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The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera)

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Abstract

Some species of parasites occur on a wide range of hosts while others are restricted to one or a few host species. The host specificity of a parasite species is determined, in part, by its ability to disperse between host species. Dispersal limitations can be studied by exploring the genetic structure of parasite populations both within a single species of host and across multiple host species. In this study we examined the genetic structure in the mitochondrial cytochrome oxidase I (COI) gene of two genera of lice (Insecta: Phthiraptera) occurring on multiple sympatric species of doves in southern North and Central America. One genus, *Columbicola*, is generally less host-specific than the other, *Physconelloides*. For both genera we identified substantial genetic differentiation between populations of conspecific lice on different host species, generally 10–20% sequence divergence. This level of divergence is in the range of that often observed between species of these two genera. We used nested clade analysis to explore fine scale genetic structure within species of these feather lice. We found that species of *Physconelloides* exhibited more genetic structure, both among hosts and among geographical localities, than did species of *Columbicola*. In many cases, single haplotypes within species of *Columbicola* are distributed on multiple host species. Thus, the population genetic structure of species of *Physconelloides* reveals evidence of geographical differentiation on top of high host species specificity. Underlying differences in dispersal biology probably explain the differences in population genetic structure that we observed between *Columbicola* and *Physconelloides*.

Keywords: cytochrome oxidase I, columbiformes, ectoparasites, genetic differentiation, lice

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Introduction

Some species of parasites occur on a wide range of hosts while others are restricted to one or a few host species. A central question in the study of host–parasite coevolution is how to explain these host specificity differences (Thompson 1994). Rigorous documentation of host specificity requires a quantitative approach in which host populations are thoroughly sampled for parasites (Tompkins & Clayton 1999). Careful delimitation of the boundaries of the parasite populations is also necessary. In some cases, host races of parasites are evident (Feder *et al.* 1990, 1993). Often these host races persist through time, eventually leading to speciation of the parasites (Bush 1969).

The genetic structure of host and parasite populations and the host specificity of parasites are important factors underlying coevolutionary history (Clayton *et al.* 2002). The level of host specificity is dictated, in part, by the parasite's ability to disperse among multiple host species. Parasites that differ in their ability to disperse among multiple hosts are expected to differ in their degree of host specificity, and ultimately in the degree of cospeciation observed between parasite and host phylogenies (Clayton *et al.* 2002). An understanding of gene flow in parasite populations is needed to predict cophylogenetic patterns across macroevolutionary timescales.

Gene flow between parasite populations on different host species can be limited even if the parasites are not

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100% host-specific (Dobler & Farrell 1999). Such structure may favour the formation of host races, or speciation. Alternatively, in the case of parasites with more transient interactions with the host, the genetic structure of parasite populations can be less than that for the host (Dybdahl & Lively 1996). In other cases, both the parasites and hosts can show geographical genetic structure and this structure may be more or less congruent (Thompson 1994). However, if factors isolating the host populations are different than those isolating the parasite populations, then the population genetic structure of host and parasite populations may show no congruence (Althoff & Thompson 1999).

Here we focus on feather lice (Phthiraptera: Ischnocera), a group of parasitic insects that show high host specificity. Species of lice are often confined to a single species or genus of host (Marshall 1981). Lice spend their entire life cycle on the body of the host, and most transmission is between host parents and offspring (Lee & Clayton 1995). Thus, in lice, species may be specific simply because they are incapable of dispersing among host taxa (Reed & Hafner 1997). Some groups of lice show substantial cospeciation with their hosts (Hafner *et al*. 1994; Page *et al*. 1998; Johnson and Clayton 2002). However, variation in the degree of cospeciation does occur, and this might be attributable to underlying differences in the ability to disperse among host species (Clayton *et al*. 2002). While the ecology and phylogeny of some groups of lice are becoming increasingly understood, little is known about dispersal and the genetic structure of louse populations, especially for species that occur on multiple host species.

In this study, we examined the population genetic structure of feather lice found on several species of doves (Aves: Columbiformes) sampled at three localities: Texas and the Mexican states of Campeche and Yucatan (both on the Yucatan peninsula). New World doves host two types of ischnoceran lice that are often called wing and body lice (Clayton 1991). Wing lice, in the genus *Columbicola*, have a long, slender body form and escape host preening defences by hiding between the barbs of the wing feathers of the host. Body lice, in the genus *Physconelloides*, have a more rounded body form and escape preening by burrowing in the downy portions of the body feathers (Clayton 1991). These two groups of lice are not closely related to each other (Cruickshank *et al*. 2001); thus they represent independent comparisons of host specificity and genetic differentiation. The taxonomy of both *Columbicola* and *Physconelloides* has been revised recently by Clayton & Price (1999) and Price *et al*. (1999), providing consistent evaluation of species limits on the basis of morphology.

For each species of louse in this study, we sequenced a portion of the mitochondrial cytochrome oxidase I (COI) gene for several individuals from each of several populations. This approach allowed us to evaluate the degree of genetic differentiation (if any) among louse populations in different geographical locations and on different species of hosts. We examined the four species of *Columbicola* and the five species of *Physconelloides* occurring on nine species of hosts over the three localities. In general, body lice are more host-specific than wing lice (see Results). Thus, we compared the genetic structure of species of *Columbicola* and *Physconelloides* across host species and geographical localities to determine if host specificity differences might reflect differences in the degree of genetic structure.

Materials and methods

Specimens

We collected lice using ethyl acetate fumigation methods (Clayton and Drown 2002). We preserved lice either by placing them in 95% ethanol or by freezing them at –70 °C. Upon return from the field, lice in ethanol were placed in a –20 °C freezer (within 30 days of collection). We collected populations of lice from doves at three major localities: southern Texas, the Mexican states of Campeche, and the Yucatan (see Table 1 for samples). All species of doves occurring at a locality were sampled for lice and included in this study with two exceptions. Pigeons in the genus *Columba* were not included in this study because they often occur in different habitats and they do not share species of lice with those species of doves included in this study. Second, at the Yucatan site, *Columbina talpacoti*, *Zenaida asiatica*, and *Z. aurita* occurred, but no lice were obtained from these species. For sequencing, we attempted to represent localities and host species as evenly as possible. Dove lice were also sampled from Arizona. However, the number of lice recovered in Arizona was exceedingly low because of a very low prevalence, so we use these lice only to fill in representation for species that were not recovered in high numbers in Texas and Mexico. In southern Texas, lice were collected from five co-occurring dove species: *C. inca*, *C. passerina*, *Leptotila verreauxi angelica*, *Z. asiatica*, and *Z. macroura*. All of these species occur in the same habitat and therefore represent one host–parasite community. In Mexico we collected lice from two more groups of doves. The first, in Campeche, included *Claravis pretiosa*, *Geotrygon montana*, *L. plumbeiceps*, *L. jamaicensis*, and *L. v. fulviventris*. The second group, in the Yucatan, consisted of *L. v. fulviventris* and *C. passerina*. In this study, we also include a few louse specimens from Arizona for *Z. macroura* and *Z. asiatica*.

Two species of *Columbicola* occur in each of the Texas and Mexico localities: *Columbicola macrourae* and *C. passerinae*. A third species, *C. gracilicapitis*, occurs at the Campeche site, and a fourth species, *C. baculoides*, occurs in both Texas and Arizona (see Table 1). For *Columbicola* we sequenced 48

Table 1 Louse samples included in study

Louse Species	Host (identification number)	Locality	GenBank No.
Columbicola gracilicapitis	*Leptotila jamaicensis* MBR4512	Campeche	AF414718
C. gracilicapitis	*L. jamaicensis* MBR4512	Campeche	AF414719
C. gracilicapitis	*L. jamaicensis* MBR4512	Campeche	AF414720
C. gracilicapitis	*Leptotila plumbeiceps* CO-40	Campeche	AF414721
C. gracilicapitis	*L. plumbeiceps* GES288	Campeche	AF414722
C. gracilicapitis	*L. plumbeiceps* GES410	Campeche	AF414723
C. gracilicapitis	*Leptotila verreauxi fulviventris* GES387	Campeche	AF414724
Columbicola passerinae	*Columbina inca* I115	Texas	AF414725
C. passerinae	*C. inca* I136	Texas	AF414726
C. passerinae	*Columbina passerina* GES233	Yucatan	AF414727
C. passerinae	*C. passerina* CO-11	Yucatan	AF414728
C. passerinae	*C. passerina* G93	Texas	AF414729
C. passerinae	*C. passerina* G181	Texas	AF414730
C. passerinae	*C. passerina* G280	Texas	AF414731
C. passerinae	*Claravis pretiosa* CO-23	Campeche	AF414732
C. passerinae	*Claravis pretiosa* CO-23	Campeche	AF414733
C. passerinae	*Claravis pretiosa* CO-14	Campeche	AF414734
Columbicola macrourae	*Geotrygon montana* CO-8	Campeche	AF414735
C. macrourae	*G. montana* GES308	Campeche	AF414736
C. macrourae	*G. montana* GES308	Campeche	AF414737
C. macrourae	*G. montana* GES308	Campeche	AF414738
C. macrourae	*G. montana* GES408	Campeche	AF414739
C. macrourae	*Leptotila plumbeiceps* MBR4464	Campeche	AF414740
C. macrourae	*L. plumbeiceps* GES410	Campeche	AF414741
C. macrourae	*Leptotila verreauxi angelica* WT16	Texas	AF414742
C. macrourae	*L. v. angelica* WT16	Texas	AF414743
C. macrourae	*L. v. angelica* WT49	Texas	AF414744
C. macrourae	*L. v. angelica* WT49	Texas	AF414745
C. macrourae	*L. v. angelica* WT22	Texas	AF414746
C. macrourae	*L. v. angelica* WT22	Texas	AF414747
C. macrourae	*L. v. angelica* WT48	Texas	AF414748
C. macrourae	*L. v. angelica* WT46	Texas	AF414749
C. macrourae	*Leptotila verreauxi fulviventris* CO-25	Yucatan	AF414750
C. macrourae	*L. v. fulviventris* CO-25	Yucatan	AF414751
C. macrourae	*Zenaida asiatica* WW358	Texas	AF414752
C. macrourae	*Z. asiatica* WW358	Texas	AF414753
C. macrourae	*Z. asiatica* WW388	Texas	AF414754
C. macrourae	*Z. asiatica* WW392	Texas	AF414755
C. macrourae	*Z. asiatica* WW380	Texas	AF414756
C. macrourae	*Z. asiatica* WW407	Texas	AF414757
C. macrourae	*Zenaida macroura* M355	Texas	AF414758
C. macrourae	*Z. macroura* M355	Texas	AF414759
C. macrourae	*Z. macroura* M387	Texas	AF414760
C. macrourae	*Z. macroura* M379	Texas	AF414761
Columbicola baculoides	*Z. macroura* M9	Arizona	AF414762
C. baculoides	*Z. macroura* M9	Arizona	AF414763
C. baculoides	*Z. macroura* M11	Arizona	AF414764
C. baculoides	*Z. macroura* M355	Texas	AF414765
Physconelloides cubanus	*Geotrygon montana* GES308	Campeche	AF414766
P. cubanus	*G. montana* GES308	Campeche	AF414767
P. cubanus	*G. montana* GES408	Campeche	AF414768
P. cubanus	*G. montana* CO-5	Campeche	AF414769
Physconelloides ceratoceps	*Leptotila jamaicensis* MBR4512	Campeche	AF414770
P. ceratoceps	*L. jamaicensis* MBR4512	Campeche	AF414771
P. ceratoceps	*L. jamaicensis* MBR4512	Campeche	AF414772
P. ceratoceps	*Leptotila plumbeiceps* GES288	Campeche	AF414773
P. ceratoceps	*L. plumbeiceps* CO-36	Campeche	AF414774
P. ceratoceps	*L. plumbeiceps* CO-39	Campeche	AF414775

Table 1 *Continued*

Louse Species	Host (identification number)	Locality	GenBank No.
P. ceratoceps	*L. plumbeiceps* CO-39	Campeche	AF414776
P. ceratoceps	*L. plumbeiceps* MBR4464	Campeche	AF414777
P. ceratoceps	*L. plumbeiceps* MBR4464	Campeche	AF414778
P. ceratoceps	*L. plumbeiceps* GES288	Campeche	AF414779
P. ceratoceps	*Leptotila verreauxi angelica* WT14	Texas	AF414780
P. ceratoceps	*L. v. angelica* WT48	Texas	AF414781
P. ceratoceps	*L. v. angelica* WT22	Texas	AF414782
P. ceratoceps	*L. v. angelica* WT49	Texas	AF414783
P. ceratoceps	*Leptotila verreauxi fulviventris* GES387	Campeche	AF414784
P. ceratoceps	*L. v. fulviventris* CO-26	Yucatan	AF414785
Physconelloides zenaidurae	*Zenaida macroura* M379	Texas	AF414786
P. zenaidurae	*Z. macroura* M379	Texas	AF414787
P. zenaidurae	*Z. macroura* M394	Texas	AF414788
Physconelloides wisemani	*Z. asiatica* WW10	Arizona	AF414789
Physconelloides eurysema	*Columbina inca* I99	Texas	AF414790
P. eurysema	*C. inca* I99	Texas	AF414791
P. eurysema	*C. inca* I136	Texas	AF414792
P. eurysema	*Columbina passerina* GES233	Yucatan	AF414793
P. eurysema	*C. passerina* GES233	Yucatan	AF414794
P. eurysema	*C. passerina* GES233	Yucatan	AF414795
P. eurysema	*C. passerina* CO-12	Yucatan	AF414796
P. eurysema	*C. passerina* CO-12	Yucatan	AF414797
P. eurysema	*C. passerina* G96	Texas	AF414798
P. eurysema	*C. passerina* G181	Texas	AF414799
P. eurysema	*C. passerina* G181	Texas	AF414800
P. eurysema	*Claravis pretiosa* GES411	Campeche	AF414801
P. eurysema	*Claravis pretiosa* RCF553	Campeche	AF414802
P. eurysema	*Claravis pretiosa* RCF553	Campeche	AF414803
P. eurysema	*Claravis pretiosa* CO-24	Campeche	AF414804
P. eurysema	*Claravis pretiosa* CO-22	Campeche	AF414805
P. eurysema	*Claravis pretiosa* GES382	Campeche	AF414806

individuals with representatives from each of the four species. For *Physconelloides*, which is more host-specific, we collected four species in Texas (*P. ceratoceps*, *P. wisemani*, *P. zenaidurae*, and *P. eurysema*), three in Campeche (*P. ceratoceps*, *P. cubanus*, and *P. eurysema*), and one in the Yucatan (*P. eurysema*) (see Table 1). For *Physconelloides* we sequenced 42 individuals with representatives from each of the five species of lice.

Sequencing

For each louse specimen we extracted DNA and then prepared it as a morphological voucher specimen. We extracted DNA by removing the head of the louse from the body. Both the head and the body were incubated in extraction buffer (Qiagen) for 42 h at 55 °C. We used a Qiagen Tissue extraction kit for the DNA extraction and followed the manufacturer's protocols. Before filtration of the digestion buffer, we removed the head and the body of the louse from the buffer and reassembled these on a microscope slide as a traditional slide mount. Thus, the voucher specimens retained all morphological features necessary for identification.

Using polymerase chain reaction (PCR), we amplified a portion of the COI gene using the primers L6625 and H7005 (Hafner *et al*. 1994). We used a Perkin Elmer Thermal Cycler 9700 with reaction conditions 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 46 °C for 30 s, 72 °C for 30 s, and 72 °C for 7 min. We purified PCR products using a Qiagen PCR Purification kit. We performed cycle DNA Sequencing with *Taq* FS DNA polymerase using either ABI dRhodamine dye terminators or ABI Prism BigDye Terminators (Perkin Elmer). In sequencing reactions, we used the PCR reaction primers. We reconciled complementary chromatograms and aligned sequences using Sequencher (GeneCodes). This produced a 379-bp fragment of COI for all individual lice (GenBank accession numbers listed in Table 1).

Analysis

We compared the specificity of columbiform wing and body lice worldwide by examining published host records

(Clayton & Price 1999; Price *et al*. 1999; R. D. Price, personal communication). We gave a species of louse a score of 0 if it occurs on only a single species of host. A louse species was given a score of 1 if it occurs on multiple host species in the same host genus. Finally, a species was given a score of 2 if it occurs on multiple host species in different host genera. We compared the overall scores for columbiform wing lice vs. body lice using a Kruskal–Wallis rank sums test.

Because of the large number of equally parsimonious trees, we constructed neighbour-joining (NJ) trees based on the COI sequences using PAUP* (Swofford 2001) for both genera of lice. For purposes of illustrating these trees, we reconstructed the number of changes over these NJ trees using parsimony. For each species of louse we also used 'statistical parsimony' to construct an unrooted haplotype cladogram (Templeton *et al*. 1992) using the computer package TCS (Clement *et al*. 2000). Statistical parsimony both estimates an unrooted haplotype tree, with linkages indicating mutational events, and provides a 95% plausible set of other possible linkages between haplotypes.

We used nested clade analysis, involving categorical tests and clade distance analysis (Templeton *et al*. 1995; Templeton 1998), to explore genetic differentiation among hosts and geographical localities. For this analysis, we considered *L. v. angelica* and *L. v. fulviventris* to be separate host taxa, because phylogenetic analysis of these hosts (unpublished data) indicates substantial genetic divergence between them, comparable to that between other species of *Leptotila*. For the nested clade analysis we first identified 1-step, 2-step, 3-step, etc. clades using the procedures outlined by Templeton *et al*. (1995). We then constructed contingency tables for the nested clades using host taxon or locality as the independent variable. The categorical test evaluates whether there is a significant association between haplotype or nested clade and either geographical location or host association. In essence, these tests reveal whether there is significant genetic structure either geographically or across host species. Significance for categorical tests of host or geographical associations was assessed by randomizing relevant individuals to cells to produce a null distribution from which a *P*-value could be assessed (Hudson *et al*. 1992; Templeton *et al*. 1995). We used the program CHIPERM (Posada 2000) with 1000 randomizations to compute these *P*-values. For these comparisons, only lice from the localities Texas, Campeche, and Yucatan were considered because sufficient numbers of lice were not recovered from Arizona. We used the relevant 1-step, 2-step, 3-step, etc. clades in these categorical tests. However, in some cases nested clades differed from each other by a large (> 20) number of steps, with no intermediate haplotypes. Because we could not resolve the nesting structure of these divergent clades with confidence, we carried out the contingency test analyses on all possible rearrangements of these higher level clades. In addition, we also performed the clade distance analyses (see below) with all possible rearrangements of these high step clades.

We performed the clade distance analyses (Templeton *et al*. 1995) across geographical localities and host species using the program GEODIS (Posada *et al*. 2000). Clade distance analysis indicates whether haplotypes and nested clades are either over or underdispersed with respect to geographical location or host genetic distance. For host 'distances' we computed the pairwise genetic distance between host species using sequences from the mitochondrial gene cytochrome *b* (Johnson & Clayton 2000a,b). We used the latitude and longitude of the collecting locality in each state to compute geographical distances in GEODIS.

Results

Analysis of host specificity scores for columbiform wing and body lice indicated that body lice are on average considerably more host-specific than wing lice worldwide (Kruskal–Wallis $P < 0.0001$). Genetic divergence in COI between species of *Columbicola* ranged from 19.5% to 25.6% uncorrected per cent sequence divergence. Genetic divergence between individuals within species of *Columbicola* ranged between 0.0% and 21.4%. Divergence between species of *Physconelloides* ranged from 8.9% to 17.7%. Genetic divergence between individuals within species of *Physconelloides* ranged from 0.0% to 17.2%.

Phylogenetic analysis of individuals revealed that for both *Columbicola* and *Physconelloides* some species contain distinctive haplotype clusters that are highly divergent from one another (Figs 1 and 2). For *Columbicola* (Fig. 1), haplotypes of *Columbicola gracilicapitis* cluster tightly in one group; maximum divergence between any haplotypes within *C. gracilicapitis* is only 0.8%. This species was found only at the Campeche locality on *Leptotila verreauxi fulviventris*, *L. jamaicensis*, and *L. plumbeiceps*. In contrast, both *C. macrourae* and *C. passerinae* show highly divergent haplotype clusters. Three such clusters are apparent in *C. macrourae* and two are evident in *C. passerinae*. *C. baculoides* was only collected from a single host species, and shows little variation among haplotypes.

In *C. passerinae* the two main haplotype groups are 11.3% divergent from each other and show an association with host species (Fig. 1). One group occurs exclusively on *Claravis pretiosa* (a species of host collected only at the Campeche locality). The other group occurs on both *Columbina inca* and *C. passerina*, and it occurs in both the Texas and Yucatan localities on *C. passerina*. Among individuals within each of these clusters, maximum divergence is only 0.8%. Within *C. macrourae*, there are three clusters of haplotypes differing from each other by between 17% and 21%. Within any of these three groups, maximum uncorrected divergence is only 1.6%. One of these groups occurs

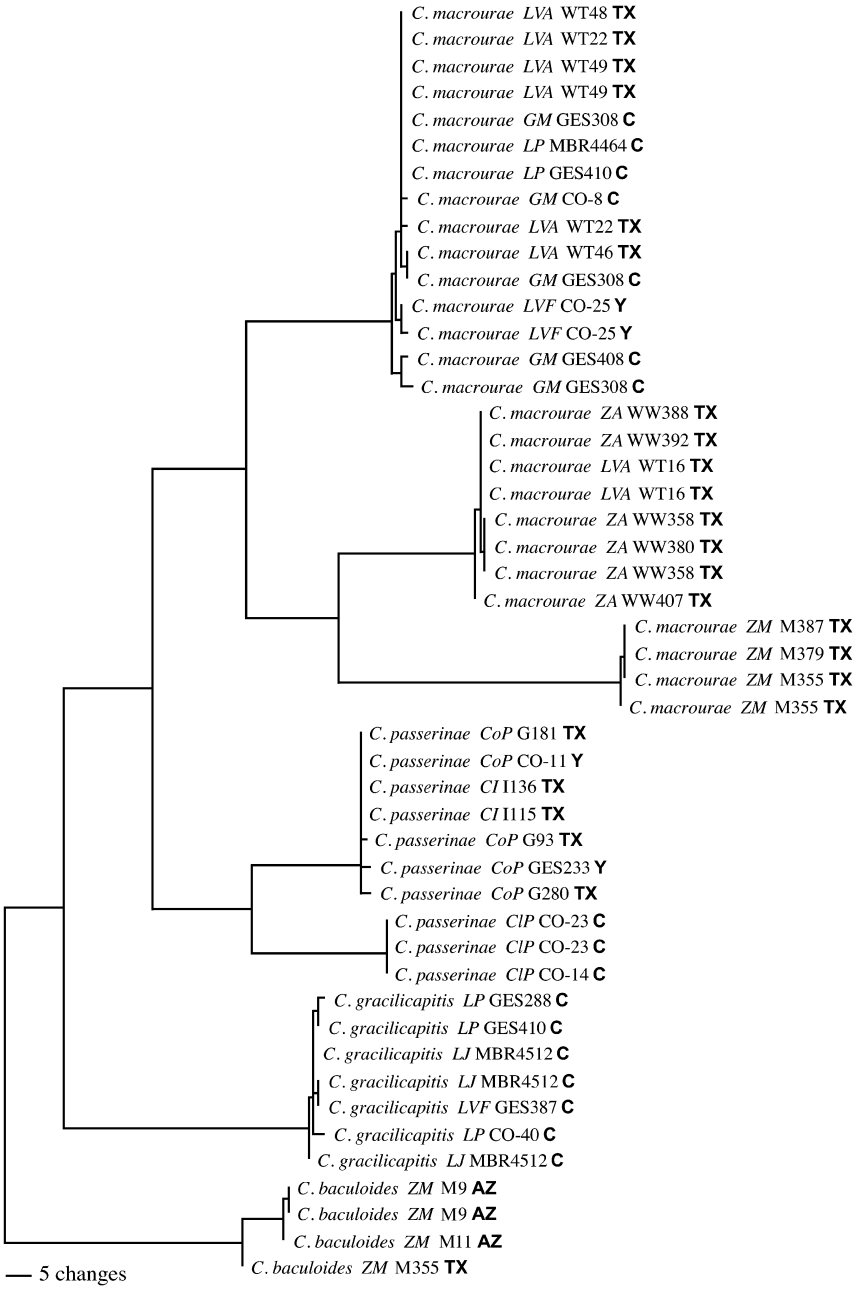

Fig. 1 Tree from neighbour-joining analysis of cytochrome oxidase I sequences for *Columbicola*. Branch lengths are proportional to changes reconstructed using parsimony (scale indicated). Tree is rooted on the midpoint. *C.* = *Columbicola*. Host abbreviations in italics are: *ClP* = *Claravis pretiosa*, *CI* = *Columbina inca*, *CoP* = *Columbina passerina*, *GM* = *Geotrygon montana*, *LJ* = *Leptotila jamaicensis*, *LP* = *Leptotila plumbeiceps*, *LVA* = *Leptotila verreauxi angelica*, *LVF* = *Leptotila verreauxi fulviventris*, *ZA* = *Zenaida asiatica*, *ZM* = *Zenaida macroura*. Host identification numbers (Table 1) are given after each host abbreviation. Localities are indicated in bold, C = Campeche, TX = Texas, Y = Yucatan, AZ = Arizona.

exclusively on *Zenaida macroura*. A second of these groups occurs only in Texas mainly on *Z. asiatica* and less frequently on *L. v. angelica*. The final cluster is found on four host taxa: *Geotrygon montana* (Campeche), *L. v. fulviventris* (Yucatan only) *L. v. angelica* (Texas), and *L. plumbeiceps* (Campeche).

Haplotypes from each of two species of *Physconelloides* (*cubanus*, *zenaidurae*) cluster tightly together with very little intraspecific differentiation (maximum intraspecific divergence 0.3%, Fig. 2). Together with *P. wisemani*, these species of lice are known from only one species of host (Price *et al*. 1999). The remaining two species, *P. eurysema* and *P. ceratoceps*, occur on multiple host species. Both of these louse species exhibit highly divergent haplotype clusters, similar to those found in *Columbicola*.

In *P. eurysema*, there are three clusters of haplotypes differing from each other by 13–17% (Fig. 2). Within each of these groups, maximum divergence is only 1.0%. One of these clusters occurs exclusively on *C. inca* in Texas. A second cluster within *P. eurysema* occurs both in Campeche and Texas, but only on the host *C. passerina*. The final group occurs on both *Claravis pretiosa* (Campeche) and *C. passerina* (Yucatan) in Mexico, but it was not collected from *C. passerina* in Texas.

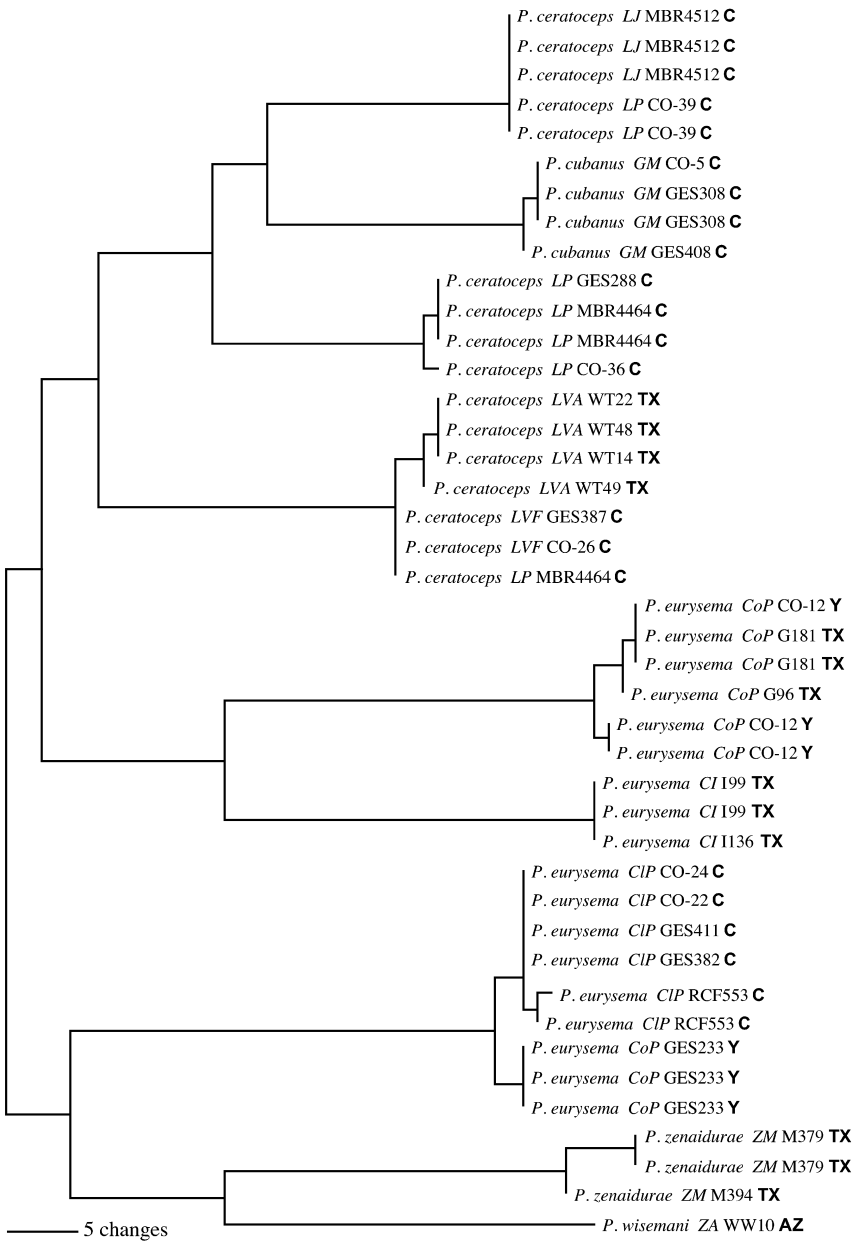

Fig. 2 Tree from neighbour-joining analysis of cytochrome oxidase I sequences for *Physconelloides*. Branch lengths are proportional to changes reconstructed using parsimony (scale indicated). Tree is rooted on the midpoint. *P.* = *Physconelloides*. Host abbreviations in italics are: *ClP* = *Claravis pretiosa*, *CI* = *Columbina inca*, *CoP* = *Columbina passerina*, *GM* = *Geotrygon montana*, *LJ* = *Leptotila jamaicensis*, *LP* = *Leptotila plumbeiceps*, *LVA* = *Leptotila verreauxi angelica*, *LVF* = *Leptotila verreauxi fulviventris*, *ZA* = *Zenaida asiatica*, *ZM* = *Zenaida macroura*. Other conventions as in Fig. 1.

Within *P. ceratoceps*, haplotypes cluster into three divergent groups differing from each other by 9–11% sequence divergence (Fig. 2). One of these groups appears to be more closely related to *P. cubanus* (8%) than it is to the other two groups of *P. ceratoceps* (10–11%), resulting in paraphyly of *P. ceratoceps* (Fig. 2). The maximum divergence within any of these three groups is 0.8%. One of the groups within *P. ceratoceps* is found only on *L. plumbeiceps* in Campeche. A second group is found both on *L. jamaicensis* and more rarely on *L. plumbeiceps* in Campeche. Finally, the third group occurs on *L. verreauxi* in Campeche, Yucatan, and Texas and infrequently on *L. plumbeiceps* in Campeche.

Haplotype networks and nested clades for the species of lice occurring on multiple species of hosts (Figs 3–7) are another way of illustrating genetic diversity and population structure. Little structure among species of hosts is evident in *C. gracilicapitis*. For example, haplotype A (Fig. 3) is found on at least two species of hosts. For *C. gracilicapitis* two categorical tests of haplotypes/clades could be made with host species (Table 2), and neither of these indicated a significant association. In this species, there was one alternative way to join together haplotypes in the parsimony network, but analyses of this alternative arrangement did not change the results. No clade distance analysis was significant for *C. gracilicapitis*.

Columbicola gracilicapitis

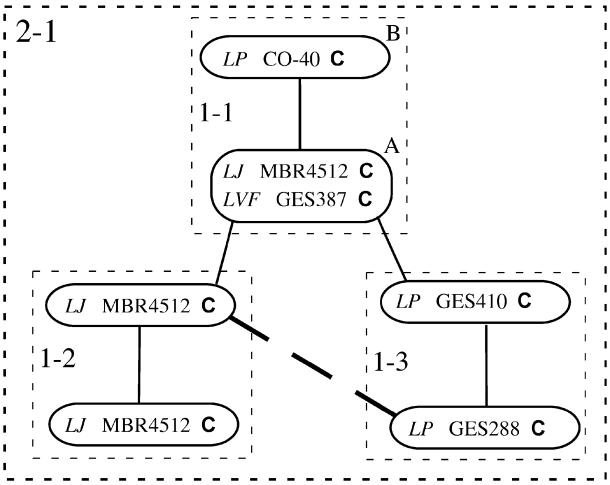

Fig. 3 Haplotype network for *Columbicola gracilicapitis*. Individuals are referenced according to their host (*LJ* = *Leptotila jamaicensis*, *LP* = *Leptotila plumbeiceps*, *LVF* = *Leptotila verreauxi fulviventris*), host identification number, and locality (C = Campeche). Circles indicate haplotypes, which are labelled with letters when necessary for identification. Solid lines connect haplotypes differing by a single step. Rectangles with dashed lines surround nested clades of increasing steps, which are indicated by the label within the relevant rectangle. The heavy dashed line indicates an alternative way of joining haplotypes together in the parsimony network, requiring removal of the connection between GES410 and GES288.

Table 2 Contingency Tables for *Columbicola gracilicapitis**

	Host		
Haplotype	*LP*	*LJ*	*LVF*
A	0	1	1
B	1	0	0
$P = 0.33$			
1–1	1	1	1
1–2	0	2	0
1–3	2	0	0
$P = 0.34$			

*Abbreviations as Fig. 3.

For *C. passerinae*, the haplotype network shows the deep break between those individuals parasitic on *Claravis* and those on *Columbina* (Fig. 4). However, within the *Columbina* clade 2–1 there was no further genetic structure, either geographically or by host (Table 3). Because *C. passerinae* from *Claravis* was collected only in Campeche, there was also a significant association between the two deeply divergent clades and geography (Table 3). Clade distance analysis also detected the geographical structure and structure by host for the two highly divergent clades (1–4 and 2–1).

Columbicola passerinae

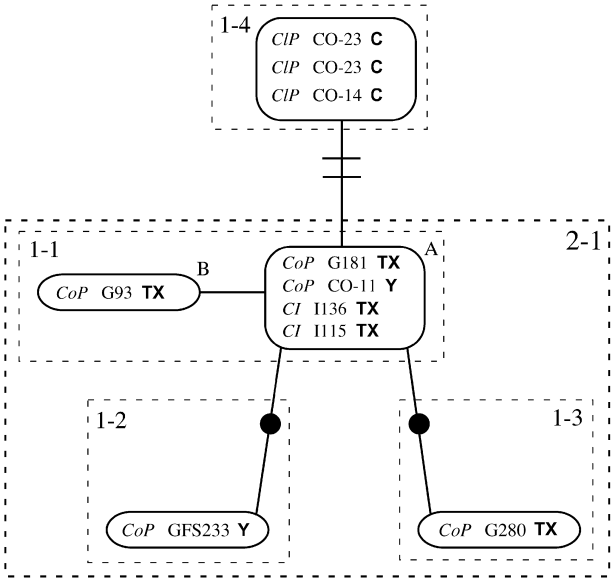

Fig. 4 Haplotype network for *Columbicola passerinae*. Individuals are referenced according to their host (*ClP* = *Claravis pretiosa*, *CI* = *Columbina inca*, *CoP* = *Columbina passerina*), host identification number, and locality (C = Campeche, TX = Texas, Y = Yucatan). Circles indicate haplotypes, which are labelled with letters when necessary for identification. Solid lines connect haplotypes differing by a single step. Solid circles represent haplotypes not observed. Hashes on a solid line indicate clades separated by many (> 20) steps. Rectangles with dashed lines surround nested clades of increasing steps, which are indicated by the label within the relevant rectangle.

C. macrourae is the most widespread species of louse in this study, occurring on six host taxa (Fig. 5). Between the major haplotype groups (3–1, 1–5, and 1–6) there was a significant categorical association with host and with locality (Table 4). However, within each of these haplotype groups there was little substructure. We detected significant structure between nested clades 1–1 and 1–2 (Fig. 5, Table 4); however, none of the seven other comparisons were significant. The most common haplotype (A) within clade 3–1 occurs on three host taxa in two localities. In clade distance analysis for clade 2–1, the clade and nested clade host distances for clade 1–1 were higher than expected by chance (both $P = 0.02$), suggesting haplotypes within 1–1 are overdispersed among host species. This result indicates that this population moves readily between distantly related hosts. For geography, clade 1–1 is also overdispersed when considering clade 1–2 ($P = 0.01$), which is underdispersed ($P = 0.01$). This result indicates the restriction of clade 1–2 to *L. v. fulviventris* in the Yucatan.

Haplotype networks for *P. eurysema*, in addition to showing a large genetic break between clades 3–1, 1–5, and 2–3 (Fig. 6), also revealed further genetic structure

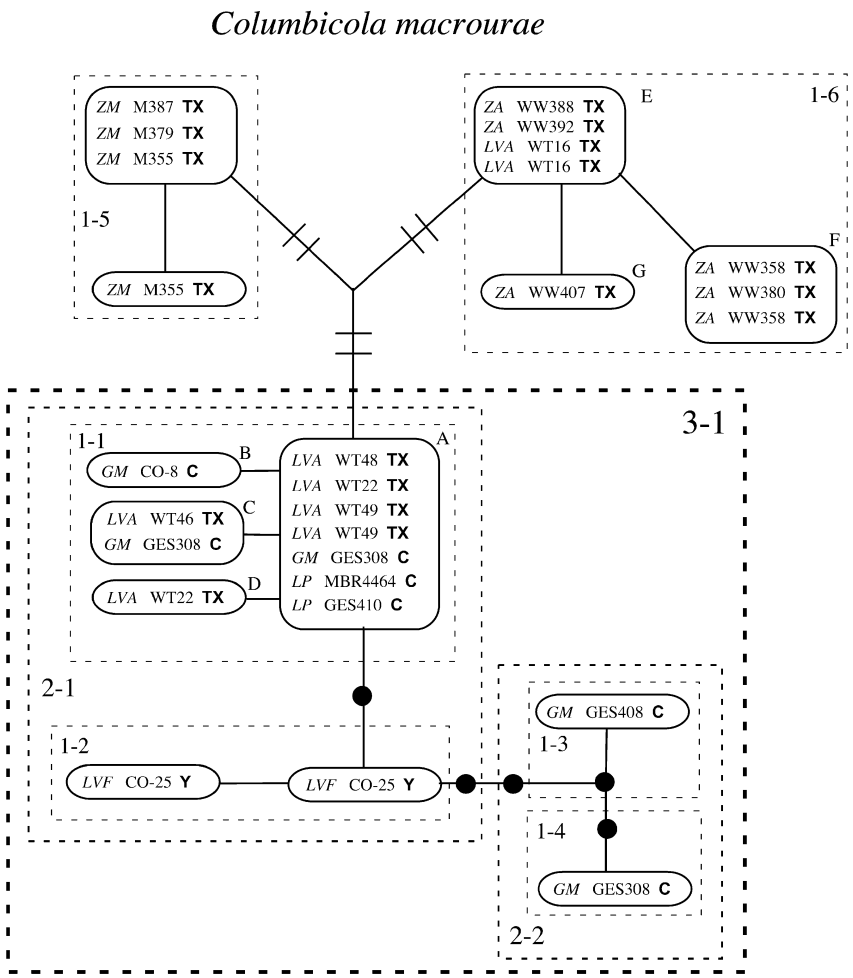

Fig. 5 Haplotype network for *Columbicola macrourae*. Individuals are referenced according to their host (*GM* = *Geotrygon montana*, *LJ* = *Leptotila jamaicensis*, *LP* = *Leptotila plumbeiceps*, *LVA* = *Leptotila verreauxi angelica*, *LVF* = *Leptotila verreauxi fulviventris*, *ZA* = *Zenaida asiatica*, *ZM* = *Zenaida macroura*), host identification number, and locality. Conventions as in Fig. 4.

Table 3 Contingency Tables for *Columbicola passerinae**

	Host				Locality		
Haplotype	*CI*	*CoP*	*ClP*	Haplotype	Texas	Yucatan	Campeche
A	2	2		A	3	1	
B	0	1		B	1	0	
$P = 1.0$				$P = 1.0$			
1–1	2	3		1–1	4	1	
1–2	0	1		1–2	0	1	
1–3	0	1		1–3	1	0	
$P = 1.0$				$P = 0.52$			
2–1	2	5	0	2–1	5	2	0
1–4	0	0	3	1–4	0	0	3
$P = 0.008$				$P = 0.004$			

*Abbreviations as Fig. 4.

(Table 5). For example, within clade 3–1, there is a three step break between lice occurring on *Claravis pretiosa* in Campeche and those on *C. passerina* in the Yucatan. Clade distance analysis also detected significant structuring both within clade 3–1 and between the major haplotype clusters.

Finally, networks of *P. ceratoceps* indicate deep genetic splits between haplotypes occurring mainly on *L. verreauxi*, *L. jamaicensis*, and *L. plumbeiceps* (Fig. 7). This deep genetic split is significantly associated with host and locality (Fig. 7, Table 6). In three cases lice from *L. plumbeiceps* fall

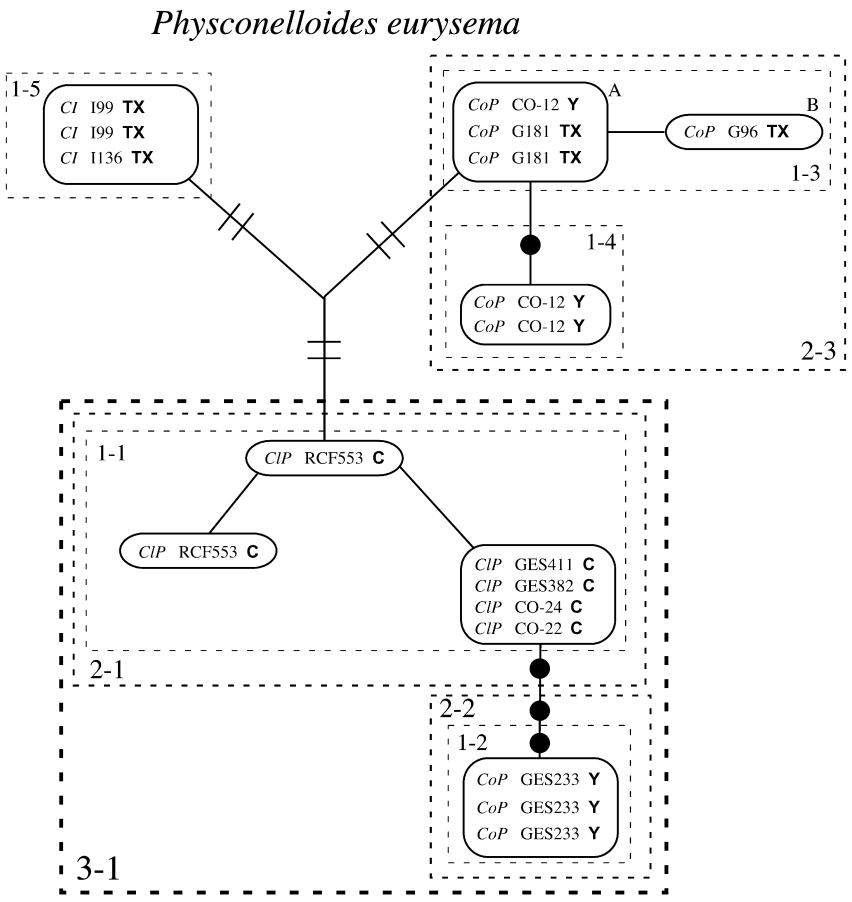

Fig. 6 Haplotype network for *Physconelloides eurysema*. Individuals are referenced according to their host (*ClP* = *Claravis pretiosa*, *CI* = *Columbina inca*, *CoP* = *Columbina passerina*), host identification number, and locality. Conventions as in Fig. 4.

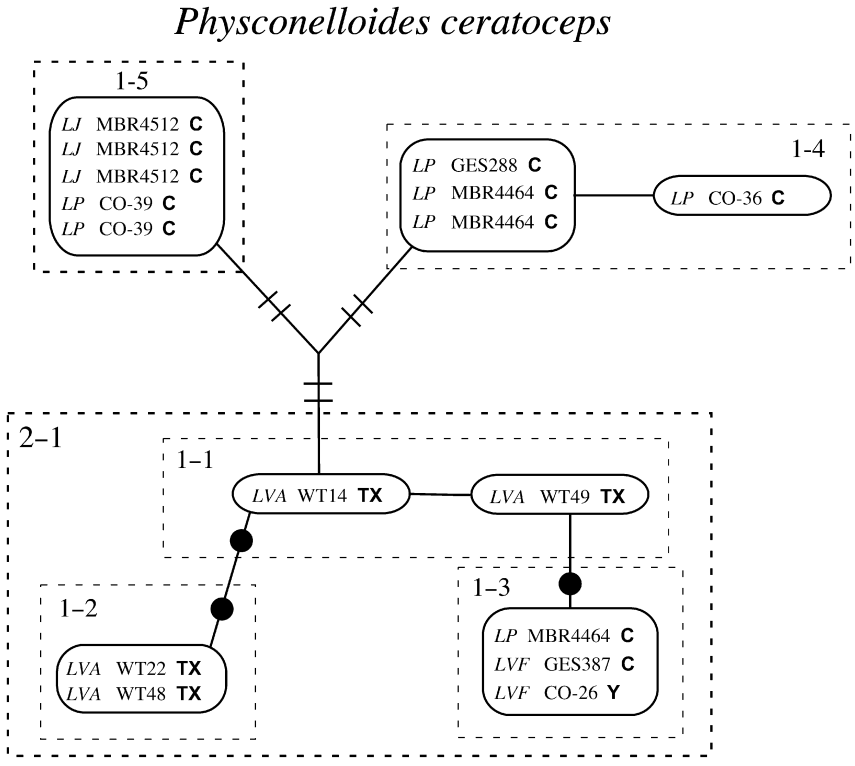

Fig. 7 Haplotype network for *Physconelloides ceratoceps*. Individuals are referenced according to their host (*LJ* = *Leptotila jamaicensis*, *LP* = *Leptotila plumbeiceps*, *LVA* = *Leptotila verreauxi angelica*, *LVF* = *Leptotila verreauxi fulviventris*), host identification number, and locality. Conventions as in Fig. 4.

Table 4 Contingency Tables for *Columbicola macrourae**

	Host							Locality		
Haplotype	*GM*	*LP*	*LVA*	*LVF*	*ZA*	*ZM*	Haplotype	Campeche	Texas	Yucatan
A	1	2	4				A	3	4	
B	0	1	0				B	1	0	
C	1	0	1				C	1	1	
D	0	0	1				D	0	1	
$P = 0.70$							$P = 1.0$			
E			2		2					
F			0		3					
G			0		1					
$P = 0.57$										
1–1	3	2	6	0			1–1	5	6	0
1–2	0	0	0	2			1–2	0	0	2
$P = 0.029$							$P = 0.011$			
2–1	3	2	6	2			2–1	5	6	2
2–2	2	0	0	0			2–2	2	0	0
$P = 0.15$							$P = 0.60$			
3–1	5	2	6	2	0	0	3–1	7	6	2
1–5	0	0	0	0	0	4	1–5	0	4	0
1–6	0	0	2	0	6	0	1–6	0	8	0
$P < 0.0001$							$P < 0.0001$			

*Abbreviations as Fig. 5.

Table 5 Contingency Tables for *Physconelloides eurysema**

	Host				Locality		
Haplotype	*ClP*	*CoP*	*CI*	Haplotype	Texas	Yucatan	Campeche
				A	2	1	
				B	1	0	
				$P = 1.0$			
				1–3	3	1	
				1–4	0	2	
				$P = 0.40$			
2–1	6	0		2–1		0	6
2–2	0	3		2–2		3	0
$P = 0.012$				$P = 0.013$			
3–1	6	3	0	3–1	0	3	6
1–5	0	0	3	1–5	3	0	0
2–3	0	6	0	1–6	3	3	0
$P < 0.0001$				$P = 0.004$			

*Abbreviations as Fig. 6.

Table 6 Contingency Tables for Physconelloides ceratoceps*

	Host				Locality			
Haplotype	*LP*	*LVA*	*LVF*	*LJ*	Haplotype	Campeche	Texas	Yucatan
1–1	0	2	0		1–1	0	2	0
1–2	0	2	0		1–2	0	2	0
1–3	1	0	2		1–3	2	0	1
$P = 0.13$					$P = 0.14$			
2–1	1	4	2	0	2–1	2	4	1
1–4	4	0	0	0	1–4	4	0	0
1–5	2	0	0	3	1–5	5	0	0
$P = 0.002$					$P = 0.02$			

*Abbreviations as Fig. 7.

in clades with either lice from *L. verreauxi* (2–1) or *L. jamaicensis* (1–5); however, all these cases involve lice from two birds in which only one or two individuals of *Physconelloides* were collected. Further sampling should reveal whether these individuals represent rare 'stragglers' (transient dispersers) or established populations on *L. plumbeiceps*. Within clade 2–1, found predominantly on *L. verreauxi*, there appears to be a clustering of haplotypes between *L. v. angelica* (Texas) and *L. v. fulviventris* (Mexico); however, there are not yet enough individuals sequenced for this trend to be significant (Table 6). Even though the contingency table analysis for clade 2–1 was not significant, clade distance analysis detected a significantly low tip vs. interior host distance for this clade ($P = 0.05$) and a significantly high host distance within the nested 1–3 clade ($P = 0.028$). This result indicates the dispersion within locality on distantly related hosts (*L. v. fulviventris* and *L. plumbeiceps*). The presence of these haplotypes on *L. plumbeiceps* may thus represent straggling, rather than established populations on *L. plumbeiceps*.

In summary, for *Columbicola* there were 16 categorical tests for host or geographical association, and only five (31%) of these were significant. In contrast, of 10 categorical tests for *Physconelloides*, six (60%) were significant. These analyses indicate that in general *Physconelloides* shows more genetic structuring than *Columbicola* and this corresponds to the observed levels of host specificity in these two genera, with *Physconelloides* being more host-specific than *Columbicola*.

Discussion

The within-species genetic structure of ischnoceran feather lice on doves in North and Central America is pronounced. Species of both wing (*Columbicola*) and body (*Physconelloides*) lice species exhibit significant population genetic structure. *Physconelloides* exhibits more within-species structure than *Columbicola*, both among localities and among hosts. For conspecific lice found on only one species of dove, mitochondrial DNA differentiation is small, typically less than 1.0% sequence divergence. In contrast, for lice found on more than one host species, differentiation among populations on different host species is often considerable (usually exceeding 10% sequence divergence). In some cases genetic differentiation corresponds closely to different host associations. In other cases, differentiation occurs both between hosts and between localities within hosts. Such dramatic genetic structuring within single morphospecies of lice has important taxonomic implications, and thus implications for assessments of host specificity. The genetic structure indicates considerably more host specificity than would be detected by collecting data on morphospecies alone.

Sequence divergences observed between populations within *Columbicola macrourae*, *C. passerinae*, *Physconelloides eurysema*, and *P. ceratoceps* are on the order of those between morphologically described species (> 10%). For example, *P. cubanus* falls within *P. ceratoceps*, making *P. ceratoceps* paraphyletic. Such differences generally reflect particular host associations and in every case correspond to significant structure as detected by nested clade analysis (Tables 2–6). These divergent lineages could appropriately be considered host races or even cryptic species.

At a finer scale, within the major haplotype clusters, genetic differentiation is still apparent. For *Physconelloides*, differentiation often exists between localities and between hosts within localities (Figs 6 and 7). For *Columbicola*, fine scale differentiation is less pronounced, either between localities or between hosts. In fact, the lack of differentiation between louse populations on different species of hosts for *Columbicola* is somewhat surprising given that the life cycle of the lice is so closely tied to that of the host, and assuming that contact between host species is rare. However, the difference in genetic structure and specificity between *Columbicola* and *Physconelloides* may have a basis in louse dispersal biology. Records of phoresis (hitch-hiking) of lice on hippoboscid flies, which are flying parasites of birds, are numerous (Keirans 1975). For lice occurring on doves, there are many records of phoresis for *Columbicola* (Keirans 1975; Adams, personal observation), however, records of phoresis of dove body lice, including *Physconelloides*, are quite rare. In general, hippoboscid flies are not known to be highly host-specific, and are substantially less specific than feather lice.

In addition to phoresis, other biological differences indicate a higher dispersal capability for *Columbicola* than *Physconelloides*. Dove wing lice can survive away from the host's body longer than can body lice (Rem & Zlotorzycka 1981; Dumbacher 1999; Clayton, unpublished data). Dove wing lice also leave the body of the dead host much more quickly than do body lice (Petryszak *et al*. 1996). In addition, all cases of dove lice occurring on hosts other than doves involve *Columbicola* (Tendeiro 1965; Teel *et al*. 1988; Aguirre & Lozoya 1991; Pavlovic *et al*. 1995). Together these observations point to a greater dispersal ability of *Columbicola* compared to *Physconelloides*. If such differences in dispersal rates exist, then genetic structure between host species should accumulate in populations of *Physconelloides* more readily than in populations of *Columbicola*. This differentiation will affect the degree of host specificity and could ultimately affect the degree of cospeciation with the hosts.

While populations of *Columbicola* can be widely distributed among host species, there is a strong pattern to these distributions. For example, even though all the dove species at each locality are sympatric and live in the same habitats, no species or haplotype of louse occurs on both small ground doves (genera *Claravis* and *Columbina*) and larger ground doves (genera *Geotrygon*, *Leptotila*, and *Zenaida*). In

addition to having quite different body sizes (30–50 g vs. 120–150 g), these two groups of hosts are only distantly related (Johnson & Clayton 2000a). Thus, either host body size or some other factor that is correlated with phylogeny may explain why species of lice are not shared by these two groups of hosts. The body size of species of *Columbicola* is strongly correlated with the body size of its host (Clayton *et al*. 2002), and this may relate to the ability of *Columbicola* to insert between the barbs of the host's wing feathers. Thus, species of *Columbicola* may not be able to establish on host species that differ dramatically in body size, because interbarb spaces are correlated with host size. While a size scaling relationship is not evident in *Physconelloides* (Johnson, unpublished data), dispersal (rather than establishment) may be limiting for these species.

In conclusion, mitochondrial genetic differentiation between populations of lice living on different species of doves is strong. Significant genetic differentiation between geographical localities can also be detected. In cases where geographical structure was detected in lice on the same host species, these hosts are sedentary. However, across the same suite of host species, the genetic structure within species of body lice, *Physconelloides*, is more pronounced than for wing lice, *Columbicola*. Combined with other correlates of dispersal capability, the genetic data suggest that *Physconelloides* is more dispersal limited than *Columbicola*, and this might partially explain differences in the degree of host specificity.

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