.59 ± 5.00	50.45 ± 6.78	10.00	0.55
Brooding	70.35 ± 5.05	41.12 ± 8.11	32.00	0.02
Standing erect in nest	2.27 ± 0.57	9.03 ± 7.46	8.00	0.64
Standing on rim of nest	7.77 ± 1.85	11.87 ± 2.65	−18.00	0.25
Nest sanitation	0.92 ± 0.43	1.24 ± 0.32	−16.00	0.31
Allopreening	15.18 ± 3.85	30.77 ± 7.36	−24.00	0.11
Provisioning behavior	3.50 ± 0.56	5.98 ± 1.04	−32.00	0.02
Medium ground finch				
Nestlings				
Begging	6.85 ± 0.90	5.53 ± 0.86	25.00	0.16
Adults				
Attendance at nest	47.29 ± 6.10	57.72 ± 9.14	−23.00	0.20
Provisioning behavior	11.05 ± 2.19	10.45 ± 4.90	27.00	0.13

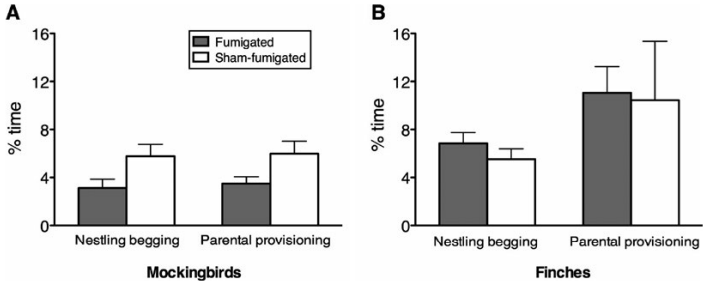

FIG. 4. Nestling and parental behavior (mean ±SE) in fumigated and sham-fumigated nests for (A) mockingbirds and (B) finches. Time allocated to nestling begging and parental provisioning behavior was significantly higher in sham-fumigated mockingbird nests than in fumigated nests. In contrast, the amount of time spent on these behaviors did not differ significantly between treatments in finch nests.

we suggest that this provides evidence that mockingbirds are tolerant hosts, whereas finches are highly vulnerable to the parasite. We then explored potential tolerance mechanisms used by mockingbirds to deal with *P. downsi*. We found that mockingbird nestlings from sham-fumigated nests begged significantly more than nestlings from fumigated nests. Greater begging was correlated with increased parental provisioning, which may have been responsible for the higher glucose concentration in nestlings in sham-fumigated nests. In contrast to mockingbirds, finch nestling begging and parental provisioning did not change in response to *P. downsi*, nor was there a difference in the plasma glucose levels of nestlings in fumigated and sham-fumigated nests. We suggest that these behavioral differences indicate adaptive tolerance of *P. downsi* by mockingbirds. The difference in the effect of *P. downsi* on tolerant vs. non-tolerant hosts motivates the question: How can hosts vary in their susceptibility to the same parasite at the same time in the same place?

Neither mockingbird nor finch nestlings produced a significant antibody-mediated immune response to *P. downsi* in our study. In fact, antibody levels in nestling finches (and mockingbirds) were nearly undetectable, compared to those measured in adult finches in an earlier study (Koop et al. 2013*a*). Captive house sparrows (*Passer domesticus*) are capable of producing independent antibody-mediated immune responses at 3 d of age when challenged with non-specific antigens (King et al. 2010). However, we found no evidence of such responses by finch or mockingbird nestlings parasitized by *P. downsi*.

It is possible that our assay was not sensitive enough to detect low concentrations of antibodies. Antibody levels did increase with nestling age, but this increase did not differ significantly between experimental treatments. One other possibility is that these hosts are exposed to relatively few native parasites in the Galápagos, meaning that non-specific antibody-mediated immune responses are not primed as much as they would be on the mainland. On the other hand, the antibodies we detected may not be specific to *P. downsi*. Instead, the antibodies may have also been influenced by other biting insects, such as mosquitoes, which have antigens in their saliva that induce similar responses to those induced by *P. downsi* (e.g., IgG) (Peng et al. 1996). Nevertheless, our results suggest that nestling immune responses do not ameliorate the effects of *P. downsi* on mockingbirds or finches.

Mockingbird parents from sham-fumigated nests brooded their nestlings less than parents from fumigated nests. Mockingbird parents were still present at the nest, but they may have been avoiding the parasites by standing on the rim of the nest. Koop et al. (2013*a*) found that adult finches in sham-fumigated nests also brood their nestlings less, and stand erect more, compared to adult finches in fumigated nests. Mockingbird parents also allopreen their nestlings; however, it was not clear from our video analyses whether allopreening removes or damages *P. downsi* (cf. Clayton et al. 2010). It is possible that allopreening does provide at least some defense against *P. downsi*. Further tests are needed to determine the extent to which mockingbirds can reduce *P. downsi* on their nestlings by allopreening them.

Mockingbirds may tolerate the effects of *P. downsi* by increasing parental provisioning of nestlings to compensate for energy lost to parasites. In other systems, parasitic flies are known to increase host metabolic rate, which depletes host energy resources (Careau et al. 2010). Several studies of other systems have also shown that parents in parasitized nests feed their nestlings more than parents in nonparasitized nests, leading to increased fledging success (Tripet and Richner 1997, Hurtrez-Bousses et al. 1998, Tripet et al. 2002). Our study suggests that increased begging by mockingbird nestlings in sham-fumigated nests led to increased provisioning by parents, which likely contributed to the improved survival of nestlings in these nests. A more definitive test of this hypothesis would involve comparison of the quality and quantity of food being delivered to nestlings between experimental treatments and species. It would also be interesting to test the begging-provisioning hypothesis by using recordings to simulate increased begging in nests to see if parents respond with the delivery of more food to the nestlings (Bengtsson and Rydén 1983, Ottosson et al. 1997).

Why do finch nestlings not beg more in sham-fumigated nests, the way that mockingbird nestlings do? The answer may lie in the smaller body size of finch nestlings, which are only half the size of mockingbird nestlings. Smaller birds require more energy per gram of body mass because they have a higher surface-to-volume ratio than larger birds (Schmidt-Nielsen 1984). Thus, small-bodied species tend to beg more than large-bodied species (Price and Ydenberg 1995, Christe et al. 1996, Leech and Leonard 1996, Kitaysky et al. 2001, Saino et al. 2001, Simon et al. 2005). As a result, they may also be fed more by their parents (Christe et al. 1996). Interestingly, nestlings in fumigated finch nests spent more than twice as much time begging as nestlings in fumigated mockingbird nests. Because begging by small birds is more energetically costly (per gram) than begging by large birds (Jurisevic et al. 1999), finch nestlings may experience an energetic ceiling beyond which they are simply incapable of additional begging. On the other hand, some small-bodied species of birds are known to increase begging in response to native parasitic flies (Christe et al. 1996). However, O'Connor et al. (2013) similarly found that *P. downsi* does not have a significant effect on another small-bodied species of Darwin's finch. This topic clearly requires further exploration.

Similar to finches, older parasitized mockingbird nestlings had lower hemoglobin and mass compared to nonparasitized nestlings. Dudaniec et al. (2006) similarly found that hemoglobin decreases as *P. downsi* abundance increases in Darwin's finches. Because hemoglobin and mass are indicators of body condition, we cannot discount the possibility that the post-fledging survival of parasitized mockingbirds was less, compared to nonparasitized mockingbirds. For example, Streby et al. (2009) found that, despite similar fledging success, parasitized ovenbirds (*Seiurus aurocapilla*) had lower post-fledging survival than nonparasitized fledglings. Alternatively, fledglings from parasitized nests may recover body mass and hemoglobin once they have left the nest, especially given that mockingbird parents continue to feed fledglings for at least a month after they leave the nest (S.A.K. personal observation). A future study could track post-fledgling survival of parasitized and nonparasitized mockingbird and finch nestlings to determine if *P. downsi* has a delayed effect on survival.

Our study is one of the first to show differential effects of an introduced parasite on different host species under natural conditions, including evidence for possible tolerance mechanisms. Only recently has the idea of animal host tolerance to parasitism become widely recognized as an important defense strategy (Read et al. 2008, Råberg et al. 2009, Baucom and de Roode 2011, Medzhitov et al. 2012, Sorci 2013). Tolerant hosts may be important ecological mediators of the effects of parasites on vulnerable hosts. Tolerant hosts may serve as parasite reservoirs, amplifying the effects of parasites on nontolerant hosts. Identifying reservoir hosts can have important conservation implications if the vulnerable host population is declining, or if the reservoir host population is increasing. However, rigorous studies of reservoir hosts are difficult because they ideally would require experimental manipulations at the population level. For example, suspected reservoir hosts could be

removed from the community, and the consequences of removal assessed for more vulnerable host species (Haydon et al. 2002, Laurenson et al. 2003). This approach is typically not feasible, particularly if the reservoir host is a protected species. In the mockingbird-finch-fly system, a future study could control or eliminate parasites from mockingbird nests at some sites, then compare the population dynamics of finches (and flies) across all sites.

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Literature Cited

Anderson, R. M., and R. M. May. 1991. Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford.

Atkinson, C. T., and D. A. Lapointe. 2009. Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. Journal of Avian Medicine and Surgery 23:53–63.

Atkinson, C. T., R. J. Dusek, K. L. Woods, and W. M. Iko. 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. Journal of Wildlife Diseases 36:197–204.

Baucom, R. S., and J. C. de Roode. 2011. Ecological immunology and tolerance in plants and animals. Functional Ecology 25:18–28.

Bengtsson, H., and O. Rydén. 1983. Parental feeding rate in relation to begging behavior in asynchronously hatched broods of the great tit *Parus major*: an experimental study. Behavioral Ecology and Sociobiology 12:243–251.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science and Business Media, Berlin, Germany.

Bush, A. O., K. D. Lafferty, J. M. Lotz and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83:575–583.

Careau, V., D. W. Thomas, and M. M. Humphries. 2010. Energetic cost of bot fly parasitism in free-ranging eastern chipmunks. Oecologia 162:303–312.

Carleton, R. E. 2008. Ectoparasites affect hemoglobin and percentages of immature erythrocytes but not hematocrit in nestling Eastern Bluebirds. Wilson Journal of Ornithology 120:565–568.

Christe, P., H. Richner, and A. Oppliger. 1996. Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. Behavioral Ecology 7:127–131.

Clayton, D. H., J. A. H. Koop, C. W. Harbison, B. R. Moyer, and S. E. Bush. 2010. How birds combat ectoparasites. Open Ornithology Journal 3:41–71.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife–threats to biodiversity and human health. Science 287:443–449.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Dodge, H. R., and T. H. G. Aitken. 1968. *Philornis* flies from Trinidad (Diptera: Muscidae). Journal of the Kansas Entomological Society 41:134–154.

Dudaniec, R. Y., S. Kleindorfer, and B. Fessl. 2006. Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's small ground finch (*Geospiza fuliginosa*). Austral Ecology 31:88–94.

Fessl, B., S. Kleindorfer, and S. Tebbich. 2006. An experimental study on the effects of an introduced parasite in Darwin's finches. Biological Conservation 127:55–61.

Fessl, B., G. H. Young, R. P. Young, J. Rodríguez-Matamoros, M. Dvorak, S. Tebbich, and J. E. Fa. 2010. How to save the rarest Darwin's finch from extinction: the mangrove finch on Isabela Island. Philosophical Transactions of the Royal Society of London B 365:1019–1030.

Grant, P. 1999. Ecology and evolution of Darwin's finches. Princeton University Press, Princeton, New Jersey, USA.

Guglielmo, C. G., P. D. O. Hara, and T. D. Williams. 2013. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free-living western sandpipers (*Calidris mauri*). Auk 119:437–445.

Hänninen, L., and M. Pastell. 2009. CowLog: open-source software for coding behaviors from digital video. Behavior Research Methods 41:472–476.

Haydon, D. T., S. Cleaveland, L. H. Taylor, and M. K. Laurenson. 2002. Identifying reservoirs of infection: a conceptual and practical challenge. Emerging Infectious Diseases 8:1468–1473.

Hudson, P. J., A. P. Rizzoli, B. T. Grenfell, J. A. P. Heesterbeek, and A. P. Dobson. 2002. The ecology of wildlife diseases. Oxford University Press, Oxford.

Hurtrez-Bousses, S., J. Blondel, P. Perret, J. Fabreguettes, and F. R. Renaud. 1998. Chick parasitism by blowflies affects feeding rates in a Mediterranean population of blue tits. Ecology Letters 1:17–20.

Jurisevic, M. A., K. J. Sanderson, and R. V. Baudinette. 1999. Metabolic rates associated with distress and begging calls in birds. Physiological and Biochemical Zoology 72:38–43.

Keesing, F., et al. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–652.

King, M. O., J. P. Owen, and H. G. Schwabl. 2010. Are maternal antibodies really that important? Patterns in the immunologic development of altricial passerine house sparrows (*Passer domesticus*). PLoS One 5:e9639.

Kitaysky, A. S., J. C. Wingfield, and J. F. Piatt. 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behavioral Ecology 12:619–625.

Kleindorfer, S., K. J. Peters, G. Custance, R. Y. Dudaniec, and J. A. O'Connor. 2014. Changes in *Philornis* infestation behavior threaten Darwin's finch survival. Current Zoology 60:542–550.

Knutie, S. A., S. M. McNew, A. W. Bartlow, D. A. Vargas, and D. H. Clayton. 2014. Darwin's finches combat introduced nest parasites with fumigated cotton. Current Biology 24:R355–R356.

Koop, J. A. H., S. K. Huber, S. M. Laverty, and D. H. Clayton. 2011. Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's finches. PLoS One 6:e19706.

Koop, J. A. H., J. P. Owen, S. A. Knutie, M. A. Aguilar, and D. H. Clayton. 2013*a*. Experimental demonstration of a parasite-induced immune response in wild birds: Darwin's finches and introduced nest flies. Ecology and Evolution 3:2514–2523.

Koop, J. A. H., C. LeBohec, and D. H. Clayton. 2013*b*. Dry year does not reduce invasive parasitic fly prevalence or abundance in Darwin's finch nests. Reports in Parasitology 3:11–17.

Laurenson, M. K., R. A. Norman, L. Gilbert, H. W. Reid, and P. J. Hudson. 2003. Identifying disease reservoirs in complex systems: mountain hares as reservoirs of ticks and louping-ill virus, pathogens of red grouse. Journal of Animal Ecology 72:177–185.

Leech, S. M., and M. L. Leonard. 1996. Is there an energetic cost to begging in nestling tree swallows (*Tachycineta bicolor*)? Proceedings of the Royal Society of London B: Biological Sciences 263:983–987.

McCallum, H. 2012. Disease and the dynamics of extinction. Philosophical Transactions of the Royal Society of London B: Biological Sciences 367:2828–2839.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution 10:190–194.

Medzhitov, R., D. Schneider, and M. Soares. 2012. Disease tolerance as a defense strategy. Science 335:936–941.

Moon, R. 1980. Effects of larval competition on face fly. Environmental Entomology 9:325–330.

Nokes, D. J. 1992. Microparasites: viruses and bacteria. Pages 349–374 *in* M. J. Crawley, editor. Natural enemies: the population biology of predators, parasites and diseases. Blackwell Scientific, Boston, Massachusetts, USA.

O'Brien, E., B. Morrison, and L. Scott Jonhson. 2001. Assessing the effects of haematophagous ectoparasites on the health of nestling birds: haematocrit vs haemoglobin levels in house wrens parasitized by blow fly. Journal of Avian Biology 32:73–76.

O'Connor, J. A., F. J. Sulloway, J. Robertson, and S. Kleindorfer. 2009. *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). Biodiversity and Conservation 19:853–866.

O'Connor, J. A., J. Robertson, and S. Kleindorfer. 2013. Darwin's finch begging intensity does not honestly signal need in parasitised nest. Ethology 119:1–10.

Ottosson, U., J. Bäckman, and H. G. Smith. 1997. Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca*. Behavioral Ecology and Sociobiology 41:381–384.

Peng, Z., M. Yang, and F. E. R. Simons. 1996. Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. Annals of Allergy, Asthma and Immunology 77:238–244.

Price, K., and R. Ydenberg. 1995. Begging and provisioning in broods of asynchronously-hatched yellow-headed blackbird nestlings. Behavioral Ecology and Sociobiology 37:201–208.

R Core Team. 2014. R: A language for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Råberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364:37–49.

Read, A. F., A. L. Graham, and L. Råberg. 2008. Animal defenses against infectious agents: is damage control more important than pathogen control. PLoS Biology 6:e4.

Saino, N., M. Incagli, R. Martinelli, R. Ambrosini, and A. P. Moller. 2001. Immunity, growth and begging behaviour of nestling Barn Swallows *Hirundo rustica* in relation to hatching order. Journal of Avian Biology 32:263–270.

Schmid-Hempel, P. 2011. Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford.

Schmidt, C. D., and R. R. Blume. 1973. Laboratory-reared horn flies: relationships between width of head and weight of pupa in both sexes and between these measurements and number of ovarioles in females. Annals of the Entomological Society of America 66:1307–1308.

Schmidt-Nielsen, K. 1984. Scaling: why is animal size so important?. Cambridge University Press, Cambridge.

Simon, A., D. W. Thomas, J. R. Speakman, J. Blondel, P. Perret, and M. M. Lambrechts. 2005. Impact of ectoparasitic blowfly larvae (*Protocalliphora* spp.) on the behavior and energetics of nestling Blue Tits. Journal of Field Ornithology 76:402–410.

Sorci, G. 2013. Immunity, resistance and tolerance in bird-parasite interactions. Parasite Immunology 35:350–361.

Streby, H. M., S. M. Peterson, and P. M. Kapfer. 2009. Fledging success is a poor indicator of the effects of bird blow flies on ovenbird survival. Condor 111:193–197.

Svensson, E. I., and L. Råberg. 2010. Resistance and tolerance in animal enemy-victim coevolution. Trends in Ecology and Evolution 25:267–274.

Tompkins, D. M., A. R. White, and M. Boots. 2003. Ecological replacement of native red squirrels by invasive greys driven by disease. Ecology Letters 6:189–196.

Tripet, F., and H. Richner. 1997. Host responses to ectoparasites: food compensation by parent blue tits. Oikos 78:557–561.

Tripet, F., M. Glaser, and H. Richner. 2002. Behavioural responses to ectoparasites: time-budget adjustments and what matters to blue tits *Parus caeruleus* infested by fleas. Ibis 144:461–469.

Wikelski, M., J. Foufopoulos, H. Vargas and H. Snell. 2004. Galápagos birds and diseases: invasive pathogens as threats for island species. Ecology and Society 9: online. http://www.ecologyandsociety.org/vol9/iss1.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1890/15-0119.1/suppinfo

APPENDIX F

THE BEAK OF THE PINCH: CONSEQUENCES OF ADAPTIVE RADIATION FOR ECTOPARASITE CONTROL

Abstract

Darwin's finches are an iconic example of adaptive radiation. The size and shape of Darwin's finch beaks are adapted for feeding on different sized seeds and other food resources. However, beaks also serve other functions, such as preening for the control of ectoparasites. In diverse groups, such as North American scrubjays, Hawaiian honeycreepers, and Peruvian songbirds, the effectiveness of preening is governed by the length of the overhanging tip of the upper mandible of the beak. This overhang functions as a template against which the tip of the lower mandible generates a pinching force sufficient to damage or kill ectoparasites. Here we show that, in contrast to the diversifying effect of foraging, the ectoparasite control function of preening appears to constrain beak morphology. Across finch species, birds with intermediate mandibular overhangs have fewer feather mites than birds with short or long overhangs. While there is little doubt that beaks are first and foremost tools for feeding, our results suggest that the evolution of beak morphology, even in well-known systems, should be evaluated with both feeding and preening in mind.

Introduction

In his Pulitzer Prize-winning book "Beak of the Finch," Jonathan Weiner (1994) features Peter and Rosemary Grant's classic work on the adaptive radiation of Darwin's finches in the Galapagos Islands (Grant and Grant 2014). As carefully demonstrated by the Grants, Darwin's finch beaks are adapted for feeding on different sized seeds and other food resources. For example, large

ground finches (*Geospiza magnirostris*) have large, deep beaks capable of crushing large, tough seeds. Medium ground finches (*G. fortis*) have intermediate beaks for feeding on smaller seeds. Small ground finches (*G. fuliginosa*) have smaller, more nimble beaks for feeding on the smallest seeds (Grant and Grant 2014). This diversification of foraging related beak morphology is a hallmark of adaptive radiation in Darwin's finches, as well as other groups of birds (Cooney *et al.* 2017; Olsen 2017).

Although beaks are first and foremost adaptations for feeding, they have other functions, as well, such as preening (Figure E.1). Preening serves to straighten and oil feathers and to combat ectoparasites. Indeed, preening is the first line of defense against several groups of ectoparasites (Clayton *et al.* 2010; Clayton *et al.* 2016). Among diverse groups of birds, such as North American scrubjays, Hawaiian honeycreepers, and Peruvian songbirds, the effectiveness of preening is governed by the overhanging tip of the upper mandible of the beak (Clayton and Walther 2001; Clayton *et al.* 2005; Freed *et al.* 2008; Clayton *et al.* 2010). Removal of the overhang in rock pigeons (*Columba livia*) triggers a dramatic increase in populations of feather lice (Clayton *et al.* 2005). When the overhang is allowed to grow back, birds regain their ability to control lice. In pigeons, the overhang functions as a template against which the tip of the lower mandible generates a pinching force sufficient to crush the lice. Interestingly, removal of the overhang has no effect on the feeding efficiency of pigeons, suggesting that it is a specific adaptation for ectoparasite control (Clayton *et al.* 2005).

Effective preening for controlling feather lice is important because they are known to have direct negative effects on host fitness (Clayton *et al.* 2008; Hoi *et al.* 2012; Clayton *et al.* 2016). Large populations of lice on birds with impaired preening cause feather damage that reduces host mating success and survival (Clayton 1990; Booth *et al.* 1993). Preening also controls feather mite populations, such that birds with impaired preening can experience dramatic increases in feather mites (Barlow 1967; Clayton 1991; Handel *et al.* 2010). The effect of such mites on host fitness has not been tested experimentally; however, feather mite increases are correlated with poor host condition, feather quality, and reduced plumage brightness (Thompson *et al.* 1997; Harper 1999). Some feather mites may be commensals with little or no effect on host fitness (Blanco *et al.* 1997; Proctor and Owens 2000; Galvan *et al.* 2012). Moreover, effects of mites may transition between parasitism and commensalism (Bronstein 1994; Jovani *et al.* 2017). Regardless of their effects, however, feather mites – like feather lice – are controlled by preening.

Here we explore the relationship between the beak morphology and ectoparasite loads of Darwin's ground finches. We compared beak size and shape to the abundance of ectoparasites among three species of ground finches, all of which have both feather mites and feather lice (Bulgarella and Palma 2017). Large, medium, and small ground finches have virtually identical mite and lice communities at our study site on Santa Cruz Island (Villa *et al.* 2013). We tested the prediction that, in contrast to the diversifying effect of feeding on beak morphology, preening is likely to have a stabilizing effect. Specifically, we

predicted that the relative length of the upper mandibular overhang, which varies within species (Figure 2.E *A*-*D*), does not differ significantly among the three species of ground finches.

Methods

We conducted field work January-April 2009 at two locations on Santa Cruz Island: a highland site near Los Gemelos (0º37’50.95’’S, 90º23’26.54’’W), and a lowland site at the Charles Darwin Research Station on Academy Bay, Puerto Ayora (0º44’27.55’’S, 90º18’10.10’’W). Finches were captured with mist-nets between 0600 and 1100 hr, and between 1600 and 1800 hr. Each bird was placed in single-use paper bag to avoid mixing parasites between birds. For each bird, we quantified body mass and beak length, width, depth, and overhang length (Clayton and Walther 2001). We then quantified the abundance of feather lice and feather mites using the dust-ruffling method (Villa *et al.* 2013). Birds were banded with metal bands and released unharmed.

Feather mites were more common than feather lice on Santa Cruz. Seven species of feather mites were collected, with the different species of finches having nearly identical feather mite communities (Villa *et al.* 2013). We tested for population level variation among the feather mites on different finch species by barcoding samples of mites with the cytochrome c oxidase subunit 1 (COI) gene. We barcoded the three most common genera of feather mites (*Mesalgoides, Trouessartia,* and *Proctophyllodes*). Because feather mites are tiny, single individuals did not yield enough DNA for reliable amplification of mitochondrial

DNA. Therefore, we pooled five individual mites, which is a common approach for small-bodied organisms (Dabert *et al.* 2010). To extract DNA, we used a Qiagen DNeasy Blood and Tissue Kit. DNA was PCR amplified using the primers bcdF05 and bcdR04, as described by Dabert *et al.* (2008). Analysis of mite COI sequences was done in Geneious v7 (Kearse *et al.* 2012). Percent sequence difference among populations of mites on the three finch species were analyzed using a one-way ANOVA in JMP v12 (SAS Institute 1985).

The relationship between beak morphology and ectoparasite abundance was analyzed using a principal component analysis (PCA) of the four beak dimensions across the three finch species (Grant and Grant 2014) in JMP v12 (SAS Institute 1985). Covariation of principal components with ectoparasite abundance was modeled using linear mixed models (LMMs). Ectoparasite abundance was log transformed (log [n+1]) to achieve normality prior to analysis. Ectoparasite abundance was strongly correlated with host body mass (linear regression; n = 88, r = 0.30, $P = 0.005$). To control for differences in the body mass of the three finch species, we calculated residuals of ectoparasite abundance plotted against finch body mass.

For each of the first two principal components (PC1 and PC2), we performed an LMM predicting residual ectoparasite abundance among the three species of ground finches by modeling the fixed effect of each principal component using the second order polynomial function with species as a random effect. Both LMMs were fit in R v3.3.1 using the “lme4” library (Bates *et al.* 2015; R Core Team 2016). Degrees of freedom and resulting p-values were estimated

with Satterwaite approximation using the lmerTest library (Kuznetsova *et al.* 2016).

Results

We processed 90 finches, including 39 small ground finches, 41 medium ground finches, and 10 large ground finches. We missed one or more measurements for two of the small ground finches, leaving a total of 88 birds with complete data sets. Feather mites were recovered from 61 of the 88 finches (69%). The overall abundance of mites ranged from 0 to 356, with a mean (± se) of 33.7 ± 7.0 mites per bird. By comparison, only 6 of the 88 birds had feather lice (8%). The overall abundance of lice ranged from 0 to 8, with a mean (± se) of 0.2 ± 0.1 lice per bird. Because the prevalence and abundance of lice were so low, we focused on the relationship between beak morphology and feather mites. However, when we included lice, the results were nearly identical.

COI comparisons were made for *Mesalgoides* mites from 2 small, 2 medium and 1 large ground finches; *Trouessartia* mites from 2 small, 2 medium, and 2 large ground finches; and *Proctophyllodes* mites from 2 small, 2 medium, and 2 large ground finches (Table E.1). Similar barcoding studies suggest that 5 to 10 barcodes should be assessed per taxon; so our sample sizes were adequate for each genus (Zhang *et al.* 2010). None of the intrageneric comparisons of mite COI sequences (Table E.1) showed more than 2% divergence among populations of mites on the different finch species (Hebert *et al.* 2003a, 2003b). In summary, the finches had essentially the same mite

populations.

Beak length, width, depth and overhang length were all correlated across the 88 finches (Table E.2). The first two principal components of beak morphology accounted for 98.8% of variation (Table E.3). PC1, which mainly reflected variation in beak length, width, and depth, was highly correlated with finch body mass (Figure E.2 *E*; linear regression; n = 88, r = 0.97, $P < 0.0001$). In contrast, PC1 was not correlated with residual mite abundance (Figure E.2 *F*; LMM; t = 0.12, $P = 0.907$).

PC2, which mainly reflected variation in overhang length (Table E.3), was not correlated with finch body mass (Figure E.2 *G*; linear regression; n = 88, r = -0.14, $P = 0.162$). However, PC2 was highly correlated with residual mite abundance (Figure E.2 *H*; LMM; t = 2.86, $P = 0.005$).

Discussion

We tested whether the foraging related diversification of beak morphology among Darwin's ground finches is related to ectoparasite load. Specifically, we compared beak size and shape to the abundance of ectoparasites on small, medium and large ground finches (Villa *et al.* 2013). Although lice on Darwin's ground finches are often relatively common (Palma and Peck 2013; Bulgarella and Palma 2017; Palma pers. comm.), the prevalence of lice on birds in our study was low (8%). One possible explanation is that feather lice are more susceptible than feather mites to abiotic factors, such as low humidity (Moyer *et al.* 2002). Thus, the low prevalence of lice in our study may have reflected

climatic conditions.

The prevalence of feather mites in our study was relatively high (69%). Barcoding revealed little or no population level differentiation among the three species of ground finches. Thus, we predicted that – in contrast to foraging – preening would not lead to patterns of diversification across ground finch species.

PC1 and PC2 explained nearly all variation (98.8%) in beak morphology across the three ground finch species (Table E.3). PC1 reflected variation in beak length, width, and depth. These three traits are the basis of adaptive differences in foraging ecology among finch species (Grant and Grant 2014). Thus, PC1 represents feeding related morphology. PC1 was highly correlated with finch body mass (Figure E.2 *E*), reflecting the fact that larger finches have larger beaks (Grant and Grant 2014). In contrast, PC1 did not correlate with feather mite abundance (Figure E.2 *F*).

PC2 summarizes mainly variation in the beak overhang. Since the beak overhang is important for controlling ectoparasites, PC2 represents preening related morphology. The relationship between PC2 and feather mite abundance was consistent with stabilizing selection, rather than diversifying selection. Across small, medium, and large ground finches, individuals with intermediate PC2 scores had the fewest mites. The 10 birds with PC2 scores nearest the mean had an average of 2 mites. By comparison, the 10 birds with PC2 scores farthest from the mean (in either direction) had more than 50-fold more mites, with an average of 110 mites. This pattern shows that individuals with extreme

overhangs (Figure E.2 *C,D*) are not as effective at controlling ectoparasites. Repeating the analysis with raw overhang length, instead of PC2, yields the same stabilizing pattern (LMM; t = 2.07, *P* = 0.042). The similarly between this more conservative analysis and our PCA approach strongly suggests that overhang length governs feather mite abundance.

Unlike PC1, PC2 was not correlated with finch mass (Figure E.2 *G*). The lack of relationship indicates that Darwin's ground finches have similar preening related beak morphology, regardless of overall differences in body size. Thus, even though the beaks of small, medium and large ground finches have diverged in size for feeding, there is an optimal relative overhang length. This fact is not surprising, given that the three finch species share the same ectoparasite species and even populations. Our results indicate that the adaptive diversification of finch beaks for feeding does not constrain the ability of finches to control ectoparasites by preening.

Our findings are consistent with earlier work examining Western scrubjays (*Aphelocoma californica*) infested with parasitic feather lice (Clayton *et al.* 2010). Analysis of overhang length and louse load in scrubjays showed a pattern consistent with stabilizing selection for beak overhang length, in which birds with intermediate overhangs had the fewest lice (Clayton *et al.* 2010). Overhangs that are "too short" are insufficient to control ectoparasites, while overhangs that are "too long" are more susceptible to breakage, as demonstrated in pigeons (Clayton *et al.* 2005). Birds with broken overhangs cannot preen well.

Our study is the first to provide evidence that aspects of the morphology of

Darwin's finch beaks may be constrained by selection independent of feeding ecology. While there is little doubt that beaks are first and foremost tools for feeding, our results suggest that the evolution of beak morphology, even in well-known systems, should be evaluated with both feeding and preening in mind.

References

Barlow, J. C. 1967. A bill deformity in a European tree sparrow, *Passer montanus* (Linnaeus). *Can. J. Zool*. 45: 889-890.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw*. 67:1-48.

Blanco, G., J. L. Tella, and J. Potti. 1997. Feather mites on group-living red-billed choughs: a non-parasitic interaction? *J. Avian Biol.* 28:197-206.

Booth, D. T., D. H. Clayton, and B.A. Block. 1993. Experimental demonstration of the energetic cost of parasitism, in free-ranging hosts. *Proc. R. Soc. Lond. B Biol. Sci.,* 253: 125-129.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol*. 9: 214-217.

Bulgarella, M., and R. L. Palma. 2017. Coextinction dilemma in the Galapagos Islands: Can Darwin's finches and their native ectoparasites survive the control of the introduced fly *Philornis downsi*? *Insect Cons. Diversity* doi:10.1111/icad.12219.

Clayton, D. H. 1990. Mate choice in experimentally parasitized rock doves: Lousy males lose. *Amer. Zool.* 30:251-262.

Clayton, D. H. 1991. Coevolution of Avian Grooming and Ectoparasite Avoidance. Pages 258-289 *in* J. E. Loye and M. Zuk, eds*. Bird-parasite Interactions: Ecology, Evolution, and Behaviour.* Oxford Univ. Press, Oxford, UK.

Clayton, D.H., and B. A. Walther. 2001. Influence of host ecology and morphology on the diversity of neotropical bird lice. *Oikos* 94:455-467.

Clayton, D.H., B. R. Moyer, S. E. Bush, T. G. Jones, D. W. Gardiner, B. B. Rhodes, and F. Goller. 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proc. R. Soc. Lond. B Biol. Sci.* 272:811-817.

Clayton, D. H., R. J. Adams, and S. E. Bush. 2008. Phthiraptera, the Chewing Lice. Pages 515-526 *in* C. T. Atkinson, N. J. Thomas, and D. B. Hunter, eds. *Parasitic Diseases of Wild Birds*. Wiley-Blackwell, Ames, Iowa.

Clayton, D. H., J. A. H. Koop, C. W. Harbison, B. R. Moyer, and S. E. Bush. 2010. How birds combat ectoparasites. *Open Ornithol. J.* 3:41-71.

Clayton, D.H., S. E. Bush, and K. P. Johnson. 2016. Coevolution of life on hosts: integrating ecology and history. Univ. Chicago Press, Chicago, IL.

Cooney, C. R., J. A. Bright, E. J. R. Capp, A. M. Chira, E. C. Hughes, C. J. A. Moody, L. O. Nouri, Z. K. Varley, and G. H. Thomas. 2017. Mega-evolutionary Dynamics of the Adaptive Radiation of Birds. *Nature* 542: 344-347.

Dabert, J., R. Ehrnsberger, and M. Dabert. 2008. *Glaucalges tytonis* sp. n. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxon descriptions in mites (Acari). *Zootaxa* 1719: 41-52.

Dabert, M., W. Witalinski, A. Kazmierski, Z. Olszanowski, and J. Dabert. 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): Strong conflict between phylogenetic signal and long-branch attraction artifacts. *Mol. Phylogenet. Evol*. 56:222-241.

Freed, L.A., C. Cann, and G. R. Bodner. 2008. Explosive increase in ectoparasites in Hawaiian forest birds. *J. Parasitol.* 94:1009-1021.

Galván, I., E. Aguilera, F. Atiénzar, E. Barba, G. Blanco, J. L. Cantó, V. Cortés, Ó. Frías, I. Kovács, L. Meléndez, A. P. Møller, J. S. Monrós, P. L. Pap, R. Piculo, J. C. Senar, D. Serrano, J. L. Tella, C. I. Vágási, M. Vögeli, and R. Jovani. Feather mites (Acari: Astigmata) and body condition of their avian hosts: a large correlative study. *J. Avian Biol.* 43:001-007.

Grant, P.R. and B. R. Grant. 2014. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. Princeton Univ. Press, Princeton, NJ.

Handel, C. M., L. M. Pajot, S. M. Matsuoka, C. V. Hemert, J. Terenzi, S. L. Talbot, D. M. Mulcahy, C. U. Meteyer, and K. A. Trust. 2010. Epizootic of beak deformities among wild birds in Alaska: an emerging disease in North America? *The Auk* 127: 882-898.

Harper, D.G.C. 1999. Feather mites, pectoral muscle condition, wing length and plumage coloration of passerines. *Animal Behavior* 58:553–562.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard. 2003a. Biological identifications through DNA barcodes *Proc. R. Soc. Lond. B Biol. Sci.* 270:313-

322.

Hebert, P.D.N, S. Ratnasingham, and J. R. deWaard. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B Biol. Sci*. 270:S96-99.

Hoi, H., J. Kristofik, A. Darolova, and C. Hoi. 2012. Experimental evidence for costs due to chewing lice in the Europefan bee-eater *(Merops apiaster)*. *Parasitology* 139:53-59.

Jovani, R., J. Dona, M. del M. Labrador, and D. Serrano. 2017. Opening the doors of parasitology journals to other symbionts. *Trends Parasitol.* https://dx.doi.org/10.1016/j.pt.2017.05.003.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics 28*:1647-1649.

Kuznetsova, A., P. B. Brockhoff, R. Haubo, and B. Christensen. 2016. lmerTest: tests in linear mixed effects models. R package version 2.0-30. https://CRAN.Rproject.org/package=lmerTest.

Moyer, B. R., D. M. Drown, and D. H. Clayton. 2002. Low humidity reduces ectoparasite pressure: Implications for host life history evolution. *Oikos* 97:223-228.

Olsen, A. M. 2017. Feeding ecology is the primary driver of beak shape diversification in waterfowl. *Funct. Ecol.* doi: 10.1111/1365-2435.12890.

Palma, R. L. and S. B. Peck. 2013. An annotated checklist of parasitic lice (Insecta: Phthiraptera) from the Galapagos Islands. *Zootaxa* 3627:001-087.

Proctor, H., and I. Owens. 2010. Mites and birds: diversity, parasitism and coevolution. *Trends Ecol. Evol.* 15:358-364

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

SAS Institute. 1985. *JMP User's Guide*. SAS Institute, Cary, N.C.

Thompson, C.W., N. Hillgarth, M. Leu, and H. E. McClure. 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *Am. Nat*. 149:270-294.

Villa, S.M., C. Le Bohec, J. A. H. Koop, H. C. Proctor, and D. H. Clayton. 2013. Diversity of feather mites (Acari: Astigmata) on Darwin's finches. *J. Parasitol.* 99:756-762.

Weiner, J. (1994). *The Beak of the Finch*. Cambridge Univ. Press. Cambridge, MA.

Zhang, A.B., L. J. He, R. H. Crozier, C. Muster, and C.D. Zhu. 2010. Estimation of sample sizes for DNA barcoding. *Mol. Phylogenet. Evol.* 54:1035-1039.

Table F.1. DNA barcoding of feather mites infesting three species of Darwin's ground finches. GenBank accession numbers correspond to the mitochondrial cytochrome c oxidase subunit 1 (COI) gene fragment (DNA barcoding region, ~630bp) of 5 pooled feather mites.

Host species	Host ID	GenBank Accession Numbers		
		Mesalgoides	*Trouessartia*	*Proctophyllodes*
Small ground finch	SH142			KY631875
	SH191		KY631881	
	SH196			KY631876
	SH201	KY631870		
	SH216	KY631871	KY631883	
Medium ground finch	SH494	KY631872	KY631884	KY631877
	SH507			KY631878
	SH583			KY631880
	SH678	KY631874		
	SH686		KY631886	
Large ground finch	SH213		KY631882	
	SH513			KY631879
	SH590	KY631873	KY631885	
Total mites		25	30	30

.

Table F.2. Coefficients of correlation (r) matrix for beak morphology measurements, all of which are significantly correlated ($P < 0.001$ for all comparisons).

Beak measurement	Length	Depth	Width	Overhang
Length	1.000			
Depth	0.964	1.000		
Width	0.977	0.986	1.000	
Overhang	0.374	0.343	0.340	1.000

Table F.3. Principal component analysis of beak morphology across three species of Darwin's ground finches.

	Beak Dimension	Principal Components			
		PC1	PC2	PC3	PC4
Eigenvalues		3.126	0.826	0.037	0.011
% Variation		78.2%	20.6%	0.9%	0.3%
Eigenvectors	Length	0.555	- 0.131	- 0.778	- 0.263
	Depth	0.554	- 0.170	0.607	- 0.544
	Width	0.556	- 0.176	0.157	0.797
	Overhang	0.276	0.961	0.030	0.014
Loadings	Length	0.981	- 0.119	- 0.149	- 0.028
	Depth	0.979	- 0.155	0.116	- 0.057
	Width	0.983	- 0.160	0.030	0.084
	Overhang	0.488	0.873	0.006	0.001

Figure F.1. Medium ground finch (*Geospiza fortis*) preening. Photographs courtesy of Kiyoko Gotanda.

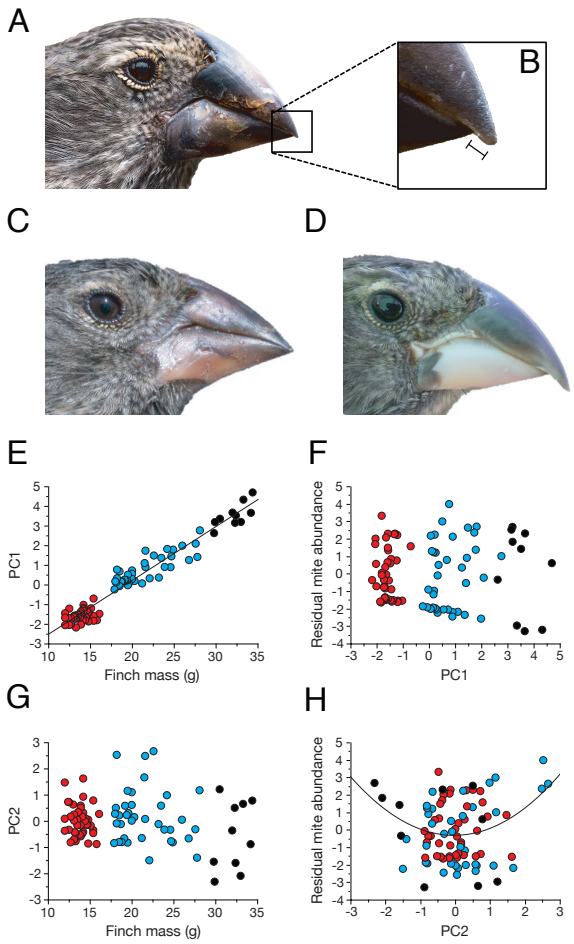

Figure F.2. Beak morphology of Darwin's finches in relation to feather mite abundance (A) Medium ground finch (*Geospiza fortis*) with (B) enlarged view of upper mandibular overhang (inset bracket); Medium ground finches with small (C) and unusually large (D) overhangs. Plots in E-H combine data from large ground finches (*G. magnirostris*; black dots), medium ground finches (blue dots), and small ground finches (*G. fuliginosa*; red dots). Across all finches, overhang length ranges from 0.0-1.1 mm, with a mean (± se) of 0.35 (± 0.03) mm. (E) The first principal component (PC1) of beak morphology, which accounts for 78.2% of variation, is highly correlated with finch body mass (linear regression; n = 88, r = 0.97, $P < 0.0001$). PC1 reflects variation in beak length, width, and depth (Table E.3). (F) Residual feather mite abundance is not significantly associated with PC1 (linear mixed model; t = 0.12, $P = 0.907$). (G) The second principal component (PC2) of beak morphology, which accounts for 20.6% of variation, is not correlated with finch body mass (linear regression; r = -0.14, $P = 0.162$). PC2 reflects variation in beak overhang length (Table E.3). (H) Residual feather mite abundance is highly significantly associated with PC2 (linear mixed model; t = 2.86, $P = 0.005$). Finches with intermediate PC2 scores had the fewest feather mites. Photographs courtesy of Kiyoko Gotanda (panel A) and Jeffery Podos (panels C and D).