ECTOPARASITES AND OTHER EPIFAUNISTIC ARTHROPODS OF SYMPATRIC COTTON MICE AND GOLDEN MICE: COMPARISONS AND IMPLICATIONS FOR VECTOR-BORNE ZOONOTIC DISEASES

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ABSTRACT: Ectoparasite and epifaunistic arthropod biodiversity and infestation parameters were compared between 2 sympatric small rodent species, the cotton mouse (Peromyscus gossypinus (Le Conte)) and golden mouse (Ochrotomys nuttalli (Harlan)), in southern Georgia from 1992 to 2003. Because the cotton mouse is known to be a reservoir of more vector-borne zoonotic pathogens than the golden mouse, we hypothesized that it would be parasitized by more ectoparasites that are known to be vectors of these pathogens. Cotton mice (n = 202) were parasitized by 19 species of arthropods, whereas golden mice (n = 46)were parasitized by 12 species. Eleven species of arthropods were recovered from both host species, whereas 7 were recorded only from cotton mice, and 1 species only from golden mice. Infestation prevalences (percent of mice parasitized) were significantly higher for 1 species of arthropod (the tropical rat mite Ornithonyssus bacoti (Hirst)) infesting cotton mice and for 4 species (the flea Peromyscopsylla scotti Fox and the mites Glycyphagus hypudaei Koch, Androlaelaps casalis (Berlese), and Androlaelaps fahrenholzi (Berlese)) infesting golden mice. Mean intensities (mean per infested mouse) were significantly higher for 2 species (the flea Orchopeas leucopus (Baker) and the blacklegged tick Ixodes scapularis Say) infesting cotton mice and for 2 species (G. hypudaei and A. fahrenholzi) infesting golden mice. Ectoparasites that are known to be vectors of zoonotic pathogens were significantly more common on cotton mice than on golden mice. These ectoparasites included the rhopalopsyllid flea Polygenis gwyni (Fox), a vector of the agent of murine typhus; I. scapularis, the principal vector of the agents of Lyme borreliosis, human granulocytic ehrlichiosis, and human babesiosis; and O. bacoti, a laboratory vector of several zoonotic pathogens. However, 2 species of ixodid ticks that can transmit zoonotic pathogens were recovered from both host species. These were the American dog tick Dermacentor variabilis (Say), the principal vector of the agent of Rocky Mountain spotted fever in eastern North America, and Ixodes minor Neumann, an enzootic vector of the agent of Lyme borreliosis. Overall, the cotton mouse was parasitized by significantly more ectoparasites that are known to be vectors of zoonotic pathogens than was the golden mouse. These data support the hypothesis that the cotton mouse has greater epidemiological importance for zoonotic vector-borne pathogen transmission than does the golden mouse.

The relationships between ectoparasites, vector-borne zoonotic diseases, and reservoir hosts can be complex (Gratz, 1999; Spielman et al., 2001). The ectoparasite-host-pathogen dynamics for some vector-borne zoonotic diseases, such as Lyme borreliosis, have been studied in more detail than those for other diseases (Gray et al., 2002). However, even the dynamics and maintenance mechanisms of Lyme borreliosis are incompletely known in some regions of North America, i.e., the southern United States (Oliver, 1996; Oliver et al., 2003). Additional ectoparasite-borne zoonotic diseases of note in the southern United States that have mammalian reservoirs include Rocky Mountain spotted fever, human granulocytic ehrlichiosis (HGE), human monocytic ehrlichiosis, and sporadic epidemic typhus (Magnarelli et al., 1999; Whitlock et al., 2000; Fang et al., 2002; Durden, 2003; Durden and Traub, 2003; Sonenshine et al., 2003). Other ectoparasite-borne, rodent-maintained zoonotic diseases that might be enzootic in this region include murine (flea borne or endemic) typhus, tularemia, Q fever, and human babesiosis (Healy et al., 1976; Adler and Wills, 2003; Durden and Traub, 2003; Sonenshine et al., 2003). Combining analyses of ectoparasites infesting sympatric mammals with knowledge of the zoonotic vector status of these ectoparasites can provide additional information for assessing the epidemiological importance of their hosts.

In the southeastern United States, the sigmodontine murid rodents *Peromyscus gossypinus* (Le Conte) (cotton mouse) and *Ochrotomys nuttalli* (Harlan) (golden mouse) are usually sympatric in deciduous woodland habitats (McCarley, 1959). The golden mouse is more arboreal than the cotton mouse; it typically builds leafy nests in tangles of vines (Linzey, 1968; Linzey and Packard, 1977), whereas the cotton mouse usually constructs nests in the ground, under logs, or in tree bases (Wolfe and Linzey, 1977). However, golden mice also occasionally nest in the ground or under logs (Morzillo et al., 2003). Both the cotton mouse and golden mouse are known to be reservoir hosts for the rickettsial agent of Rocky Mountain spotted fever (Bozeman et al., 1967; Norment et al., 1985). The cotton mouse is also known to be a reservoir host for the agents of both Lyme borreliosis and HGE (Oliver, 1996; Magnarelli et al., 1999; Oliver et al., 2003). The golden mouse is not known to be a reservoir for any other vector-borne zoonotic pathogens, although Kollars et al. (1996) reported that some live-trapped golden mice in Tennessee had seroconverted against Lyme borreliosis spirochetes. Cotton mice in Florida have also reacted seropositively for arenaviruses (2 unidentified strains) and for eastern equine encephalitis, Highlands J, and Everglades viruses, which are either known, or suspected to be, vector borne (Day et al., 1996). Analyzing the ectoparasite faunas associated with each of these rodents, with respect to the known vector capacity of these ectoparasites, will supplement information on the relative importance of each host species with respect to vector-borne zoonotic pathogen transmission. Because the cotton mouse is known to be a reservoir of more vector-borne zoonotic pathogens than is the golden mouse, we hypothesized that the cotton mouse would also be parasitized by more vectors of these pathogens. Therefore, we systematically collected and analyzed ectoparasites and other epifaunistic arthropods from sympatric cotton mice and golden mice in woodland habitats in southern Georgia with respect to their known potential for vector-borne zoonotic pathogen transmission.

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MATERIALS AND METHODS

Rodent trapping

Mice were captured in woodland habitats in 5 southern Georgia Counties (Bulloch, Candler, Chatham, Coffee, and Screven) from 1992 to 2003 using Sherman live-traps (H. B. Sherman Traps Inc., Tallahassee, Florida) (17.5 \times 6.4 \times 5.4 cm and 22.9 \times 8.9 \times 7.6 cm) baited with birdseed or oats, or both. Peanut butter was not used because red imported fire ants are common in this region; these ants are attracted to peanut butter and can kill and partially consume mammals inside traps. Captured rodents were either killed in the field with a chloroform overdose (specimens collected by T.N.) or taken to a laboratory and anesthetized with a 1:10 mixture of xylazine sulfate-ketamine hydrochloride administered intramuscularly using a 27-gauge hypodermic needle attached to a 1-cc tuberculin syringe. After removal of ectoparasites and other epifaunistic arthropods, some live rodents were released at their capture sites, whereas others were maintained in laboratory colonies or frozen for use in other studies. Trapping occurred throughout the year and was not seasonally biased. All procedures involving animals were approved by the Institutional Animal Care and use Committee (IACUC) at Georgia Southern University; trapping permits are on file with the authors.

Ectoparasite collections

Anesthetized mice were initially combed with a flea comb over a white tray to remove larger ectoparasites. Other arthropods were then recovered by systematically pushing back the host fur and removing specimens with fine forceps; this effort was aided by observation through a low-power binocular microscope. Ectoparasites and other arthropods were removed from killed mice by applying the same method and by using a "washing" technique (Henry and McKeever, 1971). Collected arthropods were stored in labeled vials containing 70% ethanol until they were identified. Some mites were cleared in lactophenol and slide-mounted in Hoyer's medium, whereas some lice and fleas were cleared in 10% potassium hydrochloride and slide-mounted in Canada balsam before identification. Voucher ectoparasites and other arthropods from this study are deposited in the Department of Biology, Georgia Southern University. Representative accession numbers of voucher specimens include L2919-L2921 and L3077-L3086. Voucher host specimens are deposited in the American Museum of Natural History (Department of Mammalogy), New York, under accession numbers AMNH 269979-AMNH 269982.

Statistical analysis

Infestation prevalences for each arthropod species infesting cotton mice versus golden mice were compared by chi-squared analysis with Bonferoni adjustment. Mean intensities were compared using the Wilcoxon/Kruskal–Wallis test. Prevalence is the percent of mice infested, and mean intensity is the mean number of ectoparasites per infested host following Bush et al. (1997).

RESULTS

Ectoparasite/arthropod diversity

Nineteen species of arthropods were recovered from 202 cotton mice, whereas 12 species were recovered from 46 golden mice (Table I). Eleven species infested both host species. Three species of fleas (*Orchopeas leucopus* (Baker), *Polygenis gwyni* (Fox), and *Stenoponia americana* (Baker)), 1 mammal nest beetle (*Lathridius liratus* Le Conte), 1 tick (*Ixodes scapularis* Say), 1 fur mite (*Radfordia subuliger* Ewing), and 1 mesostigmatid mite (*Ornithonyssus bacoti* (Hirst)) infested cotton mice but not golden mice. Conversely, 1 species of mesostigmatid mite (*Androlaelaps casalis* (Berlese)) was recovered from golden mice but not from cotton mice.

Ectoparasite/arthropod infestation parameters

Statistically significant differences between ectoparasite infestation parameters on the 2 host species involved 2 species of fleas, 1 species of tick, 1 species of fur mite, and 3 species of mesostigmatid mites. The leptopsyllid flea *Peromyscopsylla scotti* Fox, the glycyphagid fur mite *Glycyphagus hypudaei* Koch, and the laelapid mites *A. casalis* and *Androlaelaps fahrenholzi* (Berlese), all had significantly higher prevalences on golden mice (Table I). Conversely, the tropical rat mite *O. bacoti* (family Macronyssidae) was significantly more prevalent on cotton mice (Table I). Mean intensities for the ceratophyllid flea, *O. leucopus*, and the blacklegged tick, *I. scapularis*, were significantly higher on cotton mice. Conversely, both *G. hypudaei* and *A. fahrenholzi* had significantly higher mean intensities on cotton mice (Table I).

DISCUSSION

In this study, cotton mice were parasitized by more species of arthropods (19) than were golden mice (12) (Table I). Although most of the epifaunistic arthropods collected during this study are obligate ectoparasites, the beetle (L. liratus) and 1 of the fur mites (G. hypudaei) are mainly phoretic, another fur mite (R. subuliger) feeds mainly on host skin and fur, the bot (Cuterebra fontinella Clark) is a subcutaneous parasite, and 4 of the mesostigmatid mites (A. casalis, A. fahrenholzi, Eulaelaps stabularis (Koch), and Haemogamasus liponyssoides Ewing) are facultative hematophages that can also consume other resources such as host skin and other arthropods (Whitaker, 1982; Radovsky, 1985; Durden et al., 1993). All the arthropods that we recorded have been reported from these 2 host species in previous studies (Linzey, 1968; Whitaker and Wilson, 1974; Durden et al., 1993, 1999, 2000; Durden, 1995). Because we examined more than 4 times as many cotton mice as golden mice, it is possible that a few of the rarer arthropods that were recorded only from cotton mice might have also been recorded from golden mice if a larger host sample had been available for the latter host. However, at least 2 ectoparasite species, the chigger Leptotrombidium peromysci Vercammen-Grandjean and Langston and the tropical rat mite O. bacoti, were common on cotton mice but absent from golden mice, suggesting that these 2 species of ectoparasites prefer to parasitize the former host. Furthermore, the flea O. leucopus, which is known to parasitize several Peromyscus spp. mice (Durden, 1995), was a frequent parasite of cotton mice but was absent from golden mice in this study (Table I).

With respect to infestation prevalences, we cannot ascertain why 4 species of ectoparasites (1 flea, 1 fur mite, and 2 laelapid mites) infested a significantly greater proportion of golden mice than cotton mice or why 1 species (the tropical rat mite) infested significantly more cotton mice (Table I). Perhaps conditions of the host pelage or conditions inside the host nest influenced these differences. The leafy arboreal nests typically constructed by golden mice might provide a better microhabitat for the survival of the immature stages and off-host adults of this flea and of the 2 laelapid mite species. Conversely, the nonarboreal nests typically constructed by cotton mice might provide more conducive microhabitats for the survival and reproduction of tropical rat mites. Furthermore, we do not know why 2 species of ectoparasites (1 flea and the blacklegged tick) had significantly higher mean intensities on cotton mice, whereas 2 different ectoparasite species (1 fur mite and 1 laelapid mite) exhibited significantly higher mean intensities on golden

Ectoparasite	Cotton n	Cotton mice $(n = 202)$		Golden	Golden mice $(n = 46)$			P value
species	No. collected (stages)	Prevalence (%)	Mean intensity	No. collected (stages)	Prevalence (%)	Mean intensity	P value (prevalence)	(mean intensity)
Mammal nest beetle Lathridius liratus	3 (1M. 2F)	1.5	1.0				0.4056	0.0833
Sucking louse Hoplopleura hesperomydis	199 (41M, 150F, 8N)	12.4	8.0	8 (1M, 5F, 2N)	4.4	4.0	0.1146	0.5123
Botfly						< -		
Cuterebra fontinella	11(11L)	4.0	1.4	2 (2L)	4.4	1.0	0.9040	0.5930
Fleas								
Epitedia wenmanni	5 (2M, 3F)	2.5	1.0	1 (1F)	2.2	1.0	0.9044	1.0000
Orchopeas leucopus	14 (6M, 8F)	6.4	1.1				0.0771	0.0082*
Peromyscopsylla scotti	14 (2M, 12F)	3.0	2.3	10 (5M, 5F)	15.2	1.4	0.0008*	0.2141
Polygents gwynt Stenoponia americana	3 (2M, 1F) 8 (4M, 4F)	c.1 2.5	1.0				0.2810	0.0833 0.0833
Ticks								
Dermacentor variabilis	189 (29N, 160L)	31.2	3.0	37 (17N, 20L)	43.5	1.9	0.1109	0.3706
Ixodes minor	10 (9N, 1L)	3.5	1.4	2 (2L)	4.35	1.00	0.7727	0.4227
Ixodes scapularis	4 (1N, 3L)	2.0	1.0				0.3360	0.0455*
Chigger mites								
Euschoengastia peromysci	59 (59L)	2.5	11.8	3 (3L)	4.4	1.5	0.4889	0.1714
Leptotrombidium peromysci	49 (49L)	4.0	6.1				0.1700	0.1182
Fur mites								
Glycyphagus hypudaei	550 (550N)	7.4	36.7	1,664 (1,664N)	43.5	83.2	< 0.0001 *	0.0231^{*}
Radfordia subuliger	4 (4F)	1.5	1.3				0.4056	0.1573
Mesostigmatid mites								
Androlaelaps casalis				2 (2F)	2.2	2.0	0.0357*	0.3173
Androlaelaps fahrenholzi	48 (3M, 45F)	11.9	2.0	57 (52F, 5N)	23.9	5.2	0.0344^{*}	0.0124^{*}
Eulaelaps stabularis	2 (2F)	1.0	1.0	6 (6F)	4.4	3.0	0.1028	0.3173
Haemogamasus liponyssoides	6 (1M, 5F)	2.0	1.5	2 (2F)	4.4	1.0	0.3456	0.4795
Ornithonyssus bacoti	80 (31F, 49N)	13.9	2.9	Ι			0.0073*	0.0759

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* Significant difference with 95% confidence limits.

mice. Again, microhabitats inside the host nests may have influenced off-host survival and reproduction for the flea, tick, and laelapid mite. Similar to most other ixodid ticks, *I. scapularis* typically quests for hosts from vegetation or leaf litter (Durden et al., 1996); therefore, the larger proportion of time spent by cotton mice on the ground, compared with golden mice, suggests that cotton mice should be parasitized by greater numbers of ixodid ticks. Nevertheless, infestation parameters for the remaining 2 species of ixodid ticks that we collected were not significantly different between the 2 host species (Table I).

Immature stages of 2 species of ixodid ticks that are known to be vectors of zoonotic pathogens were collected from both host species, i.e., the American dog tick, Dermacentor variabilis (Say) and Ixodes minor Neumann (Table I). Dermacentor variabilis is the principal vector in eastern North America of the rickettsial agent that causes Rocky Mountain spotted fever (Norment et al., 1985; Wells et al., 2004). Both the cotton mouse and golden mouse are known to be reservoir hosts for this agent (Bozeman et al., 1967; Norment et al., 1985). Furthermore, D. variabilis can maintain the agents of both tularemia and Q fever (Sonenshine et al., 2003), and some individual engorging adult females of this tick can cause tick paralysis in both dogs and humans (Wells et al., 2004). Ixodes minor is not known to feed on humans, but is an enzootic vector of the agent of Lyme borreliosis between rodents (and possibly birds also) in nature (Oliver, 1996; Oliver et al., 2003). Bridge vectors such as I. scapularis could feed on rodents (or birds) infected by I. minor, and then, after molting to a subsequent life stage, feed on humans to transmit the pathogen.

Three species of ectoparasites that can transmit zoonotic pathogens were collected from cotton mice but not from golden mice, i.e., the rhopalopsyllid flea P. gwyni, the tick I. scapularis, and the tropical rat mite O. bacoti (Table I). Polygenis gwyni is a supplementary vector of the agent of murine typhus, which was a common zoonotic disease in the southern United States until the 1950s (Adler and Wills, 2003) and which still persists in isolated foci in parts of this region (Durden et al., 1999). Ixodes scapularis is an important vector of at least 3 zoonotic pathogens, i.e., those that cause Lyme borreliosis, HGE, and human babesiosis. The etiologic agents of the first 2 listed diseases have been isolated from, or detected in, I. scapularis from several of our study sites in southern Georgia (Oliver, 1996; Fang et al., 2002; Oliver et al., 2003). Ornithonyssus bacoti will feed on humans if given the opportunity and is a laboratory vector of the agents of murine typhus, rickettsialpox, Q fever, plague, and tularemia (Yunker, 1973). However, the importance of O. bacoti as a vector of any of these agents in nature remains to be demonstrated. Nevertheless, bites to humans by this ectoparasite can result in a pruritic skin condition called tropical rat mite dermatitis (Yunker, 1973). In this study, no ectoparasite that is known to be a vector of zoonotic pathogens was recovered exclusively from golden mice.

Overall, we found 2 species of ectoparasites (both ixodid ticks) that are known vectors of zoonotic pathogens on both cotton mice and golden mice, 3 vector species (1 flea, 1 tick, and 1 mite) exclusively on cotton mice, and no known vector species exclusively on golden mice. This supports our hypothesis that cotton mice, which are known to be reservoir hosts for several vector-borne zoonotic pathogens, are parasitized by more vectors of these pathogens than are golden mice. We conclude that cotton mice have more epidemiological importance than golden mice with respect to vector-borne zoonotic disease transmission.

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