Flea and Louse Infestations of Cotton Rats (Sigmodon Hispidus) In The Southeastern United States

Alena E. Aviles

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FLEA AND LOUSE INFESTATIONS OF COTTON RATS (*Sigmodon hispidus*)
IN THE SOUTHEASTERN UNITED STATES.

by

ALENA E. AVILES

(Under the Direction of Lance A. Durden)

ABSTRACT

Ectoparasites were collected from cotton rats (*Sigmodon hispidus*) in 20 sites in the Southeastern United States (FL, GA, MS, NC and SC). Prevalence and mean intensity of parasitism by sucking lice (Anoplura) and fleas (Siphonaptera) of cotton rats were recorded at all sites. The geographical distribution of *S. hispidus* and its main louse and flea ectoparasites range from the neotropical region to the southeastern USA. It was hypothesized that the abundance of the cotton rat associated louse (*Hoplopleura hirsuta*) and flea (*Polygenis gwyni*) would increase the further south and closer to the distribution centers of each of these ectoparasite species. In addition, it was hypothesized that male cotton rats would exhibit higher infestations (mean intensities and prevalence) by ectoparasites than females. Because males of many ectoparasites are more mobile than females and may experience more periods off the host than females, I further hypothesized that sex ratios of both flea and louse populations would be female-biased. Data collected during this study supported the hypothesis that populations of *Polygenis* fleas on *S. hispidus* increased further south (closer to the center of distribution for this flea) and thus were dependent on site location. Conversely, there was not a significant trend in abundance noted for *Hoplopleura* lice on *S. hispidus*, which was unexpected given that this ectoparasite is a more permanent ectoparasite than *P. gwyni*. Male cotton
rats were not parasitized by statistically greater numbers of *H. hirsuta* or *P. gwyni* than were female cotton rats. Thus, the male host bias hypothesis was not supported for either ectoparasite species in this study. Populations of both *H. hirsuta* and *P. gwyni* were significantly female-biased, with about twice as many females as males on cotton rats. Overall, this study provides the first evidence for larger populations of an ectoparasite (*P. gwyni*) of a vertebrate towards the geographical center of distribution of the ectoparasite. Higher on-host populations of female versus male sucking lice and fleas in this study conform to similarly sex-biased data reported for several previous studies of ectoparasites on mammals. Conversely, the lack of significant differences for louse and flea infestations on male versus female cotton rats recorded during this study differs from some previous mammal-ectoparasite studies in which male hosts were more heavily infested.

FLEA AND LOUSE INFESTATIONS OF COTTON RATS (*Sigmodon hispidus*)

IN THE SOUTHEASTERN UNITED STATES.

by

ALENA E. AVILES

B.S., Georgia Southern University, 2004

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements of the Degree

MASTER OF SCIENCE

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2009
FLEA AND LOUSE INFESTATIONS OF COTTON RATS (*Sigmodon hispidus*)

IN THE SOUTHEASTERN UNITED STATES.

by

ALENA E. AVILES

Major Professor: Lance A. Durden
Committee: William S. Irby
Alan W. Harvey

Electronic Version Approved: May 2009
DEDICATION

I would like to dedicate this thesis to my husband and children. To my husband, Carlos, who never let me stop reaching for my dreams, thank-you for always being my blessing in disguise and I will always love you. To my two beautiful children, Jerryd and Abbee, you are the light of my life; if I could offer any advice it would be to follow your dreams and never give up, remember everything happens for a reason and God closes doors in our lives so that others may open our eyes to amazing opportunities.
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Dr. Patrick Abbot of Vanderbilt University collaborated on the *Bartonella* project that included ectoparasites from this thesis research.

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CHAPTER 1
INTRODUCTION

Because some rodent ectoparasites serve as vectors of zoonotic pathogens and their rodent hosts may serve as reservoirs, it is important to record host-parasite interactions and infestation parameters for ectoparasites of rodents (Durden et al. 2000). Further, for individual species of ectoparasites, it is instructive to compare their abundance in different parts of their geographical range, to determine whether male and female hosts are differentially infested (i.e., are male hosts more heavily infested), and to determine if the on-host populations are numerically biased towards males or females.

Population densities of cotton rats can vary on a yearly basis, but in general *Sigmodon hispidus* (Rodentia: Cricetidae) is usually the most abundant small mammal species on farmlands and low scrubby habitats in the southern United States (Smith and Love 1958; Cameron and Spencer 1981). Cotton rats actively seek out food at dawn and dusk. Grassy plants including cultivated field crops are their primary source of food (Whitaker and Hamilton 1998). The distribution of *S. hispidus* (Figure 1) includes most of the Central American region, northward into the southeastern and south central United States (Cameron and Spencer 1981).

Prevalence is the number of rodent hosts infested with one or more ectoparasites of a given ectoparasite species divided by the number of cotton rats examined. This is the most commonly used descriptor of parasitic infestations because it provides a rapid and easily calculated parameter reflecting the proportion of a host population that is parasitized by a given parasite species (Bush et al. 1997). Mean intensity is the average number of a given ectoparasite among infested host species. Therefore, mean intensity is
the total number of a parasite species found at a particular sample site divided by the number of hosts infested (Bush et al. 1997). A number of factors can influence the prevalence (% of hosts infested) and mean intensity of infestation (mean number of parasites per infested host) by parasiticlice and/or fleason a host including:
environmental conditions, season, host body size, age, sex, activity level, and host body condition (Love and Smith 1958; Henry 1970; Poulin 1991; Kotiaho and Simmons 2001; Leung et al. 2001; Rolff 2001; Kelly 2005). Variations in infestation prevalence have been demonstrated for ectoparasites in previous rodent-ectoparasite surveys (e.g., Yourth et al. 2002a; Robb et al. 2003). It is important to consider differences in infestation prevalence as well as mean intensity, since together these two parameters give a reliable indication of overall parasite abundance in a host population (Rozsa et al. 2000) and, for ectoparasites this may have significance for vector-borne diseases. Although previously rarely used by parasitologists, the total number of ectoparasites of a given species on each individual host could also provide an important measure of parasite abundance. This is because just one easily plotted variable can be graphically portrayed against the other variable (geographical coordinates, etc.) as a regression and a more accurate reflection of ectoparasite populations in nature may be gained.

In this study I address three hypotheses. First, the abundance of two common species of ectoparasites of cotton rats, the flea Polygenis gwyni (Siphonaptera: Rhopalopsyllidae) (Figure 2), and the sucking louse, Hoplopleura hirsuta (Phthiraptera: Hoplopleuridae) (Figure 3), should increase the farther south (and closer to their respective centers of distribution) the study field site is sampled. Widely known as the “abundant center hypothesis”, and a “general rule” of biogeography, the general
consensus is that a species’ abundance is greatest at the center of its geographical range and lower toward the edges of its range due to environmental gradients (Brown 1984; Sagarin and Gaines 2002; Alleaume-Benharira et al. 2006; Bell 2001; Sagarin et al. 2006). Abundance gradients with respect to the center of distribution have not previously been evaluated for any species of ectoparasites associated with mammals. There are two assumptions to consider here: 1) spatial variation in local abundance is related to the likelihood of meeting a species’ niche requirements; 2) these niche requirements are geographically coordinated with the most desired conditions located near the center of the species’ distribution (Brown 1984; Kiflawi et al. 2000). Populations of both *H. hirsuta* and *P. gwyni* are close to their northern range limit in northern Georgia with the approximate centers of their ranges both being near Mexico (Figures 4 and 5). Therefore, we expected both ectoparasites to be more common closer to their centers of distribution (i.e., to the south). Both *P. gwyni* and *H. hirsuta* are very host specific and therefore should not venture outside of the host range (Figure 1). The sucking louse *H. hirsuta* is a specific ectoparasite associated with cotton rats and because of this specific host interaction it will not go beyond the range of the rat (Pfaffenberger and DeBruin 1988). The flea, *P. gwyni*, sometimes parasitizes the Virginia Opossum, *Didelphis virginiana*, and various rodents, but it cannot become established in areas without its main host, the cotton rat (Smit 1987). If both the flea and louse ectoparasites studied in this paper are reaching their northernmost boundaries at the Georgia and South Carolina trap site locations (Ferris 1921; Fox 1940; Morlan 1952; Pratt and Good 1954; Layne 1971; Benton 1980; Kim et al. 1986; Smit 1987; Durden et al. 1994, 2000), then one might
predict that the population density of ectoparasites (abundance, prevalence and/or mean intensities) would be greater the more south in latitude that the trap sites are located.

The second hypothesis for this study is that male cotton rats will be more heavily infested (measured by prevalence and mean intensity) than females by both *H. hirsuta* and *P. gwyni*. Sexual differences in parasitism by ectoparasites can be the result of differences in the intensity and prevalence of infestation based on the sex of the host, with males typically being targeted more often than females (Zuk 1990, 1992; Sheridan *et al.* 2000). The most important variables to be considered as explanations related to host sex and infestation burdens are factors such as relative size and differences in the skin and its covering (male rodents are typically larger than females), difference in blood hormonal levels due to stress or reproductive condition, and behavioral factors such as differences in grooming, nesting and mobility (Marshall 1981a). Of these, the most commonly accepted reason is known as the immunocompetence hypothesis whereby testosterone enhances the expression of male secondary sexual characters while exerting a suppressive effect on the immune system thereby predisposing male hosts to higher intensities of parasite infestations (Saino *et al.* 1995). Thus, if male rodents have a weaker immune response than do females, then males should have a greater prevalence and mean intensity of lice and fleas than females because testosterone-mediated sexual activity acts to decrease the amount of energy males can contribute to immunity.

The third hypothesis is that sex ratios of both *H. hirsuta* and *P. gwyni* collected from cotton rats during this study will be female-biased. Unequal, female-biased, parasite sex ratios have been noted in the literature for several ectoparasitic species (Marshall 1981a, b; Gorell and Schulte-Hostedde 2008). Male ectoparasites tend to have
a shorter lifespan and are smaller in average size than female ectoparasites and often less likely to stay attached to a single host. This is because males are usually more active on and off a given host, and thus more likely to be separated from the host's body or home, be more susceptible to host predation, or be killed by adverse environmental or nutritional conditions (Marshall 1981a). I further predicted that the female bias should be especially apparent in fleas because male fleas are generally more agile than females and may detach from their host (Marshall 1981a; Gorell and Schulte-Hostedde 2008) whereas sucking lice of both sexes are more heavily committed to permanent residence on their host (Durden and Loyd 2009).

In the present study, I attempted to relate flea and louse infestation parameters of cotton rats to host capture location and to the sex of the host, while also analyzing sex ratios of these two ectoparasites. This study provides statistical information concerning two species of ectoparasites and their abundance on their principal host, the cotton rat, in the southeastern United States. The two parasitic arthropods studied in this project, *Polygenis gwyni* (flea) and *Hoplopleura hirsuta* (sucking louse), are excellent subjects for this study because they are usually common, they are host specific, and they show little or no apparent seasonality (Morlan 1952; Smith and Love 1958; Henry 1970; Pfaffenerberger and DeBuin 1988). The lack of a seasonal bias is a potentially important consideration because the ectoparasites analyzed during this study were not all collected at the same time of year.

The current study of infestation parameters by *P. gwyni* fleas and *H. hirsuta* lice on cotton rats of the southeastern United States was part of a long-term investigation in collaboration with Dr. Patrick Abbot of Vanderbilt University on the evolution and co-
infection of species of *Bartonella* within this particular rodent host and its ectoparasites (see Appendix A). This rodent is an excellent reservoir for a variety of strains of *Bartonella* (Kosoy *et al.* 1997, 2004 a, b). Some blood-feeding arthropods are known to be vectors of various species of *Bartonella* (Chomel *et al.* 1996; Maurin *et al.* 1997; Karem *et al.* 2000; Chang *et al.* 2001; La Scola *et al.* 2001, Durden *et al.* 2004) and, of these, *Polygenis gwyni* has been demonstrated to be an excellent source for mixed infections of various bartonellae in the Southeastern United States (Abbot *et al.* 2007).
CHAPTER 2

MATERIALS AND METHODS

Study Sites and Trapping

Rodents were live trapped at various locations throughout the southeastern United States of America: Georgia (12 sites), Florida (4 sites), North Carolina (1 site), South Carolina (1 site) and Mississippi (2 sites) (Table 1). Rodents, mainly cotton rats (*Sigmodon hispidus*), were live trapped using Sherman live traps (H.B. Sherman Traps, Inc., Tallahassee, FL). Each field site was determined based on landowner permission as well as resources and available funding. State of Georgia county extension agents were utilized to locate willing landowners possessing appropriate habitat conditions that are associated with *S. hispidus*. Field sites used in the analysis of the current study include the following counties: Bulloch Co. (32.444N, 81.783W), Bleckley Co. (32.397N, 83.347W), Columbia Co. (33.562N, 82.175W), Decatur Co. (30.909N, 84.583W), McIntosh Co. (31.374N, 81.499W), Chatham Co. (31.942N, 81.035W), Screven Co. (32.751N, 81.604W), Lowndes Co. (30.842N, 83.306W), Candler Co. (32.318N, 82.074W), Burke Co. (32.985N, 81.978W), Jenkins Co. (32.720N, 81.979W), and Glynn Co. (31.170N, 81.499W) in Georgia; Brevard Co. (28.077N, 80.629W), Flagler Co. (29.469N, 81.364W), Bay Co. (30.169N, 85.648W), and Leon Co. (30.444N, 84.258W) in Florida; Charleston Co. (32.780N, 79.936W) in South Carolina; Jackson Co. (30.366N, 88.543W), Marion Co. (31.251N, 89.756W) in Mississippi; and Jackson Co. (35.372N, 83.199W) in North Carolina. On average, one site was sampled per county to trap rodents and collect their ectoparasites (see Appendix B). The study sites offered an array
of rodent habitat including grassland, lightly grazed pasture, and cropland. At each study site, 25-50 live traps were placed around areas of suspected rodent activity. The traps were placed approximately 10 meters apart between 1200 and 1500 hours EST and left overnight. Traps were checked the next day between 0800 and 1200 hours EST. If there was no indication of rodent activity the traps were re-baited and left for another night. Each trap was baited with oatmeal mixed with a trace of peanut butter. Cotton nests were added during winter months to prevent rodent hypothermia.

Animal Collection

Procedures for the collection and handling of captured rodents were approved by the Institutional Animal Care and Use Committee (IACUC) at Georgia Southern University (research protocol number I06003) and a Georgia State scientific collection permit (29-WCH-07-160). Trapped animals were lightly anesthetized through intramuscular administration of ketamine hydrochloride and then moved to a white tray, where they were carefully examined for ectoparasites and sexed (male rodents identified by descended testes); all procedures were done at the field site. Captured rodents were marked with a unique number using permanent ink on their dorsal surface where the fur was light colored, allowing quick identification of recaptured animals. Collected ectoparasites were placed in individually labeled vials containing 95% ethanol. Following recovery from anesthesia, all rodents were released at their capture site. Based on previously published standards, a sample size of at least 20 host rodents were collected at each field site, when possible to insure accurate host-ectoparasite interactions (Schwan 1984).
Ectoparasite Collection and Identification

Ectoparasites were collected from anesthetized rodents by combing each animal with a flea comb over a large white pan. The entire pelage was then systematically searched to collect sucking lice by the use of small forceps (Dumoxel no.5); Ectoparasites were then placed in labeled vials containing 95% ethanol, RNALater, or frozen, depending on the exact protocol needed to screen them for *Bartonella* spp. bacteria used for the pathogen genetics portion of this study. Collected ectoparasites were then transferred to a research laboratory at Georgia Southern University, identified to species, sex, and/or stage using a high power binocular microscope, then packaged and sent via FedEx to Vanderbilt University for DNA extraction and further analysis for the bartonellosis study.

Data Analysis

Rodents were characterized according to the state of their infestation. Infested rodents had one or more of the species of ectoparasites being studied (*P. gwyni* or *H. hirsuta*) while uninfested rodents had none of these particular ectoparasites. Prevalence was defined as the proportion (%) of infested individuals for each ectoparasite species. Mean intensity was defined as the mean number of an ectoparasite species (either *P. gwyni* or *H. hirsuta*) per infested rodent (Bush *et al.* 1997). Eighteen of the 20 sites sampled were considered in statistical analyses for mean intensity and prevalence’s, with Flagler Co. Florida and Jackson Co. North Carolina being excluded from analyses because only one rat per site was captured and neither rodent was infested by ectoparasites belonging to either of the species of interest in the current study.
For all 20 sampling sites, a linear regression was performed to determine if rodent infestations with fleas or lice increased further south in latitude. For this analysis raw numbers of flea and louse counts per rodent were used including rodents that had zero counts for fleas and lice (meaning these ectoparasites were absent from the host during field examination).

To compare infestation of males versus female cotton rats, I used a one way analysis of variance (ANOVA) to test whether the amount of infestation was dependent on sex of the rodent (male or female), based on mean intensity and prevalence data collected at eighteen of the 20 sample sites. To normalize the distribution of prevalence and intensity data, I performed a square root transformation (Sokal & Rohlf 1995).

Sex ratios are often expressed as the count of females per one male in the ectoparasite literature (Marshall 1981a). However, to test sex ratios of ectoparasites, I used the raw numbers of male versus female lice and male versus female fleas in a Pearson’s Chi-Square analysis. Raw data are presented in Appendix B. All statistical analyses were performed using JMP 7.0 for Windows XP.
CHAPTER 3

RESULTS

Overall, the results of this study showed that the flea *P. gwyni* was significantly more abundant with decreasing latitude (i.e., further south). There was no statistical difference between male versus female cotton rats in either louse or flea infestations. Sex ratios of both *H. hirsuta* and *P. gwyni* were significantly female-biased.

A total of 271 cotton rats were examined from 20 sites (12 in GA, 4 in FL, 2 in MS, 1 in SC and 1 in NC). One species of sucking louse (*Hoplopleura hirsuta*) and six species of fleas (*Ctenophthalmus pseudagyrtes, Orchopeas howardi, Peromyscopsylla hamifer, Peromyscopsylla scotti, Polygenis gwyni* and *Stenoponia americana*) were collected from cotton rats (Table 1). Of these flea species, only *P. gwyni* was recorded in sufficiently large numbers to warrant further analysis.

Effect of site location on ectoparasite infestation

The regression analysis revealed that the number of fleas (*P. gwyni*) on cotton rats was dependent on site location ($R^2 = 0.03$, df = 1, *p* = 0.0040, Figure 6) with significantly higher infestations recorded in more southern sites.

The abundance of the louse (*H. hirsuta*) on cotton rats did not show a comparable trend in the regression analysis to that of the flea. The noted trend actually seemed to show greater numbers of lice the higher in latitude that the trap site was located, but this was not a significant difference ($R^2 = 0.0016$, df = 1, *p* = 0.5039, Figure 7).
Effect of rodent sex on ectoparasite infestation

Male and female cotton rats did not differ in the mean intensity of either louse populations ($F_{1,38} = 1.4621, p = 0.2341, \text{Figure 8}$) or flea populations ($F_{1,38} = 0.4617, p = 0.5009, \text{Figure 8}$). Likewise, there was no difference in prevalence for either lice ($F_{1,38} = 0.0628, p = 0.8034, \text{Figure 9}$) or fleas ($F_{1,38} = 0.0478, p = 0.8281, \text{Figure 9}$) between male and female cotton rats.

Ectoparasite sex ratios

The sex ratio of the louse ($H. hirsuta$) averaged 2.6 females per male ($n= 482$) whereas the sex ratio of the flea ($P. gwyni$) averaged 1.4 females per male ($n= 471$), for all trap locations combined. The total number of female lice (349) was significantly greater than the total number if male lice (133) at the 0.05 alpha level ($x^2 = 96.796, \text{df} = 1, p = 0.001, \text{Table 2}$). Similarly, the total number of female fleas (271) was significantly greater than the total number of male fleas (200) collected ($x^2 = 10.7026, \text{df} = 1, p = 0.001, \text{Table 2}$).
CHAPTER 4
DISCUSSION

The relationships between ectoparasite infestation abundance, site location and host sex is complicated. The main finding of this study was that the likelihood of a cotton rat being parasitized by *Polygenis* fleas was dependent on the particular location in which it was sampled. However, it was interesting to see that this was not the case for *Hoplopleura* lice. Infestation was independent of rodent sex; therefore, the hypothesis predicting male biased prevalence and mean intensity of lice and fleas was not supported. Sex ratios of both fleas and lice were biased with almost 3 times as many female versus male lice and almost 1.5 times as many female versus male fleas recorded.

The prediction that the abundance of the flea studied in this project increased further south based on latitude was statistically supported. This corroborates other studies that describe the distribution of *Polygenis gwyni* as reaching its northern most boundary close to several of the trap locations stated in this study (such as Columbia County Georgia and Charleston County South Carolina) (Ferris 1921; Fox 1940; Morlan 1952; Pratt and Good 1954; Layne 1971; Benton 1980; Kim *et al.* 1986; Smit 1987; Durden *et al.* 1994, 2000). However, it is interesting to note that this was not the case for *Hoplopleura hirsuta*. This difference between the two ectoparasite species is intriguing and could be related to the fact that *H. hirsuta* is a permanent ectoparasite of cotton rats in all stages of its life cycle but that the life cycle of *P. gwyni* includes significant off-host stages (egg, larva and pupa) (Durden and Loyd 2009; Durden and Hinkle 2009). It seems plausible that the off-host stages of *P. gwyni* are influenced by some habitat gradient(s) that do not affect, or have little effect, on *H. hirsuta*. Gradients in ambient temperature
either throughout the year or during the winter are a possible cause for this phenomenon with off-host stages of *P. gwyni* showing increased survival or shorter generation times under conditions of warmer temperatures which would have occurred in the more southern locations sampled during this study. However, other factors such as humidity or precipitation gradients, soil/vegetation types, predators or competing arthropods are also feasible explanations for the observed gradient in *P. gwyni* populations. Conversely, all stages of *H. hirsuta* would presumably be buffered against these off-host factors by their permanent location on the host.

There was no difference in the infestation (as measured by prevalence and mean intensity) of the two ectoparasites studied on male versus female cotton rats. This does not corroborate some previous studies that attribute high levels of testosterone in male hosts with an increase in parasite load (Saino *et al.* 1995; Hughes and Randolph 2001). Conversely, parasite loads on some rodent hosts may depend more on the quality of the individual rodent than on rodents of different sexes (Thompson 1990); individual rodents could be affected by environmental conditions and foraging habits. Male hosts are often parasitized by greater numbers of ectoparasites of a given species than are female conspecific hosts (Marshall 1981b) for several potential reasons. In addition to the aforementioned effect of testosterone on host immunosuppression, male hosts often have larger home ranges than females and tend to accumulate more ectoparasites such as ticks, chiggers and (sometimes) fleas that can quest for hosts from vegetation or leaf litter (Mohr 1961). Male hosts also tend to have more aggressive or sexual physical encounters with other conspecific hosts which present increased opportunities for ectoparasite transfer and accumulation (Gorell and Schulte-Hostedde 2008). The fact that
neither *H. hirsuta* nor *P. gwyni* were significantly more abundant on male hosts compared to female hosts in this study, suggests that the behavior of cotton rats does not differ widely by host sex. Alternatively, some factor(s) may dictate that populations of both *H. hirsuta* and *P. gwyni* are more homogeneous within their cotton rat populations than are the populations of some other ectoparasites on other host species.

Data from this study agree with the hypothesis that female fleas and lice are more common than males on cotton rats. Marshall (1981a) evaluated this phenomenon for ectoparasites in general and suggested that there are usually two main reasons to explain this outcome. While ectoparasites emerge in approximately equal numbers, an unequal trend thereafter is nearly always found in favor of female ectoparasitic arthropods in natural populations; the result being either inadequate sampling methods or the tendency for male ectoparasites to be shorter lived than their female counterparts (Marshall 1981a). Also, male fleas and lice are more vagile and could become detached from the host (Gorell and Schulte-Hostedde 2008). Marshall (1981a) noted that solely looking at the host for ectoparasites is an adequate method for permanent ectoparasites such as lice, but that it may be necessary to also sample the nest site of the rodent in order to obtain an accurate count for certain flea species. Cotton rat nests were not examined during this study for logistical reasons of locating nests that could unequivocally be ascribed to cotton rats and not to other species of rodents. Nevertheless, the female bias for both *H. hirsuta* and *P. gwyni* on cotton rats was strongly supported for this study.

Overall, data from this study revealed significantly larger on-host populations of the flea *P. gwyni* further south and closer to the center of distribution for this flea, no significant difference between louse and flea infestations on male versus female cotton
rat hosts, and significantly female-biased on-host populations for both *P. gwyni* and the louse *H. hirsuta*. A related study (see Appendix A) assessed *Bartonella* infections of *P. gwyni* collected from the same cotton rats.
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adaptation within a species’ range: interactions between drift and gene flow.


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583.


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Sigmodon hispidus (Cricetidae), and population biology of the cotton rat louse, Hoplopleura hirsuta (Hoplopleuridae: Anoplura) in eastern New Mexico, including an annotated host-parasite bibliography. Texas Journal of Science. 40: 369-399.


Schwan, T.G. (1984) Sequential sampling to determine the minimum number of host examinations required to provide a reliable flea (Siphonaptera) index. Journal of Medical Entomology. 21: 670-674.


Table 1. Ectoparasites recovered from Cotton Rats, *Sigmodon hispidus*, at each trap site.

<table>
<thead>
<tr>
<th>State and County</th>
<th>Ectoparasites*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Georgia:</strong></td>
<td></td>
</tr>
<tr>
<td>Bleckley (32.397N, 83.347W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=14 (5M, 9F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (24M, 49F, 56N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (2M, 1F)</td>
</tr>
<tr>
<td>Columbia (33.562N, 82.175W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=15 (3M, 12F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (2M, 5F, 4N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (6M, 18F) <em>Ctenophthalmus pseudagyrtes</em> (1M) <em>Peromyscopsylla scotti</em> (1F)</td>
</tr>
<tr>
<td>Decatur (30.909N, 84.5833W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=2 (1M, 1F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (5F, 6N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (1M)</td>
</tr>
<tr>
<td>Screven (32.751N, 81.605W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=4 (1M, 3F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (3M, 6F, 7N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (16M, 16F)</td>
</tr>
<tr>
<td>Burke (32.985N, 81.978W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=7 (3M, 4F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (6M, 7F, 17N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (1M)</td>
</tr>
<tr>
<td>McIntosh (31.374N, 81.499W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=6 (3M, 3F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (8M, 13F, 38N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (3F)</td>
</tr>
<tr>
<td>Lowndes (30.842N, 83.306W)</td>
<td><em>Sigmodon hispidus</em> &lt;br&gt;n=4 (2M, 2F) &lt;br&gt;Sucking Louse: <em>Hoplopleura hirsuta</em> (1M, 1F, 1N) &lt;br&gt;Fleas: <em>Polygenis gwyni</em> (2M, 2F)</td>
</tr>
<tr>
<td>Location</td>
<td>Main Sucking Louse</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------</td>
</tr>
<tr>
<td><strong>Jenkins</strong> (32.720N, 81.979W)</td>
<td><em>Hoplopleura hirsuta</em> (8M, 16F, 63N)</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=10 (5M,5F)</td>
<td></td>
</tr>
<tr>
<td><strong>Glynn</strong> (31.170N, 81.499W)</td>
<td><em>Hoplopleura hirsuta</em> (1M, 1F, 1N)</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=5 (2M, 3F)</td>
<td></td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=10 (6M, 4F)</td>
<td></td>
</tr>
<tr>
<td><strong>Candler</strong> (32.318N, 82.074W)</td>
<td><em>Hoplopleura hirsuta</em> (5M, 17F, 6N)</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=14 (6M, 8F)</td>
<td></td>
</tr>
<tr>
<td><strong>Florida</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Brevard</strong> (28.077N, 80.629W)</td>
<td><em>Hoplopleura hirsuta</em> (1M, 5F, 17N)</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=28 (12M, 16F)</td>
<td></td>
</tr>
<tr>
<td><strong>Flagler</strong> (29.469N, 81.364W)</td>
<td></td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=1 (1M)</td>
<td></td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> n=40 (18M, 22F)</td>
<td></td>
</tr>
<tr>
<td><strong>Leon</strong></td>
<td><em>Hoplopleura hirsuta</em> (7M, 33F, 18N)</td>
</tr>
<tr>
<td><em>Sigmodon hispidus</em> (30.444N, 84.258W) n=23 (10M,13F)</td>
<td></td>
</tr>
</tbody>
</table>
## Table 1. Continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Coordinates</th>
<th>Sigmodon hispidus</th>
<th>Ectoparasites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>South Carolina:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charleston (32.780N, 79.936W)</td>
<td></td>
<td>Sigmodon hispidus</td>
<td>Sucking Louse: Hoplopleura hirsuta (6M, 19F, 2N)</td>
</tr>
<tr>
<td></td>
<td>n=18 (9M, 9F)</td>
<td></td>
<td>Fleas: Polygenis gwyni (14M, 11F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Orchopeas howardi (1F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stenoponia americana (3M, 2F)</td>
</tr>
<tr>
<td><strong>North Carolina:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackson (35.372N, 83.199W)</td>
<td></td>
<td>Sigmodon hispidus</td>
<td>Flea: Peromycopsylla hamifer (1F)</td>
</tr>
<tr>
<td></td>
<td>n=1 (1F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mississippi:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackson (30.366N, 88.543W)</td>
<td></td>
<td>Sigmodon hispidus</td>
<td>Fleas: Polygenis gwyni (1M, 4F)</td>
</tr>
<tr>
<td>Marion (31.251N, 89.756W)</td>
<td></td>
<td>Sigmodon hispidus</td>
<td>Sucking Louse: Hoplopleura hirsuta (3F)</td>
</tr>
<tr>
<td></td>
<td>n=17 (10M, 7F)</td>
<td></td>
<td>Fleas: Polygenis gwyni (11M, 17F)</td>
</tr>
</tbody>
</table>

*For each ectoparasite species, the numbers of different life stages recovered are listed (key: M, Male(s); F, Females(s), N, Nymph(s))

---

*For each ectoparasite species, the numbers of different life stages recovered are listed (key: M, Male(s); F, Females(s), N, Nymph(s))

---

25
Table 2. Sex Ratios* of *Hoplopleura hirsuta* and *Polygenis gwyni* on Cotton Rats in the southeastern United States.

<table>
<thead>
<tr>
<th>Site Location</th>
<th>Lice* (H. hirsuta)</th>
<th>n**</th>
<th>Fleas* (P. gwyni)</th>
<th>n**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulloch Co. – GA</td>
<td>1.8</td>
<td>46F;25M;150N</td>
<td>1.2</td>
<td>50F;43M</td>
</tr>
<tr>
<td>Bleckley Co. – GA</td>
<td>2.0</td>
<td>49F;24M;56N</td>
<td>0.5</td>
<td>1F;2M</td>
</tr>
<tr>
<td>Columbia Co. – GA</td>
<td>2.6</td>
<td>8F;3M;3N</td>
<td>3.2</td>
<td>16F;5M</td>
</tr>
<tr>
<td>Decatur Co. – GA</td>
<td>--</td>
<td>5F;0M;6N</td>
<td>0.0</td>
<td>0F;1M</td>
</tr>
<tr>
<td>McIntosh Co. – GA</td>
<td>1.6</td>
<td>13F;8M;22N</td>
<td>--</td>
<td>3F;0M</td>
</tr>
<tr>
<td>Chatham Co. – GA</td>
<td>4.8</td>
<td>29F;6M;17N</td>
<td>0.0</td>
<td>0F;0M</td>
</tr>
<tr>
<td>Screven Co. - GA</td>
<td>2.0</td>
<td>6F;3M;7N</td>
<td>1.0</td>
<td>16F;16M</td>
</tr>
<tr>
<td>Lowndes Co. – GA</td>
<td>1.0</td>
<td>1F;1M;1N</td>
<td>1.0</td>
<td>2F;2M</td>
</tr>
<tr>
<td>Candler Co.- GA</td>
<td>3.4</td>
<td>17F;5M;6N</td>
<td>1.4</td>
<td>13F;9M</td>
</tr>
<tr>
<td>Burke Co. – GA</td>
<td>1.2</td>
<td>7F;6M;17N</td>
<td>0.0</td>
<td>0F;1M</td>
</tr>
<tr>
<td>Jenkins Co. – GA</td>
<td>2.0</td>
<td>16F;8M;63N</td>
<td>2.0</td>
<td>2F;1M</td>
</tr>
<tr>
<td>Glynn Co. – GA</td>
<td>1.0</td>
<td>4F;4M;8N</td>
<td>1.3</td>
<td>10F;8M</td>
</tr>
<tr>
<td>Brevard Co. – FL</td>
<td>5.0</td>
<td>5F;1M;17N</td>
<td>1.2</td>
<td>57F;47M</td>
</tr>
<tr>
<td>Flagler Co. – FL</td>
<td>--</td>
<td>0F;0M;0N</td>
<td>--</td>
<td>0F;0M</td>
</tr>
<tr>
<td>Bay Co. – FL</td>
<td>3.5</td>
<td>88F;25M;176N</td>
<td>2.4</td>
<td>17F;7M</td>
</tr>
<tr>
<td>Leon Co. – FL</td>
<td>4.7</td>
<td>33F;7M;18N</td>
<td>1.6</td>
<td>52F;32M</td>
</tr>
<tr>
<td>Charleston Co. – SC</td>
<td>2.7</td>
<td>19F;7M;2N</td>
<td>0.8</td>
<td>11F;14M</td>
</tr>
<tr>
<td>Jackson Co. – MS</td>
<td>--</td>
<td>0F;0M;0N</td>
<td>4.0</td>
<td>4F;1M</td>
</tr>
<tr>
<td>Marion Co. – MS</td>
<td>--</td>
<td>3F;0M;0N</td>
<td>1.5</td>
<td>17F;11M</td>
</tr>
<tr>
<td>Jackson Co. – NC</td>
<td>--</td>
<td>0F;0M;0N</td>
<td>--</td>
<td>0F;0M</td>
</tr>
<tr>
<td>Total Sites Combined</td>
<td>2.6</td>
<td>349F;133M;428N</td>
<td>1.4</td>
<td>271F;200M</td>
</tr>
</tbody>
</table>

*expressed as number of females per one male  
**F=females  
M=males  
N=nymphs
**Figure 1.** Approximate geographical distribution of *Sigmodon hispidus* (Cotton Rat), shaded in red-modified from Hall & Kelson (1959), Cameron and Spencer (1981) and Whitaker and Hamilton (1998).
Figure 2. Male (left) and female (right) *Polygenis gwyni* flea. (Specimens cleared in Potassium hydroxide).

Figure 3. Male (left) and female (right) *Hoplopleura hirsuta* sucking louse. (Specimens cleared in Potassium hydroxide).
**Figure 4.** Approximate geographical distribution of *Hoplopleura hirsuta* (sucking louse) shaded in blue. Data compiled from Ferris (1921), Morlan (1952), Smith and Love (1958), Henry (1970), Kim et al. (1986), Pfaffenberger and DeBrian (1988), Durden *et al.* (1993, 2000) and Durden and Musser (1994).

**Figure 5.** Approximate geographical distribution of *Polygenis gwyni* (flea) shaded in green. Data compiled from Fox (1940), Morlan (1952), Pratt and Good (1954), Smith and Love (1958), Henry (1970), Layne (1971), Benton (1980), Smit (1987), Pfaffenberger and DeBrian (1988) and Durden *et al.* (1993, 2000).
Figure 6. Regression analysis of the number of fleas on cotton rats for each trap site given by latitude. The abundance of fleas per trap site was analyzed using raw numbers of fleas per rodent at each trap site including individual rodents with zero fleas recorded.
Figure 7. Regression analysis of the number of lice on cotton rats for each trap site given by latitude. The abundance of lice per trap site was analyzed using raw numbers of lice per rodent at each trap site including individual rodents with zero lice recorded.
**Figure 8.** Mean intensity of *Hoplopleura hirsuta* and *Polygenis gwyni* (only hosts with ectoparasites) infesting male and female cotton rats.

**Figure 9.** Prevalence of *Hoplopleura hirsuta* and *Polygenis gwyni* (number of hosts infested divided by the total number of hosts examined) of male and female cotton rats.
APPENDIX A

MIXED INFECTIONS, CRYPTIC DIVERSITY, AND VECTOR-BORNE PATHOGENS: EVIDENCE FROM POLYGENIS FLEAS AND BARTONELLA SPECIES

1Patrick Abbot, Alena E. Aviles, Lauren Eller, and Lance A. Durden.


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Mixed Infections, Cryptic Diversity, and Vector-Borne Pathogens: Evidence from *Polygenis* Fleas and *Bartonella* Species

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Received 29 January 2007/Accepted 26 July 2007

Coinfections within hosts present opportunities for horizontal gene transfer between strains and competitive interactions between genotypes and thus can be a critical element of the lifestyles of pathogens. *Bartonella* spp. are *Alphaproteobacteria* that parasitize mammalian erythrocytes and endothelial cells. Their vectors are thought to be various biting arthropods, such as fleas, ticks, mites, and lice, and they are commonly cited as agents of various emerging diseases. Coinfections by different *Bartonella* strains and species can be common in mammals, but little is known about specificity and coinfections in arthropod vectors. We surveyed the rate of mixed infections of *Bartonella* in flea vectors (*Polygenis gwyni*) parasitizing cotton rats (*Sigmodon hispidus*) in which previous surveys indicated high rates of infection. We found that nearly all fleas (20 of 21) harbored one or more strains of *Bartonella*, with rates of coinfection approaching 90%. A strain previously identified as common in cotton rats was also common in their fleas. However, another common strain in cotton rats was absent from *P. gwyni*, while a rare cotton rat strain was quite common in *P. gwyni*. Surprisingly, some samples were also coinfected with a strain phylogenetically related to *Bartonella clarridgeiae*, which is typically associated with felids and ruminants. Finally, a locus (pap31) that is characteristically borne on phage in *Bartonella* was successfully sequenced from most samples. However, sequence diversity in pap31 was novel in the *P. gwyni* samples, relative to other *Bartonella* previously typed with pap31, emphasizing the likelihood of large reservoirs of cryptic diversity in natural populations of the pathogen.

Most host populations harbor more than one pathogen strain at a given time, leading to mixed infections or "coinfections" in individual hosts (10, 26, 48). Unfortunately, there are gaps in our understanding of within-host pathogen interactions. The problem is particularly acute in vector-borne diseases, where little is known regarding mixed infection interactions in natural populations of the vectors themselves. Rather, with only a few notable exceptions (e.g., reference 25), most population-level or clinical data on mixed infections derive from human studies or other mammalian models. The distinction is crucial because of the role that vectors play in pathogen transmission.

The bacterial pathogen *Bartonella* sp. has become one of a few model organisms for studying the evolution and ecology of vector-borne diseases (28). This is due to diverse efforts to describe *Bartonella* biology at multiple levels, from cells and immune systems (12, 13, 14, 30), to populations and communities (31, 32), to species and clades (36, 44). The recent publication of full genome sequences is obviously key (2). *Bartonella* sp. is a short, gram-negative, fastidious bacterium belonging to the *Alphaproteobacteria* (1). Closely related to *Brucella* spp., *Bartonella* organisms are parasites of mammalian erythrocytes and endothelial cells (12, 13, 14) and are transmitted by blood-feeding insects, such as ticks, fleas, lice, and flies (9, 19, 20, 21, 23, 28). Infection of a host causes chronic bacteremia and creates a reservoir for vectors that can transmit the bacteria to new susceptible hosts. While prolonged bacteremia is normally associated with severe sickness in a susceptible host, *Bartonella*-caused bacteremia typically remains asymptomatic in the reservoir host. Some *Bartonella* are known to be transmitted by the bite (anterior station transmission) or in the feces (posterior station transmission) of insect vectors. For example, in humans, *Bartonella bacilliformis*, which causes Oroya fever (verruga peruana, or Carrion's disease) in Andean South America is transmitted by the bites of infectious sandflies (5), and *Bartonella quintana*, which causes trench fever in many parts of the world, is transmitted via the feces of infected body lice (21). Fleas infected with *Bartonella henselae* (the causative agent of cat scratch disease and of related conditions such as bacillary angiomatosis [30]) and other *Bartonella* appear to transmit these agents via their infectious feces (9, 19, 20). Current phylogenetic information indicates six distinct groups worldwide, of which all but one are found in the United States (44). Host and vector affiliations are complex, and the evidence is against strict one-to-one host specificity (28, 32, 33). A consistent trend is that groups of *Bartonella* species tend to be restricted to natural groups of mammalian hosts (rodents, cats, dogs, humans, etc.), indicating a diffuse but long-term coevolutionary history.

We surveyed the incidence of mixed *Bartonella* infections in natural populations of the flea *Polygenis gwyni* parasitizing the Eastern woodrat (*Neotoma floridana*) and the hispid cotton rat (*Sigmodon hispidus*). Previous surveys of mammalian hosts indicated that mixed infections of *Bartonella* can be common (22). An intensive survey of *S. hispidus* in the southeastern United States, for example, revealed that this host exhibits a particularly high infection prevalence overall, as well as non-

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* Published ahead of print on 10 August 2007.
TABLE 1. Loci and GenBank accession numbers used in the present study to reconstruct Bartonella phylogenetic relationships and to identify the species relationships of cloned g64 amplonoc from Polygonus fleas

<table>
<thead>
<tr>
<th>Species (strain)</th>
<th>GenBank accession no. for amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus (IBS382)</td>
<td>AF204273, AF169587, AF293937, AF67763, AY116630</td>
</tr>
<tr>
<td>B. bacilliformis (KCS8T)</td>
<td>U28076, AF169588, Z15160, AFO72566, AY26918</td>
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MATERIALS AND METHODS

Trapping and collection methods. Cotton rats and Eastern woodrats were trapped in Bullock and Searren Counties (one site in each county) in southeastern Georgia, using Sherman live traps (U. B. Sherman Traps Inc., Tallahassee, FL) baited with rolled oats and a trace of peanut butter and set near areas of rodent activity. Trapped animals were lightly anesthetized via intramuscular administration of ketamine hydrochloride and then transferred to a white tray, where they were carefully examined for ectoparasites. Retrieved ectoparasites were transferred to individually labeled vials containing 95% ethanol. Flies were later identified using the methods of Smith (52) and Lewis and Lewis (28). All flies collected were Polygonom psittaci, which is the species that typically parasitizes the cotton rat in the southern United States (48, 52). This fly species has also been reported previously from the Eastern woodrat (18). Following recovery from anesthesia, all cotton rats and woodrats were released at their capture site. Mammals were live trapped under permit 9712 issued by the Georgia Department of Natural Resources, and animal procedures were approved by the IACUC committee at Georgia Southern University (research protocol no. IACUC005). Voucher flea specimens have been deposited in the Ectoparasite Collection at Georgia Southern University under accession numbers L-358 and L-1102.

DNA methods. Whole genomic DNA from P. psittaci was extracted using a DNeasy tissue kit (QIAGEN, Inc.). Each sample was tested for the presence or absence of Bartonella by PCR amplification of an approximately 400-bp amplicon from the clades synthase g64 gene, using the universal oligonucleotides primers IBMcST(11) and IBMcST(9) (33). g64 was chosen because it is the only discriminating power for Bartonella (36), the existing coverage in GenBank of the genus using this gene, and its prior use in identifying Bartonella in the flea host, S. lusitana (32). PCR products were visualized by electrophoresis and ethidium bromide staining under UV light on 1.5% agarose gels. Samples yielding successful g64 amplicons were then restested with oligonucleotides pap1 and pap2, designed from the bacteriophage-associated gene pap1 in K. lactis (34). Both PCR amplifications were carried out at 10 μl volumes, containing 1× Invitrogen 10× buffer, 2.0 mM MgCl2, 100 μM of each deoxynucleoside triphosphate, 5 pmol of each primer, 100 ng of whole genomic DNA. Reaction conditions were as follows: 1 cycle at 94°C for 2 min and 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s, followed by 1 cycle at 72°C for 10 min. Products from both reactions were cloned via a pCR2.1 TOPO vector (Invitrogen Life Technologies, Carlsbad, CA) and TOPO TA cloning kit and Top10 competent cells, according to the manufacturer’s instructions. Positive clones for both genes were PCs amplified at 50 μl volumes, as above, purified with a Qiagen PCR Purification Kit (Qiagen, Inc.) following the manufacturer’s instructions, and sequenced at the Vanderbilt University Medical Center Sequencing Core Facility and the University of Arizona Genomic Analysis and Technology Core Facility, with either Invitrogen vector primer IV71 or M13R. Resulting sequences were then compared against known Bartonella sequences in GenBank, using default parameters in BLAST.

Phylogenetic methods. We determined the phylogenetic affinities of the g64 amplicons by first constructing a backbone phylogeny of 18 Bartonella species, isolated from a wide range of mammalian hosts from each of the five recognized host clusters (Table 1). Initially, species were selected by the availability of sequences in public databases from seven housekeeping genes commonly used in Bartonella species differentiation (168). ITS, fadZ, g64, pap1, relC, and rbcL, not all gene sequences were available for all species). However, 16S and ITS were not used because of strong phylogenetic incongruence and alignment uncertainty in these loci. The remaining genes were first aligned using a partial order alignment algorithm (implemented in the software package PAUP v.2, using default parameters and simple strict addition, the TBR swapping algorithm, and 10 random addition replicates for each search iteration. Parsimony trees were compared to those generated by a partitioned Bayesian analysis in the software package MrBayes v.3.1.2 (50), under models and parameters separately estimated via ModelTest v.3.6 (45), each conditioned on the same starting tree estimated by maximum likelihood on the entire data set using a general time-reversible model of evolu-
### Table 2. Identification of Bartonella spp. isolates cloned from each flea, based on reconstruction of gltA or pap31 phylogenies using GenBank sequences and those derived from the present study

<table>
<thead>
<tr>
<th>P. gyni sample no.</th>
<th>Host</th>
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<th>Site</th>
<th>Distinct gltA genogroup*</th>
<th>胶A GenBank accession no(s)</th>
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*The two collection sites were Candler County, GA (A) and Bulloch County, GA (B). The gltA columns represent the full diversity of positive matches based on phylogenetic reconstruction. Most matched previously undescribed "genogroups" from Sigmorhopus hirsutus, as shown in Fig. 1 and Fig. 3. The plus signs indicate a positive match. The pap31 clades are provisional designations based on the topology depicted in Fig. 3.

**Results**

Eight S. hispidus and two N. floridana rats were trapped from two sites, from which 21 P. gyni fleas were collected. Either gltA or pap31 amplicons of the expected size were detected in 20 of 21 fleas, and replicate sequences were obtained from most samples, such that any Taq error or PCR recombination could be identified and not included in the diversity estimates (Table 2). In 17 fleas, we sequenced multiple and divergent gltA or pap31 clones, permitting us to survey the frequency of single or multiple infections. Cloning efficiency varied between individual fleas, resulting in an unequal number of sequences per gene per flea (Table 2). There was some, but not perfect, overlap between gltA and pap31 as positive evidence for mixed infections. Using gltA only, mixed Bartonella infections were detected in 12 of 16 fleas positive for Bartonella and for which five or more sequence replicates were obtained (Table 2). Most gltA amplicons exhibited greater than 94% sequence similarity to undescribed Bartonella vesiculose-like genogroups previously cultured from S. hirsutus in the southeastern United States (Fig. 1 and Fig. 2) (33). However, in six fleas collected from five different S. hispidus isolates, gltA amplicons were detected with closest similarity to B. clarridgeiae, a species normally associated with fleas and ruminants (44) but which may be closely related to species with broader host ranges (18, 39). BLAST searches with the pap31 sequences resulted in highest sequence similarity scores with either B. quintana or B. henselae, although neither of these species was greater than 94% similar to any of the pap31 sequences (Fig. 3 and 4), reflecting the still-limited survey of pap31 diversity in Bartonella available in GenBank. However, the pap31 phylogeny revealed three distinct clades of P. gyni-associated Bartonella, perhaps mirroring the divergence between strains detected by gltA. One clade was characterized by a 1-bp deletion near the boundary between a putative conserved transmembrane domain and an extracellular loop sequence (Fig. 4) (42), producing a UAA stop codon downstream and thus presumably a truncated protein. This deletion was perfectly matched by an alanine-to-valine replacement downstream in a putative inner membrane loop sequence.
DISCUSSION

We surveyed the prevalence of Bartonella in a population of rodent fleas, collected from a general locale in which small mammals had been previously intensively surveyed (31, 32, 33). Because we surveyed in a manner that discriminated between single and mixed infections in fleas, we also estimated the fraction of fleas harboring more than one Bartonella isolate and the phylogenetic affinities of coinfecting isolates. We found four noteworthy results.

First, the prevalence of Bartonella was surprisingly high, exceeding characteristic records from various putative arthropod vectors (35, 49, 54). Estimating prevalence requires population-level sampling, and only in recent years have such surveys of Bartonella in presumed vectors begun to emerge. Cat fleas (Ctenocephalides felis) are important agents of zoonotic Bartonella transmission and have been examined in a number of studies sufficient to yield population-level data (41, 49). Estimates of cat-associated Bartonella prevalence (e.g., B. henselae, B. quintana, B. koehleri, and B. cimex) have ranged from 20 to 30%, although some C. felis populations may exhibit higher rates (35). Less is known about other flea or arthropod vectors from natural populations of mammals. Studies reporting nonnegligible rates of infection in fleas from various small mammals typically have ranged from 10 to 40% (41, 54). Not surprisingly, there are still few studies that report simultaneous estimates of prevalence in vectors and their mammalian hosts (54).

However, we collected fleas from S. hispidus in an area that, because of extensive prior work (31, 32, 33), corresponds to an intensively scrutinized regional population (the coastal plain and piedmont of Georgia). In one study, Kosoy et al. (32) found rates of Bartonella infection in S. hispidus in central and southern Georgia approaching 80%. Thus, the degree of Bartonella infection in the P. gyni population we surveyed is consistent with more extensive surveys of S. hispidus and lends confidence that these small sample estimates are representative.

Second, we found substantial rates of mixed Bartonella infections. More than half of the fleas we surveyed were infected by more than one Bartonella glnA genotype (Table 2). If the
paps31 screens are included, the rate is even higher. Kosoy et al. (33) originally described four broad genotypic clusters associated with various small rodents from the southeastern United States, designated A through D. Type sequences originally used to define groups A and B together form a diverse but monophyletic group, with members of some species from S. higdias, as originally indicated in the neighbor-joining distance clad tree of Kosoy et al. (33). We detected isolates similar to A and B in the surveyed fleas and, unsurprisingly, did not detect the Peromyscus-associated D group. Surprisingly, we did not detect genogroup C, previously cultured from regional samples of S. higdias (31, 32). Cluster A is, by far, the most common Bartonella genogroup isolated from cotton rats in the region (31, 32). However, C is more prevalent than B (31, 32), a pattern opposite of what we found in P. gynii from cotton rats (Table 2; Fig. 1). This pattern may simply be an artifact of small sample sizes and may not hold up to more-extensive surveys. However, one possibility is that the different P. gynii/S. higdias isolates exhibit either unequal resident times in the vectors and hosts and/or transmission biases, potentially presenting an opportunity to uncover differential adaptation and specificity in Bartonella (M. Kosoy, personal communication).

Third, we successfully amplified a fragment from the hemoglobin paps31-like isolate, which was evidently infected with B. vinsonii-like isolates (as determined by glc4). This is notable, because in both B. quintana and B. henselae, paps31 is generally known to be phage-borne and orthologous to a large family of harpin-binding protein-coding genes (hbp) critical to heme acquisition, cellular adhesion, and possibly pathogenesis (8, 11, 56). Recent work has described a paps31 homolog from bacteriophages in B. vinsonii subsp. berkhoofi (40), a species that was previously thought to lack bacteriophages and hbp4 protein homologs (8). Assuming the glc4 results are a reliable guide, we found paps31-like sequences in fleas infected by Kosoy et al. genogroups A and B; paps31 amplions were cloned in fleas apparently lacking co-infections and harboring either the A or B glc4 genogroup alone (Table 2). Although there is not yet sufficient coverage of the genus with paps31 to identify the isolates we detected, three distinct genogroups are evident (Fig. 3). Possibly, the two derived genogroups within the clade correspond to Kosoy et al.'s (33) genogroups A and B.

In B. quintana, paps31 is a member of a five-gene family, composed of three tandemly arrayed paralogs and two other homologs (42). A possible complication is the uncertain copy number of paps31 homologs across the genus. However, the clade that includes the P. gynii samples is rooted by hbp4A from B. quintana, to the exclusion of other members of the gene family, and includes orthologous sequences from B. kentense and B. vinsonii subsp. berkhoofi (Fig. 3). Moreover, the paps31 transmembrane protein includes outer membrane loops with the potential to incorporate nearly random in-frame chromosomal sequences. In B. quintana, the five homologs are difficult to align at these sites (42) (data not shown). With the exception of some isolates exhibiting distinct similarity to B. kentense (Fig. 4), the loop sequences from P. gynii isolates exhibit very little amino acid polymorphism between the conserved transmembrane domains. It is thus likely that the paps31 topology reflects orthologous sequence variation in the Bartonella isolates we surveyed. Because of the sampling design and the high rate of coinfection, it is not possible to determine the significance of the truncated hbp4 pseudogene. However, possibilities include that some isolates harbor an antigenic variant of the full hbp4 protein, similar to the msp2 locus in Anaplasma sp. (3, 71), or that the paps31 pseudogene is a loss-of-function variant derived from a cryptic strain that has undergone a change in lifestyle (23, 43).

In this vein, the fourth and perhaps most surprising result was the presence in two fleas of an isolate sharing >94% glc4 similarity to B. claridgeiae and the newly described species B. rochlamiae (the next closest relative is Bartonella henselae, at >87% sequence similarity) (Fig. 1). B. claridgeiae itself has not been described from rodents; rather, felids or canids are the primary reservoirs (49). Species near B. claridgeiae have been reported in various mammalian hosts, however, and recently, a B. claridgeiae-like isolate was identified in rat fleas from Egypt (39). Among the highest BLAST scores for the B.
FIG. 3. Consensus tree of Bartonella taxa based on partial pap31 sequences. Numbers above interior branches represent clade credibility values from the Bayesian analysis (above) and 100 maximum likelihood bootstrap replicates (below). Most amplicons were confirmed by redundant sequencing of multiple cloned products. Three distinct genotypic groups are evident from the fleas of cotton rats, as shown. The genetic distance between groups II and III, based on 125 bp of alignable transmembrane domain sequences, was approximately 1.4% (uncorrected p distance). Group I differed from both by approximately 11 to 12%. The Bartonella isolates in these groups have no clear identity based on BLAST searches of pap31 sequences. Highest BLAST scores were returned for B. henselae or B. quintana, but this probably reflects the limited taxonomic sampling of pap31 across the genus.

claridgeae-like isolates in P. oxyurus were uncultured species from rodents and other small mammals (17, 25, 46). Both fleas were coinfected with Kosoy genogroup A or B. In the case of one flea, all three principle gldA variants were detected. The significance of a B. claridgeiae species in S. oxyurus is unknown, because of the size of the survey and the absence of simultaneous information on the competence of P. oxyurus as a B. claridgeiae vector and B. claridgeiae bacteremia in cotton

FIG. 4. Amino acid fragments of pap31 homologs from various taxa and a subset amplified from representative flea samples in the present study. The fragment corresponds to an outer membrane loop sequence between conserved transmembrane domains 3 and 4, as described in reference 42. These loops may be composed of nearly random host chromosomal sequences (56), as evident in the lack of conservation between the related B. henselae and B. quintana. Seven of the nine P. oxyurus isolates exhibit strong conservation, likely indicating a recent common ancestor. Some samples align more closely to B. henselae strains, as indicated by gray shading. In one group (clade II from Fig. 3), a 1-bp deletion (grey hatched box) causes a UAA stop codon downstream. A gap has been inserted to maintain the alignment in these samples. The black box highlights an insertion of three residues. Dots indicate matches with the topmost sequence, dashes indicate gaps, and question marks indicate uncertainties due to unresolvable ambiguities in the nucleotide sequences. The black line separates the pap31 amplicons from the present study and those from known species. Distinct amplicons were cloned from the same flea samples, as illustrated by P. oxyurus numbers 1, 9, and 11.
rugs, the biological significance is difficult to judge. However, like *B. bacilliformis*, the etiological agent of bartonellosis (Carrión's disease) in humans, and *B. bovis*, *B. clarridgeiae* is one of the few flagellated bartonellae (51) and has long been a problematic species because of its uncertain phylogenetic placement and the odd host range that it shares with *B. bovis* (44). It may not be a coincidence that an isolate resembling these hypergeneralist species has been discovered in *Polynema*. Efforts to understand the molecular basis of variation in host specificity in the genus (2) would benefit from closer examination of *B. clarridgeiae* and its relatives (18).

Two opposing ecological and evolutionary processes seem to be at work in *Bartonella*. The cryptic diversity in the vectors of *Bartonella*, and the absence of strains common in mammalian hosts, may reflect an evolutionary trend towards differential adaptation to host-specific niches, in either vectors or reservoirs. If so, it seems *Bartonella* possesses a tendency towards fine-scale adaptation, ecological specialization, and divergence between essentially sympatric colonizations, despite mixed infections, close physical proximity, and generalized lifestyles. The mechanisms by which *Bartonella* genomes are protected during the process of specialization to host-associated niches, while maintaining broad host affiliations and thus mixed infections (29), are presently unknown.

ACKNOWLEDGMENTS

We thank Michael Koscoy for helpful discussion, as well as three anonymous reviewers for helpful comments on early drafts. This work was supported by NSF grant 1OB-0429400 to P.A.

REFERENCES


APPENDIX B: INDIVIDUAL MAMMAL DATA

Example entry with explanations:

1.1

*Sigmodon hispidus*

F, J

Site by State and County

1.2

*Hoplopleura hirsuta*

3F, 1N

Mammal species

1.3

*Polygenis gwyni*

1M

Mammal gender, Life Stage

1.4

*Stenoponia americana*

1F

Arthropod species

1.5

*Peromyscopsylla hamifer*

2F

Number of arthropods collected

LAD 210

Accession number

Legend:

sites:

1.1 – Georgia, Bulloch

1.2 – Georgia, Bleckley

1.3 – Georgia, Columbia

1.4 – Georgia, Decatur

1.5 – Georgia, Screven

1.6 – Georgia, Burke

1.7 – Georgia, McIntosh

1.8 – Georgia, Lowndes

1.9 – Georgia, Jenkins

1.10 – Georgia, Glynn

1.11 – Georgia, Chatham

1.12 – Georgia, Candler

2.1 – Florida, Brevard

2.2 – Florida, Flagler

2.3 – Florida, Bay

2.4 – Florida, Leon

3.1 – South Carolina, Charleston

4.1 – North Carolina, Jackson

5.1 – Mississippi, Jackson

5.2 – Mississippi, Marion
### APPENDIX B. Continued

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APPENDIX B. Continued

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