ORIGINAL PAPER

Assessing host specificity of obligate ectoparasites in the absence of dispersal barriers

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Abstract Host specificity is a characteristic property of parasite-host associations and often is high among those involving obligate or permanent parasites. While many parasites are highly host-specific under natural conditions, specificity may break down in the absence of dispersal barriers. We tested the host specificity of obligate and permanent blood-feeding bat parasites (Hemiptera: Polyctenidae) under experimental conditions where parasite dispersal barriers had been removed. Under these conditions, parasites not only readily accepted a secondary host species but also remained there when a primary host was immediately available. Experiments with bat bugs and observations of streblid bat flies suggest that specificity may at least temporarily break down when dispersal barriers are removed. To affect long-term coevolutionary patterns, such transfers would necessarily entail the establishment of viable parasite populations on secondary host species.

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Host specificity is a characteristic emergent property of parasite and host associations. Strictly host-specific parasite species associate with one host species; whereas, less specific parasites associate with a number of host species. Parasites that are obligate and permanent (i.e., all life stages on or in a particular host) generally are more specific than are facultative and transitory parasites. Assessments of ectoparasite host specificity typically pursue three lines of inquiry (Poulin and Keeney 2008). These include analyses of occurrence data from biodiversity surveys (Wenzel and Tipton 1966; Gettinger 1992; Dick 2007; Dick and Patterson 2007), molecular studies (Smith et al. 2006; Whiteman et al. 2006), and experimental pursuits (Esbérard et al. 2005; Dick and Dick 2006; Kuris et al. 2007). Each method differs in approach to the host specificity question, and sometimes in results. High host specificity for certain groups of ectoparasites is often demonstrated via survey data and under molecular scrutiny, yet experimental tests can reveal instances where specificity breaks down.

Bat bugs of the genus *Hesperoctenes* Kirkaldy (Hemiptera: Polyctenidae) are obligate, permanent, blood-feeding ectoparasite associates of Neotropical bats of the family

Molossidae. The morphology and ecology of bat bugs are highly adapted for surviving, feeding, and reproducing on the body of their nocturnal, volant hosts. Polyctenids are wingless and eyeless insects that are flattened dorsoventrally, possess sclerotized combs (ctenidea), and move quickly through host pelage propelled by long meso- and metathoracic legs. The life cycle also is adapted to permanent parasitism through viviparity, which in part facilitates the physical connection with the host (Hagan 1931). The first nymphal instars are passed within the female, while the three post-natal stages exist only on the host's body, feeding frequently and exclusively on blood. Because bug dispersal occurs only by direct host–host contact (presumably between conspecific bats), bug-bat associations are thought to be largely monoxenous (restricted to a single host species; Marshall 1980).

Host specificity assessments of bat bugs based on biodiversity studies have been inconclusive because of the high likelihood of host-to-host parasite contamination (Dick 2007) and because the bugs have been rarely collected. As of 1912, fewer than 20 specimens were known from world collections (Ferris and Usinger 1939) and by 1946, known specimens numbered under 100 (Usinger 1946). This number was substantially increased during the Smithsonian Venezuela Project, allowing Ueshima (1972) to examine >200 new specimens. Unfortunately, the group has been neglected since, and the taxonomic status of many known species is dubious (Ueshima 1972).

Although Marshall (1980) and Presley (2004) held that most species of polyctenids are monoxenous, the host associations of *Hesperoctenes fumarius* (Westwood) are questionable. Studying all New World collections available, Ueshima (1972) reported *H. fumarius* as associated with 11 species of bat, the majority, molossids, yet most of those associations were meaningless, as they were based on a single specimen or a single host individual. Many others were based on small sample sizes. The majority (78%) of the 147 collected Venezuelan *H. fumarius* were associated with *Molossus rufus* E. Geoffroy (Ueshima 1972). Although 337 Venezuelan *Molossus molossus* were sampled for ectoparasites, none yielded *H. fumarius* (Handley 1976). Autino et al. (1999) reported ten *H. fumarius* from three species of three genera of bat, the majority associated with *Eumops bonariensis* (Peters). However, based on results from Paraguay, Presley (2004) reported that *H. fumarius* was an oligoxenous parasite, with nearly 75% of 163 bugs associated with *M. molossus*, and 25% associated with *M. rufus*. Apparently, *H. fumarius* is associated with different hosts across its distribution, and no clear pattern of specificity has emerged.

In this paper, we report the results of transfer experiments with *H. fumarius*, designed to provide insights to the following questions: (1) are individuals of *H. fumarius* that naturally infest *M. rufus* so host-species specific that they immediately reject the opportunity to infest another related host species, *M. molossus* (Pallas), or will individuals readily accept the secondary host species? and (2) if experimentally placed on *M. molossus* and given the opportunity, will individuals of *H. fumarius* transfer back onto their primary host species (*M. rufus*)?

Materials and methods

The study was conducted at the Centro de Estudos Ambientais e Desenvolvimento Sustentável (CEADS-UERJ) biological station on the tropical island of Ilha Grande in southeastern Brazil. Individual *M. rufus* and *Phyllostomus hastatus* (Pallas) were collected from the attic of an old school building, which had been converted into living quarters. Daytime surveys revealed *P. hastatus* roosting in two spatially separate groups; one was a harem of females, with a single (or at most, a few) large male(s); the other consisted of only bachelor males (see also McCracken and Bradbury 1981; McCracken and Wilkinson 2000). On 30 September 2007, we visited the roost during the night (within 1 hour of dusk) when the *Phyllostomus* were out foraging. During this time, we encountered a small group of about 12 individual *M. rufus* in the precise location where the females of *Phyllostomus* were observed during daytime. Eight *M. rufus* were live-captured using long forceps. On 1 October 2007, we returned to the roost during the daytime and captured 17 individuals of *P. hastatus*. From another house-root on Ilha Grande (approximately 1 km from the schoolhouse), we captured six individuals of *M. molossus*. Individuals of each species were placed into individual cloth bags for transportation to the biological station. At the laboratory, we removed and counted the ectoparasitic insects and placed them live into glass shell vials. Permanent ectoparasites are known to die relatively quickly when removed from their host (Marshall 1981), so we carried out a short-term host-exchange experiment using adult bat bugs. For each of seven experimental replications, we randomly chose five adult bat bugs and placed them one by one onto a randomly chosen individual of *M. molossus*. Once this transfer was complete, we placed the experimentally infested *M. molossus* into a soft cloth bag with a randomly chosen individual of the bug's primary host, *M. rufus*. These two bats were maintained in immediate physical contact within the cloth bag, and host associations of the bugs were re-evaluated after 1 and 5 hours. Following each replication, bats were removed from the bag, and the bugs on each were enumerated. Counts of bugs on hosts were compared using a Chi-square test in Statistica Software (StatSoft Inc 2005).

Results

Seventeen individual *P. hastatus* roosting in association with *M. rufus* were sampled for ectoparasites and carried no polyctenid bat bugs but were infested with 105 streblid bat flies (*Trichobius longipes* (Rudow) and three *Strebla consocia* Wenzel). Furthermore, eight individuals of *M. rufus* were sampled and hosted 84 ectoparasitic arthropods—36 bat bugs (*H. fumarius*) and 48 bat flies (*T. longipes*). Six host individuals harbored both ectoparasite taxa simultaneously (Table 1). The molossid bats captured from the second roost, *M. molossus*, were not infested with bat bugs or bat flies. During the transfer experiments, bugs willingly and quickly crawled onto, and then into, the fur of their alternative host, *M. molossus*. This indicated that they at least immediately accept individuals of a secondary host species. The movement behavior of the bugs on both bat species was similar—they rapidly move within the hair, between the hair shafts, by tilting their bodies sideways and pushing with the meso- and metathoracic legs. The bugs appeared to prefer the dorsal regions of the host bat, particularly the interscapular region. Generally, once the bat bug came to rest on the host's body, it remained relatively motionless unless disturbed, when it would move quickly to avoid capture. Even though bats of two species were in physical contact with each other for at least 5 hours per replication, we observed no interspecific aggression; once they were inside the cloth bag, they remained relatively motionless.

After 1 hour, bat bugs placed experimentally on *M. molossus* generally remained with that bat species (the non-primary host); distribution of bugs upon the two bat species were decidedly non-random ($df=1$, $\chi^2=17.86$, $p<0.001$). Most bugs did not return to their primary host, *M. rufus* (Table 2). Even following five continuous hours of opportunity to switch hosts, while a few more bugs returned to their original host species (eight of 35), results were

Table 1 Infestation intensities of the bat bug *Hesperoctenes fumarius* and the bat fly *Trichobius longipes* on each of the eight freetail bats, *Molossus rufus*, captured from a roost on Ilha Grande, Brazil

Host individual (#)	*Hesperoctenes fumarius* (*n*)	*Trichobius longipes* (*n*)
1	6	2
2	0	5
3	7	7
4	7	3
5	5	8
6	8	8
7	0	10
8	3	5
	36	48

Table 2 Results of seven transfer experiment replications for the bat bug *Hesperoctenes fumarius*, allowing 1 and 5 hours, respectively, for bugs to disperse between host bats

Molossus molossus (five bugs initially)		*Molossus rufus* (zero bugs initially)	
After 1h	After 5h	After 1h	After 5h
4	4	1	1
5	4	0	1
5	5	0	0
4	3	1	2
3	3[a]	2	2
5	3	0	1
4	4	1	1[a]
30	26	5	8

[a] Between 1 and 5 hours, one bug disappeared. We assume the bug was consumed by one of the insectivorous bats

strongly non-random; the significant majority remained with the secondary host species ($df=1$, $\chi^2=9.53$, $p<0.01$; Table 2). Following the 5-hour replication, bat bugs removed from either species of bat appeared to be healthy and active. Many permanent ectoparasites die within hours of being removed from their hosts (Marshall 1981), and host blood is presumably metabolized quickly. Because the digestive tracts of bat bugs starved only a few hours after were void of fresh blood (assessed visually), the presence of fresh blood in distended guts of experimental bugs provided evidence bugs fed on both molossid bat species.

Discussion

Even though multiple bat species often forage and roost in close proximity (Goodwin and Greenhall 1961; Kunz 1982), direct physical contact between bat individuals of different species is rare. Some bats are predaceous and contact other bats as prey, but ectoparasite exchanges attributed to such dynamics have not been documented. Because bat bugs are flightless, permanent ectoparasites infesting nocturnal, volant bat hosts, interspecific host transfers depend on close spatial approximation of different bat species in roosts. Observational evidence demonstrates that molossid bats associate with other species in the same roost. In Brazil, 356 individual *M. molossus*, 25 *M. rufus*, 142 *Myotis nigricans*, five *Eptesicus brasiliensis*, and four *P. hastatus* were observed inhabiting a single roost (Esbérard 2001). Indeed, in the school roost studied on Ilha Grande, we captured individuals of *M. rufus* and *P. hastatus* inhabiting the same spatial area. Such spatial relationships might provide opportunities for polyctenid bugs and other ectoparasites to disperse from one bat species to another.

Experimental studies such as this provide important insight into the specificity of host associations. These methods allow the removal of ecological barriers to dispersal. Our limited sampling failed to reveal natural populations of *H. fumarius* on *M. molossus*, yet experimentation demonstrated that adult individuals of this bug species would readily accept that host species. Moreover, when the secondary hosts were experimentally infested with bugs, and those bats placed into immediate proximity of clean, primary hosts, the bat bugs generally failed to move back to the primary hosts.

In addition to our observations of bat bugs, we observed the streblid bat fly *T. longipes* parasitizing *M. rufus*. The flies were removed from individuals of *M. rufus* found roosting in the same spatial area in which *P. hastatus* individuals had been observed the previous day, but the reciprocal natural transfer, polyctenid bat bugs to *P. hastatus* bats, was not observed. *T. longipes* is known to be a specific parasite of *P. hastatus*; generally, Neotropical streblid bat flies are very specific, at least when such assessments are based on biodiversity survey data (Dick and Gettinger 2005; Dick 2007; Dick and Patterson 2007; Wenzel 1976). The observation of *M. rufus* relatively heavily infested with *T. longipes* is unprecedented. One possible explanation is that flies emerging from pupae during the absence of *P. hastatus* might temporarily colonize *M. rufus*, simply because they are the only bat host available, but proportions of teneral (newly emerged and unsclerotized) *T. longipes* on *P. hastatus* (12 of 105) and *M. rufus* (nine of 48) were not significantly different (Fisher exact test, $p=0.209$). These results indicate that when ecological barriers to dispersal are removed, these parasites are less specific than previously understood.

Mechanical barriers can also block the establishment of ectoparasitic arthropods on novel host species. Bush and Clayton (2006) found that bird body and feather lice depended in part on host size (feather size) for survival, and that transfers to either larger or smaller hosts were unsuccessful. Species of the genus *Molossus* vary greatly in size (Freeman 1981). *M. rufus* is the largest species, and *M. molossus* among the smallest. Though *M. molossus* is 80% the overall length of *M. rufus*, it is only 42% as massive (López-González 1998). However, body size in bats does not dictate the density or architecture of pelage in bats, as it does with birds and their feathers. Thus, we doubt that body size differences between these two molossid species could preclude establishment of ectoparasites from one to the other.

Our studies were short-term in duration. It remains unclear whether the laboratory environment biased our assessment of specificity, or whether the poorly understood ecological mechanisms that might maintain specificity in nature so easily break down in the laboratory. What happens in the short-term does not necessarily provide evidence for establishment of a reproductively viable parasite population on novel or secondary host species. Evolutionarily, a viable, long-term establishment would be necessary to enable the host switching that has been invoked to explain cases of non-reciprocal host-parasite coevolution (Johnson et al. 2002; Hafner et al. 2003). Here, we found bat flies and bat bugs adventitiously moving onto non-primary host species when the primary host was not present, and in the case of bat bugs, they did not transfer back even when given opportunity, but unsuccessful short-term transfers happening at ecological time-scales would not manifest into evolutionary-time pattern, as might be evidenced by comparative host-parasite phylogenies. More work is necessary to fully understand the dynamics that dictate and direct the host specificity of obligate parasites. These studies should include all available methods including survey sampling, experimental, and molecular assessment.

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