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ECOLOGÍA Y PERSPECTIVAS EVOLUTIVAS DE LA COEXISTENCIA DE LOS ÁCAROS DE LAS PLUMAS EN LA CURRUCA CAPIROTADA *SYLVIA ATRICAPILLA*

ECOLOGY AND EVOLUTIONARY PERSPECTIVES OF FEATHER MITES COEXISTENCE ON THE BLACKCAP *SYLVIA ATRICAPILLA*

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
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A mis padres y mi hermano

A Samuel

“¿Qué sería de la vida, si no tuviéramos el valor de intentar algo nuevo?”

(Vincent van Gogh)

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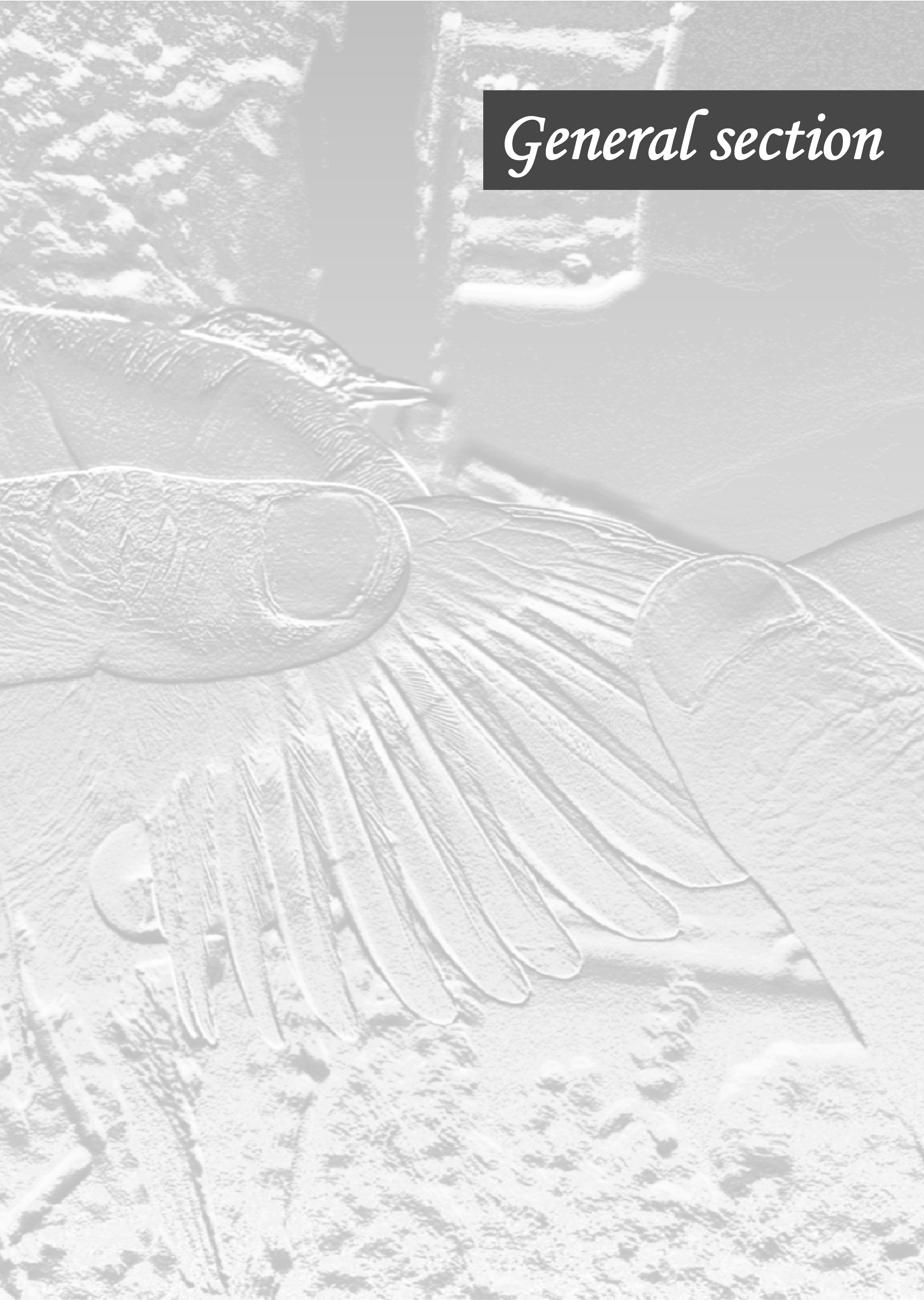
General section



Ecology and evolutionary perspectives of feather mites coexistence on the blackcap *Sylvia atricapilla*

Introduction

Nature harbours a vast diversity of organisms, many of which seem to share the same ecological niche, which is defined as a multidimensional habitat volume characterized by physical and biotic factors (Hutchison 1957). Why is there such high diversity of ecologically similar species, if just a few representatives of each kind would be enough for maintaining a functioning ecosystem? Hutchison (1961) developed this question in his paradox of the plankton, advancing some reasonable answers: environmental heterogeneity, temporal variation in competitive interactions, or variation in the impact of natural enemies may all favour different species in different ecological contexts (Chesson 1994, 2000).

Diversity of ecologically similar species is also patent in host-symbiont interactions. Symbiosis refers to the close bond among two different species, in which one of the species lives near, on or inside individuals of the other species. In the most extreme case (obligate symbionts, which are those that live permanently attached to their host), the individual host represents the only habitat available for the symbiont. Host-symbiont interactions may take different forms, ranging from negative (parasitism) to positive (mutualism), and including other intermediate interactions (such as commensalism; Douglas 2010). Nearly all species harbour some kind of symbiont at any moment of their life cycle, but symbiont diversity varies among host species, host populations and individuals within host populations (Paracer & Ahmadjian 2000).

Symbionts show a high degree of specialization in order to successfully find, colonize and grow in their hosts, which in turn will favour the existence of a wide variety of symbiotic organisms. In fact, a single host species is normally occupied by different symbiont species, which share the same ecological requirements and are even found together within the same host individual (Poulin 2007). When this occurs, symbiont infracommunities (all the individuals of all symbiont species present in a host individual; Simberloff & Moore 1997) are likely to interact with one another giving rise to different types of interspecific interactions. Firstly, one species may suffer a numerical decrease when other symbiont species is present in the same host, which in the extreme case may involve a total exclusion from the host individual (Poulin 2007). Secondly, symbiont species may shift within-host niches or the way they use resources when other symbionts species occurs in the same host. In other words, the fundamental niche, which is the potential distribution of a symbiont in a host species in the absence of competitors, predators and pathogens (Hutchison 1957, Soberón & Peterson 2005), may be reduced to its realized niche, defined as the part of the fundamental niche that is actually occupied by the symbiont species due to interspecific interactions. Niche partitioning may lead to niche specialization, which in turn may favour symbiont coexistence and the maintenance of symbiont diversity (Schluter 2000). Finally, it may also happen that two symbiont species share a host without apparent interference, for example when they show little or no niche overlap (Poulin 2007).

Interactions among symbiont species in an infracommunity (at the within-host level) may be maintained at the between-host level within host populations. Thus, within a host population, symbiont infracommunities frequently vary in composition and relative numbers of each symbiont species among host individuals. Such variation may be attributed to differences among host individuals (sex, age, quality of host services), which render different hosts better or worse habitat for symbiont species. Another factor that may underlie variation in symbiont infracommunities is the ability of symbionts to disperse and grow among host individuals. For instance, the mode of transmission plays an important role in symbiont dispersal; symbionts may be transmitted very efficiently by vectors (such as mosquitoes), they may actively disperse to new hosts that come in close contact with their current host, or they may be passively acquired by the host (*e.g.*, with food). Thus, inherent characteristics of symbionts may determine variation in the proportion of infested hosts and within-host numbers. Ultimately, symbiont exchange among host individuals will determine genetic structure of symbiont populations within a host population (Poulin 2007). Because symbionts are usually forced to mate with individuals present on the same host, symbiont populations are prone to be genetically structured. The structuring of symbiont populations may depend on the degree of isolation associated with host behaviour (which may either promote or hamper symbiont dispersal) and symbiont intrinsic transmission capabilities (Nadler 1995).

At a broader spatial scale, host populations are distributed throughout the host's distribution range. For this reason, one factor of primary importance for a symbiont to successfully thrive in a given region is the presence of the host, particularly so for obligate symbiont species. In addition, symbiont component communities (the symbiont species occurring in one host population) may also vary in composition and relative numbers of each symbiont species among host populations. If differences among individuals within a host population may create variation in symbiont environment, then differences between host populations (which are usually more evident) may expand such variation at higher geographic scales. Such differences may be caused by local adaptation giving rise to phenotypic variation among host populations, which in turn will have an impact on the distribution of symbiont populations. For example, migratory behaviour is one of the most important factors influencing symbiont distribution patterns. Many studies have suggested that migratory species may harbour richer symbiont communities simply because they are exposed to different symbiont faunas across their range (Dogiel 1964, Møller & Erritzøe 1998). Besides, migratory behaviour entails morphological, behavioural and physiological changes in the host (Berthold 1975), which may greatly influence symbiont distribution patterns by affecting dispersal or within-host growth. However, symbionts are not only dependent on host characteristics, since environmental conditions typical of a given region appear to exert a strong influence on the outcome of many host-symbiont interactions. Many investigations have reported that climatic variables such as temperature and rainfall are crucial variables shaping symbiont distribution and favouring or constraining symbiont survival, colonization success and within-host growth. Nevertheless, not all symbiont species have the same tolerance to environmental change, and some places where the host lives might be inhospitable for certain symbiont species (Malenke *et al.* 2011). Ultimately, this will create a geographic mosaic of host-symbiont interactions in which each symbiont component community will probably have different features depending on host attributes, local environmental conditions and symbiont-symbiont interactions.

The main goal of this thesis was to summarize what are the factors that may have a strong influence in the maintenance of host-symbiont interactions and the coexistence of symbionts in the same host species. The idea was to stress that not all variables having an effect on these interactions have the same importance depending on the scale of observation (see Figure 1). However, all layers participating in shaping such interactions must be taken into account. Studies of this kind provide a better knowledge of the processes involved in symbiont species diversification, symbiont community assembly and, in turn, the mechanisms through which symbiont coexistence becomes possible. In order to accomplish this goal a host species was carefully chosen that (1) is widely distributed among a broad range of environments, (2) normally harbours two ecologically similar symbiont species (two feather dwelling mites, which are potential competitors), and (3) possesses different phenotypic attributes that may create variation in individual host quality for such symbionts. The following sections present all these characters and justify this choice.

A model host species: The blackcap

The European blackcap *Sylvia atricapilla* (Linnaeus 1758; class Aves, order Passeriformes, family Sylviidae) is a widely distributed forest passerine in the Palearctic region. Blackcaps have a distinctive coloured cap extending to the eye limit, black on males, reddish-brown on females (Figure 2) and blackish-brown to yellowish-brown on juveniles (Cramp 1992). Blackcaps have a total length of 13 cm and a wing span of 20-23 cm (Cramp 1992).

Blackcaps represent a suitable model species for this study for several reasons. Firstly, it is a widely distributed species throughout the Western Palearctic (Shirihai *et al.* 2001). In the Iberian Peninsula, where these studies were carried out, blackcaps have a broad distribution range: it is a widespread species in the northern half of the Iberian Peninsula, while in the southern half its distribution is patchy (Figure 2; Carbonell 2003). Within its distribution range, blackcaps occupy a wide variety of habitats, although they clearly prefer forested areas, especially Eurosiberian woodlands with developed undergrowth. The species tends to become restricted to riparian forest towards the Mediterranean region, where blackcaps are able to cope with high temperatures and

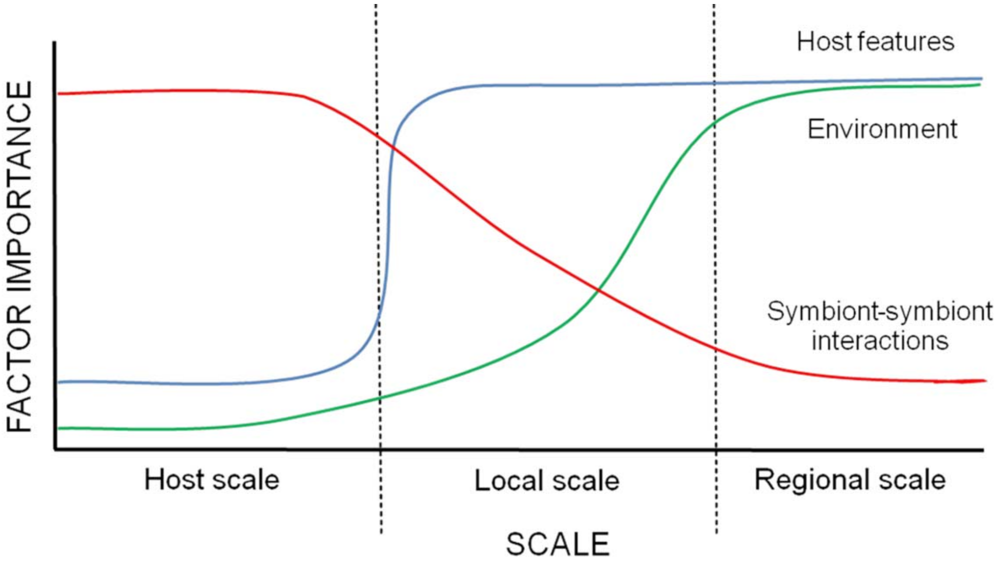


Figure 1. Factors influencing symbiont coexistence across different scales. Within a host individual (host scale) different symbiont species may co-occur and interspecific interactions between symbionts may take place, giving rise to changes in symbiont numbers, or shifts in the niches occupied or in the resources used by each symbiont species. At a local scale, where various host individuals form a host population, interspecific symbiotic interactions may be affected by variation in host phenotype. Finally, at a regional scale host presence, host phenotype, inherent characteristics of each symbiont species, as well as environmental conditions of the host's habitat have also an important role in the structuring of symbiont communities.

aridity (Carbonell 2003). In the far south blackcaps reach high density in cork-oak and Mirbeck's oak woods. The contrasting habitats and environmental conditions that the species finds in the Iberian Peninsula provide different scenarios where host-symbiont interactions may evolve.

Secondly, blackcaps have a wide variety of migratory strategies, ranging from trans-Saharan migration (northern and eastern European birds) to fully-sedentary populations (in the warmest Mediterranean sectors), and including partially migrant populations in mild areas of southern and central Europe (Berthold 2001). As a result of its variable migratory behaviour, wing morphology varies among populations (Figure 2), with migratory populations having longer and more pointed wings than sedentary populations (Tellería & Carbonell 1999, Fiedler 2005). In addition to morphological differences, bird migration entails physiological and behavioural changes (Berthold

1975, Newton 1998). Altogether, phenotypic differences associated with migratory behaviour among blackcap populations may provide different habitats for symbionts, which is likely to give rise to differences among host populations in terms of host exposure and suitability for symbionts.

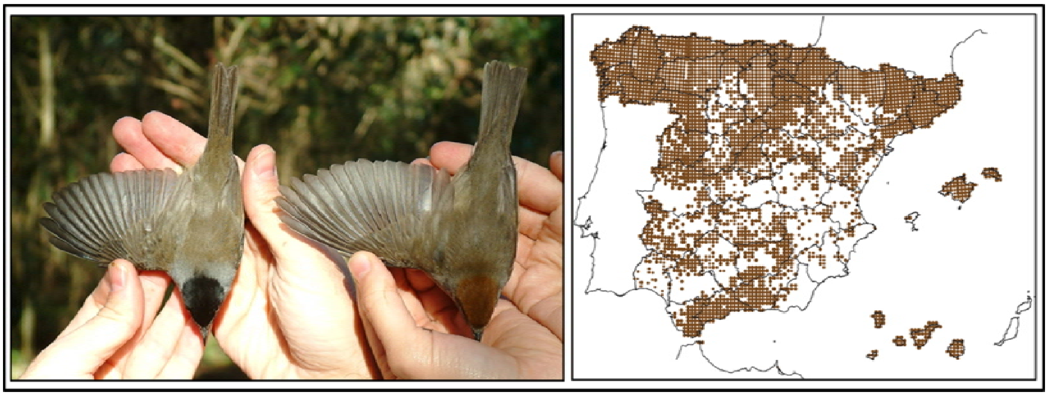


Figure 2. On the left, two blackcaps *Sylvia atricapilla*: a sedentary male (left) and a migratory female (right). On the right, map of the Iberian distribution of the blackcap. Picture credits: Javier Pérez-Tris (left) and Carbonell 2003 (right).

Finally, bird's feathers are highly specialized structures, which possess a complex architecture: flight feathers are composed of a longitudinal axis, the shaft or quill, which has a proximal part termed calamus that remains attached to bird's bones, and a distal part named the rachis or upper part of the shaft. At each side of the rachis, the vanes are formed of parallel rows of barbs which are connected to each other by means of barbules (Lucas & Stettenheim 1972, Videler 2005). Furthermore, feathers are covered by oil secretions coming from the uropygial gland, located dorsally at the base of the tail. In all, feather features have many other functions apart from their role in birds' flight, such as protection, thermoregulation or communication (Andersson 1982, Ginn & Melville 1983, Nilsson & Svensson 1996). However, feather characteristics are also favourable for a quite diverse community of symbionts. Feathers provide a suitable habitat for several arthropod species, such as lice, fleas, flies, and mites (Gaud & Atyeo 1996, Janovy 1997). Among these is the study model: mites dwelling on the feather vanes, which are widely distributed among passerines (Proctor 2003). More specifically, blackcaps harbour two common feather mite species, which are quite abundant and prevalent within and among blackcap populations. This system allows studying the possible interspecific interactions that may be taking place on blackcap feathers.

A model symbiont organism: feather mites

Astigmatid mites (subclass Acari, suborder Astigmata, superfamilies Analgoidea, Freyanoidea, Pterolichoidea) are the most important symbiont community in the plumage by their numbers (Gaud & Atyeo 1996). To date, *ca.* 2000 species (33 families and 444 genera) of feather mites have been described, which however are thought to represent less than 20% of all extant species (Gaud & Atyeo 1996). Feather mites have probably been originated from ancestors dwelling in birds’ nests 65-130 million years ago in the Cretaceous period (Atyeo & Gaud 1979). Feather mites are usually rather small, ranging from 0.3 mm to 0.7 mm (Gaud & Atyeo 1996). These organisms possess a 5-stage life cycle (egg, larva, protonymph, tritonymph and adult), which always occurs intimately linked with the host; adults present a marked sexual dimorphism (Proctor 2003). Feather mites are specialized to exploit different parts of the feathers, some living in or on the skin, others occupying the inside of the quills and finally and more importantly for this thesis, those living on the surface of feather vanes (Gaud & Atyeo 1996, Proctor 2003), which will be hereafter referred to as feather mites. The role that mites play in their hosts remains controversial. Although some studies have shown that feather mites might be detrimental to their hosts (Poulin 1991, Thompson *et al.* 1997, Harper 1999, Pérez-Tris *et al.* 2002), other authors have suggested that mites could be commensals or even mutualists (Blanco *et al.* 1997, 1999, Dowling *et al.* 2001). However, it seems more likely that mites have a positive or a neutral effect on their hosts (Blanco *et al.* 2001). Feather mites are suggested to help birds on their preening duties, by removing the old oil from the uropygial gland and detritus deposited on feather surfaces, as well as feather-degrading microorganisms (Pugh 1965, Burtt & Ichida 1999, Burtt 2009).

The distribution of feather mites is heterogeneous. Feather mite load has been shown to be positively correlated with the size of the uropygial gland (Galván & Sanz 2006). Birds with bigger glands may produce more secretions (Elder 1954), mainly composed of waxes and fatty acids (Jacob & Ziswiler 1982), and in turn provide feather mites with more food. Uropygial gland size seemingly depends on host features; it has been described that birds occurring in aquatic environments, with more needs of plumage waterproofing, tend to have greater glands and in turn higher mite loads (Dubinin 1951, Galván *et al.* 2008). Moreover, uropygial gland size seems to be closely related to host migratory behaviour. Physiological and behavioural host changes associated with migration (Berthold 1975) may have a strong effect on feather mite within-host population dynamics and dispersal among hosts (Blanco & Frías 2001). Galván *et al.* (2008) found that migratory hosts had smaller glands than sedentary birds, however, mite numbers were greater in migratory that in sedentary birds. What authors argue in response to these results is that this circumstance may be the outcome of a selective pressure for migratory birds to control high mite loads. In this thesis, the opportunity to study a single host species that spans different migratory behaviours will provide a better understanding about the effects of urogygial gland size and host phenotype on mite numbers.

Bird feathers offer different microhabitats that vary in temperature and humidity, aeration and mechanical stress, making the surface of feathers a harsh habitat for mites (Dabert & Mironov 1999). These have evolved various morphological and biological adaptations in consequence. Feather mite bodies are dorso-ventrally flattened and strongly sclerotized, which is believed to prevent water loss and bear aerodynamical forces during bird flight. Their legs are in most cases short and laterally inserted, and possess ambulacra that serve to remain attached to the barbs (Dubinin 1951, Mironov 1987, Proctor 2003).

All these morphological adaptations constrain the ability of feather mites to live off the host, where they can survive for just 3-10 days, which makes dispersal among hosts dependent on close contact between hosts (Dubinin 1951, Proctor & Owens 2000). For this reason, the best opportunity for dispersal occurs during the breeding period, when fledglings have fully developed their flight feathers (Proctor 2003). Dubinin (1951) reported that it was mostly nymphs that transferred to offspring and therefore parent’s mite load suffered an important decrease during parental care periods. Other situations where close contact between individual birds happens are courtship, mating or communal roosting (Proctor 2003). The main mode of transmission will chiefly depend on host behaviour and it will probably shape the genetic structuring of feather mite populations within host populations. If feather mites are transmitted mainly vertically (from parents to chicks), and only a few founder females are responsible for the settlement of mite populations on each host individual, then transmission bottlenecks would lead to strong among-host genetic structure of mite populations. This may render feather mites obliged to mate with close kin, thereby reducing outbreeding. However, feather mites might counteract the decrease in genetic diversity by developing transmission mechanisms that help to reduce transmission bottlenecks.

The specificity of feather mites also has a topographical aspect. In general, each species occupies a specific area of the wing plumage, or even within single feathers (Dubinin 1951, Atyeo & Windingstad 1979, Pérez & Atyeo 1984, Choe & Kim 1989, Mestre *et al.* 2011). In addition, the ventral surface of the feathers, which is less aerodynamically disturbed, gives shelter to the majority of vane-dwelling mites. One of the few exceptions is the genus *Trouessartia* (Trouessartiidae), which occupies the dorsal side of the feathers. These mites have specific adaptations to this exposed environment, such as a strong exoskeleton and dark pigmentation (Dubinin 1951). However, the causes that favour niche partitioning in feather mites remain poorly studied. In passerines, where feather morphology shows little variation, it is expected that mites show little site specificity (Dubinin 1951, Proctor 2003), although previous research has shown a different distribution of feather mite species among feather sectors (Mestre *et al.* 2011). Blackcaps often are hosts of two feather mite species (Figure 3): *Proctophyllodes sylviae* Gaud (family Proctophyllodidae) and *Trouessartia bifurcata* (Trouessart) (family Trouessartiidae). Both genera are composed of generalists and specialist species, although they probably contain many cryptic species (Atyeo & Braasch 1966, Santana 1976, Gaud & Atyeo 1996), which may change the patterns of host specialization that are currently assumed for the group.

Feather mites have raised the interest of many researchers over the last years (*e.g.*, Mestre *et al.* 2011, Galván *et al.* 2012, Soler *et al.* 2012), yet little is still known about basic aspects of the biology of feather mites, such as their exact distribution within and among host individuals, the environmental determinants of such distribution, or the genetic consequences of their obligate symbiotic life style. This thesis aims to improve the understanding of the ecology and evolution of feather mites by approaching the aforementioned questions.

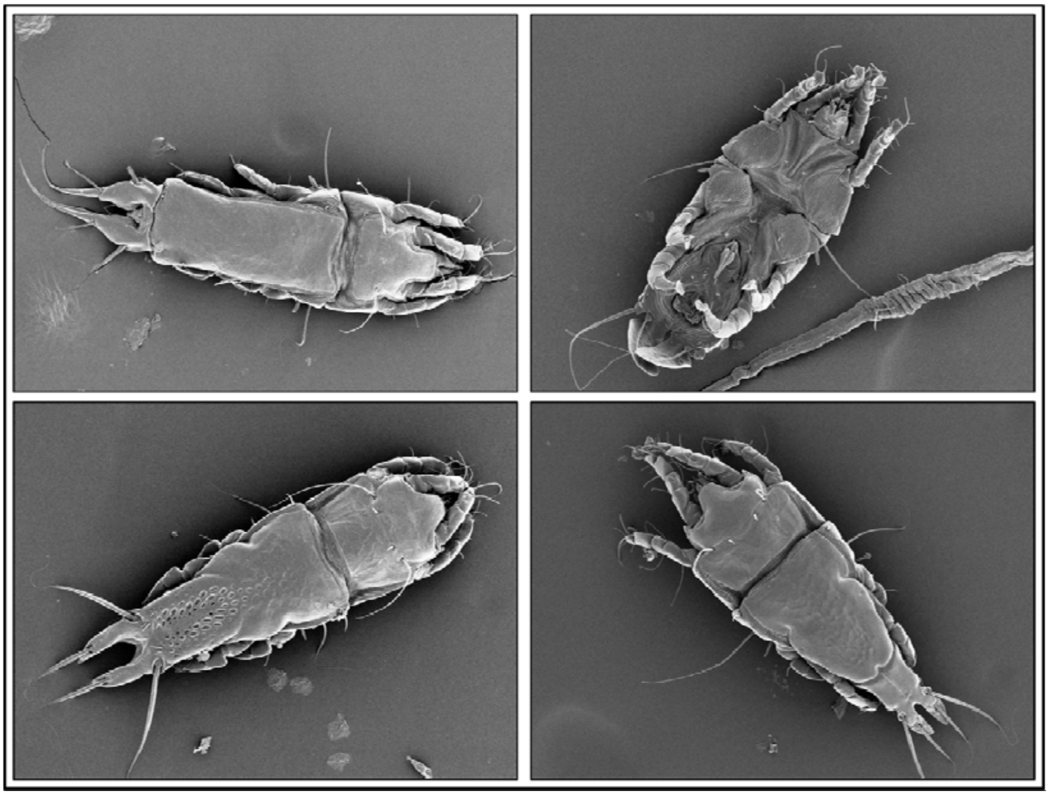


Figure 3. Scanning electron microscope (SEM) images of feather mites: *Proctophyllodes sylviae*, female (top left) and male (top right); *Trouessartia bifurcata*, female (bottom left) and male (bottom right).

Objectives

Our primary goal is to have a better understanding of the configuration of symbiotic interactions between a host species (the blackcap) and its symbionts (two feather mite species, *P. sylviae* and *T. bifurcata*) as well as the processes that favour or constrain the diversity of ecologically similar symbionts within the same host species at different geographic scales, from different locations on the host individual to the whole host species' range. To this end, this thesis has been organized in four chapters, which focus on the following specific objectives:

Chapter 1. This study examines the patterns of distribution of two feather mite species (*P. sylviae* and *T. bifurcata*), and their potential interaction in wintering blackcap populations in southern Spain. To date several studies have shown that mite numbers on the individual host and prevalence among hosts may be affected by host migratory behaviour. However, as far as it is known such analyses have not been carried out in a single species that shows different migratory behaviours. The study of mite distribution patterns at the intra-host level allows controlling for the variation created by specific features of each host species that may mask the detection of such patterns.

Chapter 2. This study investigates within-host distribution of both mite species and their interactions in the same blackcap populations investigated in Chapter 1. Thus, it will be possible to describe how these mite species share host habitat, which is a prerequisite to approach the mechanisms through which both species are able to coexist on an individual host. To this end, detailed counts of *P. sylviae* and *T. bifurcata* mites were carried out within each wing feather, obtaining a map of the distribution of each feather mite species on the wing. Hence, interspecific mite interactions could be studied on a very fine scale. Finally, the distribution of each species was analysed to study whether they have preferences for any specific sector of the feather or for any specific feather of the wing, as well as whether they follow a specific order of occupation of the different plumage areas available on the wing.

Chapter 3. This study analyses genetic diversity and genetic structure of *P. sylviae* and *T. bifurcata* in the same blackcap populations. If mite transmission from parents to offspring involves population bottlenecks, detectable genetic structure is expected to arise for both mite species. In addition, host phenotype might give rise to differences in the genetic structure of both mite species if host type creates opportunities and constraints on the distribution of each mite species. The aim of this study was to shed light on feather mite colonization strategies and their genetic consequences, which may have important implications in the context of the different distribution of these mite species among hosts investigated in the other chapters.

Chapter 4. This study analyses the distribution of the two feather mite species at a broad scale, across 37 breeding blackcap populations, in order to assess the potential influence of environmental conditions on feather mite distribution patterns (prevalence, abundance and intensity). In conjunction with differences found in population numbers and prevalence at a local scale, it is also known that feather mites are sensitive to environmental variables such as humidity and temperature. The Iberian Peninsula combines great environmental variation and reduced geographic area, thereby making an excellent scenario in which to conduct such study.

Material and methods

The general methods of this thesis are described in this section, which chiefly focuses on the study areas covered in all chapters and the protocols used for bird capture as well as for mite counting, sampling and identification. A more detailed description of the methods used will be found in each chapter.

Study areas

In order to investigate mite distribution at individual and local scales (Chapters 1-3), and to introduce variation in phenotype among host individuals, the Campo de Gibraltar area (south of Spain, Cádiz, 36°01’N, 5°36’W, open circles in Figure 4) was chosen. In this region, both sedentary and migratory blackcaps coexist during the wintering season. This region is composed of a mixture of shrublands and forests (which were sampled at 100 and 300 m a.s.l., respectively). Forests have Mirbeck’s Oaks (*Quercus canariensis*) and cork-oaks (*Quercus suber*) as the most representative tree species; these habitats are breeding grounds for sedentary blackcaps and wintering grounds for both sedentary and migratory individuals (Pérez-Tris & Tellería 2002). Shrublands, which are located at lower elevation, are dominated by fruiting shrubs such as wild olives *Olea europaea sylvestris* and lentiscs *Pistacia lentiscus*; these areas are mainly occupied by wintering migratory blackcaps (Pérez-Tris & Tellería 2002). For the study described in Chapter 1, a total of 564 birds were captured between December and February during six winters (from 2005 to 2010). A subsample of the birds captured during the two winters in 2010 was used for Chapters 2 (N = 160) and 3 (N = 27).

For the study of the general distribution of *P. sylviae* and *T. bifurcata* and the environmental factors shaping such distribution (Chapter 4) 37 populations of breeding blackcaps were sampled between the end of July and the beginning of August of 2008, 2010 and 2011 (all circles in Figure 4). This period coincides with the end of the breeding season and the beginning of autumn migration. Localities were selected to cover a broad range of climatic, geographical and landscape features within the species' Iberian range, including populations with different migratory behaviour. A total of 875 blackcaps were sampled, with an average of 24 individuals per locality.

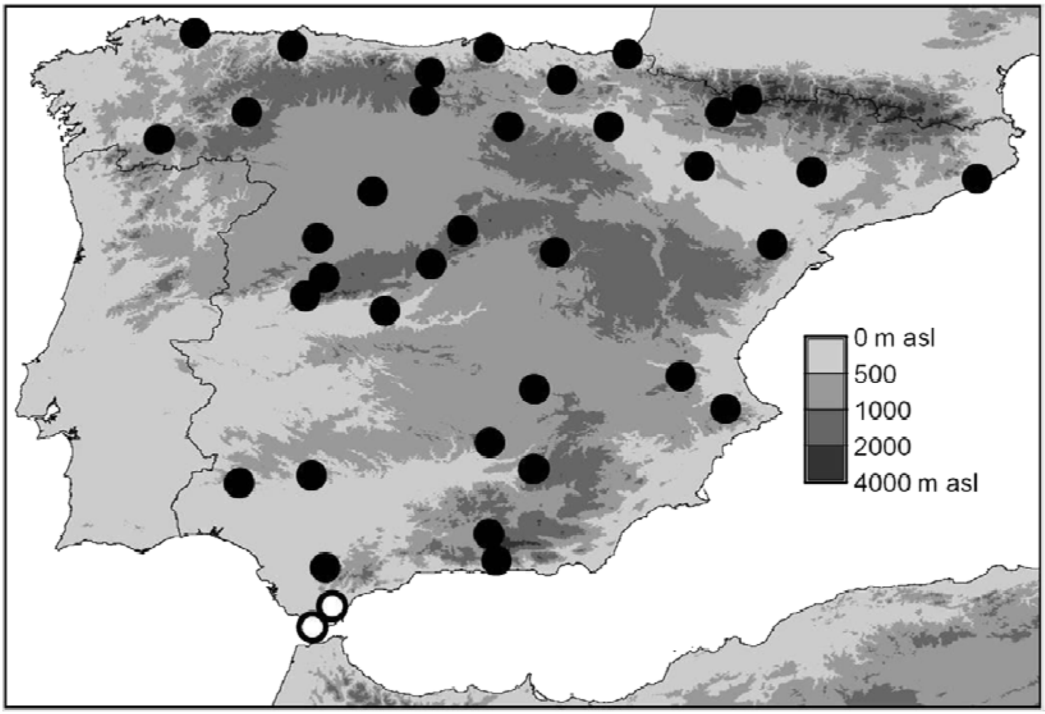


Figure 4. Map of the blackcap populations sampled in this thesis. Open circles are localities from the Campo de Gibraltar area, sampled both in winter and summer, whereas filled circles were localities sampled only in summer.

Bird sampling

Birds were mist-netted, often with the aid of a tape-lure in order to maximize capture rate. After capture an aluminium ring was fitted to each blackcap to individualise it. All individuals were aged and sexed according to their plumage characteristics (Svensson 1992). In addition, some morphological measures were taken: flattened wing chord, length of the eighth primary feather (numbered in descending order), bill and tail length. For the purpose of classifying blackcaps as migratory or sedentary individuals, a discriminant function analysis was performed, including tail length, eighth primary length and the difference between the distances to the wing tip of the primary feathers 1 and 9 (Pérez-Tris *et al.* 1999). This function correctly classifies over 90% of individuals (De la Hera *et al.* 2007). All birds were released at the site of capture after manipulation.

Feather mite sampling and identification

Each feather mite species was counted on each blackcap captured by spreading a wing towards the ambient light or a lamp when natural light was not sufficient. All large wing feathers (primaries, secondaries and tertials) were checked thoroughly. Each feather mite species is easily distinguishable owing to their different morphology and location on the wing. *T. bifurcata* is bigger and rounded in shape, and occupies the dorsal side of the feather, while *P. sylviae* is a smaller and elongated mite living on the ventral part of the wing (Atyeo & Braasch 1966, Santana 1976). A controversial issue that has been central in parasitological studies is the choice of an appropriate method of symbiont detection and counting. Regarding feather mites, many studies have compared different counting methods with visual inspection (Dowling *et al.* 2001, Pap *et al.* 2005); visual examination has been widely accepted despite the potential loss of accuracy in mite counting (*e.g.*, Galván *et al.* 2012). For this reason, the suitability of visual feather mite counts was also checked in the study model. *P. sylviae* mites were chosen because they are less visible and show greater within-host variation, which could give misleading results if the counting method is not good. These results showed that visual inspection was sufficient to capture variation in mite numbers, although mite load was underestimated. Despite underscoring, the error percentage remained constant along the range of variation in mite counts. Besides, the repeatability between wings of the same bird and that between observers was notably high (intraclass correlation coefficients, $r_i \approx 0.85$ and $r_i \approx 0.90$, respectively). Therefore, visual examination was determined to be suitable to detect biologically relevant variation in mite numbers.

Right before releasing birds, some mites were collected in 1.5 ml tubes filled with absolute ethanol to confirm field mite identifications in the laboratory; tubes were stored at -20 ºC until needed. For molecular identification the DNeasy Tissue Kit (Qiagen, USA) was used, but following a specific protocol that modified manufacturer’s instructions (Dabert *et al.* 2008, M. Dabert pers. comm.). Right after proteinase K digestion, mite exosqueletons were kept in 80% ethanol for later microscopical identification. A 661-pb fragment of the cytochrome oxidase I gene (COI), which is commonly used as a barcode for invertebrates, was amplified. This DNA region is known to be appropriate for species identification, cryptic species delimitation, and detection of geographic genetic structure, among many other applications (Roderick 1996, Roderick & Navajas 2003, Hebert *et al.* 2004, Kress & Erickson 2008). The specific PCR reaction protocol used in this thesis involved the degenerated primers bcdF05 and bcdR04, and included a denaturation step of 5 min at 95 °C, followed by 35 amplification cycles of 30 s at 95 °C, 60 s at 50 °C, and 60 s at 72 °C, with a final elongation step of 5 min at 72 °C (M. Dabert pers. comm.). PCR products were visualised on 2% agarose gels stained with GelRed™ (Biotium, USA), and bands of sufficient quality were subsequently sequenced from both ends with an ABI 3730 XL automated sequencer (Applied Biosystems, USA).

Feather mite exosqueletons were slide-mounted (those that were not used for DNA extraction went through an overnight clearing process) using polyvinyl alcohol (Bioquip Products, USA), followed by a three-day drying step. For mite identification, a light microscope with differential interference contrast (DIC) illumination was employed. Feather mite identification guides were used to determine mite species when possible (Atyeo & Braasch 1966, Santana 1976, Gaud & Atyeo 1996).

Results

Within-host feather mite distribution and interspecific interactions

As a consequence of within-host symbiont interactions, symbionts should develop strategies to avoid competition, which ultimately may allow coexistence. Among the mechanisms through which symbionts may alleviate competition, niche shifts or reduction of mite abundance may be relevant. According to results presented in Chapter 2, the two species of feather mites follow a different distribution across blackcap feathers and feather sections (Figure 5). Additionally, the filling of wing cells by feather mites was ordered, although such order was different among feather mite species. Some cells were only occupied when mite populations on the wing were large, which supports the idea that some areas of the wing are suboptimal for mites. Interestingly, the least preferred cells for one mite species ranked high in the range of cell preferences of the other species, although some areas of the wing were apparently suboptimal for both mite species. Regarding interspecific interactions, the numbers of *T. bifurcata* and *P. sylviae* were negatively correlated when both mite species co-occurred in the same wing cell. When total numbers of each mite species were taken into account, *P. sylviae* numbers (abundance and intensity) decreased when *T. bifurcata* was present on the same individual, but the contrary was not true (Chapter 1).

Host phenotype and feather mite distribution

Symbionts may share the same host species, but their success in colonization may depend on host traits, for example those traits associated with host migratory behaviour. In Chapter 1, results showed that in general, prevalence, abundance and load of both mite species considered as a whole were greater in migratory blackcaps than in sedentary blackcaps. When both mite species were taken into account separately and within-host analyses were conducted, *P. sylviae* was more abundant than *T. bifurcata* in general. Different patterns of distribution in abundance between migratory and sedentary blackcaps were also observed: *P. sylviae* was more abundant than *T. bifurcata* on migratory blackcaps, whereas both mite species converged in intermediate numbers on sedentary blackcaps.

When blackcaps were divided into migratory and sedentary individuals (Chapter 1), on sedentary blackcaps the probability of the occurrence of a mite species was higher when the other species was also present on the host. Regarding mite numbers in a between-host analysis, the interaction between host phenotype and *P. sylviae* presence had an effect on *T. bifurcata* abundance: *T. bifurcata* numbers were lower when *P. sylviae* was present, although such association was more evident in migratory blackcaps.

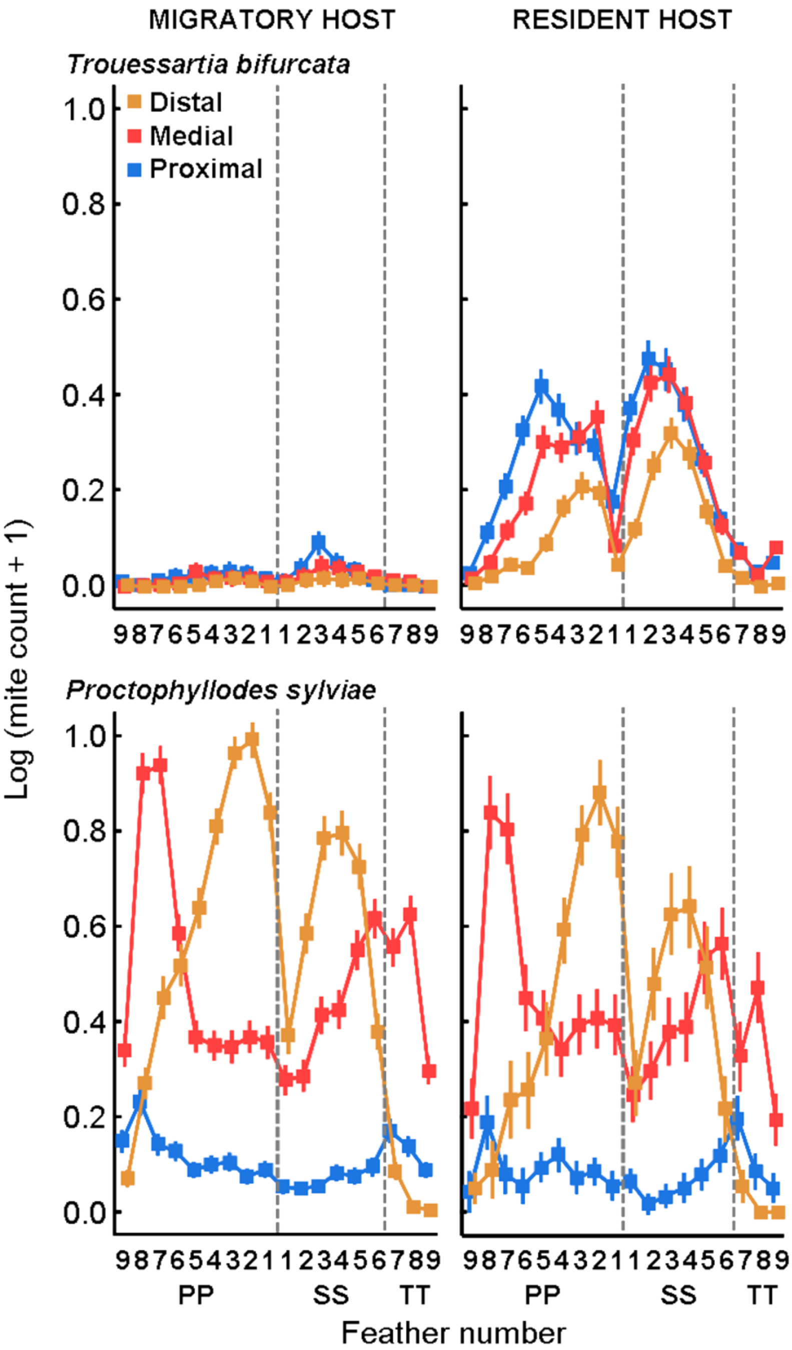


Figure 5. Variation in the abundance (mean ± SE) of the feather mites *Trouessartia bifurcata* (above) and *Proctophyllodes sylviae* (below) among blackcap feathers and feather sectors (shown in different colours). Dashed lines separate primary (PP), secondary (SS) and tertial (TT) feathers. Left and right charts show the patterns of mite distribution on migratory and resident hosts, respectively.

As described in the analysis of variation in mite numbers across the host's wing, the presence of *T. bifurcata* was associated with lower numbers of *P. sylviae* regardless of blackcap phenotype.

Finally, regarding the variation in host traits among blackcap populations (Chapter 1), different traits affected each mite species differently (Figure 6). In the case of *P. sylviae*, its load was positively correlated to host wing length (which is longer in migratory blackcaps), whereas the load of *T. bifurcata* was negatively associated with wing length and positively correlated with uropygial gland size (which is bigger in sedentary blackcaps).

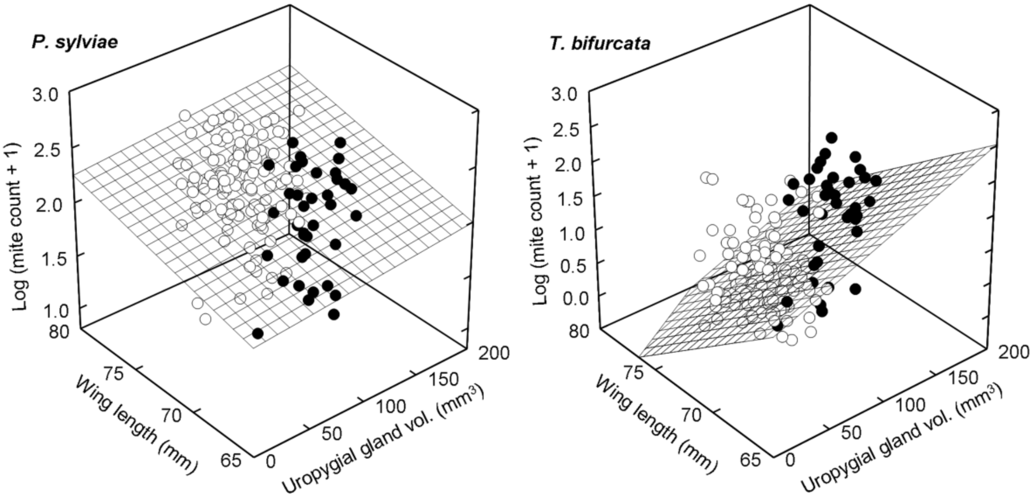


Figure 6. Relationship between uropygial gland volume, wing length and mite counts (mite abundance including mite-free hosts) of *Proctophyllodes sylviae* and *Trouessartia bifurcata*. Migratory and sedentary blackcaps are distinguished by white and filled dots, respectively. Bivariate least-squares fit surfaces are also shown.

Feather mite genetic structure and genetic diversity

Symbiont distribution patterns and population parameters (prevalence, abundance and intensity) may depend on symbiont life-history traits, such as reproductive strategies, host-to-host transmission, and within-host growth. In turn, symbiont dispersal may determine symbiont genetic structure within and between hosts. In Chapter 3 a high genetic diversity was found in within-host populations of both mite species, although it was higher in *P. sylviae*. Consequently, genetic structure of mite populations among individual hosts was weak. Any genetic structure found on blackcaps was mainly due to the fact that some mites from the same host had identical haplotypes, which was expected, but in general most mites had unique haplotypes in the host population and mite individuals that shared the same host were not more closely related from one another than expected by chance (Figure 7).

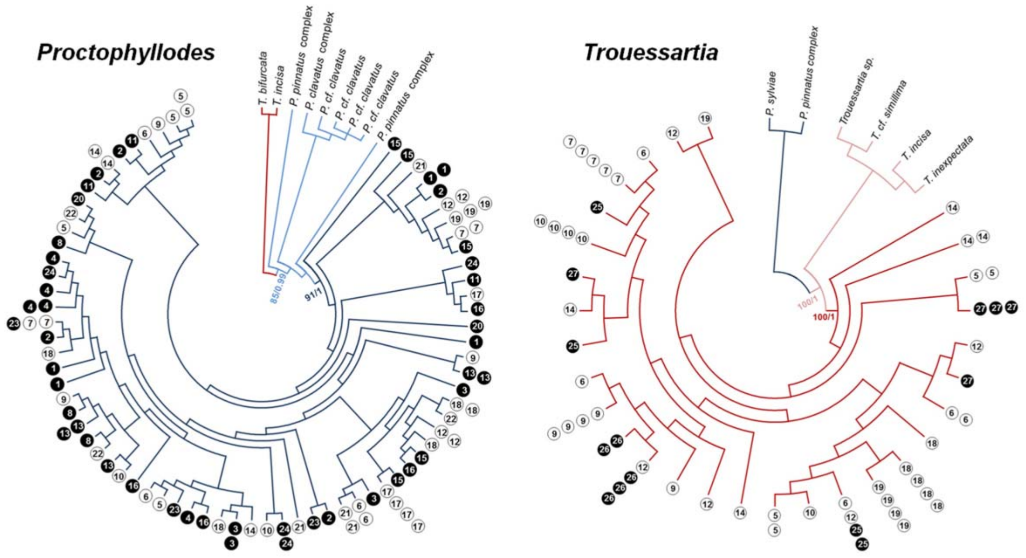


Figure 7. Majority-rule consensus phylogenetic tree of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* haplotypes of the cytochrome oxidase I (COI) mitochondrial gene. Topology was rooted with species of the other genera from different hosts. Numbers on branch nodes indicate >80% support for 1000 bootstrap replicates in a maximum likelihood analysis, and >0.90 posterior probabilities extracted from 45,500 trees obtained with Bayesian methods.

Feather mite distribution among host populations

One of the main factors limiting the distribution of a given symbiont species is simply the presence or absence of its host. However, this factor is not sufficient for a symbiont to settle in a given region. For instance, host features associated with host migration have an important influence on symbiont establishment. In addition, local environmental conditions have a great impact on the distribution of all living organisms. It also may occur that symbionts sharing the same host may have different ecological requirements as well as a different tolerance to climate and host habitat. In Chapter 4, *P. sylviae* was found in all sites included in this study, while *T. bifurcata* was detected in 65% of localities (Figure 8). In general, *P. sylviae* reached higher prevalence, abundance and intensity than *T. bifurcata* within each locality. In addition, mite numbers (abundance and intensity) of both mite species showed no correlation among blackcap populations, whereas prevalence had a significant (but weak) association between both species.

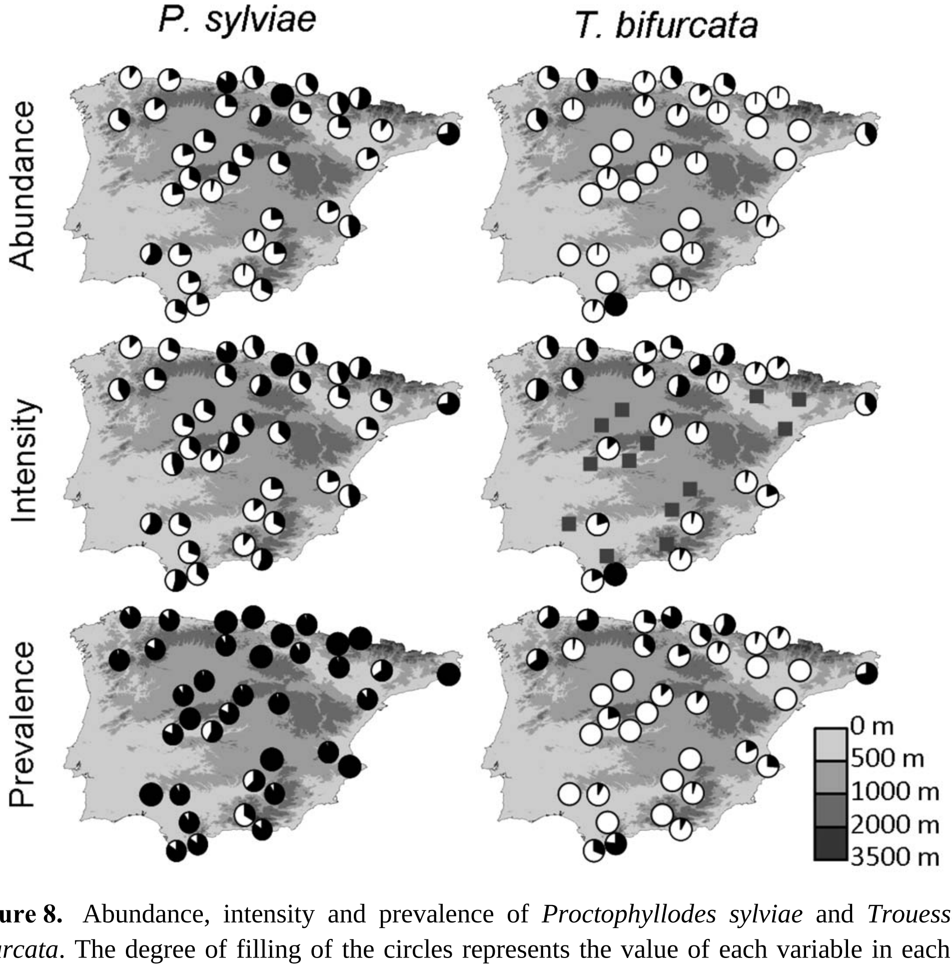


Figure 8. Abundance, intensity and prevalence of *Proctophyllodes sylviae* and *Trouessartia bifurcata*. The degree of filling of the circles represents the value of each variable in each site, expressed as the percentage of the maximum value observed across sites. Squares represent sites where the mite was absent, and therefore lacked data for intensity of infestation. Colour map represents altitude (metres above sea level).

P. sylviae prevalence and abundance were poorly modelled with the variables included in the study in comparison to *T. bifurcata*. However, the factors extracted for intensity of both mite species did not explain much variance. In every case, the variables that played the most important role in explaining differences in mite population patterns were temperature and precipitation variables: dry areas with a marked seasonality had a detrimental effect on both mite species. Furthermore, migration had a positive effect on *P. sylviae* prevalence.

Discussion

The study of interspecific interactions among coexisting symbionts ideally requires a multi-scale approach, which considers the distribution of different symbionts within the same host, among hosts in the same population, and among populations. Such analysis should enlighten what processes take part in the maintenance of diversity of symbiont species within the same host species. However, investigating host-symbiont interactions from such an integrative perspective is difficult, and consequently the patterns of distribution of coexisting symbionts are virtually unknown for most host-symbiont systems (Poulin 2007, Morand & Krasnov 2010). For this reason, this thesis is expected to contribute to increase the knowledge about symbiont distributions and interspecific interactions.

This study is not the first to show that feather mites are able to coexist within a single host; however, it is novel in describing how host-sharing may come at a price. *P. sylviae* and *T. bifurcata* frequently co-occur on blackcap wings, and there is compelling evidence that they interact, as shown by the fact that one mite species is not freely distributed with respect to the distribution of the other. At the host level, both mite species might exert a negative effect on each other, an idea which is supported by a negative correlation between the mite numbers of each species when they coexist on the same host. A possible strategy that mites may have followed to relax this competition is spatial segregation (Poulin 2007), beyond the fact that one species dwells on the dorsal side of feathers while the other lives on the ventral side. Thus, each mite species occupies a distinct location on the wing: *P. sylviae* preferentially occupies distal and medial sectors of the feather, whereas *T. bifurcata* preferably chooses internal and medial sections of the feathers. This niche partitioning supports the idea that, in the past, mites specialized in the exploitation of different parts of the wing, either because micro-habitat preferences evolved in each species associated with their modes of host exploitation (different sectors of the wing plumage might be optimal for the settlement of dorsal and ventral mites), or as a consequence of niche partitioning due to competition (Pritchard & Schluter 2001, Poulin 2007). Anyway, these diverging patterns of microhabitat selection on the host wing may facilitate coexistence of ecologically similar mite species on the same host species, often on the same individual. Even so, these results suggest that competition may still operate among coexisting feather mites. When total numbers are considered, interactions between mite species seem to be asymmetrical, with *T. bifurcata* apparently playing a dominant role over *P. sylviae*. To date it has not been possible to unravel the determinants of the distribution of different mite species on bird wings; thus, experimental studies would be an excellent way to have a deeper knowledge about this interesting system.

Interactions between mite species seem to be asymmetrical when host phenotype is taken into account at a local scale. Only in migratory blackcaps *T. bifurcata* seems to suffer a reduction in numbers when *P. sylviae* is present, but this reduction may be due to specific colonization problems (probably because migratory blackcaps represent poor-quality hosts for *T. bifurcata* mites), rather than to a negative interaction with *P. sylviae*. However, perhaps as a means of compensation, *P. sylviae* is much more abundant and prevalent, especially in migratory blackcaps, which seem to be less accessible to *T. bifurcata*. Generalist symbiont species are capable of colonising a wider range of hosts and/or a wider variety of habitats as opposed to more specialist species (Fox & Morrow 1981). This suggests that *P. sylviae* is more generalist than its putative competitor, which may be troubled to settle on migratory hosts or be more specialised in the exploitation of sedentary hosts. In sum, each host phenotype seemingly favours specifically a certain mite species (*P. sylviae* is more abundant on migratory blackcaps and *T. bifurcata* on sedentary ones), which is probably associated with specific host traits linked to habitat quality for mites (wing length in migratory blackcaps and uropygial gland size in sedentary blackcaps). Nevertheless, sedentary blackcaps seem to offer a more suitable scenario for mite coexistence than migratory blackcaps, according to the observation of both mite species co-occurring more frequently on this type of hosts.

As mentioned before, inherent characteristics of symbionts may condition their opportunities to encounter different host types or their success to establish on encountered hosts, and this might be a cause of the different patterns of distribution found for both mite species. Besides, the mode of transmission to new hosts may determine the genetic structure of within-host mite populations. In both mite species, the analyses of genetic structure showed that mite populations are genetically highly diverse within host individuals. Slight differences found in the degree of genetic structuring between the two mite species might be due to different reproductive strategies or dispersal opportunities (Barrett *et al.* 2008). Although vertical transmission apparently is the main mechanism for mite transmission, by no means bottleneck events seem to take place during transmission. As a consequence, outbreeding is guaranteed to mites within a single host, which is relevant to the understanding of within-host population dynamics of feather mites (Thornhill 1993, Keller & Waller 2002). More importantly from the perspective of this thesis, the fact that conspecific mites sharing a host individual are not close kin might create a competitive scenario, in which mites that are forced to occupy the least preferred sectors on the host wing do not obtain inclusive fitness returns from having relatives occupying the most favourable sectors. From this point of view, the comparison of phenotypic and genetic attributes of mites found on preferred *vs.* disfavoured sectors would provide much insight on mite-mite interactions. For example, the competitive scenario promoted by the coexistence of unrelated mites on the same wing, is a prerequisite for variation in body condition or age structure among mite populations occupying wing sectors of variable quality to have microevolutionary implications.

At a regional scale, as shown in this thesis, there are other factors shaping feather mite distribution and population parameters apart from the unavoidable presence of the host (Gray *et al.* 1992, Proctor 2003, Giorgi *et al.* 2004). Climatic conditions, as well as other variables participating in the characterization of a given area such as landscape and geographical features, exert a strong impact on feather mite distribution and numbers (Pérez-Rodríguez *et al.* 2013). As expected, drought may be a limiting factor for feather mites to thrive since they might have difficulties in capturing moist from the environment (Gaede & Knülle 1987). On the other hand, despite the fact that both mite species have similar requirements (food, space…) they seem to have different tolerance to changes in environmental conditions in the same geographical context. This suggests that these feather mites have developed different ecological specializations (Evangelista *et al.* 2008, Malenke *et al.* 2011); in other words, despite temperature and rainfall variables may exert a strong impact on the distribution of both mite species, the magnitude of such influence varies between species. *P. sylviae* has succeeded in thriving in all populations sampled for this thesis, giving evidence that this mite may be a more generalist species compared to *T. bifurcata* (which was apparently absent from a relatively large number of Iberian blackcap populations). This generalist behaviour of *P. sylviae* may counteract the numerical decrease of this species associated with coexistence with *T. bifurcata* at the within-host level. Conversely, *T. bifurcata* may compensate the potential limitations associated with environmental (Chapter 4) and host specialization (Chapters 1 and 2) by being more able than *P. sylviae* to maintain within-host population levels in the face of coexistence with other mites. Despite host phenotype apparently determines symbiont distribution and population size at a local scale, no evidence was found that host phenotype has an important effect in shaping such distributions at this level.

In conclusion, each mite species apparently has advantages over the other under some circumstances, and disadvantages in other circumstances. This in turn may contribute to favour the maintenance of these two ecologically similar species coexisting on the same host, at different scales from the host individual to the host species' range. Such scenario sheds light on the putative mechanisms through which coexistence of symbiont species in the same host species may be possible. Host-symbiont interactions may evolve in different ways across populations of the same host species, usually influenced by variation in host traits and changing environmental conditions across the host species' range (Malenke *et al.* 2011). The diversity of biotic and abiotic influences on symbiont distributions creates a mosaic of host-symbiont interaction outcomes among populations within the host's distribution range (Thompson 2005).

Conclusions

1. Feather mites are able to coexist on the same host. *P. sylviae* and *T. bifurcata* are the most common feather mite species occurring on blackcaps; both mite species may appear alone or sharing the same host individual. The type of mite infestation (single or multiple) may be influenced by a wide array of circumstances, ranging from differences in host attributes to contrasting host environments.

2. Different feather mite species occupy different parts of the wing. *P. sylviae* lives on the ventral side while *T. bifurcata* occupies the dorsal part of the wing. In addition, mites occupy different areas of the wing as well as different sectors within a single feather: *P. sylviae* preferentially occupies medial-outer regions of the feather while *T. bifurcata* appears in medial-inner sections. Besides, the order of cell occupation follows a different sequence in each feather mite species, hence the most preferred cells for one mite are not the most preferred for the other.

3. Host-sharing comes at a cost. Despite apparent niche partitioning, when both mite species coincide in the same cell, they experience a reduction in numbers. When mite numbers of each mite species on the wing are taken as a whole, *T. bifurcata* seems to play a dominant role when both mite species coexist on the same host individual, given that the presence of that mite is associated with lower numbers of *P. sylviae*. However, *T. bifurcata* mites apparently have more difficulties in colonizing as many hosts and to reach as large population sizes within hosts, compared to *P. sylviae*.

4. Host phenotype creates opportunities and constraints on feather mite distribution and population size. *P. sylviae* is favoured on migratory blackcaps, where it is more prevalent and abundant, whereas *T. bifurcata* shows greater prevalence and abundance on sedentary blackcaps. Interestingly, sedentary blackcaps offer a suitable habitat for both mite species, where coexistence becomes more frequent than on migratory blackcaps. Finally, certain host traits may favour an increase in mite load: *P. sylviae* load was positively correlated with host wing length (wings were longer in migratory blackcaps), while *T. bifurcata* load was positively correlated to uropygial gland size (sedentary blackcaps had bigger glands).

5. Local environmental conditions create a mosaic of outcomes across the host species' range. Climate factors (most notably temperature and precipitation) exert a strong impact on feather mite distribution; high temperatures, dry conditions and a marked seasonality have a detrimental effect on feather mite presence and abundance across the Iberian Peninsula. However, different feather mite species are unequally influenced by such variables: most remarkably, *T. bifurcata* is absent from the driest habitats, whereas *P. sylviae* is able to exist in all populations although it decreases in prevalence and abundance in the least favourable areas.

6. Feather mite coexistence might be explained by the advantages one species has over the other at different scales. At the within-host scale *T. bifurcata* may reduce *P. sylviae* numbers. However, *P. sylviae* is able to colonise migratory and sedentary hosts alike, and reaches much higher numbers than *T. bifurcata* (both within hosts and at higher geographic scales). At a regional scale, *P. sylviae* is more tolerant to environmental conditions than *T. bifurcata*, which is absent from some localities. This suggests that *P. sylviae* is a more generalist mite than *T. bifurcata*, which seems to suffer greater constraints associated with host attributes and environmental conditions.

Future perspectives

One approach that may help to understand the processes underlying mite distribution and coexistence would be conducting experiments. For example, an interesting experiment would be one in which mite numbers were manipulated, thus creating populations with different population sizes of each mite, either in single infestation or with the two species coexisting on the same host individual. This approach would make possible to know to what extent competition is taking an active part in regulating the populations of coexisting mites. However, such experiments are difficult to conduct (birds tend to drop feather mites in captivity) and many researchers have failed in their attempts.

It would be very interesting to unravel why certain mite species perform differently in hosts with different phenotype. It has been suggested that uropygial gland and wing length play an important role in regulating within-host mite population numbers. However, mites are also dependent on food availability on bird feathers, and blackcaps may have different resources depending on their phenotype. Microbiological techniques are currently being developed in order to know the microbiota (fungi, bacteria and yeast) on blackcap feathers, with the general goal of finding the links between mite numbers and the resources available for them on different hosts or parts of the host plumage.

The molecular ecology of feather mites still is a largely unexplored field. In this thesis it has been found that feather mites may have an enormous genetic diversity even within a single host individual, let alone within one host species. This observation supports the existence of important gene flow among mite infrapopulations (the populations existing on each host individual). An appealing follow-up question would be whether the weak genetic structure found among mite infrapopulations within the same blackcap population persists or turns into more perceptible structure at a broader scale, for example among host populations across the Iberian blackcap species’ range.

References

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature*, 299: 818-820.

Atyeo, W. T. & Braasch, N. L. 1966. The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). *Bulletin of the University of Nebraska State Museum*, 5: 1-354.

Atyeo, W. T. & Gaud, J. 1979. *Feather mites and their hosts.* In: Rodríguez, J. G. (ed.). Recent Advances in Acarology. Academic Press, New York: 355-361.

Atyeo, W. T. & Windingstad, R. M. 1979. Feather mites of the Greater Sandhill Crane. *Journal of Parasitology*, 65: 650-658.

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 in Ecology and Evolution*, 23: 678-685.

Berthold, P. 1975. *Migration: control and metabolic physiology*. In: Farner, D. S. & King, J. R. (eds.). Avian biology. Academic Press, New York, V: 77-128.

Berthold, P. 2001. *Bird migration: a general survey*. Oxford Ornithology Series, Oxford University Press, Oxford.

Blanco, G. & Frías, O. 2001. Symbiotic feather mites synchronize dispersal and population growth with host sociality and migratory disposition. *Ecography*, 24: 113-120.

Blanco, G., Tella, J. L. & Potti, J. 1997. Feather mites on group-living Red-billed Choughs: a non-parasitic interaction? *Journal of Avian Biology*, 28: 197-206.

Blanco, G., Seoane, J. & De la Puente, J. 1999. Showiness, non-parasitic symbionts, and nutritional condition in a passerine bird. *Annales Zoologici Fennici*, 36: 83-91.

Blanco, G., Tella, J. L. & Potti, J. 2001. Feather mites on birds: costs of parasitism or conditional outcomes? *Journal of Avian Biology,* 32: 271-274.

Burtt, E. H., Jr. 2009. A future with feather-degrading bacteria. *Journal of Avian Biology*, 40: 349-351.

Burtt, E. H., Jr. & Ichida, J. M. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. *The Auk*, 116: 364-372.

Carbonell, R. 2003. *Curruca capirotada Sylvia atricapilla*. In: Martí, R. & del Moral, J. C. (eds.). Atlas de las aves reproductoras de España. Dirección General de Conservación de la Naturaleza - SEO/BirdLife, Madrid: 484-485.

Chesson, P. 1994. Multispecies competition in variable environments. *Theoretical Population Biology*, 45: 227-276.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 31: 343-366.

Choe, J. C. & Kim, K. C. 1989. Microhabitat selection and coexistence in feather mites (Acari: Analgoidea) on Alaskan seabirds. *Oecologia,* 79: 10-14.

Cramp, S. (ed.). 1992. *Handbook of the birds of Europe, the Middle East, and North Africa. The Birds of the Western Palearctic, Vol. VI, Warblers*. Oxford University Press, Oxford.

Dabert, J., Ehrnsberger, R. & 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, J. & Mironov, S. V. 1999. Origin and evolution of feather mites (Astigmata). *Experimental and Applied Acarology*, 23: 437-454.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 2007. Testing the validity of discriminant function analyses based on bird morphology: the case of migratory and sedentary blackcaps *Sylvia atricapilla* wintering in southern Iberia. *Ardeola*, 54: 81-91.

Dogiel, V. A. 1964. *General Parasitology*. Oliver and Boyd, Edinburgh.

Douglas, A. E. 2010. *The symbiotic habit*. Princeton University Press, Princeton.

Dowling, D. K., Richardson, D. S. & Komdeur, J. 2001. No effects of a feather mite on body condition, survivorship, or grooming behavior in the Seychelles warbler, *Acrocephalus sechellensis*. *Behavioral Ecology and Sociobiology*, 50: 257-262.

Dubinin, V. B. 1951. Feather mites (Analgesoidea). Part I. Introduction to their study. *Fauna SSSR, Paukoobraznye*, 6: 1-363.

Elder, W. H. 1954. The oil gland of birds. *Wilson Bulletin*, 66: 6-31.

Evangelista, P. H., Kumar, S., Stohlgren, T. J., Jarnevich, C. S., Crall, A. W., Norman, J. B., III & Barnett, D. T. 2008. Modelling invasion for a habitat generalist and a specialist plant species. *Diversity and Distributions*, 14, 808-817.

Fiedler, W. 2005. Ecomorphology of the external flight apparatus of Blackcaps (*Sylvia atricapilla*) with different migration behavior. *Annals of New York Academy of Sciences*, 1046: 253-263.

Fox, L. R. & Morrow, P. A. 1981. Specialization: species property or local phenomenon? *Science*, 211: 887-893.

Gaede, K. & Knülle, W. 1987.Water vapour uptake from the atmosphere and critical equilibrium humidity of a feather mite. *Experimental and Applied Acarology*, 3: 45-52.

Galván, I. & Sanz, J. J. 2006. Feather mite abundance increases with uropygial gland size and plumage yellowness in Great Tits *Parus major*. *Ibis*, 148: 687-697.

Galván, I., Barba, E., Piculo, R., Cantó, J. L., Cortés, V., Monrós, J. S., Atiénzar, F. & Proctor, H. 2008. Feather mites and birds: an interaction mediated by uropygial gland size? *Journal of Evolutionary Biology*, 21: 133-144.

Galván, I., Aguilera, E., Atiénzar, F., Barba, E., Blanco, G., Cantó, J. L, Cortés, V., Frías, O., Kovács, I., Meléndez, L., Møller, A. P., Monrós, J. S., Pap, P. L., Piculo, R., Senar, J. C., Serrano, D., Tella, J. L., Vágási, C. I., Vögeli, M. & Jovani, R. 2012. Feather mites (Acari: Astigmata) and body condition of their avian hosts: a large correlative study. *Journal of Avian Biology*, 43: 273-279.

Gaud, J. & Atyeo, W. T. 1996. Feather mites of the world (Acarina, Astigmata): The supraspecific taxa. Musée Royale de l'Afrique Centrale, Annales Sciences Zoologiques, 277(Part I, Text): 1-193.

Ginn, H. B. & Melville, D. S. 1983. *Moult in birds*. BTO, Tring.

Giorgi, M. S., Arlettaz, R., Guillaume, F., Nusslé, S., Ossola, C., Vogel, P. & Christe, P. 2004. Causal mechanisms underlying host specificity in bat ectoparasites. *Oecologia*, 138: 648-654.

Gray, J. S., Kahl, O., Janetzki, C. & Stein, J. 1992. Studies on the ecology of Lyme disease in a deer forest in county Galway, Ireland. *Journal of Medical Entomology*, 29: 915-920.

Harper, D. G. C. 1999. Feather mites, pectoral muscle condition, wing length and plumage coloration of passerines. *Animal Behaviour*, 58: 553-562.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 14812-14817.

Hutchinson, G. E. 1957. Concluding remarks. *Cold Spring Harbour Symposia*, 22: 415-427.

Hutchinson, G. E. 1961. The paradox of the plankton. *The American Naturalist*, 95: 137-145.

Jacob, J. & Ziswiler, V. 1982. *The uropygial gland*. In: Farner, D. S., King, J. R. & Parkes, K. C. (eds.). Avian Biology. Academic Press, New York, VI: 199-324.

Janovy, J., Jr. 1997. *Protozoa, helminths, and arthropods of birds*. In: Clayton, D. H. & Moore, J. (eds.) Host-parasite evolution: general principles and avian models. Oxford University Press, New York: 303-337.

Keller, L. F. & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17: 230-241.

Kress, W. J. & Erickson, D. L. 2008. DNA barcodes: genes, genomics, and bioinformatics. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 2761-2762.

Lucas, A. M. & Stettenheim, P. R. 1972. *Avian anatomy, Integument, Part I*. Agriculture Handbook 362. United States Government Printing Office, Washington.

Malenke, J. R., Newbold, N. & Clayton, D. H. 2011. Condition-specific competition governs the geographic distribution and diversity of ectoparasites. *The American Naturalist*, 177: 522-534.

Mestre, A., Mesquita-Joanes, F., Proctor, H. & Monrós, J. S. 2011. Different scales of spatial segregation of two species of feather mites on the wings of a passerine bird. *Journal of Parasitology*, 97: 237-244.

Mironov, S.V. 1987. Morphological adaptations of feather mites to different types of plumage and skin of birds. *Parazitologicheskii Sbornik*, 34: 114-132.

Møller, A. P. & Erritzøe, J. 1998. Host immune defense and migration in birds. *Evolutionary Ecology*, 12: 945-953.

Morand, S. & Krasnov, B. R. (eds.). 2010. *The biogeography of host-parasite interactions*. Oxford University Press, New York.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. *Journal of Parasitology*, 81: 395-403.

Newton, I. 1998. *Population limitation in birds*. Academic Press, London.

Nilsson, J.-A. & Svensson, E. 1996. The cost of reproduction: a new link between current reproductive effort and future reproductive success. *Proceedings of the Royal Society of London B*, 263: 711-714.

Pap, P. L., Tökölyi, J. & Szép, T. 2005. Host-symbiont relationship and abundance of feather mites in relation to age and body condition of the barn swallow (*Hirundo rustica*): an experimental study. *Canadian Journal of Zoology*, 83: 1059-1066.

Paracer, S. & Ahmadjian, V. 2000. *Symbiosis: an introduction to biological associations*. Oxford University Press, Oxford.

Pérez, T. M. & Atyeo, W. T. 1984. *Site selection of feather and quill mites of Mexican parrots*. In: Griffiths, D. A. & Bowman, C. E. Acarology VI. Ellis Horwood, Chichester: 563-570.

Pérez-Rodríguez, A., Fernández-González, S., De la Hera, I. & Pérez-Tris, J. 2013. Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Global Change Biology*, doi: 10.1111/gcb.12226.

Pérez-Tris, J. & Tellería, J. L. 2002. 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., Carbonell, R., Tellería, J. L. 1999. A method for differentiating between sedentary and migratory Blackcaps *Sylvia atricapilla* in wintering areas of southern Iberia. *Bird Study*, 46: 299-304.

Pérez-Tris, J., Carbonell, R., Tellería, J. L. 2002. Parasites and the blackcap’s tail: implications for the evolution of feather ornaments. *Biological Journal of the Linnean Society*, 76: 481-492.

Poulin, R. 1991. Group-living and infestation by ectoparasites in passerines. *Condor*, 93: 418-423.

Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, Princeton.

Pritchard, J. R. & Schluter, D. 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. *Evolutionary Ecology Research*, 3: 209-220.

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

Proctor, H. & Owens, I. 2000. Mites and birds: diversity, parasitism and coevolution. *Trends in Ecology and Evolution*, 15: 358-364.

Pugh, G. J. F. 1965. Cellulolytic and keratinophilic fungi recorded on birds. *Sabouraudia*, 4: 85-91.

Roderick, G. K. 1996. Geographic structure of insect populations: gene flow, phylogeography, and their uses. *Annual Review of Entomology*, 41: 325-352

Roderick, G. K. & Navajas, M. 2003. Genes in new environments: genetics and evolution in biological control. *Nature Reviews Genetics*, 4: 889-899.

Santana, F. J. 1976. A review of the genus *Trouessartia*. *Journal of Medical Entomology*, 1: S1-S128.

Shirihai, H., Gargallo, G. & Helbig, A. J. 2001. *Sylvia warblers: identification, taxonomy and phylogeny of the genus Sylvia*. Christopher Helm, London.

Simberloff, D. & Moore, J. 1997. *Community ecology of parasites and free-living animals*. In: Clayton, D. H. & Moore, J. (eds.) Host-parasite evolution: general principles and avian models. Oxford University Press, New York: 174-197.

Schluter, D. 2000. *The ecology of adaptive radiation.* Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Soberón, J. & Peterson, A. T. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, 2: 1-10.

Soler, J. J., Peralta-Sánchez, J. M., Martín-Platero, A. M., Martín-Vivaldi, M., Martínez-Bueno, M. & Møller, A. P. 2012. The evolution of size of the uropygial gland: mutualistic feather mites and uropygial secretion reduce bacterial loads of eggshells and hatching failures of European birds. *Journal of Evolutionary Biology*, 25: 1779-1791.

Svensson, L. 1992. *Identification guide to European passerines*. L. Svensson, Stockholm.

Tellería, J. L. & Carbonell, R. 1999. Morphometric variation of five Iberian Blackcap *Sylvia atricapilla* populations. *Journal of Avian Biology*, 30: 63-71.

Thompson, J. N. (ed.). 2005. *The geographic mosaic of coevolution*. University of Chicago Press, Chicago.

Thompson, C. W., Hillgarth, N., Leu, M. & McClure, H. E. 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *The American Naturalist*, 149: 270-294.

Thornhill, N. W. (ed.). 1993. *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*. University of Chicago Press, Chicago.

Videler, J. J. 2005. Avian flight. Oxford Ornithology Series, Oxford University Press, Oxford.

Chapter 1

This chapter is based on the manuscript:

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Divergent host phenotypes create opportunities and constraints on the distribution of two wing-dwelling feather mites

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The diversity of symbionts (commensals, mutualists or parasites) that share the same host species may depend on opportunities and constraints on host exploitation associated with host phenotype or environment. Various host traits may differently influence host accessibility and within-host population growth of each symbiont species, or they may determine the outcome of within-host interactions among coexisting species. In turn, phenotypic diversity of a host species may promote divergent exploitation strategies among its symbiotic organisms. We studied the distribution of two feather mite species, *Proctophyllodes sylviae* and *Trouessartia bifurcata*, among European blackcaps *Sylvia atricapilla* wintering in southern Spain during six winters. The host population included migratory and sedentary individuals, which were unequally distributed between two habitat types (forests and shrublands). Visual mite counts showed that both mite species often coexisted on sedentary blackcaps, but were seldom found together on migratory blackcaps. Regardless of host habitat, *P. sylviae* was highly abundant and *T. bifurcata* was scarce on migratory blackcaps, but the abundance of both mite species converged in intermediate levels on sedentary blackcaps. Coexistence may come at a cost for *P. sylviae*, whose load decreased when *T. bifurcata* was present on the host (the opposite was not true). *P. sylviae* load was positively correlated with host wing length (wings were longer in migratory blackcaps), while *T. bifurcata* load was positively correlated to uropygial gland size (sedentary blackcaps had bigger glands), which might render migratory and sedentary blackcaps better hosts for *P. sylviae* and *T. bifurcata*, respectively. Our results draw a complex scenario for mite co-existence in the same host species, where different mite species apparently take advantage of, or are constrained by, divergent host phenotypic traits. This expands our understanding of bird-mite interactions, which are usually viewed as less dynamic in relation to variation in host phenotype, and emphasizes the role of host phenotypic divergence in the diversification of symbiotic organisms.

Introduction

Ever since Hutchinson (1961) introduced his “paradox of the plankton”, identification of mechanisms that allow coexistence of species with apparently equivalent functional roles in ecosystems has been central to understanding the evolution and maintenance of biodiversity (Chesson 2000, Fox *et al.* 2010). If different species occupy the same ecological niche, any competitive advantage for one species should drive all others to extinction. However, diversity is the rule rather than the exception in nature, a circumstance which is usually attributed to environment heterogeneity, temporal variation in competitive interactions, or variation in the impact of natural enemies (Chesson 1994, 2000).

Within-host coexistence of symbionts (commensals, mutualists or parasites) may be particularly intricate, because a host may accommodate various symbiont species with apparently the same resources, while symbionts often share the same mode of host exploitation (Poulin 2007). For an obligate symbiont, the population of hosts may be broadly viewed as the fundamental niche, *i.e.*, the habitat that provides conditions and resources for the species to exist in the absence of competitors, predators and pathogens (Hutchinson 1957, Soberón & Peterson 2005). Such a habitat is divided into spatially limited patches (individual hosts), which are ephemeral and may be difficult to access (Schmid-Hempel 2011). In this context, whether a symbiont species is abundant or not depends on its ability to successfully colonize new hosts and to increase population size in newly colonized hosts (Clayton & Moore 1997, Poulin 2007).

Different characteristics of the host-symbiont relationship may determine the proportion of individual hosts that are occupied by the symbiont (symbiont prevalence) and within-host number of symbionts (symbiont load). With regard to prevalence, host population density and exposure to symbionts facilitate symbiont spread, while symbiont species may show variable degrees of host specificity (Poulin 1991, Poulin *et al.* 2011). With regard to load, within-host number of symbionts primarily depends on quality, quantity or accessibility of the host resource under exploitation (Kelly & Thompson 2000, Krasnov *et al.* 2005). Finally, interactions with other symbionts may greatly determine which individuals in a host population are exploited by a particular symbiont species (Poulin 2007). For instance, when two different symbiont species coexist on the same host, the abundance of each species may decrease in presence of the other (Poulin 2007). Alternatively, competition may trigger niche shifts instead of changes in relative numbers of symbionts (Poulin 2007), including segregation of food, space or time (Schoener 1974, Mestre *et al.* 2011). Knowledge of the demographic consequences of symbiont coexistence is central to our understanding of the evolution of symbiont diversity, yet how within-host co-occurrence affects prevalence and load of coexisting symbionts remains unknown for most host-symbiont systems (Schmid-Hempel 2011).

We studied the environmental determinants and the population consequences of coexistence of two feather mite species, *Proctophyllodes sylviae* and *Trouessartia bifurcata*, that often co-occur on European blackcaps *Sylvia atricapilla* wintering in southern Spain. *P. sylviae* and *T. bifurcata* mites provide an excellent opportunity to explore the determinants and consequences of within-host mite coexistence because of two reasons. Firstly, they are distinct enough to be easily told apart in the field. *P. sylviae* are small elongate mites which occupy the ventral side of wing feathers, while *T. bifurcata* mites are larger, more rhomboidal in shape, and live on the dorsal side of wing feathers (Atyeo & Braasch 1966, Santana 1976). Secondly, the two mites feed on uropygial gland oil and particles contained within (pollen, fungi, yeast, bacteria, etc.; Proctor 2003). Therefore, although competition between these mites may be somewhat prevented because they occupy different spatial location on the host (Mestre *et al.* 2011), they still could compete for resources if uropygial oil seeping through feathers can be depleted from the ventral or dorsal sides of the wing.

Blackcaps wintering in southern Spain make an interesting scenario in which the distribution of different mite species could be subjected to different constraints and opportunities, which ultimately might determine the outcomes of interactions between mites. Mites are influenced both by host characteristics and by different components of the host environment, such as temperature and humidity (Dubinin 1951, Blanco & Frías 2001). Interestingly, blackcap populations wintering in southern Spain are composed of a mixture of local sedentary individuals and overwintering migratory individuals arrived from further north (primarily from western Central Europe; Pérez-Tris & Tellería 2002). The coexistence of two host types in the same population introduces variation in host characteristics and host environments that might affect the context in which *P. sylviae* and *T. bifurcata* mites interact. In the first place, sedentary birds are nearly restricted to the forests where they breed during the summer, while migratory blackcaps are common both in these forests and in the surrounding shrublands. Compared to forests, shrublands are located at lower elevation (and consequently are drier and warmer than forests), and they are more exposed to sunlight due to reduced vegetation cover (Pérez-Tris &

Tellería 2002). These characteristics of the host's habitat may differently affect each mite species (Dowling *et al.* 2001, Krasnov *et al.* 2008), thereby creating patterns of variation in prevalence or mite load between habitat types that may interact with the different distribution of migratory and sedentary blackcaps in these habitats.

Migratory and sedentary blackcaps also show different characteristics that may affect both their exposure to mites and their suitability as hosts for different mite species. Various comparative studies have found that migratory bird species have more abundant feather mites than sedentary bird species (Galván *et al.* 2008), although there seems to be little variation in mite prevalence in relation to host migration (Figuerola 2000). Whether bird migration promotes mite species coexistence remains unknown. Migratory birds have physiological and behavioural adaptations for migration (Piersma *et al.* 2005), which may affect their profitability as mite hosts (Blanco & Frías 2001, Galván *et al.* 2008). For example, migration promotes an acceleration of moult (De la Hera *et al.* 2009) that can impair the expression of feather characteristics such as structure or colour (Dawson *et al.* 2000, Griggio *et al.* 2009). In fact, migratory blackcaps moult faster and invest less material per feather than do sedentary blackcaps (De la Hera *et al.* 2009), although their feathers end up showing increased bending stiffness (a trait which improves feather aerodynamics; De la Hera *et al.* 2010a). Variation in plumage attributes may involve different feather maintenance needs, although we do not know whether sedentary blackcaps devote more efforts to maintain their more densely constructed feathers, or whether migratory blackcaps devote greater efforts to maintain their lighter but stiffer feathers in good shape for migration. In any case, given that feather maintenance greatly depends on uropygial oil secretions, we might expect migratory and sedentary blackcaps to differ in the size of their uropygial glands (as a correlate of their secretory capacity; Bhattacharyya & Chowdhury 1995, Møller *et al.* 2009), potentially resulting in habitats of different nutritional quality for feather mites.

Intrinsic and extrinsic differences (associated with habitat use) between sedentary and migratory blackcaps could differently affect *P. sylviae* and *T. bifurcata* mite populations, and therefore may determine the outcomes of interactions between these species. Based on six years of feather mite population monitoring on migratory and sedentary blackcaps wintering in sympatry, we set out to test several questions relevant to our understanding of the causes and consequences of mite coexistence:

What determines variation in mite distribution among individual blackcaps?

The distribution of *P. sylviae* and *T. bifurcata* feather mites (abundance, prevalence and mite load) on blackcaps wintering in southern Spain might vary between habitat types (forests and shrublands), between blackcap populations (sedentary or migratory), or among years. In addition, individual host traits may help to explain variation (if any) between migratory and sedentary hosts in the structure of mite populations. In particular, the amount of habitat available for mites to occupy may depend on host's wing size (Jovani & Blanco 2000), which greatly varies among individual blackcaps (because migratory blackcaps have longer wings as an adaptation to long-distance flight, resulting in increased wing area; Tellería & Carbonell 1999, Pérez-Tris & Tellería 2001). In addition, birds may vary in the size of the uropygial gland, which may also differ between migratory and sedentary blackcaps if the variation in plumage structure described above involves different oil demands.

How does the distribution of each mite species affect within-host mite coexistence?

Whether *P. sylviae* and *T. bifurcata* mites have similar or different distribution between forests and shrublands, host phenotypes (migratory or sedentary) or years may determine the chances of finding both mite species co-occurring on the same host individual. We identified factors that may favour or prevent mite coexistence by analysing the distribution of each mite species in relation to the occurrence of the other. Because the distribution of each mite species may vary between habitats or host phenotypes, we tested for variation in the frequency of within-host mite coexistence between habitat types (forests or shrublands) and host phenotypes (migratory or sedentary), controlling for possible variation among years.

What are the consequences of coexistence for mite populations?

If *P. sylviae* and *T. bifurcata* share host resources, their coexistence on the same host individual might affect population growth rate of one or both mite species. Also, presence of one species on a particular host individual might reduce the likelihood of members of the other species colonizing that host. As a consequence, both the frequency of occurrence and the load of a given mite species are expected to vary in relation to the occurrence of the other on the same host. However, the outcome of these interactions between mite species may depend on individual host phenotype. In our study, host-specific outcomes of mite coexistence may be particularly variable between migratory and sedentary hosts. If mite populations are limited by habitat size, migratory blackcaps may be better hosts because they have larger wings. Different outcomes could be expected if mite populations are limited by food availability, depending on which type of blackcaps (sedentary or migratory) provides more abundant oil secretions. In turn, we expect the impact of competition on mite populations to be greater on the least rewarding host phenotype, according to the observed variation in the abundance of resources that may limit mite populations (habitat or food).

Material and methods

Study area and field methods

Between December and February during six winters (from 2005 to 2010), we sampled blackcaps both in forests and in shrublands in the Campo de Gibraltar area (southern Spain). We captured birds using mist nets and we kept them in individual cloth bags fitted with coffee filters, which were originally used to collect faecal samples of the birds but gave us the opportunity to evaluate the chances of mites being artificially transported among birds kept in the same bags. We never found mites of any kind in the analysis of 760 faecal samples of blackcaps inspected under the microscope (including most of the birds used in this study), although we thoroughly searched for arthropod items (IDH & JPT unpubl.). Therefore, the chances are very slim that mites remained in the bags and could thus be transported among birds. We sexed and aged birds according to plumage (Svensson 1992). We distinguished between first winter and older blackcaps, although ten birds could not be unambiguously aged. We measured tarsus length and bill length to the nearest 0.01 mm, and the length of the flattened wing, the eighth primary feather and the tail to the nearest 0.5 mm. We also measured distances from the wing tip to the tip of each primary feather 1 to 9 (primary distances, 0.5-mm precision). We fitted all birds with a standard aluminium ring to avoid repeatedly sampling the same individual, and we released them at the site of capture after manipulation. In all, we studied 564 individual blackcaps during the six study winters.

To count mites of each species, we exposed one spread wing towards the ambient light or a lamp, and counted all mites visible on the vanes of primary, secondary and tertial feathers (Jovani & Serrano 2004). For heavily infested birds (scoring mite counts in the hundreds) we determined the area of the wing occupied by ten mites and counted the number of groups of similar size on the whole wing to obtain an approximate mite count. Between-observer repeatability, as computed from data of mite numbers that were blindly assessed by two of us, was very high (ri > 0.88).

Mites of the genera *Proctophyllodes* and *Trouessartia* were distinguished by eye according to their size, shape and location on the ventral or dorsal side of feathers, respectively. Microscope examination of a random sample of 203 *Proctophyllodes* and 32 *Trouessartia* mites obtained from 14 blackcaps (including migratory and sedentary individuals) confirmed field identification (according to Atyeo & Braasch 1966, Santana 1976), with *P. sylviae* and *T. bifurcata* as the only two species of vane-dwelling feather mites found. We also found a few representatives of other mite genera (*Analges* and *Strelkoviacarus*), which together accounted for less than 1% of all mites observed. Therefore, we are confident that our data represented variation in the distribution of the aforementioned two mite species.

During the last two winters (February and December 2010), we completed our sample with the aim of analysing relationships between individual host traits (wing length and size of uropygial glands) and mite occurrence and load. We took the same morphological measurements and counted mites on all birds included in this new dataset (*n* = 160) as described above. In addition, we measured the length, width and depth of their uropygial glands to the nearest 0.01 mm. We used the product of the three metrics as a measure of uropygial gland volume (Galván & Sanz 2006, Galván *et al.* 2008).

We used a discriminant function analysis based on the length of the eighth primary, tail length and the difference between primary distances 1 and 9 to classify blackcaps as migratory or sedentary (Pérez-Tris *et al.* 1999). Great morphological differences related to migration allows for the correct classification of over 90% of blackcaps using this method (De la Hera *et al.* 2007).

Statistical analyses

The distribution of mite abundances among hosts depends on the proportion of occupied hosts and within-host mite numbers. We used mite prevalence (proportion of hosts that had at least one mite) as a measure of the distribution of mite occurrence among hosts. Mite load (number of mites counted on hosts that had at least one mite) represented within-host mite population size. The combined variation in mite prevalence and mite load generate variation in mite abundance, which we define here as the average number of mites per host including mite-free birds. We analysed variation in abundance of each mite species using repeated measures Generalised Linear Models (GLZ, in which individual host was included as a within-subject factor) with a Poisson error structure and Log link function (GENMOD procedure implemented in SAS 2008). We used log-linear analysis to model variation in prevalence of either *P. sylviae* or *T. bifurcata* in relation to year, habitat type, host phenotype and presence or absence of the other mite species on the host, using the hierarchical method for model building implemented in STATISTICA 7.0 (StatSoft 2004).

We used GLZ with a Poisson error structure and Log link function to analyse variation in mite counts in relation to year, habitat type, host phenotype and presence or absence of the other mite species on the host. We run the same analysis using mite abundance of the other mite species as a covariate instead of mite presence or absence. We conducted separate analyses of mite abundance (considering all hosts) and mite load (excluding mite-free hosts). For the analyses of abundance and load of *T. bifurcata* and *P. sylviae* presence/absence as a classification factor, we excluded the last three years (which reduced sample size to $n = 366$), because we found only one blackcap free of *P.*

sylviae (the absence of birds not infested with this mite species produced empty cells in the statistical design, which prevented us from testing for variation in numbers of *T. bifurcata* in relation to coexistence with *P. sylviae*).

We are aware that mite prevalence and load may be affected by host sex and age (Proctor 2003), although including these variables as factors would fragment our statistical designs making it difficult to test for the relevant effects in our study. Nevertheless, we made sure that sex and age classes were homogeneously distributed between habitat types and in relation to host phenotype (log-linear model of the associations among sex, age, host phenotype, habitat type and year of capture of blackcaps: goodness of fit maximum likelihood chi-square test: $\chi^2_{(70)} = 59.90$, $P = 0.80$, all two-way associations involving the relevant factors with $P > 0.05$). We therefore excluded sex and age effects from our analyses.

Results

General patterns of distribution of mite abundance

Mite populations on infested hosts ranged 2-1000 mites for *P. sylviae* and 1-217 mites for *T. bifurcata*. We did not find consistent effects of habitat type (shrubland or forest) on mite abundance or load (either considering all mites together or distinguishing between mite species) measured in migratory blackcaps, the only ones that regularly occur in shrublands. Only the abundance of *T. bifurcata* changed between habitats for one of the six study years (all other effects of habitat type or its interaction with other factors in GLZ models with $P > 0.10$). We therefore excluded habitat type from the analyses of these variables, which allowed for a better estimation of the effects of host phenotype by avoiding including cells with too small a sample size in our statistical designs (due to the scarcity of sedentary blackcaps in shrublands).

Considering both mite species together (as in most studies of feather mites conducted so far), mites were more abundant on migratory than on sedentary blackcaps (mean abundance ± SE: migratory blackcaps = 98.9 ± 0.07 mites per host; sedentary blackcaps = 42.0 ± 0.15 mites per host; $\chi^2_{(1)} = 32.73$, $P < 0.001$), after controlling for a significant effect of year on total mite abundance ($\chi^2_{(5)} = 46.63$, $P < 0.001$). Mite load (excluding mite-free birds) was also higher on migratory than on sedentary blackcaps (mean load ± SE: migratory blackcaps = 112.8 ± 0.05 mites per infested host; sedentary blackcaps = 81.1 ± 0.13 mites per infested host; $\chi^2_{(1)} = 6.73$, $P = 0.009$), after controlling for a significant effect of year on total mite load ($\chi^2_{(5)} = 45.88$, $P < 0.001$). The best log-linear model to explain variation in mite occurrence in relation to habitat type, host phenotype and year (goodness of fit maximum likelihood χ^2-test: $\chi^2_{(22)} = 19.50$, $P = 0.61$) showed that total mite prevalence varied among years (partial association: $\chi^2_{(5)} = 34.52$, $P < 0.001$; marginal association: $\chi^2_{(5)} = 31.48$, $P < 0.001$) and depended on host phenotype (partial association: $\chi^2_{(1)} = 25.05$, $P < 0.001$; marginal association: $\chi^2_{(1)} = 26.96$, $P < 0.001$), but did not change among habitats ($P > 0.60$), controlling for significant variation in the proportion of sedentary and migratory blackcaps captured each year or in each habitat type (effects not reported but qualitatively equal to those shown in Table 1.1). In all, migratory blackcaps had higher prevalence of feather mites (97.2%) than sedentary blackcaps (83.9%).

Table 1.1. Log-linear analysis of mite prevalence (*P. sylviae* or *T. bifurcata*) according to host habitat, host phenotype (migratory or sedentary), year, and occurrence of the other mite species in the same host. From the top downwards, the table shows the fit to the null hypothesis that all interactions of the corresponding order (only the relevant ones are shown) are simultaneously equal to zero, the goodness of fit of the final model, and the contribution of each interaction included in the model. Partial associations are computed by evaluating the gain of fit of the model that includes the corresponding interaction with the model that excludes it. Marginal associations are computed by comparing the fit of the model including all effects of lower order than the one of interest with the model including that interaction instead (StatSoft 2004).

		Maximum likelihood chi-square			
	df	χ^2	P		
Order of interactions					
No fourth-order interactions	21	10.99	0.963		
No third-order interactions	34	53.80	0.017		
Test of fit of the final model:	50	27.18	0.997		
		Partial association		Marginal association	
	df	χ^2	P	χ^2	P
Interactions in the model					
Habitat × host phenotype	1	35.26	< 0.001	50.58	< 0.001
Winter × host phenotype	5	25.54	< 0.001	43.21	< 0.001
Proctophyllodes × habitat × winter	5	6.04	0.303	12.12	0.033
Proctophyllodes × *Trouessartia* × winter	5	15.10	0.010	15.60	0.008
Proctophyllodes × *Trouessartia* × host phenotype	1	7.07	0.008	12.11	< 0.001

Abundance distribution of each mite species

P. sylviae and *T. bifurcata* showed different patterns of variation in abundance between migratory and sedentary hosts. In a repeated-measures GLZ with the individual host as a within-subject factor, *P. sylviae* was more abundant than *T. bifurcata* overall (within-host difference in abundance between mite species: $\chi^2_{(1)} = 82.55$, $P < 0.001$), but this effect changed in relation to host phenotype (mite species × host phenotype: $\chi^2_{(1)} = 59.71$, $P < 0.001$). *P. sylviae* was much more abundant than *T. bifurcata* on migratory blackcaps, while *T. bifurcata* increased abundance and *P. sylviae* decreased abundance on sedentary blackcaps, so that both mites reached similar abundance on this type of hosts (Figure 1.1). This pattern was consistent among years, although mite numbers on migratory and sedentary hosts greatly varied among study seasons (year × host phenotype: $\chi^2_{(5)} = 21.76$, $P < 0.001$; Figure 1.1). In general, the different distribution of *P. sylviae* and *T. bifurcata* between migratory and sedentary blackcaps created a slight but significant negative correlation between the abundance of the two mite species among hosts (beta = -0.18, $F_{1,562} = 17.98$, $P < 0.001$).

Patterns of mite co-occurrence

The above results were partly explained by different patterns of occurrence of each mite species between migratory and sedentary blackcaps. The best log-linear model to explain the frequency of occurrence of the two mite species in relation to year and host phenotype took into account among-year changes in both the proportion of migratory and sedentary blackcaps and the relative prevalence of *T. bifurcata* and *P. sylviae* mites (Table 1.1). Controlling for these effects, the frequency of co-occurrence of the two species depended on host phenotype (leading to a significant interaction between presence of *T. bifurcata*, presence of *P. sylviae* and host phenotype; Table 1.1). The prevalence of a mite given species was higher among host individuals that were infested by the other species in sedentary blackcaps, but did not vary in relation to the occurrence of the other species in migratory blackcaps (Figure 1.2).

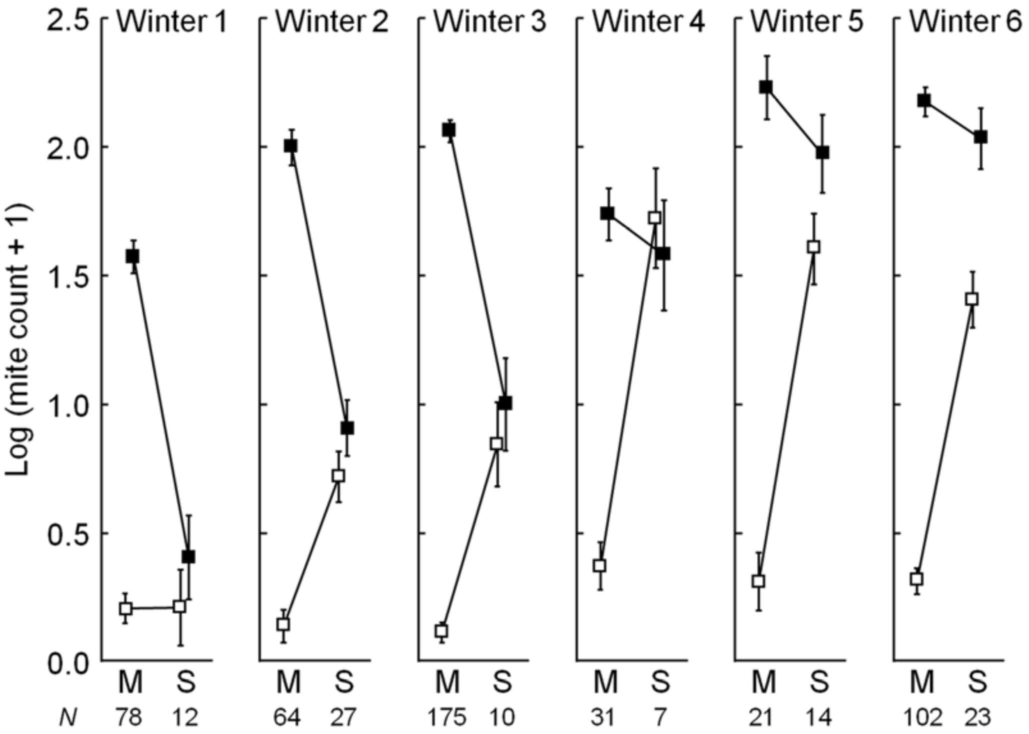

Figure 1.1. Variation in the total number of *Trouessartia bifurcata* (white squares) and *Proctophyllodes* sylviae (filled squares) mites counted on migratory (M) and sedentary (S) blackcaps for each study year (means ± SE and sample sizes).

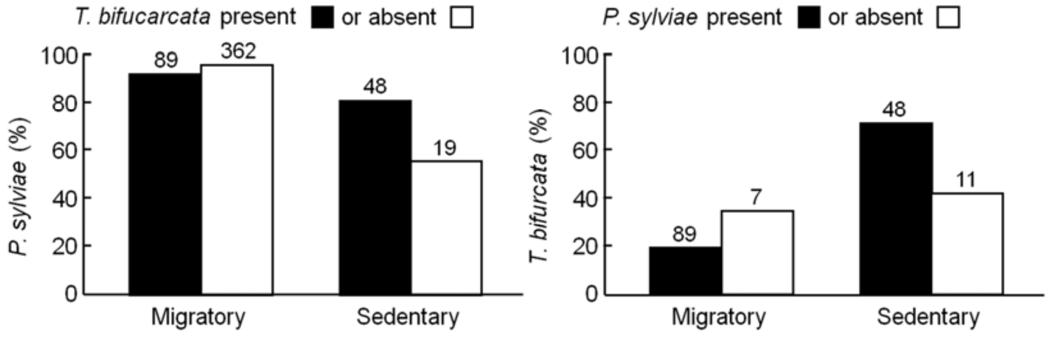

Figure 1.2. Prevalence of each mite species in migratory and sedentary blackcaps in relation to the presence or absence of the other mite species. Sample sizes are indicated on top of bars.

Population consequences of mite coexistence

We conducted GLZ models of variation in abundance and load of each mite species, among years and in relation to host phenotype and presence (or abundance) of the other mite species on the same host. To build the models, we included all effects and two-way interactions, but excluded higher order interactions because biased distribution of mite species between migratory and sedentary blackcaps (see above) produced too many missing cells. The models revealed complex interactions between *P. sylviae* and *T. bifurcata*, which changed among years and depended on host phenotype (Table 1.2).

Controlling for the effects of year and host phenotype, the abundance of *P. sylviae* tended to decrease when *T. bifurcata* was present, and the effect was only clearly observed on migratory blackcaps (Figure 1.3a), although such an interaction did not reach statistical significance (Table 1.2). The same was observed for the abundance of *T. bifurcata* in relation to the presence of *P. sylviae* on the host, but in this case the interaction was significant (Table 1.2, Figure 1.3a). However, such effects seemed influenced by the fact that co-occurrence of the two mite species is more common on sedentary blackcaps (Figure 1.2). The load of *P. sylviae* was lower when *T. bifurcata* was present on the host, an effect which seemed more evident in migratory blackcaps although no interaction between presence of *T. bifurcata* and host phenotype was found (Table 1.2, Figure 1.3b). However, the load of *T. bifurcata* did not significantly vary in relation to the presence of *P. sylviae* on the host (Figure 1.3d), although it varied among years following different patterns in migratory and sedentary blackcaps (Table 1.2).

We repeated the above analyses using abundance instead of presence of the other mite as correlates of *P. sylviae* and *T. bifurcata* numbers, and our results did not change qualitatively, although we found a significant decrease in both abundance and load of *P. sylviae* as *T. bifurcata* numbers increased (estimates: abundance = -0.10, load = -0.06), and higher load of *T. bifurcata* on sedentary blackcaps observed in other analyses was also supported (Table 1.2). As in the other analysis, the abundance of *T. bifurcata* was negatively associated with *P. sylviae* numbers on migratory (estimate = -0.64) but not on sedentary blackcaps (estimate = 0.13), leading to a significant

Table 1.2. Results of generalised linear models of variation in abundance (number of mites including non-infested birds) and load (number of mites including only infested birds) of *Proctophyllodes sylviae* and *Trouessartia bifurcata*, in relation to the presence (above) or the abundance (below) of the other mite. For *Trouessartia*, the effects of presence of the other mite were estimated in winters 1 to 4 alone, because the prevalence of *Proctophyllodes* reached 100% in the winters 5 and 6.

Models with presence of the other mite as a classification factor

	Mite abundance				**Mite load**			
	df	Log-lik.	χ^2	*P*	df	Log-lik.	χ^2	*P*
***Proctophyllodes*:**								
Trouessartia	1	-1458.1	2.29	0.130	1	-4143.3	5.16	0.023
Winter	5	-1510.0	106.03	< 0.001	5	-4161.5	41.59	< 0.001
Host phenotype	1	-1480.8	47.67	< 0.001	1	-4145.2	9.01	0.003
Trouessartia × winter	5	-1474.2	34.45	< 0.001	5	-4153.9	26.36	< 0.001
Trouessartia × host phenotype	1	-1458.8	3.63	0.057	1	-4141.8	2.20	0.138
Winter × host phenotype	5	-1468.9	23.95	< 0.001	5	-4142.1	2.72	0.743
***Trouessartia*:**								
Proctophyllodes	1	-139.4	2.29	0.130	1	-239.4	0.19	0.664
Winter	2	-139.7	3.01	0.221	2	-244.4	10.08	0.006
Host phenotype	1	-139.9	3.42	0.065	1	-239.4	0.09	0.765
Proctophyllodes × winter	2	-143.7	10.92	0.004	2	-240	1.36	0.506
Proctophyllodes × host phenotype	1	-141.3	6.12	0.013	1	-240.1	1.47	0.226
Winter × host phenotype	2	-139.3	2.15	0.341	2	-242.9	7.20	0.027

Models with abundance of the other mite as a covariate

	Mite abundance				**Mite load**			
	df	Log-lik.	χ^2	*P*	df	Log-lik.	χ^2	*P*
Trouessartia	1	-1469.3	5.37	0.021	1	-4102	5.60	0.018
Winter	5	-1497.1	60.89	< 0.001	5	-4104.6	10.85	0.054
Host phenotype	1	-1481.6	29.95	< 0.001	1	-4103.7	8.97	0.003
Trouessartia × winter	5	-1485.6	37.95	< 0.001	5	-4109.4	20.3	0.001
Trouessartia × host phenotype	1	-1467.4	1.46	0.227	1	-4100.3	2.20	0.138
Winter × host phenotype	5	-1479.5	25.7	< 0.001	5	-4099.9	1.46	0.918
***Trouessartia*:**								
Proctophyllodes	1	-329.5	2.97	0.085	1	-720.8	0.11	0.735
Winter	5	-338.1	20.03	0.001	5	-728.3	15.13	0.010
Host phenotype	1	-328.1	0.13	0.714	1	-723.4	5.36	0.021
Proctophyllodes × winter	5	-339.7	23.34	< 0.001	5	-723.6	5.84	0.322
Proctophyllodes × host phenotype	1	-333.0	9.90	0.002	1	-721.1	0.83	0.363
Winter × host phenotype	5	-329.8	3.43	0.634	5	-731.4	21.30	< 0.001

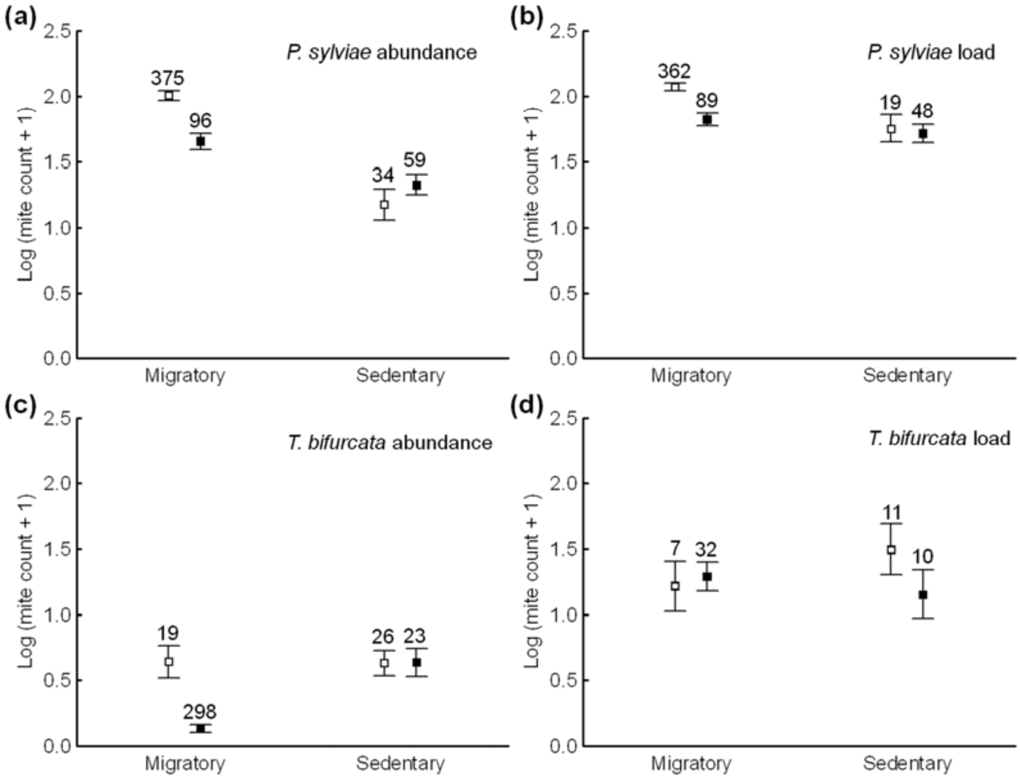

Figure 1.3. Variation in the abundance (number of mites including non-infested birds) (a, c) and load (number of mites including only infested birds) (b, d) of each mite species (*P. sylviae* and *T. bifurcata*) in relation to host phenotype (migratory or sedentary) and the absence (open squares) or presence (filled squares) of the other mite species on the same host (means ± SE and sample sizes).

interaction between host phenotype and *P. sylviae* numbers, which was not found for *T. bifurcata* load (Table 1.2).

Host traits and mite distribution

Both wing length and uropygial gland volume varied between migratory and sedentary blackcaps, which could help to explain the patterns described above. We first conducted a Principal Components Analysis with the length of tarsus, bill, wing and tail, which extracted two principal components of blackcap morphology. The PC1 accounted for 37.9% of variance in the correlation matrix (eigenvalue = 1.52) and was interpreted as an index of body shape, with positive loading for wing and tail length (factor loadings: wing = 0.797, tail = 0.478) and negative loading for tarsus and bill length (tarsus = -0.560, bill = -0.583). Therefore, birds with high positive PC1 scores had longer wings and tails but short legs and bills, thereby showing the typical body structure of migratory blackcaps (sedentary blackcaps scored negative values on PC1, results not shown). The PC2 was an index of structural body size independent of body shape, as all body dimensions were positively correlated with PC2 scores (factor loadings: tarsus = 0.544, bill = 0.517, wing = 0.310, tail = 0.751, eigenvalue = 1.22, variance explained = 30.6%).

Controlling for a positive effect of structural body size (beta = 0.44, $F_{1,157} = 87.8$, $P < 0.001$); migratory blackcaps had longer wings (adjusted mean ± SE = 74.3 ± 0.13 mm) than sedentary blackcaps (70.1 ± 0.23 mm; $F_{1,157} = 258.6$, $P < 0.001$). Variation in wing length between migratory and sedentary blackcaps was also significant when variation in body size was not controlled for (the wings of migratory blackcaps were on average 5.4% longer than the wings of sedentary blackcaps; $F_{1,158} = 138.7$, $P < 0.001$). The size of the uropygial gland of blackcaps was also positively correlated with structural body size (beta = 0.22, $F_{1,156} = 5.84$, $P = 0.017$), but it did not depend on wing length ($F_{1,156} = 0.01$, $P = 0.904$). Controlling for these effects, sedentary blackcaps showed larger uropygial glands (mean ± SE = 110.4 ± 5.0 mm^3) than migratory blackcaps (91.3 ± 2.1 mm^3; $F_{1,156} = 9.56$, $P = 0.002$). The difference between migratory and sedentary blackcaps became more evident when structural body size was not controlled for in the analysis, as sedentary blackcaps are bigger than migratory blackcaps (the uropygial glands of sedentary blackcaps were on average 23.8% bigger than the glands of migratory blackcaps; $F_{1,158} = 32.22$, $P < 0.001$).

All blackcaps inspected during the last two seasons were infested by *P. sylviae*, and therefore abundance and load of this mite species (or of both species together) were equivalent in this analysis. When we analysed variation in total mite load among individual blackcaps, we did not find any effect of wing length ($\chi^2_{(1)} = 0.09$, $P = 0.770$) or size of the uropygial gland ($\chi^2_{(1)} = 2.54$, $P = 0.111$). However, such negative results masked different patterns of correlation between mite load and host wing length or uropygial gland size for each mite species. Thus, *P. sylviae* load was positively correlated with host wing length (estimate = 0.014; $\chi^2_{(1)} = 5.22$, $P = 0.022$), but not with uropygial gland size ($\chi^2_{(1)}$= 0.49, *P* = 0.485, Figure 1.4). Conversely, the abundance of *T. bifurcata* was positively associated with uropygial gland size (estimate = 0.011; $\chi^2_{(1)}$= 6.24, *P* = 0.012), and it was negatively associated with wing length (estimate = -0.25; $\chi^2_{(1)}$= 42.26, *P* < 0.001, Figure 1.4). The same pattern was found for the load of *T. bifurcata* (effect of uropygial gland size: estimate = 0.008; $\chi^2_{(1)}$= 8.78, *P* = 0.003; effect of wing length: estimate = -0.10; $\chi^2_{(1)}$= 24.84, *P* < 0.001).

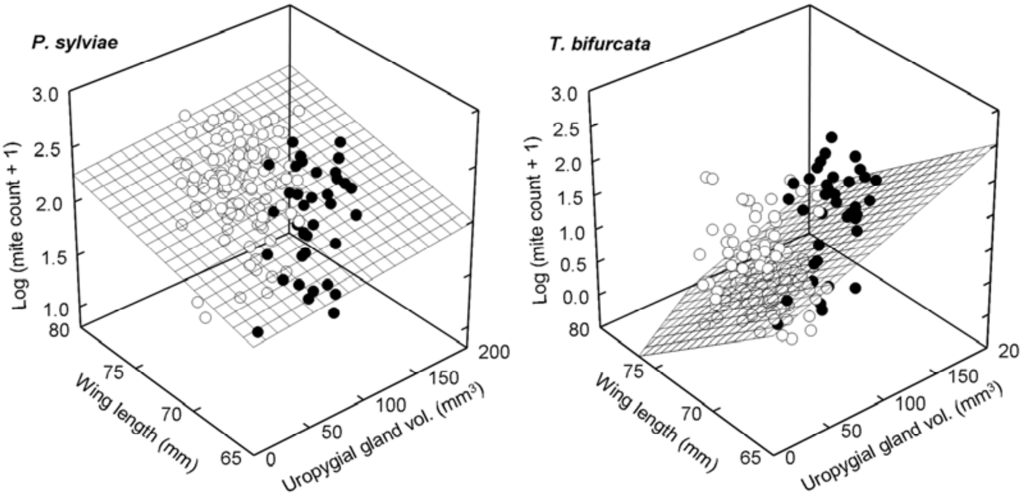

Figure 1.4. Relationship between uropygial gland volume, wing length and mite counts (mite abundance including mite-free hosts) of *Proctophyllodes sylviae* and *Trouessartia bifurcata*. Migratory and sedentary blackcaps are distinguished by white and filled dots, respectively. Bivariate least-squares fit surfaces are also shown.

Discussion

The distribution of feather mites among individual bird hosts may be influenced by host habitat choice, phenotypic differences among hosts, mite-specific strategies of host exploitation, and competition among mite species sharing the same individual host. These factors may determine the frequency of within-host co-occurrence of different mite species, and therefore the opportunities for mite behavioural interactions to occur. In our study, *P. sylviae* mites were generally more abundant than *T. bifurcata* mites (total prevalence: *P. sylviae* = 91.7%, *T. bifurcata* = 27.5%) and reached higher within-host population size on average (mite load, mean ± SE: *P. sylviae* = 111.4 ± 2.04, *T. bifurcata* = 18.4 ± 2.11). However, controlling for variation in the abundance of both mite species among years (which probably arose as a consequence of inter-year changes in environmental conditions; Gaede & Knülle 1987, Krasnov *et al.* 2008, Malenke *et al.* 2011), we found that variation in host phenotype was a key factor associated with mite distribution. Migratory and sedentary blackcaps had different prevalence of each mite species, harboured mite populations of different sizes, and offered different scenarios for interspecific interactions between mites. In fact, most of the difference in abundance between mite species could be attributed to the presence of migratory blackcaps wintering in our study area. *P. sylviae* mites were more abundant on migratory than on sedentary blackcaps (on which the two mites showed very different abundances), while *T. bifurcata* mites were more abundant on sedentary than on migratory blackcaps (on which both mite types showed more similar abundance). Importantly, these patterns of distribution of *P. sylviae* and *T. bifurcata* rendered coexistence of the two mite species more frequent on sedentary blackcaps, which therefore played a more relevant role than migratory blackcaps as arenas for mite interactions. Finally, our analysis of putative components of habitat quality for mites of individual blackcaps helped us to identify some host features that could help to explain the opportunities and constraints faced by each mite species on migratory and sedentary hosts. Altogether, these findings suggested possible mechanisms facilitating the coexistence of the two mite species in the same host population, despite suggestive signs of competition between them.

A negative correlation between the abundance of *P. sylviae* and *T. bifurcata* among individual blackcaps suggested that negative ecological interactions may play a role in finely tuning the distribution of these two mite species. Thus, the load of *P. sylviae* decreased when *T. bifurcata* was present or more abundant, more clearly on migratory hosts than on sedentary ones (although the interaction did not reach statistical significance), while *T. bifurcata* maintained similar population size regardless of the presence or numbers of *P. sylviae*. However, disputable outperformance of *T. bifurcata* on co-infested hosts was far from suggesting a clear competitive advantage for this mite species, which in fact reached lower prevalence and average load than *P. sylviae* in the whole host population. Mite abundance patterns depend on host colonization success and within-host growth rate, two ways to increase population size that might be differently exploited by *P. sylviae* and *T. bifurcata*. *P. sylviae* may easily disperse among individual blackcaps reaching high prevalence, but its great variation in within-host population size might reflect high variance in population growth rate on the host. Meanwhile, the distribution of *T. bifurcata* seems to be more limited by host accessibility, with low prevalence (overall and on migratory blackcaps, which are the most abundant in the study area), but also less variable load among infested hosts. Importantly, both within-host population size of *P. sylviae* and colonization success of *T. bifurcata* are strongly correlated with blackcap migration pattern. Such a role of host phenotype in determining the success of alternative host exploitation strategies of feather mites might be common in other bird-mite systems, and may have contributed to the evolution and maintenance of feather mite diversity.

We further explored which individual traits may be associated with the value of migratory and sedentary blackcaps as hosts for different mites. We found correlational evidence that both wing length and uropygial gland size may be key traits of migratory and sedentary blackcaps, respectively, which may favour either mite species in each type of host. Sedentary blackcaps had shorter wings but larger uropygial glands than migratory blackcaps. Short wings may limit the space available for mites to settle on a host (Jovani & Blanco 2000), which may explain why mite load was generally low in sedentary blackcaps despite their being potentially more rewarding hosts than migratory blackcaps from a nutritional perspective (assuming that birds with larger uropygial glands produce larger amounts of oil secretion). However, the evolution of blackcap migration may have constrained the distribution of *T. bifurcata*, rendering migratory blackcaps poor hosts for this species possibly because they do not produce as much oil secretion. In addition, the dorsal feather surfaces of migratory blackcaps could be less favourable for the settlement of *T. bifurcata* mites (Proctor 2003) if the wings of migratory blackcaps are subjected to higher mechanical stress than the wings of sedentary blackcaps, or if there are microstructural differences in the feather surface that makes it more difficult to hold on to migratory birds than to sedentary ones. Conversely, migration might have created an opportunity for niche expansion of *P. sylviae* mites, which may freely settle on migratory blackcaps (where they often remain free of *T.*

bifurcata putative competitors and may reach large population size taking advantage of the large space available for their expansion on the ventral wing surface). There is also a possibility that migration per se, rather than morphological correlates of migratory behaviour, constrains the distribution of mites, for example if *T. bifurcata* has problems coping with seasonal movement between habitat types or fails to thrive as well as *P. sylviae* in the breeding habitats of migratory blackcaps.

Several comparative studies have analysed the relationships between bird migration and the distribution of feather mites among bird species. While mite prevalence seems not influenced by host migration when species with different body size, habitat preferences, or social systems are compared (Figuerola 2000), mite numbers per host individual are larger in migratory than in sedentary bird species (Galván *et al.* 2008). Our comparison of migratory and sedentary individuals of the same bird species produced similar results, except that we not only observe greater mite load, but also higher mite prevalence in migratory compared to sedentary hosts. Therefore, our study adds to existing evidence that variation in host migration may influence feather mite populations. However, the divergence between migratory and sedentary blackcap populations (which most likely occurred during the last glaciation; Pérez-Tris *et al.* 2004) was much more recent than the divergence between migratory and sedentary species compared in interspecific studies (Piersma *et al.* 2005). Migratory and sedentary blackcaps share the same mite species probably because the evolution of migration in blackcaps is too recent to have allowed mite specialization, which is probably not true for most interspecific comparisons (Proctor 2003). Because of this reason, our intraspecific study makes an important contribution to our understanding of the evolutionary opportunities and constraints faced by different feather mites in relation to the evolution of diverse host migration patterns.

How host migration influences mite distribution is a debated issue. In addition to different movement patterns, migratory and sedentary birds differ in many morphological, physiological and behavioural traits (Piersma *et al.* 2005). Variation in plumage quality (as measured by the amount of material per feather), which is associated with time constraints on moult faced by migratory populations (De la Hera *et al.* 2009), is a putative cause for divergence in the size of the uropygial gland between migratory and sedentary blackcaps, and could also drive the evolution of uropygial gland sizes among species. Interestingly, reduced plumage quality associated with migratory behaviour has been found in comparative analyses of passerine species (De la Hera *et al.* 2010b), and parallel studies with overlapping species lists have found that migratory species have smaller uropygial glands than sedentary species (Galván *et al.* 2008). It remains an open question why sedentary birds have better constructed feathers and invest more oil secretions in plumage maintenance than migratory birds (both among species and in blackcaps), despite their having reduced flight requirements. Nevertheless, our results show that whether or not uropygial gland size is associated with mite load depends on the mite species considered. In fact, the abundance of the most common mite species in our study system, which was also the one showing highest prevalence and load on migratory hosts (*P. sylviae*), was apparently independent of host secretory capacity, and was instead positively correlated with host wing size. Clearly, further intraspecific and comparative studies are needed to understand the role of host migration on the distribution of *T. bifurcata* mites and their interactions with co-existing mites such as *P. sylviae*.

Species interactions involve complex combinations of negative and positive effects that can be either direct or indirect, all of which end up influencing variation in relative abundance of the different species in the community. Such complexity is revealed in our study by an apparently direct impact of within-host coexistence on mite populations (*P. sylviae* reached smaller population size when both mite species coexist) and, more importantly, by indirect effects illustrated by different mites thriving on migratory and sedentary hosts. To add complexity, different host phenotypes provided different scenarios for between-mite interactions. These results add up to growing evidence that symbiont coexistence may be favoured in some instances but niche partitioning may be favoured in others (Poulin 2007), and the outcomes of symbiont interactions also depend on host phenotype (Wille *et al.* 2002, De Roode *et al.* 2004). In turn, host phenotypic diversity creates opportunities and constraints on the distribution of different symbiont species, even though these may obtain the same host resources and share modes of host exploitation. In such circumstances, host-phenotype-dependent symbiont distribution and coexistence may facilitate the maintenance of symbiont species diversity within the same host species.

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References

Atyeo, W. T. & Braasch, N. L. 1966. The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). *Bulletin of the University of Nebraska State Museum*, 5: 1-354.

Bhattacharyya, S. P. & Chowdhury, S. R. 1995. Seasonal variation in the secretory lipids of the uropygial gland of a sub-tropical wild passerine bird, *Pycnonotus cafer* (L) in relation to the testicular cycle. *Biological Rhythm Research*, 26: 79-87.

Blanco, G. & Frías, O. 2001. Symbiotic feather mites synchronize dispersal and population growth with host sociality and migratory disposition. *Ecography*, 24: 113-120.

Chesson, P. 1994. Multispecies competition in variable environments. *Theoretical Population Biology*, 45: 227-276.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 31: 343-366.

Clayton, D. H. & Moore, J. (eds.). 1997. *Host-Parasite evolution. General principles and avian models*. Oxford University Press, Oxford.

Dawson, A., Hinsley, S. A., Ferns, P. N., Bonser, R. H. C. & Eccleston, L. 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Proceedings of the Royal Society of London B*, 267: 2093-2098.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 2007. Testing the validity of discriminant function analyses based on bird morphology: the case of migratory and sedentary blackcaps *Sylvia atricapilla* wintering in southern Iberia. *Ardeola*, 54: 81-91.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 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., Hedenström, A., Pérez-Tris, J. & Tellería, J. L. 2010a. Variation in the mechanical properties of flight feathers of the blackcap *Sylvia atricapilla* in relation to migration. *Journal of Avian Biology*, 41: 342-347.

De la Hera, I., Pérez-Tris, J. & Tellería J. L. 2010b. Relationships among timing of moult, moult duration and feather mass in long-distance migratory passerines. *Journal of Avian Biology*, 41: 609-614.

De Roode, J. C., Culleton, R., Cheesman, S. J., Carter, R. & Read, A. F. 2004. Host heterogeneity is a determinant of competitive exclusion or coexistence in genetically diverse malaria infections. *Proceedings of the Royal Society of London B*, 271: 1073-1080.

Dowling, D. K., Richardson, D. S., Blaakmeer, K. & Komdeur, J. 2001. Feather mite loads influenced by salt exposure, age and reproductive stage in the Seychelles Warbler *Acrocephalus sechellensis*. *Journal of Avian Biology*, 32: 364-369.

Dubinin, V. B. 1951. Feather mites (Analgesoidea). Part I. Introduction to their study. *Fauna SSSR, Paukoobraznye*, 6: 1-363.

Figuerola, J. 2000. Ecological correlates of feather mite prevalence in passerines. *Journal of Avian Biology*, 31: 489-494.

Fox, J. W., Nelson, W. A. & McCauley, E. 2010. Coexistence mechanisms and the paradox of the plankton: quantifying selection from noisy data. *Ecology*, 91: 1774-1786.

Gaede, K. & Knülle, W. 1987. Water vapour uptake from the atmosphere and critical equilibrium humidity of a feather mite. *Experimental and Applied Acarology,* 3: 45-52.

Galván, I. & Sanz, J. J. 2006. Feather mite abundance increases with uropygial gland size and plumage yellowness in Great Tits *Parus major*. *Ibis*, 148: 687-697.

Galván, I., Barba, E. Piculo, R., Cantó, J. L., Cortés, V., Monrós, J. S., Atiénzar, F. & Proctor, H. 2008. Feather mites and birds: an interaction mediated by uropygial gland size? *Journal of Evolutionary Biology*, 21: 133-144.

Griggio, M., Serra, L., Licheri, D., Campomori, C., & Pilastro, A. 2009. Moult speed affects structural feather ornaments in the blue tit. *Journal of Evolutionary Biology*, 22: 782-792.

Hutchinson, G. E. 1957. Concluding remarks. *Cold Spring Harbor Symposia*, 22: 415-427.

Hutchinson, G. E. 1961. The paradox of the plankton. *The American Naturalist*, 95: 137-145.

Jovani, R. & Blanco, G. 2000. Resemblance within flocks and individual differences in feather mite abundance on long-tailed tits, *Aegithalos caudatus* (L.). *Ecoscience*, 7: 428-432.

Jovani, R. & 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.

Kelly, D. W. & Thompson, C. E. 2000. Epidemiology and optimal foraging: modelling the ideal free distribution of insect vectors. *Parasitology*, 120: 319-327.

Krasnov, B. R., Mouillot, D., Shenbrot, G. I., Khokhlova, I. S. & Poulin, R. 2005. Abundance patterns and coexistence processes in communities of fleas parasitic on small mammals. *Ecography*, 2: 453-464.

Krasnov, B. R., Korallo-Vinarskaya, N. P., Vinarski, M. V., Shenbrot, G. I., Mouillot, D. & Poulin, R. 2008. Searching for general patterns in parasite ecology: host identity versus environmental influence on gamasid mite assemblages in small mammals. *Parasitology*, 135: 229-242.

Malenke, J. R., Newbold, N. & Clayton, D. H. 2011. Condition-specific competition governs the geographic distribution and diversity of ectoparasites. *The American Naturalist*, 177: 522-534.

Mestre, A., Mesquita-Joanes, F., Proctor, H. & Monrós, J. S. 2011. Different scales of spatial segregation of two species of feather mites on the wings of a passerine bird. *Journal of Parasitology*, 97: 237-244.

Møller, A. P., Czirjak, G. A. & Heeb, P. 2009. Feather micro-organisms and uropygial antimicrobial defences in a colonial passerine bird. *Functional Ecology*, 23: 1097-1102.

Pérez-Tris, J. & Tellería, J. L. 2001. Age-related variation in wing shape of migratory and sedentary Blackcaps *Sylvia atricapilla*. *Journal of Avian Biology*, 32: 207-213.

Pérez-Tris, J. & Tellería, J. L. 2002. 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., Carbonell, R. & Tellería, J. L. 1999. A method for differentiating between sedentary and migratory Blackcaps *Sylvia atricapilla* in wintering areas of southern Iberia. *Bird Study*, 46: 299-304.

Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A. J. & Tellería, J. L. 2004. Historical diversification of migration patterns in a passerine bird. *Evolution*, 58: 1819-1832.

Piersma, T., Pérez-Tris, J., Mouritsen, H., Bauchinger, U. & Bairlein, F. 2005. Is there a “migratory syndrome” common to all migrant birds? *Annals of the New York Academy of Sciences*, 1046: 282-293.

Poulin, R. 1991. Group-living and infestation by ectoparasites in passerines. *Condor*, 93: 418-423.

Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, Princeton.

Poulin, R., Krasnov, B. R. & Mouillot, D. 2011. Host specificity in phylogenetic and geographic space. *Trends in Parasitology*, 27: 355-361.

Proctor, H. C. 2003. Feather mites (Acari: Astigmata): ecology, behaviour, and evolution. *Annual Review of Entomology*, 48: 185-209.

Santana, F. J. 1976. A review of the genus *Trouessartia*. *Journal of Medical Entomology*, 1: S1-S128.

SAS 2008. *SAS/STAT® 9.2 User’s Guide. The GENMOD Procedure*. SAS Institute Inc, Cary.

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

Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science*, 185: 27-39.

Soberón, J. & Peterson, A. T. 2005. Interpretation of models of fundamental ecological niches and species’ distributional areas. *Biodiversity Informatics*, 2: 1-10.

StatSoft 2004. *STATISTICA version 7.0*. Statsoft, Tulsa.

Svensson, L. 1992. *Identification guide to European passerines*. L. Svensson, Stockholm.

Tellería, J. L. & Carbonell, R. 1999. Morphometric variation of five Iberian Blackcap *Sylvia atricapilla* populations. *Journal of Avian Biology*, 30: 63-71.

Wille, P., Boller, T. & Kaltz, O. 2002. Mixed inoculation alters infection success of strains of the endophyte *Epichloë bromicola* on its grass host *Bromus erectus*. *Proceedings of the Royal Society of London B*, 269: 397-402.

Chapter 2

This chapter is based on the manuscript:

Fernández-González, S., De la Hera, I., Pérez-Rodríguez, A., Proctor, H. C. & Pérez-Tris, J. Dissimilar space preferences and within-host competition promote spatial niche partitioning between coexisting feather mites. *In preparation.*

Dissimilar space preferences and within-host competition promote spatial niche partitioning between coexisting feather mites

Sofía Fernández-González, Iván de la Hera, Antón Pérez-Rodríguez, Heather C. Proctor and Javier Pérez-Tris

Obligate symbionts (parasites, commensals or mutualists) often share host species and host exploitation mechanisms. Such symbionts may distribute unequally among hosts with different phenotypic features, or occupy different regions on a host, yet the processes leading to distinct symbiont distributions are generally unknown. We studied the distribution among and within individual hosts of two species of feather dwelling mites (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) in a population of European blackcap *Sylvia atricapilla* that includes migratory and resident individuals. We aimed to investigate whether distinct mite distributions arise as the outcome of habitat preferences or competition for space, and how variation in host phenotype influences such distributions. *T. bifurcata* was mostly restricted to resident blackcaps, while *P. sylviae* was abundant on both host types. *P. sylviae* was more abundant towards distal feather sectors, while *T. bifurcata* occupied proximal sectors. Both species spread over the wing following ordered but opposite patterns of wing filling, supporting the view that spatial segregation was the outcome of dissimilar space preferences, probably associated with mechanical limitations. However, when *P. sylviae* increased its abundance, it also expanded its range on the host wing towards the range of *T. bifurcata*. Then, competition between mites was evidenced by a negative correlation between their abundances within shared areas of the wing, which was stronger in the preferred range of *T. bifurcata*. In addition, the presence of *T. bifurcata* on its preferred hosts (resident blackcaps) was associated with a contracted distribution of *P. sylviae* on the wing. Our results show that both mite preferences and interspecific interactions may contribute to shape among and within-host mite distributions, thereby improving our knowledge of the mechanisms that promote the evolution and maintenance of symbiont diversity.

Introduction

Most species are not found in all places where they could possibly thrive. The ecological niche of a species can be defined as the combination of conditions of the physical environment, resource requirements and biological interactions (with competitors, mutualists, predators and pathogens) that allow its existence (Hutchinson 1957, Chase & Leibold 2003). In principle, the various species that live in the same space cannot occupy the same ecological niche, because the slightest advantage for one competitor will eventually drive others to extinction (MacArthur & Levins 1967, Amarasekare

2007, Schoener 2009). Therefore, ecologically similar species are expected to partition their ecological niche, showing differences in some niche dimensions such as diet, spatial distribution or phenology, which in turn make their coexistence possible (Schoener 1974, Chesson 2000). These processes promote niche specialization by phenotypic and ecological divergence, which is central to the evolution and maintenance of biological diversity (Chesson 2000, Schluter 2000, Pfenning & Pfenning 2010).

For obligate symbionts (those that live permanently attached to their hosts, such as many parasites, commensals and mutualists), the host population represents the fundamental niche (*i.e.*, the habitat that provides conditions and resources for the species to exist in the absence of competitors, predators and pathogens; Hutchinson 1957, Soberón & Peterson 2005). This is in turn divided into patches (the individual hosts) which in many cases are shared by different types of symbionts with overlapping niches. In these cases, the realized niche of each symbiont may be the outcome of specific habitat preferences or interspecific interactions, which may result in a different distribution of each symbiont species on the host (Holmes 1973, Sousa 1994).

However, the distribution of symbionts within the host may also be determined by intraspecific interactions (Kuris & Lafferty 1994, Friggens & Brown 2005). Within the space occupied by one symbiont species, variation in different elements of habitat quality (food availability, exposure to mechanical or chemical stress, etc.) may render some habitat patches preferred above others. Symbionts may aggregate in the best patches, but when population density increases, some individuals may be forced to poorer habitats, where fitness may be lower (Pulliam 1988, Rodenhouse *et al.* 1997). The dynamics of space occupation within the host may create a "buffer effect" (Brown 1969), when population fluctuations involve great changes in abundance in low-quality sites (which are typically occupied only when population abundance is high), but "buffered" fluctuation in high-quality sites (which typically are the first to be occupied and remain occupied in the face of population fluctuation; Brown 1969, Gill *et al.* 2001). From the perspective of symbiont interspecific interactions within a host, the displacement of surplus individuals into less preferred habitats may lead to contact with competitors, if the least preferred habitats for one symbiont are favoured by others. Therefore, variation in the quality of different host habitats and interspecific interactions between coexisting symbionts may shape their patterns of distribution within the host (Pulliam 2000). However, within-host symbiont distributions, and the way these vary in relation to changes in species' abundance or species co-occurrence among individual hosts, are poorly known for most host-symbiont systems, despite their importance to our understanding of the processes that promote and maintain the diversity of symbiotic organisms (Poulin 2007).

We studied the distribution of two feather-dwelling mite species (order Astigmata: *Proctophyllodes sylviae* and *Trouessartia bifurcata*) on European blackcap *Sylvia atricapilla* wintering in southern Spain. *P. sylviae* and *T. bifurcata* are the most common mites of blackcaps, and often coexist on the same host (Fernández-González *et al*. 2013). These mites feed upon the uropygial gland secretions that cover the feathers, also taking embedded particles and microorganisms (such as bacteria, yeast and fungi; Proctor 2003). Spatial niche partitioning between these species is evident, as *P. sylviae* occupies the ventral side of the wing feathers, while *T. bifurcata* lives on the dorsal side. These different distributions, and the morphological traits associated with them (Atyeo & Braasch 1966, Santana 1976), are to be interpreted as the outcome of specialization during the evolutionary divergence of the two genera. In addition, the two species show a different distribution among host individuals, which is associated with variation in host phenotype between the migratory and resident blackcaps that spend the winter in the same areas. Thus, *P. sylviae* is more abundant but *T. bifurcata* is rare on migratory blackcaps, while both mite species reach intermediate abundance on resident blackcaps (Fernández-González *et al*. 2013). Such differences may be associated with phenotypic divergence between migratory and resident blackcaps, which differ in wing morphology (migratory blackcaps possess a greater wing surface area) and food availability (resident blackcaps have larger uropygial glands, and therefore may produce more abundant oil secretions; Fernández-González *et al*. 2013).

Specialization in relation to within-host microhabitat and spatial segregation in relation to host phenotype may reduce direct competition between *P. sylviae* and *T. bifurcata*. Still, there is evidence of negative interactions between both mite species when they coexist on the same host: *P. sylviae* reaches lower abundance when *T. bifurcata* is present on the host, although the opposite is not true (Fernández-González *et al.* 2013). Such observations suggest that variation in mite abundance in the presence of competitors may be associated with changes in mite distribution on the host, and may be influenced by the degree of overlap between the distributions of different mite species, thus determining competition outcomes. Previous research has found that mites are not randomly distributed across the host's plumage (Jovani & Serrano 2004), and coexisting mite species may show distinct distributions on the wing surface (Mestre *et al.* 2011). Aggregation of each mite species in distinct areas of the plumage may decrease the opportunity for competition (Holmes 1973, Morand *et al.* 1999), but an increase in population density in the preferred sectors may cause the spread of the species over the plumage to overlap with the range of other species.

We investigated the distribution of *P. sylviae* and *T. bifurcata* on the wing plumage of blackcaps, aiming to test predictions derived from a scenario of mite competition and divergent habitat preferences. Firstly, we tested whether *P. sylviae* and *T. bifurcata* follow a random distribution on the wing, are regularly distributed, or rather they tend to crowd in distinct sectors of the plumage, as would be expected if they partitioned the space available on the wing as a consequence of competition or different microhabitat preferences. If different areas of the plumage differ in quality, a nested pattern of filling of the available areas of the plumage is expected, according to which the best habitat patches will be the first to be occupied, and habitats of progressively worse quality will be occupied only when better habitats are already filled. Competition would then be promoted if both mite species preferred the same areas of the wing, while dissimilar space preferences of the two mite species would support a scenario of niche partitioning with relaxed competition. Nevertheless, even if each mite species preferred different parts of the plumage, competition might still occur in areas of the wing that end up occupied by both species, for example if surplus individuals of one species expand and overlap with the habitat range of the other species on the wing. We therefore analysed whether a negative correlation between the numbers of the two species could be detected, controlling for putative variation in population size of each mite species across the host's plumage associated with specific site preferences. In all these analyses, we took into account host's phenotypic diversity (migratory or resident blackcaps), which may influence on the patterns of distribution of the two mite species and the outcome of their interspecific interactions.

Material and methods

Study area and field methods

Our study was conducted in the Campo de Gibraltar region (southern Spain) during two consecutive winters, in February and December 2010. Blackcaps (n = 160) were captured using mist nets and kept in individual cloth bags until manipulation. All birds were individually identified by an aluminium ring; after manipulation birds were released at the site of capture.

Birds were measured for the length of the eighth primary feather and the tail length to the nearest 0.5 mm. Primary feather distances (the distances from the wing tip to the tip of each primary feather 1 to 9) were also measured to the nearest 0.5 mm. The length of the eighth primary and the tail, and the difference between primary distances 1 and 9 were used to classify blackcaps as migratory or resident according to a discriminant function analysis (Pérez-Tris *et al*. 1999). Morphological differences existing between migratory and resident blackcaps allow for the correct classification of over 97% of blackcaps using this method (De la Hera *et al*. 2007, 2012).

To count mites of each species, we exposed one spread wing towards the ambient light or a lamp, and counted all mites of each species visible on the vanes of primaries 1-9 (the tenth primary is too small in blackcaps and never has mites attached to it), the six secondaries, and the three tertial feathers (Jovani & Serrano 2004). We counted mites on the proximal, medial and distal thirds of each feather, thereby defining a spatial grid with 54 wing cells (3 sectors × 18 feathers) in which to study mite distributions. Mites of the genera *Proctophyllodes* and *Trouessartia* were easily distinguished by their size, shape and location on the ventral or dorsal side of feathers, respectively (Atyeo & Braasch 1966, Santana 1976). We examined a random sample of 188 *Proctophyllodes* (from 25 different hosts) and 27 *Trouessartia* (from 10 different hosts), obtained from 29 blackcaps (15 migratory and 14 resident) using light microscope with differential interference contrast (DIC) illumination. Morphological identifications (according to Atyeo & Braasch 1966, Santana 1976, Gaud & Atyeo 1996) confirmed our field identification, with *P. sylviae* and *T. bifurcata* as the only species of their genera found on blackcaps. Other species (of the genera *Analges* and *Strelkoviacarus*) accounted for less than 1% of the observed mites.

General patterns of mite distribution

We used Generalized Linear Mixed-effects Models (GLMMs) with Poisson error distribution and Log link function included in the lme4 package (Bates *et al.* 2012) of R 2.15.2 (http://www.rproject.org) to analyse variation in the patterns of mite distributions on blackcap wings. We simultaneously tested for differences in mite abundance between mite species and host phenotypes (migratory or resident), and among feathers (9 primaries, 6 secondaries and 3 tertials) and feather sectors (proximal, medial, or distal thirds of each feather). We used a split-plot design to account for the fact that we simultaneously tested a between-subject factor (host phenotype) and two within-subject factors (feather identity and feather sector; individual blackcaps were the subjects in the analysis). Firstly, we used the Laplace approximation to find the optimal structure for the random error term, for which we compared models with different random parameters but the same fixed structure (all the main effects and their interactions, the so called "beyond optimal model"; Zuur *et al.* 2009). We tested random structures including differences among individual blackcaps, or among blackcaps nested within phenotypes (because a blackcap is either migratory or resident), which were compared to each other using the Akaike Information Criterion (AIC). Once the most appropriate random structure was found, we compared 95 different models, each containing a different subset of the "beyond optimal model" for the fixed structure, with the Laplace approximation procedure. The model with the lowest AIC score was selected as the best one.

To further investigate the exact patterns of distribution of mites across the host's wing, we also examined the distribution of mites using SADIE (Spatial Analysis by Distance Indices; Perry 1995). This program analyses count data with many zeroes and Poisson fit, in order to determine general spatial patterns arising in a grid of cells (in our case the 54 wing cells resulting from dividing 18 feathers into three sectors), specifically aiming to identify possible local aggregation of the mites. SADIE uses a mathematical algorithm of transport to calculate such aggregation, which is the minimum distance across the grid (D) needed to obtain regularity, which is attained when all cells have the same average number of individuals after transportation of individuals from overpopulated cells to less crowded cells (Perry 1998). The statistical significance of the distribution is obtained through Monte Carlo permutation analyses, where observed values are randomly distributed (Perry *et al.* 1999). The observed D value, divided by the average values obtained in the permutations, gives an aggregation index (Ia). Values of Ia > 1 mean that individuals are spatially aggregated, Ia = 1 indicates a random distribution of the observations, and Ia < 1 indicates a regular distribution. On the other hand, a clustering index (*v*) is calculated, which represents local clusters (cells that exceed the average number of mites) and gaps (cells below the average), thereby providing information about how each cell contributes to the general distribution pattern. Cluster cells that are close to other clusters have a higher clustering index compared to cluster cells that are located near gaps (Perry *et al.* 1999). Again, statistical significance is calculated by comparing the average value of clusters and gaps with those obtained in the permutations.

We studied the degree of aggregation of mite distributions using two approaches. We first computed aggregation indices for the average distribution of mites of each species (taking within-cell averages as the data for each cell in the grid), conducting separate analyses for migratory and resident blackcaps. We then computed the aggregation indices for each individual blackcap and tested for possible relationships between aggregation and abundance of mites on the host's wing.

Patterns of wing filling by mites

In order to investigate the patterns of mite spread across the wing surface, we examined the degree of nestedness of mite distributions among wing cells. Our matrices included wing cells in columns, individual blackcaps in rows, and the occurrence of each mite as the data. We quantified the degree of nestedness by means of the matrix temperature (T), which is a measure of deviation of the observed distribution from perfect nestedness (Atmar & Patterson 1993). In a perfectly nested matrix (T = 0), all matrix presences are in the upper left corner of a theoretical isocline (a curvature of maximum packing given the size and filling of the matrix). We used BINMATNEST, which uses an improved algorithm for matrix packing (Rodríguez-Gironés & Santamaría 2006) designed for calculation of T. Furthermore, BINMATNEST uses three null hypotheses to obtain the significance of T: H1 fixes the number of presences to equal observed values, leaving row and column totals unconstrained (Atmar & Patterson 1993); H2 fills cells in the simulated matrices according to presences in columns (Fischer & Lindenmayer 2002); and H3 populates cells proportionately to row and column totals (Bascompte *et al.* 2003). Once we determined that mites filled wing cells according to a nested pattern, we ranked each wing cell according to its nestedness order in the matrix, from the first cells to be occupied (lowest rank indicating strong mite preference for that cell) to the latest occupied cells (highest rank indicating low or no preference).

Feather mite interactions

In order to investigate the possible within-host interaction between *P. sylviae* and *T. bifurcata* we used Generalized Linear Mixed-effects Models (GLMMs) with Poisson error distribution and Log link function, using the lme4 module in R with the same procedure described above. For this analysis, we used blackcaps that were infested by the two mite species, and for each individual blackcap we only included wing cells occupied by at least one mite. A model was built for each mite species, including its abundance as the dependent variable. Predictor variables were feather identity, feather sector, host phenotype, and the abundance of the other mite species, as well as all two-way interactions between these variables. In this case, we chose one among four possible random structures (variation in the abundance of the focal mite species among individual blackcaps, or among blackcaps nested within phenotype, in each case assuming that within-host relationships between the abundance of the two mites had either constant or changing slope among blackcap individuals). Once the best random structure was obtained, we tested 79 subsets of the model that included all main effects and two-way interactions, and selected the one that best fitted to the data according to AIC scores.

We used the nestedness ranks of wing cells to test (1) whether mite distributions were broader (*i.e.*, they spanned more wing cells and these reached higher ranks in the nested pattern of wing filling) when the size of mite populations increased on the host wing, (2) if the size of mite distributions changed in relation to host phenotype, and (3) whether the distribution of one mite species contracted when the other species was also present on the host.

Results

General patterns of mite distribution

The best model of variation in mite abundance across the wing included a random component that considered that individual blackcaps had different abundance of mites regardless of their phenotype. The model (Table S2.1) included mite species, feather identity, sector location (proximal, medial or distal), host phenotype and their two-way and three-way interactions as significant predictors of local mite numbers. The two mite species followed different patterns of distribution among and within host individuals (Figure 2.1). *P. sylviae* was abundant on migratory and resident blackcaps alike, while *T. bifurcata* was extremely rare on migratory hosts. The distribution of *P. sylviae* was similar on both types of host, with low numbers on the proximal sector of the wing feathers, and reaching maximum abundance on medial and distal feather sectors. Medial feather sectors were more populated than distal sectors towards the outer primaries and at the inner wing, and the feathers had fewer mites near the limit between primary and secondary feathers (with a minimum abundance on the innermost primary, where *P. sylviae* dropped in numbers especially on the distal sector; Figure 2.1). *T. bifurcata* followed roughly the opposite pattern of distribution among feather sectors, reaching highest abundance towards proximal and medial feather sectors. The outer primaries harboured few mites of this species, which increased abundance towards inner feathers, reaching local maxima in areas of the wing that coincided chiefly with those where *P. sylviae* was most abundant on distal feather sectors, but decreasing abundance at the inner wing. *T. bifurcata* also decreased in numbers at the limit between primary and secondary feathers, reaching minimum abundance on the first secondary (Figure 2.1).

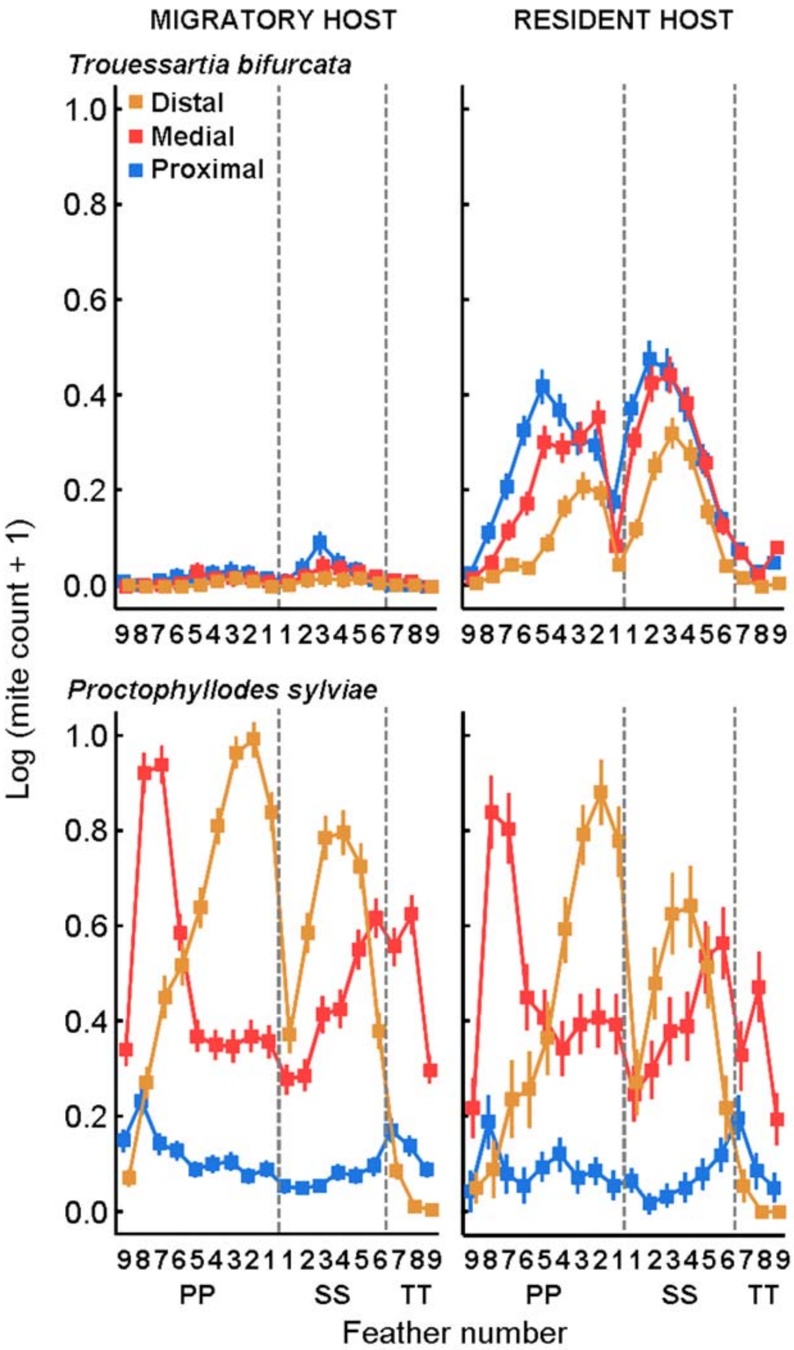

Figure 2.1. Variation in the abundance (mean ± SE) of the feather mites *Trouessartia bifurcata* (above) and *Proctophyllodes sylviae* (below) among blackcap feathers and feather sectors (shown in different colours). Dashed lines separate primary (PP), secondary (SS) and tertial (TT) feathers. Left and right charts show the patterns of mite distribution on migratory and resident hosts, respectively.

The distribution of mite average abundance across the wing showed aggregation indices above 1, although the degree of aggregation varied between mite species and in relation to host phenotype (Figure 2.2). The distribution of within-cell mean abundances of *P. sylviae* showed no significant aggregation across the wing on migratory (Ia = 1.103, *P* = 0.28) or resident blackcaps (Ia = 1.137, *P* = 0.26). However, the aggregation of the distribution of *T. bifurcata* mean abundances was close to statistical significance on migratory blackcaps (Ia = 1.467, *P* = 0.099), and it was significant on resident blackcaps (Ia = 1.99, *P* = 0.015).

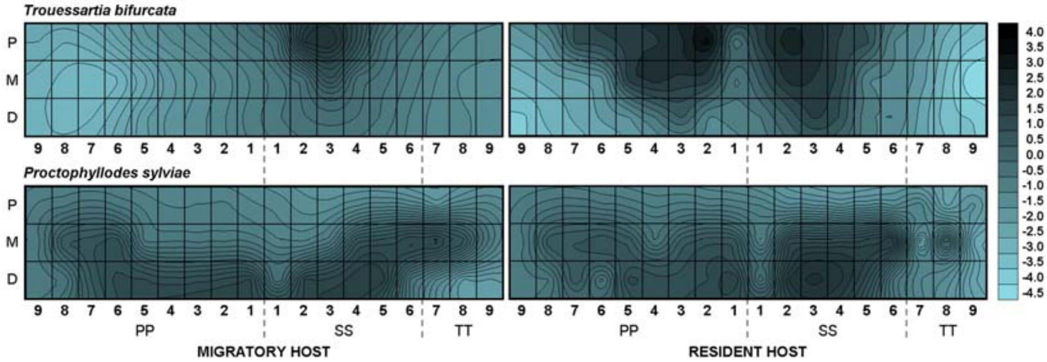

Figure 2.2. Patterns of aggregation of mite numbers across 54 wing cells resulting from dividing 18 feathers (columns; PP: primaries, SS: secondaries, TT: tertials) into three sectors (rows; P: proximal, M: medial, D: distal). The colour scale represents values of the clustering index (v) for each wing cell. Left and right charts show the patterns of aggregation of *Trouessartia bifurcata* (above) and *Proctophyllodes sylviae* (below) on migratory and resident hosts, respectively.

When aggregation indices were computed for the distribution of mites within each individual blackcap, the aggregation of *P. sylviae* was independent of its abundance (R^2 = 0.009, *P* = 0.24). However, *T. bifurcata* showed more aggregated distributions on the most populated hosts (R^2 = 0.45, *P* < 0.001). The same results were obtained (qualitatively) when individual blackcaps whose mite distributions scored Ia ≤ 1 were excluded from the analyses.

Patterns of wing filling by mites

Our analysis of the order in which different cells of the wing were occupied by mites showed a moderate degree of nestedness for *P. sylviae*, with similar values of T for matrices including all blackcaps in the sample (T = 21.4) or migratory and resident blackcaps tested separately (T = 21.6 and T = 22.4, respectively). We detected a higher degree of nestedness for *T. bifurcata*, but T values differed depending on the blackcaps considered in the matrix (T = 10.4 for all blackcaps, T = 10.0 for migratory blackcaps, and T = 21.2 for resident blackcaps). The observed matrices were significantly more nested than expected by chance under all three null hypotheses (all analyses with $P < 0.001$), but the pattern of wing filling differed between mite species. Thus, the wing cells that were first to be occupied by one mite species were not the first occupied by the other (Figure 2.3): *P. sylviae* favoured medial and distal feather sectors, which ranked comparatively high in the order of cell occupation by *T. bifurcata*. Conversely, *T. bifurcata* settled first on proximal feather sectors, which *P. sylviae* resisted to occupy. Nevertheless, both mite species agreed to a large extent on which wing cells were least preferred, as some medial and distal sectors of wing feathers were the last to be occupied by both species. The dissimilar patterns of wing filling found in each mite species, and their agreement on which cells were to be avoided, was evident from the U-shaped relationship between the ranks of wing cells in the order of wing filling by each species (Figure 2.3).

When the total abundance of a mite species increased on the host wing, that mite species occupied more wing cells (*P. sylviae*: $R^2 = 0.63$, $P < 0.001$; *T. bifurcata*: $R^2 = 0.88$, $P < 0.001$), and the cells that were occupied by the mite scored higher nestedness ranks (*i.e.*, mites progressed further in the ordered sequence of wing cell occupation; *P. sylviae*: $R^2 = 0.22$, $P < 0.001$; *T. bifurcata*: $R^2 = 0.07$, $P = 0.017$). However, host phenotype had an influence on these patterns for *T. bifurcata*, which increased the number of occupied wing cells as its abundance increased faster in resident blackcaps than in migratory blackcaps (Table 2.1). In addition, the relationship between the total abundance of *T. bifurcata* and the mean nestedness rank of occupied cells was no longer significant when host phenotype was controlled for in the analysis (Table 2.1). Such influences of host phenotype on the number of cells occupied or their nestedness ranks were not observed for *P. sylviae* (Table 2.1).

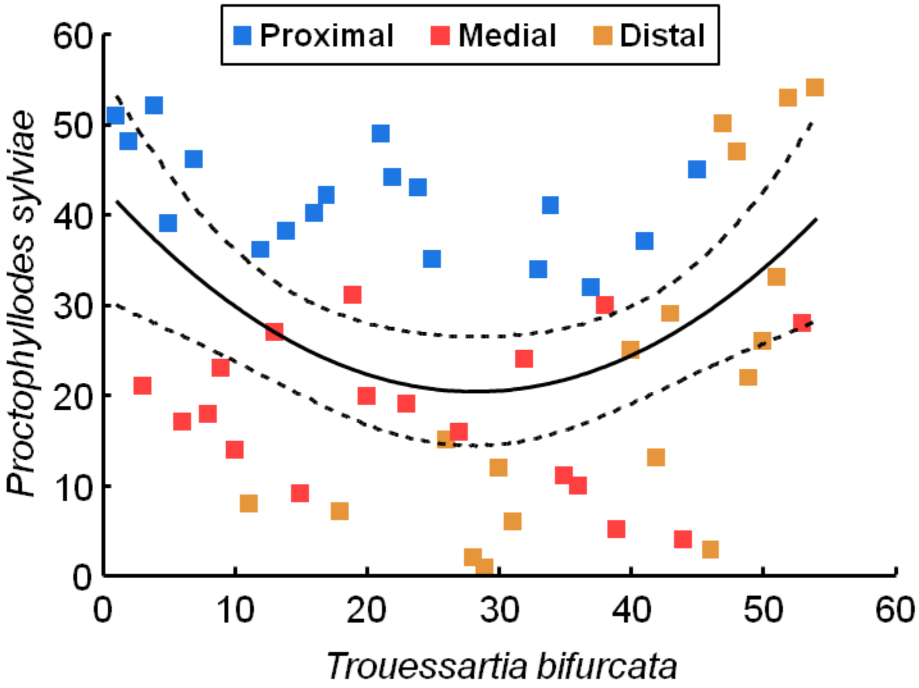

Figure 2.3. Relationship between the rank of 54 wing cells (18 feathers × 3 sectors) in the nested order of wing filling found for the feather mites *Trouessartia bifurcata* and *Proctophyllodes sylviae*. The first cells to be occupied score the lowest rank values for each mite species, so that the points located to the lower left corner of the graph represent wing cells that are preferred by both mite species, and the points located at the upper right corner represent cells avoided by both mite species. The colours indicate different sector locations of the wing cells (proximal, medial or distal). The best fit ($y = 43.24 - 1.61x + 0.03x^2$) is shown with 95% confidence bands (dashed lines).

Feather mite interactions

For both mite species, the best models of variation in abundance across the wing in relation to the abundance of the other mite species included a random component that considered a variable intercept (individual blackcaps differ in the abundance of mites they harbour, regardless of their phenotype) and variable slopes of the relationships between the abundances of the two mites across individual blackcaps. For both mite species, the models included the effects of abundance of the other mite, feather identity, feather sector (proximal, medial or distal) and their two-way interactions as significant predictors of mite numbers. The models also included a significant interaction between

Table 2.1. Results of general linear models for variation in the number of wing cells occupied by each mite species and the mean rank order reached by such cells in the nested pattern of wing filling, in relation to variation in mite abundance and host phenotype. When the slope of the abundance effect depended on host phenotype, separate slopes were estimated for migratory (M) and resident (R) hosts.

	Number of cells occupied			**Mean nestedness order**		
T. bifurcata	**beta**	**$F_{1,75}$**	**P**	**beta**	**$F_{1,76}$**	**P**
Host phenotype		6.85	0.012		2.65	0.11
Mite abundance	M: 0.57 R: 1.58	141.53	< 0.001		0	0.97
P. sylviae	**beta**	**$F_{1,157}$**	**P**	**beta**	**$F_{1,157}$**	**P**
Host phenotype		0.28	0.62		0.58	0.45
Mite abundance	0.79	254.21	< 0.001	0.46	40.42	< 0.001

feather identity and host phenotype. Still, there were some differences between the models obtained for each species (Tables S2.2 and S2.3). Firstly, the abundance of *P. sylviae* was similar in migratory and resident blackcaps, but significantly differed between host types for *T. bifurcata*, which was virtually absent from migratory hosts (see Figure 2.1). In addition, the pattern of variation in the abundance of mites among feather sectors was similar in migratory and resident blackcaps for *P. sylviae*, while in *T. bifurcata* such differences varied in relation to host phenotype.

The effects described above captured the heterogeneous distribution of mites among wing cells and its variation between host types that had already been retrieved by our analysis with all blackcaps (see Figure 2.1). Controlling for these effects, we found a significant negative relationship between the numbers of the two mite species across wing cells (Tables S2.2 and S2.3). Moreover, the slope of such relationships significantly varied among feathers (with beta ± SE ranging between -0.21 ± 0.02 and -0.03 ± 0.02) and sector locations: the slope was steepest on proximal feather sectors (-0.29 ± 0.02), shallower on medial sectors (-0.22 ± 0.02), and shallowest on distal sectors (-0.10 ± 0.02).

In addition, *P. sylviae* had a different pattern of distribution across the wing when it occurred alone or coexisted with *T. bifurcata*. Thus, controlling for the effect of its abundance ($F_{1,155}$ = 267.08, P < 0.001), *P. sylviae* occupied more wing cells when *T.*

bifurcata was present on migratory hosts (mean ± SE: *T. bifurcata* present = 26.3 ± 0.77; absent = 24.0 ± 0.62), but it filled less wing cells on resident hosts (*T. bifurcata* present = 23.9 ± 0.97; absent = 27.2 ± 2.21; ANOVA results, host phenotype: $F_{1,155}$ = 0.08, P = 0.78; coexistence: $F_{1,155}$ = 0.16, P = 0.69; phenotype × coexistence: $F_{1,155}$ = 4.80, P = 0.030). Similar results were found for the average nestedness rank attained by *P. sylviae* populations in relation to coexistence with *T. bifurcata* and host phenotype (migratory blackcaps: *T. bifurcata* present = 18.3 ± 0.46; absent = 17.0 ± 0.37; resident blackcaps: *T. bifurcata* present = 16.7 ± 0.57; absent = 19.1 ± 1.31; ANOVA results, mite abundance: $F_{1,155}$ = 44.42, $P < 0.001$; host phenotype: $F_{1,155}$ = 0.09, P = 0.76; coexistence: $F_{1,155}$ = 0.54, P = 0.46; phenotype × coexistence: $F_{1,155}$ = 5.88, P = 0.016).

Discussion

The distribution of feather mites on the wing may be influenced by preferences of each mite species (which may vary in relation to host type), but also by intra and interspecific interactions among mites that live on the same host. Our results show that two feather mite species that have a different pattern of distribution among host types (with *T. bifurcata* being almost absent from migratory blackcaps), also show different distributions across wing patches within the host. These different patterns of distribution were the outcome of dissimilar orders of wing filling by each species, so that the wing cells most preferred by one mite species were not the most preferred by the other. As a consequence of these unequal patterns of wing filling, each species tended to increase its abundance on wing locations where the other species was relatively scarce, which led to effective spatial segregation between the two species. However, when *P. sylviae* reached high abundance on the host wing, its population spread towards the least preferred sectors, with individuals often settling in areas of the plumage where *T. bifurcata* (which had an aggregated distribution on the wing) was abundant. Spatial overlap between the two species was relevant, as shown by a negative correlation in the local abundance of the two mite species, which emerged controlling for the fact that each species has a distinct distribution on the host’s wing. These results contribute to a better understanding of the distribution of feather mites within their hosts, and the population consequences of the coexistence of different mite species on the same host individual.

Within-host distribution patterns of coexisting mites

Apart from the fact that each mite species attaches to a different side of the wing (dorsal or ventral), the two species also illustrated different spatial distributions on the wing surface. *P. sylviae* was more abundant towards distal feather sectors, as opposed to *T. bifurcata* which was more abundant towards proximal sectors. Besides, each species also reached its highest abundance on different feathers (Figure 2.1). These results show that mites are not randomly nor homogenously distributed across the space available to them on the wing. Most importantly, our results suggest that there are areas of the plumage that are commonly exploited by one mite but not the other, despite the fact that the whole wing surface is, in principle, available to both species. Supporting this view, both species occurred at least once in any of the wing cells defined in our study. We also found variation between species in the degree of aggregation of their populations. Thus, *P. sylviae* had a broader distribution on the wing with little evidence of aggregation, while the distribution of *T. bifurcata* was regularly aggregated. This difference suggests that *T. bifurcata* has stronger spatial preferences; in fact, its distribution became more aggregated when its abundance increased, which was not observed for *P. sylviae*.

The evolution of distinct space preferences in these two mite species could be the consequence of competition in the past (during the divergence of the two genera) resulting in niche partitioning (Chesson 2000, Pfenning & Pfenning 2010). Alternatively, *P. sylviae* and *T. bifurcata* could be simply occupying different optimal locations on the wing surface associated with their distinct attachment to the ventral or the dorsal side of the wing feathers, respectively. During bird flight, airflow runs faster on the dorsal than on the ventral wing surface, creating a zone of reduced pressure which could render dorsal wing surfaces subject to stronger aerodynamic stress (Pennycuick 2008). If mite attachment becomes more unstable due to aerodynamic forces near feather tips (where vibration is stronger; Videler 2005), such effect might favour more proximal locations for mites that live on the dorsal surface of wings, such as *T. bifurcata*. Interestingly, if dorsal mites are more sensitive to mechanical stress associated with flight, this might explain why *T. bifurcata* is so rare among migratory blackcaps but common on resident blackcaps. Conversely, if ventral surfaces are aerodynamically less stressful, *P. sylviae* may be less compromised by distal sectors of the feathers. However, even though *P. sylviae* may thrive on distal feather sectors, our results support the idea that they are not free from mechanical constraints. For example, the distal end of feathers is the most susceptible to wear and vibration, and it is responsible for producing lift during flapping flight (Videler 2005). These circumstances may explain why *P. sylviae* shifted its range from distal to medial feathers sectors at the tip and the leading edge of the wing (primaries 7-9, Figure 2.1).

We made other observations that could be interpreted as evidence of mechanical constraints on the distribution of each feather mite. *P. sylviae* avoided the outermost secondary feather, a behaviour that Jovani & Serrano (2004) interpreted as a way to avoid friction between the ventral surface of that feather and the dorsal surface of the innermost primary when the bird folds the wings. Such interpretation predicted a similar behaviour in *T. bifurcata*, which should then avoid the innermost primary (as this mite settles on the dorsal surface of the wing); a prediction which was clearly supported by our data.

Patterns of wing filling by mites

The fact that *P. sylviae* and *T. bifurcata* occupied their hosts roughly following opposite orders of wing filling supports the view that their distinct patterns of distribution on the wing are the outcome of habitat preference, rather than the consequence of interspecific interactions on the host. The sequences of wing cell occupation identified for these two species suggest that not all parts of the wing are habitats of the same quality, and that the parts of the wing that are most valued by one mite species are not necessarily the most important for the other species. However, although the two mites occupied different habitats, we identified areas that were equally rated as poor habitat by both species. Such areas of the plumage may be unfavourable for mites because they are too small or have loose feather structure (two features of tertial feathers, which were largely avoided by both mite species), or because they are subject to more intense mechanical stress (as may occur in the distal sectors of the outermost wing feathers).

If mites are to maximize their own fitness, competition for the best habitats may drive the patterns of wing filling by mites (Pulliam 1988, Rankin *et al.* 2007). Then, poor sites would act as buffer habitats, which are occupied by the individuals that are unable to settle on the most preferred patches. We found evidence of within-host variation in mite habitat quality consistent with the existence of buffer habitats on the host's wing, because the least-valued wing patches were unlikely to be filled unless mite population size was large. In these circumstances, individual preferences for optimal sites may increase the probability of population persistence, because any reduction in numbers at core habitats will induce individuals living in poor habitats to fill the gaps left in high quality habitats (Brown 1969, Gill *et al*. 2001).

Mite-mite interactions

Aside from showing dissimilar habitat preferences of *P. sylviae* and *T. bifurcata*, our results provide evidence that these two species may compete for resources when they coexist on the same host. Interspecific competition was supported by a negative correlation between the numbers of each species within the wing patches where both species co-occurred (a relationship which was detected when controlling for the different patterns of variation in numbers of each species across the wing surface or between host types). Interestingly, the slope of such relationships increased from distal to proximal sectors. This may be evidence that interspecific interactions between the two mite species are strongest in the areas of the wing that are preferred by *T. bifurcata*.

Interspecific interactions between these two species arise at different levels. At the between-host level, our results reveal an important constraint on the distribution of *T. bifurcata*, which apparently has difficulty in successfully colonizing migratory hosts. This observation may be interpreted as the consequence of variation in blackcap features that may determine host quality; for instance, *T. bifurcata* is more abundant on blackcaps with larger uropygial glands, and resident blackcaps have larger glands than migratory blackcaps (Fernández-González *et al*. 2013). However, at the within-host level *T. bifurcata* seems more capable of maintaining itself on its preferred habitat in the face of competition with *P. sylviae*. Thus, apart from the general negative correlation between the abundances of the two mites, *P. sylviae* contracted its range on resident blackcaps when *T. bifurcata* was present on the same host. Given that *T. bifurcata* may have difficulty in colonizing migratory blackcaps (arguably due to their poor quality as hosts for this species; Fernández-González *et al*. 2013), the fact that *P. sylviae* expanded its range on the host wing when it coexisted with *T. bifurcata* on migratory hosts (where *T. bifurcata* was always scarce and localised; Figure 2.1) may be interpreted as the consequence of the improved host quality of some migratory blackcaps, which may favour both the settlement of a few *T. bifurcata* and the expansion of *P. sylviae* through the host's wing.

In summary, our results depict a scenario in which within-host mite distributions have evolved as the outcome of diverging habitat preferences, which to a large extent may prevent interspecific competition. However, competition for space may still be important, especially when increased population size of competitors puts individuals of both species in contact in the same space. The impact of coexistence with competitors on mite populations may be stronger in areas of the host wing that are preferred by the most competitive mite, and it may have different consequences depending on host phenotype. In turn, variation in host phenotype may also create opportunities and constrains on the distribution of different mite species among hosts, thereby promoting different outcomes of mite-mite competition at the between-host level. Considering all these interactions at different spatial scales may be critical for understanding the outcomes of host-symbiont interactions, and will in turn improve our knowledge of the mechanisms that promote the evolution and maintenance of symbiont diversity.

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References

Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters*, 6: 1109-1122.

Atmar, W. & Patterson, B. D. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia*, 96: 373-382.

Atyeo, W. T. & Braasch, N. L. 1966. The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). *Bulletin of the University of Nebraska State Museum*, 5: 1-354.

Bascompte, J., Jordano, P., Melián, C. J. & Olesen, J. M., 2003. The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, 100: 9383-9387.

Bates, D., Maechler, M. & Bolker, B. 2012. Linear mixed-effects models using S4 classes. R package version 0.999999-0. http://lme4.r-forge.r-project.org/

Brown, J. L. 1969. The buffer effect and productivity in tit populations. *The American Naturalist*, 103: 347-354.

Chase, J. M., Leibold, M. A. 2003. *Ecological niches: linking classical and contemporary approaches.* University of Chicago Press, Chicago.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 31: 343-366.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 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. & Tellería, J. L. 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.

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

Fischer, J. & Lindenmayer, D. B. 2002. Treating the nestedness temperature calculator as a “black box” can lead to false conclusions. *Oikos*, 99: 193-199.

Friggens, M. M. & Brown, J. H. 2005. Niche partitioning in the cestode communities of two elasmobranchs. *Oikos*, 108: 76-84.

Gaud, J. & Atyeo, W. T. 1996. Feather mites of the world (Acarina, Astigmata): The supraspecific taxa. Musée Royale de l'Afrique Centrale, Annales Sciences Zoologiques, 277(Part I, Text): 1-193.

Gill, J. A., Norris, K., Potts, P. M., Gunnarsson, T. G., Atkinson, P. W. & Sutherland, W. J. 2001. The buffer effect and large-scale population regulation in migratory birds. *Nature*, 412: 436-438.

Holmes, J. C. 1973. Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology*, 51: 333-347.

Hutchinson, G. E. 1957. Concluding remarks. *Cold Spring Harbour Symposia*, 22: 415-427.

Jovani, R. & 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.

Kuris, A. M. & Lafferty, K. D. 1994. Community structure: larval trematodes in snail hosts. *Annual Review of Ecology, Evolution, and Systematics*, 25: 189-217.

MacArthur, R. & Levins, R. 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, 101: 377-385.

Mestre, A., Mesquita-Joanes, F., Proctor, H. & Monrós, J. S. 2011. Different scales of spatial segregation of two species of feather mites on the wings of a passerine bird. *Journal of Parasitology*, 97: 237-244.

Morand, S., Poulin, R., Rohde, K. & Hayward, C. 1999. Aggregation and species coexistence of ectoparasites of marine fishes. *International Journal for Parasitology*, 29: 663-672.

Pennycuick, C. J. 2008. *Modelling the flying bird*. Theoretical Ecology Series, Academic Press, Burlington.

Pérez-Tris, J., Carbonell, R., Tellería, J. L. 1999. A method for differentiating between sedentary and migratory Blackcaps *Sylvia atricapilla* in wintering areas of southern Iberia. *Bird Study*, 46: 299-304.

Perry, J. N. 1995. Spatial analysis by distance indices. *Journal of Animal Ecology*, 64: 303-314.

Perry, J. N. 1998. Measures of spatial pattern for counts. *Ecology*, 79: 1008-1017.

Perry, J. N., Winder, L., Holland, J. M. & Alston, R. D. 1999. Red-blue plots for detecting clusters in count data. *Ecology Letters*, 2: 106-113.

Pfenning, D. W. & Pfenning, K. S. 2010. Character displacement and the origins of diversity. *The American Naturalist*, 176: S26-S44.

Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, Princeton.

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

Pulliam, H. R. 1988. Sources, sinks, and population regulation. *The American Naturalist*, 132: 652-661.

Pulliam, H. R. 2000. On the relationship between niche and distribution. *Ecology Letters*, 3: 349-361.

Rankin, D. J., Bargum, K. & Kokko, H. 2007. The tragedy of the commons in evolutionary biology. *Trends in Ecology and Evolution*, 22: 643-651.

Rodenhouse, N. L., Sherry, T. W. & Holmes, R. T. 1997. Site-dependent regulation of population size: a new synthesis. *Ecology*, 78: 2025-2042.

Rodríguez-Gironés, M. A. & Santamaría, L. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. *Journal of Biogeography*, 33: 924-935.

Santana, F. J. 1976. A review of the genus *Trouessartia*. *Journal of Medical Entomology*, 1: S1-S128.

Schluter, D. 2000. *The ecology of adaptive radiation.* Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science*, 185: 27-39.

Schoener, T. W. 2009. *Ecological niche.* In: Levin, S.A. (ed.). The Princeton Guide to Ecology. Princeton University Press, Princeton: 3-13.

Soberón, J. & Peterson, A. T. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, 2: 1-10.

Sousa, W. P. 1994. Patterns and processes in communities of helminth parasites. *Trends in Ecology and Evolution*, 9: 52-57.

Videler, J. J. 2005. Avian flight. Oxford Ornithology Series, Oxford University Press, Oxford.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. & Smith, G. M. 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York.

Supplementary material

Table S2.1. Results of the generalized linear mixed-effect model for variation in number of mites among 18 wing feathers (9 primaries, 6 secondaries and 3 tertials) and three feather sectors (proximal, medial and distal), taking into account differences between mite species (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) and host phenotype (migratory or resident) and controlling for host identity (individual blackcaps as a random factor). Significant codes are: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Random effects	**Variance**	**Std. Dev.**			
Blackcap (Intercept)	0.599	0.774			
Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Intercept	-2.671	0.290	-9.206	< 0.001	***
Type					
Proctophyllodes	1.549	0.304	5.097	< 0.001	***
Feather					
P2	0.320	0.359	0.892	0.372	
P3	0.594	0.344	1.726	0.084	
P4	0.434	0.350	1.243	0.214	
P5	0.453	0.351	1.291	0.197	
P6	0.042	0.394	0.107	0.914	
P7	-0.597	0.460	-1.298	0.194	
P8	-1.256	0.579	-2.168	0.030	*
P9	-0.979	0.544	-1.801	0.072	
S1	-0.710	0.443	-1.602	0.109	
S2	0.892	0.337	2.648	0.008	**
S3	1.649	0.310	5.324	< 0.001	***
S4	0.949	0.331	2.871	0.004	**
S5	0.568	0.341	1.666	0.096	
S6	-0.413	0.408	-1.010	0.312	
T7	-1.556	0.534	-2.911	0.004	**
T8	-1.234	0.564	-2.186	0.029	*
T9	-18.962	230.120	-0.082	0.934	
Sector					
B	-1.467	0.397	-3.698	< 0.001	***
C	-2.530	0.506	-4.996	< 0.001	***
Phenotype					
SED	2.271	0.347	6.544	< 0.001	***
Type x Feather					
Proctophyllodes x P2	-0.518	0.388	-1.334	0.182	

Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)	
Proctophyllodes x P3	-0.375	0.373	-1.006	0.314	
Proctophyllodes x P4	-0.267	0.376	-0.710	0.478	
Proctophyllodes x P5	-0.538	0.380	-1.416	0.157	
Proctophyllodes x P6	0.492	0.420	1.172	0.241	
Proctophyllodes x P7	1.275	0.480	2.658	0.008	**
Proctophyllodes x P8	2.650	0.592	4.474	< 0.001	***
Proctophyllodes x P9	1.823	0.561	3.249	0.001	**
Proctophyllodes x S1	0.184	0.463	0.397	0.691	
Proctophyllodes x S2	-1.595	0.394	-4.051	< 0.001	***
Proctophyllodes x S3	-2.119	0.365	-5.799	< 0.001	***
Proctophyllodes x S4	-0.948	0.366	-2.587	0.010	**
Proctophyllodes x S5	-0.722	0.369	-1.956	0.051	
Proctophyllodes x S6	0.518	0.428	1.211	0.226	
Proctophyllodes x T7	2.363	0.545	4.334	< 0.001	***
Proctophyllodes x T8	1.803	0.580	3.107	0.002	**
Proctophyllodes x T9	18.976	230.120	0.082	0.934	
Type x Sector					
Proctophyllodes x B	3.200	0.387	8.275	< 0.001	***
Proctophyllodes x C	5.710	0.499	11.442	< 0.001	***
Type x Phenotype					
Proctophyllodes x SED	-2.656	0.381	-6.967	< 0.001	***
Feather x Sector					
P2 x B	1.322	0.464	2.851	0.004	**
P3 x B	0.899	0.459	1.960	0.050	*
P4 x B	1.094	0.454	2.408	0.016	*
P5 x B	1.114	0.455	2.447	0.014	*
P6 x B	-0.096	0.525	-0.183	0.855	
P7 x B	0.071	0.522	0.136	0.892	
P8 x B	0.076	0.523	0.145	0.884	
P9 x B	-0.216	0.806	-0.268	0.788	
S1 x B	1.683	0.513	3.282	0.001	**
S2 x B	0.507	0.483	1.050	0.294	
S3 x B	0.684	0.440	1.552	0.121	
S4 x B	0.969	0.452	2.142	0.032	*
S5 x B	1.649	0.445	3.705	< 0.001	***
S6 x B	1.733	0.492	3.522	< 0.001	***
T7 x B	2.593	0.574	4.516	< 0.001	***
T8 x B	1.689	0.678	2.491	0.013	*
T9 x B	1.673	0.740	2.261	0.024	*

Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)	
P2 x C	1.556	0.561	2.773	0.006	**
P3 x C	1.463	0.552	2.650	0.008	**
P4 x C	1.539	0.554	2.778	0.005	**
P5 x C	0.931	0.581	1.601	0.109	
P6 x C	-0.544	0.688	-0.791	0.429	
P7 x C	0.347	0.639	0.543	0.587	
P8 x C	-0.207	0.805	-0.257	0.797	
P9 x C	-0.292	1.042	-0.280	0.780	
S1 x C	1.069	0.619	1.727	0.084	
S2 x C	0.865	0.577	1.498	0.134	
S3 x C	0.915	0.541	1.689	0.091	
S4 x C	1.365	0.548	2.492	0.013	*
S5 x C	2.118	0.545	3.883	< 0.001	***
S6 x C	1.564	0.636	2.460	0.014	*
T7 x C	1.482	0.806	1.840	0.066	
T8 x C	1.395	1.222	1.142	0.254	
T9 x C	8.989	167.474	0.054	0.957	
Feather x Phenotype					
P2 x SED	0.485	0.392	1.238	0.216	
P3 x SED	0.320	0.380	0.843	0.399	
P4 x SED	0.562	0.382	1.470	0.142	
P5 x SED	0.827	0.382	2.164	0.030	*
P6 x SED	0.811	0.429	1.891	0.059	
P7 x SED	1.036	0.492	2.106	0.035	*
P8 x SED	1.305	0.609	2.144	0.032	*
P9 x SED	-1.114	0.684	-1.629	0.103	
S1 x SED	1.697	0.467	3.637	< 0.001	***
S2 x SED	0.537	0.373	1.440	0.150	
S3 x SED	-0.246	0.349	-0.703	0.482	
S4 x SED	0.079	0.369	0.215	0.829	
S5 x SED	-0.016	0.376	-0.043	0.966	
S6 x SED	0.069	0.438	0.158	0.874	
T7 x SED	0.249	0.530	0.469	0.639	
T8 x SED	-1.182	0.644	-1.834	0.067	
T9 x SED	17.433	230.119	0.076	0.940	
Sector x Phenotype					
B x SED	0.856	0.340	2.514	0.012	*
C x SED	0.871	0.351	2.482	0.013	*

Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Type x Feather x Sector					
Proctophyllodes x P2 x B	-1.101	0.451	-2.443	0.015	*
Proctophyllodes x P3 x B	-1.065	0.447	-2.383	0.017	*
Proctophyllodes x P4 x B	-1.365	0.439	-3.110	0.002	**
Proctophyllodes x P5 x B	-1.002	0.439	-2.284	0.022	*
Proctophyllodes x P6 x B	0.334	0.511	0.654	0.513	
Proctophyllodes x P7 x B	0.874	0.506	1.728	0.084	
Proctophyllodes x P8 x B	0.174	0.509	0.342	0.732	
Proctophyllodes x P9 x B	-0.577	0.797	-0.724	0.469	
Proctophyllodes x S1 x B	-1.338	0.490	-2.733	0.006	**
Proctophyllodes x S2 x B	0.062	0.489	0.128	0.898	
Proctophyllodes x S3 x B	0.197	0.451	0.436	0.663	
Proctophyllodes x S4 x B	-0.505	0.447	-1.128	0.259	
Proctophyllodes x S5 x B	-0.735	0.433	-1.698	0.090	
Proctophyllodes x S6 x B	-0.877	0.477	-1.837	0.066	
Proctophyllodes x T7 x B	-2.533	0.563	-4.503	< 0.001	***
Proctophyllodes x T8 x B	-1.256	0.672	-1.869	0.062	
Proctophyllodes x T9 x B	-1.939	0.717	-2.706	0.007	**
Proctophyllodes x P2 x C	-1.077	0.551	-1.955	0.051	
Proctophyllodes x P3 x C	-1.523	0.543	-2.805	0.005	**
Proctophyllodes x P4 x C	-1.817	0.542	-3.351	< 0.001	***
Proctophyllodes x P5 x C	-1.229	0.569	-2.159	0.031	*
Proctophyllodes x P6 x C	-0.465	0.677	-0.687	0.492	
Proctophyllodes x P7 x C	-1.645	0.626	-2.625	0.009	**
Proctophyllodes x P8 x C	-2.689	0.795	-3.380	< 0.001	***
Proctophyllodes x P9 x C	-3.685	1.031	-3.575	< 0.001	***
Proctophyllodes x S1 x C	-1.663	0.598	-2.782	0.005	**
Proctophyllodes x S2 x C	-0.732	0.581	-1.260	0.208	
Proctophyllodes x S3 x C	-0.417	0.549	-0.760	0.447	
Proctophyllodes x S4 x C	-1.299	0.543	-2.391	0.017	*
Proctophyllodes x S5 x C	-2.099	0.535	-3.922	< 0.001	***
Proctophyllodes x S6 x C	-2.811	0.624	-4.507	< 0.001	***
Proctophyllodes x T7 x C	-5.241	0.792	-6.615	< 0.001	***
Proctophyllodes x T8 x C	-6.983	1.271	-5.495	< 0.001	***
Proctophyllodes x T9 x C	-15.408	167.473	-0.092	0.927	
Type x Feather x Phenotype					
Proctophyllodes x P2 x SED	-0.113	0.440	-0.257	0.797	
Proctophyllodes x P3 x SED	-0.315	0.438	-0.720	0.471	
Proctophyllodes x P4 x SED	-0.030	0.437	-0.068	0.946	

Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Proctophyllodes x P5 x SED	-0.176	0.443	-0.398	0.691	
Proctophyllodes x P6 x SED	-1.616	0.536	-3.014	0.003	**
Proctophyllodes x P7 x SED	-1.608	0.568	-2.830	0.005	**
Proctophyllodes x P8 x SED	-1.330	0.655	-2.030	0.042	*
Proctophyllodes x P9 x SED	-0.304	0.761	-0.400	0.689	
Proctophyllodes x S1 x SED	-1.212	0.500	-2.424	0.015	*
Proctophyllodes x S2 x SED	-0.796	0.457	-1.740	0.082	
Proctophyllodes x S3 x SED	-0.133	0.424	-0.313	0.755	
Proctophyllodes x S4 x SED	-0.296	0.429	-0.690	0.490	
Proctophyllodes x S5 x SED	1.055	0.422	2.496	0.013	*
Proctophyllodes x S6 x SED	0.717	0.475	1.510	0.131	
Proctophyllodes x T7 x SED	0.410	0.557	0.737	0.461	
Proctophyllodes x T8 x SED	0.891	0.668	1.333	0.183	
Proctophyllodes x T9 x SED	-17.735	230.119	-0.077	0.939	
Type x Sector x Phenotype					
Proctophyllodes x B x SED	-0.141	0.157	-0.899	0.368	
Proctophyllodes x C x SED	-0.677	0.188	-3.608	< 0.001	***
Feather x Sector x Phenotype					
P2 x B x SED	-0.393	0.408	-0.965	0.335	
P3 x B x SED	-0.259	0.401	-0.645	0.519	
P4 x B x SED	-0.786	0.391	-2.011	0.044	*
P5 x B x SED	-0.861	0.395	-2.177	0.029	*
P6 x B x SED	0.132	0.459	0.288	0.773	
P7 x B x SED	0.012	0.431	0.028	0.978	
P8 x B x SED	-0.406	0.363	-1.120	0.263	
P9 x B x SED	0.625	0.526	1.189	0.234	
S1 x B x SED	-1.198	0.456	-2.626	0.009	**
S2 x B x SED	0.120	0.439	0.273	0.785	
S3 x B x SED	-0.125	0.397	-0.316	0.752	
S4 x B x SED	-0.254	0.403	-0.631	0.528	
S5 x B x SED	-1.343	0.380	-3.536	< 0.001	***
S6 x B x SED	-1.270	0.390	-3.257	0.001	**
T7 x B x SED	-1.948	0.377	-5.164	< 0.001	***
T8 x B x SED	-0.724	0.430	-1.684	0.092	
T9 x B x SED	-0.523	0.523	-1.000	0.318	
P2 x C x SED	-0.406	0.399	-1.016	0.310	
P3 x C x SED	-0.255	0.391	-0.653	0.514	
P4 x C x SED	-0.922	0.380	-2.425	0.015	*
P5 x C x SED	-1.279	0.393	-3.257	0.001	**

Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
P6 x C x SED	-0.112	0.462	-0.242	0.808	
P7 x C x SED	-0.012	0.435	-0.027	0.979	
P8 x C x SED	-0.628	0.394	-1.596	0.111	
P9 x C x SED	1.134	0.602	1.883	0.060	
S1 x C x SED	-0.662	0.452	-1.466	0.143	
S2 x C x SED	0.111	0.438	0.253	0.801	
S3 x C x SED	0.222	0.395	0.563	0.573	
S4 x C x SED	0.139	0.397	0.351	0.726	
S5 x C x SED	-1.068	0.374	-2.859	0.004	**
S6 x C x SED	-1.047	0.396	-2.644	0.008	**
T7 x C x SED	-0.825	0.456	-1.808	0.071	
T8 x C x SED	-13.310	400.598	-0.033	0.973	
T9 x C x SED	-9.280	167.471	-0.055	0.956	

Table S2.2. Results of the generalized linear mixed-effect model for variation in number of *Proctophyllodes sylviae* in relation to the numbers of *Trouessartia bifurcata*, taking into account the different mite numbers among 18 wing feathers (9 primaries, 6 secondaries and 3 tertials), three feather sectors (proximal, medial and distal) and phenotype (migratory or resident), and controlling for host identity (individual blackcaps as a random factor). Significant codes are: * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

Random effects	**Variance**	**Std. Dev.**	**Corr.**		
Blackcap (Intercept)	0.280	0.529			
Trouessartia	0.163	0.404	0.279		
Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Intercept	0.464	0.201	2.309	0.021	*
Trouessartia	-0.933	0.097	-9.580	< 0.001	***
Feather					
P2	-0.116	0.263	-0.441	0.659	
P3	-0.063	0.241	-0.263	0.792	
P4	0.238	0.241	0.989	0.323	
P5	0.061	0.245	0.250	0.802	
P6	0.175	0.229	0.762	0.446	
P7	0.263	0.226	1.162	0.245	
P8	0.910	0.202	4.505	< 0.001	***
P9	0.954	0.225	4.231	< 0.001	***
S1	-0.074	0.276	-0.268	0.789	
S2	-0.716	0.325	-2.200	0.028	*
S3	-0.420	0.333	-1.262	0.207	
S4	-0.700	0.307	-2.285	0.022	*
S5	-0.079	0.262	-0.302	0.762	
S6	-0.052	0.258	-0.202	0.840	
T7	0.563	0.219	2.568	0.010	*
T8	0.380	0.233	1.627	0.104	
T9	-0.175	0.268	-0.654	0.513	
Sector					
B	0.708	0.196	3.617	< 0.001	***
C	1.688	0.188	8.987	< 0.001	***
Phenotype					
SED	0.069	0.166	0.417	0.677	
Trouessartia x Feather					
Trouessartia x P2	0.096	0.065	1.480	0.139	
Trouessartia x P3	0.092	0.064	1.434	0.152	
Trouessartia x P4	0.250	0.067	3.710	< 0.001	***

Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Trouessartia x P5	0.187	0.066	2.845	0.004	**
Trouessartia x P6	0.234	0.066	3.523	< 0.001	***
Trouessartia x P7	0.232	0.066	3.523	< 0.001	***
Trouessartia x P8	0.427	0.071	6.041	< 0.001	***
Trouessartia x P9	0.485	0.123	3.927	< 0.001	***
Trouessartia x S1	-0.103	0.095	-1.085	0.278	
Trouessartia x S2	0.030	0.065	0.462	0.644	
Trouessartia x S3	0.056	0.065	0.863	0.388	
Trouessartia x S4	0.104	0.065	1.599	0.110	
Trouessartia x S5	0.002	0.070	0.034	0.973	
Trouessartia x S6	-0.057	0.091	-0.628	0.530	
Trouessartia x T7	-0.721	0.168	-4.289	< 0.001	***
Trouessartia x T8	-0.411	0.199	-2.060	0.039	*
Trouessartia x T9	-1.132	0.379	-2.989	0.003	**
Trouessartia x Sector					
Trouessartia x B	0.554	0.060	9.169	< 0.001	***
Trouessartia x C	0.582	0.061	9.520	< 0.001	***
Feather x Sector					
P2 x B	0.013	0.279	0.047	0.962	
P3 x B	-0.084	0.258	-0.324	0.746	
P4 x B	-0.484	0.258	-1.875	0.061	
P5 x B	0.050	0.262	0.192	0.847	
P6 x B	0.366	0.242	1.510	0.131	
P7 x B	0.930	0.236	3.940	< 0.001	***
P8 x B	0.289	0.213	1.360	0.174	
P9 x B	-0.658	0.242	-2.718	0.007	**
S1 x B	-0.273	0.293	-0.930	0.352	
S2 x B	0.595	0.340	1.749	0.080	
S3 x B	0.672	0.343	1.959	0.050	
S4 x B	0.996	0.318	3.134	0.002	**
S5 x B	0.715	0.272	2.626	0.009	**
S6 x B	0.502	0.266	1.887	0.059	
T7 x B	0.098	0.231	0.423	0.672	
T8 x B	0.324	0.246	1.316	0.188	
T9 x B	0.018	0.285	0.063	0.949	
P2 x C	0.347	0.265	1.309	0.191	
P3 x C	0.153	0.244	0.626	0.532	
P4 x C	-0.477	0.244	-1.951	0.051	
P5 x C	-0.346	0.251	-1.379	0.168	

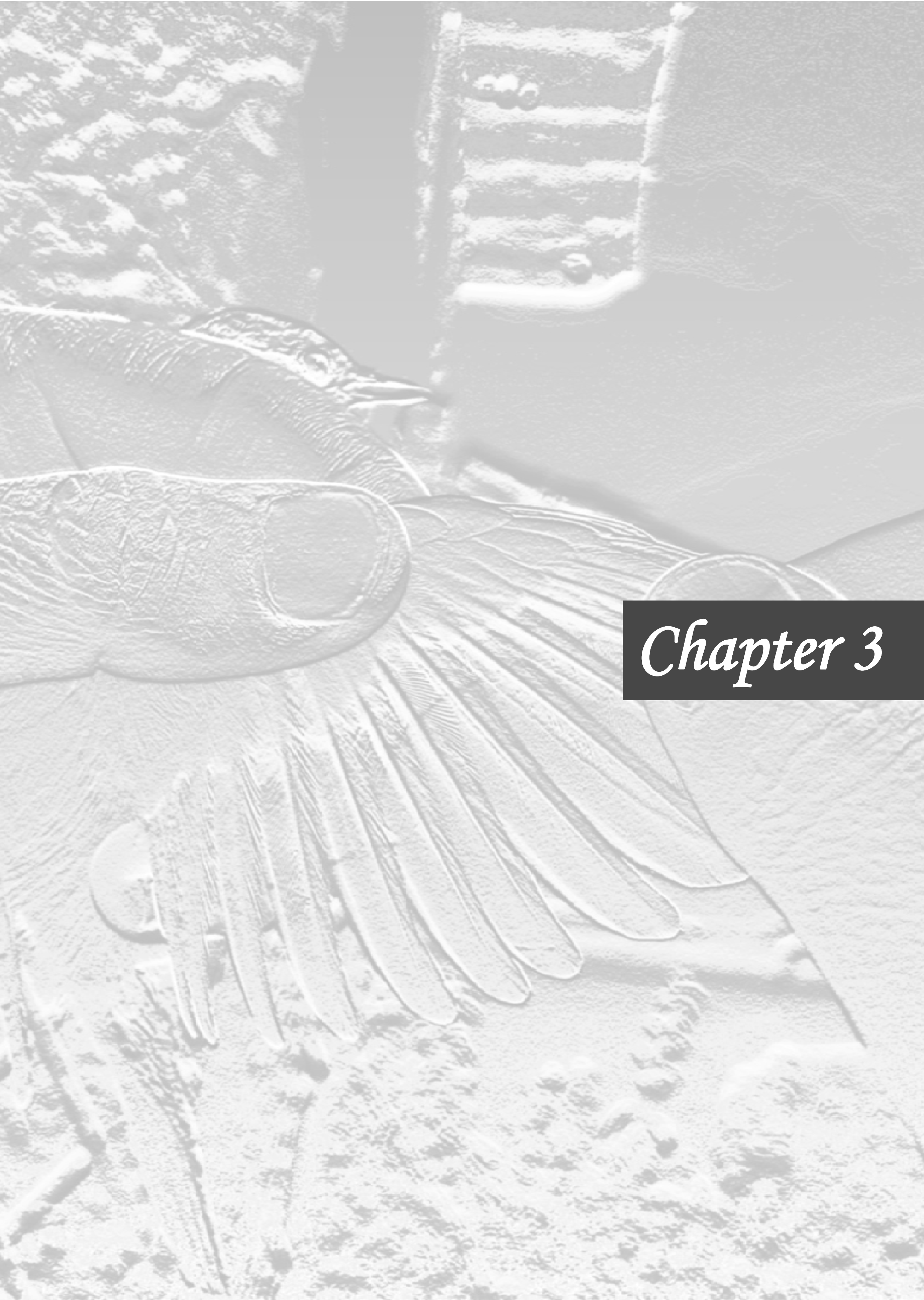
Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
P6 x C	-0.402	0.237	-1.693	0.090	
P7 x C	-0.361	0.235	-1.537	0.124	
P8 x C	-1.525	0.222	-6.881	< 0.001	***
P9 x C	-1.937	0.283	-6.832	< 0.001	***
S1 x C	-0.803	0.281	-2.857	0.004	**
S2 x C	0.362	0.329	1.100	0.271	
S3 x C	0.600	0.335	1.789	0.074	
S4 x C	0.653	0.309	2.112	0.035	*
S5 x C	0.047	0.265	0.177	0.859	
S6 x C	-0.784	0.266	-2.950	0.003	**
T7 x C	-1.661	0.273	-6.078	< 0.001	***
T8 x C	-0.917	0.475	-1.930	0.054	
T9 x C	-2.507	1.037	-2.418	0.016	*
Feather x Phenotype					
P2 x SED	-0.005	0.096	-0.056	0.955	
P3 x SED	-0.221	0.102	-2.176	0.030	*
P4 x SED	-0.351	0.115	-3.056	0.002	**
P5 x SED	-0.424	0.120	-3.519	< 0.001	***
P6 x SED	-0.771	0.115	-6.700	< 0.001	***
P7 x SED	-0.469	0.100	-4.700	< 0.001	***
P8 x SED	-0.403	0.102	-3.939	< 0.001	***
P9 x SED	-0.684	0.180	-3.800	< 0.001	***
S1 x SED	0.013	0.140	0.090	0.928	
S2 x SED	-0.051	0.119	-0.428	0.669	
S3 x SED	-0.342	0.109	-3.141	0.002	**
S4 x SED	-0.294	0.107	-2.744	0.006	**
S5 x SED	-0.073	0.101	-0.719	0.472	
S6 x SED	0.124	0.112	1.111	0.267	
T7 x SED	-0.570	0.136	-4.203	< 0.001	***
T8 x SED	-0.763	0.137	-5.582	< 0.001	***
T9 x SED	-0.272	0.216	-1.258	0.209	
Sector x Phenotype					
B x SED	0.174	0.091	1.908	0.056	
C x SED	-0.051	0.096	-0.528	0.597	

Table S2.3. Results of the generalized linear mixed-effect model for variation in number of *Trouessartia bifurcata* in relation to the numbers of *Proctophyllodes sylviae*, taking into account the different mite numbers among 18 wing feathers (9 primaries, 6 secondaries and 3 tertials), three feather sectors (proximal, medial and distal) and phenotype (migratory or resident), and controlling for host identity (individual blackcaps as a random factor). Significant codes are: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Random effects	**Variance**	**Std. Dev.**	**Corr.**		
Blackcap (Intercept)	0.454	0.674			
Proctophyllodes	0.024	0.154	0.200		
Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	
Intercept	-0.237	0.319	-0.743	0.458	
Proctophyllodes	-0.508	0.068	-7.458	< 0.001	***
Feather					
P2	-0.080	0.353	-0.227	0.820	
P3	-0.087	0.344	-0.254	0.799	
P4	0.062	0.351	0.178	0.859	
P5	0.216	0.351	0.615	0.538	
P6	-0.335	0.410	-0.816	0.415	
P7	-0.841	0.482	-1.744	0.081	
P8	-1.214	0.598	-2.032	0.042	*
P9	-0.843	0.635	-1.329	0.184	
S1	-0.505	0.412	-1.227	0.220	
S2	0.301	0.342	0.879	0.379	
S3	0.669	0.317	2.111	0.035	*
S4	0.242	0.333	0.728	0.467	
S5	0.439	0.332	1.320	0.187	
S6	-0.304	0.402	-0.757	0.449	
T7	-0.392	0.508	-0.771	0.441	
T8	-0.400	0.558	-0.717	0.473	
T9	-13.320	333.130	-0.040	0.968	
Sector					
B	-1.590	0.316	-5.030	< 0.001	***
C	-3.278	0.574	-5.714	< 0.001	***
Phenotype					
SED	0.798	0.352	2.263	0.024	*
Proctophyllodes x Feather					
Proctophyllodes x P2	-0.036	0.039	-0.920	0.358	
Proctophyllodes x P3	0.003	0.043	0.069	0.945	
Proctophyllodes x P4	-0.022	0.041	-0.525	0.600	

Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)	
Proctophyllodes x P5	-0.025	0.042	-0.609	0.542	
Proctophyllodes x P6	-0.014	0.043	-0.339	0.735	
Proctophyllodes x P7	-0.048	0.042	-1.154	0.249	
Proctophyllodes x P8	-0.047	0.043	-1.103	0.270	
Proctophyllodes x P9	0.146	0.095	1.534	0.125	
Proctophyllodes x S1	-0.227	0.078	-2.932	0.003	**
Proctophyllodes x S2	-0.036	0.041	-0.879	0.380	
Proctophyllodes x S3	-0.043	0.039	-1.109	0.268	
Proctophyllodes x S4	0.010	0.038	0.261	0.794	
Proctophyllodes x S5	-0.038	0.039	-0.968	0.333	
Proctophyllodes x S6	-0.072	0.049	-1.473	0.141	
Proctophyllodes x T7	-0.077	0.094	-0.816	0.415	
Proctophyllodes x T8	-0.228	0.154	-1.484	0.138	
Proctophyllodes x T9	-0.526	0.271	-1.944	0.052	
Proctophyllodes x Sector					
Proctophyllodes x B	0.374	0.054	6.881	< 0.001	***
Proctophyllodes x C	0.388	0.055	7.042	< 0.001	***
Feather x Sector					
P2 x B	1.051	0.332	3.170	0.002	**
P3 x B	0.751	0.336	2.236	0.025	*
P4 x B	0.657	0.336	1.954	0.051	
P5 x B	0.622	0.333	1.871	0.061	
P6 x B	0.140	0.372	0.377	0.706	
P7 x B	0.466	0.430	1.083	0.279	
P8 x B	-0.661	0.557	-1.187	0.235	
P9 x B	-1.044	0.894	-1.168	0.243	
S1 x B	1.114	0.340	3.277	0.001	**
S2 x B	0.901	0.321	2.808	0.005	**
S3 x B	0.809	0.319	2.535	0.011	*
S4 x B	1.001	0.328	3.049	0.002	**
S5 x B	0.868	0.348	2.495	0.013	*
S6 x B	0.910	0.426	2.139	0.032	*
T7 x B	0.749	0.511	1.467	0.142	
T8 x B	0.843	0.714	1.180	0.238	
T9 x B	1.332	0.584	2.279	0.023	*
P2 x C	1.957	0.599	3.269	0.001	**
P3 x C	1.741	0.603	2.888	0.004	**
P4 x C	1.208	0.609	1.985	0.047	*
P5 x C	0.593	0.627	0.945	0.345	

Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)	
P6 x C	0.194	0.704	0.275	0.783	
P7 x C	1.469	0.635	2.313	0.021	*
P8 x C	0.440	0.817	0.539	0.590	
P9 x C	2.130	0.987	2.158	0.031	*
S1 x C	2.017	0.602	3.348	< 0.001	***
S2 x C	1.829	0.576	3.176	0.001	**
S3 x C	2.000	0.570	3.507	< 0.001	***
S4 x C	1.650	0.581	2.841	0.004	**
S5 x C	2.000	0.590	3.388	< 0.001	***
S6 x C	1.686	0.667	2.526	0.012	*
T7 x C	2.031	0.815	2.493	0.013	*
T8 x C	3.054	1.266	2.413	0.016	*
T9 x C	2.354	1.210	1.946	0.052	
Feather x Phenotype					
P2 x SED	0.583	0.361	1.613	0.107	
P3 x SED	0.644	0.356	1.809	0.071	
P4 x SED	0.457	0.361	1.266	0.206	
P5 x SED	0.618	0.363	1.702	0.089	
P6 x SED	0.784	0.423	1.853	0.064	
P7 x SED	1.170	0.491	2.383	0.017	*
P8 x SED	1.781	0.614	2.901	0.004	**
P9 x SED	-0.082	0.662	-0.123	0.902	
S1 x SED	0.920	0.421	2.184	0.029	*
S2 x SED	0.576	0.352	1.636	0.102	
S3 x SED	0.204	0.331	0.617	0.537	
S4 x SED	0.247	0.345	0.716	0.474	
S5 x SED	-0.367	0.348	-1.054	0.292	
S6 x SED	-0.026	0.411	-0.064	0.949	
T7 x SED	-0.529	0.497	-1.065	0.287	
T8 x SED	-1.010	0.633	-1.596	0.110	
T9 x SED	12.285	333.130	0.037	0.971	
Sector x Phenotype					
B x SED	0.653	0.128	5.119	< 0.001	***
C x SED	0.865	0.184	4.688	< 0.001	***



Chapter 3

This chapter is based on the manuscript:

Fernández-González, S., Proctor, H. C., Pérez-Rodríguez, A., De la Hera, I. & Pérez-Tris, J. High diversity and low genetic structure of populations of feather mites with phenotypically divergent bird hosts. *In preparation.*

High diversity and low genetic structure of populations of feather mites with phenotypically divergent bird hosts

Sofía Fernández-González, Heather C. Proctor, Antón Pérez-Rodríguez, Iván de la Hera and Javier Pérez-Tris

Populations of obligate symbionts may be genetically structured among host individuals (if transmission bottlenecks render in-host populations formed by the descendants of one or a few colonizers) and among phenotypically distinct hosts, if differences in host phenotype promote symbiont specialization. Such processes may in turn determine the amount of genetic diversity of within-host symbiont populations, which is relevant for understanding symbiont population dynamics. We analysed the genetic structure of populations of two species of feather-dwelling mites (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) in migratory and resident European blackcaps *Sylvia atricapilla* wintering in sympatry in southern Spain. We found high genetic diversity of within-host populations for both mite species, and no sign of genetic structure of mite populations between migratory and resident hosts. Our results show that mite populations are not limited by transmission bottlenecks reducing genetic diversity among mite individuals that share a host. In addition, 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. In fact, the mixing of mite haplotypes among migratory and sedentary hosts rather supports the view that mites may disperse among hosts with distinct geographic origin, behaviour and physiology. These results provide insight into the likely mechanisms that allow symbiotic organisms to avoid endogamy and to persist in the face of habitat heterogeneity in phenotypically diverse host populations.

Introduction

Population genetic structure is a common phenomenon in nature, which typically arises as the outcome of restricted gene flow and lineage divergence during periods of population isolation (Avise 2000, Hartl & 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 temporarily limited habitat (Price 1980, Poulin 2007, Barrett *et al.* 2008). This circumstance forces symbionts to continuously colonize new habitat patches, thereby creating opportunity for population structuring via transmission bottlenecks, especially if populations established on one individual are composed of the descendants of a few colonizers (Hedrick 2000).

The genetic structuring of symbiont infrapopulations (the stock of symbionts that become temporarily isolated in a single host individual; Poulin 2007) may be relevant for understanding the population dynamics of symbiotic organisms. Individual symbionts may differ in their ability to access host resources or to occupy the best habitat within the host (Mideo 2009). In these circumstances, individual symbionts may benefit from occupying the best host microhabitat, a situation which may render poor competitors, leading to low individual fitness (Fretwell & Lucas 1970). However, fitness differences among symbionts that share a host are expected to only have evolutionary consequences in genetically diverse symbiont infrapopulations (Rigaud *et al.* 2010): if all symbionts in the infrapopulation 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). Nevertheless, low genetic diversity of the infrapopulation may force symbionts to mate with more closely related individuals than is optimal, a circumstance which may favour transmission mechanisms that promote outbreeding (Keller & Waller 2002).

For most symbiont species, host demography, movements and distribution may arise as major factors determining host accessibility, and consequently the genetic structuring of their populations (Nadler 1995, Criscione *et al.* 2005, Huyse *et al.* 2005, Whiteman & Parker 2005, Barrett *et al.* 2008). In some instances, symbionts may exploit different host types, which may differ in their spatio-temporal distribution or in their compatibility to 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 alternative host types (Nadler 1995, Nosil *et al.* 2002, Rigaud *et al.* 2010). Despite the fact that knowledge of the patterns of population genetic structure of symbionts should form the basis of our understanding of the ecology and evolution of host-symbiont interactions (Hewitt 2001), the genetic composition of symbiont populations within host individuals and the genetic structuring of such populations amongst individuals and higher geographic scales remains obscure for most host-symbiont systems (Nadler 1995, McCoy *et al.* 2003, Álvarez *et al.* 2010).

We studied the genetic structuring of populations of two species of feather-dwelling mites (Astigmata: *Proctophyllodes sylviae* and *Trouessartia bifurcata*) among European blackcaps (*Sylvia atricapilla*) wintering in Southern Spain. In this area, resident and migratory blackcaps share the same habitat (Pérez-Tris & Tellería 2002a), thereby creating opportunities for mites to spread amongst hosts from different geographic origins (see Pérez-Tris & Bensch 2005 for an example of the same process with blood parasites). However, feather mite transmission requires close physical contact between hosts (Proctor 2003), which in the blackcap is likely to take place mostly during mating or parental care (Mason 1995). This circumstance may decrease transmission opportunities for mites during winter, and consequently may promote genetic structure of mite populations between migratory and resident blackcaps. For example, mite populations that have coevolved with resident blackcaps might have diverged from those populations that co-expanded with their migratory hosts across Europe after the last glaciation (Pérez-Tris *et al.* 2004). In fact, the resident blackcaps studied here form the most unique blackcap population evolutionarily within the species' continental range, showing evidence of genetic isolation from other populations (some of which cohabitate during winter; Pérez-Tris *et al.* 2004) and signs of local adaptation associated to a sedentary life style. Some of the attributes that make resident blackcaps distinct may be relevant for feather mites. Thus, long life expectancy makes residents more durable hosts (Pérez-Tris & Tellería 2002b), and year-round site tenacity provides mites with stable habitat (migrants move between contrasting regions biannually; Pérez-Tris & Tellería 2002a). Finally, large size of uropygial glands is likely to make residents more nutritionally rewarding hosts (Fernández-González *et al.* 2013).

The coexistence of migratory and resident blackcaps may provide opportunities for mite dispersal among hosts with different phenotype and geographic origins. Whether such dispersal events have microevolutionary consequences depends on the potential for mite transmission outside of the host breeding season, the ability of mites to successfully disperse among distantly located regions taking advantage of host migratory movements, or the degree of host specialization of different mite populations. In addition, transmission bottlenecks might reduce genetic diversity of mite infrapopulations, rendering the whole population of mites further structured in consequence (Nadler 1995). The genetic consequences of all these processes may differ between the two mite species despite the fact that they have virtually identical life styles and share hosts. In fact, there is evidence that *T. bifurcata* thrives on resident blackcaps while *P. sylviae* is equally frequent on both types of host, although it is more abundant 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 genetic structure between mite species in the same host population.

Our study aimed to investigate the genetic implications for symbiotic feather mites of (1) the coexistence of different host phenotypes in the same population (*i.e.*, sympatric migratory and resident blackcaps), and (2) the population bottlenecks that mites might undergo during transmission among hosts. 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 subpopulations (with migratory or resident blackcaps as types of hosts that may harbour genetically distinct mite infrapopulations). If the transmission of these mites takes place through host familial contact alone (which is likely since the blackcap hardly shows any sociality outside the breeding period; Figuerola 2000, Blanco & Frías 2001, Proctor 2003), then our analysis of the genetic structure of mite populations should answer whether or not host philopatry promotes the isolation of feather mite populations (McCoy *et al.* 2003). In addition, our analysis will answer if population bottlenecks play a relevant role in shaping the genetic composition of mite populations within a host. Among other implications, knowing whether the mites that live on the same host form genetically diverse populations, or if they are close kin is pivotal in understanding the evolutionary implications of competitive asymmetries among mites that share an individual host (Fernández-González *et al.* 2013).

Material and methods

Study site and field methods

Blackcaps were captured during two winter seasons (February and December 2010) in the Campo de Gibraltar area (southern Spain). A total of 160 birds were mist-netted with the aid of a tape-lure in order to increase capture rate. Birds were fitted with aluminium rings, and their age and sex were determined according to plumage traits (Svensson 1992). 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. These variables were used to classify blackcaps as migratory or resident using a discriminant function (Pérez-Tris *et al.* 1999), which correctly assigns > 97% of individuals (De la Hera *et al.* 2007, 2012).

Mites were removed from the host by introducing various wing feathers in tubes filled with absolute ethanol, trying to sample from the whole area of the wing that was populated by mites. The samples were stored at -20 ºC until analysed. A sample of the blackcaps that harboured a sufficient number of *Proctophyllodes* mites was selected to analyse the population genetic structure of mites in migratory and resident blackcaps. Five individuals of each mite species were randomly selected from each host for genetic analyses. We chose 24 blackcaps (12 of each type) with at least 5 male *Proctophyllodes*, because female *Proctophyllodes* cannot be reliably determined to the species level based on morphology. These birds included 9 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); the remaining resident and all of the migratory blackcaps sampled for *Proctophyllodes* were randomly selected among those that had enough mites of that species. We also included three migratory blackcaps that harboured *Trouessartia* mites (we did not have any migratory individuals with a sufficient number of mites of the two species), so that the final number of blackcaps sampled for *Trouessartia* was 12 (9 resident and 3 migratory), and total number of hosts sampled was 27 hosts. 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). For the remaining blackcaps sampled for *Trouessartia*, we retrieved less than 5 mite individuals; therefore they were not considered for the analyses.

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 manufacturer's instructions (Dabert *et al.* 2008, M. Dabert pers. comm.). Individual mites were transferred from the stock preserved in ethanol to tubes containing 180 µl of ATL lysis buffer with 20 µl of Proteinase K (Qiagen, USA), which were incubated at 57 °C with 500 rpm shaking in a thermoshaker (GRANT ®) for 72 h, vortexing the samples thoroughly 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 ºC until used for microscopy.

The ecdysozoan 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). PCR or sequence failure produced variable sample sizes in the analyses.

Genetic analyses

In order 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), as implemented in Arlequin 3.5.1.2 (Excoffier & 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 α = 0.24 for *P. sylviae*). This was the 6th best model according to the Akaike Information Criterion implemented in JModelTest, and according to model parameters it was the closest to the best models among the available in Arlequin. We tested statistical significance of population genetic structure using 1,000 permutations.

The evolutionary relationships among all unique haplotypes of *P. sylviae* and *T. bifurcata* were estimated separately for each genus. The trees included COI sequences of various species of the corresponding genus obtained from our mite collection. We also included some species of the other genus in order to confirm the monophyly of each mite group. Maximum-likelihood phylogenetic analyses were carried out using PhyML 3.0 (Guindon & Gascuel 2003). A heuristic search with the Nearest Neighbour Interchange algorithm for branch swapping was conducted under the most appropriate substitution model in each case (HKY+I+G for *Proctophyllodes* and *Trouessartia*), as estimated by jModelTest. Support for internal nodes was derived from a bootstrap resampling with 1,000 repetitions. In addition, Bayesian phylogenetic reconstructions were performed using MrBayes version 3.2.1 (Ronquist *et al.* 2012), which were sampled using one cold and three heated Markov-Coupled Monte Carlo chains (MCMC), with temperature of the chains set to T = 0.1. The most appropriate substitution model obtained from MrModeltest 2.3 (Nylander 2004) was HKY+I+G for both mite species. Trees were sampled every 200 steps to obtain 50,000 trees, and the first 10% were discarded as burn-in. Posterior probabilities were used to assess clade support.

A haplotype network was built for each mite species with the software NETWORK (Fluxus Technology), using Median-Joining algorithm. For each mite species, we computed the mean genetic distance between haplotypes on the same host in order to better assess the degree of within-host genetic resemblance among mites.

Results

According to microscopic examination, all genotyped mites belonged to *Proctophyllodes sylviae* or *Trouessartia bifurcata* (Table S3.1). A total of 72 haplotypes were found among 93 sequenced *P. sylviae* individuals. Among 58 *T. bifurcata* individuals, we found 29 haplotypes. 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 sequences were deposited in Genbank with accession numbers KF613605 - KF613716 (Table S3.1).

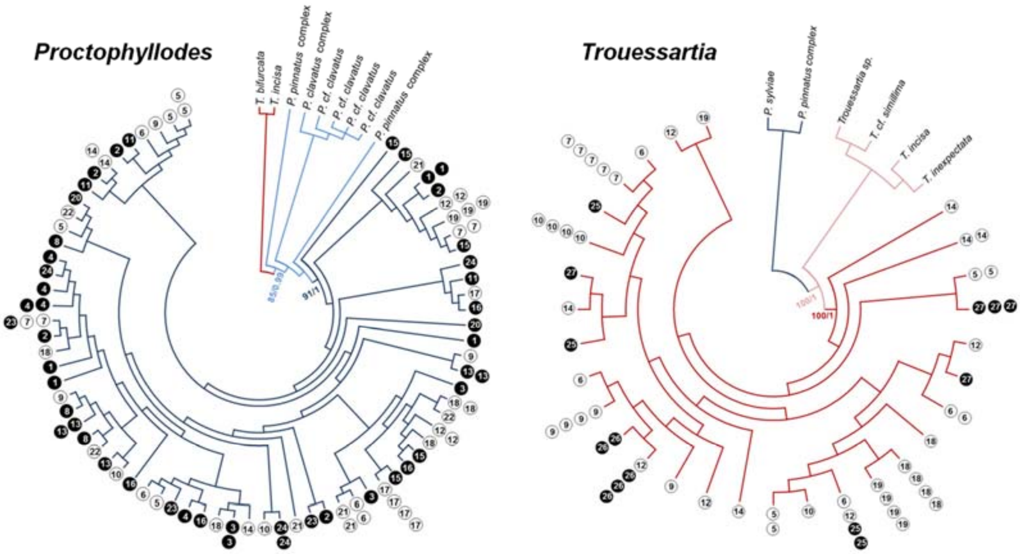


Figure 3.1. Phylogenetic relationships of cytochrome oxidase I (COI) haplotypes of feather mites sampled from wintering blackcaps. In both trees, blue colours represent *Proctophyllodes* and red colours represent *Trouessartia*. Each tree contains several haplotypes retrieved from individuals of other species of the same genus (shown in light colour on the tree). The trees have been rooted with two haplotypes of the other mite genus (shown in the opposite colour). Statistical support for the monophyly of each mite genus, and for the clade formed by mites identified as *P. sylviae* and *T. bifurcata* under the microscope (shown in dark colour in the corresponding tree), are shown with numbers on the relevant nodes (bootstrap support in maximum likelihood trees, and posterior probabilities in Bayesian trees). The numbers in the circles indicate individual hosts of origin of each haplotype (open circles represent resident blackcaps and filled circles migratory blackcaps).

Both Maximum Likelihood and Bayesian Inference methods of phylogenetic reconstruction placed the haplotypes of *P. sylviae* and *T. bifurcata* forming monophyletic clades within the genera *Proctophyllodes* and *Trouessartia*, which were supported as reciprocally monophyletic (Figure 3.1). Genetic diversity of mite populations was extremely high. For *P. sylviae*, we only found one mite haplotype shared by two host individuals, while for *T. bifurcata* we found one haplotype shared by three blackcaps and another shared by two. However, different mites shared the same haplotype in the infrapopulations sampled on the same host individual. Consequently, a simple AMOVA analysis revealed significant structure of *P. sylviae* and *T. bifurcata* populations among individual hosts, with around 20% and 50% of genetic variance being explained by differences among mite infrapopulations, respectively (Table 3.1). 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 population (Table 3.1). However, no genetic structure was detected between populations of mites sampled on different host groups (Table 3.1).

Table 3.1. Results of AMOVA for population genetic structure of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* in migratory and resident blackcap populations. The analyses partition total molecular variance into different components, whose significance was obtained by randomization after 1000 permutations.

	P. sylviae				***T. bifurcata***			
Population structure tested	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
Within infrapopulations	69	6.184	81.21		46	2.400	51.71	
Between host populations:								
Between host types	1	-0.011	0	0.421	1	-0.137	0	0.663
Among infrapopulations	22	1.437	18.88	< 0.001	10	2.296	50.36	< 0.001
Within infrapopulations	69	6.184	81.27		46	2.400	52.64	

The haplotype networks supported the idea that both *P. sylviae* and *T. bifurcata* populations were unstructured between migratory and resident blackcaps (Figure 3.2).

The distribution of average within-host pairwise genetic distances among feather mites revealed that blackcaps harboured mites with intermediate genetic distances as a rule (Figure 3.3). Only one blackcap had all sampled mites of one species (*T. bifurcata*) sharing the same haplotype.

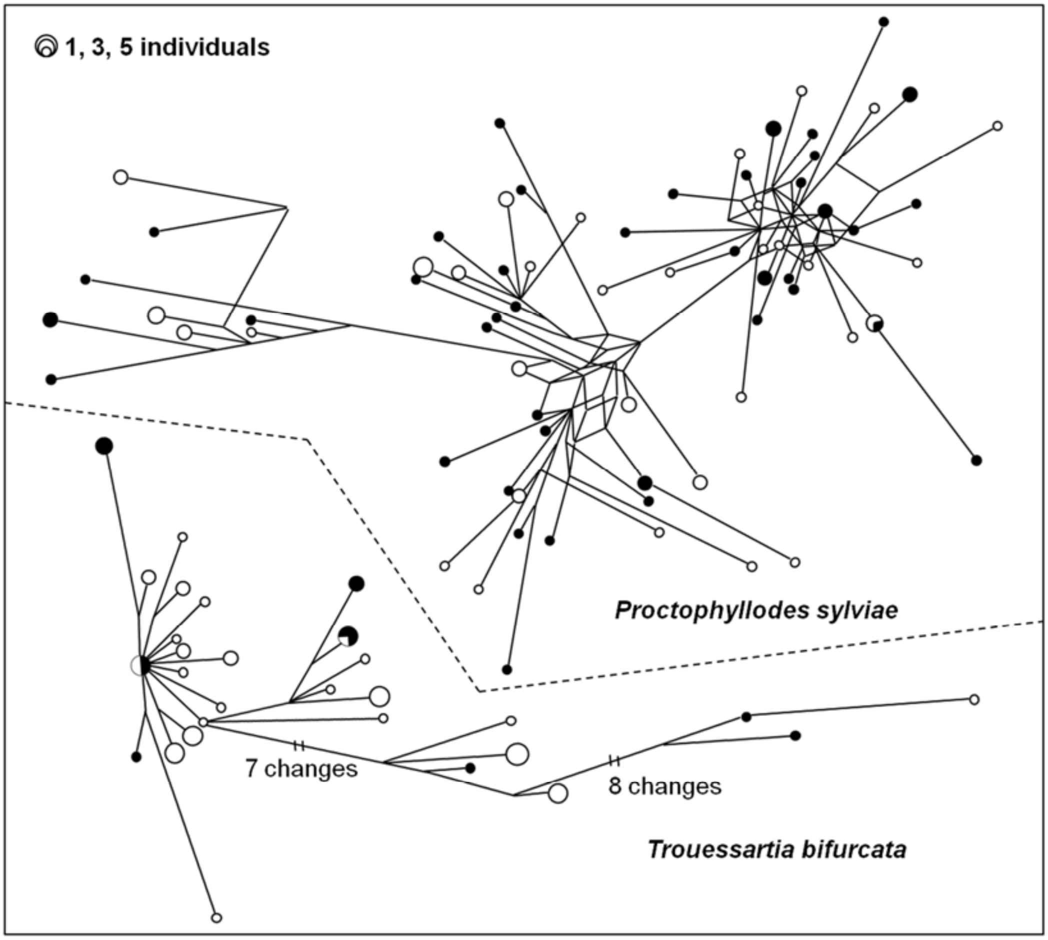


Figure 3.2. Haplotype networks for the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from wintering blackcaps. Colours in the circles indicate resident (white) or migratory (black) blackcaps. The shortest link between haplotypes sets the scale for 1 bp sequence difference, and the size of circles is proportional to haplotype frequency.

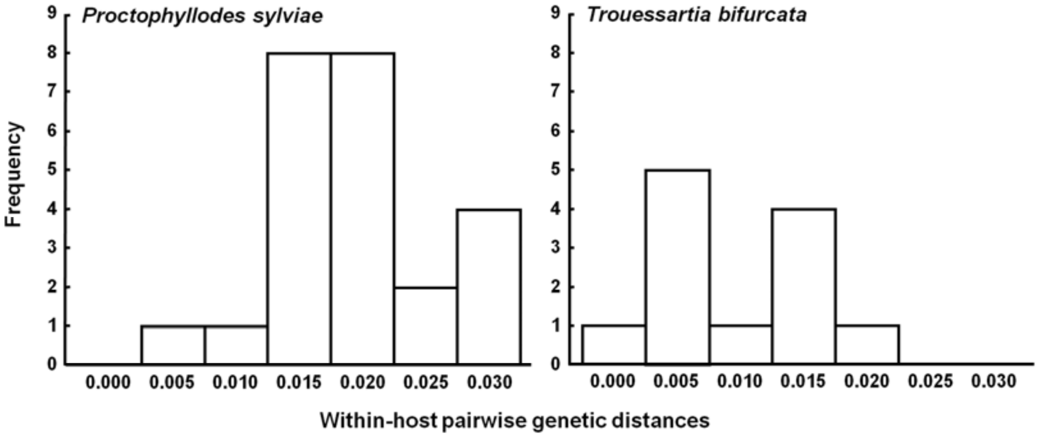


Figure 3. 3. Frequency distribution of average within-host pairwise genetic distances among haplotypes of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from blackcaps.

Discussion

Our results reveal high genetic diversity and weak genetic structure among infrapopulations of two feather mite species in the same avian host population, despite the fact that the two species have a different distribution among host types (migratory or resident), and the two host types have different population histories and phenotypic attributes (Pérez-Tris *et al.* 2004, Fernández-González *et al.* 2013).

According to morphological identifications, all the mites analysed in our study belonged to the species *P. sylviae* or *T. bifurcata*. In addition, our phylogenetic analyses placed all haplotypes retrieved from conspecific mites in a well-supported monophyletic group within the lineage diversity known for each genus. This result supports our identifications using microscopy, and rules out the possibility that our samples contained a mix of mite species. Moreover, the genetic differences observed among haplotypes within each mite species were much smaller than the differences observed between other species that have been sequenced. In addition, the internal clades within each species were randomly distributed among host individuals and host types, showing no structure beyond that expected from haplotypes shared between mites of the same host (which is explained by close relatives having been often sampled on each bird host). Therefore, we can safely assume that *P. sylviae* and *T. bifurcata* of blackcaps, as identified by microscopy, are two mite species that show no evidence of significant intraspecific subdivision.

In the two mite species investigated, some individuals sampled on the same host shared the COI haplotype. However, we generally found great genetic diversity of mite infrapopulations, despite the fact that our reduced sample size (five mites per host) somewhat limits our capacity to detect many different genotypes in the same infrapopulation. This result clearly supports the view that feather mite infrapopulations are built by the combination of successful immigration of unrelated mites and within-host reproduction of colonizing mites (Nadler 1995). The high genetic diversity of mite infrapopulations revealed in our study is incompatible with the existence of severe bottlenecks during mite transmission. This result, together with the absence of genetic structure among host types, rules out the possibility that dispersal of mite genetic lineages among hosts may be limited.

Obligate symbionts may reduce inbreeding by means of dispersal and avoidance of mating with relatives (Thornhill 1993, Futuyma 2005). According to our results, mite populations found on the same host are most likely built through multi-founder events, which may promote exogamy. The blackcap shows no social behaviour outside the breeding season, which means that transmission from both parents to their offspring, and transmission within host breeding pairs, may provide enough opportunities for mites to keep up the genetic diversity of their infrapopulations. Nevertheless, the possibility that transmission outside the hosts' breeding season is also relevant for non-social species such as the blackcap is open by our observation of a complete absence of genetic structure among mite populations sampled on migratory and resident blackcaps. While the exact patterns of mite transmission remain to be investigated, our results already reveal a scenario in which genetic lineages are frequently reshuffled in feather mite infrapopulations, a process which may have erased any sign of mite population subdivision, despite the fact that their hosts have different evolutionary history, geographic origins and ecological attributes (Pérez-Tris & Tellería 2002a, Pérez-Tris *et al.* 2004, Fernández-González *et al.* 2013).

In a scenario of free interchange of mites among hosts, mites with different ancestry will frequently end up sharing a host, which may have important implications in intra-host mite interactions. Previous research has revealed a non-random distribution of feather mites among wing feathers in blackcaps (Jovani & Serrano 2004), and even among sectors of the same feather in other birds (Mestre *et al.* 2011), suggesting that some areas of the bird plumage may be preferred and others may be avoided by different species (Proctor 2003). If competition determines the distribution of mites among host microhabitats of different quality, our results suggest a scenario in which mites may fail 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 they freely mix among host microhabitats, remains an open question for future research.

In our study, genetic structure of mite populations was solely associated with some mites of the same host frequently sharing haplotypes, but no structure was revealed among populations infesting resident and migratory blackcaps. It is important to recall that the resident blackcaps sampled in this study represent a distinct population in the host's range, which shows evidence of isolation from other populations (Pérez-Tris *et al.* 2004). Therefore, the absence of genetic structure among mite populations infecting migratory and resident blackcaps wintering in sympatry may have two explanations. First, mites may attain greater gene flow through the host's range than from the hosts themselves, thanks to a larger population size and a shorter generation time (Avise 2000). Second, dispersal may have erased the genetic footprint of putative periods of mite population isolation in glacial refugia, though it remains visible in host populations (Pérez-Tris *et al.* 2004). Alternatively, the coexistence of blackcaps from different geographic origins in sympatric wintering areas could promote the interchange of mites among hosts that hardly ever interbreed (because blackcaps are highly philopatric; Mason 1995, Bearhop *et al.* 2005). This interpretation involves winter transmission of blackcap feather mites taking place in some instances, although it does not need to be a common occurrence; in fact, a few dispersal events each winter might represent a number of migrant mites per generation large enough to erase any structure associated to host population. After all, the population density of blackcaps in wintering grounds is very high (sometimes reaching >200 individuals/10 ha; Tellería *et al.* 2008), and the fact that coexisting mites may compete for space within the host (Fernández-González *et al.* 2013) might promote dispersal when there is a chance to find a new host (Poulin 2007).

Because feather mites are obligate symbionts, their populations are subjected to environmental changes associated with host phenotypic diversity and host habitat use (Proctor 2003). For blackcap mites, our results suggest a scenario in which a mite that lives on a resident (which has constant habitat, long life expectancy, and large production of uropygial oil secretions; Pérez-Tris & Tellería 2002a, 2002b, Fernández-González *et al.* 2013) may sometimes have its offspring living on a migratory host (which will migrate seasonally between different habitats and undergo profound physiological changes, Kullberg *et al.* 1996, Berthold 2001). In principle, high genetic diversity may facilitate persistence of mite populations in the face of frequent habitat change (Keller & Waller 2002), since subdivided populations are more vulnerable to the extinction of particular genetic variants in stochastic environments (Barrett *et al.* 2008). Interestingly, we found stronger genetic structure of infrapopulations in the most host specialist of the two mite species investigated. Thus, variation among infrapopulations explained twice as much genetic variance in the population of *T. bifurcata*, which is abundant on resident hosts alone. *T. bifurcata* was also less genetically diverse than *P. sylviae*, which is found on migratory and resident blackcaps alike (Fernández-González *et al.* 2013). Nevertheless, the different patterns of genetic structure observed in these two mite species on the same host population may be explained by different breeding systems and dispersal capabilities (Johnson *et al.* 2002, Barrett *et al.* 2008, Toon & Hughes 2008). In turn, whether the amount of genetic variation observed in different mite species may be associated with their degree of host specialization may only be answered with broader comparative analyses.

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References

Álvarez, N., McKey, D., Kjellberg, F. & Hossaert-McKey, M. 2010. *Phylogeography and historical biogeography of obligate specific mutualisms*. In: Morand, S. & Krasnov, B. R. (eds.). The biogeography of host-parasite interactions. Oxford University Press, Oxford: 31-39.

Avise J. C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge.

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 in Ecology and Evolution*, 23: 678-685.

Bearhop, S., Fiedler, W., Furness, R. W., Votier, S. C., Waldron, S., Newton, J., Bowen, G. J., Berthold, P. & Farnsworth, K. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science*, 310: 502-504.

Berthold, P. 2001. *Bird migration: a general survey*. Oxford Ornithology Series, Oxford University Press, Oxford.

Blanco, G. & Frías, O. 2001. Symbiotic feather mites synchronize dispersal and population growth with host sociality and migratory disposition. *Ecography*, 24: 113-120.

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

Dabert, J., Ehrnsberger, R. & 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.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9: 772.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 2007. Testing the validity of discriminant function analyses based on bird morphology: the case of migratory and sedentary blackcaps *Sylvia atricapilla* wintering in southern Iberia. *Ardeola*, 54: 81-91.

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

Emlen, S. T. 1995. An evolutionary theory of the family. *Proceedings of the National Academy of Sciences of the United States of America*, 92: 8092-8099.

Excoffier, L. & Lischer, H. E. L. 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. & Pérez-Tris, J. 2013. Divergent host phenotypes create opportunities and constraints on the distribution of two wing-dwelling feather mites. *Oikos*, 122: 1227-1237.

Figuerola, J. 2000. Ecological correlates of feather mite prevalence in passerines. *Journal of Avian Biology*, 31: 489-494.

Fretwell, S. D. & Lucas, H. L., Jr. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Part I: theoretical development. *Acta Biotheoretica*, 19: 16-36.

Futuyma, D. J. 2005. *Evolution*. Sinauer Associates, Inc., Sunderland.

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52: 696-704.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95-98.

Hartl, D. L. & Clark, A. G. 2007. *Principles of population genetics*. Sinauer Associates, Inc., Sunderland.

Hedrick, P. W. 2000. *Genetics of populations*. Jones and Bartlett Publishers, Sudbury.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, 10: 537-549.

Huyse, T., Poulin, R. & Théron, A. 2005. Speciation in parasites: a population genetics approach. *Trends in Parasitology*, 21: 469-475.

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). *Molecular Ecology*, 11: 25-38.

Jovani, R. & 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.

Keller L. F. & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17: 230-241.

Kullberg, C., Fransson, T. & Jakobsson, S. 1996. Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society of London B*, 263: 1671-1675.

Mason, C. F. 1995. *The blackcap*. Hamlyn Species Guides, London.

McCoy, K. D., Boulinier, T., Tirard, C. & Michalakis, Y. 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. *Evolution*, 57: 288-296.

Mestre, A., Mesquita-Joanes, F., Proctor, H. & Monrós, J. S. 2011. Different scales of spatial segregation of two species of feather mites on the wings of a passerine bird. *Journal of Parasitology*, 97: 237-244.

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

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. *Journal of Parasitology*, 81: 395-403.

Nosil, P., Crespi, B. J. & Sandoval, C. P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, 417: 440-443.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pérez-Tris, J. & Tellería, J. L. 2002a. 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. & Tellería, J. L. 2002b. Regional variation in seasonality affects migratory behaviour and life-history traits of two Mediterranean passerines. *Acta Oecologica*, 23: 13-21.

Pérez-Tris, J. & Bensch, S. 2005. Dispersal increases local transmission of avian malarial parasites. *Ecology Letters*, 8: 838-845.

Pérez-Tris, J., Carbonell, R. & Tellería, J. L. 1999. A method for differentiating between sedentary and migratory Blackcaps *Sylvia atricapilla* in wintering areas of southern Iberia. *Bird Study*, 46: 299-304.

Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A. J. & Tellería, J. L. 2004. Historical diversification of migration patterns in a passerine bird. *Evolution*, 58: 1819-1832.

Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, Princeton.

Price, P. W. 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton.

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

Rigaud, T., Perrot-Minnot, M-J. & Brown, M. J. F. 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*, 277: 3693-3702.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 539-542.

Svensson, L. 1992. *Identification guide to European passerines*. L. Svensson, Stockholm.

Tellería, J. L., Ramírez, A. & Pérez-Tris, J. 2008. Fruit tracking between sites and years by birds in Mediterranean wintering grounds. *Ecography*, 31: 381-388.

Thornhill, N. W. (ed.). 1993. *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*. University of Chicago Press, Chicago.

Toon, A. & Hughes, J. M. 2008. Are lice good proxies for host history? A comparative analysis of the Australian magpie, *Gymnorhina tibicen*, and two species of feather louse. *Heredity*, 101: 127-135.

Whiteman, N. K. & Parker, P. G. 2005. Using parasites to infer host population history: a new rationale for parasite conservation. *Animal Conservation*, 8: 175-181.

Supplementary material

Table S3.1. Morphological identification of *Proctophyllodes* and *Trouessartia* species sequenced and the bird host species on which they were collected. Host ID and phenotype (1 = migratory, 2 = sedentary) are presented in the case of *Sylvia atricapilla*. The designation of the different haplotypes and their accession numbers are also given.

Mite species	Host species	Blackcap ID	Blackcap phenotype	Haplotype	Genbank reference
P. sylviae	*S. atricapilla*	1	1	PROCTO_001	KF613605
P. sylviae	*S. atricapilla*	1	1	PROCTO_002	KF613606
P. sylviae	*S. atricapilla*	1	1	PROCTO_003	KF613607
P. sylviae	*S. atricapilla*	1	1	PROCTO_004	KF613608
P. sylviae	*S. atricapilla*	2	1	PROCTO_005	KF613609
P. sylviae	*S. atricapilla*	2	1	PROCTO_006	KF613610
P. sylviae	*S. atricapilla*	2	1	PROCTO_007	KF613611
P. sylviae	*S. atricapilla*	2	1	PROCTO_008	KF613612
P. sylviae	*S. atricapilla*	2	1	PROCTO_009	KF613613
P. sylviae	*S. atricapilla*	3	1	PROCTO_010	KF613614
P. sylviae	*S. atricapilla*	3	1	PROCTO_011	KF613615
P. sylviae	*S. atricapilla*	3	1	PROCTO_012	KF613616
P. sylviae	*S. atricapilla*	4	1	PROCTO_013	KF613617
P. sylviae	*S. atricapilla*	4	1	PROCTO_014	KF613618
P. sylviae	*S. atricapilla*	4	1	PROCTO_015	KF613619
P. sylviae	*S. atricapilla*	4	1	PROCTO_016	KF613620
P. sylviae	*S. atricapilla*	5	2	PROCTO_017	KF613621
P. sylviae	*S. atricapilla*	5	2	PROCTO_018	KF613622
P. sylviae	*S. atricapilla*	5	2	PROCTO_019	KF613623
P. sylviae	*S. atricapilla*	5	2	PROCTO_020	KF613624
P. sylviae	*S. atricapilla*	6	2	PROCTO_021	KF613625
P. sylviae	*S. atricapilla*	6	2	PROCTO_022	KF613626
P. sylviae	*S. atricapilla*	6	2	PROCTO_023	KF613627
P. sylviae	*S. atricapilla*	7	2	PROCTO_024	KF613628
P. sylviae	*S. atricapilla*	7	2	PROCTO_025	KF613629
P. sylviae	*S. atricapilla*	8	1	PROCTO_026	KF613630
P. sylviae	*S. atricapilla*	8	1	PROCTO_027	KF613631
P. sylviae	*S. atricapilla*	8	1	PROCTO_028	KF613632
P. sylviae	*S. atricapilla*	9	2	PROCTO_029	KF613633
P. sylviae	*S. atricapilla*	9	2	PROCTO_030	KF613634
P. sylviae	*S. atricapilla*	9	2	PROCTO_031	KF613635
P. sylviae	*S. atricapilla*	10	2	PROCTO_032	KF613636
P. sylviae	*S. atricapilla*	10	2	PROCTO_033	KF613637
P. sylviae	*S. atricapilla*	11	1	PROCTO_034	KF613638
P. sylviae	*S. atricapilla*	11	1	PROCTO_035	KF613639
P. sylviae	*S. atricapilla*	11	1	PROCTO_036	KF613640

Mite species	Host species	Blackcap ID	Blackcap phenotype	Haplotype	Genbank reference
P. sylviae	*S. atricapilla*	12	2	PROCTO_038	KF613641
P. sylviae	*S. atricapilla*	12	2	PROCTO_039	KF613642
P. sylviae	*S. atricapilla*	13	1	PROCTO_040	KF613643
P. sylviae	*S. atricapilla*	13	1	PROCTO_041	KF613644
P. sylviae	*S. atricapilla*	13	1	PROCTO_042	KF613645
P. sylviae	*S. atricapilla*	14	2	PROCTO_043	KF613646
P. sylviae	*S. atricapilla*	14	2	PROCTO_044	KF613647
P. sylviae	*S. atricapilla*	15	1	PROCTO_045	KF613648
P. sylviae	*S. atricapilla*	15	1	PROCTO_046	KF613649
P. sylviae	*S. atricapilla*	15	1	PROCTO_047	KF613650
P. sylviae	*S. atricapilla*	15	1	PROCTO_048	KF613651
P. sylviae	*S. atricapilla*	15	1	PROCTO_049	KF613652
P. sylviae	*S. atricapilla*	16	1	PROCTO_050	KF613653
P. sylviae	*S. atricapilla*	16	1	PROCTO_051	KF613654
P. sylviae	*S. atricapilla*	16	1	PROCTO_052	KF613655
P. sylviae	*S. atricapilla*	16	1	PROCTO_053	KF613656
P. sylviae	*S. atricapilla*	17	2	PROCTO_054	KF613657
P. sylviae	*S. atricapilla*	17	2	PROCTO_055	KF613658
P. sylviae	*S. atricapilla*	18	2	PROCTO_056	KF613659
P. sylviae	*S. atricapilla*	18	2	PROCTO_057	KF613660
P. sylviae	*S. atricapilla*	18	2	PROCTO_058	KF613661
P. sylviae	*S. atricapilla*	18	2	PROCTO_059	KF613662
P. sylviae	*S. atricapilla*	19	2	PROCTO_060	KF613663
P. sylviae	*S. atricapilla*	20	1	PROCTO_061	KF613664
P. sylviae	*S. atricapilla*	20	1	PROCTO_062	KF613665
P. sylviae	*S. atricapilla*	21	2	PROCTO_063	KF613666
P. sylviae	*S. atricapilla*	21	2	PROCTO_064	KF613667
P. sylviae	*S. atricapilla*	21	2	PROCTO_065	KF613668
P. sylviae	*S. atricapilla*	22	2	PROCTO_066	KF613669
P. sylviae	*S. atricapilla*	22	2	PROCTO_067	KF613670
P. sylviae	*S. atricapilla*	22	2	PROCTO_068	KF613671
P. sylviae	*S. atricapilla*	23	1	PROCTO_069	KF613672
P. sylviae	*S. atricapilla*	23	1	PROCTO_070	KF613673
P. sylviae	*S. atricapilla*	24	1	PROCTO_071	KF613674
P. sylviae	*S. atricapilla*	24	1	PROCTO_072	KF613675
P. sylviae	*S. atricapilla*	24	1	PROCTO_073	KF613676
P. pinnatus complex	*Carduelis cannabina*			F004	KF613677
P. clavatus complex	*Cettia cetti*			F007	KF613678
P. pinnatus complex	*Emberiza cirlus*			F010	KF613679
P. cf. *clavatus*	*S. borin*			F037	KF613680
P. cf. *clavatus*	*S. borin*			F039	KF613681
P. cf. *clavatus*	*S. borin*			F043	KF613682
P. cf. *clavatus*	*S. borin*			F045	KF613683

Mite species	Host species	Blackcap ID	Blackcap phenotype	Haplotype	Genbank reference
T. bifurcata	*S. atricapilla*	5	2	TROUE_001	KF613684
T. bifurcata	*S. atricapilla*	5	2	TROUE_002	KF613685
T. bifurcata	*S. atricapilla*	7	2	TROUE_003	KF613686
T. bifurcata	*S. atricapilla*	9	2	TROUE_004	KF613687
T. bifurcata	*S. atricapilla*	9	2	TROUE_005	KF613688
T. bifurcata	*S. atricapilla*	6, 12, 25	2, 2, 1	TROUE_006	KF613689
T. bifurcata	*S. atricapilla*	6	2	TROUE_007	KF613690
T. bifurcata	*S. atricapilla*	6	2	TROUE_008	KF613691
T. bifurcata	*S. atricapilla*	6	2	TROUE_009	KF613692
T. bifurcata	*S. atricapilla*	10	2	TROUE_010	KF613693
T. bifurcata	*S. atricapilla*	10	2	TROUE_011	KF613694
T. bifurcata	*S. atricapilla*	12	2	TROUE_012	KF613695
T. bifurcata	*S. atricapilla*	12	2	TROUE_013	KF613696
T. bifurcata	*S. atricapilla*	12	2	TROUE_014	KF613697
T. bifurcata	*S. atricapilla*	12, 26	2, 1	TROUE_015	KF613698
T. bifurcata	*S. atricapilla*	18	2	TROUE_016	KF613699
T. bifurcata	*S. atricapilla*	18	2	TROUE_017	KF613700
T. bifurcata	*S. atricapilla*	14	2	TROUE_018	KF613701
T. bifurcata	*S. atricapilla*	14	2	TROUE_019	KF613702
T. bifurcata	*S. atricapilla*	14	2	TROUE_020	KF613703
T. bifurcata	*S. atricapilla*	14	2	TROUE_021	KF613704
T. bifurcata	*S. atricapilla*	25	1	TROUE_022	KF613705
T. bifurcata	*S. atricapilla*	25	1	TROUE_023	KF613706
T. bifurcata	*S. atricapilla*	26	1	TROUE_024	KF613707
T. bifurcata	*S. atricapilla*	27	1	TROUE_025	KF613708
T. bifurcata	*S. atricapilla*	27	1	TROUE_026	KF613709
T. bifurcata	*S. atricapilla*	27	1	TROUE_027	KF613710
T. bifurcata	*S. atricapilla*	19	2	TROUE_028	KF613711
T. bifurcata	*S. atricapilla*	19	2	TROUE_029	KF613712
T. incisa	*Turdus merula*			T001	KF613713
Trouessartia sp.	*Erithacus rubecula*			T002	KF613714
T. cf. simillima	*Muscicapa striata*			T003	KF613715
T. inexpectata	*S. melanocephala*			T005	KF613716

Chapter 4

This chapter is based on the manuscript:

Fernández-González, S., Pérez-Rodríguez, A., De la Hera, I. & Pérez-Tris, J. Environmental heterogeneity favours different obligate symbiotic mites across their common host's range: implications for the evolution of symbiont diversity. *In preparation.*

Environmental heterogeneity favours different obligate symbiotic mites across their common host's range: implications for the evolution of symbiont diversity

Sofía Fernández-González, Antón Pérez-Rodríguez, Iván de la Hera and Javier Pérez-Tris

The geographic distribution of obligate symbionts is restricted to areas where their host species is present. However, not all symbionts will thrive in the habitat occupied by the host. For example, symbionts sharing the same host may have different ecological requirements, or a different tolerance to environmental conditions. If dissimilar symbiont distributions make symbiont assemblages vary locally across the host species' range, the environmental constraints affecting symbiont distributions would play a relevant role in shaping the geographic mosaic of host-symbiont coevolution. We analysed the distribution of two feather-dwelling mite species (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) among 37 Iberian populations of the European blackcap *Sylvia atricapilla*. We used PLS regression models to assess which among 48 environmental variables (including climate, landscape physiognomy, or host migratory behaviour) best explained the geographic distribution of prevalence, intensity of infestation and abundance of each mite species. The distributions of both mite species were primarily dependent on climatic variables: the probability of their occurrence and their abundance decreased in the driest sectors of the Iberian Peninsula. However, prevalence or within-host numbers of the two mites were largely uncorrelated across blackcap populations, revealing that sites that were favourable for one species were not necessarily favourable for the other. Differences in the amount of variance explained in the models provided evidence that the two mite species exhibit different degrees of specialization in relation to variation in host environment. Our results reveal that geographic variation in host environment represents a different set-up of opportunities and constraints for different symbiont species, even if these share most elements of their life-style. This idea may help us to understand the ecological factors that promote and maintain symbiont diversity within a host species.

Introduction

Understanding which processes underlie the distribution of biodiversity has been a central issue in ecology. Species' ranges, and the patters of variation in abundance within them, are shaped by the interplay among inherent species properties (such as body size, dispersal capability or metabolic rate; Blackburn & Gaston 2001, López-Sepulcre & Kokko 2005, Krasnov & Poulin 2010), and local biotic and abiotic conditions that determine where each species can thrive (Rosenzweig 1981, Morris 1987, Newton 1998).

For many species, important factors limiting geographical distributions may be evident. For example, obligate symbionts (mutualists, commensals or parasites) depend on the presence of their hosts to exist (Gray *et al.* 1992, Proctor 2003, Giorgi *et al.* 2004). However, although the presence of the host is a necessity for symbionts to thrive in a given habitat, by no means is it sufficient. The probability of presence or prevalence (the proportion of host individuals harbouring the symbiont in a host population) of a symbiont, may vary considerably across the host’s range. Moreover, within a host population, symbionts may be unevenly distributed among hosts (few hosts harbouring the majority of symbionts; Wilson *et al.* 2002, Poulin 2007). Geographic variation in prevalence and within-host abundance of symbionts are important components of symbiont distributions, which depend on many factors affecting symbiont local establishment, transmission success, and on-host population growth (Fox & Morrow 1981). Such processes may greatly depend on local environmental factors affecting both host and symbiont populations, which therefore may shape the assembly of symbiont communities across host geographical ranges.

Many environmental factors also play a role in shaping symbiont distributions (Pietrock & Marcogliese 2003). However, such factors may differently affect symbionts exploiting the same host species. For example, ectosymbionts are likely to be more vulnerable than endosymbionts to ambient temperature and humidity (Krasnov & Poulin 2010), and therefore these variables have been shown to affect a wide array of ectosymbionts of vertebrates, such as feather-dwelling lice and mites, ticks or fleas (Rudolph 1983, Gaede & Knülle 1987, Wiles *et al.* 2000, Moyer *et al.* 2002, Randolph 2004, Benoit & Denlinger 2010). Together with host habitat, host population attributes may further enrich the range of host environments faced by symbionts; for instance, host migration may involve variation in seasonal availability, morphology or physiology of the host (Berthold 1975), all of which may affect symbiont colonization success.

However, it remains unclear whether ecologically very similar symbionts (those of the same taxonomical group that share host-species, life-style, host-to-host transmission mechanisms, etc.) may be differently affected by the host environment. In principle, the ecological niches of such species should differ in some way to make their coexistence possible (Hutchinson 1957, Schoener 1974, Chase & Leibold 2003, Schoener 2009). In many cases, niche differences are evidenced by symbionts exploiting different microhabitats within the host (Poulin 2007). But given that symbionts also depend on the host environment, local conditions could favour different symbiont species in different environments (Malenke *et al.* 2011), a possibility which remains largely unexplored. Filling this gap in our knowledge is important, because variable symbiont distributions associated with changing host environments may be evidence of symbiont niche specialization (beyond within-host microhabitat choice; (Brown 1995, Peterson *et al.* 1999), a process which may promote and maintain symbiont diversity within the same host species (Schluter 2000).

Here we investigate which environmental factors determine heterogeneity in the geographic distribution of two feather-dwelling mite species (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) within the range of their common host species (the European blackcap *Sylvia atricapilla*) in the Iberian Peninsula. Iberian blackcaps include migratory and resident populations, and are broadly distributed across different regions, occupying a wide variety of habitats (Carbonell 2003, Tellería *et al.* 2001). This allows for studying feather mite distributions in a diverse, yet geographically restricted area. By studying a single host species, we aim to analyse variation in feather mite distributions due to differences in host environment, by removing the confusion attributable to host identity (Krasnov *et al.* 2008). According to previous studies, *P. sylviae* and *T. bifurcata* are the two most common feather mite species found on blackcaps. Both mite species live on large wing feathers; but while *P. sylviae* occupies the ventral part of the wing, *T. bifurcata* settles on its dorsal side (Proctor 2003, Fernández-González *et al.* 2013). Furthermore, *P. sylviae* is more abundant on migratory blackcaps while *T. bifurcata* more commonly occupies the feathers of resident blackcaps in wintering areas where both host types occur in sympatry (Fernández-González *et al.* 2013). Such distribution differences between host types could be due to differences in characteristics of migratory *vs.* resident blackcaps, or to the different environments exploited by each type of host during its life.

We specifically tested which environmental factors best explain variation in prevalence and numbers of *P. sylviae* and *T. bifurcata* among Iberian blackcap populations, and if such factors are the same (or the magnitude and direction of their influences are similar) for both mite species. In the Iberian Peninsula, summer drought may be a major limiting factor for the distribution of feather mites through its impact on their water balance. Feather mites obtain water directly from the atmosphere (Gaede & Knülle 1987), so we expect high temperatures to have a negative impact on feather mite growth, through decreasing available atmospheric water vapour and increasing mite exposure to water loss. Conversely, precipitation should favour water balance of mites by increasing humidity of the environment. However, mite distributions may be shaped by a wide array of environmental influences other than climate. These may include local effects of landscape features or land uses (which may affect local microclimate or host body condition), or population attributes that may affect host physiology or habitat choice (*e.g.*, host migratory behaviour; Fernández-González *et al.* 2013). We therefore hypothesize that broad-scale patterns of variation in climate on the one hand, and host population attributes (migration pattern) on the other hand, most influence the distribution (prevalence and abundance) of *P. sylviae* and *T. bifurcata* among Iberian blackcap populations. Yet the most important question we set to answer is whether these two ecologically similar species of obligate symbionts show different patterns of distribution, faced with heterogeneity of host environments within the range of their common host species. A positive answer to that question would expand the role of host environment in shaping the geographic mosaic of host-symbiont coevolution (Thompson 2005) by creating geographical variation in the opportunities and limitations for different symbionts to colonize the same host species.

Materials and methods

Study area and field methods

We sampled blackcaps in 37 localities that were selected to cover the range of environmental conditions in which the species may be found in the Iberian Peninsula (Carbonell 2003; Figure S4.1, Table S4.1). Our field work took place from 2008 to

2011, between mid July and early August to guarantee sampling local birds before migrant birds from other populations could be accidentally captured. We captured 875 blackcaps in total (mean n = 24 per site, range 11-43), using mist nets and tape-luring birds to increase capture rate. Each bird was kept in a cloth bag until manipulation. We sexed and aged blackcaps based on plumage characteristics (Svensson 1992), and measured wing length, tail length and distances from the wing tip to the primary feathers 1 and 9 (primary distances P1 and P9; all measurements to the nearest 0.5 mm).

Feather mites were counted by spreading the bird's wings against light. Mite species were differentiated based on *T. bifurcata* mites being larger, more rounded and occupying the dorsal side of the feather vanes, whereas *P. sylviae* were smaller, elongated and dwell on the ventral side of feathers (Atyeo & Braasch 1966, Santana 1976). We checked both wings in order to better assess mite prevalence, and counted mites on the wing these were first located. A sample of feather mites from several feathers of the wing was collected and stored in absolute ethanol at ambient temperature during fieldwork and then at -80 ºC until identification. We only found *P. sylviae* and *T. bifurcata* in our samples, which supports previous research that sets them as the most common mite species on blackcaps (Atyeo & Braasch 1966, Santana 1976, Fernández-González *et al.* 2013). Once processed, blackcaps were fitted an aluminium ring to avoid sampling the same bird repeatedly, and then released unharmed at the site of capture.

Characterization of sampling localities

We delimited an area of 10 Km^2 around each sampling location, which we characterized using different environmental variables. For each site, we scored mean, maximum and minimum monthly temperatures, and monthly rainfall. We also obtained 19 bioclimatic variables (BIOCLIM variables from WorldCLim; Hijmans *et al.* 2005), altitude and slope (MDE GETOPO30; Smith & Sandwell 1997), eight descriptors of land use (CORINE 2000 Land Cover, http://www.eea.europa.eu) and a proxy for levels of primary production (mean monthly NDVI index during the 1982-2000 period; Tucker *et al.* 2005). Monthly measures were combined in order to obtain both annual and breeding season (March to June) means. We also included latitude and longitude, and sampling year. All GIS analyses were done using ESRI® ArcMap™ 9.3 (2008). In all, we used 48 predictor variables, which are all listed in Table S4.2.

We determined the migratory behaviour of each blackcap population based on the morphology of birds captured at each locality. We used the length of the eighth primary feather, tail length and a wing-tip shape index (P9-P1) to classify blackcaps as migratory or resident according to a discriminant function (Pérez-Tris *et al.* 1999). This method successfully distinguishes migratory birds from northern Europe from Iberian residents, but it has a substantial error rate (around 10%) when applied to Iberian blackcap populations, whose morphological variation in relation to migration pattern is less pronounced (Pérez-Tris *et al.* 1999, De la Hera *et al.* 2007). Therefore, not all birds captured in each locality were classified in the same group, which is to be interpreted as the likely effect of classification errors. Although some populations might be partially migratory (a type of behaviour we cannot reliably identify with our data), we assigned single behaviours (migratory or resident) to each locality based on the proportion of birds scoring migratory-like or resident-like morphology. We thus determined 30 populations to be migratory and seven to be resident.

Statistical analyses

For each population, we computed prevalence of each mite species as the proportion of blackcaps harbouring that species. We distinguished between intensity of infestation (number of mites counted per infested host) and abundance of mites (number of mites per host, regardless of infestation status). We log-transformed our data to improve the fit to normality before computing averages. Note that mite abundance, as we define it in our study, is a composite variable that depends on the frequency of mite occurrence among hosts (prevalence) and the average size of in-host mite populations (intensity). Abundance and intensity of mites were computed as numbers per wing, and the mean of all individuals in a locality was used as the value for the population. We are aware that prevalence, intensity and abundance estimates may have different measurement error among localities as a consequence of variable sample size. We therefore computed bootstrap estimates of each variable (with 1000 replicates) for each locality, which we compared with our observations. Correlations between observed and bootstrapped estimates were significant and very high (in all cases, r > 0.99 and slope not significantly different from one), meaning that sampling bias did not affect our estimates.

We used Partial Least Squares Regression analysis (PLS hereafter) to test the importance of each independent variable as a predictor of prevalence, intensity and abundance of feather mites. PLS works extracting latent factors (linear combinations of predictor and dependent variables) that maximize explained variance in the dependent variable. The multidimensionality of the data is thus reduced into a small number of orthogonal factors, which account for consecutively smaller proportions of the original variance. Within each factor, predictor variables are assigned a sign (indicating the direction of effects) and a weight (representing the magnitude of effects). To perform the PLS analyses we used the NIPALS algorithm with seven-fold cross-validation implemented in STATISTICA 7.0 (StatSoft 2004). The relative contribution (and the statistical significance) of each predictor variable within each factor is given by the square of its predictor weight: the sum of all the square predictor weights equals 100% of the explained variance, so that any variable with a square weight greater than 1/k (where k is the number of predictor variables) significantly contributes to the factor. The R^2 of each factor can be decomposed among predictors, as they contribute to it proportionally to their square predictor weights.

For a study like ours, PLS has a number of advantages compared to other multiple regression techniques; most importantly, it allows for analyzing many predictor variables (even more than sample units), which may be highly correlated to one another (Carrascal *et al.* 2009). These properties make it possible to reliably identify partial variable contributions when the effects investigated are multidimensional (as it happens with environmental influences, which represent the joint effect of many correlated variables) and sample size is relatively small (as often happens in biogeographical studies, especially if localities have specifically been sampled for the purpose of the study; see Carrascal *et al*. 2009, Pérez-Rodríguez *et al.* 2013).

We transformed variables to meet statistical requirements (log-transformation for numerical data and arcsin-transformation for proportions). Because our predictor variables do not necessarily follow a linear relationship with dependent variables, we repeated our analyses including quadratic terms of all variables. However, the amount of variance explained remained very similar, and both linear and quadratic terms scored similar square weights. Therefore, we consider non-linear effects to be irrelevant compared to the corresponding linear effects, and only included the original variables, thereby simplifying the analyses and allowing for a straightforward interpretation of the effects they revealed.

Results

The prevalence of *P. sylviae* ranged from 33-100% (Figure 4.1, Table S4.1), while *T. bifurcata* reached lower prevalences (0-81%; within-subjects ANOVA for differences between mite species within localities: $F_{1,36} = 242.2$, $P < 0.001$). *T. bifurcata* mites were absent from 13 (35%) of the 37 study sites. The intensity of infestation also varied among populations, ranging from 6.9-65.5 for *P. sylviae* and 1.0-30.7 for *T. bifurcata* per infested bird (within-subjects ANOVA with sites where both mites were present: $F_{1,23} = 60.3$, $P < 0.001$). Mean abundance per site ranged from 1.0-65.4 for *P. sylviae* and from 0.0-13.3 for *T. bifurcata* per inspected host (within-subjects ANOVA: $F_{1,36} = 253.0$, $P < 0.001$). In general, prevalence, intensity and abundance of the two mites depicted different patterns of geographical variation: intensity and abundance of the two mites were uncorrelated among study sites, and prevalence of the two mites showed a weak correlation which was clearly due to the low prevalence of *P. sylviae* in various sites where *T. bifurcata* was absent, with no clear pattern of association elsewhere (Figure 4.2).

The distribution of *P. sylviae* among study sites was more difficult to model than the distribution of *T. bifurcata* based on the variables used in this study. Thus, the latent factors extracted for both prevalence and abundance of *P. sylviae* explained a lower amount of variance (R^2 = 28.3% and 29.2% respectively; Table 4.1) in comparison to those extracted for prevalence and abundance of *T. bifurcata* (R^2 = 68.2% and 68.6% respectively). A second latent factor was extracted for prevalence and abundance of *T. bifurcata*, but its contribution to explaining variance was negligible in both cases (R^2 = 0.05% and 0.03% respectively); therefore we did not further consider these factors. Geographic variation in the intensity of infestation was difficult to explain with the variables considered, for the two mite species alike (*P. sylviae*: $R^2 = 28.0$; *T. bifurcata*: $R^2 = 31.6$).

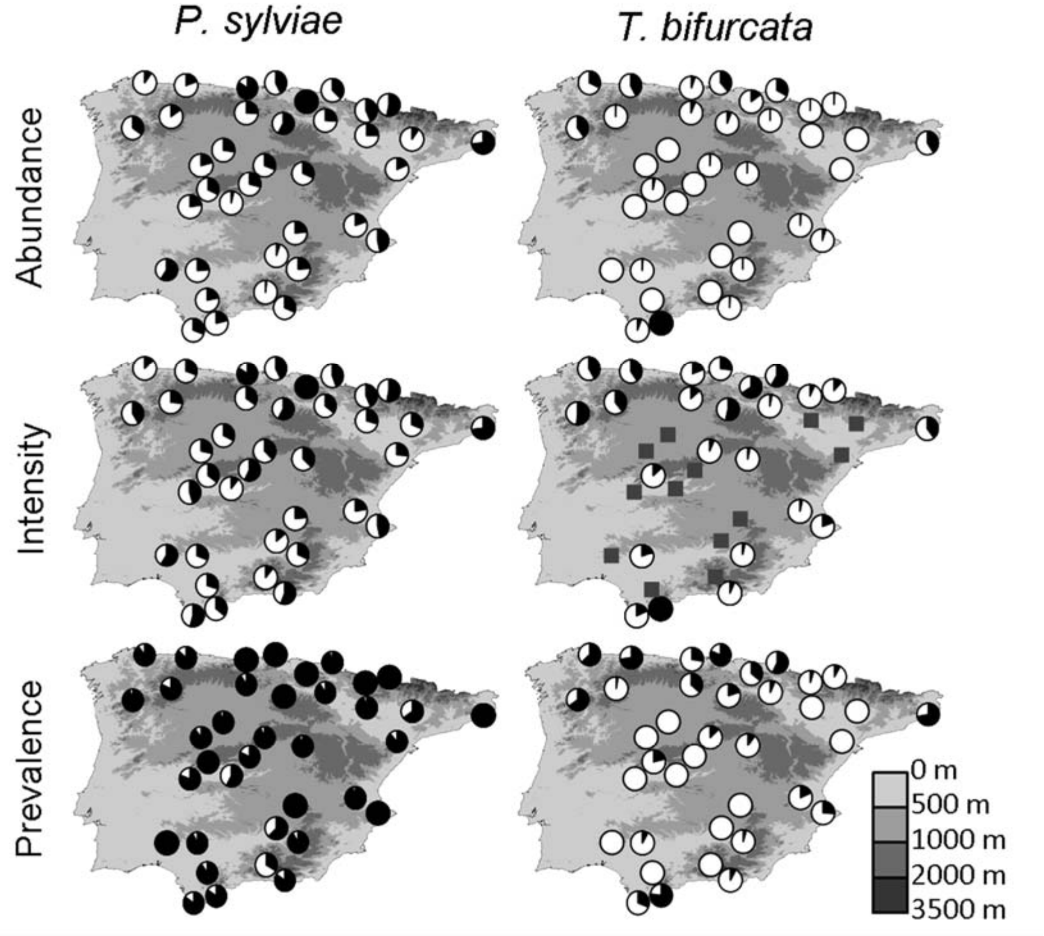

Figure 4.1. Abundance, intensity and prevalence of *Proctophyllodes sylviae* and *Trouessartia bifurcata*. The degree of filling of the circles represents the value of each variable in each site, expressed as the percentage of the maximum value observed across sites. Squares represent sites where the mite was absent, and therefore lacked data for intensity of infestation. Colour map represents altitude (metres above sea level).

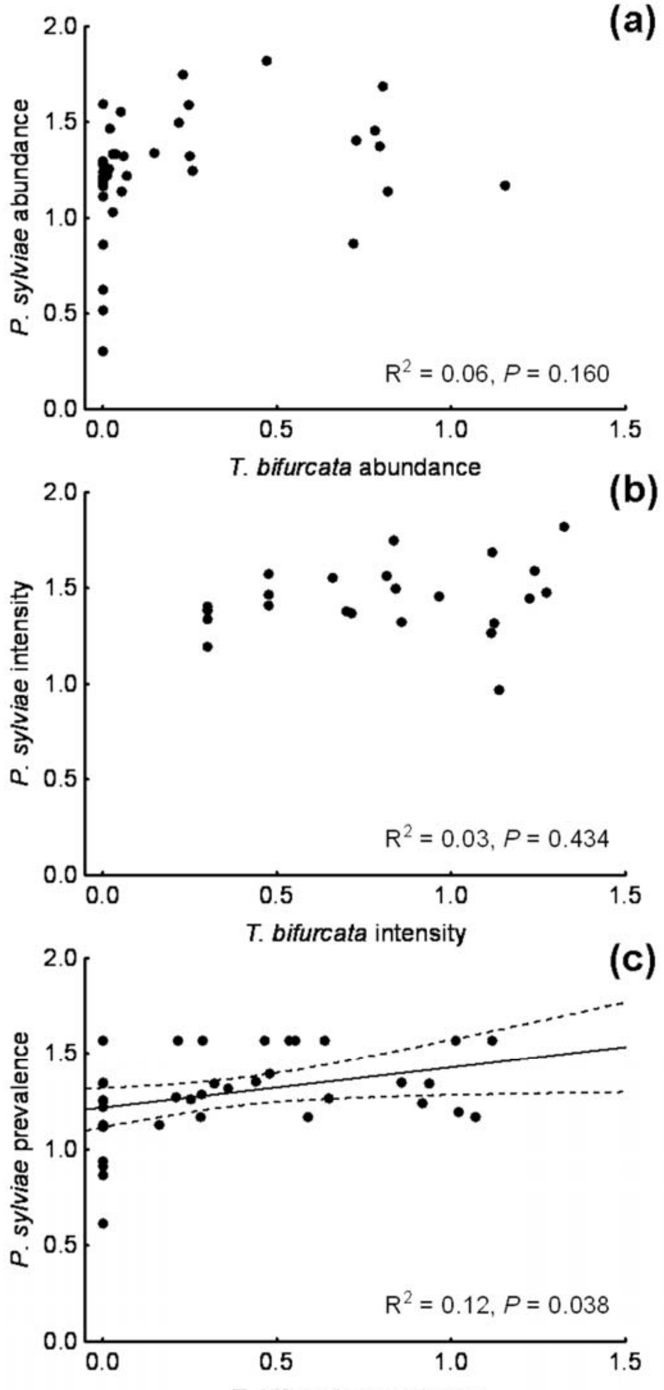

Figure 4. 2. Relationship between abundance (a), intensity (b) and prevalence (c) of *Proctophyllodes sylviae* and *Trouessartia bifurcata*.

Table 4.1. Results of the Partial Least Squares Regression analyses for prevalence, abundance and intensity of *Proctophyllodes sylviae* and *Trouessartia bifurcata*. Numbers represent the weight of each predictor variable that showed a significant effect on some of the dependent variables. Predictor variables have been divided into six different groups for the clarity of the reader. R^2 values of each model are also shown.

	Proctophyllodes sylviae			***Trouessartia bifurcata***		
Predictor variables (grouped by type):	**Prevalence**	**Abundance**	**Intensity**	**Prevalence**	**Abundance**	**Intensity**
Year						
2008						0.184
Geographical effects						
Latitude (Y)	0.198	0.165				
X·Y			0.202			
$X \cdot Y^2$						-0.167
X^3			0.145			
Y^3	0.184					
Temperature						
Annual mean temperature			-0.154			
Maximum annual mean temperature	-0.186	-0.206	-0.215			
Minimum annual mean temperture					0.148	
Breeding mean temperature		-0.155	-0.165			
Maximum breeding mean temperature	-0.201	-0.224	-0.234			
Maximum temperature of the warmest month	-0.257	-0.270	-0.258	-0.209	-0.172	
Minimum temperature of the coldest month				0.161	0.189	0.168
Temperature annual range	-0.195	-0.205	-0.184	-0.300	-0.287	-0.231
Temperature mean diurnal range	-0.209	-0.230	-0.223	-0.292	-0.281	-0.218
Temperature seasonality (SD of isothermality)	-0.152	-0.158		-0.294	-0.283	-0.238
Mean temperature of the warmest quarter	-0.207	-0.221	-0.219			
Mean temperature of the coldest quarter					0.172	

	Proctophyllodes sylviae			*Trouessartia bifurcata*		
Predictor variables (grouped by type):	**Prevalence**	**Abundance**	**Intensity**	**Prevalence**	**Abundance**	**Intensity**
Rainfall						
Annual rainfall	0.158	0.196	0.212	0.250	0.246	0.252
Breeding rainfall	0.153	0.186	0.208	0.207	0.200	0.195
Rainfall of the wettest month		0.160	0.179	0.263	0.267	0.267
Rainfall of the driest month	0.190	0.159				
Rainfall seasonality (coefficient of variation)	-0.226	-0.195	-0.163			
Rainfall of the wettest quarter			0.162	0.242	0.250	0.269
Rainfall of the driest quarter	0.231	0.197	0.159			
Rainfall of the warmest quarter	0.243	0.215	0.183	0.149		
Rainfall of the coldest quarter			0.145	0.208	0.218	0.247
Landscape features						
Altitude				-0.206	-0.209	-0.155
Mean annual NDVI				0.221	0.227	0.257
Mean breeding NDVI			0.173	0.212	0.221	0.257
Percentage of broadleaf forest			0.147			0.181
Percentage of shrubland	-0.214	-0.268	-0.245			
Percentage of open spaces		0.147	0.186	0.166		
Percentage of water bodies	0.168					-0.171
Behaviour						
Migration: Migration	0.200					
Migration: Sedentarism	-0.200					
R2 of the model						
(% variance explained)	**28.27**	**29.21**	**28.00**	**68.16**	**68.55**	**31.62**

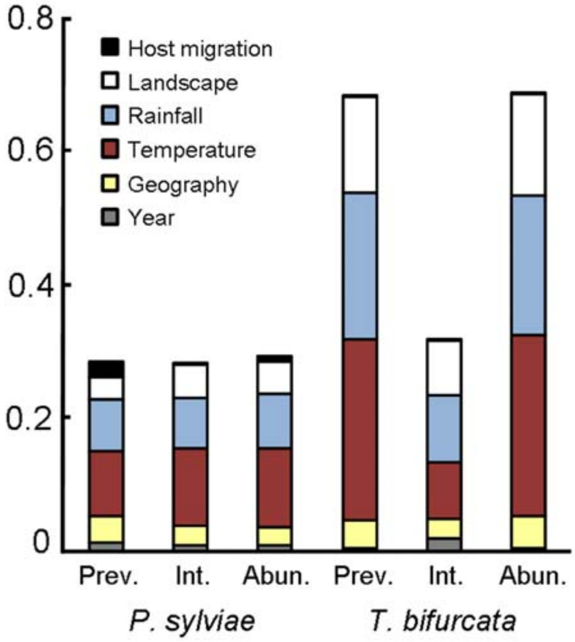

Figure 4.3. Percentage of partial contribution of each group of predictor variables to the total variance explained (R^2) in each model: Prevalence (Prev.), Intensity (Int.) and Abundance (Abun.) of *Proctophyllodes sylviae* and *Trouessartia bifurcata*.

The groups of variables that had the strongest effect in all models were related to temperature, followed by rainfall-related variables (Figure 4.3). In general, prevalence, intensity and abundance of both mite species increased where temperatures were low and showed little variation, either during the day or seasonally. In addition, they were positively correlated with variables describing localities that were moist around the year, even during the warmest months (Table 4.1). Landscape variables played a secondary role in explaining variation in mite occurrence and numbers, with no clear patterns being identified in the set of correlations defining the latent PLS factors. Finally, *P. sylviae* reached higher prevalence in migratory than in resident blackcap populations.

Discussion

Our results show that two mite species that share most elements of their life-style (including their main host species) differ from each other in prevalence, intensity and, in turn, abundance within host populations (for a similar result at a local scale, see Fernández-González *et al.* 2013). But most importantly, they also follow different patterns of geographical variation in the probability of their occurrence (prevalence) or the size of their populations within individual hosts (intensity of infestation). The distribution patterns of *P. sylviae* and *T. bifurcata* were primarily influenced by broad-scale patterns of climate variation, which were driven by differences among study sites in annual temperature and precipitation regimes. We also found an effect of host population attributes on mite distributions, as shown by an increased prevalence of *P. sylviae* in migratory blackcap populations. Remarkably, the distribution of *T. bifurcata* was more limited by variation in host environment than that of *P. sylviae*, thereby revealing differences between the two mite species in their degree of ecological specialization, this being seldom investigated in obligate symbionts (Poulin 2007). The fact that the distribution of feather mites may be influenced by elements of the host environment, as well as by host attributes (such as migratory behaviour), supports the idea that the presence of the host is necessary, yet insufficient for obligate symbionts to thrive, and help to expand our understanding of the realised niche of these organisms within their host species' ranges.

The different patterns of distribution of the two mite species depicted a complex geographic context of interactions between blackcaps and feather mites. In the first place, *P. sylviae* was always more abundant than *T. bifurcata*: *P. sylviae* occurred in all study sites (*T. bifurcata* was absent from 35% of localities), always scored higher prevalence, and reached higher numbers within infested hosts than *T. bifurcata*. These differences may be the outcome of inherent attributes of each mite species (such as body size, growth rate, or breeding investment), which may influence their prospects of being successfully transmitted among host individuals, and consequently their degree of ecological dominance (in terms of host monopolization) within host populations (Magurran & Henderson 2011). In addition, across the gradient of host environments that both mite species faced among Iberian blackcap populations, *P. sylviae* showed less variable prevalence, intensity or abundance than *T. bifurcata*, an observation which supports the idea that *P. sylviae* may be ecologically more versatile than *T. bifurcata* (Fernández-González *et al.* 2013). However, prevalence, intensity or abundance of the two mite species was largely uncorrelated across sites. In fact, *T. bifurcata* thrived in some localities where *P. sylviae* seemed not particularly favoured. As a consequence, the complex set up of environmental conditions of the Iberian Peninsula provided opportunities for each mite species in different regions (Pérez-Rodríguez *et al.* 2013). Such circumstances may be general to other bird species, and may contribute to maintaining the diversity of feather mites within the same host species.

Environmental determinants of feather mite distribution

Our results show that variation in prevalence, rather than intensity of infestation, shape the geographical patterns of distribution of feather mite abundance among blackcap populations. This result may be evidence that local environment has a strong influence on the ability of mites to colonize new host individuals, but it barely affects the capability of mite populations to grow up on infested hosts. Climatic variables, especially temperature and precipitation, accounted for the greatest proportion of explained variance of *P. sylviae* and *T. bifurcata* distributions, although geographical and landscape variables also had a significant influence (Pérez-Rodríguez *et al.* 2013). Both mite species decreased in overall abundance in areas where temperature was high and varied seasonally, while precipitation was low and concentrated outside the warmest months. These conditions describe areas where the summer drought typical of Mediterranean environments has its greatest impact, and create a gradient along which habitat suitability for feather mites decreases in the Iberian Peninsula from the Eurosiberian Atlantic belt towards the warmest sectors of the Mediterranean (Font 1983).

It may be argued that the conditions that mites face in dry Mediterranean areas negatively affect them because they are exposed to dehydration on the feathers they occupy (Proctor 2003). However, mites are known to live on birds from arid zones (*e.g.* Gaud & Mouchet 1958, Atyeo & Braasch 1966, Santana 1976, Manilla *et al.* 1994, Mironov & Kopij 2000, O'Connor *et al.* 2005), meaning that they may possess mechanisms to avoid dehydration. As an alternative explanation for our results, blackcaps inhabiting the driest sectors of the Iberian Peninsula score the lowest abundance and body condition among Iberian blackcap populations (Pérez-Tris & Tellería 2002, Carbonell *et al.* 2003). Birds in poor body condition may make poor hosts for feather mites (Blanco *et al.* 1997) if the capacity of individuals to produce uropygial oils is condition-dependent. This problem may be particularly important for *T. bifurcata*, which seems more dependent than *P. sylviae* on uropygial oil production (Fernández-González *et al.* 2013), an interpretation which also follows from the comparison of the two mite species in our study. If host abundance or body condition influences host quality for mites, then the distribution of feather mites may be driven by environmental factors affecting host attributes, thereby revealing new links between host population ecology and the biogeography of host-symbiont interactions. Finally, migration only seems to have an effect on *P. sylviae* prevalence, which decreased in residents compared to migratory blackcaps, although these mites are still present in all localities as opposed to *T. bifurcata*. For this reason, this may support that, at least at this level, host features other than migration might be shaping variation in prevalence and mite numbers.

Environmental variation, habitat specialization, and the diversity of feather mites

Environmental variables explained nearly twice as much variation in mite prevalence and abundance for *T. bifurcata* than for *P. sylviae*. In fact, although dry Iberian areas could be viewed as poor habitat for both mite species, *P. sylviae* showed little variation in abundance among Iberian blackcap populations compared to *T. bifurcata*. This may be evidence that *P. sylviae* is a habitat generalist species, while *T. bifurcata* is more specialized (see Evangelista *et al.* 2008, Pérez-Rodríguez *et al.* 2013 for similar examples in other taxa). These results support the idea that the two species face different environmental constraints in the habitat occupied by their common host (Malenke *et al.* 2011). Previous studies have suggested that *P. sylviae* may be more easily dispersed than *T. bifurcata* among individual hosts within blackcap populations (Fernández-González *et al.* 2013), and accessibility to new hosts may be an advantage in terms of monopolization of a shared resource (the host population). This idea is supported by our analysis of the distribution of the two mite species at a broad geographic scale. From that perspective, the apparent habitat specialization of *T. bifurcata* (which thrives in moist Iberian areas) may be the unavoidable consequence of restrictions imposed by the host environment on mite dispersal among hosts. This fact may be due to *T. bifurcata* failing to establish on poor quality hosts (if blackcaps living in dry environments are in poor body condition and produce little uropygial oil; Fernández-González *et al.* 2013), to reduced blackcap abundance in dry Iberian habitats (constraining dispersal success of the mite species with the lowest dispersal capacity), or simply to physiological mechanisms of *T. bifurcata* not being adapted to face strong humidity restrictions.

The results described above immediately suggest that *P. sylviae* is favoured over *T. bifurcata* in most host environments, which drives us to an important question: what mechanisms allow the coexistence of both mite species in the same host species? The key for understanding the coexistence of these two ecologically similar species may be the scale at which one species is favoured over the other. Although *P. sylviae* may be superior to *T. bifurcata* in colonizing new hosts in a wide range of host environmental conditions, *T. bifurcata* not only thrives in presence of *P. sylviae* on the few individual hosts it manages to settle on, but it may actually outcompete *P. sylviae* when both mite species co-occur on the same individual host (Fernández-González *et al.* 2013). Therefore, superior performance of *T. bifurcata* over *P. sylviae* on shared host individuals may help the more specialized mite species (*T. bifurcata*) persist in the face of host monopolization by the generalist mite (*P. sylviae*).

To sum up, the presence of the host is not a sufficient condition for the successful establishment of symbiont populations in a given geographic region. Our results show that local host environments influence colonization of the host by feather mites, and to some extent also affect the capacity of mite populations to grow up on infested hosts. However, different mite species may show varying degrees of specialization in relation to variation in host environment, despite the fact that they may share most elements of their life histories (Malenke *et al.* 2011). Importantly, the mite species whose geographic distribution is least constrained by host environment (*P. sylviae*), may conversely be in disadvantage in the context of within-host interactions between mite species (Fernández-González *et al.* 2013). Therefore, opportunities and constraints affecting the distribution of symbionts among individual hosts and host populations may operate at different scales for different symbiont species (even the most ecologically similar ones), an idea which may help us to understand the ecological factors that promote and maintain symbiont diversity within a host species.

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References

Atyeo, W. T. & Braasch, N. L. 1966. The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodidae). *Bulletin of the University of Nebraska State Museum*, 5: 1-354.

Benoit, J. B. & Denlinger, D. L. 2010. Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *Journal of Insect Physiology*, 56: 1366-1376.

Berthold, P. 1975. *Migration: control and metabolic physiology*. In: Farner, D. S. & King, J. R. (eds.). Avian biology. Academic Press, New York, V: 77-128.

Blackburn, T. M. & Gaston, K. J. 2001. Linking patterns in macroecology. *Journal of Animal Ecology*, 70: 338-352.

Blanco, G., Tella, J. L. & Potti, J. 1997. Feather mites on group-living Red-billed Choughs: a non-parasitic interaction? *Journal of Avian Biology*, 28: 197-206.

Breiman, L. 2001. Random forests. *Machine Learning*, 45: 5-32.

Brown, J. H. 1995. *Macroecology*. University of Chicago Press, Chicago.

Carbonell, R. 2003. *Curruca capirotada Sylvia atricapilla*. In: Martí, R. & del Moral, J. C. (eds.). Atlas de las aves reproductoras de España. Dirección General de Conservación de la Naturaleza - SEO/BirdLife, Madrid: 484-485.

Carbonell, R., Pérez-Tris, J. & Tellería, J. L. 2003. Effects of habitat heterogeneity and local adaptation on the body condition of a forest passerine at the edge of its distributional range. *Biological Journal of the Linnean Society*, 78: 479-488.

Carrascal, L. M., Galván, I. & Gordo, O. 2009. Partial least squares regression as an alternative to current regression methods used in ecology. *Oikos*, 118: 681-690.

Chase, J. M., Leibold, M. A. 2003. *Ecological niches: linking classical and contemporary approaches.* University of Chicago Press, Chicago.

De la Hera, I., Pérez-Tris, J. & Tellería, J. L. 2007. Testing the validity of discriminant function analyses based on bird morphology: the case of migratory and sedentary blackcaps *Sylvia atricapilla* wintering in southern Iberia. *Ardeola*, 54: 81-91.

ESRI® 2008. *ArcGIS Desktop release 9.3.* Environmental Systems Research Institute, Redlands.

Evangelista, P. H., Kumar, S., Stohlgren, T. J., Jarnevich, C. S., Crall, A. W., Norman, J. B., III & Barnett, D. T. 2008. Modelling invasion for a habitat generalist and a specialist plant species. *Diversity and Distributions*, 14, 808-817.

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

Font, I. 1983. *Climatología de España y Portugal*. Instituto Nacional de Meteorología, Madrid.

Fox, L. R. & Morrow, P. A. 1981. Specialization: species property or local phenomenon? *Science*, 211: 887-893.

Gaede, K. & Knülle, W. 1987.Water vapour uptake from the atmosphere and critical equilibrium humidity of a feather mite. *Experimental and Applied Acarology*, 3: 45-52.

Gaud, J. & Mouchet, J. 1958. Acariens plumicoles (Analgesoidea) des oiseaux du Cameroun I. Proctophyllodidae (suite). *Annales de Parasitologie Humaine et Comparée*, 33: 36-68.

Giorgi, M. S., Arlettaz, R., Guillaume, F., Nusslé, S., Ossola, C., Vogel, P. & Christe, P. 2004. Causal mechanisms underlying host specificity in bat ectoparasites. *Oecologia*, 138: 648-654.

Gray, J. S., Kahl, O., Janetzki, C. & Stein, J. 1992. Studies on the ecology of Lyme disease in a deer forest in county Galway, Ireland. *Journal of Medical Entomology*, 29: 915-920.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25: 1965-1978.

Hutchinson, G. E. 1957. Concluding remarks. *Cold Spring Harbour Symposia*, 22: 415-427.

Krasnov, B. R. & Poulin, R. 2010. *Ecological properties of a parasite: species-specific stability and geographical variation*. In: Morand, S. & Krasnov, B. R. (eds.). The biogeography of host-parasite interactions. Oxford University Press, New York: 99-113.

Krasnov, B. R., Korallo-Vinarskaya, N. P., Vinarski, M. V, Shenbrot, G. I., Mouillot, D. & Poulin, R. 2008. Searching for general patterns in parasite ecology: host identity versus environmental influence on gamasid mite assemblages in small mammals. *Parasitology*, 135: 229-242.

López-Sepulcre, A., Kokko, H. 2005. Territorial defense, territory size, and population regulation. *The American Naturalist*, 166: 317-329.

Magurran, A. E. & Henderson, P. A. 2011. *Commonness and rarity*. In: Magurran, A. E. & McGill, B. J. (eds.). Biological diversity: frontiers in measurement and assessment. Oxford University Press, New York: 97-104.

Malenke, J. R., Newbold, N. & Clayton, D. H. 2011. Condition-specific competition governs the geographic distribution and diversity of ectoparasites. *The American Naturalist*, 177: 522-534.

Manilla, G., Demartis, A. M., Montemaggiori, A., Spina, F. & Zullo, T. 1994. Acari plumicoli nuovi per la fauna d'Italia (Acari: Sarcoptiformes). *Avocetta*, 18: 29-35.

Mironov, S. V. & Kopij, G. 2000. New feather mite species of the genus *Trouessartia* (Acari: Analgoidea: Trouessartiidae) from South African passerines (Aves: Passeriformes). *Mitteilungen aus den Hamburgischen Zoologischen Museum und Institut*, 97: 99-115.

Morris, D. W. 1987. Ecological scale and habitat use. *Ecology*, 68: 362-369.

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.

Newton, I. 1998. *Population limitation in birds*. Academic Press, London.

O'Connor, B. M., Foufopoulos, J., Lipton, D. & Lindström, K. M. 2005. Mites associated with the small ground finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae), from the Galapagos Islands. *Journal of Parasitology*, 91: 1304-1313.

Pérez-Rodríguez, A., Fernández-González, S., De la Hera, I. & Pérez-Tris, J. 2013. Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Global Change Biology*, doi: 10.1111/gcb.12226.

Pérez-Tris, J. & Tellería, J. L. 2002. 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., Carbonell, R., Tellería, J. L. 1999. A method for differentiating between sedentary and migratory Blackcaps *Sylvia atricapilla* in wintering areas of southern Iberia. *Bird Study*, 46: 299-304.

Peterson, A. T., Soberón, J. & Sánchez-Cordero, V. 1999. Conservatism of ecological niches in evolutionary time. *Science*, 285: 1265-1267.

Pietrock, M. & Marcogliese, D. J. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. *Trends in Parasitology*, 19: 293-299.

Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, Princeton.

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

Randolph, S. E. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology*, 129: S37-S65.

Rosenzweig, M. L. 1981. A theory of habitat selection. *Ecology*, 62: 327-335.

Rudolph, D. 1983. The water-vapour uptake system of the Phthiraptera. *Journal of Insect Physiology*, 29: 15-25.

Santana, F. J. 1976. A review of the genus *Trouessartia*. *Journal of Medical Entomology*, 1: S1-S128.

Schluter, D. 2000. *The ecology of adaptive radiation.* Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science*, 185: 27-39.

Schoener, T. W. 2009. *Ecological niche*. In: Levin, S.A. (ed.). The Princeton Guide to Ecology. Princeton University Press, Princeton: 3-13.

Smith, W. H. F. & Sandwell, D. T. 1997. Global seafloor topography from satellite altimetry and ship depth soundings. *Science*, 277: 1956-1962.

StatSoft 2004. *STATISTICA version 7.0*. Statsoft, Tulsa.

Svensson, L. 1992. *Identification guide to European passerines*. L. Svensson, Stockholm.

Tellería, J. L., Pérez-Tris, J., Carbonell, R. 2001. Seasonal changes in abundance and flight-related morphology reveal different migration patterns in Iberian forest passerines. *Ardeola*, 48: 27-46.

Thompson, J. N. (ed.). 2005. *The geographic mosaic of coevolution*. University of Chicago Press, Chicago.

Tucker, C. J., Pinzón, J. E., Brown, M. E., Slayback, D. A., Pak, E. W., Mahoney, R., Vermote, E. F. & El Saleous, N. 2005. An extended AVHRR 8-km NDVI dataset compatible with

MODIS and SPOT vegetation NDVI data. *International Journal of Remote Sensing*, 26: 4485-4498.

Wiles, P. R., Cameron, J., Behnke, J. M., Hartley, I. R., Gilbert, F. S., McGregor, P. K. 2000. Season and ambient air temperature influence the distribution of mites (*Proctophyllodes stylifer*) across the wings of blue tits (*Parus caeruleus*). *Canadian Journal of Zoology*, 78: 1397-1407.

Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Randolph, S. E., Read, A. F., & Skorping, A. 2002. *Heterogeneities in macroparasite infections: patterns and processes*. In: Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H. & Dobson, A. P. (eds.). The ecology of wildlife diseases. Oxford University Press, New York: 6-44.

Supplementary material

Table S4.1. Prevalence, mean abundance and mean intensity values of *Proctophyllodes sylviae* and *Trouessartia bifurcata* for each sampled locality. Host phenotype (1 = migratory, 2 = sedentary) and number of blackcap individuals captured in each locality are also presented.

				Proctophyllodes sylviae			***Trouessartia bifurcata***		
	Locality	**Host**	**N**	**Prevalence Ab**	**undance**	**Intensity**	**Prevalence Ab**	**undance**	**Intensity**
1.	Aguilar de Campoo (P)	1	22	90.91	16.60	22.45	36.36	0.82	4.16
2.	Alájar (H)	1	25	100	38.51	38.51	0.00	0.00	
3.	Aldeaquemada (J)	1	24	62.50	3.23	9.03	0.00	0.00	
4.	Alfarràs (L)	1	20	65.00	6.31	20.33	0.00	0.00	
5.	Barreiros (LU)	1	19	89.47	6.37	8.32	63.16	4.23	12.74
6.	Barrundia (VI)	1	31	100	65.48	65.48	35.48	1.95	20.07
7.	Bera (NA)	1	21	95.24	24.44	28.91	57.14	4.35	17.83
8.	Cazalla de la Sierra (SE)	2	38	92.11	15.60	20.11	7.89	0.17	6.19
9.	Cocentaina (A)	1	27	100	30.43	30.43	25.93	0.65	5.93
10.	Cofrentes (V)	1	22	95.45	12.75	14.57	18.18	0.13	1.00
11.	El Bosque (CA)	2	31	90.32	14.25	19.41	0.00	0.00	
12.	Gilbuena (AV)	1	33	96.97	20.83	23.04	21.21	0.41	4.01
13.	Grado (O)	1	22	86.36	12.81	19.90	72.73	5.58	12.34
14.	Güéjar-Sierra (GR)	2	15	33.33	1.00	6.97	0.00	0.00	
15.	Hermandad de Campoo de Suso (S)	1	29	100	55.09	55.09	27.59	0.70	5.86
16.	Jaca (HU)	1	22	100	28.36	28.36	4.55	0.05	2.00
17.	Jerte (CC)	1	26	80.77	14.88	29.67	0.00	0.00	
18.	Limpias (S)	1	21	100	27.70	27.70	80.95	5.06	8.25
19.	Los Barrios (CA)	2	13	84.62	13.86	23.27	76.92	13.26	30.66
20.	Molinaseca (LE)	1	38	81.58	9.78	17.44	2.63	0.07	12.00

				Proctophyllodes sylviae			*Trouessartia bifurcata*		
	Locality	**Host**	**N**	**Prevalence Ab**	**undance**	**Intensity**	**Prevalence Ab**	**undance**	**Intensity**
21.	Pampaneira (GR)	2	13	84.62	20.53	36.62	7.69	0.09	2.00
22.	Panticosa (HU)	1	25	100	34.78	34.78	8.00	0.13	3.58
23.	Pinilla del Valle (M)	1	16	93.75	20.05	24.79	12.50	0.15	2.00
24.	Pradoluengo (BU)	1	20	100	37.85	37.85	20.00	0.77	16.36
25.	Ruidera (CR)	1	25	100	15.44	15.44	0.00	0.00	
26.	San Adrián (NA)	1	32	90.63	16.98	23.25	6.25	0.04	1.00
27.	San Lorenzo de El Escorial (M)	1	11	81.82	18.83	37.52	0.00	0.00	
28.	Santa Marta de Tormes (SA)	1	30	90.00	13.57	18.62	0.00	0.00	
29.	Santiago-Pontones (J)	2	23	91.30	15.63	20.73	4.35	0.03	1.00
30.	Talavera de la Reina (TO)	1	19	57.89	2.30	6.87	0.00	0.00	
31.	Tarifa (CA)	2	13	84.62	20.00	35.53	30.77	0.78	5.54
32.	Tordera (B)	1	25	100	47.81	47.81	72.00	5.38	12.12
33.	Tordesillas (VA)	1	20	95.00	17.86	21.02	0.00	0.00	
34.	Trillo (GU)	1	20	95.00	20.60	24.39	10.00	0.07	1.00
35.	Valderrobres (TE)	1	43	88.37	12.03	17.26	0.00	0.00	
36.	Vilar de Barrio (OR)	1	20	95.00	22.68	26.97	65.00	5.26	15.82
37.	Zuera (Z)	1	21	95.24	16.47	19.16	0.00	0.00	

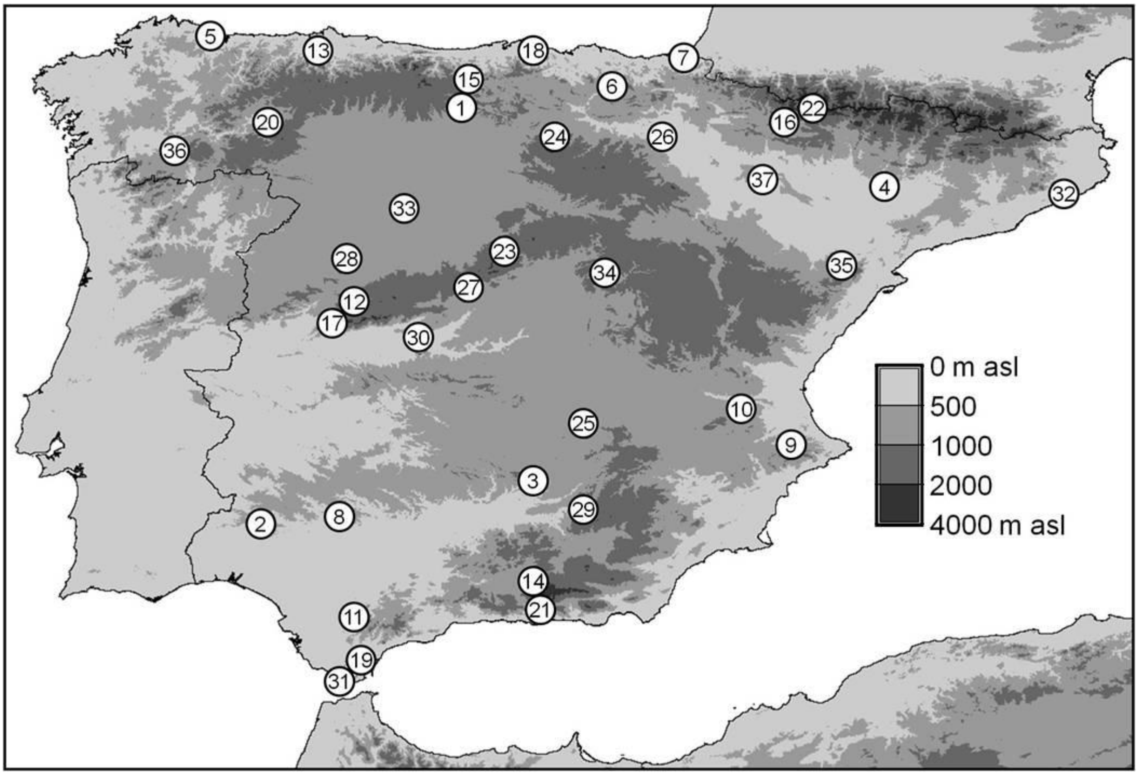
Table S4.2. Complete results of the Partial Least Squares Regression analyses for prevalence, abundance and intensity of *Proctophyllodes sylviae* and *Trouessartia bifurcata*.

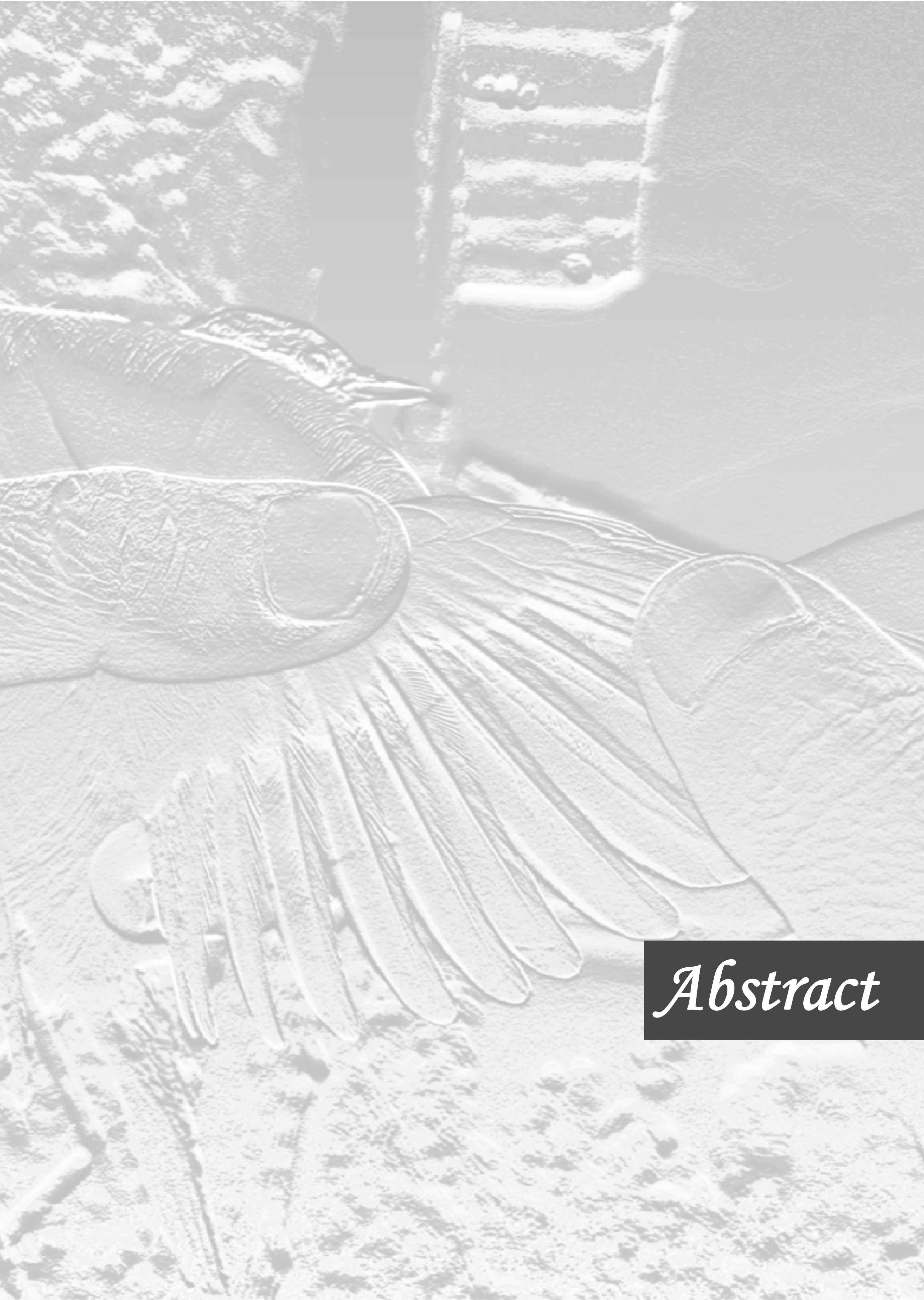
Predictor variables (grouped by type):	***Proctophyllodes sylviae***			***Trouessartia bifurcata***		
	Prevalence	**Abundance**	**Intensity**	**Prevalence**	**Abundance**	**Intensity**
Year						
2008	-0.062	0.028	0.109	0.027	0.057	**0.184**
2009	-0.117	-0.129	-0.132	-0.054	-0.022	-0.001
2010	0.090	0.071	0.049	0.019	-0.021	-0.070
2011	0.141	0.075	0.009	0.022	-0.022	-0.143
Geographical effects						
Longitude (X)	0.130	0.105	0.111	-0.026	-0.047	-0.124
Latitude (Y)	**0.198**	**0.165**	0.114	0.115	0.093	0.090
X·Y	0.056	0.131	**0.202**	-0.054	-0.027	0.001
X^2	0.102	0.089	0.081	0.094	0.115	0.119
Y^2	-0.097	-0.037	0.019	0.122	0.137	0.126
$X^2 \cdot Y$	0.023	-0.011	-0.036	0.103	0.110	0.091
$X \cdot Y^2$	0.111	0.082	0.076	-0.092	-0.123	**-0.167**
X^3	0.100	0.114	**0.145**	0.028	0.026	-0.014
Y^3	**0.184**	0.098	0.014	0.039	0.012	0.004
Temperature						
Annual mean temperature	-0.129	-0.142	**-0.154**	0.044	0.082	0.062
Maximum annual mean temperature	**-0.186**	**-0.206**	**-0.215**	-0.044	-0.006	-0.004
Minimum annual mean temperture	-0.070	-0.073	-0.087	0.114	**0.148**	0.118
Breeding mean temperature	-0.141	**-0.155**	**-0.165**	0.024	0.065	0.055

	Proctophyllodes sylviae			*Trouessartia bifurcata*		
Predictor variables (grouped by type):	**Prevalence**	**Abundance**	**Intensity**	**Prevalence**	**Abundance**	**Intensity**
Maximum breeding mean temperature	**-0.201**	**-0.224**	**-0.234**	-0.080	-0.036	-0.025
Minimum breeding mean temperature	-0.080	-0.084	-0.099	0.097	0.134	0.111
Maximum temperature of the warmest month	**-0.257**	**-0.270**	**-0.258**	**-0.209**	**-0.172**	-0.134
Minimum temperature of the coldest month	-0.021	-0.018	-0.039	**0.161**	**0.189**	**0.168**
Temperature annual range	**-0.195**	**-0.205**	**-0.184**	**-0.300**	**-0.287**	**-0.231**
Temperature mean diurnal range	**-0.209**	**-0.230**	**-0.223**	**-0.292**	**-0.281**	**-0.218**
Isothermality (mean diurnal range/annual range)	-0.025	-0.057	-0.101	0.051	0.047	0.071
Temperature seasonality (SD of isothermality)	**-0.152**	**-0.158**	-0.135	**-0.294**	**-0.283**	**-0.238**
Mean temperature of the wettest quarter	0.070	-0.007	-0.053	0.043	0.050	-0.033
Mean temperature of the driest quarter	0.020	-0.019	-0.094	0.006	0.007	-0.035
Mean temperature of the warmest quarter	**-0.207**	**-0.221**	**-0.219**	-0.096	-0.056	-0.054
Mean temperature of the coldest quarter	-0.056	-0.064	-0.085	0.142	**0.172**	0.142
Rainfall						
Annual rainfall	**0.158**	**0.196**	**0.212**	**0.250**	**0.246**	**0.252**
Breeding rainfall	**0.153**	**0.186**	**0.208**	**0.207**	**0.200**	**0.195**
Rainfall of the wettest month	0.117	**0.160**	**0.179**	**0.263**	**0.267**	**0.267**
Rainfall of the driest month	**0.190**	**0.159**	0.128	0.097	0.066	0.031
Rainfall seasonality (coefficient of variation)	**-0.226**	**-0.195**	**-0.163**	-0.019	0.021	0.058
Rainfall of the wettest quarter	0.094	0.139	**0.162**	**0.242**	**0.250**	**0.269**
Rainfall of the driest quarter	**0.231**	**0.197**	**0.159**	0.119	0.085	0.053
Rainfall of the warmest quarter	**0.243**	**0.215**	**0.183**	**0.149**	0.115	0.073
Rainfall of the coldest quarter	0.066	0.119	**0.145**	**0.208**	**0.218**	**0.247**

Predictor variables (grouped by type):	*Proctophyllodes sylviae* Prevalence	 Abundance	 Intensity	*Trouessartia bifurcata* Prevalence	 Abundance	 Intensity
Landscape features						
Altitude	-0.029	-0.034	-0.029	**-0.206**	**-0.209**	**-0.155**
Slope	-0.057	-0.037	0.006	-0.041	-0.049	-0.062
Mean annual NDVI	0.073	0.123	0.141	**0.221**	**0.227**	**0.257**
Mean breeding NDVI	0.062	0.135	**0.173**	**0.212**	**0.221**	**0.257**
Percentage of coniferous forest	-0.044	-0.051	-0.049	-0.026	-0.046	-0.095
Percentage of broadleaf forest	0.083	0.119	**0.147**	0.111	0.143	**0.181**
Percentage of shrubland	**-0.214**	**-0.268**	**-0.245**	-0.144	-0.116	-0.038
Percentage of open spaces	0.074	**0.147**	**0.186**	**0.166**	0.137	0.133
Percentage of wooded croplands	-0.026	-0.005	0.028	-0.098	-0.105	-0.082
Percentage of arable land	0.096	0.077	0.022	0.003	-0.019	-0.071
Percentage of water bodies	**0.168**	0.100	0.021	-0.054	-0.091	**-0.171**
Percentage of urban areas	0.080	0.076	0.073	0.000	-0.007	-0.037
Behaviour						
Migration: Migration	**0.200**	0.123	0.056	0.010	-0.001	0.026
Migration: Sedentarism	**-0.200**	-0.123	-0.056	-0.010	0.001	-0.026
R2 of the model						
(% variance explained)	**28.27**	**29.21**	**28.00**	**68.16**	**68.55**	**31.62**

Figure S4.1. Map of the sampling sites in the Iberian Peninsula.





Abstract

Introduction

Why are there so many functionally equivalent species in ecosystems, if apparently the latter could work as well with just one or a few species of each kind? Evolutionary biologists have devoted a large body of research to answer this question, which is in the core of the understanding of the evolution of biodiversity. A clear example of this paradox is provided by symbionts (symbiosis refers to the close bond among two different species, in which one of the species lives near, on or inside individuals of the other species), which show a high degree of specialization in order to successfully find, colonize and grow in their hosts, which in turn will favour the existence of a wide variety of symbiotic organisms.

A single host species may be normally occupied by different symbiont species, which share the same ecological requirements and are even found together within the same host individual. As a consequence, symbiont infracommunities (all the individuals of all symbiont species present in a host individual) are likely to interact with one another giving rise to different types of interspecific interactions such as a numerical decrease of one or more of the interacting species, or a shift in their ecological niches within the host. This niche partitioning may lead to niche specialization, which in turn may favour symbiont coexistence and the maintenance of symbiont diversity.

Within a host population, the composition of symbiont infracommunities and their relative numbers may vary among host individuals. Such variation may be due to differences in host characteristics that may indicate a better or worse habitat for symbionts and/or the ability of symbionts to disperse and grow among host individuals. In the last term, symbiont exchange among hosts will determine the genetic structure of symbionts populations within a host population, which will depend on the degree of isolation associated with host behaviour and symbiont intrinsic transmission capabilities.

Symbiont component communities (the symbiont species occurring in one host population) may also vary in composition and relative numbers among host populations across the host’s distribution range. Such differences may be caused by local adaptation giving rise to phenotypic variation among host populations. For example, migratory behaviour may expose host individuals to different symbiont faunas across their range and also entails morphological, behavioural and physiological changes in the host which may greatly influence symbiont distribution patterns by affecting dispersal or within-host growth. Moreover, environmental conditions typical of a given region appear to exert a strong influence on the outcome of many host-symbiont interactions. It has been reported that climatic variables such as temperature and rainfall are crucial variables shaping symbiont distribution and favouring or constraining symbiont survival, colonization success and within-host growth. Nevertheless, not all symbiont species have the same tolerance to environmental change, and some places where the host lives might be inhospitable for certain symbiont species. Ultimately, this will create a geographic mosaic of host-symbiont interactions in which each symbiont component community will probably have different features depending on host attributes, local environmental conditions and symbiont-symbiont interactions.

The main goal of this thesis was to summarize what are the factors that may have a strong influence in the maintenance of host-symbiont interactions and the coexistence of symbionts in the same host species. The idea was to stress that not all variables having an effect on these interactions have the same importance depending on the scale of observation. Studies of this kind provide a better knowledge of the processes involved in symbiont species diversification, symbiont community assembly and, in turn, the mechanisms through which symbiont coexistence becomes possible. In order to accomplish this goal a host species was carefully chosen, the European blackcap *Sylvia atricapilla*, which (1) is widely distributed among a broad range of environments, (2) normally harbours two ecologically similar symbiont species (two feather dwelling mites, *Proctophyllodes sylviae* and *Trouessartia bifurcata*, which are potential competitors), and (3) possesses different phenotypic attributes that may create variation in individual host quality for such symbionts.

Objectives

Chapter 1. This study examines the patterns of distribution of two feather mite species (*P. sylviae* and *T. bifurcata*), and their potential interaction in wintering blackcap populations in southern Spain. To date several studies have shown that mite numbers on the individual host and prevalence among hosts may be affected by host migratory behaviour. However, as far as it is known such analyses have not been carried out in a single species that shows different migratory behaviours. The study of mite distribution patterns at the intra-host level allows controlling for the variation created by specific features of each host species that may mask the detection of such patterns.

Chapter 2. This study investigates within-host distribution of both mite species and their interactions in the same blackcap populations investigated in Chapter 1. Thus, it will be possible to describe how these mite species share host habitat, which is a prerequisite to approach the mechanisms through which both species are able to coexist on an individual host. To this end, detailed counts of *P. sylviae* and *T. bifurcata* mites were carried out within each wing feather, obtaining a map of the distribution of each feather mite species on the wing. Hence, interspecific mite interactions could be studied on a very fine scale. Finally, the distribution of each species was analysed to study whether they have preferences for any specific sector of the feather or for any specific feather of the wing, as well as whether they follow a specific order of occupation of the different plumage sectors available on the wing.

Chapter 3. This study analyses genetic diversity and genetic structure of *P. sylviae* and *T. bifurcata* in the same blackcap populations. If mite transmission from parents to offspring involves population bottlenecks, detectable genetic structure is expected to arise for both mite species. In addition, host phenotype might give rise to differences in the genetic structure of both mite species if host type creates opportunities and constraints on the distribution of each mite species. The aim of this study was to shed light on feather mite colonization strategies and their genetic consequences, which may have important implications in the context of the different distribution of these mite species among hosts investigated in the other chapters.

Chapter 4. This study analyses the distribution of the two feather mite species at a broad scale, across 37 breeding blackcap populations, in order to assess the potential influence of environmental conditions on feather mite distribution patterns (prevalence, abundance and intensity). In conjunction with differences found in population numbers and prevalence at a local scale, it is also known that feather mites are sensitive to climatic variables such as humidity and temperature. The Iberian Peninsula combines

great environmental variation and reduced geographic area, thereby making an excellent scenario in which to conduct such study.

Results

Within-host feather mite distribution and interspecific interactions

According to results presented in Chapter 2, the two species of feather mites follow a different distribution across blackcap feathers and feather sections. Additionally, the filling of wing cells by feather mites was ordered, although such order was different among feather mite species. Some cells were only occupied when mite populations on the wing were large, which supports the idea that some areas of the wing are suboptimal for mites. Interestingly, the least preferred cells for one mite species ranked high in the range of cell preferences of the other species, although some areas of the wing were apparently suboptimal for both mite species. Regarding interspecific interactions, the numbers of *T. bifurcata* and *P. sylviae* were negatively correlated when both mite species co-occurred in the same wing cell. When total numbers of each mite species were taken into account, *P. sylviae* numbers (abundance and intensity) decreased when *T. bifurcata* was present on the same individual, but the contrary was not true (Chapter 1).

Host phenotype and feather mite distribution

In Chapter 1, results showed that in general, prevalence, abundance and load of both mite species considered as a whole were greater in migratory blackcaps than in sedentary blackcaps. When both mite species were taken into account separately and within-host analyses were conducted, *P. sylviae* was more abundant than *T. bifurcata* in general. Different patterns of distribution in abundance between migratory and sedentary blackcaps were also observed: *P. sylviae* was more abundant than *T. bifurcata* on migratory blackcaps, whereas both mite species converged in intermediate numbers on sedentary blackcaps.

When blackcaps were divided into migratory and sedentary individuals (Chapter 1), on sedentary blackcaps the probability of the occurrence of a mite species was higher

when the other species was also present on the host. Regarding mite numbers in a between-host analysis, the interaction between host phenotype and *P. sylviae* presence had an effect on *T. bifurcata* abundance: *T. bifurcata* numbers were lower when *P. sylviae* was present, although such association was more evident in migratory blackcaps. As described in the analysis of variation in mite numbers across the host's wing, the presence of *T. bifurcata* was associated with lower numbers of *P. sylviae* regardless of blackcap phenotype.

Finally, regarding the variation in host traits among blackcap populations (Chapter 1), different traits affected each mite species differently. In the case of *P. sylviae*, its load was positively correlated to host wing length (which is longer in migratory blackcaps), whereas the load of *T. bifurcata* was negatively associated with wing length and positively correlated with uropygial gland size (which is bigger in sedentary blackcaps).

Feather mite genetic structure and genetic diversity

In Chapter 3 a high genetic diversity was found in within-host populations of both mite species, although it was higher in *P. sylviae*. Consequently, genetic structure of mite populations among individual hosts was weak. Any genetic structure found on blackcaps was mainly due to the fact that some mites from the same host had identical haplotypes, which was expected, but in general most mites had unique haplotypes in the host population and mite individuals that shared the same host were not more closely related from one another than expected by chance.

Feather mite distribution among host populations

In Chapter 4, *P. sylviae* was found in all sites included in this study, while *T. bifurcata* was detected in 65% of localities. In general, *P. sylviae* reached higher prevalence, abundance and intensity than *T. bifurcata* within each locality. In addition, mite numbers (abundance and intensity) of both mite species showed no correlation among blackcap populations, whereas prevalence had a significant (but weak) association between both species.

P. sylviae prevalence and abundance were poorly modelled with the variables included in the study in comparison to *T. bifurcata*. However, the factors extracted for intensity of both mite species did not explain much variance. In every case, the variables that played the most important role in explaining differences in mite population patterns were temperature and precipitation variables: dry areas with a marked seasonality had a detrimental effect on both mite species. Furthermore, migration had a positive effect on *P. sylviae* prevalence.

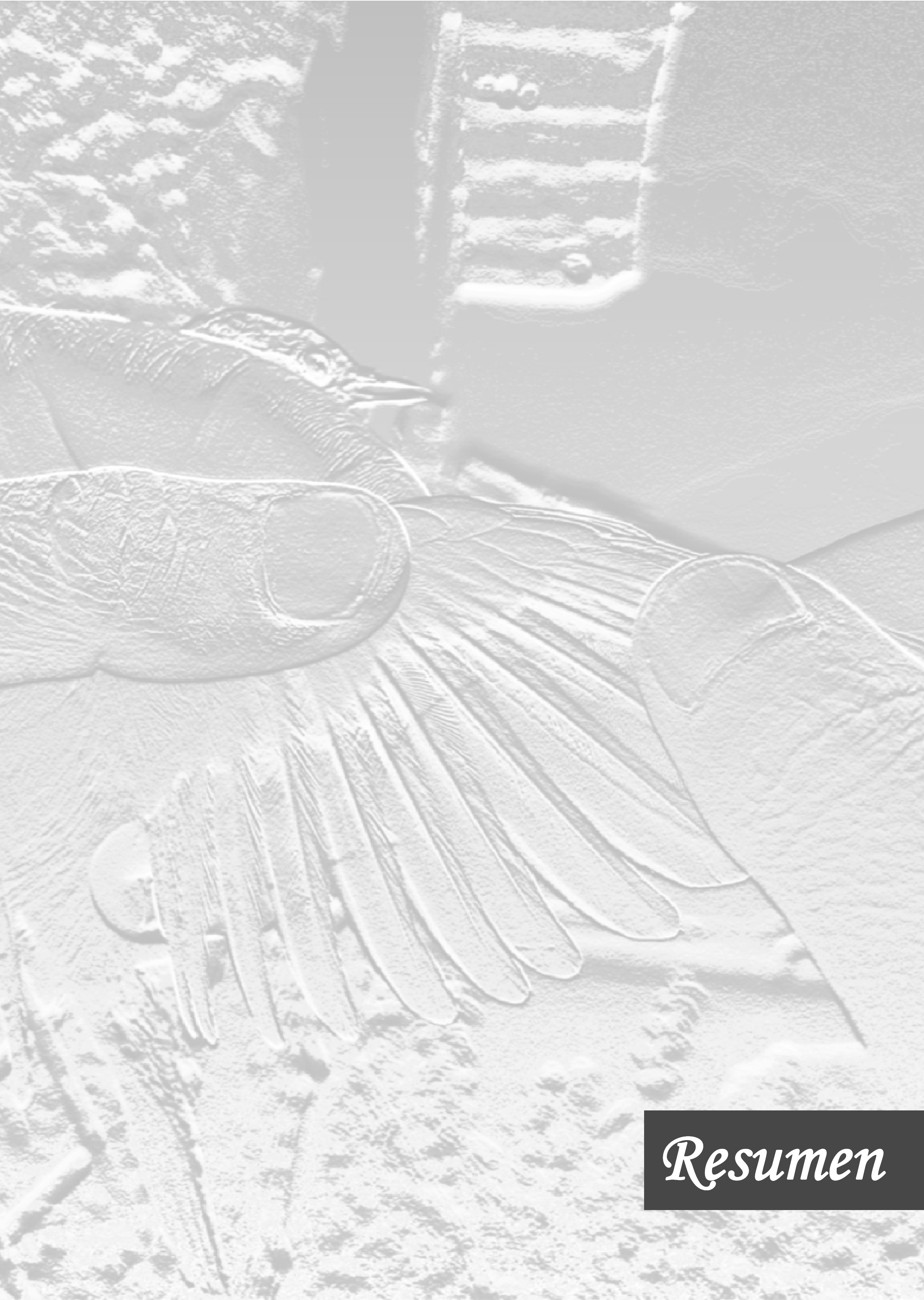
Conclusions

1. **Feather mites are able to coexist on the same host.** *P. sylviae* and *T. bifurcata* are the most common feather mite species occurring on blackcaps; both mite species may appear alone or sharing the same host individual. The type of mite infestation (single or multiple) may be influenced by a wide array of circumstances, ranging from differences in host attributes to contrasting host environments.
2. **Different feather mite species occupy different parts of the wing.** *P. sylviae* lives on the ventral side while *T. bifurcata* occupies the dorsal part of the wing. In addition, mites occupy different areas of the wing as well as different sectors within a single feather: *P. sylviae* preferentially occupies medial-outer regions of the feather while *T. bifurcata* appears in medial-inner sections. Besides, the order of cell occupation follows a different sequence in each feather mite species, hence the most preferred cells for one mite are not the most preferred for the other.
3. **Host-sharing comes at a cost.** Despite apparent niche partitioning, when both mite species coincide in the same cell, they experience a reduction in numbers. When mite numbers of each mite species on the wing are taken as a whole, *T. bifurcata* seems to play a dominant role when both mite species coexist on the same host individual, given that the presence of that mite is associated with lower numbers of *P. sylviae*. However, *T. bifurcata* mites apparently have more difficulties in colonizing as many hosts and to reach as large population sizes within hosts, compared to *P. sylviae*.
4. **Host phenotype creates opportunities and constraints on feather mite distribution and population size.** *P. sylviae* is favoured on migratory blackcaps, where it is more prevalent and abundant, whereas *T. bifurcata* shows greater prevalence and abundance on sedentary blackcaps. Interestingly, sedentary blackcaps offer a suitable habitat for both mite species, where coexistence becomes more frequent than on

migratory blackcaps. Finally, certain host traits may favour an increase in mite load: *P. sylviae* load was positively correlated with host wing length (wings were longer in migratory blackcaps), while *T. bifurcata* load was positively correlated to uropygial gland size (sedentary blackcaps had bigger glands).

5. Local environmental conditions create a mosaic of outcomes across the host species' range. Climate factors (most notably temperature and precipitation) exert a strong impact on feather mite distribution; high temperatures, dry conditions and a marked seasonality have a detrimental effect on feather mite presence and abundance across the Iberian Peninsula. However, different feather mite species are unequally influenced by such variables: most remarkably, *T. bifurcata* is absent from the driest habitats, whereas *P. sylviae* is able to exist in all populations although it decreases in prevalence and abundance in the least favourable areas.

6. Feather mite coexistence might be explained by the advantages one species has over the other at different scales. At the within-host scale *T. bifurcata* may reduce *P. sylviae* numbers. However, *P. sylviae* is able to colonise migratory and sedentary hosts alike, and reaches much higher numbers than *T. bifurcata* (both within hosts and at higher geographic scales). At a regional scale, *P. sylviae* is more tolerant to environmental conditions than *T. bifurcata*, which is absent from some localities. This suggests that *P. sylviae* is a more generalist mite than *T. bifurcata*, which seems to suffer greater constraints associated with host attributes and environmental conditions.



Resumen

Introducción

¿Por qué existen en los ecosistemas tantas especies funcionalmente similares, si estos podrían funcionar también con una o unas pocas especies de cada tipo? Los biólogos evolutivos han dedicado grandes esfuerzos a responder a esta pregunta, la cual supone la clave para entender la evolución de la biodiversidad. Un claro ejemplo de esta paradoja lo constituyen los simbiontes (la simbiosis se refiere a la unión estrecha entre dos especies de organismos, en la cual una de las especies vive cerca, sobre o dentro de individuos de la otra especie), los cuales muestran un alto grado de especialización a la hora de encontrar, colonizar y establecerse en el hospedador, mecanismos por los cuales se favorece la existencia de una gran variedad de organismos simbiontes.

Normalmente, dentro de una especie de hospedador pueden encontrarse distintos tipos de simbiontes que pueden poseer los mismos requerimientos ecológicos e incluso puede darse el caso en que ocupen el mismo individuo. Como resultado, puede ocurrir que las infracomunidades de simbiontes (todos los individuos de todas las especies de simbiontes presentes en un individuo) puedan interaccionar entre ellas dando lugar a distintos tipos de interacciones interespecíficas, como por ejemplo una disminución en el número de individuos de alguna de las especies participantes en la interacción o un desplazamiento de los nichos ecológicos que ocupan dentro del hospedador. Dicho desplazamiento puede dar lugar a una especialización del nicho, que a su vez puede favorecer la coexistencia de simbiontes y el mantenimiento de la diversidad.

La composición de las infracomunidades de simbiontes así como la abundancia e intensidad de las especies, pueden variar entre individuos que pertenecen a la misma población hospedadora. Esta variación puede deberse a características de los hospedadores que podrían traducirse en hospedadores de mejor o peor calidad para los simbiontes y/o a la capacidad de los simbiontes para dispersarse y crecer en los hospedadores. En última instancia, el intercambio de individuos simbiontes entre hospedadores determinará la estructura genética de las poblaciones de simbiontes dentro de una población hospedadora, estructura que dependerá del grado de aislamiento determinado a su vez por el comportamiento del hospedador y de la capacidad de transmisión de los simbiontes.

Las comunidades componentes de simbiontes (las especies de simbiontes presentes en una población de hospedadores) pueden variar también en su composición, abundancia e intensidad entre poblaciones de hospedadores a lo largo de su rango de distribución. Dichas diferencias pueden estar causadas por adaptaciones locales de los hospedadores que generan variación fenotípica entre sus poblaciones. Por ejemplo, el comportamiento migratorio de los hospedadores puede exponerlos a distintos tipos de simbiontes a lo largo de su rango de distribución. También puede dar lugar a cambios morfológicos, comportamentales y fisiológicos en el hospedador que pueden tener gran influencia en los patrones de distribución de los simbiontes ya que pueden afectar a su dispersión y a su crecimiento dentro del hospedador. Por otra parte, las condiciones ambientales de una región pueden afectar a la interacción entre simbionte y hospedador. Por ejemplo, se ha demostrado que variables climáticas como la temperatura y la precipitación pueden afectar a la distribución de los simbiontes además de favorecer o restringir su supervivencia, éxito de colonización y crecimiento dentro del hospedador. Sin embargo, no todas las especies tienen la misma tolerancia frente a estas variables, ya que en algunos lugares donde el hospedador se establece hay especies de simbiontes que no son capaces de desarrollarse. En conjunto, todos estos aspectos darán lugar a un mosaico geográfico de interacciones simbionte-hospedador donde cada comunidad componente de simbiontes estará condicionada por características de los hospedadores, las condiciones ambientales locales y las interacciones interespecíficas entre simbiontes.

El objetivo de esta tesis ha sido analizar qué factores pueden influir en el mantenimiento de las interacciones simbionte-hospedador y en la coexistencia de especies simbiontes en el mismo hospedador. También se ha querido remarcar que la importancia de los factores que influyen sobre estas interacciones varía dependiendo de la escala de observación. Estudios de este tipo ayudan a comprender mejor los procesos implicados en la diversificación de especies de simbiontes y en el ensamblaje de comunidades de simbiontes, así como los mecanismos a través de los cuales la coexistencia es posible. Para poder alcanzar este objetivo se ha elegido una especie hospedadora, la curruca capirotada *Sylvia atricapilla*, la cual (1) se distribuye en un rango amplio de ambientes, (2) alberga normalmente dos especies de simbiontes ecológicamente similares (dos especies de ácaros de las plumas, *Proctophyllodes sylviae* y *Trouessartia bifurcata*, los cuales son competidores potenciales), y (3) posee características fenotípicas que pueden dar lugar a diferencias en la calidad de cada hospedador para dichos simbiontes.

Objetivos

Capítulo 1. En este estudio se investigan los patrones de distribución de dos especies de ácaros de las plumas (*P. sylviae* and *T. bifurcata*) y su posible interacción en poblaciones invernantes de curruca capirotada en el sur de España. Varios estudios han demostrado que el comportamiento migratorio puede afectar al número de ácaros en un hospedador y a la prevalencia entre hospedadores. Sin embargo, no se conocen trabajos en los que se hayan realizado estudios de este tipo dentro de una especie hospedadora con distintos comportamientos migratorios. El estudio de los patrones de distribución de los ácaros a nivel intraespecífico ayuda a controlar la variación debida a la especie de hospedador que puede enmascarar la detección de dichos patrones.

Capítulo 2. Este estudio analiza la distribución de ambas especies de ácaros de las plumas dentro del hospedador así como sus interacciones en las mismas poblaciones de curruca capirotada examinadas en el Capítulo 1. De este modo, se podría averiguar cómo ambas especies comparten el hábitat (hospedador), lo cual es un prerrequisito para conocer los mecanismos a través de los cuales ambas especies son capaces de coexistir en un hospedador. Para ello, se realizaron conteos detallados de *P. sylviae* y *T. bifurcata* en cada pluma del ala, obteniendo así un mapa de la distribución de cada especie. De esa manera se han podido estudiar las interacciones interespecíficas a pequeña escala. Por último, se analizó si las especies tienen preferencia por algún sector de la pluma o por alguna pluma del ala, así como si siguen un patrón de ocupación específico de los sectores disponibles en el ala.

Capítulo 3. En este estudio se examina la diversidad genética y la estructura genética de *P. sylviae* y *T. bifurcata* en las mismas poblaciones de curruca capirotada analizadas en los capítulos 1 y 2. Si la transmisión de los ácaros de padres a hijos implica cuellos de botella, se esperaría detectar estructura genética en ambas especies de ácaros. Además, el fenotipo del hospedador podría dar lugar a diferencias en la estructura genética de cada especie si el tipo de hospedador favorece o restringe su distribución. De esta manera, se podrán conocer mejor las estrategias de colonización de los ácaros de las plumas y sus consecuencias genéticas, lo cual puede tener importantes implicaciones en la distribución de estos ácaros analizada en los capítulos anteriores.

Capítulo 4. En este estudio se analiza la distribución de ambas especies de ácaros a mayor escala, en 37 poblaciones reproductoras de curruca capirotada, para así evaluar la posible influencia de las condiciones ambientales en los patrones de distribución de los ácaros de las plumas (prevalencia, abundancia e intensidad). Además de las diferencias que pueden existir en el número de ácaros y la prevalencia a escala local, variables climáticas como la humedad y la temperatura pueden tener efecto en los ácaros. La Península Ibérica combina una gran variación ambiental con un área geográfica reducida, lo que la hace un excelente escenario donde realizar este estudio.

Resultados

Distribución e interacciones interespecíficas de los ácaros en el hospedador

En el Capítulo 2 se observó que los ácaros de las plumas se distribuyeron de forma distinta entre plumas y sectores de las plumas. Además, la ocupación de las celdas fue ordenada, aunque dicho orden fue distinto entre ambas especies de ácaros. Algunas celdas sólo se ocuparon cuando el número de ácaros fue alto, lo que apoya la idea de que algunas áreas del plumaje son subóptimas para los ácaros. Por otra parte, las celdas menos preferidas por una de las especies de ácaro fueron las primeras en ser seleccionadas por la otra especie, aunque algunas áreas del ala no fueron ocupados por ninguna de las dos especies. Respecto a las interacciones interespecíficas, cuando *T. bifurcata* y *P. sylviae* se encontraron en la misma celda, sus números se correlacionaron negativamente. Al analizar el número total de ácaros de cada especie, la abundancia e intensidad de *P. sylviae* disminuyeron cuando *T. bifurcata* coincidía en el mismo hospedador, pero no se observó el efecto inverso (Capítulo 1).

Fenotipo del hospedador y distribución de los ácaros de las plumas

Los resultados del Capítulo 1 revelaron que en general la prevalencia, abundancia e intensidad de ambas de especies de ácaros en conjunto fueron mayores en las currucas migradoras que en las sedentarias. Cuando ambas especies fueron examinadas por separado y se realizaron análisis dentro de sujetos, los resultados indicaron que *P. sylviae* fue más abundante que *T. bifurcata* en general. También observamos distintos patrones de distribución en la abundancia de los ácaros entre currucas migradoras y sedentarias: *P. sylviae* fue más abundante que *T. bifurcata* en currucas migradoras, mientras que ambas especies alcanzaron números parecidos e intermedios en currucas sedentarias.

Cuando las currucas migradoras y sedentarias fueron consideradas por separado (Capítulo 1), se encontró que en las currucas sedentarias la probabilidad de aparición de una de las especies de ácaros era mayor cuando la otra estaba presente en el hospedador. En cuanto al número de ácaros en los análisis entre sujetos, la interacción entre el fenotipo del hospedador y la presencia de *P. sylviae* tuvo efecto en la abundancia de *T. bifurcata*: el número de ácaros de *T. bifurcata* fue menor cuando coincidía con *P. sylviae*, siendo esta asociación más patente en currucas migradoras. Como se observó en el análisis de variación en el número de ácaros a través del ala del hospedador, la presencia de *T. bifurcata* se relacionó con un menor número de ácaros de *P. sylviae* independientemente del fenotipo de la curruca.

Por último, cuando se analizó la variación en los atributos de las poblaciones de currucas (Capítulo 1), encontramos que las características de los hospedadores afectaron de distinta manera a cada especie de ácaro. En el caso de *P. sylviae*, su intensidad se relacionó positivamente con la longitud del ala del hospedador (la cual es mayor en currucas migradoras), mientras que la carga de *T. bifurcata* se relacionó negativamente con la longitud del ala y positivamente con el tamaño de la glándula uropigial (la cual es mayor en currucas sedentarias).

Estructura genética y diversidad genética de los ácaros de las plumas

En el Capítulo 3 se observó que la diversidad genética de cada población de ácaros dentro del hospedador fue muy alta, siendo esta mayor en *P. sylviae*. Por consiguiente, la estructura genética de las poblaciones de ácaros entre hospedadores fue muy débil. La estructura genética encontrada en las currucas se debió principalmente al hecho de que los ácaros dentro de un mismo hospedador compartían haplotipos. Sin embargo, la mayoría de ácaros poseían haplotipos únicos y los ácaros que se encontraron en el mismo hospedador no estaban más emparentados entre ellos que con los de otros hospedadores.

Distribución de los ácaros de las plumas entre poblaciones de hospedadores

En el Capítulo 4 se encontró que *P. sylviae* estaba presente en todos las localidades estudiadas, mientras que *T. bifurcata* se encontró en el 65% de las localidades. En general, *P. sylviae* alcanzó prevalencias, abundancias e intensidades mayores que *T. bifurcata* dentro de cada localidad. Además, el número de ácaros de ambas especies (tanto abundancia como intensidad) no estaba correlacionado entre poblaciones de currucas, mientras que la prevalencia estaba correlacionada positivamente (aunque de forma débil) entre ambas especies.

Los modelos en los que se analizaron la prevalencia y abundancia de *P. sylviae* explicaron un menor porcentaje de la varianza que en el caso de *T. bifurcata*. Por otra parte, los factores extraídos en el análisis de la intensidad de ambas especies no consiguieron explicar un alto porcentaje de la varianza. En cualquier caso, las variables que jugaron un papel más importante en las diferencias entre los patrones poblacionales de los ácaros fueron la temperatura y la precipitación: las áreas más secas con una estacionalidad marcada ejercieron un efecto negativo en ambas especies de ácaros. Además, la migración tuvo un efecto positivo en la prevalencia de *P. sylviae*.

Conclusiones

1. Los ácaros de las plumas pueden coexistir en el hospedador. *P. sylviae* y *T. bifurcata* son las especies de ácaros encontradas con mayor frecuencia en la curruca capirotada; ambas especies pueden aparecer solas o compartiendo hospedador. El tipo de infestación (simple o múltiple) puede deberse a múltiples factores, que incluyen desde características del hospedador a diferencias ambientales en el hábitat de los hospedadores.

2. Las distintas especies de ácaros ocupan distintas partes del ala. *P. sylviae* ocupa la parte ventral del plumaje mientras que *T. bifurcata* se encuentra en la parte dorsal. Además, las especies ocupan distintas áreas del ala así como distintos sectores dentro de una misma pluma: *P. sylviae* ocupa preferentemente partes medio-externas de las plumas mientras que *T. bifurcata* se encuentra en zonas medio-internas. Por otra parte, el orden de ocupación de las celdas sigue una secuencia distinta en cada especie, de tal manera que las celdas preferidas por una especie son las últimas en ocuparse por la otra.

3. La coexistencia en el mismo hospedador tiene un precio. A pesar de que existe un reparto del hábitat, cuando ambas especies coinciden en la misma celda, se produce una reducción en el número de ácaros. Al considerar el número total de ácaros de cada especie, cuando ambas especies coexisten en el mismo hospedador, *T. bifurcata* parece jugar un papel dominante, ya que su presencia está relacionada con un descenso en el número de ácaros de *P. sylviae*. Sin embargo, *T. bifurcata* parece tener mayores dificultades para colonizar tantos hospedadores y alcanzar tamaños de población tan grandes como *P. sylviae*.

4. El fenotipo del hospedador crea oportunidades y restricciones en la distribución y el tamaño de las poblaciones de ácaros. *P. sylviae* parece estar favorecida por las currucas migradoras, donde alcanza mayor prevalencia y abundancia, mientras que *T. bifurcata* muestra mayor prevalencia y abundancia en las currucas sedentarias. Por otra parte, las currucas sedentarias ofrecen un hábitat propicio para ambas especies de ácaros, donde la coexistencia ocurre con mayor frecuencia. Por último, algunos rasgos de los hospedadores favorecen el incremento de la intensidad de ácaros: la carga de *P. sylviae* se relacionó positivamente con la longitud del ala (la cual es mayor en currucas migradoras), mientras que la carga de *T. bifurcata* se asoció positivamente con el tamaño de la glándula uropigial (la cual es mayor en las currucas sedentarias).

5. Las condiciones ambientales locales crean un mosaico de escenarios a lo largo del rango de distribución del hospedador. Los factores ambientales (sobre todo la temperatura y precipitación) ejercen un impacto en la distribución de los ácaros; temperaturas altas, condiciones de sequía y una estacionalidad marcada afectan de forma negativa a la presencia y a la abundancia de los ácaros en la Península Ibérica. Sin embargo, dichas variables no afectan de la misma manera a ambas especies: *T. bifurcata* no aparece en las regiones más secas, mientras que *P. sylviae* es capaz de establecerse en todas las poblaciones de currucas estudiadas aunque su prevalencia y abundancia disminuyen en las áreas menos favorables.

6. La coexistencia de las dos especies de ácaros puede explicarse por las ventajas que tiene una sobre la otra a distintas escalas. A escala intra-hospedador *T. bifurcata* puede reducir el número de ácaros de *P. sylviae*. Sin embargo, *P. sylviae* es capaz de colonizar tanto currucas migradoras como sedentarias, y alcanza tamaños de población mayores que *T. bifurcata* (tanto dentro de hospedadores como a escalas geográficas mayores). A escala regional, *P. sylviae* es más tolerante frente a las condiciones ambientales que *T. bifurcata*, la cual no aparece en algunas localidades. Este hecho sugiere que *P. sylviae* es una especie más generalista que *T. bifurcata,* la cual parece sufrir mayores restricciones relacionadas con los rasgos del hospedador y las condiciones ambientales.