Polymerase chain reaction (PCR) identification of rodent blood meals confirms host sharing by flea vectors of plague

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ABSTRACT: Elucidating feeding relationships between hosts and parasites remains a significant challenge in studies of the ecology of infectious diseases, especially those involving small or cryptic vectors. Black-tailed prairie dogs (Cynomys ludovicianus) are a species of conservation importance in the North American Great Plains whose populations are extirpated by plague, a flea-vectored, bacterial disease. Using polymerase chain reaction (PCR) assays, we determined that fleas (Oropsylla hirsuta) associated with prairie dogs feed upon northern grasshopper mice (Onychomys leucogaster), a rodent that has been implicated in the transmission and maintenance of plague in prairie-dog colonies. Our results definitively show that grasshopper mice not only share fleas with prairie dogs during plague epizootics, but also provide them with blood meals, offering a mechanism by which the pathogen, Yersinia pestis, may be transmitted between host species and maintained between epizootics. The lack of identifiable host DNA in a significant fraction of engorged Oropsylla hirsuta collected from animals (47%) and prairie-dog burrows (100%) suggests a rapid rate of digestion and feeding that may facilitate disease transmission during epizootics but also complicate efforts to detect feeding on alternative hosts. Combined with other analytical approaches, e.g., stable isotope analysis, molecular genetic techniques can provide novel insights into host-parasite feeding relationships and improve our understanding of the role of alternative hosts in the transmission and maintenance of disease. Journal of Vector Ecology 35 (2): 363-371. 2010.

Keyword Index: Cynomys ludovicianus, host-parasite feeding interactions, multi-host pathogens, Onychomys leucogaster, Yersinia pestis.

INTRODUCTION

A central problem in the study of the ecology of infectious diseases within diverse communities is determining the roles of different vector and host species (Fenton and Pederson 2005, Keessing et al. 2006). A complete understanding of host-vector feeding relationships is critical for determining the likelihood of an outbreak of disease (epizootic), as well as the rate and extent of its spread. Detailed investigations of feeding relationships between cryptic vectors and hosts often reveal the involvement of less common or previously unrecognized hosts (e.g., Kilpatrick et al. 2006, Brisson et al. 2008). For most diseases, however, including many zoonoses and diseases of conservation importance, these relationships remain poorly understood.

Plague is a disease of wild rodents caused by the bacterium Yersinia pestis and transmitted primarily by the bites of fleas (Gage and Kosoy 2005). The introduction of plague to the United States in the early 20th century has had important conservation consequences because the black-tailed prairie dog (Cynomys ludovicianus), a species that profoundly affects the functioning and biodiversity of grassland ecosystems, is highly susceptible to the disease (Stapp et al. 2004). Because mortality is so high (>90%; Cully and Williams 2001), it is unlikely that prairie dogs can maintain the pathogen locally between epizootics, leading some to argue that another rodent species that is resistant to plague may act as an enzootic host (Anderson and Williams 1997, Cully et al. 1997, Gage and Kosoy 2005, but see Pauli et al. 2006). Several rodent species found in prairie-dog colonies show resistance to plague mortality (Holdenried and Quan 1956, Lechleitner et al. 1968, Anderson and Williams 1997), but to date there is little direct evidence that these species are fed upon by fleas that transmit Y. pestis to prairie dogs (Gage and Kosoy 2005, Salkeld and Stapp 2008a).

The northern grasshopper mouse (Onychomys leucogaster; Figure 1) is found throughout western North America, including much of the area where plague occurs (Thomas 1988). Grasshopper mice use burrows of other animals and are common in prairie-dog colonies (Stapp 2007). They are resistant to plague mortality in the laboratory (Holdenried and Quan 1956, Thomas et al. 1988), and individuals with serological evidence of exposure to Y. pestis have been captured alive in the field (Stapp et al. 2008). Grasshopper mice are also unusual because, across their range, they harbor an extremely high diversity of fleas (57 species, Thomas 1988). Most rodent fleas are associated with one or a few closely related primary hosts (Marshall 1981, Traub 1985). A primary host is defined as a host species that provides appropriate conditions for a flea to successfully reproduce (Krasnov 2008); however, in practical terms, the determination of a flea’s primary host is usually based on repeated field collections of the flea from a particular host, rather than on laboratory trials. A secondary host is defined as a species that occurs in the same area as the flea’s primary
host and is exposed to that flea species on a regular basis (Krasnov 2008). When a flea feeds from a secondary host, its feeding success may be altered, resulting in a smaller blood meal and a slower rate of engorgement and digestion, which may ultimately reduce survival and reproduction compared to feeding on a primary host (Marshall 1981, Krasnov 2008). The ability of grasshopper mice to serve as a secondary host for fleas associated with prairie dogs could indicate an important role in the spread of plague.

In northern Colorado, the black-tailed prairie dog is the primary host of *Oropsylla hirsuta*; these fleas are routinely collected from prairie dogs (Brinkerhoff et al. 2006, Tripp et al. 2009) and their burrows (Salkeld and Stapp 2008b). The thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), another rodent common in prairie-dog colonies (Stapp 2007), is the primary host of *Thrassis fotus*, a flea typically associated with ground-dwelling sciurids (Traub 1985, Thomas 1988). Neither rodent species harbors many flea species, and they rarely share fleas (Figure 2). In contrast, grasshopper mice captured in prairie-dog colonies are infested with their primary flea, *Pleochaetis exilis* (Hubbard 1968, Thomas 1988), and also significant numbers of *T. fotus* and *O. hirsuta* (Figure 2; Salkeld and Stapp 2009, Stapp et al. 2009). Interestingly, *O. hirsuta* loads increase markedly during epizootics, suggesting that fleas switch to grasshopper mice upon the death of their primary hosts (Stapp et al. 2009). Using polymerase chain reaction (PCR) assays, Stapp et al. (2009) detected *Y. pestis* in *P. exilis* and *O. hirsuta* combed from grasshopper mice during plague epizootics, suggesting that mice could be infected by or be a source of, *Y. pestis* infection for *O. hirsuta*, and therefore prairie dogs. However, stable isotope analysis of gut contents of *O. hirsuta* collected from grasshopper mice suggested that they mostly contained prairie-dog blood (Stapp and Salkeld 2009), which implies that grasshopper mice may transport *O. hirsuta* between burrows but not provide significant blood meals.

Molecular genetic techniques, including PCR, have been increasingly used to identify the sources of blood meals in arthropod vectors, such as mosquitoes and ticks (e.g., Humair et al. 2007, Kilpatrick et al. 2006, Kent and Norris 2005, Wickramasekara et al. 2008), but until recently (Woods et al. 2009) have not been used to identify blood meals of fleas. We used PCR and gel electrophoresis to identify blood meals of fleas collected from primary and secondary rodent hosts on prairie-dog colonies. Specifically, we examined blood meals of *O. hirsuta* (hereafter, *Oropsylla*), *T. fotus* (*Thrassis*), and *P. exilis* (*Pleochaetis*), combed from black-tailed prairie dogs, thirteen-lined ground squirrels and northern grasshopper mice, respectively. We also identified blood meals of *Oropsylla* and *Thrassis* combed from grasshopper mice to determine if these fleas feed upon grasshopper mice. Additionally, we examined blood meals of *Oropsylla* collected from prairie-dog burrows to further determine if *Oropsylla* feeds on other rodents, which may increase its survival when prairie dogs are absent. The presence of grasshopper-mouse blood in *Oropsylla* would provide evidence that *Oropsylla* is a source of *Y. pestis* infection for grasshopper mice and a bridging vector for interspecific transmission of plague.

**MATERIALS AND METHODS**

To identify the type of host blood found in fleas, DNA in flea blood meals was compared to DNA from blood...
(genomic DNA) of each of the three focal rodent species using PCR. Oropsylla, Pleochaetis, and Thrassis were tested in three groups: individual fleas combed from their primary hosts; individual Thrassis and Oropsylla combed from grasshopper mice; and pooled Oropsylla collected from burrows prior to and during plague epizootics, when large numbers of fleas and hosts were still present. Included in the samples were 15 Pleochaetis and Oropsylla (combed from eight different grasshopper mice) that had previously tested positive for Y. pestis (Stapp et al. 2009). When blood meals could not be identified using species-specific primers, a generalized primer was used to screen for DNA of other potential hosts at our study site, which were expected to be uncommon. Ground squirrels and grasshopper mice made up 65% of rodent captures and often were the only species captured at our sites (Stapp et al. 2009).

Collection and identification of fleas
Field work was conducted at the Pawnee National Grasslands, located 61 km northeast of Fort Collins, CO, U.S.A. Fleas were collected from live-trapped animals and prairie-dog burrows between May-September 2004-2007 (see Stapp et al. 2009 for methods). Fleas were combed from anesthetized animals or swabbed from burrows and placed in cryotubes containing a mixture of Tween detergent and saline solution. Blood was collected from the retro-orbital sinus (small rodents) or by tail venipuncture (prairie dogs). Flea and blood samples were stored frozen at -80°C. Animal handling procedures were approved by the Institutional Animal Care and Use Committee (O6-R-03) at California State University Fullerton, following American Society of Mammalogists guidelines (Gannon et al. 2007).

Fleas were identified under a dissecting microscope and given a blood meal score from 0-4, with 0 indicating no blood meal and 4 indicating a fully engorged flea. Only fleas with scores of 2-4 were included in our analyses. Fleas from animals were analyzed individually, whereas those from burrows were analyzed in pools of two to five fleas from the same burrow.

Molecular assays
To extract DNA from flea blood meals, fleas were tritutrated by using a MM301 mixer mill (Retsch). Fleas were placed in a 2 ml boiling tube containing 100µl of brain-heart infusion broth (Becton, Dickinson and Company) and three 3-mm sterile Pyrex glass beads. Fleas were milled for 3.5 min at 20 beats/s. Ground flea samples were then incubated at 96°C for 10 min and stored at -20°C. Genomic DNA samples were obtained by extracting pure DNA from rodent blood samples with a Qiagen DNeasy kit (Qiagen) and stored at -20°C.

Three sets of species-specific primers were designed to target the cytochrome b (cyt b) gene of each rodent species. This region of mitochondrial DNA was chosen because it was highly conserved, but also contained several areas that differed among the three rodent species. Cyt b sequences were obtained through the National Center for Biotechnology Information (NCBI) database (Table 1). Once sequences were obtained, all three were aligned in the program ClustalX (Conway Institute). Regions of significant differences (differences between at least four nucleotides) were located and used to design a species-specific primer set (Table 1). Each species-specific primer set targeted areas with at least one difference in nucleotides at both the 5’ and 3’ ends, as well as one to two differences in the middle of the sequence. Species-specific primer sets were designed to produce a specific-sized PCR product (Table 1). A generalized primer was designed to produce a same-sized DNA fragment for the three focal rodent species (165 bp; Figure 3). In addition, when run in a NCBI BLAST search, the generalized primer was 94-98% similar to other possible mammalian hosts at our site, including the deer mouse (Peromyscus maniculatus), Ord’s kangaroo rat (Dipodomys ordii), silky pocket mouse (Perognathus flavus), hispid pocket mouse (Chaetodipus hispidus), red fox (Vulpes vulpes), coyote (Canis latrans), and desert cottontail (Sylvilagus audubonii). We therefore assumed that the generalized primer would allow us to detect DNA of other possible hosts. Although we have no data on rabbits or coyotes, O. hirsuta are absent or rare (<2% of fleas on other rodent species, P. Stapp, unpublished data). If no band was produced using one of the species-specific primers but one was detected for the generalized primer, we would interpret this result as evidence that the flea fed on an unidentified mammal. Absence of a band for the generalized primer was considered evidence that there was no detectable host DNA in the flea. All primers were synthesized by Sigma-Genosys.

PCR reactions contained 20 µl of a mixture consisting of 1X GoTaq Flexi PCR buffer and 5 U/µl GoTaq Flexi DNA polymerase (Promega) 5 mM MgCl₂, 200 µM deoxynucleotide triphosphates (Promega), 1.2 µl of a given flea blood-meal sample, and 5 µM of each primer (forward and reverse). Three negative controls (two with genomic DNA of the two rodent species not being tested and one with master mix reagents only) and one positive control (genomic DNA of the rodent species being tested) were included in each test. All reactions that included genomic DNA contained a final concentration of 40 ng/µl. DNA was amplified using a Techgene TC-312 thermocycler (Techne). Reactions with prairie-dog and grasshopper-mouse primers were carried out with an initial denaturation step of 94°C for 1 min, followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. Ground-squirrel and generalized primers were run with an initial denaturation step of 94°C for 1 min, followed by 35 cycles of 1 min at 95°C, 1 min at 45°C, 1 min at 72°C, and a final extension step of 10 min at 72°C.

PCR products were separated on Ethidium Bromide (EtBr)-stained 2% agarose gels in a 1X TBE buffer solution at 85 volts for 115 min. PCR products were visualized using a Kodak Gel Logic 100 imaging system (Eastman Kodak Company). The presence of DNA from a given species was determined by visualization of a specific-sized, fluorescent band on the gel, using a 25-bp DNA step ladder (Promega) for reference. Each flea DNA sample was tested with the three species-specific primers. Samples that did not yield
Table 1. Sequences and location of the species-specific forward and reverse primer sets for the cyt b gene of three focal rodent species, and the generalized primer used to detect the DNA of unidentified mammalian hosts in flea blood meals.

<table>
<thead>
<tr>
<th>Target host species</th>
<th>NCBI GenBank Accession Number</th>
<th>Fragment size (bp)</th>
<th>Direction</th>
<th>Sequence (5’ - 3’)</th>
<th>Location in Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black-tailed prairie dog</td>
<td>AF157892</td>
<td>191</td>
<td>forward</td>
<td>AACACTCGAAAAACCCAT</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>reverse</td>
<td>ACTGATGAGAAGGCTGTTGTG</td>
<td>177</td>
</tr>
<tr>
<td>Northern grasshopper mouse</td>
<td>AY195794</td>
<td>171</td>
<td>forward</td>
<td>CCCATCCAACATCTCAT</td>
<td>69</td>
</tr>
<tr>
<td>(Onychomys leucogaster)</td>
<td></td>
<td></td>
<td>reverse</td>
<td>TCGAATTAAACCATCCGAT</td>
<td>222</td>
</tr>
<tr>
<td>Thirteen-lined ground squirrel</td>
<td>AF157870</td>
<td>162</td>
<td>forward</td>
<td>CTTATCTAAACACGTAGCG</td>
<td>924</td>
</tr>
<tr>
<td>(Spermophilus tridecemlineatus)</td>
<td></td>
<td></td>
<td>reverse</td>
<td>GATGATGGTGAAGTATAGG</td>
<td>1068</td>
</tr>
<tr>
<td>All species (generalized)</td>
<td></td>
<td>165</td>
<td>forward</td>
<td>AAACATGAAACATTGGA</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>reverse</td>
<td>CCTCAAATTCATTCTACT</td>
<td>480</td>
</tr>
</tbody>
</table>

Table 2. Identification of host DNA in fleas combed from rodents associated with prairie dog colonies on the Pawnee National Grasslands, Colorado. Values are the percentage of fleas containing DNA of a particular rodent host based on PCR assays with cyt b species-specific primers, with the number of fleas containing DNA of a particular host given in parentheses. Primary host feeding relationships are shown in boldface, whereas entries in italics represent secondary host relationships for grasshopper mice. None of the fleas that were negative with a species specific primer set tested positive for DNA from other potential mammalian hosts in the study area, based on PCR assays with a generalized primer. N denotes sample size.

<table>
<thead>
<tr>
<th>Source of fleas</th>
<th>Flea species</th>
<th>N</th>
<th>Black-tailed prairie dog</th>
<th>Northern grasshopper mouse</th>
<th>Thirteen-lined ground squirrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern grasshopper mouse</td>
<td><em>Plecochaetis exilis</em></td>
<td>46</td>
<td>0</td>
<td>83 (38)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Oropsylla hirsuta</em></td>
<td>62</td>
<td>0</td>
<td>53 (33)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Thrassis fotus</em></td>
<td>54</td>
<td>0</td>
<td>52 (28)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Black-tailed prairie dog</td>
<td><em>Oropsylla hirsuta</em></td>
<td>38</td>
<td><strong>53 (20)</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thirteen-lined ground squirrel</td>
<td><em>Thrassis fotus</em></td>
<td>39</td>
<td>0</td>
<td>0</td>
<td>72 (28)</td>
</tr>
</tbody>
</table>
a band when tested with the species-specific primers were tested with the generalized primer.

RESULTS

Species-specific primers for the cyt b gene were designed to produce a band that differed by approximately 10-20 bp between the three focal rodent species when visualized on an agarose gel. When tested with purified genomic DNA collected from the three species, each species-specific primer produced a band of the expected size for that species (Figure 3, Table 1), and failed to amplify a cyt b fragment of genomic DNA from the other two species.

Fleas collected from their primary host contained only the DNA of that host. Most Pleocharaetes (83%) combed from grasshopper mice contained grasshopper-mouse DNA (Table 2), as evidenced by a band at 171 bp (Figure 4 top, lanes 3-11). No Pleocharaetes tested positive using the prairie-dog or ground-squirrel primers. Similarly, most Thrassis (72%) combed from ground squirrels contained ground-squirrel DNA (Table 2), based on the presence of a band at 162 bp when tested with thirteen-lined ground-squirrel primers (Figure 4 bottom, lanes 3-11). No amplified DNA fragments were observed when DNA from the blood meals of these fleas was tested with the prairie-dog and grasshopper-mouse primers. A much smaller fraction of Oropsylla (53%) combed from prairie dogs contained prairie-dog DNA (Table 2), based on the presence of a band at 191 bp (Figure 4 middle, lanes 3-11). Reactions that employed grasshopper-mouse or ground-squirrel primers failed to amplify DNA fragments. For all three rodent species, none of the fleas that were negative in tests using species-specific primers tested positive using the generalized primer.

Patterns of feeding on secondary hosts were similar to those seen on primary hosts: fleas fed on the host that carried them. Many (53%) Oropsylla combed from grasshopper mice contained only grasshopper-mouse DNA (Table 2), as evidenced by a band at 171 bp when tested with grasshopper-mouse primers (Figure 5 top, lanes 3-11), and no amplification of a DNA fragment when tested with the prairie-dog or ground-squirrel primer. None of the fleas that were negative in tests using grasshopper-mouse primers tested positive using the generalized primer. A similar fraction (52%) of the Thrassis from grasshopper mice contained grasshopper-mouse DNA (Table 2, Figure 5 bottom). One Thrassis contained DNA of both grasshopper mice and ground squirrels (Table 2, Figure 6 lane 9). No Thrassis contained DNA from prairie dogs or other mammalian hosts.

Most Pleocharaetes (86%; N = 7) that had previously tested positive for the plague bacterium Y. pestis (Stapp et al. 2009) contained grasshopper-mouse DNA. None of these fleas produced bands when tested with prairie-dog or ground-squirrel primers, and none of the fleas that were negative in tests using grasshopper-mouse primers tested positive using the generalized primer. A smaller fraction (25%; N = 8) of Oropsylla combed from grasshopper mice that tested positive for Y. pestis (Stapp et al. 2009) were positive for grasshopper-mouse DNA. None of these fleas contained DNA from prairie dogs, ground squirrels, or other mammalian hosts. All three of the grasshopper mice with Y. pestis-positive Pleocharaetes were or eventually tested seropositive for Y. pestis exposure using antibody tests (0, 31, 41 days after positive fleas collected), whereas only one of the five grasshopper mice with Y. pestis-positive Oropsylla tested seropositive (42 days later), although three of these mice were only captured once (P. Stapp, unpublished data).

In contrast, none of the fed Oropsylla swabbed from prairie-dog burrows (N = 230 fleas, tested in 94 pools) contained any detectable host DNA, based on the absence of bands on gels from tests with the three species-specific or generalized primers (data not shown). The fact that all these fleas had feeding status scores of 2-4, presumably as a result of feeding on prairie dogs, suggests that their blood meals had already been digested or degraded.

DISCUSSION

Our study demonstrates that PCR can be used to identify the source of host blood meals in fleas. We found that fleas usually fed upon the rodent that carried them, regardless of whether the host was considered a primary or secondary host. Pleocharaetes (83%) and Thrassis (72%) that were combed from their primary host consistently had blood meals containing DNA of northern grasshopper mice and thirteen-lined ground squirrels, respectively (Table 2). When the remaining flea samples were tested for the presence of other potential hosts using the generalized primer, none of these samples yielded a detectable DNA fragment, suggesting that these fleas contained blood meals...
Figure 4. Species-specific primers for the cyt $b$ gene amplify unique DNA fragments of flea blood meals from their primary rodent hosts. Top: Pleochaitis exilis collected from northern grasshopper mice. Middle: Oropsylla hirsuta collected from blacktailed prairie dogs. Bottom: Thrassis fotus collected from thirteen-lined ground squirrels. For all three gels: lane 1, 25 bp DNA step ladder, with arrows on the left indicating fragments of 150 bp and 300 bp; lane 2, genomic DNA of the target host species; lanes 3-11, flea blood meal DNA; lane 12, negative control (no DNA). The arrows to the right of each gel indicate the size of the DNA fragment generated with a species-specific primer set.

Figure 5. DNA fragments from flea blood meals of Oropsylla hirsuta (top) and Thrassis fotus (bottom) after feeding on a secondary host, the northern grasshopper mouse. DNA fragments were amplified using the grasshopper mouse cyt $b$ primer set; the arrows to the right indicate the size of the DNA fragment associated with the grasshopper mouse (171 bp). For both gels: lane 1, 25 bp DNA step ladder (with arrows on the left indicating fragments of 150 bp and 300 bp); lane 2, grasshopper-mouse genomic DNA; lanes 3-11, flea blood meal DNA; lane 12, negative control (no DNA).

Figure 6. DNA fragments from blood meals of Thrassis collected from northern grasshopper mice tested with the northern grasshopper-mouse cyt $b$ primer set (top) or the thirteen-lined ground squirrel cyt $b$ primer set (bottom). For both gels: lane 1, 25 bp DNA step ladder, with arrows on the left indicating fragments of 150 bp and 300 bp; lane 2, grasshopper-mouse genomic DNA (171 bp; top) or ground-squirrel genomic DNA (162 bp; bottom); lanes 3-11, Thrassis blood meal DNA; lane 12, negative control (no DNA). The sample positive for grasshopper mouse and ground squirrel DNA is indicated with a downward arrow (lane 9).
that had already been digested. A much smaller fraction (53%) of blood meals of Oropsylla contained DNA of black-tailed prairie dogs, with 47% containing no detectable host DNA (Table 2). The absence of amplifiable host DNA in so many fleas, especially for Oropsylla, was unexpected, and suggests differences in the rate of digestion of the blood meal among flea species. Woods et al. (2009) showed that the amount of detectable host DNA in the guts of the Asian rat flea Xenopsylla cheopis Rothschield declined considerably after about 72 h. They identified approximately 80% of the blood meals of five different flea species, which is similar to our findings for Pleocheatis and Thrassis. Thus, the lower percentage of identifiable blood meals in Oropsylla (53%) suggests that this species of flea either feeds less frequently or takes smaller meals than Pleocheatis and Thrassis, or that it digests host blood more quickly. Wilder et al. (2008) reported high mortality of Oropsylla in the laboratory when they were starved for more than two days, which suggests a rapid rate of blood meal digestion. The need to feed often implies that Oropsylla must quickly abandon a dead host for a living one, perhaps indiscriminately, which could make it an effective vector for epizootic spread. A rapid digestive rate could also explain why no DNA was detected in any Oropsylla collected from prairie-dog burrows, which presumably had not fed as recently as the fleas that were combed from live hosts. The fact that some fleas that previously tested positive for Y. pestis contained no detectable host DNA suggests that the plague pathogen may remain in a flea’s gut after digestion of the blood meal, and possibly serve as a source of infection.

Both Thrassis and Oropsylla, fleas associated with ground squirrels and prairie dogs, respectively, but combed from grasshopper mice, contained grasshopper-mouse DNA. The proportion of fleas with detectable blood meals (52%, 53%) was similar to or lower than that for these fleas on their primary hosts (Table 2), perhaps indicating a reduced feeding rate. The ability of northern grasshopper mice to harbor and, presumably, provide blood meals to such a wide variety of flea species (Stapp et al. 2009) underscores this species’ reputation as a highly generalized host, although the exact mechanism by which grasshopper mice tolerate infestation by so many flea species remains unknown. However, in a separate analysis, we found that 79% (11/14) of Thrassis and 33% (5/15) of Pulex sp. combed from prairie dogs contained DNA from the prairie dogs but not from any other mammals1. These results suggest that feeding by fleas upon a secondary host may be common, although it is not clear if blood meals obtained from such hosts are adequate to ensure survival and reproduction.

On the surface, our results appear to differ from those of Stapp and Salkeld (2009), who found, using stable nitrogen isotope analysis, that Oropsylla combed from grasshopper mice contained mostly prairie-dog blood. Based on a mixing model, grasshopper-mouse blood contributed 42% to the average nitrogen isotope signature of Oropsylla collected from grasshopper mice. In the current study, the absence of detectable prairie-dog DNA in all Oropsylla collected from grasshopper mice may further support our contention that a previous blood meal from a prairie dog would likely have been digested by the time the flea had jumped to a mouse. PCR may therefore be useful for identifying recent blood meals, whereas stable isotope analysis is more effective at detecting degraded or digested blood meals (Rasgon 2008), e.g., in burrow fleas, or meals integrated over longer time periods. Ideally, a combination of molecular-genetic methods and stable isotope analysis will provide the most complete picture of a flea’s host-feeding relationships.

Lastly, our study demonstrates that grasshopper mice can provide blood meals to Oropsylla, the primary flea of prairie dogs, and thus serve as an important vector of plague in our system. Several fleas collected from grasshopper mice were positive for Y. pestis (Stapp et al. 2009), suggesting that mice may be capable of infecting fleas and passing them (and therefore, Y. pestis) to individuals of different species. Combined with the observations that grasshopper mice are common in prairie-dog colonies (Stapp 2007), share burrows with prairie dogs2, and forage across social boundaries of prairie-dog coteries (Stapp et al. 2009), it seems increasingly likely that these mice are involved in epizootic transmission of plague. In addition, because grasshopper mice are clearly fed upon by Oropsylla and show heterogeneity to plague mortality (Thomas et al. 1988, Stapp et al. 2008), they could also serve as enzootic hosts to help maintain Y. pestis in the absence of prairie dogs. The fact that grasshopper mice and prairie dogs are fed upon by the same fleas provides a mechanism by which Y. pestis can be transmitted from grasshopper mice back to prairie dogs once a colony has recovered. The relative rarity of Oropsylla on grasshopper mice outside of epizootics (Stapp et al. 2009) suggests that blood meals from mice may be inadequate to permit long-term survival and reproduction, but mice could provide maintenance meals to fleas and contribute to long-term persistence of Y. pestis, either in rodent or flea populations, or other reservoirs. Future studies should clarify the roles of different reservoirs in the maintenance of plague at the landscape scale.

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REFERENCES CITED


Thomas, R.E., A.M. Barnes, T.J. Quan, M.L. Beard, L.G. Carter, and C.E. Hopla. 1988. Susceptibility to Yersinia pestis in the northern grasshopper mouse (Onychomys