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ECOLOGY OF A HOST-PARASITE SYSTEM
A STUDY IN TEMPERATE CAVE-DWELLING BATS

SOFIA ISABEL CASTRO GIL LOURENÇO

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BOLSEIRA DA FUNDAÇÃO PARA A CIÊNCIA E TECNOLOGIA NO
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NOTA PRÉVIA

A presente tese apresenta resultados de trabalhos já publicados ou a submeter para publicação, que correspondem aos capítulos 2, 3, 4 e 5. Uma vez que os referidos trabalhos foram realizados em colaboração, e de acordo com o disposto no nº 1. do artigo 41º do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República II série nº 209 de 30 de Outubro de 2006, a candidata esclarece que liderou e participou activamente em todas as fases dos trabalhos, incluindo obtenção, análise e discussão dos resultados, bem como na redacção dos manuscritos.

Lisboa, 20 de Setembro de 2008

Sofia Isabel Lourenço

Aos meus pais e ao Sergio

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Resumo

Os parasitas englobam, segundo algumas estimativas, cerca de metade das espécies conhecidas do planeta. Pela sua vasta distribuição e diversidade, associada ao elevado grau de adaptação ao meio onde vivem (i.e. os seus hospedeiros), o parasitismo pode ser considerado uma das formas de vida mais bem sucedidas. A transição de um estilo de vida livre para o parasitismo trouxe inúmeras vantagens a estes organismos, nomeadamente o uso de um ambiente mais estável, uma maior mobilidade, e a redução dos custos relacionados com várias funções vitais como a digestão e a procura de alimento, que passaram a ser maioritariamente desempenhados pelos seus hospedeiros.

No entanto, no decorrer da evolução para esta forma de vida, os parasitas enfrentaram diversos desafios adaptativos, relacionados com as características intrínsecas dos seus hospedeiros. Neste contexto, a imprevisibilidade espacial e temporal de algumas espécies hospedeiras constitui um dos maiores obstáculos à conclusão do ciclo de vida de um parasita, dado dificultar significativamente a sua dispersão. Paralelamente, estes organismos tiveram de se adaptar à presença frequente de outras espécies de parasitas num mesmo hospedeiro, e explorando os mesmos recursos. Adicionalmente, devido à sua dependência em relação ao hospedeiro para uma reprodução e dispersão bem sucedidas, a maioria dos parasitas necessitou de evoluir de maneira a maximizar a exploração dos recursos do hospedeiro e ao mesmo tempo evitar a sua morte.

A presente tese teve como principal objectivo contribuir para o conhecimento sobre os mecanismos desenvolvidos por alguns ectoparasitas para contornarem estes desafios e se adaptarem com sucesso à vida nos seus hospedeiros. Para tal, usaram-se duas espécies de morcegos de regiões temperadas, o morcego-de-peluche *Miniopterus schreibersii* e o morcego rato-grande *Myotis myotis,* e os seus ectoparasitas como sistemas modelo. Apesar dos morcegos se encontrarem geralmente fortemente parasitados, pouco se sabe sobre a dinâmica e o funcionamento destes sistemas parasita-hospedeiro. Para alcançar o objectivo delineado, foram estabelecidos quatro objectivos específicos, cada qual abordado num capítulo distinto da presente tese.

Capítulo 2. Localizar um hospedeiro

O principal objectivo deste capítulo foi determinar as pistas sensoriais pelas quais os ectoparasitas de morcegos conseguem localizar os seus hospedeiros à distância, de modo a maximizarem a sua dispersão. Para tal, realizaram-se pela primeira vez experiências laboratoriais que testaram as respostas comportamentais de duas espécies de nicteribideos (*Penicillidia conspicua* e *Penicillidia dufourii*) a diversas pistas sensoriais potencialmente emitidas pelos seus hospedeiros primários (*M. schreibersii* e *M. myotis*, respectivamente). Testou-se ainda a capacidade destes parasitas localizarem os seus hospedeiros específicos à distância. Surpreendentemente, observou-se que os nicteribideos se orientaram principalmente por pistas gerais (i.e. dióxido de carbono e temperatura) potencialmente emitidas pela respiração de qualquer espécie de morcego, ao invés de pelos odores corporais emitidos pelos seus hospedeiros específicos. Adicionalmente, ambas as espécies de nicteribideos localizaram mais eficazmente pistas combinadas (e.g. dióxido de carbono associado a temperatura) do que pistas emitidas isoladamente. As experiências revelaram que ambas as espécies de nicteribideos desenvolveram estratégias comportamentais muito semelhantes para localizar os seus hospedeiros à distância. Esta estratégia consiste na detecção de um conjunto de pistas que lhes permite localizarem uma colónia de morcegos na sua proximidade, onde a probabilidade de encontrarem um hospedeiro específico, para o qual possam dispersar, é elevada.

Capítulo 3. Maximizar a reprodução

O principal objectivo deste capítulo foi determinar quais as estratégias reprodutivas usadas pelos ectoparasitas de morcegos para aumentar a sua probabilidade de dispersão. Neste contexto, determinou-se qual a influência de factores ambientais e de factores relacionados com o hospedeiro na reprodução destes parasitas. A actividade reprodutiva dos ectoparasitas de *M. schreibersii* foi determinada ao longo das épocas mais importantes do ciclo de vida deste hospedeiro (i.e. gravidez, criação, acasalamento e hibernação). Este morcego foi maioritariamente parasitado por quatro parasitas ao longo do seu ciclo anual: o ácaro *Spinturnix psi*, os nicteribideos *Nycteribia schmidlii* e *P. conspicua* e a carraça *Ixodes simplex simplex.* Estes parasitas mostraram um padrão reprodutivo bastante semelhante, reproduzindo-se principalmente durante a época de gravidez ou criação do hospedeiro, e em particular nas fêmeas grávidas e em morcegos juvenis. O padrão foi também evidente durante o Inverno, quando as baixas temperaturas das grutas e a hibernação dos morcegos contribuíram para a cessação ou redução significativa da actividade reprodutiva de todos os parasitas. Os resultados permitiram concluir que a reprodução dos ectoparasitas de *M. schreibersii* é fortemente regulada pelo ciclo reprodutivo do hospedeiro, mas também limitada pelas baixas temperaturas presentes nos abrigos durante o Inverno. O clima das regiões temperadas parece impor um constrangimento sazonal à disponibilidade de hospedeiros e consequentemente um desafio sazonal à dispersão dos parasitas de morcegos. Neste contexto, a sincronização da reprodução destes parasitas com o período de reprodução de *M. schreibersii* revela-se uma estratégia eficaz, dado facilitar a sua dispersão na altura de maior disponibilidade de hospedeiros.

Capítulo 4. Coexistir com outras espécies de parasitas

Um morcego alberga geralmente diversas espécies de ectoparasitas que podem eventualmente interagir entre si. O principal objectivo deste capítulo foi identificar possíveis interacções entre estas espécies e averiguar qual a influência destas interacções na estruturação das suas comunidades. Para tal, mediram-se as abundâncias dos quatro ectoparasitas específicos de *M. schreibersii* anteriormente mencionados e os seus padrões de co-ocorrência nas épocas mais importantes do ciclo anual deste hospedeiro (ver capítulo 3). Os padrões de co-ocorrência entre as diferentes espécies foram averiguados através de análises por modelos nulos e da correlação de abundâncias entre todos os possíveis pares de parasitas. Os resultados mostraram que as abundâncias de destes parasitas variaram sazonalmente, sendo consistentemente mais elevadas durante o período de gravidez ou criação do morcego, e em particular nas fêmeas grávidas e nos juvenis. Por seu lado, os padrões de co-ocorrência mostraram que as espécies de parasitas geralmente não interagem entre si, provavelmente devido às suas baixas abundâncias observadas. No entanto, este cenário alterou-se durante o período de gravidez de *M. schreibersii*, quando as duas espécies de nicteribideos (*P. conspicua* e *N. schmidlii*) foram observadas a co-ocorrer nos morcegos grávidos significativamente menos do que o esperado. A causa mais provável para estas interacções é a competição directa por espaço, dado que ambos os nicteribideos vivem em grande proximidade no pêlo dos seus hospedeiros. Os resultados permitiram assim concluir que, em determinadas circunstâncias, elevadas abundâncias de ectoparasitas podem levar à competição entre espécies ecologicamente similares, tendo estas interacções um papel importante na estruturação das suas comunidades.

Capítulo 5. Manter o hospedeiro vivo

O principal objectivo deste capítulo foi avaliar se os ectoparasitas de morcegos afectam a condição corporal dos seus hospedeiros, usando o *M. schreibersii* e o seu ácaro parasita *S. psi* como modelo de estudo. Para tal, mediu-se a condição corporal dos hospedeiros e as suas cargas parasíticas ao longo das quatro fases importantes do seu ciclo de vida (ver capítulo 3). Os ácaros foram menos abundantes durante a hibernação, começando a aumentar na Primavera, até atingir um pico de abundância na época de criação dos morcegos. Durante o período reprodutivo dos morcegos, as cargas parasíticas foram particularmente elevadas nos juvenis e nas fêmeas lactantes. Os resultados permitiram concluir que as variações sazonais na abundância de *S. psi* são controladas pelo ciclo reprodutivo deste ácaro, que por sua vez é regulado pela reprodução do hospedeiro (capítulo 3). Adicionalmente, as fortes correlações negativas encontradas entre as cargas de ácaros e a condição dos morcegos durante a sua época de criação, sugerem que estes parasitas poderão afectar a condição dos seus hospedeiros, embora outras interpretações sejam legítimas para este resultado. Este potencial efeito reflecte-se numa perda de 10% do peso corporal dos morcegos, que poderá ser prejudicial para estes hospedeiros, especialmente porque ocorre num período em que as fêmeas estão sob stress devido ao aleitamento e os juvenis estão sujeitos à pressão de um desenvolvimento rápido. O provável efeito de *S. psi* no seu hospedeiro parece abranger mais do que a sua condição corporal. De facto, é sugerido que a ausência dos morcegos machos das colónias de criação poderá ser uma estratégia comportamental para evitar os potenciais custos do

parasitismo por *S. psi*, sugerindo que o ectoparasitismo poderá desempenhar um papel importante na regulação das populações de morcegos e na determinação da sua organização social.

Em conclusão, a informação recolhida no decorrer da presente tese permitiu conhecer melhor os mecanismos pelos quais os ectoparasitas se adaptaram às características dos morcegos de regiões temperadas. Alguns dos resultados e conclusões discutidos na presente tese poderão ser extrapolados para outros sistemas parasita-hospedeiro envolvendo espécies de morcegos de regiões temperadas.

Palavras-chave: ectoparasitismo, morcegos, coevolução, mecanismos de localização de hospedeiros, estratégias reprodutivas, comunidades de ectoparasitas

Abstract

Parasitism is one of the most successful modes of life displayed by living organisms. The transition to a parasitic lifestyle brought many advantages to parasites, namely a stable environment, mobility and a lesser investment in nutricional functions. However, it also entailed a set of adaptative challenges that had to be met by parasites. These had to find a way of dispersing between hosts often discontinuously distributed in space and time. Moreover, they had to adapt to the frequent presence of other potential competing parasites within the host's body. Lastly, due to their dependence on hosts for successfully reproducing and dispersing, parasites had to balance the exploitation of resources of a host with the need to keep it alive. The main aim of this thesis was to determine how bat ectoparasites have overcome some of these challenges and successfully adapted to their hosts, using two temperate-zone cave dwelling bats (*Miniopterus schreibersii* and *Myotis myotis*) and its ectoparasites as model systems. Results showed that a group of specific bat ectoparasites, the nycteribiids, was able to overcome the spatial unpredictability of its hosts within caves by evolving efficient sensorial mechanisms to locate them from a distance. In addition, some parasitic mites, ticks and nycteribiids were found to deal with the temporal unpredictability of their bat hosts, by maximising their reproduction during the reproductive period of bats, when more hosts were more available and particularly vulnerable. Results also showed that competition is likely to occur among bat parasite species, even if for short-term periods, influencing the structure of their communities. Finally, a suggestion was found of potential costs induced by a parasitic mite on the body condition of its bat host. It is discussed how these potential costs may play a role in the social structure of the bat. Overall, this study provided evidences that bat parasites have tightly coevolved with their hosts. Some of conclusions discussed here are likely to apply to other host-parasite systems involving bats in temperate-zones.

Key-words: ectoparasitism, bats, coevolution, host location mechanisms, reproductive strategies, ectoparasite communities

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Chapter 1

General Introduction

"A walk through the woods or along the creek side reveals the living world as we commonly think about it-the flash of a colourful bird, all the shades of photosynthesis, the near frantic activity of insects as the day warms up…Dwelling inside (an on) most of these organisms are other populations, other communities."

Moore 1993 *in* Combes 2001

1.1. The nature of parasitism

The oldest records of parasites go back several million years to the Cretaceous Period (Klompen & Grimaldi, 2001). Since then, parasites have diversified greatly and nowadays about half of the known species on earth have evolved a parasitic lifestyle (Price, 1980; Windsor, 1998; Poulin & Morand, 2000; Poulin, 2006). Parasitism can therefore be considered as one of the most widespread and successful modes of life displayed by living organisms.

The term “parasite” has its origin on the Greek word *parasitos*, meaning “the one who eats at the table of someone else” (Harper, 2001). It is broadly defined as an organism that lives in close association with another one, living on its resources, and depending on it for the relationships with the external environment (Marshall, 1981). The fact that parasitic organisms divert resources that could otherwise be used for host growth, maintenance and reproduction led many researchers, since early times, to intuitively define parasites as organisms that have a negative impact on the fitness of their hosts. The manner by which parasites profoundly influence their hosts was clearly demonstrated by May & Anderson (1978) and Anderson & May (1978), who argued that an organism should only be classified as a parasite if it had a detrimental effect on the intrinsic growth of its host population. Despite this general perception, not all researchers consider pathogenicity as an essential characteristic of parasitism, since parasitic organisms can at different times be harmful or innocuous depending on the site they inhabit and the stimulus they produce (e.g. Munger & Holmes, 1988; Baker, 1994). Recently, Combes (1995; 2001) added the term “durable interactions” to the concept of parasitism, referring to it as an evolutionary strategy in which organisms usurp resources from other organisms throughout an intimate, long-life relationship. Overall, parasitism can be considered as a relationship that is not discrete but instead merges into other interspecific relationships, and therefore it remains vague to define.

Because of its vague definition, the term “parasite” has been applied to a wide diversity of living organisms, ranging from viruses and bacteria to eukaryotic organisms such as protozoa, helminths (e.g. flatworms, roundworms), arthropods (e.g. lice, mites, fleas) or even some vertebrates such as vampire bats or cuckoos. A dichotomous way for classifying parasites is based on their size. Generally, microparasites *sensu* Anderson & May (1979) are small, multiply within the hosts and have unstable populations (e.g. viruses, bacteria and protozoa), whereas macroparasites are large, do not multiply within their hosts and have stable populations (e.g. helminths, arthropods). Parasites that are confined within the body of hosts are known as endoparasites (e.g. flatworms, roundworms), while those that live on their outer surface are called ectoparasites (e.g. most arthropods, monogeneans). This distinction reflects adaptations made by the parasites to overcome certain barriers to parasitism (Marshall, 1981). Moreover, regarding their relation with the host, parasites can be classified as temporary, when only spending part of their lives in or on their hosts (e.g. ticks, fleas), or permanent (e.g. nycteribiids, mites). They can also be classified regarding their dependence on host resources, as facultative parasites (e.g. most protozoans, some nematodes), when relying on other resources for survival, or obligate parasites (e.g. ticks), when totally depending upon the resources of their hosts (Marshall, 1981; Bush *et al.,* 2001).

Parasites show tremendous variation in the types of life cycles they exhibit, which can be direct (i.e. require one host for completion) or indirect (i.e. require multiple host species for completion). They also developed a variety of mechanisms to disperse between hosts. The extraordinary diversity of parasite adaptations to their environment is probably the reason for their widespread success (Combes, 2001).

1.2. Major challenges for parasites

The transition from a free-living to a parasitic lifestyle brought many advantages to parasites. Among others, they benefited from occupying a more stable environment created by the body of their hosts, since living organisms possess mechanisms to ensure homeostasis or the consistency of a number or psycochemical parameters. Moreover, they acquired a greater capacity to disperse by associating themselves to mobile hosts. Also, by becoming successful in using the resources of their hosts, they decreased the allocation of its own resources to nutricional functions. Indeed, functions such as digestion and locomotion were in some parasites left almost entirely to the host (Marshall, 1981; Combes, 2001; Lehane, 2005).

However, the passage to a parasitic lifestyle entailed a new set of adaptative challenges that had to be met by parasites (Poulin, 2006). In the course of evolution, parasites had to find ways of dispersing between hosts which are generally discontinuously distributed in space and time (Marshall, 1981; Combes, 2001; Poulin, 2006). This constraint led to drastic adjustments in their biology, with natural selection favouring parasites that evolved effective means of dispersing and locating new hosts, and that maximized their reproduction in order to compensate for the enormous losses incurred while locating and establishing within hosts (Marshall, 1981). Moreover, because individual hosts often harbour several parasitic species exploiting the same resources, parasites are likely to interact with parasites of other species, and therefore have to find a way to co-exist or exclude competing species. Finally, despite controversial, it is often considered that, because parasites depend on host resources to reproduce and disperse to new hosts, they face the challenge of exploiting their hosts as much as possible, while at the same time keeping them alive (Combes, 2001).

The selective pressures of a parasitic lifestyle have therefore led to drastic adjustments in the biology of organisms adopting them. A number of questions can be posed on how parasites have evolved to successfully adapt to their hosts: 1) which mechanisms do parasites use to locate their hosts from a distance? 2) how do parasites maximize their reproduction to compensate for high dispersal losses? 3) how do parasites interact with other parasitic species? And 4) how detrimental are parasites to their hosts?

1.2.1. Locating a host

Dispersal may be defined as the process by which individuals escape from their natal environment and move to other locations. It is considered a fundamental aspect of an organism life history because of its significant effect on the dynamics and genetic structure of its populations (Price, 1980). Virtually all species disperse, but this process is particularly important for parasites because of their continual danger of over infecting their hosts (Rea &

Irwin, 1994). Dispersal is often a difficult task for parasites since their hosts are unpredictably distributed in time and space (Combes, 2001). The probability of contact between a parasite and a host (i.e. encounter filter *sensu* Euzet & Combes, 1980) is therefore generally low. Moreover, even when a potential host is found, it may not be totally compatible for a parasite to live and reproduce (compability filter *sensu* Euzet & Combes, 1980) due to a number of host defences such as behavioural and immunological ones (Figure 1).

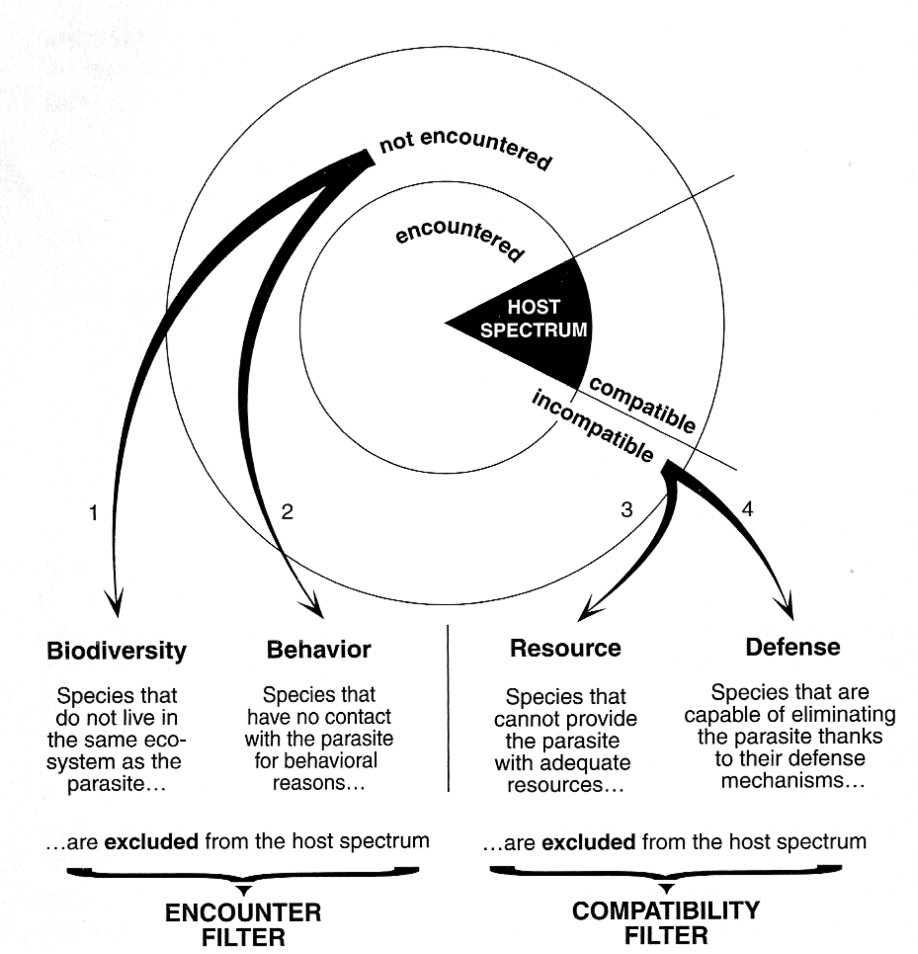

Figure 1. Parasite dispersal (based on the proposal of Euzet & Combes, 1980). The intersection of encountered with compatible hosts constitutes the host spectrum (in black). (Adapted from Combes, 2001).

To compensate for this, some parasites evolved adaptive strategies in which they disperse through vectors, through intermediate hosts, by direct body contact between hosts or even by the use of the same host *habitat* such as nest burrows or roost places (Marshall, 1981; Rea & Irwin, 1994; Bush *et al,* 2001; Combes, 2001). However, for parasites with free-living short-lived stages, dispersal to a new host become one of its most difficult and costly activities, since these had to evolve complex and efficient behavioural strategies to quickly locate suitable hosts from a distance (Lehane, 2005).

The ecology of host-finding behaviour began receiving attention mainly as a mean to develop efficient strategies to control parasites (or its vectors) directly responsible for devastating diseases or significant damages in animal husbandry. The first studies published on this area allowed Sutcliffe (1987) to develop a behavioural framework for host location by parasites, which was recently reviewed by Lehane (2005). These authors postulated that host location consists in a complex series of behavioural responses to environment and host sensory cues that gain momentum as the host is tracked down. A great number of authors have been focusing on the role of host-derived cues in the location behaviour of parasites. They concluded that parasites are able to exploit a wide variety of sensory cues to locate their hosts at some distance, ranging from general cues, delivered by all potential hosts, such as light, vibration, heat, and carbon dioxide (e.g. badger fleas, Cox *et al.*, 1999; chicken mites, Kilpinen & Mullens, 2004; nematodes, Torr *et al.,* 2004; fish lice, Bandilla *et al.,* 2007), to specific cues like particular chemical compounds present in host body odours (e.g. rodent fleas, Krasnov *et al.,* 2002).

Despite the substantial amount of information available on the host location behaviour of parasites, the majority of relevant studies have as subjects a small number of species with economic, medical and veterinary importance. Knowledge in other parasitic groups, namely bat ectoparasites, is still fragmentary and mostly limited to small descriptions in broader autoecology studies of these parasitic species (e.g. Marshall, 1970; Overal, 1980). An accurate assessment of the host location behaviour of bat parasites is therefore essential to understand how some of these species found an effective way to disperse between hosts and complete their life-cycles. This issue is addressed in chapter 2.

1.2.2 Maximising reproduction

For the vast majority of parasites, the transmission between hosts is a time of tremendous loss because of the unpredictability and incompatibility of host resources in their environment (see 1.2.1.). To overcome these barriers and increase their chance of transmission, parasites invested much in reproduction and evolved specialized reproductive strategies, such as more complex life cycles, with intermediate hosts and resistant dispersive free-living stages, and high fecundity (Combes, 2001; but see Trouvé & Morand, 1998). Other parasites have developed a more selective strategy, in which they narrow their reproductive activity to particular periods when the opportunity for transmission is higher. For instance, during periods when hosts aggregate, are particularly vulnerable, or when host progeny appears (Marshall, 1981; Combes, 2001).

Stearns (1992), among other authors, incorporated the reproductive strategies of parasites in the development of life history theories, but empirical studies detailing the factors that regulate the parasitic reproductive processes are greatly missing. An extensive revision of the role of environmental factors in the reproductive processes of parasites was done by Marshall (1981), who provided large evidence of the influence of temperature in the survival and development of all parasitic stages. Nevertheless, the role of host-dependent factors as regulators of parasite reproduction remains largely to be determined.

Regarding the reproduction of parasites of temperate-zones bats, almost nothing is known. Some authors provided contributions on the processes and factors influencing reproduction of some bat ectoparasite species (e.g. Marshall, 1970; Marshall, 1971; Overal, 1980; Christe *et al.,* 2000; Reckardt & Kerth, 2006). However most of these were conducted in parasites of tropical bats, and therefore results cannot be directly extrapolated to temperate-zone species. Chapter 3 addresses the factors influencing reproductive processes of bat parasites, and how these contribute to their effective dispersal.

1.2.3. Coexisting with other parasites

Few species live alone, but instead are part of communities where they are subjected to several types of interspecific interactions (Marshall, 1981). Parasites are no exception. Because of the remarkable number of parasite species (Price, 1980; Poulin & Morand, 2000), individual hosts often harbour several species, providing resources for parasite communities of considerable complexity (Marshall, 1981). These parasites, therefore, may at least periodically have to interact among themselves, and ultimately find a way to co-exist or exclude each other (Poulin, 2006).

The relative frequency of interspecific interactions among parasites, how these affect the structure of parasite communities, and how these may ultimately influence host-parasite interactions, have become central issues in community ecology. Despite its vast interest, it was only in the mid seventies that researchers begun taking a community approach to parasite ecology. The pioneering works in this area were developed by Kisielewska *et al* (1973) and Holmes (1973) with their studies on the structure of helminth communities. Since then, there is vigorous discussion about the importance of interspecific interactions, namely competition, in natural parasite communities. Price (1980) considered that parasite communities are mostly not interactive, with parasite populations only rarely attaining values above the threshold where regulation would be triggered. Many authors (e.g. Mouillot *et al*., 2003; Krasnov *et al*., 2005) agree on his view and provided large evidence that vacant niches (i.e. empty hosts) often exist for parasites, that niches of coexisting parasites are mostly non-overlapping, that parasite densities are often low, and finally that host resources are generally not limiting, overall suggesting that interactions are not important among parasites. On the other hand, some authors contest it, showing that competition and other interactions among parasite species do occur, and play a major role in structuring parasite communities (e.g. Marshall, 1981 and references therein, Holland, 1984; Patrick, 1991).

Studies on parasite communities are common, but on a limited set of parasitic groups (e.g. helminths, monogeneans). Bat ectoparasite communities, for instance, have been widely neglected. Nevertheless, these provide excellent candidates to learn how interspecific interactions shape parasite communities (see 1.3.1). Moreover, such studies can contribute with much information about the ecology of the hosts in which bat parasites are found. Currently, apart from the results of the study by Tello *et al.* (2008), virtually nothing is known about the possible role of species interactions in the structuring of the communities of bat parasites. Chapter 4 provides fundamental data on this issue.

1.2.4. Keeping the host alive

The question of whether parasites affect the health of their hosts has been widely debated for a long time. Until some decades ago, most researchers accepted the theoretical perspective that the most successful parasite in evolutionary terms would be the least pathogenic (Combes, 2001). Behind this assumption laid the fact that parasites totally depend on host fitness to reproduce and disperse, and that if their actions lead to the death of the host, than the parasite would also die. Therefore, it was frequently argued that throughout coevolution, natural selection should have pointed towards lower levels of virulence, which would lead to lower effects on host fitness, opening the possibility to stability in the host-parasite system. However, two early theoretical papers by Anderson & May (1978) and May & Anderson (1978), and many thereafter questioned this assumption. According to them, since the lifetime reproductive success of parasites does not depend upon survival alone, but on the interactions between survival, reproduction and transmission, parasites can indeed have highly detrimental effects on their hosts. Their general assumption was therefore that host-parasite associations did not always evolve towards minimal virulence.

The empirical studies detailing the effects of parasites both in natural hosts and in laboratory model systems are abundant, but equivocal. Indeed, there is a large body of evidence that supports the adverse effects of parasites on their hosts (see examples hereafter), but other researchers demonstrated that in some systems, the presence of the parasite had no detectable effect on the host (e.g. monogeneans on rainbow trouts, Cusack,1986; lice on alpine swifts, Tella *et al.*,1995).

Despite the divergent theories, the effects of parasites on their hosts have been well documented at all levels of the host-parasite interaction. Ecologists have become increasingly aware of the profound affects these organisms have on host communities, regulating host population sizes (Goater & Holmes, 1997), driving host population cycles (Dobson & Hudson, 1992), and mediating host community structure (Minchella & Scott, 1991). At the individual level, parasites can reduce the fitness of their hosts under a diversity of ways, namely influencing host physiological condition (Goater & Holmes, 1997; Brown & Brown, 2004), immune responses (e.g. John, 1994), energy budgets (Lehmann, 1993), and behaviour (Mooring & Samuel, 1999). Parasites also impose a strong pressure upon the overall reproductive success and individual reproductive value of their hosts (Lehmann, 1993; Giorgi *et al.,* 2001), thereby affecting one of the most critical aspects of their life histories.

Despite the large amount of information on this subject, knowledge remains biased towards the medical and veterinary fields and to certain host groups (e.g. birds). Very little is known about the impact of parasitic organisms in bat populations, despite their often heavy parasite loads (but see Lewis, 1996; Christe *et al.,* 2000; Giorgi *et al.,* 2001; Zahn & Rupp, 2004; Lucan, 2006). An objective assessment of the impact of parasites on the fitness of bats is thus important, not only because of the broad range of interesting biological questions related to it, but also because many bat species are presently endangered. Parasites, combined with other biological and environmental factors, may have the potential to affect susceptible bat populations (Altringham, 1999). Chapter 5 provides new data on the effects of ectoparasites on bat hosts.

1.3. Study organisms

1.3.1. Temperate-zone bats as hosts

Bats are the most widely distributed and the second most diverse order of mammals, comprising more than 1000 species worldwide (Altringham, 1999; Hutson *et al.,* 2001). They belong to the order Chiroptera, which is separated into two distinct sub-orders, the Megachiroptera and the Microchiroptera. Megachiropterans include the larger frugivorous bats, while the microchiropterans are much smaller and are generally insectivorous, echolocating to capture their prey (Altringham, 1999).

Flight gave bats the opportunity to become widespread. They can be found worldwide, appart from the highest latitudes of the northern and southern hemispheres, the most inhospitable deserts or some remote oceanic islands (Altringham, 1999; Hutson *et al.,* 2001). The most diverse bat fauna is that of the neotropics of South and Central America (over 200 species), followed by the paleotropical regions of Asia and then Africa. In comparison, temperate-zone regions have a substantially lower diversity, with about 40 species in North America and around 85 species in northern Eurasia (Altringham, 1999).

Bats exhibit a remarkable ecological diversity. They are known to explore a wide range of roosting places, such as holes in trees, rock crevices, caves, mines and artificial structures. Also, they explore diverse foraging niches, as they can be insectivorous, carnivorous, piscivorous, frugivorous, nectarivorous or pollinivorous, although temperate-zone species are mainly insectivorous (Altringham, 1999; Hutson *et al.,* 2001).

Tropical and temperate-zone species differ in their reproductive strategies. Temperate-zone bats typically exhibit a well-marked seasonal cycle (Figure 2), since they are tightly constrained by climate and insect availability during winter (Altringham, 1999). In vespertilionids and rhinolophids, mating occurs mostly in autumn, but sperm is retained within the females throughout the hibernation period, which in southern Europe usually lasts from December to February. Ovulation and fertilization then occur in early spring, when females arise from hibernation. A variant of this pattern is observed in the Schreiber´s bat (*Miniopterus schreibersii*) in which copulation, ovulation, and early development of the embryo occur prior to hibernation, but embryonic development is further suspended until the climate becomes suitable in the spring. Pregnant females start gathering in nursing colonies in spring to give birth and raise their young. At this time, males and non-pregnant females may roost in the same caves, but usually away from nursing colonies (Palmeirim & Rodrigues, 1995).

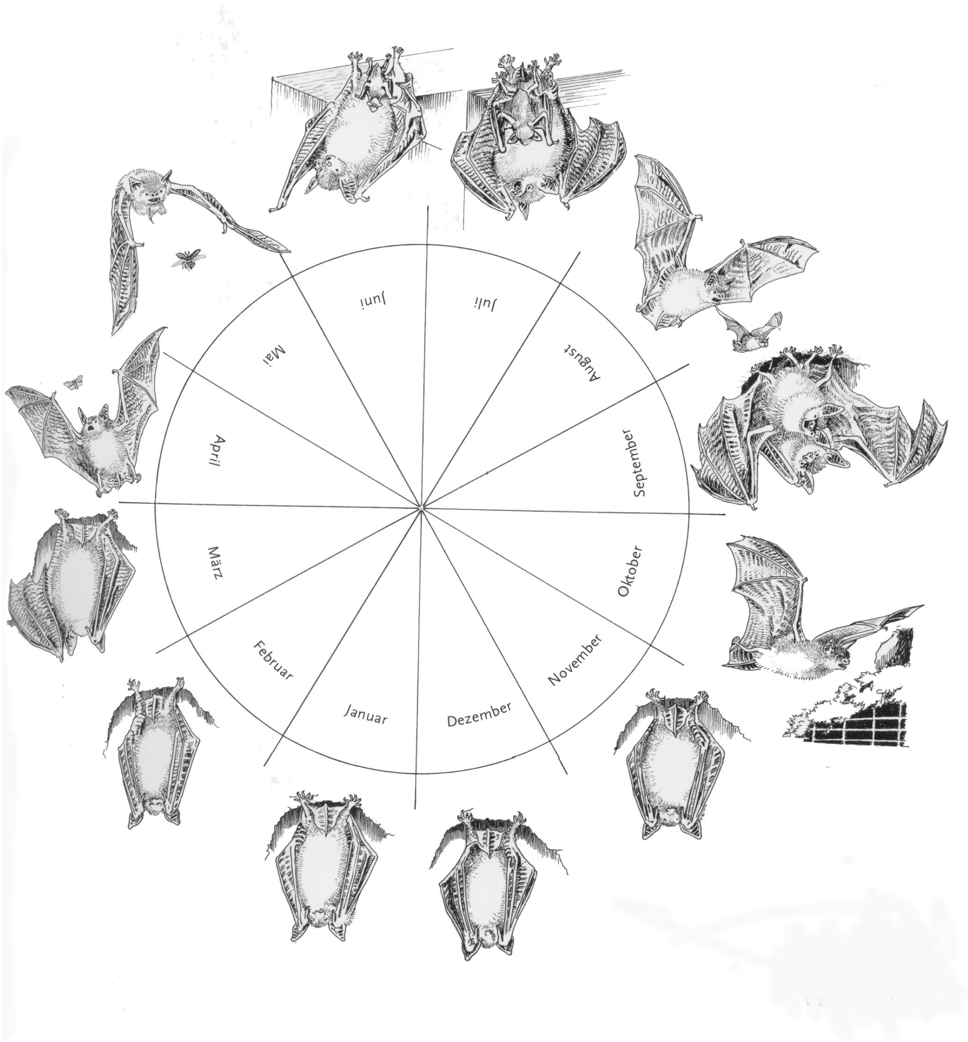

Figure 2. a) Typical life cycle of temperate-zone bat species. *(Adapted from Dietz et al. 2007)*

Temperate-zone bats and their ectoparasites provide exceptional opportunities to study several ecological aspects of parasitism. They are generally highly gregarious, particularly cave-dwelling species, forming large colonies that are known to favour the transmission and reproduction of ectoparasites (Christe *et al.*, 2000; ter Hofstede & Fenton, 2005). This trait may partially explain why bats often host heavy loads and a wide range of ectoparasities (Marshall, 1981; Zahn & Rupp, 2004). Through the course of evolution, most of these bat parasites acquired a high degree of specificity regarding their hosts (Giorgi *et al.,* 2004), and as such, they are probably strongly influenced by the pronounced seasonal cycles of temperate-zone bats. This feature creates an interesting setting to study how the reproductive events of hosts affect parasite reproduction. Moreover, the hibernating habits of temperate bats allow assessing how parasites respond to challenging environmental conditions.

The Schreiber's bat (*Miniopterus schreibersii*) and the Greater mouse-eared bat (*Myotis myotis*) are among the most abundant cave-dwelling species present in Portugal (Palmeirim & Rodrigues, 1992) and they often carry significant loads of ectoparasites. Their colonial nature, along with the fact that there are several known roosts in the country, makes these species particularly suitable for this study.

Schreiber's bat. *Miniopterus schreibersii* (Kuhl, 1817)

The Schreiber's bat (Figure 3) is a microchiropteran traditionally considered as a vespertilionid species included in the subfamily Miniopterinae (Koopman, 1994). However, recent molecular and morphological information have questioned this classification, and strongly support the view that the genus *Miniopterus* is sufficiently distinct from Vespertilionidae to be placed in a separate family, the Miniopteridae (Miller-Butterworth *et al.,* 2005).

The Schreiber's bat is a medium-sized bat typically weighing between 9 g and 16 g (Hutson *et al.,* 2001). Its fur is greyish and very dense, fading to slightly lighter grey in the ventral side. The wings are darker than the fur and its ears are very short and have a characteristic square shape (Palmeirim, 1990).

Figure 3. Schreiber’s bat (Photo: Ana Rainho)

This species was once described as the most widespread of all bat species (Hutson *et al.,* 2001), but its status as a single species or as a species complex has increasingly been debated. While some authors treat the entire *M. schreibersii* complex as a single species with several subspecies (e.g. Wilson & Reeder, 1993), others divide it into several species (Maeda, 1982). According to the molecular data of Tian *et al.* (2004), which corroborates the previous data of skull measurements presented by Maeda (1982), *M. schreibersii* can be divided into three species, namely *M. schreibersii* in Europe and Northern Africa*, M. fuliginosus* in Asia and *M. oceanensis* in Australia. In Europe, *M. schreibersii* is distributed throughout the Mediterranean region (Palmeirim, 1990).

One of the main characteristics of *M. schreibersii* is its cave-dwelling nature, roosting mainly in caves and abandoned mines throughout southern Europe (Palmeirim & Rodrigues, 1992). This species is also known for its strong gregarious behaviour, generally gathering in large colonies during the hibernation and breeding seasons, where it can mix with other species such as *Myotis myotis* (Palmeirim, 1990).

In Portugal, *M. schreibersii* is considered the most abundant cave-dwelling bat species (Palmeirim & Rodrigues, 1992). Because of its strong gregarious behaviour, this species is highly vulnerable to a number of serious threats, the most important of which are the disturbance and destruction of their roosts (Palmeirim & Rodrigues, 1992).The species is currently classified as “Vulnerable” in the Portuguese Red Data Book (Cabral *et al.,* 2005) and as “Near Threatened” by the IUCN (2007).

Greater mouse-eared bat. *Myotis myotis* (Borkhausen, 1797)

The Greater mouse-eared bat is a microchiropteran belonging to the family Vespertilionidae. It has a wide distribution, being present in most Europe, only excluding Iceland and Scandinavia. The lack of records of this species in early Holocene cave deposits of central Europe led some authors to suggest that this bat may have been absent from this region during most of the Holocene, due to the thermal unsuitability of potential underground roosts. In fact, Zahn (1999) suggested that the present wide range of *M. myotis* in Europe is likely the result from a northward expansion due to the availability of man-made roosts.

The greater mouse-eared bat is the largest of European cave-dwelling bats, weighting between 28 g and 40 g (Hutson *et al.,* 2001). The fur is short and dense being greyish-brown on the back, contrasting with the whitish-gray ventral side (figure 4). The face is bare and light in colour and the ears are long and broad, with pointy tragus. The wings are large and broad and grey in colour (Palmeirim, 1990).

Figure 4. Greater mouse-eared bat (Photo: Ana Rainho)

Like *M. schreibersii*, this species is gregarious, forming colonies throughout much of the year, but particularly during the breeding season, when it is sometimes found in mixed colonies with *M. schreibersii*, *Rhinolophus mehelyi* and *Myotis blythii* (Palmeirim & Rodrigues, 1992). It shows distinct ecological habits in central and southern Europe, probably resulting from the distinct environmental conditions in these regions. For instance, while this species is mainly a cave-dweller during its breeding period in southern Europe, it strictly reproduces in buildings north of the Alps (Zahn, 1999).

The Portuguese populations of *M. myotis* are composed of about 10.000 individuals gathered in less than 20 colonies (Cabral *et al.,* 2005). Therefore, the loss of a single roost would mean a significant local decline. This is one of the reasons why this species is considered “Vulnerable” in the Portuguese Red Data Book (Cabral *et al.,* 2005). After a sharp decline between 1950 and 1970, probably due to *habitat* loss and the uses of pesticide, the populations of *M. myotis* in Europe seem to have stabilized, and the species is now considered as “Near Threatened” on a global level (IUCN, 2007).

1.3.2. Bat ectoparasites

The ectoparasites of bats comprise a wide diversity of arthropods, with more than 1400 arachnid species and around 350 insect species described so far worldwide (Estrada-Peña *et al*., 1991a). According to Baker & Craven (2003), among the arachnid class, bats often harbour a diverse number of mites (Orders Astigmata, Mesostigmata, Oribatida and Prostigmata) and ticks (Order Ixodida). Regarding the insect group, bats are parasitized by two families of bat flies (Diptera: Nycteribiidae and Strebliidae), a family of bat fleas (Siphonaptera: Ischnopsyllidae) and a family of bat bugs (Heteroptera: Cimicidae) (Estrada-Peña *et al*., 1991a).

Overall, bat ectoparasites are characterized by a remarkable diversity in their life-cycles, ecology, adaptations and relationships with their bat hosts. While species of some families are obligate parasites, spending their entire life-cycles in the body of bats (e.g. mites), others live in their roosts and are able to survive away from hosts for long periods of time (e.g. cimicids). Also, some families are considered highly host-specific, usually parasitizing a single bat species (e.g. mites, ticks, nycteribiids), whereas other families are generalist, with species parasitizing bats but also other mammal species (e.g. some species of cimicids). Depending on their life-cycle requirements, bat parasites also greatly diverge on the type of bat they use as hosts, with some families mostly associated with bat species dwelling in crevices of trees or buildings (e.g. bugs and bat fleas) and others mainly parasitizing cave-dwelling species (e.g. nycteribiids).

During the present study, a total of 10 haematophagous ectoparasite species were found on *M. schreibersii*, the most common of which were the wing mite *Spinturnix psi* (Acari: Spinturnicidae), the hard tick *Ixodes simplex simplex* (Acari: Ixodidae) and two nycteribiid flies *Penicillidia conspicua* and *Nycteribia schmidlii* (Diptera, Nycteribiidae). The remaining species (i.e. the mites *Spinturnix myoti*, *Macronyssus granulosus* and *Macronyssus longimanus*; the nycteriibids *Penicillidia dufourii and Nycteribia latreilii* and the streblid

Nycteribosca kolenatti) were only sporadically found on *M. schreibersii*, and always in low numbers. The ectoparasite fauna of *M. myotis* was not deeply studied, but a particular nycteribiid *Penicillidia dufourii* was used as a model to answer specificity and host-location questions.

Mites

In terms of both morphology and *habitat*, mites are the most diverse of the arthropods carried by bats. They include obligate blood or lymph-feeding ectoparasites, scavengers of skin or hair debris, and also endoparasites that live within the skin or mucus membranes of body cavities such as the nostrils and mouth (Baker & Craven, 2003). They are also the most abundant ectoparasites on cave-dwelling bats (Zahn & Rupp, 2004).

The mite *Spintunix psi* Kolenati, 1856 (Acari: Mesostigmata, Spinturnicidae) is an obligate parasite which spends its entire lifecycle on the wings and tail membranes of its bat host (Rudnick, 1960). It is a highly host-specific species, exhibiting marked morphological, physiological and behavioural adaptations to *M. schreibersii* (Estrada- Peña *et al*., 1991b; Lourenço & Palmeirim, 2007). These mites are dorso-ventrally flattened and have four pairs of legs equipped with strong claws so that they can easily hold to the smooth hairless wing and tail membranes, even while the bat is flying (figure 5). Their thin chelicerae are adapted for sucking and are primarily used to stab into the wing near a capillary bed. They move poorly when not on these areas, and die within one to two days when off the host (Estrada-Peña *et al*., 1991b).

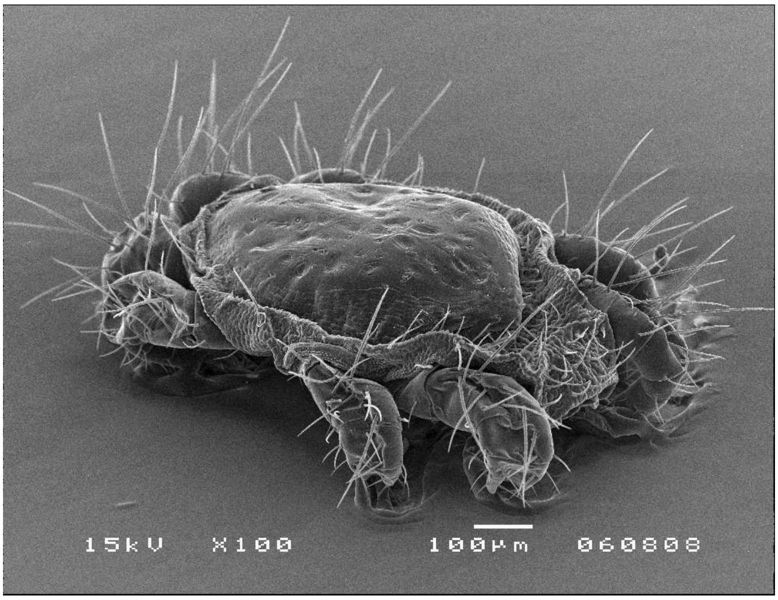

Figure 5. Bat parasitic mite *Spinturnix psi*. Scanning electron microscope

The life cycle of *S. psi* is greatly reduced. It is a viviparous species, with the development of the egg and pre-larval stages occurring inside the female mite (Rudnick, 1960; Evans, 1968). The female then gives birth to a single protonymph, which moults once into a female or male deutonymph with an adult appearance (Evans, 1968). Each type of deutonymph will then moult into its respective sexually mature adult form.

Ticks

Ticks are obligate parasites. They spend part of their life cycle off of the host, and are able to survive for large periods away from it (Oliver, 1989). Despite their diversity worldwide, with more than 850 known species, there is only a small number of ticks exclusively parasitizing bats (Oliver, 1989). Estrada-Peña *et al.* (1991a) mentions five distinct species in the Iberian Peninsula and two more in other parts of Europe, belonging to the families Argasidae and Ixodidae.

The tick *Ixodes simplex simplex* Neumann, 1906 (Acari: Ixodida: Ixodidae) is a highly specific parasite of *M. schreibersii* (Arthur, 1956; Estrada-Peña *et al*., 1991a), being usually found attached around the ears and lower lip of bats, where they cannot be easily reached. It belongs to the *I. simplex* complex: while *I. s. africanus* occurs in Central and Southern

Africa, *I. s. simplex* is widespread in Central and Eastern Europe (Estrada-Peña *et al.* 1991a). The body of *I. simplex* is small and relatively flat, to facilitate host attachment, but it can swell considerably after a large blood meal. Adult ticks have four pairs of legs covered by short spiny hairs and with a claw at the end that allows them to grasp their hosts (Figure 6). Moreover, their mouthparts are well adapted to pierce through the skin of bat hosts. Although there is no detailed information about this particular species, it is likely that *I. simplex* has developed chemosensory, behavioural and physiological adaptations to live off the host and locate it from a distance.

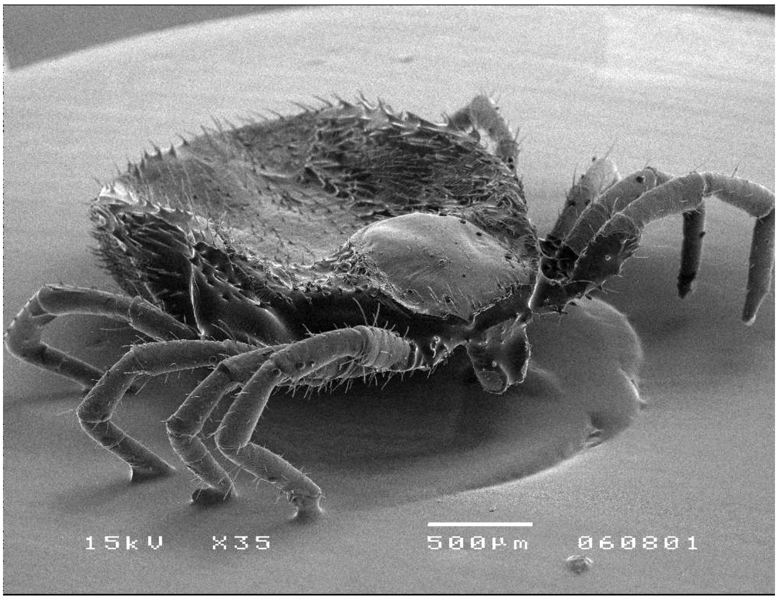

Figure 6. Bat ectoparasitic tick. *Ixodes simplex simplex.* Scanning electron microscope

The life cycle of *I. simplex* is characterized by free-living and parasitic stages. After engorging on large amounts of bat blood, females drop off and deposit hundreds of eggs in a sheltered microenvironment. These will later moult into a 6-legged larva that returns to the bat and, after feeding, moults into an 8-legged nymph (Travassos-Dias, 1994). Nymphs also require a blood meal before moulting into a female or male adult.

Nycteribiids

The Nycteribiidae are a distinctive family of haematophagous ectoparasites exclusively associated with bats (Dick & Patterson, 2006). They include 3 subfamilies, 12 genera, and

275 described species (Dick & Patterson, 2006). Although nycteribiids are found worldwide, they are much more diverse in the tropics than in temperate regions.

Among the Diptera, nycteribiids are considered the most specialized group, exhibiting a remarkable adaptation to their parasitic lifestyle on the fur of their bat hosts (Figure 7). They are wingless, have reduced compound eyes, and have a spider-like appearance adapted to an easy movement through dense pelage (Dick & Patterson, 2006). Their legs and small heads protrude from the dorsal thoracic surface for protection and when feeding, the head rotates nearly 180º forward and downward (Marshall, 1981). In addition, like fleas, nycteribiids possess several ctenidea or combs, which help them to anchor to the hair of bats (Marshall, 1981; Lehane, 2005).

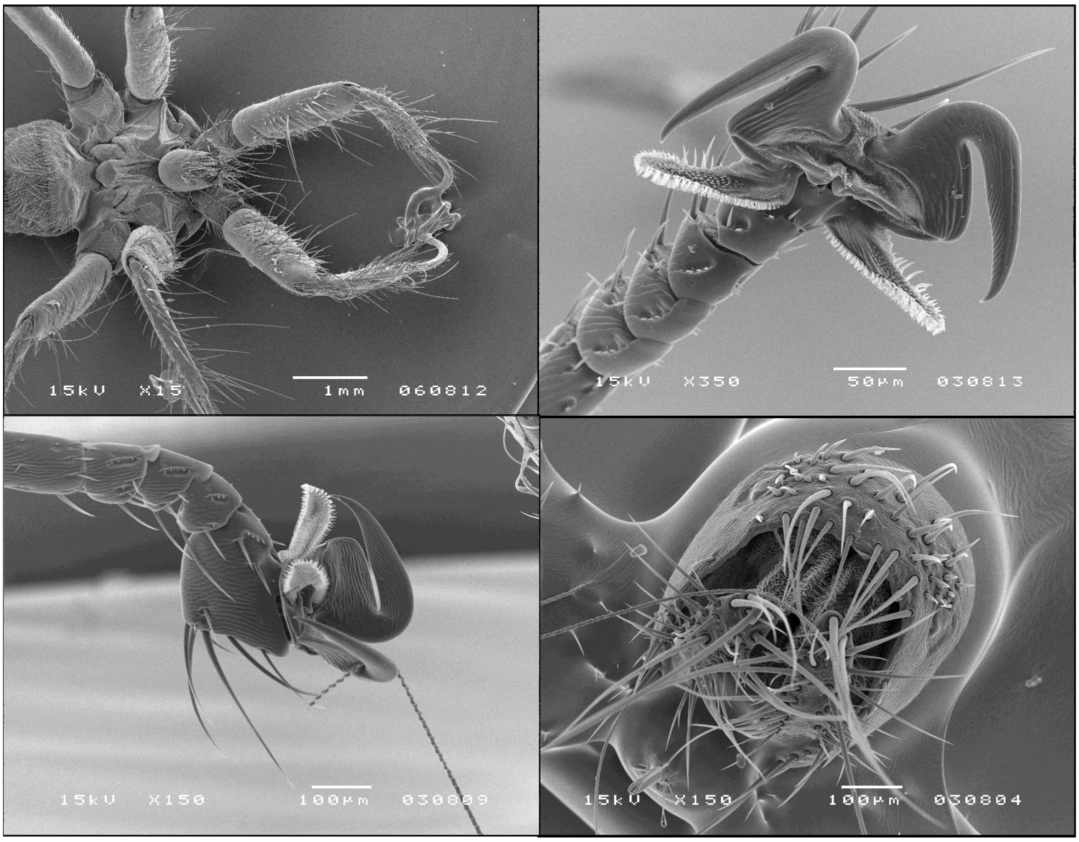

Figure 7. Bat parasitic nycteribiid belonging to the genus *Penicillidia.* Several adaptations to a parasitic life can be observed at distinct parts of the nycteribiid body.

The life cycle of nycteribiids is rather uniform. They undergo adrenotrophic vivaparity (i.e., the complete development of three nymphal instars occurs within the female uterus). Females, which spent most of their lives on the bat fur, leave it to deposit the mature larva on the walls of the roost in order to protect the larva from host-induced mortality (Marshall, 1981; Dick & Patterson, 2006). The larva immediately pupates, metamorphoses, and emerges later as an adult nycteribiid ready to look for a new host (Marshall, 1970).

The nycteribiid *N. schmidlii,* Schiner, 1853 is highly specific, and considered as part of the typical parasitic fauna of *M. schreibersii* (Estrada-Peña *et al*., 1991a). It is a small-sized species, with short legs adapted to its movement under the fur of the host. The nycteribiids *P. conspicua* Speiser, 1901 and *P. dufourii*, Westwood, 1935 are morphologically similar species, characterized by the presence of a pair of ocelli and by atypical large bodies and long legs, which allow them to live mainly on the surface of the fur of the bat (Marshall, 1981). Both species are considered to be host specific; *P. conspicua* has a clear preference for *M. schreibersii*, although it can sporadically be found on *M. myotis* (Estrada-Peña *et al*., 1991a; Lourenço & Palmeirim, 2008a), whereas *P. dufourii* is mostly associated with *M. myotis* and *M. blythii*, although it can sporadically be found on other *Myotis sp.* and on *M. schreibersii* (Lourenço & Palmeirim, 2008a). These three nycteribiid species can often be found cohabiting in the same bat colonies, because their hosts often cluster together (Palmeirim, 1990; Rodrigues *et al.,* 2003).

1.4. Aims and structure of the thesis

This thesis focused on the ecology of ectoparasites of temperate-zone bats. Its main aim was to contribute to the understanding of how some ecological traits of bat parasites have evolved in order to successfully adapt to their hosts. In this context, its general objectives were:

- To determine the range of sensorial cues involved in host location by bat parasites, and their role in a successful parasite dispersal;
- To identify the reproductive strategies of bat parasites to facilitate their dispersal, and the factors that regulate their reproduction;

- To determine if bat parasite species interact among themselves and if these interactions have a role in the structuring of their communities;
- To evaluate if bat parasites are detrimental to their hosts.

This thesis is structured to address the main objectives outlined above. Each of the following chapters (2 to 5) was prepared as a paper to be submitted for publication or already published in a scientific journal. This section summarizes each chapter, evidencing their specific objectives and main methodological approaches.

Chapter 2: Locating a host

How do ectoparasitic nycteribiids locate their bat hosts?

Published in Parasitology (2008) 135: 1205-1213

The major aim of this study was to determine the sensory cues involved in the host location behaviour by nycteribiids. In addition, it was tested whether these parasites were able to discriminate between their primary bat host species and an alternative bat host from some distance. Two nycteribiids (*P. conspicua* and *P. dufourii*) and their primary bat hosts (*M. schreibersii* and *M. myotis*, respectively) were used as model systems.

To conduct this study, bats and their nycteribiids had to be caught in caves and brought to laboratory. The behavioural responses of nycteribiids to a range of sensory cues, namely body heat, vibration and olfactory cues, including carbon dioxide and host specific odours, was experimentally tested. The responses of *P. dufourii* and *P. conspicua* to odour cues and to the bats were tested in a glass Y-tube olfactometer, while the responses of these nycteribiids to heat, vibration and carbon dioxide were tested in an experimental arena. All the experiments were video-recorded continuously and the recordings were used to quantify the responses of nycteribiids to the different cues.

Chapter 3: Maximising reproduction

Which factors regulate the reproduction of ectoparasites of temperate zone cave-dwelling bats?

Published in Parasitology Research (2008) DOI: *10.1007/s00436-008-1170-6*

The main aim of this study was to determine the factors which regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats, using the bat *M. schreibersii* and its ectoparasites as a model. In particular, the potential roles of the reproductive cycle of the bat host and of ambient roost temperature were studied.

To achieve this, the seasonal dynamics of the reproductive activity of all ectoparasites was determined, along with their rates of reproduction on different classes of hosts (i.e. sex, age, and reproductive condition) and at different roost temperatures. Bats were captured during visits to caves during important phases of the host's annual cycle (i.e. pregnancy, nursing, mating, and hibernation). In each of these visits, roost temperatures were measured. All ectoparasites were collected from bats and brought to laboratory, where they were sexed and identified to the species level. Moreover, their reproductive activity was assessed either by the proportion of pregnant parasites (for mites and nycteribiids) or by the proportion of immature stages (for ticks). The differences in reproductive rates of parasites across host groups and seasons and the relationship between reproductive activity and roost temperatures were determined through statistical analyses.

Chapter 4: Coexisting with other parasites

Structuring of the ectoparasite community in bats: evidence for a seasonal role of competition.

This study set out to determine the role of positive and negative interactions among bat parasite species in the structuring of its communities, using the bat *M. schreibersii* and its ectoparasites as a model. The patterns of abundance and co-occurrence of ectoparasite species on hosts were studied over the entire annual cycle.

The patterns of parasite abundances were determined by capturing bats and their ectoparasites seasonally, and analysing ectoparasites in laboratory as described in chapter 3. The co-occurrence patterns along the yearly cycle of the host and on different host groups were tested using null model analysis. Whenever evidences of co-occurrence were found at a particular season or on a particular host group, it became important to determine which species were interacting. This was done through correlations between abundances of all parasite species pairs.

Chapter 5: Keeping the host alive

Can mite parasitism affect the condition of bat hosts? Implications for the social structure of colonial bats

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This study assessed the influence of ectoparasitic loads on the corporal condition of bats, and evaluated parasitic pressure throughout their yearly cycle, using the colonial bat *M. schreibersii* and its parasitic mite *S. psi* as a model.

To achieve this, the host body condition and mite loads were measured throughout the annual cycle of bats, by capturing bats and their mites seasonally, as described in chapter 3. The body condition of all captured bats was measured through the ratio of body weight to forearm length. Mites were brought to laboratory where their loads were described taking into account the parameters of prevalence, mean intensity, median intensity and mean abundance.

Chapter 2

Locating a host

How do ectoparasitic nycteribiids locate their bat hosts?

Lourenço, S.I. & Palmeirim, J.M. 2008. Parasitology 135: 1205-1213.

Abstract

Nycteribiids (Diptera: Nycteribiidae) are specific haematophagous ectoparasites of bats, which spend nearly all their adult lives on hosts. However, females have to leave bats to deposit their larva on the walls of the roosts, where they later emerge as adult flies. Nycteribiids had thus to evolve efficient sensorial mechanisms to locate hosts from a distance. We studied the sensory cues involved in this process, experimentally testing the role of specific host odours, and general cues such as carbon dioxide, body heat, and vibrations. As models we used two nycteribiids (*Penicillidia conspicua* and *Penicillidia dufourii*) and their primary bat hosts (*Miniopterus schreibersii* and *Myotis myotis*, respectively). Carbon dioxide was the most effective cue activating and orientating the responses of nycteribiids, followed by body heat and body odours. They also responded to vibration, but did not orientate to its source. In addition, sensory cues combined (carbon dioxide and body heat) were more effective in orientating nycteribiids than either cues delivered alone. Results suggest that nycteribids have some capacity to distinguish specific hosts from a distance, probably through their specific body odours. However, the strong reliance of nycteribiids on cues combined indicates that they follow these to orientate to nearby multispecies bat clusters, where chances of finding their primary hosts are high. The combination of sensory cues seems therefore an effective strategy used by nycteribiids to locate bat hosts at a distance.

Introduction

Nycteribiid flies (Diptera: Nycteribiidae) are a distinctive family of widespread haematophagous ectoparasites exclusively associated with bats (Marshall, 1970, 1981; Dick & Patterson, 2006). They exhibit a high degree of host specificity, with most species parasitizing a single bat species (monoxenous), or a group of phylogenetically close bat species, usually from the same genus (stenoxenous) (Marshall, 1981; ter Hofstede, 2004; Dick & Patterson, 2006).

Through evolution, these animals acquired a high degree of morphological specialization to their parasitic lifestyle: they are wingless, have reduced compound eyes and dorso-ventrally flattened bodies with combs, which help them to anchor to the hair of bats (Marshall, 1981; Lehane, 2005). Moreover, as with most obligate parasites, the life cycles of nycteribiids became intimately associated with those of their hosts (Lehane, 2005; Dick & Patterson, 2006). Adult individuals spend nearly all their lives on the fur of their bat hosts, where they feed on blood, encounter mates and reproduce (Marshall, 1970, 1981; Lehane, 2005). However, nycteribiids have a viviparous reproduction, and females often have to leave their hosts to deposit a full-grown larva on the walls of bat roosts, one at a time (e.g. Ryberg, 1947; Ching & Marshall, 1968; Marshall, 1970). This larva immediately pupates, metamorphoses attached to the walls, and emerges about four weeks later as an adult fly (e.g. Ryberg, 1947; Ching & Marshall, 1968; Marshall, 1970). This phase of reproduction away from the host required the evolution of effective sensorial mechanisms by nycteribiids to locate and recognize suitable hosts from a distance. However, this may be a complex task because bats are highly mobile and nycteribiids perish quickly when away from hosts (*unpublished personal observations*). Furthermore, because many bat species regularly roost in multi-species aggregations (Palmeirim, 1990), nycteribiids have to be able to distinguish them. Survival of nycteribiids thus largely depends on their ability to efficiently locate suitable hosts in a vast environment and in a limited amount of time.

A substantial amount of information is available on the host location behaviour of some groups of ectoparasites. These are known to exploit a wide variety of sensory cues to locate and recognize their hosts at some distance, ranging from general cues, delivered by all potential hosts, like light (e.g. Humphries, 1968; Mikheev *et al*., 1998; Bandilla *et al.*, 2007), vibration (e.g. Lawrence, 1981), heat (e.g. Wigglesworth, 1941; Meyrowitsch *et al.*, 1991; Kilpinen & Mullens, 2004), and carbon dioxide (e.g. Gillies, 1980; Takken & Knols, 1999; Guerestein & Hildebrand, 2008), to specific cues like particular chemical compounds present in host body odours (e.g. Vaughan & Mead-Briggs, 1970; Osterkamp *et al.*, 1999; Costantini *et al.*, 2001; Krasnov *et al.*, 2002; Smallegange *et al.*, 2005). However, knowledge on the mechanisms involved in host location by nycteribiids is scarce, and mostly limited to small descriptions in broader autoecology studies of these parasitic species (e.g. Ryberg, 1947; Ching & Marshall, 1968; Marshall, 1970). According to these authors, mechanical vibrations, carbon dioxide and heat are likely to play a role in the emergence behaviour of nycteribiids. However, these same authors do not mention what cues are involved in host location after emergence, apart from Marshall (1970) which briefly states that host location is done by random movements.

To our knowledge, this is the first experimental study that attempts to determine how nycteribiids locate their hosts at some distance. For this, we tested the role of sensory cues known to be involved in host searching behaviour by other haematophagous ectoparasites, namely body heat, vibration and olfactory cues, including carbon dioxide and host specific odours. In addition, we tested whether these parasites are able to discriminate between their primary bat host species and an alternative bat host from some distance. As models we used two nycteribiid species from the same genus, *Penicillidia conspicua* and *Penicillidia dufourii*, and their primary bat host species, *Miniopterus schreibersii* and *Myotis myotis*, respectively.

Methods

Study species

The studied hosts are two temperate zone bats, the Schreibers´ bat (*M. schreibersii*) (Kuhl, 1817) (Chiroptera: Miniopteridae) and the greater mouse-eared bat (*M. myotis*) (Borkhausen, 1797) (Chiroptera: Vespertilionidae). Both bats usually carry heavy loads of ectoparasites (Lourenço & Palmeirim, 2007). In the Mediterranean region, they roost almost exclusively in caves and mines, but further north *M. myotis* roosts mostly in buildings (Palmeirim, 1990; Rodrigues *et al.*, 2003). In southern Europe, the two bats are highly gregarious, forming large nursing colonies, where individuals of both species often mix (Palmeirim, 1990; Rodrigues *et al*., 2003).

The nycteribiids *P. conspicua* Speiser, 1901 and *P. dufourii* (Westwood, 1935) (Diptera, Nycteribiidae) are morphologically similar species, characterized by the presence of a pair of ocelli and by atypical large bodies and long legs which allow them to live largely on the surface of the fur of the bat (Marshall, 1981). Both species are considered to be host specific; *P. conspicua* has a clear preference for *M. schreibersii*, although it can sporadically be found on *M. myotis* (Estrada-Peña *et al.*, 1991a; Imaz *et al.*, 1999), whereas *P. dufourii* is mostly associated with *M. myotis* and *M. blythii*, although it can sporadically be found on other *Myotis sp.*, and on *M. schreibersii* (Estrada-Peña *et al.*, 1991a; *unpublished personal observations*). These two parasites species can often be found cohabiting in the same bat colonies, because their hosts often cluster together (Palmeirim, 1990; Rodrigues *et al*., 2003).

Data collection

Bats and their nycteribiids were collected during the spring and summer of 2006 in four roosts of the region of Moura in southern Portugal (38º 08´N, 7º 26´W)_We captured an average of 15 bats of each species per visit to a roost. These were caught with the help of a harp trap placed at the entrance of roosts (Lourenço & Palmeirim, 2007), and under a permit (57/2006/CAPT) issued by Instituto para a Conservação da Natureza e da Biodiversidade. Captures took place early in the morning (~7.00) and bats were released 12 hours later (~19.00), so that they did not miss any foraging night. Each bat was placed in a separate cotton bag, to avoid the mixing of their parasites, and brought to captivity where experiments took place. These hosts had to be held in captivity to prevent starvation from the flies. While in captivity, bats were kept in total darkness under ambient conditions, similar to those of their roosts (~17º C and ~75% relative humidity). Collection of nycteribiids for the experiments was made by directing them into a plastic tube, avoiding the use of forceps, since these can harm their hind legs and hence affect their behaviour. After collection, nycteribiids were sexed and identified. All nycteribiids used in the experiments were adult females and no individual was used more than once.

Experimental setup and testing procedures

We design distinct experiments to test the responses of nycteribiids to potential sensory cues (specific host body odours, heat, vibration, and carbon dioxide), and to test whether they can discriminate between their primary host and an alternative bat host at a distance. All experiments took place between 9.00 and 17.00, since this is the time when nycteribiids deposit their larva on the cave walls and need to locate suitable roosting bats (Marshall, 1970). Experiments were conducted in total darkness and under controlled ambient conditions (~17º C and ~75% relative humidity). The activity of nycteribiids during tests was recorded continuously with a digital camcorder (Panasonic NV-15) using reflected infra-red illumination (not thermal infra-red), which allowed filming without disturbing their behaviour.

Response of nycteribiids to host body odours

To test the responses of *P. dufourii* and *P. conspicua* to body odours of their primary hosts we used a glass Y-tube olfactometer (stem length 10 cm and internal diameter 2cm, each arm length 12 cm and internal diameter 2cm) (Fig. 1).

Air flowed from an aquarium pump into two separate flowmeters (Matheson FM-1000 flowmeter) adjusted to 1000ml/min flow. From there, airflow moved into a stimulus chamber (containing an odour cue) and a control chamber (without an odour cue), and subsequently into two choice arms (Fig. 1). The air then converged into the test arm, at the end of which there was a holding chamber where nycteribiids were placed prior to each test.

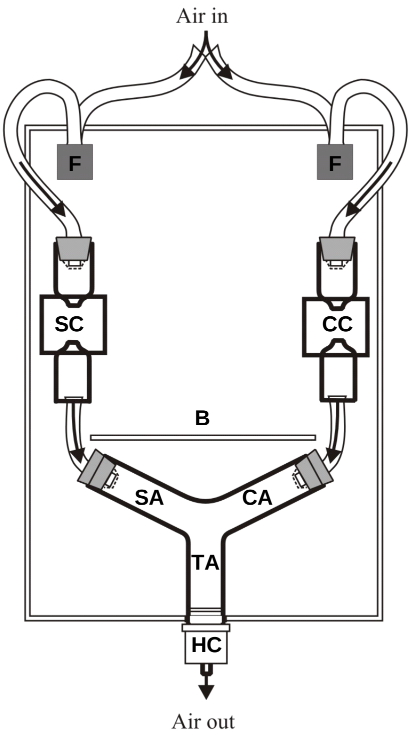

Figure 1. Y-tube olfactometer (adapted from Jackson *et al.* 2000) (not drawn to scale). Arrows indicate the path of air flow. F - flowmeters, SC - stimulus chamber, CC - control chamber, B- opaque barrier to prevent nycteribiids from seeing odour cues, SA - stimulus arm, CA - control arm, TA - test arm, HC - holding chamber.

The olfactometer was surrounded by a white frame to minimize visual distractions from the room (Fig. 1). 10 min before each test begun, the air pump was turned on, and we placed an odour cue - a piece of cotton rubbed on the urine and fur of the host - in the stimulus chamber, and a piece of clean cotton in the control chamber. A single nycteribiid was then placed in the holding chamber and 5 min later the net separating the holding chamber from the test arm was removed. A test began in the moment the net was removed and lasted for 10 min. For each test, we randomly switched the position of the stimulus and control chambers in order to avoid directional biases. After each test, the olfactometer was dismantled and cleaned with 80% alcohol and distilled water (Jackson *et al*., 2002), to prevent any potential influences from traces of previously tested parasites.

Response of nycteribiids to heat

The responses of nycteribiids to heat were tested in an experimental arena (Fig. 2A).

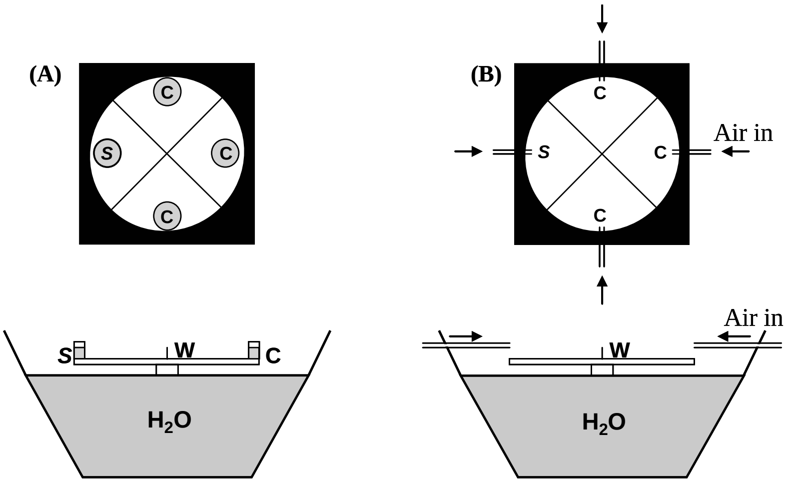

Figure 2. Top and lateral views of the arena used to test the responses of nycteribiids to (A) heat and (B) carbon dioxide. (A) *S*- stimulus container (at 35º C), C- control containers (at room temperature), W- wire. (B) *S*-stimulus tube (with flow of carbon dioxide), C- control tubes (with flow of air), W- wire.

This consisted of a circular glass (30 cm diameter) covered with white paper. The arena was placed inside a black plastic box (50 x 100 cm and 40 cm high) filled with distilled water to prevent nycteribiids from escaping. The plastic box was covered with a black lid fitted with a transparent window through which the arena could be viewed and filmed. The arena was divided in four equal quadrants; one was used as a stimulus quadrant and the remaining as controls (Fig. 2A). The heat cue was simulated using a glass container (6 cm diameter, 7 cm high) filled with water at 35º C (±1) to replicate bat body temperature, and sealed with parafilm ®. Prior to each test, we placed this container, hereafter named stimulus container, in the middle of the stimulus quadrant, and three identical control containers with water at room temperature (~17º C) in the three control quadrants (Fig. 2A). After all containers were positioned in the arena, a nycteribiid was placed at the top of a wire (0.3 cm diameter, 5 cm long) in its centre (Fig. 2A), so that it could choose any direction on its way down. Tests started immediately and lasted for 10 min. The positions of the stimulus and control quadrants were switched between tests to exclude directional bias. After each test, the white paper surface was replaced to prevent any chemical traces of previously tested parasites.

Response of nycteribiids to carbon dioxide

The response of nycteribiids to carbon dioxide was tested in the described arena. We inserted four identical plastic tubes (5 mm diameter) through holes in the outer box, which delivered air to the centre of each quadrant of the arena (Fig. 2B). During tests, the tubes in the control quadrants delivered charcoal–filtered air at 1000ml/min (flowmeters Matheson FM-1000 flowmeter). The tube of the stimulus quadrant delivered air at the same rate, but with a concentration of carbon dioxide above the normal atmospheric concentration of 350 ppm (Gillies, 1980). We tried increasing concentrations until reaching a level to which nycteribiids responded (~2000 ppm above atmospheric concentration). Carbon dioxide was delivered from a pressurized cylinder with outflow pressure regulated by a manometer (RBD-30 Carburos Metalicos), and mixed with charcoal-filtered air in an airtight box. All other test procedures were analogous to those described for the heat tests.

Response of nycteribiids to vibrations

The response of nycteribiids to substrate vibrations was tested in the described arena. The vibration stimulus was a gentle continuous scratching of the edge of one of its quadrants with a piece of wire (0.3 cm diameter, 50 cm long). Each test consisted of a control period of 5 min without any vibration stimulus, and a 5 min period during which the edge of the arena was scratched as uniformly as possible. The position of the scratched quadrant (i.e. stimulus quadrant) varied between tests. All other test procedures were analogous to those described for the heat tests.

Response of nycteribiids to carbon dioxide and heat combined

Bat hosts always provide more than one potential sensory cue simultaneously, such as body heat and exhaled carbon dioxide. To determine how nycteribiids responded to this particular combination of cues we ran experiments in an arena in which we provided them simultaneously. For this, we combined the procedures already described in the heat and carbon dioxide tests. Thus, while the stimulus quadrant delivered a temperature around 35º C and a concentration of carbon dioxide above the normal atmospheric one, the control quadrants delivered room temperature and charcoal–filtered air.

Ability of nycteribiids to discriminate their primary host

We used the olfactometer previously described (Fig. 1) to test whether nycteribiids were able to discriminate their primary host from an alternative bat host at a distance. The methodology was similar to that employed to test the responses to host body odours, but this time we placed two live bats in the chambers of the olfactometer (i.e., the primary bat host species of the tested nycteribiid in one chamber, and its alternative host in the other). Each of these bats thus delivered a combination of the potential cues we tested previously (body odour, heat, and carbon dioxide).

Data analysis

All the experiments were video recorded as described above, and the recordings were used to quantify the behavioural responses of nycteribiids to the different cues. We used the following parameters to quantify the responses: (1) latency of movement (time from beginning of the test to the first movement); (2) latency of choice (time from first movement to the choice of a host); and (3) choice (considered as the arm or quadrant where the nycteribiid spent most time, and at least one continuous minute). The sample sizes (*n*) values given in the figures and in Table 1 only included the individuals that responded to the cues.

We calculated confidence intervals of 95 % for percentages using the Wilson score method (Newcomb, 1998). Chi-square goodness of fit tests (Sokal & Rohlf, 1995) were used to analyse the choices of nycteribiids. Between-species comparisons were analysed with non parametric U-Mann Whitney tests (Sokal & Rohlf, 1995). All statistical analyses were performed using Excel (2002) and SPSS (version 12). P-values ≤0.05 were considered to be statistically significant.

Results

Response of nycteribiids to host body odours

About half of the tested individuals (47 % of *P. conspicua* and 53 % *P. dufourii*) responded to host odours by moving in the olfactometer. In both species, the responsive individuals showed a slight tendency to prefer the arm with the odour cue, although this was not statistically significant (*P. conspicua*, χ^2_1=1.2, d.f. = 1, *P*=0.27; *P. dufourii*, χ^2_1=2.9, d.f. = 1, *P*=0.09; Fig. 3). Both nycteribiid species took a similar amount of time to initiate their movements towards the odour cues (U=249, *P*=0.57), and to choose between one of the arms (U= 212.5, *P*=0.17) (Table 1).

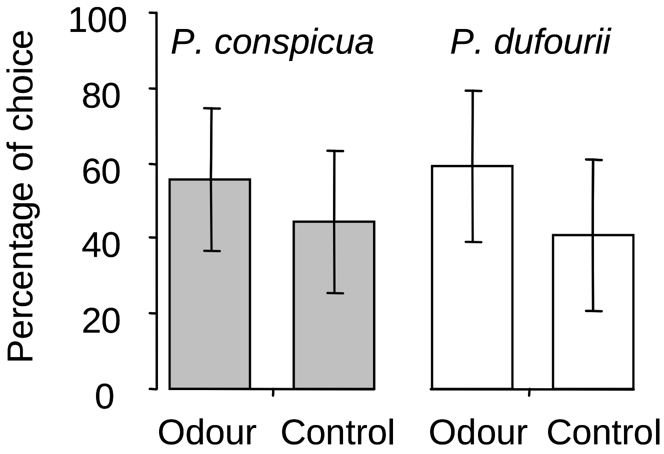

Figure 3. Percentage (± 95% confidence intervals) of *P. conspicua* (*n*=27) and *P. dufourii* (*n*=32) that chose the odour arm versus the control arm in olfactometer tests.

Table 1. Time lag (mean±s.d.) (in sec.) between the beginning of the test to first movement of parasite (latency of movement), and from time of first movement to choice of a host (latency of choice).

Cues	***P. conspicua***			***P. dufourii***		
	Movement	**Choice**	***n***	**Movement**	**Choice**	***n***
Primary host	10± 8	182± 121	42	8± 5	14± 7	39
Host odour	102± 41	173± 108	27	88± 40	143± 114	32
Heat	58± 23	92± 33	29	70± 42	109± 52	24
CO_2	17± 9	10± 7	41	24± 10	21± 14	39
CO_2 + Heat.	11± 7	6± 5	40	13± 10	12± 10	39

Response of nycteribiids to heat

Heat was more effective than odours in stimulating the movements of *P conspicua* (64%) and *P. dufourii* (54%). These nycteribiids were attracted to the stimulus quadrant about twice as often as to any of the control quadrants (*P. conspicua*, χ^2_3=23.7, d.f. = 3, *P*=0.009; *P. dufourii*, χ^2_3=9.4, d.f. = 3, *P*=0.02) (Fig. 4). Both nycteribiids species spent a similar amount of time to start moving towards heat (Table 1), and these responses were quicker than the ones observed to host odours. Likewise, they took similar time to make their choice for a host (Table 1).

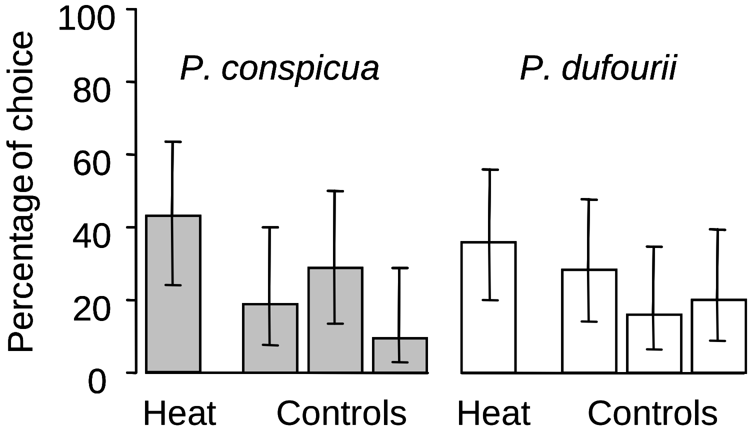

Figure 4. Percentage (± 95% confidence intervals) of *P. conspicua* (*n*=29) and *P. dufourii* (*n*=24) that chose the stimulus quadrant (releasing heat) versus the control quadrants in arena tests.

Response of nycteribiids to vibration

The large majority of *P. conspicua* (80%) and *P. dufourii* (85%) exhibited a behavioural response to vibration, waving their frontlegs up in the air, but remained at the top of the wire. This behaviour was not detected in the absence of the vibration stimulus. Only 22% of *P. conspicua* and 14% of *P. dufouri* exposed to vibrations descended from the wire but remained near its base and did not walk towards any of the quadrants.

Response of nycteribiids to carbon dioxide

Carbon dioxide was effective in activating movements from most tested individuals (*P. conspicua*, 91%, *P. dufourii*, 86.6%). Moreover, the large majority of these were attracted to the quadrant where the increased concentration of carbon dioxide was being released (*P. conspicua*, $\chi^2_3=86.0$, d.f. = 3, *P*=0.001; *P. dufourii*, $\chi^2_3=130.1$ *P*=0.001) (Fig. 5). Additionally, both species were equally fast in activating their movements to carbon dioxide (U=122, *P*=0.44) and when choosing the carbon dioxide quadrant (U=408, *P*=0.45) (Table 1).

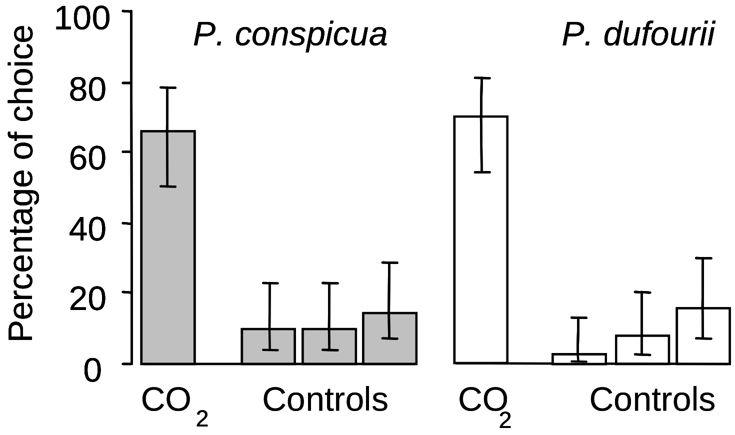

Figure 5. Percentage (± 95% confidence intervals) of *P. conspicua* (*n*=41) and *P. dufourii* (*n*=39) that chose the stimulus quadrant (releasing carbon dioxide) versus the control quadrants in arena tests.

Response of nycteribiids to carbon dioxide and heat combined

The combination of carbon dioxide and heat resulted in a very high proportion of active responses (*P. conspicua*, 93%. *P. dufourii*, 88%). In addition, both species chose the quadrant which delivered heat and carbon dioxide combined far more often than the remaining quadrants (*P. conspicua*, χ^2_3=123.9, *P*=0.0001; *P. dufourii*, χ^2_3= 162.2, *P*=0.002) (Fig. 6). Also, the number of correct choices made by *P. dufourii* and *P. conspicua* when carbon dioxide and heat were delivered combined, increased by about 10%, compared to carbon dioxide delivered alone (Fig. 5,6) and about 100% compared to heat delivered alone (Fig. 4,6). *P. conspicua* and *P. dufourii* showed a similar latency to move (U=334, *P*=0.67), and to choose a quadrant (U=438, *P*=0.54). Both species took less time to respond and to make a choice when both cues were delivered together than when delivered alone (Table 1).

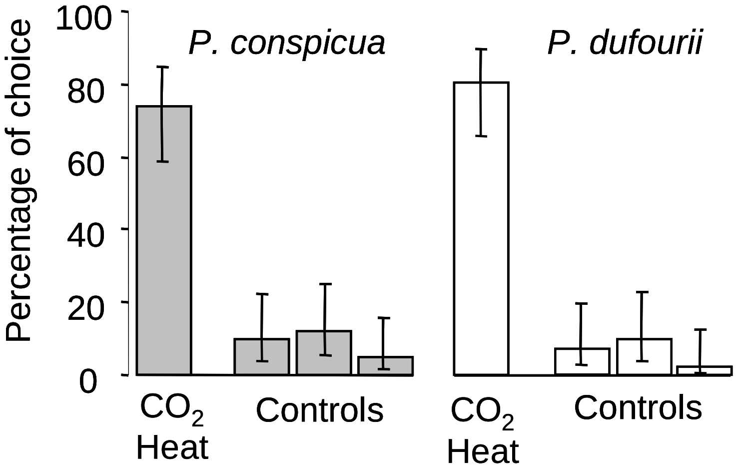

Figure 6. Percentage (± 95% confidence intervals) of *P. conspicua* (*n*=40) and *P. dufourii* (*n*=39) that chose the stimulus quadrant (releasing carbon dioxide and heat) versus the control quadrants in arena tests.

Ability of nycteribiids to discriminate their primary host

All tested individuals of both nycteribiid species were able to choose a host within the time of experiment. However, the rate of correct choices differed between the species (Fig. 7).

Indeed, *P. dufourii* consistently chose the arm of the olfactometer with cues of its primary host, *M. myotis* (χ^2_1=15.22, *P*<0.001). In contrast, *P. conspicua* chose more frequently the arm of its alternative host, although this was not significant (χ^2_1=0.82, *P*=0.36) (Fig. 7). *P. conspicua* and *P. dufourii* were equally fast in moving in the presence of bats (U=674.5, *P*=0.92) (Table 1). However, they differed in their latency of choice (U=98, *P*<0.001), with *P. dufourii* rapidly choosing a bat and *P. conspicua* exhibiting a long exploratory behaviour at the junction of the arms before making a choice (Table 1).

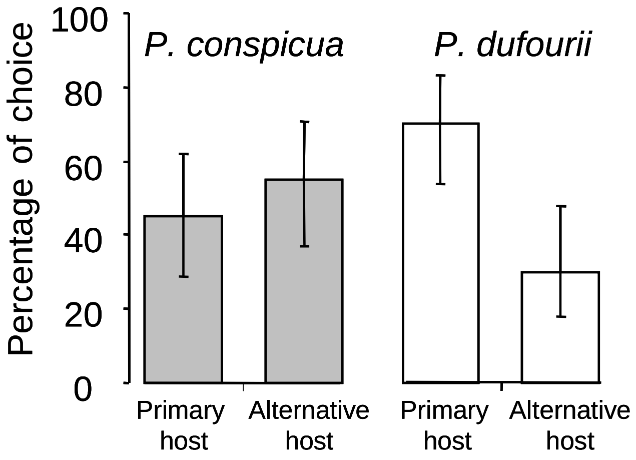

Figure 7. Percentage (± 95% confidence intervals) of *P. conspicua* (*n*=42) and *P. dufourii* (*n*=39) that chose their primary host species versus the alternative species in olfactometer tests.

Discussion

Which sensory cues are involved in host location by nycteribiids at a distance?

Our results suggest that carbon dioxide is the most efficient cue used by nycteribiids to locate their hosts from a distance. This cue on its own promptly activated the movements from both *P. conspicua* and *P. dufourii* (Table 1), and clearly attracted them (Fig. 5). The decisive role of carbon dioxide in the activation and orientation behaviour of haematophagous ectoparasites is widely recognized (Marshall, 1981; Lehane, 2005; Guerenstein & Hildebrand, 2008). This is considered a long-range cue, delivered in high amounts by the breathing of vertebrates, and also through their skin. For example, the human breath contains levels of carbon dioxide of about 45 000 ppm per exhalation, against the atmospheric concentration of 350 ppm (Barrozo & Lazzari, 2004). Our tests revealed that nycteribiids of both species are very sensitive to small increases of carbon dioxide, responding to concentrations as low as 2000 ppm above those of normal atmospheric levels. Lower concentrations did not evoke behavioural responses. This is the first demonstration of the importance of carbon dioxide as a long-range cue for adult nycteribiids.

Although it seems that nycteribiids are able to find hosts using just carbon dioxide to guide them, our results suggest that other cues, such as body heat (Fig. 4) and host odours (Fig. 3) are also be involved in the process. In fact, simulated body heat alone activated the movements in both *P. conspicua* and *P. dufourii*. In addition, they appeared to be attracted to the heated container (Fig. 4). The use of host body heat as a cue by nycteribiids is not surprising, because even though heat is generally considered a close-range stimulus (Lehane, 2005), some other small ectoparasites use it from as far as 2 m (e.g. Wigglesworth, 1941). Furthermore, the thermally stable and homogeneous conditions found in underground roosts are presumably suitable for the use of this sensory cue. Specific host odours were also able to activate the movement of nycteribiids, but were less successful than heat or carbon dioxide at directing them (Fig. 3). Such a weak directional response to the odours of their primary hosts is not in line with findings for other host specific haematophagous parasites (e.g. Vaughan & Mead-Briggs, 1970; Osterkamp *et al.*, 1999; Costantini *et al.*, 2001; Krasnov *et al.*, 2002; Smallegange *et al.*, 2005), including species of the closely related family Streblidae (Overal, 1980). Why don't nycteribiids rely more on specific odour cues, which would orientate them directly to their specific hosts? These two host species, like many other cave bats, often form dense mixed clusters (Palmeirim, 1990), which release large amounts of different sensory cues. We presume that the specific body odours of the various bat species present in these clusters blend, and consequently these might not be such efficient host discriminating cues for nycteribiids. Vibration was the only tested cue that elicited a behavioural response of nycteribids but did not orientate their responses. Marshall (1970) and Ryberg (1947) had already noted that touching the pupa caused the adult nycteribiids to emerge, and suggested that at least the pupae are sensitive to mechanical vibration.

Our results also revealed that nycteribiids rely on a combination of sensory cues to enhance their ability to locate bats. In fact, they responded stronger and more efficiently to the combination of carbon dioxide and heat than to either of the stimuli alone (Figs. 4-6), and table 1). This capacity of nycteribiids to take advantage of cues combined for locating hosts has also been described for many other haematophagous parasites (e.g. Gillies, 1980; Osterkamp *et al.*, 1999; Takken & Knols, 1999; Barrozo & Lazzari, 2004; Smallegange *et al.*, 2005). Lehane (2005) suggested that this strategy increases the certainty of the presence and nature of a host, since one cue alone has a higher chance of not being host originated, and therefore maximizes the chances of host encounter while minimizing energy consumption.

Are nycteribiids able to discriminate their primary hosts from other bats at a distance?

The nycteribids species responded differently when exposed to their primary hosts and an alternative bat in the olfactometer. *P. dufourii* tended to quickly select the side of its primary host (*M. myotis*), while *P. conspicua* spent far more time in exploratory behaviour at the junction of the arms of the olfactometer, and in the end was unable to choose its primary host (*M. schreibersii*). This apparent difference in host location behaviour between the two nycteribiids is surprising, because *P. conspicua* and *P. dufourii* are closely related species and exhibited similar responses to all sensory cues. How can this difference be explained?

Body heat and carbon dioxide are general cues, released by all vertebrates (Lehane, 2005), and therefore have a low potential to allow discrimination between host species. However, hosts with greater body masses or metabolic rates are likely to emit these general cues in larger amounts. Thus, as *M. myotis* has approximately twice the body mass of *M. schreibersii* (Palmeirim *et al.*, 1999), it presumably delivers stronger general cues than the latter. We presume that in the experiments, when *P. dufourii* reached the junction of the arms of the olfactometer, it received from the side of its primary bat host (*M. myotis*) both specific odour cues and a great amount of general cues. All cues combined might have been responsible for the strong attraction of this nycteribiid towards the *M. myotis* side, explaining the high percentage of correct choices and the short time needed to make them.

In contrast, when *P. conspicua* reached the junction of the stimulus and control arms, it probably received contradictory cues: specific odour cues from the side of its primary host (*M. schreibersii*), but stronger general cues from the side of its alternative host *M. myotis*. This conflict may explain why *P. conspicua* did not significantly choose any of the arms and took so much time at their junction. Hence, the most parsimonious interpretation for these results is that nycteribiids have some capacity to discriminate their primary bat hosts from other bats at some distance, probably by their odours. However, specific cues seem to be unable to counter the attraction of general cues combined, to which we found nycteribids to be very sensitive. Nevertheless, these results can not be considered entirely conclusive and the issue deserves further research.

Altogether, our results suggest that in order to find a host, nycteribids initially rely on the combination of several cues, such as carbon dioxide and body heat, rather than only on specific host odour cues. These general cues may orientate them to individual bats or to large multispecies clusters, where the chance of finding their primary hosts is high. Even if nycteribiids do not directly find their primary bat hosts, this may not be a major problem, as they are most likely able to survive on alternate hosts, presumably until they have an opportunity to change to their preferred bat species. We assume that for *P. conspicua* and *P. dufourii* these opportunities might be common, as their hosts often form mixed clusters in southern Europe. In this work, we have only considered the cues important in host location. Once in physical contact with potential hosts, nycteribiids might use different cues. In fact, these may discriminate their primary hosts mainly through specific bat skin emanations, which they likely recognize by tarsal contact (Marshall, 1981).

Chapter 3

Maximising reproduction

Which factors regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats?

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Abstract

We studied the factors which regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats, using the Schreiber's bat (*Miniopterus schreibersii*) and its ectoparasites as a model. For this, we searched 969 bats during 2003 and 2004 at important stages of the bat yearly cycle and found four ectoparasite species regularly occurring on them: two nycteribiids (*Nycteribia schmidlii* and *Penicillidia conspicua*, one wing mite (*Spinturnix psi*) and one hard tick (*Ixodes simplex simplex*). These parasites were present throughout the yearly cycle, but their reproductive activity greatly fluctuated seasonally. Also, we found that sex, age and reproductive status of the host strongly influenced the reproductive activity of parasites. Overall, the four parasite species had a similar reproductive pattern, reproducing more intensively during the pregnancy and nursing seasons of *M. schreibersii*, mainly on pregnant and juvenile bats. Moreover, parasites greatly reduced reproductive activity during winter, while bats were in deep torpor or hibernating. We conclude that reproduction in ectoparasites of *M. schreibersii* and of many other temperate cave-dwelling bats is mostly regulated by the reproductive cycle of their bat hosts, but also limited by roost temperatures during winter. The fact that the reproductive cycle of parasite species of such distinct taxonomic groups are similarly adjusted to that of their bat host, suggests that this adjustment is a highly adaptive trait.

Introduction

The intimate association which evolved between parasites and their hosts is reflected in a number of key parasitic features, for instance their specialized reproductive biology (Marshall, 1981; Combes, 2001). Reproduction is the most energetically demanding phase of a parasitic life cycle (Combes, 2001; Tinsley, 2004), and since parasites entirely depend on the amount and quality of nutrients provided by their hosts, it is expectable that host-dependent factors play an important role in regulating the parasitic reproductive processes. Indeed, the reproductive activity of parasites has been shown to be greatly affected by the general quality of their hosts, on the basis of host age, size, diet, immune status or condition (e.g. Ito *et al.*, 1986; Crompton, 1987; Quinnell, 1988; Poulin, 1996; Tsai *et al.*, 2001; Seppälä *et al.*, 2008). On the other hand, because hosts are often scarce or widely distributed, parasites had to evolve a number of strategies to reproduce successfully, for instance, a high reproductive rate that compensates for their enormous losses while locating and establishing within a host (Kennedy, 1975; Combes, 2001; Tinsley, 2004). Other parasites, however, have developed a more selective strategy in which they reproduce in low numbers, but in particular periods during which their hosts aggregate or are particularly vulnerable (Kennedy, 1975; Marshall, 1981). The life cycle of such parasites is thus adjusted to those of their hosts, so that the infective stages are produced or released when hosts aggregate or when their progeny appears (Kennedy, 1975; Marshall, 1981).

Parasite reproduction can also be regulated by environmental conditions, particularly ambient temperature (Marshall, 1981; Combes, 2001; Lehane, 2005). Among other features, temperature is known to influence the copulation behaviour of parasites (e.g. Yuval, 2006; Bartonička & Gaisler, 2007), the deposition and development of its immature instars (e.g. Marshall, 1970; Overal, 1980; Tinsley, 2004; Reckardt & Kerth, 2006; Shan *et al.*, 2006; Bartonička & Gaisler, 2007; Minato *et al.*, 2008) and adult emergence (e.g. Marshall, 1970). This influence may be particularly relevant in temperate zones, where temperature substantially fluctuates throughout the seasons (Marshall, 1981).

Despite the increasing interest in the reproductive strategies of parasites, knowledge on the factors regulating these remains scarce, and generalizations are based on studies of a few parasite groups. Moreover, most data available comes from laboratory studies. In this context, temperate zone bats and its ectoparasitic fauna provide a valuable model-system to study parasite reproduction and the factors regulating it under natural conditions. Bats are parasitized by a wide variety of ectoparasitic groups including mites and ticks (arachnid: Acari), fleas (Siphonaptera), bat-flies (Diptera, Nycteribiidae and Streblidae) and bat-bugs (Hemiptera, Cimicidae). Most of these ectoparasites (hereafter designated as parasites) are exclusively associated with bats, and therefore strictly depend on these hosts for reproducing. In addition, temperate-zone bats exhibit a defined seasonal cycle, characterized by a well-marked reproductive season (Altringham, 1999), which allows determining if parasite reproduction is in part regulated by the reproductive cycle of the bat host. Finally, temperate bats generally hibernate or enter deep torpor in cold roosts during winter, which likely exposes their parasites to particularly challenging environmental conditions. As far as we know, only few authors mentioned the relative role of host reproduction or ambient temperature in regulating the reproductive activity of bat parasites (e.g. Ryberg, 1947; Marshall, 1970; 1971; Christe *et al.*, 2000; Reckardt & Kerth, 2006; Bartonička & Gaisler, 2007).

The main objective of this study was to understand which factors regulate the reproduction of ectoparasites living on temperate zone bats, using the cave-dwelling Schreiber’s bat (*Miniopterus schreibersii*) and its ectoparasites as a model. We studied the seasonal dynamics of parasite reproduction, and determined their rates of reproduction on different classes of hosts (i.e. sex, age, and reproductive condition) and at different roost temperatures. Using this information, we tested the potential roles of the reproductive cycle of the bat host and ambient roost temperature in the regulation of parasite reproduction.

Methods

The model system

The Schreiber's bat *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Miniopteridae) is a highly gregarious cave-dwelling species (Lourenço & Palmeirim, 2007). Its life cycle, like those of most temperate zone bat species, is characterized by well-defined seasons (Altringham, 1999). Mating occurs in autumn and fertilization soon follows, but embryonic development is delayed during the period of hibernation. In the Mediterranean region, these bats usually hibernate together in tight clusters from December to February. In late spring, pregnant females form large nursing colonies, where they give birth to a single offspring. Males and non-pregnant females usually roost within the same caves, but away from these large colonies (Rodrigues & Palmeirim, 2008).

In Southern Europe, *M. schreibersii* can harbour a wide range of haematophagous ectoparasites, the most common of which are two nycteribiid flies (Diptera, Nycteribiidae) *Penicillidia conspicua* Speiser, 1901 and *Nycteribia schmidlii*, Schiner, 1853, one wing mite (Acari, Mesostigmata, Spinturnicidae) *Spinturnix psi* Kolenati, 1856, and one hard tick (Acari: Ixodidae) *Ixodes* (Eschatocephalus) *simplex simplex* Neumann, 1906 (unpublished personal observations). All these parasitic groups have distinctive life cycles.

Both nycteribiid species spend most of their adult lives in the fur of *M. schreibersii*, and they are host specific; *N. schmidlii* is part of the typical parasitic fauna of *M. schreibersii* and *P. conspicua* is only accidentally found on bats of other species (Balcells, 1968; Imaz *et al.*, 1999; Estrada-Peña *et al.*, 1991a, Lourenço & Palmeirim, 2008a). Nycteribiids undergo adrenotrophic vivaparity (i.e., the complete larval life cycle occurs within the female uterus), and have a single offspring which develops though three nymphal instars (Marshall, 1981). Females leave the host to deposit the third instar on the walls of the roost where it pupates within minutes of deposition (Marshall, 1981). After 3-4 weeks, new nycteribiids emerge from the pupa and look for a new host. Their life cycle is of about one month, allowing up to 12 generations per year in the tropical regions (Marshall, 1970).

The mite *S. psi* spends its entire life on the wings and tail membranes of *M. schreibersii* (Rudnick, 1960), and exhibits a marked morphological and physiologically adaptation to its primary bat host (Estrada-Peña *et al.*, 1991a; 1991b; Imaz *et al.*, 1999; Lourenço & Palmeirim, 2007). It is viviparous, with the development of the egg and pre-larval stages taking place inside the female mite (Rudnick, 1960; Evans, 1968). The female then gives birth to a single protonymph, which molts once into a female or male deutonymph with an adult appearance (Evans, 1968). Each type of deutonymph will then molt into its respective sexually mature adult form.

The tick *I. simplex* is considered highly specific of *M. schreibersii* (Arthur, 1956; Estrada-Peña *et al.*, 1991a), and is normally found around its ears and lower lip. After engorging on bat blood, females drop off and deposit hundreds of eggs in a sheltered microenvironment. These will later moult into a 6-legged larva which after feeding moult into an 8-legged nymph (Travassos-Dias, 1994). Nymphs reattach and feed, later moulting into a female or male adult.

Data collection

The present study was conducted during 2003 and 2004 in several caves of Southern Portugal. We mostly visited the same four caves throughout the study, as they tended to harbour bats throughout most of their annual cycle. However, on some occasions, bats moved to alternative roosts, so using knowledge of the movement patterns of *M. schreibersii* in the south of Portugal (Palmeirim & Rodrigues, 1995), we sampled these roosts instead. We captured the bats and its associated ectoparasites on a seasonal basis, at important phases of the host's annual cycle, namely late pregnancy (April–May), nursing (June–July), mating (October–November) and hibernation (December–February) (Lourenço & Palmeirim, 2007). Usually, we captured bats with a harp trap at cave entrances, but during the hibernation season we captured them by hand in the roosts. Captured bats were placed individually in clean cotton bags to avoid ectoparasites contamination (i.e., assignment of ectoparasites to the wrong host). Details about handling of bats can be found in Lourenço & Palmeirim (2007).

We visually inspected bats (i.e. wing and tail membranes, ears, face and fur) for ectoparasites, which were collected with tweezers. While this procedure is effective for collecting ticks and mites which are easily seen, it is unsuccessful when collecting nycteribiids since they move very quickly through the bat fur. Thus, to assure that these parasites were collected, we sprayed bats with a non-toxic insecticide for vertebrates (Selamectin) and kept them in their bags for 30 min, after which they were inspected again and released in their roosts. Each bag was later searched for dead parasites, which were placed in vials containing glycerine and 70% alcohol.

All ectoparasites were sexed and identified to the species level. Mites had to be cleared and mounted before identification, which was done following the protocol described in Dias (1982). All identifications were done in a compound light microscope, with the help of descriptions of several authors including Theodor & Moscona (1954) for the nycteribiids, Travassos-Dias (1994) and Arthur (1956) for the ticks, and Radovsky (1967) for the mites. Moreover, we determined the development stages (i.e. immature instars, adults) and reproductive status of all collected parasites.

To determine a possible relation between the reproductive activity of parasites and roost temperatures, we measured cave temperatures in each visit to a roost. These were taken near the bat roosting areas using a digital thermometer with a precision of 0.1 ºC.

Statistical analyses

We assessed the reproductive activity of parasites using the proportion of pregnant parasites found on bats. We easily identified pregnant nycteribiids and mites (i.e. pregnant nycteribiids were identified by the round swelling of the abdomen, and pregnant mites by observing the cleared specimens with a microscope). Pregnancy in ticks was not as easy to determine, so we assessed the reproductive activity of ticks using the proportion of its immature stages (i.e. larvae and nymphs) found on bats. The reproductive activity of the parasite species was determined for different seasons and for different sexes, ages and reproductive status of bat hosts. The 95% confidence interval for all proportions was calculated using the Wilson score method (Newcombe, 1998). We tested for differences in reproductive rates of parasites across seasons, sexes, ages and reproductive status of hosts using Chi-square goodness of fit tests (Sokal & Rohlf, 1995).

The relationship between reproductive activity of parasite species and roost temperatures was investigated using the Spearman's rank correlation coefficient (rs) (Sokal & Rohlf, 1995). Statistical analyses were performed using Excel (version 2002) and SPSS (version 13). P-values ≤0.05 were considered to be statistically significant.

Results

A total of 969 bats were searched for parasites during 25 visits to caves. In total, we identified 11 ectoparasitic species, of which only four were commonly found parasitizing bats throughout their entire annual cycle: the nycteribiids *N. schmidlii* and *P. conspicua*, the mite *S. psi*, and the hard tick *I. simplex*. The remaining species were only sporadically found on *M. schreibersii* in low numbers, so they were excluded for further analysis.

At which roost temperatures do parasites reproduce?

The roosting areas occupied by bats within caves show a great thermal inertia, with temperatures ranging from 8 to 15ºC during the entire study period (Fig. 1). Temperatures registered in the bat roosting areas during the winter visits to caves were the lowest, varying between 8 and 11º C, but all temperatures registered during the remaining seasons were above 11º C. We found that the reproductive activity of all parasite species ceased or was greatly reduced in roosts with temperatures bellow 11ºC. In contrast, parasites reproduced with roosts temperatures between 11ºC and 15ºC (Fig. 1). Additionally, excluding temperatures bellow 11ºC (i.e. during winter), the reproductive activity did not significantly increase with rising temperatures for any of the parasite species (Spearman rank correlation: *S.psi* r_s= 0.48, *n*=19, *P*=0.07; *N. schmidlii* r_s= 0.33, *n*=19, *P*=0.15; *P.conspicua* r_s= -0.30, *n*=19, *P*=0.16; *I. simplex* r_s=-0.09, *n*=19, *P*=0.68). In fact, as we can observe in Fig. 1, the reproductive activity of parasites varied widely within the short-temperature intervals considered.

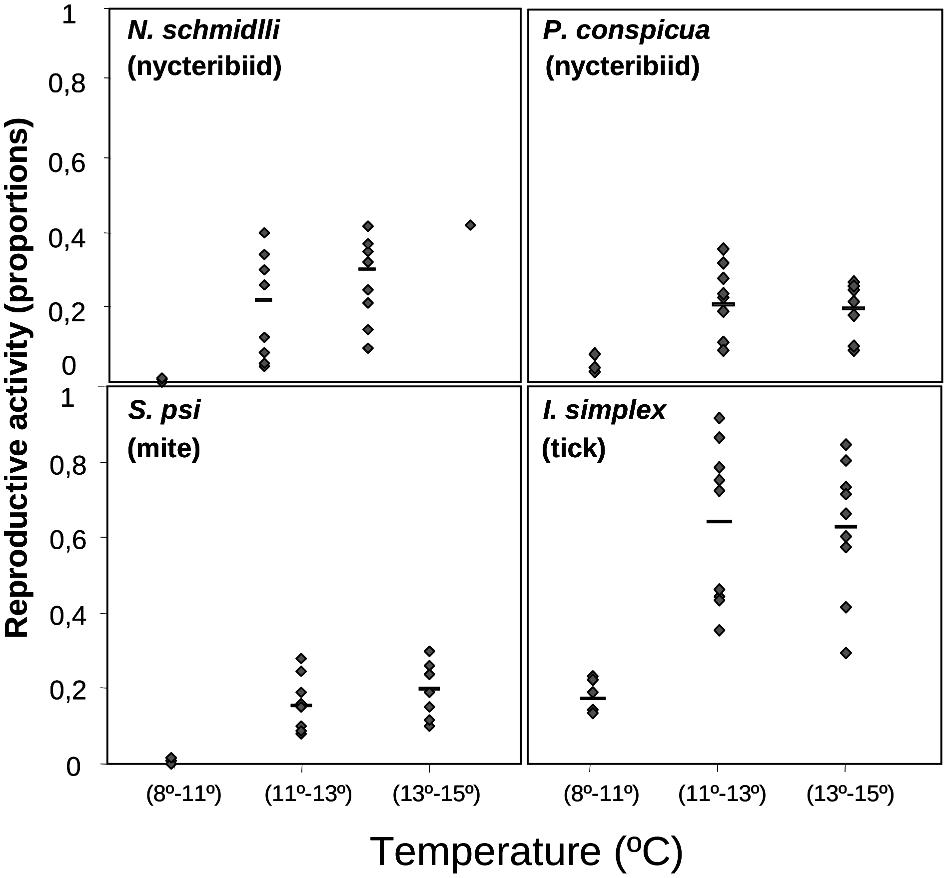

Figure 1 Reproductive activity of the four parasite species in relation to roost temperatures (at intervals of two degrees celsius). Each dot represents the proportion of pregnant parasites (for *S.psi, N. schmidlii* and *P. conspicua*) or immature stages (for *I. simplex*) observed in a visit to a cave (*n*=25), to which there is a correspondent temperature (some dots are overlapping). Horizontal bars represent means

When and on which bat hosts do parasites reproduce?

The mite *S.psi*

Pregnancy rates varied greatly throughout the yearly cycle (χ = 20.6, d.f.= 2, *P*=0.01). We found pregnant mites during most of the year, but not in the bat hibernation season (Fig. 2). The first pregnancies were observed in the spring, mostly on pregnant hosts (χ = 15.7, d.f.=2, *P*=0.01). The highest proportion of pregnant mites was observed during the nursing season of bats, particularly parasitizing flying juveniles (χ =27.9, d.f.=2, *P*<0.001). Overall, mite reproduction during this season was double that of other seasons (Fig. 2). The numbers then declined towards autumn, at which point the proportion of pregnant mites on male and female bats did not differ (χ= 0.8, d.f.=1, *P*=0.34).

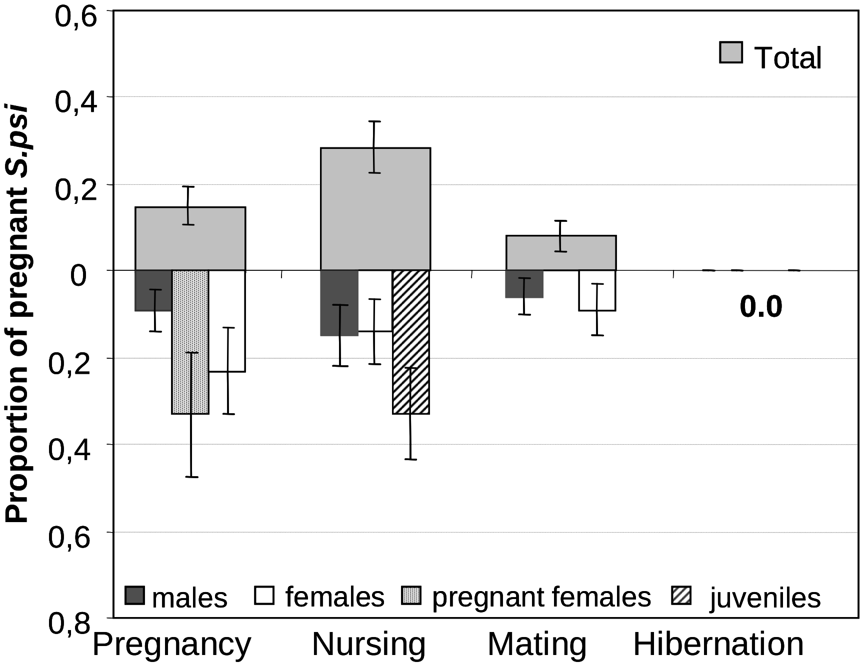

Figure 2. Seasonal variation in the proportion of pregnant *S. psi* (with 95 % confidence intervals). Upper columns represent the total proportion of pregnant mites found in each season. Lower columns represent the proportion of pregnant mites in each host group. (*adapted from Lourenço and Palmeirim 2007).*

The nycteribiid *N. schmidlii*

The rate of pregnancies of *N. schmidlii* showed considerable seasonal variation (χ=63.7, d.f.=2, *P*<0.001). We recorded pregnant individuals throughout the yearly cycle, except during hibernation (Fig. 3). During the host pregnancy season, about a third of all *N. schmidlii* females were pregnant, and these were mainly concentrated on pregnant bats, followed by non pregnant females bats (χ=24.3, d.f.=2, *P*<0.001). *N. schmidlii* reproduction then grew slightly during the bat nursing season, with pregnant nycteribiids mostly occurring on juvenile bats (χ =15.2, d.f.=2, *P*=0.01). Pregnancies felled markedly in autumn, when only around 10% of these nycteribiids were reproducing, on both male and female bats (χ =1.1, d.f.=1, *P*=0.31) (Fig. 3).

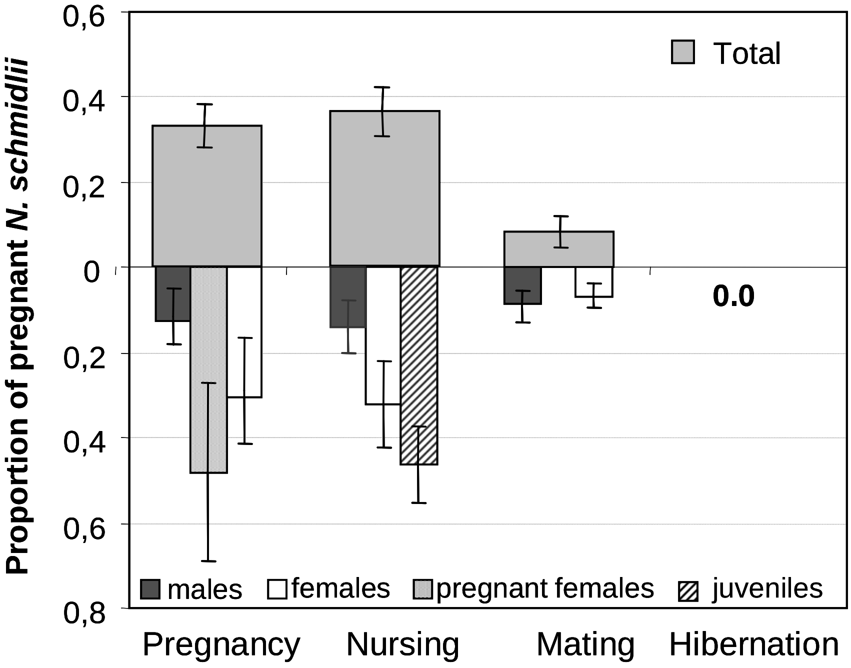

Figure 3. Seasonal variation in the proportion of pregnant *N schmidlii* (with 95 % confidence intervals). Upper columns represent the total proportion of pregnant nycteribiids found in each season. Lower columns represent the proportion of pregnant nycteribiids in each host group.

The nycteribiid *P. conspicua*

We found pregnant *P. conspicua* throughout the entire yearly cycle, but their proportions varied significantly (χ =40.7, d.f.=3, *P*<0.001). The lowest numbers were found when bats were hibernating, and at this time the rates of pregnancies on both sexes of the host did not differ (χ =0.2, d.f.=1, *P*=0.67) (Fig. 4). The proportion of pregnancies then greatly increased during the host pregnancy season, particularly on pregnant and non-pregnant female bats (χ =9.1, d.f.=2, *P*=0.07). After that, pregnancies declined slightly during the nursing season of bats, becoming mostly concentrated on juvenile bats (χ =19.8, d.f.=2, *P*<0.001) (Fig. 4). Reproduction was much less frequent during the host mating season, and almost nil during the winter.

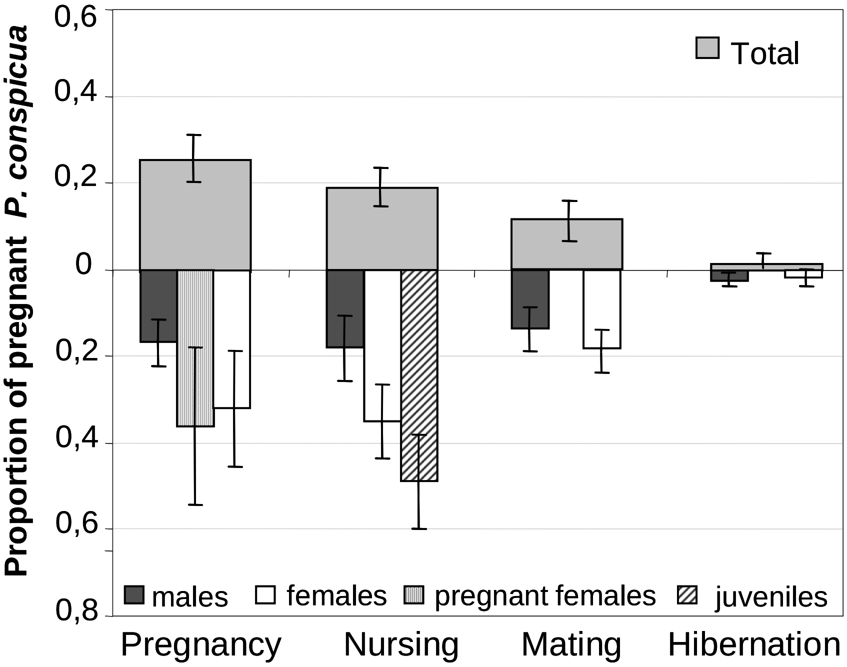

Figure 4. Seasonal variation in the proportion of pregnant *P. conspicua* (with 95 % confidence intervals). Upper columns represent the total proportion of pregnant nycteribiids found in each season. Lower columns represent the proportion of pregnant nycteribiids in each host group

The tick *I. simplex*

We found both larvae and nymphal stages of *I. simplex* throughout the entire yearly cycle, but their proportion varied significantly along it (χ =115.6, d.f.=3, *P*<0.001) (Fig. 5). Both larvae and nymphs occurred simultaneously, and were more abundant during the pregnancy and nursing seasons of bats, when they were mainly found on females (pregnant and non-pregnant) (χ =6.4, d.f.=2, *P*=0.04), and juvenile bats (χ =23.3, d.f.=2, *P*<0.001), respectively (Fig. 5). The proportion of larvae and nymphs then significantly decreased during the mating season, when they were concentrated on male and female bats (χ =4.8, d.f.=1, *P*=0.06). The lowest numbers of immature stages were observed while bats were hibernating, with similar proportions of larvae and nymphs occurred on male and female bats (χ =1.9, d.f.=1, *P*=0.16).

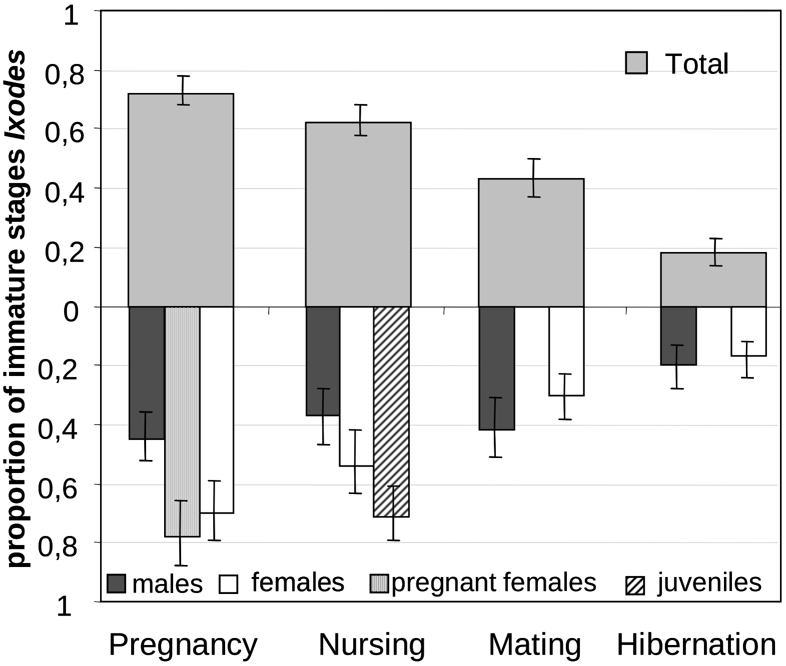

Figure 5. Seasonal variation in the proportion of immature stages of *I. simplex* (with 95 % confidence intervals). Upper columns represent the total proportion of larvae and nymphs found in each season. Lower columns represent the proportion of larvae and nymphs in each host group.

Discussion

Dynamics of parasite reproduction

Our data provide detailed information about the reproductive activity of four parasite species of *M. schreibersii* under natural conditions. In spite of their distinctive reproductive strategies, a main pattern is visible in our data. Overall, the four parasite species reproduced more intensively during the pregnancy and nursing seasons of *M. schreibersii*, much less during the subsequent host mating season, and very little or nothing during winter, while bats were hibernating or in torpor. Moreover, parasites tended to reproduce more heavily on pregnant bats and on flying juveniles (Fig. 2-5).

In accordance with observations of Balcells (1968), our data show that *N. schmidlii* concentrates its reproduction during the pregnancy and nursing season of the bat host, while *P. conspicua* frequently reproduces during the later seasons but also during autumn. Also, we did not observe any pregnant *N. schmidlii* during the hibernation period, suggesting that this species overwinters in a state of reproductive cessation. In contrast, we observed pregnant *P. conspicua* while bats hibernated. Likewise, we observed that *S. psi* mainly reproduce during spring and summer months, supporting previous punctual observations from Estrada-Peña *et al.* (1991a; 1991b) and Imaz *et al.* (1999). Moreover, we did not collect any pregnant *S. psi* during the hibernation season, which was expected as most spintunicids are reproductively inactive during winter (Rudnick, 1960; Deunff & Beaucornu, 1981). Regarding *I. simplex*, the abundance of immature stages recovered from *M. schreibersii* is a new finding, since this species was given as rare for the Iberian Peninsula until now (see Imaz *et al.*, 1999; Estrada-Peña *et al.*, 1991a). The presence of larvae and nymphal stages throughout the entire annual cycle confirms its continuous reproductive activity. However, the low proportions of immature stages observed during winter reveal that the species greatly reduces reproduction during this period.

Which factors regulate parasite reproduction?

The role of roost temperature

Our results suggest that the roost temperature does not influence the reproductive activity of the bat parasites throughout most of the yearly cycle, apart from the winter period when temperatures bellow ~11º C apparently limit it (Fig. 1). Generally, reproduction of parasites of temperate regions is greatly influenced by the wide seasonal thermal fluctuations present throughout the year (Marshall, 1981). However, in the particular case of parasites of cave-dwelling bats, these are subjected to rather constant roost temperatures throughout the year (~7ºC) (Fig. 1), when compared with the much wider thermal fluctuations outside caves (~20º C) (www.meteo.pt).

The lower roost temperatures registered during winter visits, however, seem to play an important role in limiting the parasite reproductive activity (Fig. 1). The cessation or significant decrease of the reproductive activity of bat parasites during winters in temperate regions is well-documented (e.g. Ryberg, 1947; Reisen *et al.*, 1976; Deunff & Beaucournu, 1981; Reckard & Kerth, 2006; Bartonička & Gaisler, 2007; Lourenço & Palmeirim, 2007). In contrast, in tropical and subtropical regions, where temperature remains fairly constant, bat parasite species are reported to be able to reproduce all year round (e.g. Marshall, 1970; 1971).

One of the reasons for the decline in parasite reproduction during winter may be the fact that some free-living stages (i.e. eggs and immature instars) are highly vulnerable to low temperatures and unable to develop or even survive below a certain temperature threshold. For instance, Reckardt & Kerth´s (2006) experiments showed that the pupa of the nycteribiid *Basilia nana* is not able to survive the low winter temperatures in central Europe. But in a number of other studies, this threshold is mentioned to occur at more moderate temperatures such as the pupa of *Trichobius major*, a species closely related to nycteribiids, which requires temperatures above 15º C to develop (Overal, 1980). Additionally, even if the free-living stages (i.e. eggs of *I. simplex* and pupae of *N. schmidlii* and *P.conspicua*) are able to survive temperatures bellow 11ºC, they likely require rising temperatures to act as external stimulus for developing (i.e. hatching of eggs or emergence of pupas), as described for many parasitic groups (Marshall, 1981).

The vulnerability of free-living parasitic stages to temperature bellow 11ºC may be a plausible explanation for the low reproductive activity observed in *N. schmidlii*, *P. conspicua* and *I. simplex* during winter, but it does not explain the absence of reproduction in *S. psi*, since mites complete their entire life cycle on the body of the bat host (Rudnick, 1960), and are therefore subjected to higher temperatures. The low winter temperatures might therefore also profoundly affect the parasite stages which remain on bat hosts, by directly influencing the thermoregulatory behaviour of bats. Indeed, to adapt to low temperatures and limited food resources during winter, most temperate-zone bat species hibernate, during which their body temperature typically falls to near ambient temperature (Altringham, 1999). In addition, hibernation is characterized by peripherical vasoconstriction in the body of bats, which decreases the blood supply to the bat skin (Altringham, 1999) and may limit the access of parasites to its food resources. Both factors combined may therefore affect the capacity of parasites to reproduce during winter, because of their need of optimum temperatures and frequent feeding when reproductively active (Ryberg, 1947; Lehane, 2005).

Altogether, our results suggest that roost temperatures do not play an important role in regulating parasite reproduction, apart from the winter ones bellow 11ºC which appear to limit it. Parasites presumably do not have any fitness advantage of reproducing during winter months, so they cease or greatly reduce it to ensure later reproductive success when more moderate temperatures and adequate blood supply is present. Inevitably, this adaptive strategy is likely to have significant effects on life-cycle dynamics and the size of reproducing parasite populations.

The role of the reproductive cycle of the bat host

Winter roost temperatures may limit parasite reproduction, but the fairly constant roost temperatures present during the rest of the year fail to explain the significant seasonal fluctuations in the reproductive activity of the parasite species (Fig. 2-5). This strongly suggests that factors other than temperature may regulate parasite reproduction. In fact, tropical parasites also show seasonal peaks of reproduction (e.g. Marshall, 1971) which cannot be explained by the fairly constant temperatures present in these regions.

Our results show that *P. conspicua* and *I. simplex* (Fig. 4, 5) intensify their reproduction soon after hibernation, during the pregnancy season of the host, while *S. psi* and *N. schmidlii* exhibit the peak of reproduction somewhat later, during the host nursing season (Fig. 2, 3). In addition, a general pattern can be observed, in which all parasite species reproduce more intensively on pregnant females and young bats (Fig. 2-5). Overall, these results support the idea that the reproductive activity of these parasites is strongly regulated by the seasonal reproduction of their bat host. Indeed, all parasite species synchronized their reproduction with that of *M. schreibersii*, mainly parasitizing bat nurseries where pregnant bats aggregate to give birth and rear their young. The adjustment of the reproductive cycles of parasites to those of their bat hosts was previously documented for other bat-parasite models (e.g. Christe *et al.*, 2000; Bartonička & Gaisler, 2007). However, this is the first study in which such synchronization was observed for a range of parasite species with distinctive reproductive strategies but a common host. Indeed, the fact that the life cycles of all studied parasite species showed this adjustment suggests that a strong adaptive force might have intervene in this model system.

What do parasite species gain from reproducing during bat reproduction? They can benefit from the appearance of a great number of new hosts when births take place (Christe *et al.*, 2000). Also, the high density of hosts in nursing colonies and the fact that bats cluster tightly, increases the opportunities for parasites to disperse horizontally after reproducing (Christe *et al.*, 2000). Moreover, during the pregnancy and nursing periods, parasites presumably have susceptible wider availability of susceptible hosts, because pregnant female bats are known to have a lower immune defence, which may result from endocrine changes related to reproduction (Grossman, 1985). Likewise, the immune defences of young bats are probably lower than those of adult ones and these are poorer self-groomers (Christe *et al.*, 2000), which makes them particularly attractive to parasites.

In conclusion, our study suggests that the reproductive activity of parasites of *M. schreibersii* is strongly regulated by the reproductive cycle of their host, but also limited by temperature during winter. Alongside these, it is possible that other factors not addressed here might also contribute to the regulation of parasite reproduction. Nevertheless, the combination of temperature and host-imposed control we observed is likely to be common across parasites of all temperate cave-dwelling bats, because these bats have similar life-cycles and may be subjected to identical seasonal thermal patterns within their roosts.

Chapter 4

Coexisting with other parasites

Structuring of the ectoparasite community in bats: evidence for a seasonal role of competition.

Manuscript

Abstract

Parasite communities are generally considered unstructured assemblages of species due to the lack of interactions among them. However, some studies demonstrated that both positive and negative interspecific interactions among parasites can occur, leading to structured communities. We used null model analysis to study the seasonal structure of the ectoparasite community of the Schreiber’s bat (*Miniopterus schreibersii*) and identify potential interspecific interactions between its parasites. We searched 969 bats at important phases of its yearly cycle (i.e. late pregnancy, nursing, mating and hibernation) and found four haematophagous ectoparasite species regularly occurring on them: the nycteribiids *Nycteribia schmidlii* and *Penicillidia conspicua,* the wing mite *Spinturnix psi* and the hard tick *Ixodes simplex simplex*. The same parasite species were found along the yearly cycle, but their abundances varied among seasons, and depended on the sex, age and reproductive status of the host. Random co-occurrence patterns were observed during most of the yearly cycle, suggesting that this parasite community is generally unstructured. However, we detected a structured parasite community during the pregnancy season of the host, with parasite species co-occurring on pregnant females much less than expected by chance. An analysis of density-compensation between parasite species pairs indicated that the observed structure is likely a result of negative interactions between the nycteribiid species. A plausible explanation is that these nycteribiid species directly compete with each other for space, due to their close *habitat* requirements. Overall, results allowed us to conclude that, under certain circumstances, competition between ectoparasites of bats might occur, playing a role in shaping bat parasite communities.

Introduction

Some animal communities appear to be simple random assemblages of species, whereas others have been found to have a marked structure (Mourin, 1999; Bush *et al.,* 2001). Such structuring in the way species assemble is usually interpreted as evidence that biotic interactions are important determinants of the organization of communities (e.g. Menge, 2000; Stubbs & Wilson, 2004), although other factors may also be involved in this process (e.g. Gonzalez & Poulin, 2005). The characterisation of the structure of natural communities, and the identification of the processes that influence that structure, have been for a long time central issues in ecology (Ulrich, 2004; Gonzalez & Poulin, 2005).

Parasite infracommunities, i.e. those inhabiting individual hosts, are good model systems for testing hypothesis in community ecology because host organisms provide a set of discontinuous *habitat* patches that can support discrete assemblages of parasites (Gotelli & Rohde, 2002; Tello *et al*., 2008). Moreover, such communities can easily be sampled in many individual hosts, thus allowing robust statistical analyses (Holmes & Price, 1986).

Most studies of parasite communities have found simple stochastic assemblages of species (e.g. Haukisalmi & Henttonen, 1998; Morand *et al*., 1999; Gotelli & Rhode, 2002; Mouillot *et al*., 2003), which suggest an absence of interspecific interactions among them (Price, 1980). However, there are also studies which report either positive (e.g. Bush & Holmes, 1986; Krasnov *et al*., 2005, 2006; Brinkerhoff *et al.,* 2006) or negative interactions (e.g. Dawson *et al.,* 2000; Mouillot *et al*., 2005), which indicate facilitating or competitive relationships between parasite species, respectively. Consequently, the debate on the extent to which parasite species interact and on the relevance of these interactions as determinants of the structure of species assemblages on hosts remains open.

In many circumstances, both the environment and the parasite populations vary throughout the year, which may consequently lead to seasonal variations in the parasite community structure and in the factors influencing it. The knowledge of these seasonal patterns is important to detect short term processes potentially relevant for community structuring (Poulin & Valtonen, 2001). However, relatively few studies have addressed this issue (e.g.

Timi & Poulin, 2003; Vidal-Martinez & Poulin, 2003; Gonzalez & Poulin, 2005), presumably due to the considerable effort required to sample multiple seasons.

Temperate zone bats are well suited to address the variations of parasite community structuring over time, because they live in highly seasonal environments and have a marked annual cycle, which is known to strongly influence the reproduction of their parasites (Lourenço & Palmeirim, 2008b). In this context, seasonality may influence the abundance of individual parasite species and thus affect the structure of the communities and the potential interactions among its species. The ectoparasite assemblages of bats may include mites (e.g. Spinturnicidae Macronyssidae, Sarcoptidae), ticks (Ixodidae and Argasidae), batflies (Nycteribiidae and Streblidae), bat bugs (Cimicidae) and fleas (Ischnopsyllidae) (Zahn & Rupp, 2004). A single bat may often harbour several ectoparasite species, and since they are all haematophagous and live in close proximity on the small bodies of bats, they are likely to occupy similar niches. This similarity sets the stage for negative (competitive) interactions, even between species that belong to very different taxonomic groups. However, such systems involving infections by different parasites may also include positive interactions between them. In fact, it has been suggested that the effectiveness of host defences may decrease as the diversity of parasites increases, so parasitism by one species may facilitate the installation of others (Bush & Holmes, 1986; Jokela *et al*., 2000).

Our general objective was to contribute to the clarification of the possible role played by positive and negative interactions among bat parasite species in the structuring of its communities, using the cave-dwelling bat *Miniopterus schreibersii* and its ectoparasites as a model. To identify the potential role of factors that vary seasonally, we studied the patterns of abundance and co-occurrence of ectoparasite species on hosts over the entire annual cycle.

The role of interactions among parasite species in the shaping of their assemblages was tested using a set of predictions: If there are positive interactions between species then these will co-occur on the same individual hosts more often than expected by chance, and their abundance on hosts will be positively correlated. Conversely, if there are competitive interactions between parasites, they will co-occur less than expected by chance, and their abundances will be negatively related. In addition, we predicted that if interactions are indeed relevant in the shaping of the studied parasite assemblages, then the consequences of these interactions should be particularly evident during the periods of the annual cycle when parasites are particularly abundant.

Although in recent years the role of species interactions in the structuring of parasitic assemblages has received much attention, studies are mainly focused on endoparasites and ectoparasites of aquatic animals (e.g. Bush & Holmes, 1986; Morand *et al*., 1999; Poulin & Valtonen, 2001; Gotelli & Rhode, 2002). The only studies deeply focused on the ectoparasite assemblages of terrestrial animals are those of Krasnov *et al.* (2005, 2006), and Brinkerhoff *et al.* (2006), all on fleas, and that of Tello *et al.* (2008), on streblid batflies, so there is very little understanding about the potential role of species interactions on the assembly of ectoparasitic communities.

Methods

Study species

The Schreiber's bat *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Miniopteridae) is a gregarious cave-dwelling species, which often carries multiple species of ectoparasites (Lourenço & Palmeirim, 2008b). Its life cycle, like those of most temperate-zone bats, has well defined seasons (Altringham, 1999). Mating occurs in autumn and fertilization follows immediately, but embryonic development is delayed during the period of hibernation (Altringham, 1999), which in Southern Europe usually lasts from December to February. During spring, pregnant females form large nursing colonies, where they later give birth. Males and non-breeding females may roost within the same caves, but usually away from the large clusters of females and young of the year (Rodrigues & Palmeirim, 2008). During late autumn, bats usually congregate in large clusters in cool caves while preparing for hibernation.

Data Collection

The study was carried out in eight caves of Southern Portugal in 2003 and 2004. During this period, we captured bats seasonally, at the most important phases of their life-cycle, namely during late pregnancy (April-May), nursing (June-July), mating (October-November), and hibernation (December-February). Most bats were captured with a harp trap as they left the caves at dusk, but hibernating individuals were captured by hand in the roosts. After capture, they were placed individually in clean cotton bags, to avoid contamination of ectoparasites between hosts. We recorded the sex, age and reproductive status of bats (for details see Lourenço & Palmeirim, 2007). Bats were then inspected for ectoparasites, which were collected with tweezers. Following this initial inspection, they were sprayed with an insecticide not toxic for mammals (*Selamectin*), and then kept in their holding bags for 30 minutes. After this, bats were inspected again and then released in their roosts. The holding bags were brought to lab and searched for dead ectoparasites, which were preserved in 70% alcohol and glycerine. Due to this combination of techniques, we consider that substantial errors in estimates of the abundance of ectoparasites are unlikely.

All ectoparasite species were identified to the species level. Mite species had to be clear and mounted prior to identification, as described in Lourenço and Palmeirim (2007). Parasite identifications were mostly done with the help of the keys and descriptions in Theodor & Moscona (1954) and Falcoz (1926) for the nycteribiids; Travassos-Dias (1994) and Arthur (1956) for the ticks and Radovsky (1967) for the mites.

Statistical analyses

Ectoparasite abundances on *M. schreibersii*

We calculated mean abundance of each parasite species according to Bush *et al.* (1997). Confidence intervals were calculated using the bootstrap confidence interval (*BCa*) of Efron & Tibshirani (1993). Differences in mean abundance among seasons and among host groups (i.e. according to host sex, age and reproductive status) were tested using distribution-free two-sample bootstrap t-tests (each with 2000 replicates) (Reiczigel *et al.,* 2005).

Ectoparasite community structure and species interactions

We used null model analysis (EcoSim, Gotelli & Entsminger 2008, version 7), to test for nonrandomness in the structure of the ectoparasite community of *M. schreibersii* along its yearly cycle (i.e. pregnancy, nursing, mating and hibernation) and on different host groups. Data was organized in presence-absence matrices, in which each row represented a different parasite species and each column represented an individual host. The entries in the matrices then represent the absence (0) or presence (1) of a particular parasite species in an individual host. We used a total of 14 different matrices, which corresponded to the different seasons (4 matrices) and to the distinct host groups available within each season, regarding sex, age, and reproductive status (10 matrices). We used the most conservative algorithm available in EcoSim (i.e. fixed rows and fixed columns) to generate randomly constructed assemblages, because of its good Type I properties and high power to detect non-random patterns in noisy data sets (Gotelli & Entsminger, 2008).

Among the several indices available to quantify co-occurrence patterns in the presence-absence matrices (i.e. C-score, number of checkerboards, number of species combinations, V-ratio), we chose the C-score (Stone & Roberts, 1990), because of its good Type 1 properties (i.e. low chance of falsely rejecting the null hypothesis) and the greater statistical power for detecting non-randomness (Gotelli & Entsminger, 2008). In a community structured by competition, the C-score should be significantly larger than expected by chance. Conversely, in a community influenced by positive interactions between species the C-score value should be lower than expected by chance (Stone & Roberts, 1990).

To test for departures of randomness in our 14 matrices, we calculated the observed C-score, and then compared it with the simulated C-scores of 5000 randomly assembled null communities created with EcoSim (Gotelli & Entsminger, 2008). Whenever, we found non-random assemblages of species at a particular season or on a particular host group, it became important to determine which species were interacting. Thus, in these cases, we examined interspecific interactions for all possible pair-wise combinations of species using non-parametric Spearman correlations (Sokal & Rohlf, 1995).

Statistical analyses were performed using Quantitative Parasitology (Reiczigel & Rósza, 2001, version 3.0), EcoSim (Gotelli & Entsminger 2008, version 7), and SPSS (version 12). All P-values less than 0.05 were considered statistically significant.

Results

Ectoparasite abundances on *M. schreibersii*

A total of 969 bats were captured in 25 sampling visits to caves. We collected 10 ectoparasite species, of which four were common: the nycteribiids *Nycteribiia schmidlii* and *Penicillidia conspicua*; the mite *Spinturnix psi* and the hard tick *Ixodes simplex simplex*. The remaining species (i.e. the mites *Spinturnix myoti*, *Macronyssus granulosus, Macronyssus longimanus*; the nycteribiids *Penicillidia dufourii* and *Nycteribia latreilii,* and the streblid *Nycteribosca kolenatti*) were only found sporadically and in very low numbers, so we excluded them from the analysis.

Although we recorded *P. conspicua*, *N. schmidlii*, *I. simplex* and *S. psi* throughout the entire yearly cycle, their abundances showed considerable seasonal variations (Fig.1). Overall, mean abundances of all parasite species were highest during the pregnancy or nursing season of bats, declined towards the bat mating season, and were lowest during hibernation (Fig.1).

Parasite mean abundances differed between hosts of different sexes, ages or reproductive status (Fig.1). The tendency of the differences was the same for all the parasite species. Abundances were similar on male and female bats in their mating and hibernation seasons, but they were consistently greater on female bats during the rest of the yearly cycle. During the bat pregnancy season, pregnant females tended to harbour the greatest numbers of ectoparasites, and after the bat births, the peak of parasite abundance was observed on juveniles (Fig. 1).

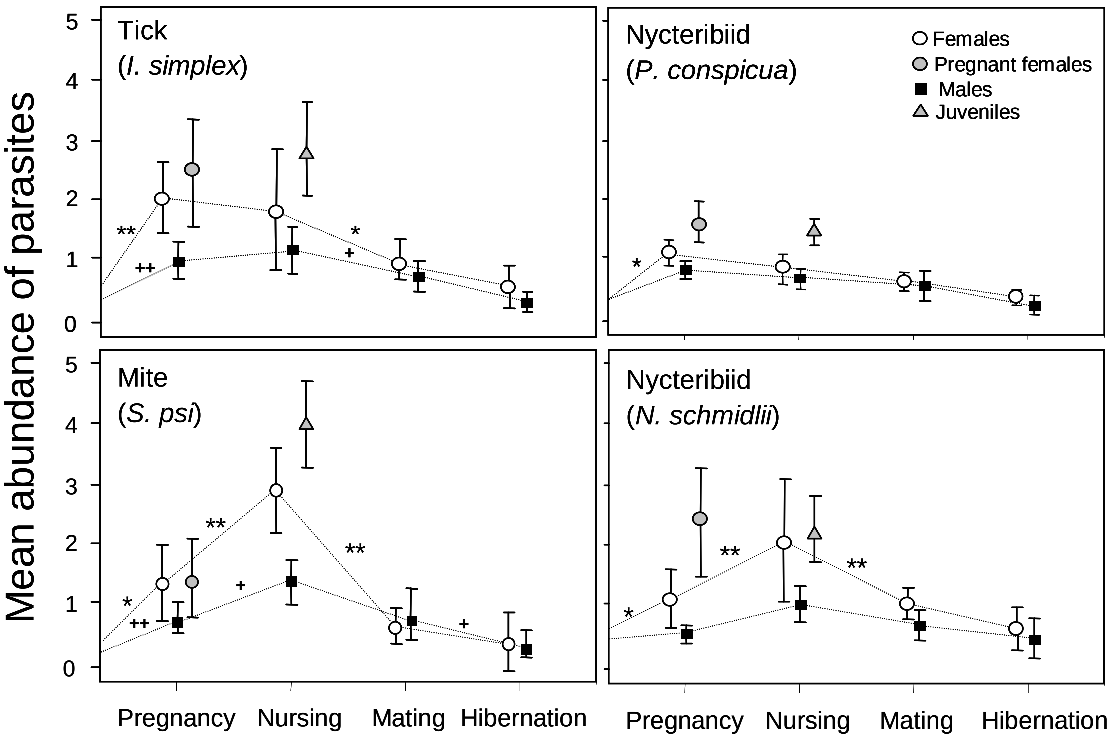

Figure 1 Mean abundances of ectoparasites on *M. schreibersii* throughout the yearly cycle and in different host groups. For the pregnancy season, we indicate pregnant and non-pregnant females separately. Vertical lines represent 95% confidence intervals. Significant differences between mean abundances of subsequent seasons are shown both for female *$P<0.05$, **$P<0.001$, and male bats $^{+}$P<0.05, $^{++}$ P<0.001 (two-sample bootstrap t-tests).

Ectoparasite community structure and species interactions

In the null model analyses, the observed C-scores did not significantly differ from the simulated C-scores in the nursing, mating and hibernation seasons of bats, indicating an unstructured parasite community throughout most of the host yearly cycle (table 1). However, in the bat pregnancy season, the observed C-score was significantly greater than that resulting from the simulations. This indicates that during this particular season, parasite species co-occur less often than expected by chance, and therefore that negative interactions between some species are present (Table 1). The results of the null models applied to the matrices within each host group were statistically significant only in the case of pregnant bats. In this case, the observed C-score was larger than the simulated C-cores, indicating fewer parasite species co-occurrences than expected by chance, and therefore a parasite community structured by competition (Table 1).

Table 1 Null model analysis. Observed and simulated co-ocurrence indices (C-scores) of ectoparasite species on *M. schreibersi.*

	Observed C-score	Simulated C-score	*P*
Pregnancy	1.03	1.02	**0.05**
Pregnant females	1.26	1.17	**0.004**
Females	1.24	1.21	0.98
Males	1.08	1.07	0.11
Nursing	0.80	0.79	0.08
Females	0.73	0.73	0.58
Males	0.78	0.78	0.26
Juveniles	0.81	0.80	0.23
Mating	0.80	0.81	0.27
Males	0.88	0.89	0.10
Females	0.67	0.68	0.35
Hibernation	0.64	0.65	0.22
Males	0.72	0.73	0.41
Females	0.55	0.56	0.42

To identify the pairs of species potentially competing on pregnant bats, we calculated pair-wise correlations between all possible combinations of parasite species abundances (Table 2). None of the correlations between distantly related parasites were significant, so there is no evidence that they interact. However, the abundances of the two nycteribiid species showed a highly significant negative correlation, suggesting that there is competition between them.

Table 2. Spearman correlation coefficients between pairs of ectoparasite species of *M. schreibersii* P-values have been adjusted with the Holm-Bonferroni procedure (Ludbrook, 1988).

	Pregnancy	
Species pairs	**r**	***P***
Nycteribiid vs nycteribiid (*N. schmidlii* vs *P. conspicua)*	-0.26	**<0.001**
Nycteribiid vs tick (*N. schmidlii* vs *I. simplex)*	-0.11	0.32
Nycteribiid vs mite (*N. schmidlii* vs *S. psi)*	0.115	0.35
Nycteribiid vs tick (*P. conspicua* vs *I. simplex)*	-0.008	0.90
Nycteribiid vs mite (*P. conspicua* vs *S. psi)*	-0.012	1.00
Tick vs mite (*I. simplex* vs *S. psi)*	0.086	0.57

Discussion

Ectoparasite abundances on *M. schreibersii*: influence of season and host type

The most striking conclusion of our results in the abundance of parasites is the similarity of the patterns across very distinct taxonomic groups – ticks, mites, and nycteribiids (Fig. 1). With minor variations, the studied parasites (i) tended to be abundant during the breeding season of the host (i.e. pregnancy and nursing season), and decline substantially towards its hibernation. During the host breeding seasons, parasites were (ii) first more abundant on pregnant females, (iii) and later on juveniles. During these seasons (iv) bat females tended to be more parasitized than males, even when not pregnant. This great convergence suggests that the main determinants of abundance are similar for the four parasites, and that the fitness advantages of these patterns are very high.

The greater abundance of all parasites during the breeding seasons of the bats is probably mostly a consequence of the intensification of their reproduction. In fact, Lourenço & Palmeirim (2008b) found that these four parasites intensify their reproduction during the pregnancy and nursing of their bat hosts. Host reproduction may facilitate the built up of heavy populations of parasites in multiple ways: availability of pregnant hosts, availability of juvenile hosts, and host aggregation in large and packed clusters.

In our sample, pregnant bats harboured many parasites, presumably because they have lower immune defences (Grossman, 1985). In addition, it should be adaptive for parasites to build up their populations on pregnant females, because this will position them well to colonize the juveniles after the births (Christe *et al.,* 2000). The great abundance of parasites on juvenile bats could be a consequence of lower immune defences (Grossman, 1985) or of a lower efficiency getting rid of parasites through grooming (Christe *et al.,* 2000). It has also been suggested that young host are particularly suitable for haematophagous ectoparasites because their thinner skin facilitates feeding (Marshall, 1981).

Finally, during the breeding seasons, females and juveniles of *M. schreibersii*, like in most bats species, congregate in large clusters (Rodrigues & Palmeirim, 2008). Such concentrations of hosts facilitate horizontal dispersal of parasites (Clayton & Tompkins, 1994; Christe *et al*., 2000; ter Hofstede & Fenton, 2005), and are conspicuous and attractive targets for parasites looking for hosts (Lourenço & Palmeirim, 2008a). This situation may explain why during the nursing season females tend to have higher parasitic loads than males, which usually do not roost in the nursing clusters (Lourenço & Palmeirim, 2007).

Once the young bats become independent of their mothers, all bats abandon the nursing clusters. These later regroup for hibernation, together with the males, but this does not result in an increased abundance of ectoparasites, presumably because the low temperatures of the roosts and of the body of bats seem to prevent their reproduction (Lourenço & Palmeirim, 2008b).

Ectoparasite community structure and species interactions

The ectoparasite communities of *M. schreibersii* were generally unstructured, with no evidence of interactions among parasites species during most of the bat annual cycle and on most types of hosts. This is in line with the view that interspecific interactions usually are not major forces in shaping the structure of parasitic communities (e.g. Mouillot *et al.,* 2003;

Poulin, 2007). However, our results suggest that under certain circumstances, negative interactions among distinct ectoparasites of bats can occur, playing a role in shaping their communities. The fact that we only detected evidences of these interactions during one of the four studied seasons highlights the importance of carrying out studies that take into consideration the temporal variation of the communities of parasites (Poulin & Valtonen, 2001; Krasnov *et al.,* 2006).

One of the reasons for the general lack of negative interactions found among the ectoparasite species might be the low abundances observed during most of the yearly cycle and on most host groups (Fig. 1). Indeed competition is unlikely to have consequences if the populations of parasites on individual hosts are too low (Combes, 2001, Tello *et al*., 2008). However, the higher parasite abundances present on pregnant bats may have created the ideal setting for competition to occur, and consequently, for the appearance of a structured community (Fig.1).

Parasites in situations of high abundances do not always compete. In fact, there are cases in which they can show a tendency for forming interspecific aggregations, in which two or more species occur together more often than expected. Brinkerhoff *et al.* (2006) and Krasnov *et al.* (2006) reported such cases in fleas parasitizing rodents, but we did not find any positive associations in our system. One common explanation for these aggregations is interspecific facilitation, mediated via the host (Bush & Holmes, 1986). According to it, a host already challenged by multiple parasites may be easily infected by a new species, because he is incapable of mounting one more type of response to a different parasite. Why is this not occurring in our system? One possible explanation is that the infections are generally not severe enough to incapacitate the immune system of the bat host, so parasitied and non-parasitized animals are equally likely to resist to new infections. A second explanation would be cross-resistance, in which the host develops defence mechanisms that are effective against more than one parasite (McTier *et al*., 1981; Krasnov *et al*., 2006), thus minimizing the total challenge posed by multiple species infections. In addition, grooming is a first line of defence against ectoparasites that may reduce their pressure on host's immune system, and there is evidence suggesting that bats are very efficient groomers (ter Hofstede *et al.,* 2004). These animals have very flexible bodies and can reach the full body surface either with their mouth or with the strong comb formed by the claws of the hind feet. In bats, the grooming activity is known to increase with the level of parasitism (Giorgi *et al.*, 2001), so it is likely that the presence of one ectoparasite may affect other species of ectoparasites. In this context, grooming can function as a cross-defence mechanism likely effective against multiple ectoparasites (but not necessarily all).

In our study, the assemblage of parasites on pregnant bats seemed to be structured by competition. But what were the factors driving it? All the studied parasite species feed on the blood of the host and can thus be considered part of the same guild. Since parasite species of the same guild are likely to negatively interact with one another (Poulin, 2006), one could expect competition to occur. However, we did not find any evidence of competitive interactions between the three major taxonomic groups (mites, ticks, and nycteribiids). This might be because at the level of the infracommunity, the blood of the host is not limited, so competition driven by the exploitation of limiting resources (i.e. exploitative competition) would not be likely to occur (Ricklefs & Miller, 1999). In fact, the consumption of blood by one parasite species is not likely to limit the abundance of other parasites, nor prevent their establishment by reducing the availability of food resources.

Since the conditions for exploitative competition for blood are not met, we are probably in the presence of interference competition for space (i.e. in which one species interferes with the capacity of the other to access a resource) (Park, 1962; Poulin, 2006). This scenario could explain why we only found evidences of competition among the two nycteribiids, since these species have similar *habitat* requirements, inhabiting closely in the fur of bats. In a situation of interference competition, one of the nycteribiid species must either be able to kill or expel the other species. Although such behaviours were never described for nycteribids, there is evidence that these species can be aggressive. For instance, Ryberg (1947) reported that in overcrowding situations, repeated copulations may kill nycteribiid females, and we observed cases of aggression between captive nycteribiids of different species. A similar scenario is described by Sousa (1993) who demonstrated that large larval trematodes parasitic of snails prey on smaller species, and considered that these antagonistic interactions are the primary mechanisms by which they exclude some species from host snails.

Because *P. conspicua* is much larger than *N. schmidlii,* it would be more likely to be the dominant species in an aggressive interaction. However, the smaller *N. schmidlii* may be able to co-exist with the larger species by living and moving well buried in the long fur of *M. schreibersii*, whereas *P. conspicua* usually moves on the fur surface (unpublished personal observations). Such differences in the micro*habitat* preferences of both species may indeed be an evolutionary adaptation to minimize interference competition.

Although evidence of competition among nycteribiids was only detected in pregnant bats, it is possible that these parasites might compete at lower abundances than those observed on these hosts. However, if this is the case, the consequences of competition at the level of community structure were not sufficiently strong to show statistical significance. The occurrence of competition, even if just during a particular period, may have long lasting consequences for the populations of the species involved. In our model system, the potential constraints of competition occurred at the peak of the reproduction of the two competing nycteribiids, so this could have significant effects on the population of one or both species throughout the year.

Competition, however, is not the only possible explanation for the type of results we obtained. In fact, if individual hosts were sufficiently dissimilar to represent different *habitats* in the perspective of the two nycteribiids, then some hosts could be more suitable for one species than for the other, as suggested by ter Hofstede *et al.* (2004) for a similar situation. However, we attempted to eliminate this confounding factor by running the tests on homogeneous host groups. Indeed, the host group in which the interactions were detected is uniquely composed of adult pregnant females, all roosting in large and dense groups in which there are no barriers for nycteribids to disperse. Consequently, competition remains the most parsimonious explanation for the results observed. The literature on this subject is very scarce, but a few other instances of possible competition between ectoparasite species have been reported for bats (Wenzel & Tipton, 1966; Komeno & Linhares, 1999; Tello *et al.,* 2008).

Overall, our results allowed us to conclude that generally there is little interaction between bat ectoparasites throughout the host yearly cycle. However, under certain circumstances related with higher parasite abundances, competition between ectoparasites of bats might occur, playing a role in shaping bat parasite communities. This also supports the idea that in the past, competition may have been an important driver in the evolution of bat ectoparasites, resulting in species that can now avoid competition either through host specificity or by exploiting different parts of the body of bat hosts, two features common in bat ectoparasites.

Chapter 5

Keeping the host alive

Can mite parasitism affect the condition of bat hosts? Implications for the social structure of colonial bats.

Lourenço, S.I. & Palmeirim, J.M. 2007. Journal of Zoology 273: 161-168.

Abstract

Ectoparasitism is recognized as one of the main costs of coloniality, but little is known about how it affects fitness and social structure of bats, the most gregarious of mammals. We studied these issues using the colonial bat *Miniopterus schreibersii* and its hematophagous parasitic mite *Spinturnix psi* as a model. Body condition is an important determinant of individual fitness that is potentially affected by ectoparasitism. Thus, we measured host body condition and mite loads in a total of 969 bats throughout the annual cycle. Mites were rare while hosts hibernated, increased in abundance in spring, and peaked during nursing season of bats, when they were particularly abundant on lactating females and young bats. This strong seasonal variation in mite loads is related to the reproductive cycle of mites, which in turn appears to be synchronized with the reproductive cycle of their hosts. Mite loads and the condition of bats were negatively correlated, and information available suggests that this can due to an effect of parasitism. However, this effect was only observed during the bat's nursing season, when mites were most abundant, and heavily parasitised bats lost about 10% of their weight. Such a decline in condition is potentially detrimental to bats. Mite parasitism did not seem to be a significant disadvantage of coloniality, except in nurseries, where it seems to impose significant costs. However, since females and young usually aggregate in these colonies, we presume that for them such costs are probably offset by advantages of group living. Adult males, however, are usually absent from nurseries, which may be a strategy to minimise mite parasitism. Overall, results suggest that ectoparasitism may play a significant role in determining the social structure of *Miniopterus schreibersii* and of many other temperate bats that have similar life cycles and ectoparasitic loads.

Introduction

Parasites can affect the fitness of their hosts by influencing traits like their immune responses, energy budgets, behaviour and body condition (Hart, 1997; Giorgi *et al.*, 2001; Khokhlova *et al.*, 2002; Brown & Brown, 2004; Møller & Saino, 2004). The effect of parasitism on the condition of hosts has been the focus of many studies. However, while in some circumstances authors found a significant effect (e.g. Neuhaus, 2003; Brown & Brown, 2004; Whiteman & Parker, 2004), others concluded that body condition of hosts was independent of parasitic load (e.g. Johnson & Albrecht, 1993; Tompkins *et al.*, 1996; Perez-Orella & Schulte-Hostedde, 2005). This issue is particularly relevant because a poor condition may increase the susceptibility to predation, diseases, and starvation, and consequently reduce the survival or reproductive success of hosts (Brown & Brown, 2004).

Bats comprise one-fourth of the world's species of mammals (Altringham, 1999) and often host heavy loads of ectoparasitic arthropods (e.g. bat bugs, bat flies, fleas, ticks, and mites) (Zahn & Rupp, 2004). Despite this, very little is known about the effect of ectoparasites on the condition of these hosts.

The knowledge on how well bats cope with their ectoparasites is of particular interest, since they are the most gregarious of mammals (McCracken & Wilkinson, 2000), frequently forming colonies with thousands of individuals (Altringham, 1999). In most of these colonies bats cluster tightly, favouring parasites that are transmitted horizontally (i.e. among individuals) which tend to be more virulent than those transmitted vertically (i.e. from mother to offspring) (Clayton & Tompkins, 1994; Christe *et al.*, 2000; ter Hofstede & Fenton, 2005). Indeed, ectoparasitism is known to be one of the most important costs of coloniality in a wide range of taxa (Coté & Poulin, 1995; Brown & Brown, 2004; Ezenwa, 2004). It is therefore highly likely that bats in colonies incur similar costs.

If ectoparasitism does affect colonial bats, then their social structure may be influenced by the balance of parasitic costs with the benefits of group-living, such as reduced thermoregulatory costs (Hamilton & Barclay, 1994). Moreover, costs induced by ectoparasites may vary during the host annual cycle: ectoparasite abundance is likely to be influenced by the complex life cycle of bats, which exposes parasites to marked seasonal variations in factors like host immunocompetence (Nelson & Demas, 1996), environmental and host body temperature and grooming activity. Consequently, the balance between costs and benefits of group living may vary during the year, potentially influencing the seasonal changes in the structure of bat colonies.

In Bradbury's (1977) classification of bat social structures, most temperate and many tropical species follow the "temperate cycle" pattern; they form sexually mixed groups during most of the year, but sexually segregate during nursing season, with males tending to be absent from the nurseries. As far as we known, nobody has evaluated if ectoparasites may play a role in the determination of this social structure.

Among bat ectoparasites, mites tend to be the most diverse (Baker & Craven, 2003) and abundant (Zahn & Rupp, 2004) on cave dwelling bats. These hematophagous parasites spend their entire lifecycle on hosts and tend to remain on the same individuals for long periods (Christe *et al.*, 2000; ter Hofstede & Fenton, 2005), thus, intuitively it seems likely that they impose a continuous pressure upon their hosts.

Giorgi *et al.* (2001) have demonstrated that mite parasitism has energetic costs for bats but, to our knowledge, nobody found a clear link between ectoparasitic load and condition in free-living bats. In this study we determined if ectoparasitism can influence the body condition of bats, and evaluated if parasitic pressure varies throughout the yearly cycle of bats. Based on these results and on knowledge of colony composition throughout the annual cycle, we discuss if ectoparasitism is likely to have implications for the social structure of colonial temperate zone bats. As a model system, we used the bent-winged-bat (*Miniopterus schreibersii*) and its specific mite *Spinturnix psi.*

Methods

Study species

The bent-winged bat *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Miniopteridae) is a highly colonial cave dwelling species that often carries heavy loads of ectoparasites. Like most bat species in the temperate regions, it has a well-marked seasonal cycle (Altringham, 1999). Mating occurs in autumn and is immediately followed by fertilization, but embryonic development is suspended during hibernation (Altringham, 1999). In southern Europe these bats usually hibernate in tight clusters of thousands of individuals from December to February. Pregnant females start gathering in nursing colonies in spring to give birth and raise their young. At this time, males and non-pregnant females may roost in the same caves, but usually away from nursing colonies (Palmeirim & Rodrigues, 1995).

The mite *Spinturnix psi*, Kolenati, 1856 (Acari: Mesostigmata, Spinturnicidae) is a hematophagous ectoparasite specific of the bent-winged bat (Deunff & Beaucournu, 1981; Peribañez-Lopez *et al.*, 1993). The lifecycle of this species occurs entirely on the host, where they are normally found on wing and tail membranes (Peribañez-Lopez *et al.*, 1993).

Data collection

The study was carried out in several caves of Southern Portugal during 2003 and 2004. Mite loads were measured seasonally, at important phases of the annual cycle of the bent-winged bat, namely late pregnancy (April-May), nursing, which is the only time when host juveniles are easily distinguishable from adults (June-July), mating (October-November), and hibernation (December-February). We sampled bats in four caves that were visited once in each of the above seasons. Sampling was mostly done in these four caves throughout the study. However, on a few occasions bats had moved to alternative caves, so using knowledge of the movement patterns of *M. schreibersii* in the south of Portugal (Palmeirim & Rodrigues, 1992), these roosts were also sampled. The studied caves harboured a few thousand bats, but we only sampled about 70 individuals in each visit, so the chances of relevant pseudoreplication are minimal.

Most bats were captured with a harp trap at the entrance of caves, but hibernating individuals were captured by hand in the interior. Bats were placed individually in clean cotton bags to avoid contamination of mite loads. We measured forearm length with callipers and weighed the animals with a Pesola spring-scale with a precision of 0.25 g. During the pregnancy season, we used palpation to distinguish between pregnant and non-pregnant females. In the nursing season we only included in the analyses females that were nursing, which were identified by their well developed nipples. In the same season, juveniles of the year were identified by the level of ossification of the hand bones and fur colour.

There is no consensus on the best method to measure body condition (e.g. Green, 2001; Schulte-Hostedde *et al.*, 2005), so we used the two most common approaches: the ratio of body mass to body size, and the residuals of a linear regression of body mass on body size. However, both methods resulted in the same conclusions, so we only present the results of the ratio index because of its simplicity. Body weight was divided by the length of forearm, as done by Zahn & Rupp (2004) and Siemers *et al.* (2005). The index was not calculated for pregnant females.

All bats were visually screened for mites, which were collected with tweezers. Bats were then sprayed with a non-toxic insecticide commonly used in pets (Selamectin) and were kept in their holding bags for 30 minutes, after which they were released in their roost. The bags were later searched for dead mites. All mites were preserved in 70% alcohol and glycerine.

We followed the protocol described in Dias (1982) to clear and mount the specimens on slides prior to identification. Mites were boiled in Marc-Andre liquid for about four minutes, placed dorsally on a slide with a drop of Hoyer medium, and covered with a cover glass. The preparations were dried in an oven at 30º degrees Celsius for 15 days.

The identification of mite species, age (adult, nymph stage), reproductive status (pregnant, non-pregnant), and sex, was made in a Compound Light microscope, using the identification keys and descriptions in several papers (e.g. Radovsky, 1967; Dias, 1982).

Statistical analyses

To describe mite loads we used the parameters of prevalence (percentage of infected hosts), mean intensity (mean number of parasites found in infected hosts), and mean abundance (mean number of parasites found in all hosts examined) (Bush *et al.*, 1997).

We calculated confidence intervals to indicate the accuracy of the above parameters. For the prevalence we used Sterne´s exact interval (Reiczigel, 2003) and for the mean intensity and mean abundance we used the bootstrap confidence interval (BC_a) of Efron & Tibshirani (1993).

We tested for differences in mite load between seasons, sexes and ages of the hosts. To test differences in the prevalence of mites among groups we used Fisher's exact test (Rósza *et al.*, 2000). To compare mean mite intensities and mean mite abundances among host groups we used distribution-free two-sample bootstrap t-tests (each with 2000 replicates) (Reiczigel *et al.*, 2005). Statistical analyses were performed using Quantitative Parasitology (Reiczigel & Rósza, 2001, version 3.0) and SPSS (version 12). P-values less than 0.05 were considered statistically significant.

We pooled the results of the caves sampled in each season, because there were no statistical differences in mite loads among them. For the same reason, data of corresponding periods of 2003 and 2004 were also pooled. Finally, we pooled mite loads of male and female juveniles, because there were no differences between them.

Results

Seasonal variation of *Spinturnix psi*

Parasites were searched on a total of 969 bats captured in 25 visits to caves, and we collected 1350 mites of several species: *S. psi* Kolenati, 1856 (93 %), *S. myotis* Kolenati, 1856 (2 %), *Macronyssus granulosus* Kolenati, 1856 (4 %) *and Macronyssus longimanus* Kolenati, 1856 (1 %).

We recorded *S. psi* on the bent-winged bat throughout the entire yearly cycle, but both prevalence and mean intensity of its loads showed considerable seasonal variation (Table 1). Consequently, mean abundance also varied seasonally (Fig. 1, Table 2). The overall pattern of this variation was similar for male and female bats, but females carried significantly more mites than males during pregnancy (bootstrap *t* test, t= 2.11, P= 0.04) and nursing season (bootstrap *t* test, t= 3.81, P< 0.001, Fig. 1).

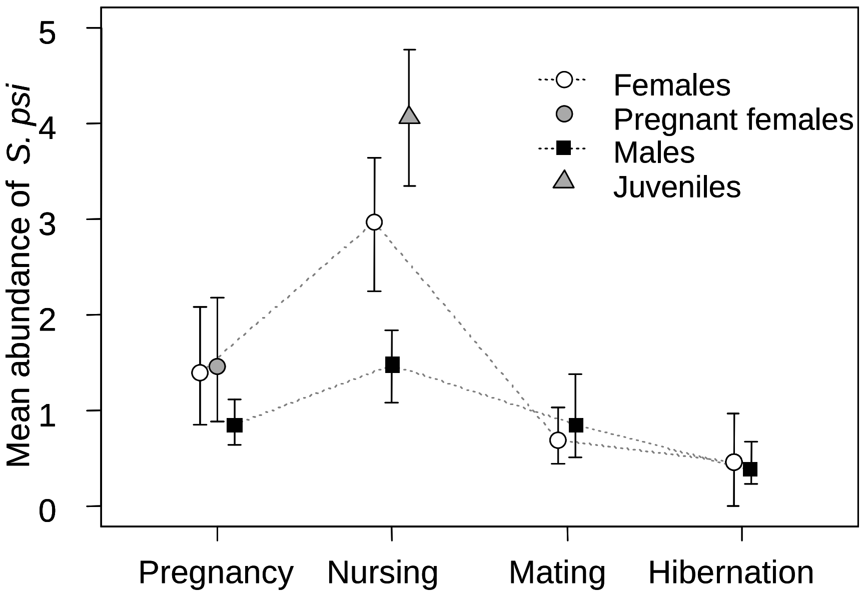

Figure 1. Seasonal variation of the mean abundance (± 95% bootstrap confidence intervals) of *S. psi* on the bent-winged bat. For the pregnancy season we indicate separately pregnant and non-pregnant females.

The lowest abundance in mites was found during winter, when bats were hibernating (Fig. 1). Loads then started to increase on hosts of both sexes, reaching a peak during the nursing season (Table 1, Fig. 1). In fact, during this season, mite abundance was almost double that of the other periods (Fig. 1). At this time, flying juveniles harboured the highest mite loads, significantly greater than those of adult males and females (bootstrap *t* test, t= 6.13, P< 0.001). In autumn, during the mating season of bats, mite loads declined considerably to less than one mite per host (Fig. 1).

Table 1. Host sample size (n), prevalence (%), and mean intensity of *S. psi* found on the different host groups in each season. Bootstrap confidence intervals (95%) for prevalence and mean intensity are represented in parenthesis.

Hosts		Pregnancy	Nursing	Mating	Hibernation
Females	n	68	82	88	99
	prevalence	56 (36-74)	62 (51-72)	30 (20-40)	14.5 (8-24)
	mean int.	2.5 (1.79-3.29)	4.76 (3.9-5.51)	2.62 (2.08-3.15)	3.18 (1.73-5.45)
Pregnant females	n	42	-	-	-
	prevalence	57.1 (38-74)	-	-	-
	mean int.	2.56 (2.00-3.44)	-	-	-
Males	n	133	99	123	119
	prevalence	39 (30-48)	53 (43-63)	29 (22-38)	16 (10-23)
	mean int.	2.19 (1.83-2.65)	2.58 (2.13-3.23)	3.47 (2.61-4.97)	2.42 (1.63-3.68)
Juveniles	n	-	116	-	-
	prevalence	-	73 (63-79)	-	-
	mean int.	-	5.67 (4.88-6.53)	-	-

Table 2. Results of two sample bootstrap *t*-tests used to compare mean abundances of *S. psi* between subsequent seasons. Mite abundances of different host sexes were tested separately.

	Males	Females
Pregnancy-Nursing	t= 2.28, P= 0.02	t= 3.12, P< 0.001
Nursing-Mating	t= -1.26, P= 0.2	t= -5.52, P< 0.001
Mating-Hibernation	t= -2.54, P= 0.01	t= 1.29, P= 0.2
Hibernation-Pregnancy	t= -2.68, P< 0.001	t= -2.41, P= 0.01

We found pregnant mites during most of the yearly cycle, except for hibernation season (Fig. 2). They started to appear in spring, mostly on pregnant female hosts. The highest proportion of pregnant mites was observed during the bats' nursing season, particularly on flying juveniles (Fig. 2). Numbers then drastically declined during autumn, when the few pregnant mites similarly parasitised male and female bats.

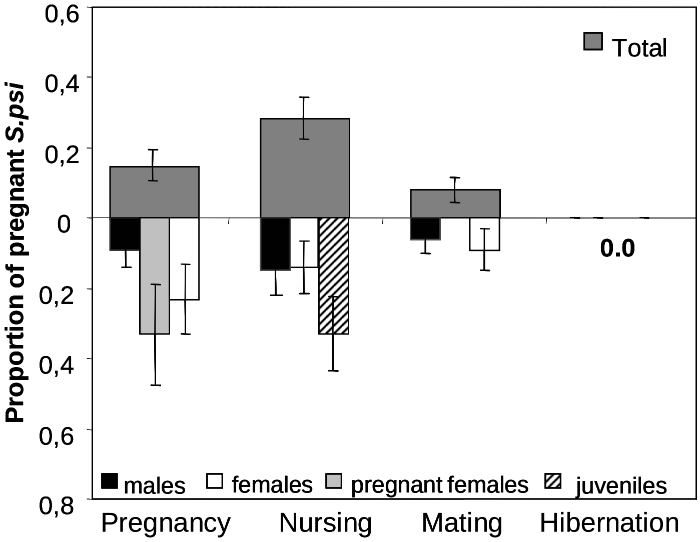

Figure 2. Seasonal variation in the proportion of pregnant *S.psi* (with confidence intervals). Upper columns represent the total proportion of pregnant mites found in each season. Lower columns represent the proportion of pregnant mites found in each host group. For the pregnancy season we indicate separately pregnant and non-pregnant females.

Effect of *Spinturnix psi* on the condition of the bent-winged bat

The index of condition of bats varied along the annual cycle, with bats in worse condition having been found in the nursing season (Fig. 3). Furthermore, during the nursing season there was a clear decline in the condition of bats with increasing mite loads (Fig. 3). This pattern was statistically significant for both males (r_s= -0.71, n= 12, P=0.004) and females (r_s= -0.55, n=12, P=0.006), and for juveniles (r_s= -0.41, n= 12, P= 0.031, Fig. 3). In the remaining seasons we did not detect any significant relationship between mite loads and body condition.

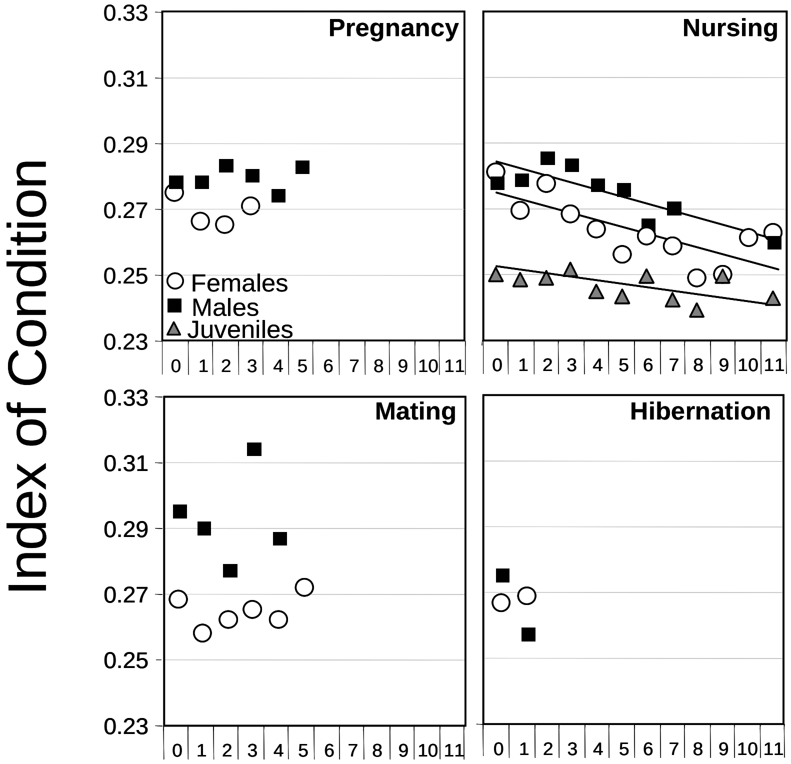

Figure 3. Relationship between mite loads and the Index of Condition of hosts. Symbols represent the mean condition of hosts with the same mite loads. Numbers along the horizontal axis show the size of mite loads. Regression lines are shown only when statistically significant.

To evaluate the relevance for bats of the decline of body condition caused by mite loads, we compared this decline to the observed amplitude of the seasonal variation in condition of these individuals (Fig. 4). We found the variation in host condition caused by mite parasitism to be of similar magnitude to that observed throughout the studied annual cycle, both for male and female bats.

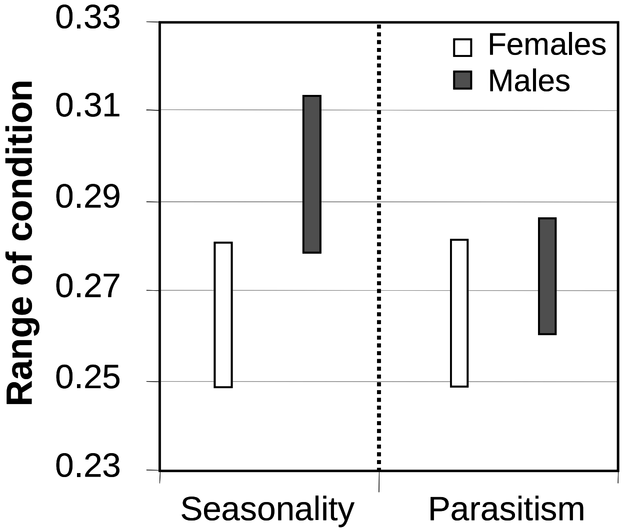

Figure 4. Range of condition caused by seasonality (using maximum and minimum mean values from Fig. 3), and range of condition caused by different levels of mite parasitism during nursing season alone (using end points of regression lines in Fig. 3). Parasitism caused a variation in condition similar to that of seasonality.

Discussion

Mite loads vary throughout the yearly cycle of the bent-winged bat

Our results reveal strong seasonal fluctuations in *S. psi* loads throughout the annual cycle of the bent-winged bat. Furthermore, they corroborate the idea that *S. psi* has its life cycle synchronized with that of the bent-winged bat (Peribañez-Lopez *et al.*, 1993). The dramatic seasonal fluctuations observed in mite abundance can therefore be mainly explained by variations in its reproductive cycle, which are adjusted to that of the host.

Winter was the period when mite abundance was lowest (Fig. 1) and no pregnant mites were found (Fig. 2). This interruption of mite breeding while bats hibernate can be explained by the fact that mite reproduction is affected by environmental temperature (Møller, 2000).

Mite abundance then increased during spring, particularly on female hosts, following the appearance of the first pregnant mites. Several factors may explain the onset of mite reproduction at this time: (i) female bats leave their cold hibernation sites and start forming colonies in warmer caves (Palmeirim & Rodrigues, 1995), where they are able to maintain higher body temperatures, which facilitate mite reproduction and (ii) mites, through their hematophagous feeding, may get hormonal signs about the impending births of hosts, which may stimulate their own reproduction (Deunff & Beaucornu, 1981). The latter explanation is consistent with the observed higher abundance of reproducing mites on pregnant bats (Fig. 2). The increase of mite abundance may also be facilitated by the availability of numerous pregnant female hosts with depressed immune systems (Nelson & Demas, 1996; Christe *et al.*, 2000).

Mite abundance continued to grow into the bat's nursing season, when it peaked (Fig. 1). This is a likely consequence of a boom in mite reproduction during the same period, caused by the sudden appearance of large numbers of young bats. Indeed, it was on these vulnerable hosts that mite reproduction was most intense and loads heavier. Mites are likely to breed more easily on young bats because they possess a naïve immune system and are weak self-groomers (Christe *et al.*, 2000).

Strong declines in mite pregnancies (Fig. 2) and abundance (Fig. 1) were observed in autumn. These may be due to the dispersal of nursing colonies, as bats tend to scatter for mating (Palmeirim & Rodrigues, 1995), In fact, when hosts are isolated or in small groups, mites can no longer benefit from the advantages of horizontal transmission provided by large colonies. In addition, roosts used during the mating season are usually colder than those occupied by nurseries.

Bat condition declines with increasing mite loads: cause or consequence?

We found strong negative correlations between mite loads and body condition of the bent-winged bat. Zahn & Rupp (2004) also found some bats in poor condition and with heavy ectoparasitic loads, and interpreted this as a consequence rather than a cause of host's condition. In fact, one should not exclude the possibility that some bats get heavily parasitised because they are weak. However, there is good evidence that parasitic mites have a detrimental effect on their bat hosts. Under laboratory conditions, Giorgi *et al.* (2001)

found that grooming activity and overall metabolism of *Myotis myotis* - a bat that often roosts together with *M. schreibersii* - increased drastically with loads of *Spinturnix myoti* (a close relative to our mite study species). As a consequence, in their experiment, individuals with heavy mite loads lost more energy and weight than those without mites. These authors also suggested that the impact of mites might be even more marked under natural conditions. Moreover, a negative effect of mites on body condition has been documented for other vertebrates (e.g. Merino & Potti, 1995; Møller, 2000; Weddle, 2000). Altogether, this information indicates that a negative effect of mite load on the condition of their hosts is a parsimonious explanation for the correlation that we observed.

How relevant is the loss of condition due to mite parasitism?

The effect of mites on the condition of bats appeared to be restricted to the nursing season. This is probably a consequence of the heavier loads observed at this time of the year. In addition, it is possible that mites feed more frequently during this period, because of their intense reproduction and high environmental temperatures.

Heavy mite parasitism during the nursing season resulted in a loss of about 10% of body weight of bats. This was similar to the seasonal variations in body weight observed throughout the entire study period (Fig. 4). Such a large decline in condition is potentially detrimental to bats, particularly if coincident with periods of resource limitation or stress, when parasitism tends to be most harmful (Zahn & Rupp, 2004). This is the case of the nursing season of temperate bats, which is their most costly period in terms of allocation of resources (Racey & Entwistle, 2000). In fact, lactating females are known to have particularly high energy demands because they need to feed and groom their young (Hamilton & Barclay, 1994; Altrigham, 1999). Likewise, this season is critical for juveniles, because in a short period they have to grow, learn to fly, and store enough fat reserves prior to hibernation (Altringham, 1999; Racey & Entwistle, 2000). Delays in this process can result in high juvenile over-winter mortality in bats (Rodrigues *et al.*, 2003).

An effect similar to that reported here for *M. schreibersii* is quite likely to occur in many other temperate zone bats, which have comparable seasonal cycles, and in some cases heavier mite loads (e.g. Christe *et al.*, 2000; Zahn & Rupp, 2004).

Can mite parasitism influence the social structure of colonial bats?

Our results showed that mites might affect the condition of the bent-winged bat. This species forms colonies during most of the year (Palmeirim & Rodrigues, 1995), and parasites tend to benefit from the aggregation of hosts in colonies (e.g. Coté & Poulin, 1995; Brown & Brown, 2004; Ezenwa, 2004). Consequently, mite parasitism could be expected to be a significant cost of coloniality in the bent-winged bat during most of its yearly cycle.

However, we did not find this to always be the case. During hibernation, the bent winged-bat forms compact clusters that could favour ectoparasitism. In this energetically demanding period, thriving populations of mites could have serious consequences: blood feeding by mites could drain resources from hosts and cause frequent arousals from torpor, leading to the depletion of energy reserves that could compromising their over-winter survival. However, we found that mite populations were low during hibernation, not affecting host condition (Fig. 4). Consequently, we infer that mite parasitism is not a drawback of colonial social behaviour of temperate bats during winter.

In contrast, during the nursing period, the high loads observed in young and lactating female bats and their effect on host condition suggest that mite parasitism represents an important cost of joining nurseries for the bent-winged bat. However, the cost of mite parasitism seems to be outweighed by the advantages of coloniality, because females and young of this species, like those of many other bats, usually form compact nurseries.

Males from most species, however, tend to be absent from nurseries, although they can show intraspecific flexibility in this behaviour (e.g. Entwistle *et al.*, 2000; Rodrigues *et al.*, 2003). Can mite parasitism help explain this sexual segregation?

Sexual segregation is typical of the social structure that Bradbury (1977) classified as the “temperate cycle”. It is usually explained by constraints of behavioural thermoregulation;

lactating females may cluster to maintain a high body temperature for rearing their young (Lourenço & Palmeirim, 2004), whereas males may stay in cooler places to enter torpor and thus minimize energy expenditure (Hamilton & Barclay, 1994). However, thermoregulation alone does not always explain this pattern of social structure; (i) males are often absent from nurseries in the tropics, where their roosts can be as warm as nurseries (Cheng & Lee, 2004), so it is unlikely that they are trying to minimize energy expenditures, (ii) in the temperate zones, males would gain from joining nurseries because this would facilitate spermatogenesis and increase the opportunity of finding mates (Entwistle *et al.*, 2000).

Our results suggest that mite parasitism can help explain the absence of male bats from nursing colonies. In fact, we found that (i) mite parasitism has the potential to be detrimental to bats during this season, and (ii) higher mite loads are associated with nurseries. Consequently, we believe that the avoidance of nurseries may be a behavioural defence of males to minimise the costs of parasitism.

Overall, we conclude that mite parasitism can be detrimental to the bent-winged bat and may play an important role in the organization of its social structure. This is likely to be the case in other temperate bat species, because most of them are colonial, carry heavy loads of ectoparasites and have similarly complex annual life cycles.

Chapter 6

General Discussion

This thesis is a comprehensive study focused on the ecology of parasitism of temperate-zone bats, and is largely directed towards fundamental ecological questions. In particular, the thesis addressed 1) the host-location behaviour of bat parasites, 2) the reproductive processes of bat parasites and the factors affecting these, 3) the way by which bat parasite species interact among themselves and how these interactions determine the structure of their communities, and 4) the effect of bat parasites on the condition of their bat hosts.

Data obtained throughout the development of this study were thoroughly discussed in chapters 2 through 5 of the thesis. The main objectives of this chapter are to make an integrated discussion of the results presented in the previous chapters, and to set them in an evolutionary framework. The results obtained raised new questions, some of which are partly addressed in the present chapter, while others are presented as suggestions for future research.

Chapter 2. Locating a host

For horizontally-transmitted parasites, which rely on free-living short-lived stages to find a new host at a distance, dispersal is a difficult challenge. This is the case for the highly host-specific nycteribiids, which have to quickly find a specific bat host to return to after depositing pupae on the walls of their roost, or immediately after emerging from them. The second chapter of this thesis addressed the host location behaviour of nycteribiids. Results showed that these parasites do not find hosts randomly, as previously suggested by Marshall (1970), but instead rely on efficient sensorial mechanisms to respond to a combination of host-emitted cues which guide them towards the bats. Without these finely tuned host location mechanisms, their dispersal ability and consequent reproductive success would probably be nil.

Previous studies addressing this subject indicate that horizontally-transmitted parasites use a vast number of host location strategies to facilitate parasite dispersal, adapted to their local probability of finding a host. In this context, parasites are known to use a wide combination of environmental or host-emitted cues to locate their host at a distance. The cues used by nycteribiids proved to be similar to those commonly used by several other types of arthropods parasitizing vertebrates. In general, these locate their hosts using mainly body heat and olfactory cues, such as carbon dioxide and other compounds, which are present in the breath and body of all vertebrates (e.g. Cox *et al.,* 1999; Krasnov *et al.,* 2002; Kilpinen & Mullens, 2004). Additionally, the ability of nycteribiids to respond more efficiently to combinations of cues is shared by almost all parasites of vertebrates. Lehane (2005) interprets this ability as an optimization strategy, because it increases the certainty of the presence and nature of a host, and therefore maximizes the chances of host encounter, while minimizing energy consumption.

The fact that carbon dioxide was the most effective cue used in the location behaviour of nycteribiids, and that body odours elicited weaker responses, was a surprising finding. Indeed, most published studies report that other host-specific parasites strongly rely on specific host odours to locate them, as in the case of the rabbit flea *Spilopsyllus cuniculi* (Vaughan & Mead-Briggs, 1970), the cattle tick *Boophilus microps* (Ostercamp *et al.,* 1999), or the streblid batfly *Megistopoda aranea* (Overal, 1980). This usual preference for specific cues is explained by the higher fitness advantages that parasites have in evolving traits allowing the effective location of specific hosts (Rea & Irwin, 1994). In the particular case of cave-dwelling bats, however, different species often mix in large colonies, which might not favour the use of specific bat odours as host discriminating cues. Therefore, the best host locating strategy for nycteribiids probably consists of following several cues combined to orientate themselves to nearby multispecies bat colonies, where the chances of finding their primary hosts are high. In this context, the present study provided evidences that highly-specific parasites, such as nycteribiids, do not always completely rely on specific body odours as their most rewarding strategy to locate and disperse to new hosts.

The responses of nycteribiids to distinct host-emitted cues suggest that these possess a variety of highly sensitive sensory receptors, but information on these is scarce, as is the one regarding the receptors of their related parasitic species, the streblids and hippoboscids (but see Frantsevich & Gladun, 2002). Therefore, it would be very interesting to investigate the sensorial physiology of nycteribiids and, in this context, the way by which sensory receptors changed in the course of evolution to a parasitic lifestyle.

Also, it remains unclear what are the mechanisms used by nycteribiids for host recognition when in physical contact with them. These mechanisms would be especially interesting to investigate, because these parasites frequently have to discriminate between distinct potential host species located within the same dense clusters. Such a study would contribute to obtain a more complete picture of the host location behaviour and the dispersal abilities of nycteribiids. Among other factors, it would be important to study the possible influence of chemical cues present on the skin of bats and of the components in host blood. Moreover, future studies could focus on possible morphological adaptations of nycteribiids to certain host characteristics (e.g. distance between bat hairs, hair shape, etc.), because such adaptations have been previously observed in other parasites of vertebrates (Lehane, 2005).

Chapter 3. Maximising reproduction

The second chapter showed how a group of specific bat parasites, the nycteribiids, overcome the spatial unpredictability of bat hosts within their environment, in order to disperse successfully. However, suitable hosts are also known to be patchily distributed in time. This is especially true for bats in the temperate-zones, which hibernate during winter and therefore expose parasites to seasonal challenging conditions for dispersal. In this context, the third chapter showed that the four specific ectoparasites of *M. schreibersii* increase their likelihood of successful reproduction and dispersal by adjusting their reproduction to the annual cycle of the host. They cease reproduction when environmental temperatures are low and vulnerable hosts were not available (i.e. during winter), and maximize it during the reproductive seasons of bats, when these are aggregated and particularly vulnerable.

Researchers have been increasingly focusing on parasite reproduction because of its role in host-to-host transmission, but there is still a great lack of detailed information on this subject. Until recently, a high reproductive rate was often considered as the main reproductive strategy of parasites to counter the hazards of parasite transfer between hosts (Marshall, 1981; Bush *et al.*, 2001). However, this trait is only one among the several strategies evident in parasite life-history patterns (Poulin, 2006). The present study is one of the first to reveal a full assemblage of taxonomically distinct parasites with their reproductive cycles similarly adjusted to that of their bat host, suggesting that this adjustment is a highly adaptive trait. Nevertheless, some differences were found among the reproductive strategies of the four studied parasites. The tick *Ixodes simplex* has a higher fecundity than the nycteribiids *P. conspicua* and *N. schmidlii,* and the mite *S. psi.* Moreover, the reproductive activity of this tick was slightly more spread throughout the bat annual cycle than the reproduction of the remaining parasites, which were more concentrated on the pregnancy and nursing season of bats. The higher fecundity of the tick is presumably a strategy to compensate for its losses of infective stages during periods when dispersal is not successful, as observed in other parasites (Bush *et al*., 2001; Combes, 2001). In turn, viviparous nycteribiids and mites exhibit significant lower numbers of offspring. Marshall (1981) discussed how viviparity has allowed a reduction in fecundity, by eliminating the exposed egg and immature stages. According to him, this form of reproduction in parasites reflects a tight coevolution between a parasite and its host.

The synchronization of the reproductive processes between parasites and their hosts was previously documented for other specific parasites of vertebrate species (e.g. Mead-Briggs, 1964; Randolph, 1975; Brown & Brown, 2004; Calero-Torralbo & Valera, 2008), including two other bat parasites (Christe *et al*., 2000; Bartonička & Gaisler, 2007). Most of these studies suggest that the synchronization of reproductive events between the parasite and its host mainly occurs in the temperate regions. These studies and our results corroborate the idea that, in general, host-specific parasites of the temperate regions greatly benefit from synchronizing their reproduction with that of their hosts, because the temporal window of opportunity for a successful dispersal is very narrow, due to the environmental constraints during winter and the well marked seasonal reproduction of most hosts. In contrast, parasites of tropical regions, where tempearature remain fairly stable, are able to reproduce all year long.

Although this study allowed determining the factors influencing reproduction of the bat parasites, it did not reveal the mechanisms by which parasites are able to recognize the best time for reproduction. Roost temperatures do not seem to be directly associated with it, so it is likely that parasites rely on host-emitted signs, such as hormones, as already observed for other species (Mead-Briggs, 1964). Therefore, it would be interesting to assess the capacity of these parasites to exploit the hormonal microenvironment within their hosts to favour their own reproduction.

Chapter 4. Coexisting with other parasites

Dispersal is not the only challenge faced by parasites. These often have to deal the presence of other parasitic species exploiting the same resources within a host. The fourth chapter of the thesis addressed the identification of interactions among bat ectoparasite species and the understanding of how these interactions can influence the structure of their communities, using *M. schreibersii* and its ectoparasites as a model.

Most studies report parasite communities as unstructured assemblages, where interactions among parasitic species do not play a major structuring role (e.g. Haukisalmi & Henttonen, 1998; Morand *et al.*, 1999; Gotelli & Rhode, 2002; Mouillot *et al.*, 2003). The present study revealed a similar lack of structure in bat parasite communities throughout most of the year, but evidences were found of competitive interactions during the bat pregnancy season. This indicates that the communities of bat parasites are dynamic, and can be both interactive or isolationist at different periods of the year. Seasonal variations in the structure of parasite communities were seldom reported before, mainly because most of the studies lack a temporal component, which allows detecting short term events responsible for species interactions (but see Timi & Poulin, 2003; Vidal-Martinez & Poulin, 2003 and Gonzalez & Poulin, 2005). In this context, this study strongly emphasizes the need to carry out community studies over larger periods of time.

The seasonal analysis of co-occurrence showed that competition among parasite species coincided with the pregnancy period of bats, which was generally when mites, ticks and nycteribiids reproduced more intensively (see chapter 3) and parasitic loads were heavier. Moreover, competition was only observed in pregnant bats, on which parasite reproduction is also particularly intensive. This host group appears to create a particularly favourable environment for the occurrence of competition among parasites, since parasite populations are much denser than in other host groups (i.e. males and non pregnant females) and therefore more likely to reach values above which regulation is triggered. In this sense, the coincidence of the reproductive activity of parasites with that of the bat host seems to partly result in a disadvantage for parasites, since it increases the likelihood of negative interactions among them. Because host-specific parasites of temperate regions likely synchronize their reproduction with those of their hosts (chapter 3), it is probable that parasite interactions occur more often than usually reported. Although evidence of competition among ectoparasites is difficult to obtain, it would be interesting to infer if reproduction of hosts often sets a stage for interactions among parasites in other host-parasite systems.

The nycteribiid, mite and tick species composing the parasite community of *M. schreibersii* are all haematophagous, and could potentially exploit the same food and space resources within the bat host. However, it is highly probable that the host blood is not a limited resource for parasites, so these are most likely to directly compete for space. This could explain why evidences of competition were only found among the two nycteribiid species. Mites and ticks might provide a fine example of competition avoidance by niche shifts, as reported in other ectoparasite species (Marshall, 1981, and references therein). In fact, these species may have competed for space in the past, but might have been able to avoid it by specializing in different parts of the bat body (see 1.3.2.). In contrast, both nycteribiids live in the fur of bats, so their spatial niches greatly overlap, promoting direct competition for space. An interesting theory is that competition among closely related ectoparasites (i.e. from the same or related genus) may result in their increased host specificity (ter Hofstede *et al*., 2004). Competition could then partly explain why nycteribiids of the same genus (e.g. *P. conspicua* and *P. dufourii*) are highly specific of different bat hosts (*M. schreibersii* and *M. myotis*, respectively), even though their hosts live side by side in the same colonies. Although this issue was beyond the scope of the present thesis, it would be an interesting question to address in future work.

Chapter 5. Keeping the host alive

The fitth chapter of this thesis assessed the effects of mite loads on the body condition of their bat hosts. Results suggested that mites may have a detrimental effect in their hosts, influencing their body condition. However, this potencial effect appeared to be limited to specific periods during which mite loads are heavier and hosts are particularly susceptible to infections in dense nurseries. Moreover, such an effect on bat condition may in part explain the usual absence of males from nurseries as a behavioral defence to minimize parasitism, which can ultimately influence the social behaviour of these bats.

These findings support the widespread theory that parasites can be detrimental to their hosts at various levels. Moreover, they are in accordance with a wide number of studies that demonstrate that social hosts suffer significantly from the detrimental effects of parasites, and consequently invest more in behavioural defences (e.g. yellow baboons, Hausfater & Meade, 1982; great tits, Christe *et al*., 1994; european badgers, Butler & Roper, 1996; rats, Roper *et al.*, 2002; cliff swallows, Brown & Brown, 2004).

Apart from the present work, only three other studies focused on the impact of bat mite species (Spinturnicidae) on the fitness of their hosts, but yielded contradictory results. Indeed, while the related mite *Spinturnix myotis* was found to be clearly detrimental to its bat host (Giorgi *et al.,* 2001), no evidence of effects of other similar mites were observed by Zahn & Rupp (2004) or Lucan (2006). A possible explanation for these divergent results might be that mites usually have low virulence levels, only becoming costly to their hosts at particular periods when heavy loads coincide with host periods of resource limitation or stress, such as the bat pregnancy and nursing periods (see also chapter 3). In addition, it is usually accepted that vertically transmitted parasites (i.e. those who passed from host parents to offspring), such as mites, are usually less harmful to their hosts than horizontally-transmitted ones (i.e. those who passed among host individuals), since their fitness greatly depends on the successful reproduction of their hosts (Clayton & Tompkins, 1994).

In this context, it would be interesting to assess the effects of horizontally-transmitted parasites on several aspects of the fitness of bat hosts. The nycteribiids can clearly be considered within this category, given their high mobility in nurseries, and their good capacity to locate new hosts (chapter 2). Moreover, their reproduction also coincides with that of the bat host (chapter 3), which translates into heavy nycteribiid loads in bat nurseries. Although an assessment was done on the effects of nycteribiids on their host body condition during the present study, results were not conclusive. It is assumed that this was mainly because these parasites are highly mobile, and consequently their parasitic pressure is likely to be homogenous upon all bats in a colony. Nevertheless, a recent study by Reckardt & Kerth (2007) revealed a possible detrimental effect of nycteribiids, by demonstrating that Bechstein's bats change their roosting behaviour in order to decrease their contact with the nycteribiid *Basilia nana*. Consequently, further studies on the effects of nycteribiids and other horizontally-transmitted parasites of temperate-zone bats would be useful.

Although this study revealed a potencial effect of mite loads on the condition and social structure of bats in nurseries, it is very likely that the overall cumulative effect of ectoparasitism is greater than the one measured. In fact, some of the heaviest loads of mites, nycteribiids and ticks were also recorded in bats during the pregnancy period, due to their synchronized reproduction with that of the host (chapter 3). This simultaneous increase in the pressure by various parasites, along with the fact that this period is one of the most sensitive in the life-cycle of bats, can translate in significant parasite-associated costs for these. In addition, the endoparasites may also exert some costs upon their hosts. Finally, the possible role of spinturnicid mites and other bat parasites in the transmission of blood parasites might be of great importance. As such, further studies would contribute to better assess the overall role of bat parasites on the fitness of their hosts.

Concluding remarks

The results obtained in this thesis contributed towards the understanding on how parasite species adapted to the particular characteristics of temperate-zone bats as hosts. Overall, results provided evidence that the studied parasites have tightly coevolved with their bat hosts. The complex host location behaviour of nycteribiids can be considered as an evidence of a long coevolutionary history in the studied host-parasite systems. Moreover, the close reproductive synchronization of all ectoparasites with that of their hosts provides a fine example on how selection has favoured efficient reproductive mechanisms to maximise parasite dispersal on hosts patchly distributed in time. In turn, as a response to the potential pressures imposed by mite parasitism, bats (i.e. *M. schreibersii*) seemed to have evolved behavioural defences, which ultimately may have influenced their social structure. Results also showed that competition can occur among bat parasite species. The pressures imposed by competitive interactions between parasites may have been an important driver in the evolution of a high degree of specificity and morphological speciation observed in these bat parasites.

Some of the results and conclusions discussed here are likely to apply to other host-parasite systems involving cave-dwelling bats in the temperate-zones, because they are all submitted to comparable constraints imposed by the seasonality of the environment, and the life cycle of most temperate-zone bat hosts tends to be very similar.

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