Parasitology

cambridge.org/par

High diversity and low genetic structure of feather mites associated with a phenotypically variable bird host

Sofía Fernández-González[1], Antón Pérez-Rodríguez[1,*], Heather C. Proctor[2], Iván De la Hera[1,†] and Javier Pérez-Tris[1]

[1]Departamento de Biodiversidad, Ecología y Evolución, Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain and [2]Department of Biological Sciences, University of Alberta, T6G 2E9 Edmonton, Alberta, Canada

Research Article

*Current address: Department of Zoology and Entomology, Faculty of Natural Sciences, University of the Free State, 9301 Bloemfontein, South Africa.

†Current address: School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland.

Cite this article: Fernández-González S, Pérez-Rodríguez A, Proctor HC, De la Hera I, Pérez-Tris J (2018). High diversity and low genetic structure of feather mites associated with a phenotypically variable bird host. *Parasitology* **145**, 1243–1250. https://doi.org/10.1017/S0031182017002360

Received: 20 October 2017
Revised: 12 December 2017
Accepted: 14 December 2017
First published online: 17 January 2018

Key words:
Astigmata; COI gene; DNA-barcoding; migratory behaviour; symbiont dispersal; symbiont genetic diversity

Author for correspondence:
Javier Pérez-Tris, E-mail: jperez@bio.ucm.es

Abstract

Obligate symbionts may be genetically structured among host individuals and among phenotypically distinct host populations. Such processes may in turn determine within-host genetic diversity of symbionts, which is relevant for understanding symbiont population dynamics. We analysed the population genetic structure of two species of feather mites (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) in migratory and resident blackcaps *Sylvia atricapilla* that winter sympatrically. Resident and migratory hosts may provide mites with habitats of different qualities, what might promote specialization of mite populations. We found high genetic diversity of within-host populations for both mite species, but no sign of genetic structure of mites between migratory and resident hosts. Our results suggest that, although dispersal mechanisms between hosts during the non-breeding season are unclear, mite populations are not limited by transmission bottlenecks that would reduce genetic diversity among individuals that share a host. Additionally, there is no evidence that host phenotypic divergence (associated with the evolution of migration and residency) has promoted the evolution of host-specialist mite populations. Unrestricted dispersal among host types may allow symbiotic organisms to avoid inbreeding and to persist in the face of habitat heterogeneity in phenotypically diverse host populations.

Introduction

Genetic structuring is the outcome of restricted gene flow and lineage divergence during periods of population isolation (Hartl and Clark, 2007). For some organisms, such as obligate symbionts (parasites, mutualists and commensals), population isolation events may take place at very small spatial and temporal scales, because individual hosts represent a patchy and ephemeral habitat (Poulin, 2007; Barrett *et al.* 2008). This forces symbionts to colonize continuously new habitat patches, thereby creating opportunities for population structuring via founder effects, especially if populations established on one individual host originate in a low number of colonizers (Hedrick, 2000). Host population size and symbiont dispersal ability can also influence symbiont genetic structuring (Johnson *et al.* 2002; Whiteman *et al.* 2007; Dabert *et al.* 2015; Martinu *et al.* 2015). Moreover, geographic structuring of host populations themselves, either because of low host vagility, or because of host population isolation in discrete habitat patches, may result in the further genetic structuring of symbiont populations (Harper *et al.* 2015).

The genetic structuring of symbiont infrapopulations (the stock of symbionts that become temporarily isolated in a single host individual; Poulin, 2007) can determine several aspects of their ecological and evolutionary interactions. Regarding within-host population dynamics, individual symbionts may differ in their ability to access host resources, or to occupy the best habitat within the host (Mideo, 2009). Poor competitors may be displaced to areas of inferior quality and have reduced individual fitness (Fretwell and Lucas, 1970). However, within-host fitness differences among symbionts will have evolutionary consequences only in genetically diverse symbiont infrapopulations (Rigaud *et al.* 2010): if all symbionts are close kin because of intense transmission bottlenecks, individuals that occupy poorer microhabitats within the host may still obtain fitness returns from close kin occupying the best habitat (Emlen, 1995). Among-host population dynamics can also be dependent on genetic structuring, because reduced infrapopulation genetic diversity might entail inbreeding costs (Keller and Waller, 2002), while too much gene flow might hamper local adaptation to specific host types (Lenormand, 2002). Host specificity is thus a further element driving the genetic structuring of symbiont populations: some symbionts may exploit different host types (either different host species, or host populations of the same species occupying contrasting environments), which may differ in their spatiotemporal distribution or in their suitability for the symbionts. For example, alternative host types may offer different quantity or quality of resources to the symbiont (Fernández-González *et al.* 2013), which may promote specialization of symbionts, and ultimately genetic isolation among populations of symbionts associated with different host types (Nadler, 1995; Rigaud *et al.* 2010).

© Cambridge University Press 2018

CAMBRIDGE UNIVERSITY PRESS

Population genetic structure of symbionts should inform our understanding of their ecology and evolution (Hewitt, 2001), but the genetic composition of symbiont populations within host individuals, and the genetic structuring of such populations among individual hosts and host types, remain obscure for most host–symbiont systems (Nadler, 1995; McCoy *et al.* 2003; Doña *et al.* 2015*a*). We studied the genetic structuring of two species of astigmatan feather mites, *Proctophyllodes sylviae* Gaud (Proctophyllodidae) and *Trouessartia bifurcata* (Trouessart) (Trouessartiidae) sampled from resident and migratory blackcaps, *Sylvia atricapilla* (L.) (Sylviidae), coexisting in winter in Southern Spain. Feather mites are a very diverse and broadly distributed group of avian mutualists, composed mostly of host specialists (Proctor, 2003). Estimates of genetic diversity calculated for mitochondrial DNA show that it depends to some extent on average infrapopulation size (Doña *et al.* 2015*a*), which is in turn roughly repeatable for a given species of feather mite (Díaz-Real *et al.* 2014). As for most feather mites, *P. sylviae* and *T. bifurcata* are only known to be transmitted by direct contact between hosts (Proctor, 2003), so that their genetic diversity on a single-host individual might be very reduced if the number of founders is low.

Blackcaps are the main host of the two studied species of feather mite, which live on the flight feathers of the wing (Doña *et al.* 2016). Blackcaps breeding in medium and high latitudes of Western Europe typically winter around the Mediterranean region, where they coexist with resident blackcap populations (Pérez-Tris and Tellería, 2002*a*). The extreme SW of Spain, where this study was conducted, offers an exceptional opportunity to study the symbiont communities of birds that, although temporally sympatric during winter, are effectively segregated during the breeding period and have undergone contrasting evolutionary paths (Pérez-Tris *et al.* 2004). Resident birds provide mites with more stable environmental conditions, while migrants move between contrasting regions biannually. Resident and migratory blackcaps also differ as 'habitats' for feather mite infracommunities. Residents are longer-lived (Pérez-Tris and Tellería, 2002*b*), have differently shaped wings and structurally different feathers (De la Hera *et al.* 2009). They also possess larger uropygial glands, which likely make them more rewarding hosts from a nutritional perspective (vane-dwelling feather mites feed on the oil of the uropygial gland that covers the feathers, and on the particles embedded within; Proctor, 2003).

In bird species with no social behaviour (as blackcaps), feather mite transmission is posited to be reduced to male–female and parent-nestling contact during the breeding season (Proctor, 2003; Doña *et al.* 2017*a*). It is thus likely that if the differences between migratory and resident blackcaps have promoted mite specialization, mite populations may have a detectable genetic structure related to host type. However, the coexistence of migratory and resident blackcaps may provide opportunities for mite dispersal among hosts with different phenotype and geographic origins, thereby preventing genetic structuring of mite populations.

These processes may further differentiate between the two species studied, which are unequally distributed between host types: whereas *P. sylviae* occurs with similar prevalence on migratory and resident blackcaps, it is more abundant on migratory blackcaps. In contrast, *T. bifurcata* is rarely found on migratory blackcaps (Fernández-González *et al.* 2013). Such a pattern of segregated distribution may be associated with differences between mite species in host preference, dispersal capabilities, or in-host population dynamics, all of which could lead to variation in the patterns of genetic structure between mite species. In addition, the coexistence of both mite species happens at the expense of the smaller species (*P. sylviae*), which on resident hosts is displaced by *T. bifurcata* from preferred wing areas and attains lower population size (Fernández-González *et al.* 2015). Because of these interspecific interactions, if within-host genetic diversity of mites depends on population size (Doña *et al.* 2015*a*), the genetic diversity of *P. sylviae* could be lower on resident than on migratory hosts, where infrapopulation size is not limited by the existence of the other species.

Our study aims to elucidate: (1) the genetic implications for symbiotic feather mites of the coexistence of different host phenotypes in the same habitat (i.e., sharing of wintering grounds by migratory and resident blackcaps), and (2) whether the pattern of genetic diversity differs between the two species of symbionts. To this end, we analysed genetic structure among mite infrapopulations (with the host individual as the habitat patch for mites), both within and between host groups (with migratory or resident blackcaps as types of hosts that may harbour genetically distinct mite infrapopulations).

Materials and methods

Study site and field methods

Blackcaps were captured during two winter seasons (February and December 2010) in a forest of the Campo de Gibraltar region (extreme south of Spain: 36°9′33.98″N, 5°34′50.0″W). A total of 160 birds were mist-netted and individualized with metal rings. We measured the length of the eighth primary, tail length and the difference between the distances from primary feathers 1 and 9 to the wing tip to classify blackcaps as migratory or resident, using a discriminant function that correctly assigns >97% of individuals (De la Hera *et al.* 2012).

Blackcaps were held individually until processing in bird bags to prevent mite cross-contamination. Mites were retrieved by immersing several wing feathers in tubes filled with absolute ethanol, trying to sample from the whole area of the wing that was populated by mites. Blackcaps were subsequently released unharmed. The samples were stored at −20 °C until analysed. We aimed to obtain five individuals of each mite species from each host that could be typed for genetic analyses and slide-mounted for species identification by microscopy. *Proctophyllodes* females cannot be reliably determined to the species level based on morphology (Atyeo and Braasch, 1966), so that although no other species of *Proctophyllodes* than *P. sylviae* are known from *S. atricapilla*, we erred on the side of caution by only sampling male mites. We sampled as many males as it was possible for *Trouessartia* (which was far less abundant), but some female individuals were included in our analyses as females can be identified to species (Santana, 1976). Among the blackcaps that harboured a sufficient number of male *Proctophyllodes* mites, we selected 24 individuals (12 migratory and 12 resident). These birds included nine resident blackcaps that also harboured *Trouessartia* mites (so that the sample of hosts used to test for population structure of the two mite species overlapped as much as possible). We also included three migratory blackcaps that harboured *Trouessartia* mites (we did not have any migratory individuals with a sufficient number of mites of both species). Therefore, the total number of hosts sampled was 27.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from individual mites using a DNeasy Blood and Tissue Kit (Qiagen, USA), following a specific protocol that modified the manufacturer's instructions (Dabert *et al.* 2008; M. Dabert, personal communication). Individual mites were transferred from the original stock to tubes containing 180 μL of ATL lysis buffer with 20 μL of Proteinase K (Qiagen, USA), which were then incubated at 57 °C

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

with 500 rpm shaking in a thermoshaker (GRANT®) for 72 h, vortexing thoroughly and spinning down the samples every day. After digestion, the sample was mixed by vortexing for 10 s and spun down. The supernatant was transferred to a new tube for DNA isolation, and the exoskeleton of the mite was stored in 80% ethanol at −20 °C until mounted on PVA (polivynil alcohol) and used for species identification with a Leica DM 2500/BF with Differential Interference Contrast, following the keys of Atyeo and Braasch (1966) and Santana (1976). All mites employed in our analyses were morphologically confirmed to be either *P. sylviae* or *T. bifurcata*.

The metazoan DNA barcoding fragment [661 bp near the 5′ end of the cytochrome oxidase I (COI)] mitochondrial gene] was amplified by PCR with the degenerated primers bcdF05 and bcdR04 (Dabert *et al.* 2008). PCR reactions were carried out in 10 μL total volume, and contained 5 μL of Type-it Microsatellite PCR Kit (Qiagen, USA), 5 pmoles of each primer, and 4 μL of template DNA (undiluted DNA extract). Reaction conditions consisted of one initial step of 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 60 s at 50 °C, 60 s at 72 °C, with a final extension step of 5 min at 72 °C. After amplification, 5 μL of purified water was added to PCR products, and 5 μL of the diluted PCR product was visualized on 2% agarose gels stained with GelRed™ (Biotium, USA) under UV light. After electrophoresis, 5 μL of purified water was added to the remaining PCR product. Bands of sufficient quality were sequenced from both ends with an ABI 3730 XL automated sequencer (Applied Biosystems) using 1–1.5 μL of diluted PCR product and 50 pmoles of each primer. Sequences were edited manually using BioEdit 7.0.5.3 (Hall, 1999) and aligned using ClustalW as implemented in the same program.

Genetic analyses

Since there are several cases of cryptic speciation described among feather mites (Doña *et al.* 2015*b*), before performing our analyses of population genetic structure, we wanted to ensure that we were working with samples pertaining to a single species. To do so, we compared our mite sequences with all the other haplotypes of COI amplified from feather mites of the same two species available in GenBank (downloaded the 27 September 2016). We included as well COI haplotypes of the mite species most closely related to our two focal ones (according to Doña *et al.* 2017*b*). After discarding the sequences with low coverage or ambiguous nucleotides, our final working file (including our sequences) was composed of 150 sequences of *Proctophyllodes* spp. belonging to five morphospecies, and of 46 sequences of *Trouessartia* spp. belonging to four morphospecies. Sequences were trimmed to a final length of 505 bp, which included the hypervariable minibarcoding sequence of 200 bp identified by Doña *et al.* (2015*b*) as a good marker of feather mite species limits. To estimate the relationships among sequences, we conducted a neighbour-joining phylogenetic analysis using MEGA7 (Kumar *et al.* 2016) using the Kimura two-parameter (K2P) substitution model. Support for internal nodes was derived from a bootstrap resampling with 1000 replications.

After establishing that our sequences of *P. sylviae* and *T. bifurcata* pertained to well-defined species (see Results), to estimate population genetic structure of mites between migratory and resident blackcaps, and among host individuals within blackcap populations, we conducted simple and hierarchical analyses of molecular variance (AMOVA) using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). We used jModelTest 2.1.4 (Darriba *et al.* 2012) to infer the most appropriate model of nucleotide substitution for the COI gene in each mite species (TPM2uf + I + G for *P. sylviae* and HKY + I for *T. bifurcata*). However, given that the Arlequin software does not implement these models, we used the Tamura and Nei model for both mite species (with $\alpha = 0.24$ for *P. sylviae*). This was the 6th best model according to the Akaike Information Criterion implemented in jModelTest, and according to the model parameters it was the closest to the best models among the available in Arlequin. We tested the statistical significance of population genetic structure using 1000 permutations. To be sure that potential differences between mite species were not affected by the mix of *T. bifurcata* males and females if mite dispersal is somehow sex-biased, we also repeated the AMOVA analysis using only male mites (although at the expense of sample size and statistical power).

The evolutionary relationships among all our unique haplotypes of *P. sylviae* and *T. bifurcata* were reassessed separately for each species (alignments of our sequences were 661 bp long). We performed a neighbour-joining analysis using MEGA7 (Kumar *et al.* 2016) and the K2P substitution model. Support for internal nodes was derived from a bootstrap resampling with 1000 replications. Furthermore, to better visualize the patterns of population structure of mites among host groups, a haplotype network was built for each mite species with the software NETWORK (Fluxus Technology), using the Median-Joining algorithm. For each mite species, we computed the mean genetic distance between haplotypes on the same host individual in order to better assess the degree of within-host genetic resemblance among mites.

Results

We found 72 haplotypes among 93 sequenced *P. sylviae* individuals, and 29 haplotypes among the 58 *T. bifurcata* individuals (Table S1, on Supporting Information). For an alignment length of 661 bp, the number of polymorphic sites was 108 for *P. sylviae* and 58 for *T. bifurcata*, and nucleotide diversity was 0.020 and 0.014, respectively. Sequence data met the assumption of selective neutrality for both species, as shown by non-significant Tajima's *D* statistics (in both cases with $P > 0.05$). All haplotypes were deposited in GenBank with accession numbers KF613605–KF613676 (*P. sylviae*), and KF613684–KF613716 (*T. bifurcata*).

In relation to the sequences assigned to other feather mite morphospecies, our sequences of *P. sylviae* were recovered as components of a single clade, amongst sequences of the same morphospecies retrieved from other studies (99% bootstrap support) and with no evident internal structure. The same was true for *T. bifurcata* (73% bootstrap support), although there were only two sequences available for comparison.

Genetic diversity among hosts was very high: for *P. sylviae*, we only found one haplotype shared by mites on two host individuals, whereas for *T. bifurcata* we found one haplotype shared by mites on three hosts and another shared by mites on two hosts. In contrast, individual mites more frequently shared the same haplotype at the infrapopulation level (Fig. 1). This trend was stronger for *T. bifurcata*: an AMOVA analysis revealed that, whereas in *P. sylviae* more than 80% of the genetic variance could be explained by differences within mite infrapopulations, this value was only roughly 50% for *T. bifurcata* (Table 1). This pattern held true when analysing only *T. bifurcata* males (Table 1).

When the same test was conducted with individual hosts classified as migratory or resident in a hierarchical AMOVA, a similar amount of genetic variance was explained by differences among infrapopulations of the same host group (Table 1). However, no genetic structure was detected between populations of mites sampled on migratory or resident blackcaps (Table 1). This was supported also by the phylogenetic reconstructions at the mite species level and the haplotype networks, where haplotypes coming from mites sampled on resident or migratory hosts do not

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

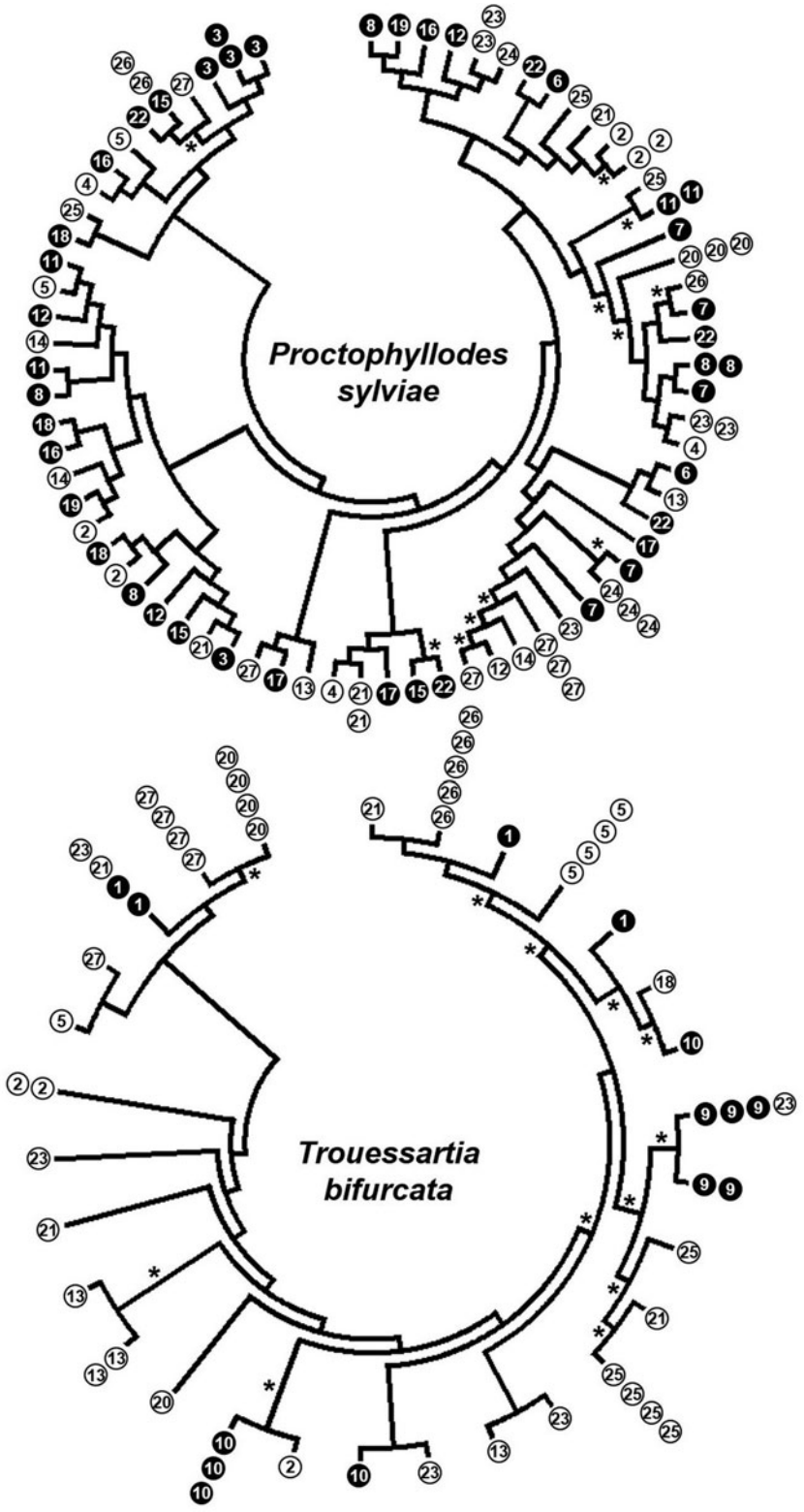


Fig. 1. Evolutionary relationships among the cytochrome oxidase I (COI) haplotypes of feather mites sampled from wintering blackcaps. Optimal neighbour-joining (NJ) trees of the haplotypes of *Proctophyllodes sylviae* and *Trouessartia bifurcata* found in this study, calculated after 1000 bootstrap replications. * indicates bootstrap support on nodes > 50%. The numbers in the circles indicate individual hosts of origin of each haplotype (open circles represent resident blackcaps and filled circles migratory blackcaps). The connections between haplotype and host ID's can be seen in Table S1.

form any sort of clustering (Figs 1 and 2). The distribution of average within-host pairwise genetic distances among feather mites revealed that blackcaps harboured unrelated mites as a rule (Fig. 3).

Discussion

Our analyses unveiled high within-host genetic diversity of two feather mite species living on migratory and resident blackcaps. Remarkably, this genetic diversity was not structured between host types, despite the fact that resident and migratory birds have contrasting population histories and phenotypic attributes. The specific identity of all the sampled mites was ascertained both by microscopy and by molecular means, ruling out the possibility that our samples contained a mix of cryptic mite species that could distort our estimates of genetic diversity. At the infrapopulation level, genetic diversity was significantly greater for one of the two mite species (*P. sylviae*), what shows that the pattern of genetic structuring of mite populations may differ among mite species that share hosts in the same environment.

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

Table 1. Results of analyses of molecular variance (AMOVA) for population genetic structure of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* in migratory and resident blackcap populations

	P. sylviae				*T. bifurcata*				*T. bifurcata* males only			
Population structure tested	d.f.	Var. comp.	% Var.	*P*	d.f.	Var. comp.	% Var.	*P*	d.f.	Var. comp.	% Var.	*P*
No grouping												
Among infrapopulations	23	1.431	18.79	<0.001	11	2.241	48.29	<0.001	10	1.881	36.81	<0.001
Within infrapopulations	69	6.184	81.21		46	2.400	51.71		16	3.229	63.19	
Between host populations												
Between host types	1	−0.011	0	0.421	1	−0.137	0	0.663	1	−0.201	0	0.663
Among infrapopulations	22	1.437	18.88	<0.001	10	2.296	50.36	<0.001	9	1.980	39.54	<0.001
Within infrapopulations	69	6.184	81.27		46	2.400	52.64		16	3.229	64.49	

The analyses partition total molecular variance into different components, whose significance was obtained by randomization after 1000 permutations.

Large levels of intraspecific COI gene genetic diversity had already been reported by Doña *et al.* (2015*a*) for *P. sylviae*, as well as several other species of European feather mites, although their sampling was not designed to detect genetic structuring among discrete bird populations (samples of *P. sylviae* included in the analyses, for instance, came from breeding birds sampled in Russia and from wintering birds of unknown origin captured in Spain; R. Jovani, personal communication). It is important to recall that the resident blackcaps sampled in this study represent a distinctive population within the host's range. They show genetic evidence of reproductive isolation from other populations, and a number of phenotypical traits of potential relevance for feather mite biology that make them different from migratory blackcaps (Pérez-Tris and Tellería, 2002*a*; Pérez-Tris *et al.* 2004; Fernández-González *et al.* 2013). Contrary to our expectations, we did not find any genetic structuring of mites inhabiting resident blackcaps in relation to those sampled from migratory hosts: terminal nodes within each mite species' COI tree were randomly distributed among host individuals and host types. Neither was there any trace of a founder effect in the haplotype networks, what on the contrary is clearly shown by mites sampled elsewhere (Dabert *et al.* 2015; Doña *et al.* 2015*a*).

The absence of mite genetic structure between host types suggests that the coexistence of blackcaps from different geographic origins in sympatric wintering areas promotes the interchange of mites outside the host's breeding season. Nevertheless, mechanisms of transmission different from parent–offspring transmission, which could explain the exchange of mites between hosts outside the breeding season, are yet to be explored. Several groups of feather symbionts (some groups of feather lice and mites) are known to be transmitted phoretically by louse flies (Hippoboscidae), but this behaviour has not been found for the mites under study (Jovani *et al.* 2001). In our study region, blackcaps do protect small feeding territories around particularly attractive bushes, and do engage in the odd fight (personal observation). Fighting has been posited by Dabert *et al.* (2015) as a potential mechanism of mite exchange between skua species, despite the fact that feather mites display a number of morphological adaptations directed to avoid the dislodging of feather mites from their hosts (Doña *et al.* 2017*a*).

Incomplete lineage sorting could also explain this pattern, because the actual haplotype sharing between resident and migratory blackcaps is reduced to one haplotype of *P. sylviae* and two of *T. bifurcata*. Still, a few dispersal events each winter might represent a number of migrant mites per generation large enough to erase any structure associated with host population (Slatkin, 1987). On the other hand, the lack of genetic clustering found among the mites of some fully sedentary birds species studied by Doña *et al.* (2015*b*) suggest that in spite of the apparent genetic isolation of sedentary blackcaps, a stepwise transmission of mites following short-distance host dispersal could be sufficient to dilute the genetic structuring of mite populations. Actually, differences between the effective population sizes of both blackcaps and mites might explain that the degree of genetic structuring found among blackcaps is not mirrored by their symbionts (Criscione, 2008).

Once acquired, the high genetic diversity of mite infrapopulations revealed in our study seems incompatible with the existence of severe bottlenecks during mite transmission. Fledglings are colonized by large numbers of mite nymphs coming from their parents (Doña *et al.* 2017*a*), so that mite infrapopulation genetic diversity would likely be preserved across bird generations. At the infrapopulation level, some individuals sampled on the same host shared COI haplotypes, which is to be expected if related mites from the same founder stock are sampled. Some degree of genetic homogeneity at the infrapopulation level has been described

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

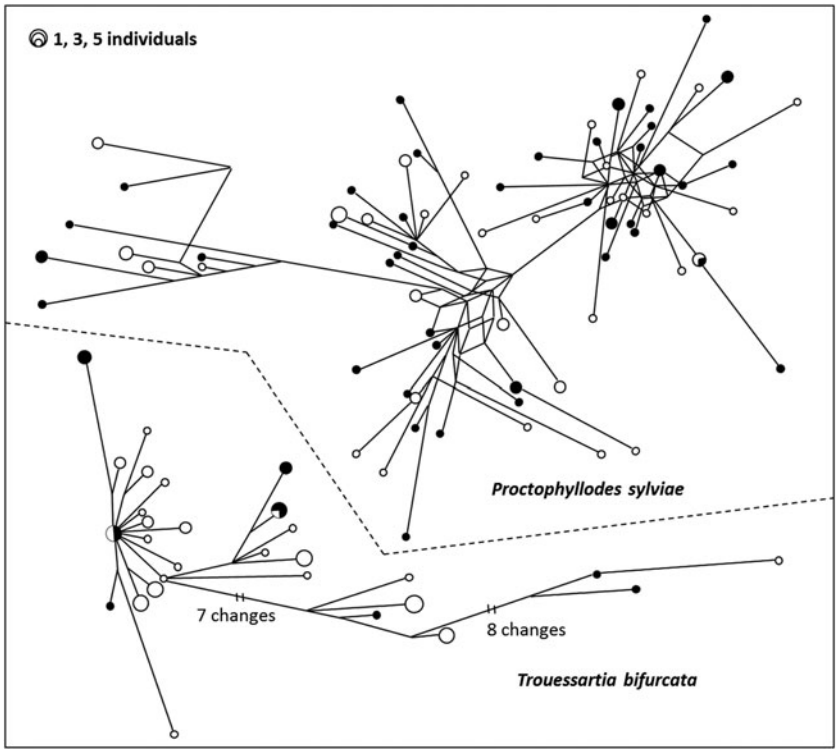


Fig. 2. Haplotype networks for the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from wintering blackcaps. Shading in the circles indicates resident (white) or migratory (black) blackcaps. The shortest link between haplotypes sets the scale for 1 bp sequence difference, and the size of circles is proportional to haplotype frequency.

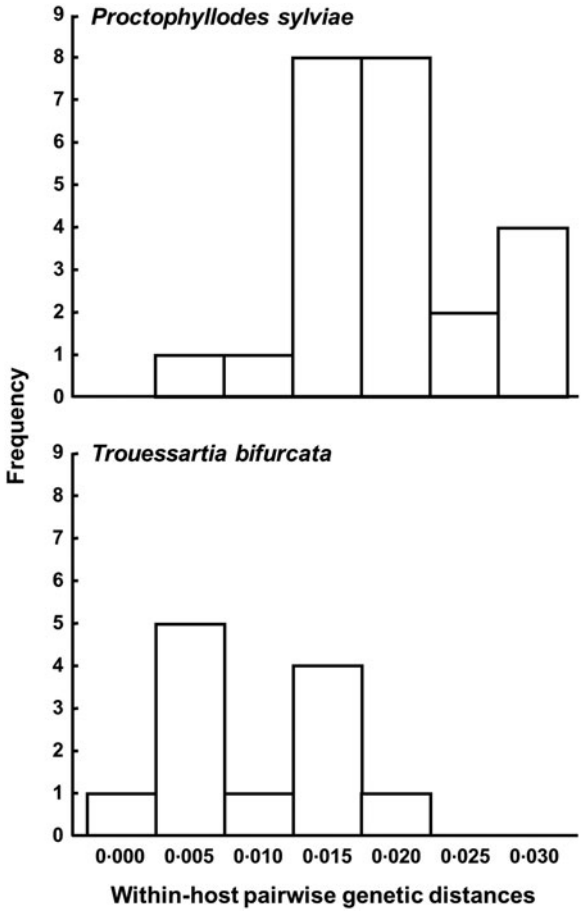


Fig. 3. Frequency distribution of average within-host pairwise genetic distances among haplotypes of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from blackcaps.

recently in other feather mite species (Dabert *et al.* 2015; Doña *et al.* 2015*a*); however, we still found a great genetic diversity of mite infrapopulations, even if our small sample size (five mites per host) somewhat limits our capacity to detect many different haplotypes in the same infrapopulation.

Differences in infrapopulation genetic diversity between both mite species also call for an explanation. *Trouessartia bifurcata* is much more mobile along the feathers than *P. sylviae* (personal observation), so that in theory it could switch between individual hosts more easily, yet it is the species with the lower infrapopulation genetic diversity. In line with the findings of Dabert *et al.* (2015), differences between the infrapopulation genetic diversity of the two mite species could be related to their degree of host specificity. *Trouessartia bifurcata* occurs very rarely on migratory hosts, while *P. sylviae* is equally frequent in both host types (Fernández-González *et al.* 2013; 2015). Even though *P. sylviae* is much more abundant in migratory hosts (Fernández-González *et al.* 2013, 2015), its infrapopulation genetic diversity did not change between host types. Furthermore, even in sedentary birds, the average number of *T. bifurcata* individuals per host is lower than that of *P. sylviae*, so that differences between the effective population sizes could be behind these differences in genetic diversity (Criscione, 2008). Although we are aware of the problems of interpreting two-species comparisons from an adaptive perspective (e.g. Garland and Adolph, 1994), our results show that peculiarities of each symbiont species may shape the patterns of genetic structuring of different symbionts that share host species.

Since feather mites are obligate symbionts, their populations are subjected to environmental changes associated with host phenotypic diversity and host habitat use (Proctor, 2003). Our results suggest a scenario in which a mite that lives on a resident blackcap may sometimes have its offspring living on a migratory host, which differs in evolutionary history, geographic origins and ecological attributes. This scenario in which mites with different ancestry can end up sharing a host may have important implications in intra-host mite interactions. Previous research on the

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

same study system analysed here has revealed a non-random distribution of feather mites among wing feathers and sectors of the same feather in blackcaps (Jovani and Serrano, 2004; Fernández-González *et al.* 2015), suggesting that some areas of the bird plumage may be preferred and others may be avoided by different species. If competition determines the distribution of mites among host microhabitats of different quality, our results suggest a scenario in which mites are unable to compensate for the costs of occupying poor sectors through inclusive fitness returns, because in genetically diverse mite populations there is no guarantee that the best sectors will always be occupied by close kin. Still, whether different mite families segregate among sectors of the host plumage, or freely mix among host microhabitats, remains an open question for future research.

As a final remark, it should be taken into account that both *P. sylviae* and *T. bifurcata* have been reported from several other host species (see records in Doña *et al.* 2016). Whether the amount of genetic variation observed in our study may be associated with their degree of host specialization can only be answered with broader comparative analyses. Since the very few studies analysing genetic diversity within feather mite species have documented cases of both cryptic speciation and of mites with no genetic structuring whatsoever between different hosts (Dabert *et al.* 2015; Doña *et al.* 2015*a*; Szudarek *et al.* 2017), the exploration of how population structure varies among different mite species on different host species is a promising research field. This study makes thus a significant contribution to our understanding of the evolutionary implications of competitive asymmetries among symbionts that share an individual host, as well as of the factors that may promote or hamper the genetic structuring of symbiont communities among different host populations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182017002360.

Acknowledgements. We thank Miroslawa and Jacek Dabert for advice on DNA extraction and genotyping of mites, Roger Jovani for comments on the sampling of his papers, and Alejandro Llanos for inspiring discussions. Kaylee Byers revised our English. The comments of Staffan Bensch, Jorge Doña and three anonymous reviewers improved earlier versions of this manuscript. All samples were collected under license from Junta de Andalucía (SGYB-AFRCMM). This is a contribution from the Moncloa Campus of International Excellence of the Complutense and the Polytechnic Universities of Madrid.

Financial support. This study was funded by the Ministry of Science and Innovation (grants CGL2007-62937/BOS, CGL2010-15734/BOS and CGL2013-41642-P/BOS, and a FPI studentship to SFG), the Ministry of Education (FPU studentship to APR), the Basque Government (BFI04-33 and 09-13 studentships to IH) and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to HP.

References

Atyeo WT and Braasch NL (1966) The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). *Bulletin of the University of Nebraska State Museum* **5**, 1–354.

Barrett LG, Thrall PH, Burdon JJ and Linde CC (2008) Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends in Ecology & Evolution* **23**, 678–685.

Criscione CD (2008) Parasite co-structure: broad and local scale approaches. *Parasite-Journal de la Societé Francaise de Parasitologie* **15**, 439–443.

Dabert J, Ehrnsberger R and Dabert M (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, Coulson SJ, Gwiazdowicz DJ, Moe B, Hanssen SA, Biersma EM, Pilskog HE and Dabert J (2015) Differences in speciation progress in feather mites (Analgoidea) inhabiting the same host: the case of *Zachvatkinia* and *Alloptes* living on Arctic and long-tailed skuas. *Experimental and Applied Acarology* **65**, 163–179.

Darriba D, Taboada GL, Doallo R and Posada D (2012) Jmodeltest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

De la Hera I, Pérez-Tris J and Tellería JL (2009) Migratory behaviour affects the trade-off between feather growth rate and feather quality in a passerine bird. *Biological Journal of the Linnean Society* **97**, 98–105.

De la Hera I, Pérez-Tris J and Tellería JL (2012) Habitat distribution of migratory and sedentary blackcaps *Sylvia atricapilla* wintering in southern Iberia: a morphological and biogeochemical approach. *Journal of Avian Biology* **43**, 333–340.

Díaz-Real J, Serrano D, Pérez-Tris J, Fernández-González S, Bermejo A, Calleja JA, De la Puente J, De Palacio D, Martínez JL, Moreno-Opo R, Ponce C, Frías O, Tella JL, Moller AP, Figuerola J, Pap PL, Kovacs I, Vagasi CI, Meléndez L, Blanco G, Aguilera E, Senar JC, Galván I, Atienzar F, Barba E, Cantó JL, Cortés V, Monros JS, Piculo R, Vogeli M, *et al.* (2014) Repeatability of feather mite prevalence and intensity in Passerine birds. *PLoS ONE* **9**, 12.

Doña J, Moreno-García M, Criscione CD, Serrano D and Jovani R (2015*a*) Species mtDNA genetic diversity explained by infrapopulation size in a host-symbiont system. *Ecology and Evolution* **5**, S801–S809.

Doña J, Díaz-Real J, Mironov S, Bazaga P, Serrano D and Jovani R (2015*b*) DNA barcoding and minibarcoding as a powerful tool for feather mite studies. *Molecular Ecology Resources* **15**, 1216–1225.

Doña J, Proctor H, Mironov S, Serrano D and Jovani R (2016) Global associations between birds and vane-dwelling feather mites. *Ecology* **97**, 3242.

Doña J, Potti J, De La Hera I, Blanco G, Frías O and Jovani R (2017*a*) Vertical transmission in feather mites: insights into its adaptive value. *Ecological Entomology* **42**, 492–499.

Doña J, Sweet AD, Johnson KP, Serrano D, Mironov S and Jovani R (2017*b*) Cophylogenetic analyses reveal extensive host-shift speciation in a highly specialized and host-specific symbiont system. *Molecular Phylogenetics and Evolution* **115**, 190–196.

Emlen ST (1995) An evolutionary theory of the family. *Proceedings of the National Academy of Sciences USA* **92**, 8092–8099.

Excoffier L and Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

Fernández-González S, De la Hera I, Pérez-Rodríguez A and Pérez-Tris J (2013) Divergent host phenotypes create opportunities and constraints on the distribution of two wing-dwelling feather mites. *Oikos* **122**, 1227–1237.

Fernández-González S, Pérez-Rodríguez A, de la Hera I, Proctor HC and Pérez-Tris J (2015) Different space preferences and within-host competition promote niche partitioning between symbiotic feather mite species. *International Journal for Parasitology* **45**, 655–662.

Fretwell SD and Lucas HL Jr. (1970) On territorial behavior and other factors influencing habitat distribution in birds. Part I: theoretical development. *Acta Biotheoretica* **19**, 16–36.

Garland T and Adolph SC (1994) Why not to do two-species comparative studies – limitations on inferring adaptation. *Physiological Zoology* **67**, 797–828.

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Harper SE, Spradling TA, Demastes JW and Calhoun CS (2015) Host behaviour drives parasite genetics at multiple geographic scales: population genetics of the chewing louse, *Thomomydoecus minor*. *Molecular Ecology* **24**, 4129–4144.

Hartl DL and Clark AG (2007) *Principles of Population Genetics*. Sunderland, MA: Sinauer Associates Inc.

Hedrick PW (2000) *Genetics of Populations*. Burlington, MA: Jones and Bartlett Publishers.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* **10**, 537–549.

Johnson KP, Williams BL, Drown DM, Adams RJ and Clayton DH (2002) The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). *Molecular Ecology* **11**, 25–38.

Jovani R and Serrano D (2004) Fine-tuned distribution of feather mites (Astigmata) on the wing of birds: the case of blackcaps *Sylvia atricapilla*. *Journal of Avian Biology* **35**, 16–20.

Jovani R, Tella JL, Sol D and Ventura D (2001) Are hippoboscid flies a major mode of transmission of feather mites? *Journal of Parasitology* **87**, 1187–1189.

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press

Keller LF and Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**, 230–241.

Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution* **17**, 183–189.

Martinu J, Sychra O, Literák I, Capek M, Gustafsson DL and Stefka J (2015) Host generalists and specialists emerging side by side: an analysis of evolutionary patterns in the cosmopolitan chewing louse genus *Menacanthus*. *International Journal for Parasitology* **45**, 63–73.

McCoy KD, Boulinier T, Tirard C and Michalakis Y (2003) Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution* **57**, 288–296.

Mideo N (2009) Parasite adaptations to within-host competition. *Trends in Parasitology* **25**, 261–268.

Nadler SA (1995) Microevolution and the genetic structure of parasite populations. *Journal of Parasitology* **81**, 395–403.

Pérez-Tris J and Tellería JL (2002*a*) Migratory and sedentary blackcaps in sympatric non-breeding grounds: implications for the evolution of avian migration. *Journal of Animal Ecology* **71**, 211–224.

Pérez-Tris J and Tellería JL (2002*b*) Regional variation in seasonality affects migratory behaviour and life-history traits of two Mediterranean passerines. *Acta Oecologica-International Journal of Ecology* **23**, 13–21.

Pérez-Tris J, Bensch S, Carbonell R, Helbig AJ and Tellería JL (2004) Historical diversification of migration patterns in a passerine bird. *Evolution* **58**, 1819–1832.

Poulin R (2007) *Evolutionary Ecology of Parasites*. Princeton, NJ: Princeton University Press.

Proctor HC (2003) Feather mites (Acari: Astigmata): ecology, behavior, and evolution. *Annual Review of Entomology* **48**, 185–209.

Rigaud T, Perrot-Minnot M-J and Brown MJF (2010) Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proceedings of the Royal Society of London B: Biological Sciences* **277**, 3693–3702.

Santana FJ (1976) A review of the genus *Trouessartia*. *Journal of Medical Entomology* **1**, S1–S128.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787–792.

Szudarek N, Kanarek G and Dabert J (2017) The genetic structure of hypoderatid mites (Actinotrichida: Astigmata) parasitizing great cormorant (*Phalacrocorax carbo*) during host post-breeding dispersal in Milicz, SW Poland. *Acta Parasitologica* **62**, 76–89.

Whiteman NK, Kimball RT and Parker PG (2007) Co-phylogeography and comparative population genetics of the threatened Galapagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Molecular Ecology* **16**, 4759–4773.

https://doi.org/10.1017/S0031182017002360 Published online by Cambridge University Press