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SHORT COMMUNICATION

Pocket gophers and chewing lice: a test of the maternal transmission hypothesis

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Abstract

The life-history traits of pocket gophers and their chewing lice suggest that there is little opportunity for transmission of parasites among pocket gophers, with the exception of transmission from mother to offspring. Herein, we test the hypothesis that lice are transmitted maternally by using an indirect approach that compares the distribution of louse populations to the distribution of mitochondrial DNA haplotypes in the pocket gophers. Comparison of the chewing louse distributions to the distribution of mtDNA haplotypes for the gophers revealed no significant concordance, and thus falsifies the maternal transmission hypothesis.

Keywords: coevolution, Geomyidae, mtDNA, parasite, RFLP, Trichodectidae

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Introduction

Pocket gophers (Rodentia: Geomyidae) and their chewing lice (Pthiraptera: Trichodectidae) exhibit a coevolutionary pattern known as cospeciation, wherein the phylogenies of the two groups are more similar than would be expected by chance (Hafner & Nadler 1988; Demastes & Hafner 1993; Hafner *et al.* 1994). Despite repeated documentation of widespread cospeciation in multiple lineages of gophers and lice, little is known about the mechanism(s) responsible for generating this macroevolutionary pattern. Perhaps the most basic question about the gopher–louse system focuses on mode of transmission of chewing lice from one pocket gopher to another. In this study, we examine mode of louse transmission among pocket gophers to gain a better understanding of potential underlying factors that may influence, and perhaps even determine, patterns at higher levels of biological organization within this host–parasite assemblage.

All chewing lice of the genera *Geomydoecus* and *Thomomydoecus* (Pthiraptera: Trichodectidae) exclusively parasitize pocket gophers (Hellenthal & Price 1984). Because trichodectid lice are wingless insects that cannot survive for prolonged periods off their host (Scott 1950; Hopkins 1970), louse transmission presumably occurs only during direct physical contact between hosts (Kellogg 1913; Hopkins 1942; Rust 1974; Timm 1983). However, pocket gophers are asocial animals that rarely come into contact (Howard & Childs 1959), meaning that transfer of lice between adults must be rare (Patton *et al.* 1984). Thus, mother-to-offspring transfer of lice during the relatively long period of suckling is thought to be the predominant mode of louse transmission among pocket gophers (Rust 1974). Maternal transmission also was hypothesized for the chewing louse, *Pitrufquenia coypus*, of the European nutria, *Myocastor coypus* (Newson & Holmes 1968). This maternal-transmission hypothesis is supported further by observations that the density of chewing louse populations on female pocket gophers declines by 55–66% shortly after a female gives birth to a litter (Rust 1974). This decline in louse density on the mother probably is caused by dispersal of lice to her offspring.

A study of the distribution of chewing lice at a pocket gopher hybrid zone (Patton *et al.* 1984) suggested some degree of nonmaternal transmission of lice. Herein, we test the hypothesis that lice are transmitted maternally by using an indirect approach that compares the distribution of louse populations to the distribution of mitochondrial DNA (mtDNA) haplotypes in the pocket gophers. With few exceptions (Gyllensten *et al.* 1991), mtDNA is known to be maternally inherited in mammals (Brown 1983). Thus, if lice are transmitted among pocket gophers exclusively from mother to offspring, then the distribution of

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chewing louse populations should mirror that of the mtDNA haplotypes of their hosts.

The study site (Fig. 1) is located at a narrow constriction of the Rio Grande Valley near San Acacia, New Mexico. At this narrow constriction, two highly differentiated subspecies of pocket gophers (*Thomomys bottae connectens* and *T.b. opulentus*) come into contact and hybridize (Smith *et al.* 1983). Pocket gophers on opposite sides of the constriction exhibit striking allozymic differentiation, with genetic distances exceeding those found between many pairs of mammalian species (Smith *et al.* 1983; Demastes 1990). These two subspecies of gophers also show marked morphological and chromosomal differentiation (Smith *et al.* 1983). Gene flow between the two populations of gophers is severely restricted because of limited suitable habitat at the constriction, which results in low density of pocket gophers near the constriction.

The two subspecies of pocket gophers that meet at the San Acacia constriction host two different species of chewing lice of the genus *Geomydoecus* (Price & Hellenthal 1981). An earlier study revealed that the chewing lice, unlike the pocket gophers, do not meet precisely at the constriction (Demastes 1990). The northern species of louse, *G. aurei*, comes into contact with the southern species, *G. centralis*, 6 km south of the midpoint of the gopher contact zone (locality 4 on Fig. 1). There is no evidence of hybridization between the two louse species (Demastes 1990). Thus, gophers from localities 2 and 3 (Fig. 1) belong to the southern subspecies (*T.b. opulentus*), but host northern lice (*G. aurei*). This distributional discontinuity presents an ideal opportunity to study the transmission of lice among pocket gophers.

For clarity of discussion, the zone is divided into three regions (Fig. 1): region A contains only northern pocket gophers (*T.b. connectens*) parasitized by northern lice (*Geomydoecus aurei*); region B contains only southern pocket gophers (*T.b. opulentus*) parasitized by southern lice (*G. centralis*); and region C contains only southern gophers parasitized by northern lice. All louse and gopher identifications were made by use of diagnostic allozyme markers (Smith *et al.* 1983; Demastes 1990). If chewing lice are transmitted maternally, then the pocket gophers in region C (which are southern, based on their nuclear DNA, but host northern lice) should exhibit mtDNA haplotypes that are characteristic of northern gophers, such as those in region A (Fig. 1). Discordance between the positions of a nuclear DNA

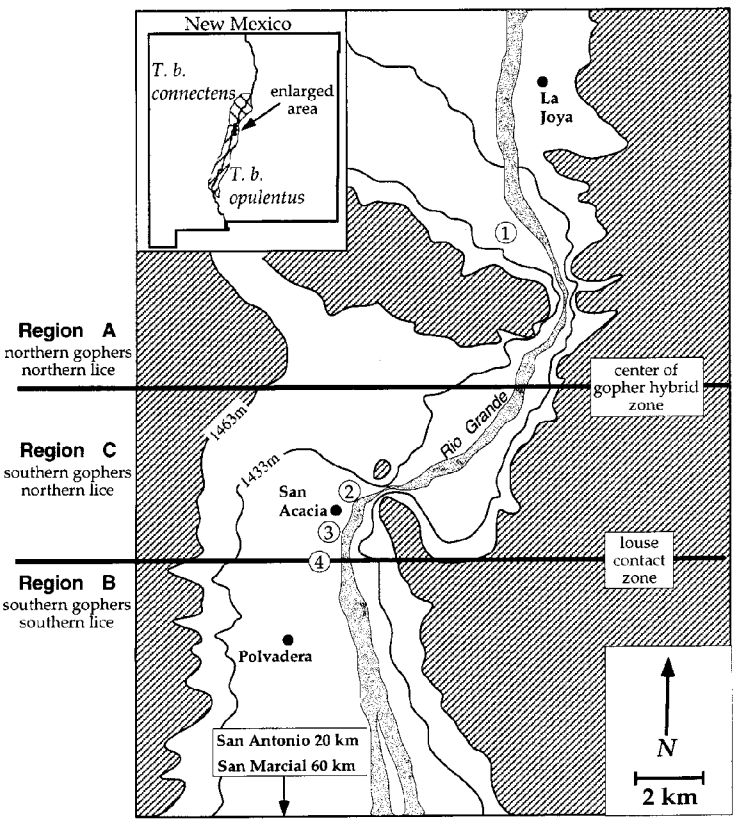

Fig. 1 Map of the study area near San Acacia, New Mexico. The three study regions are delineated (A, B, and C), and circled numbers indicate collecting localities listed in the Materials and methods.

contact zone and an mtDNA contact zone can be explained by past hybridization events and subsequent lineage sorting of the mtDNA haplotypes (Avise *et al.* 1984). Such discordance is not uncommon (Barton & Hewitt 1989) and has been demonstrated in mammals, including *Mus* (Gyllensten & Wilson 1987) and *Thomomys* (Patton & Smith 1994).

Materials and methods

Carcasses of freshly captured specimens were exposed to chloroform for 3–5 min to facilitate collection of ectoparasites by brushing the pelage. Whole lice and tissue samples of pocket gophers were frozen immediately in liquid nitrogen. Pocket gophers were characterized as to their nuclear DNA and chewing louse population identities by examination of diagnostic allozyme loci (Smith *et al.* 1983; Demastes 1990) visualized by use of horizontal starch-gel electrophoresis. The diagnostic loci used for identification of pocket gophers were glucose-6-phosphate dehydrogenase (EC 1.1.1.49; Harris & Hopkinson 1978) and mannose phospate isomerase (EC 5.3.1.8). The diagnostic locus used for identification of the louse species was glucose phosphate isomerase (EC 5.3.1.9).

MtDNA was purified from liver tissue by ultracentrifugation in caesium chloride gradients (Lansman *et al.* 1981). The resulting closed-circular mtDNA was then digested by one of 13 restriction enzymes: *Ava*I, *Ava*II, *Bam*HI, *Bgl*I, *Bcl*I, *Eco*RI, *Hin*cII, *Hin*dIII, *Nco*I, *Nde*I, *Pst*I, *Stu*I, and *Xho*I. These enzymes have 6-base recognition sequences, with the exception of *Ava*II, which has five. The resulting fragments were end-labelled with ^{35}S radionuclides and separated in agarose gels (0.8%). Restriction fragments were visualized by autoradiography of vacuum-dried gels and compared to a 1-kb standard (Bethesda Research Laboratories, Gaithersburg, MD, USA). Individuals sharing a unique combination of fragment patterns for the suite of all 13 restriction enzymes were designated as a haplotype. Restriction sites were inferred from the fragment data (Dowling *et al.* 1990) and used in subsequent analyses. Estimated percentage sequence divergence between haplotypes (δ) was calculated by the method of Nei & Tajima (1983).

Phylogenetic analyses were used to test the monophyly of mtDNA haplotype groups. Inferred restriction sites were treated as discrete characters and analysed with both maximum-likelihood (Felsenstein 1993) and parsimony (Swofford 1993) methods. The g_1 statistic was calculated and used to examine the data set for presence of phylogenetic signal (Hillis & Huelsenbeck 1992).

Specimens examined

Numbers in parentheses refer to localities (Fig. 1), and letters refer to the general region of the contact zone (Fig. 1). The number of pocket gophers examined at each locality is indicated by *n*. 1A, New Mexico, Socorro Co., 5.64 km south of La Joya, west side of Rio Grande ($n = 6$); 2C, New Mexico, Socorro Co., San Acacia, ($n = 4$); 3C, New Mexico, Socorro Co., 1.13 km south, 0.32 km east of San Acacia ($n = 1$); 4B, New Mexico, Socorro Co., 3.22 km north, 0.81 km east Polvadera ($n = 2$). Additional specimens examined from region B were collected 50–80 km south of the contact zone to insure minimal genetic introgression from the northern subspecies of gopher. These localities (not shown in Fig. 1) are: New Mexico, Socorro Co., San Marcial ($n = 1$); and New Mexico, Socorro Co., San Antonio ($n = 2$). The outgroup in the phylogenetic analyses was *Thomomys umbrinus* (Mexico: Mexico; 34 km east of Zitácuaro, Bosencheve). Voucher specimens of lice are deposited in the Entomology Collection of the University of Minnesota. Pocket gopher specimens are housed in the Museum of Natural Science, Louisiana State University.

Results and Discussion

Allozyme data confirmed that the pocket gophers examined from region A were of the northern subspecies, and individuals from regions B and C belonged to the southern subspecies. Electrophoretic examination of individual chewing lice from these pocket gophers (region A, $n = 64$; region B, $n = 74$, and region C, $n = 94$) confirmed that the pocket gopher individuals from regions A and C hosted northern lice, and gophers from region B hosted southern lice.

Twelve of the 13 restriction enzymes that were used to diagnose the mtDNA of pocket gophers revealed variation in restriction-fragment size. Excluding the outgroup, 10 unique haplotypes were determined (Table 1). A cursory examination of Table 1 is sufficient to see the marked differences in fragment patterns between the two subspecies of pocket gophers examined. These fragment patterns were used to infer maps of 61 individual restriction sites. All subsequent analyses are based on these restriction-site data.

Estimates of percentage sequence divergence coincide with what is apparent by visual examination of Table 1. Average sequence divergence is $\approx 0.5\%$ within each region and between regions B and C (Fig. 1). However, sequence divergence between regions A and B and between regions A and C is $\approx 5.0\%$.

Parsimony analysis of these restriction-site data yields a significantly skewed distribution of trees ($g_1 = -0.88$, $P < 0.01$), indicating that the data contain phylogenetic signal (Hillis & Huelsenbeck 1992). Parsimony analysis yields 11 shortest trees (18 steps). All of these trees depict two major monophyletic clades: A and B + C (Fig. 2). Maximum-likelihood analysis also indicated that each of these clades represent monophyletic groups. Therefore, the pocket gopher mtDNA cline is concordant with the nuclear DNA

cline (i.e. gophers in region A are of the northern subspecies and also have northern mtDNA haplotypes, whereas those in regions B and C are of the southern subspecies and have southern mtDNA haplotypes). Because gophers in region C host northern lice, yet have southern mtDNA haplotypes, the strict maternal-transmission hypothesis is falsified. In other words, the distribution of the two louse species does not mirror that of the pocket gopher mtDNA haplotypes, Indicating that it is unlikely that the two were transmitted in the same manner (i.e. maternally).

Importantly, the distributional pattern of chewing lice at San Acacia (this study) and northeastern California

	Zone Region										
	A			C				B			OG
Haplotype	1	2	3	4	5	6	7	8	9	10	11
Restriction enzyme*											
*Ava*I	B	B	B	A	A	A	A	A	A	A	C
*Ava*II	B	B	B	A	C	A	A	A	A	D	B
*Bam*HI	B	B	B	A	A	A	A	A	A	A	–
*Bcl*I	B	B	B	A	A	A	A	A	A	A	C
*Bgl*I	B	C	B	A	A	A	A	A	A	A	–
*Bgl*II	C	C	C	A	A	A	A	A	B	A	–
*Eco*RI	B	B	B	A	C	A	A	A	A	A	D
*Hin*dIII	A	A	A	A	A	A	A	A	B	A	C
*Nci*I	B	B	B	C	A	A	A	A	A	A	D
*Nco*I	C	B	B	A	A	A	D	A	A	A	–
*Pst*I	A	A	A	A	B	A	B	B	B	B	A
*Pvu*II	A	A	A	A	A	A	A	A	A	A	B
*Stu*I	B	B	B	A	A	A	A	A	A	A	–

Table 1 MtDNA restriction-fragment patterns revealed using 13 restriction enzymes in a survey of pocket gophers (*Thomomys bottae*) and the outgroup (OG = *Thomomys umbrinus*). Zone regions (A, B, and C) refer to Figs 1 and 2. Missing data for the outgroup (designated by –) were the result of fragment patterns too divergent to allow confident inference of restriction sites

*Estimated fragment sizes (kb) produced for each fragment pattern listed above: *Ava*I: A-7.1, 5.2, 4.7; B-11.8, 5.2; C-10.0, 5.2, 1.8; *Ava*II: A-9.0, 2.7, 2.2, 1.5, 0.9, 0.5; B-8.1, 4.2, 2.2, 0.9, 0.9, 0.5; C-9.0, 4.2, 2.2, 0.9, 0.5; D-9.0, 2.3, 2.0, 1.5, 0.9, 0.6, 0.5; *Bam*I II: A-7.0, 5.2, 3.1, 1.4; B-10.0, 3.2, 2.1, 1.4; *Bcl*I: A-5.1, 4.1, 3.1, 1.3, 1.3, 0.7, 0.6, 0.25, 0.25; B-5.1, 4.1, 3.1, 2.8, 0.9, 0.7; C-7.5, 5.1, 1.1; *Bgl*I: A-17.0, B-9.2, 7.1, 0.7; C-6.0, 2.5, 2.5, 2.0, 1.8, 1.1, 1.1; *Bgl*II: A-9.5, 7.5; B-9.5, 4.0, 3.5; C-17.0; *Eco*RI: A-3.3, 2.6, 2.5, 2.4, 1.9, 1.7, 1.0, 0.6, 0.5, 0.2; B-8.2, 2.6, 1.9, 1.7, 1.2, 0.6, 0.5; C-3.3, 2.6, 2.5, 2.3, 1.9, 1.7, 1.0, 0.6, 0.5, 0.3; D-7.7, 6.0, 1.9, 0.6, 0.5; *Hin*dIII: A-11.0, 6.0; B-17.0; C-9.0, 6.0, 2.0; *Nci*I: A-8.9, 6.3, 1.8; B-7.1, 6.1, 2.1, 1.7; C-7.1, 5.1, 2.1, 1.7, 1.0; D-8.0, 6.1, 2.0, 0.9; *Nco*I: A-10.0, 2.9, 2.0, 1.8; B-5.7, 5.0, 5.0, 1.0; C-10.0, 5.7, 1.0; D-5.0, 5.0, 2.9, 2.0, 1.8; *Pst*I: A-14.4, 2.5; B-10.3, 4.1, 2.5; *Pvu*II: A-12.0, 5.0; B-12.0, 4.5, 0.5; *Stu*I: A-8.3, 4.3, 1.7, 1.4, 1.2; B-9.9, 7.0.

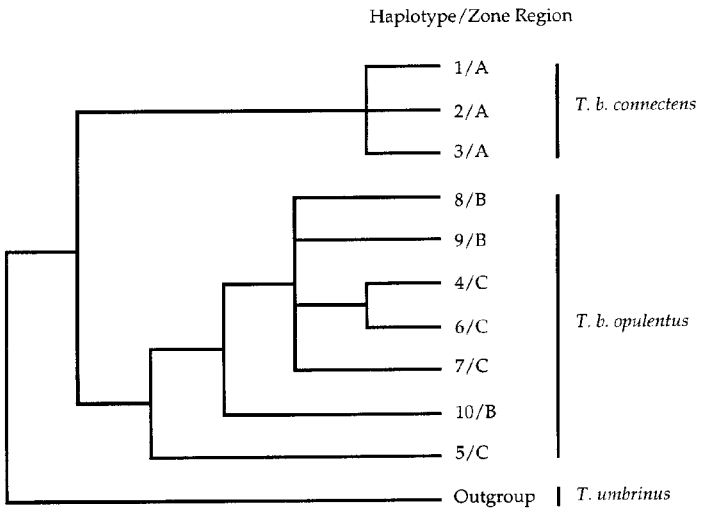

Fig. 2 Strict consensus tree of mitochondrial DNA haplotypes of *Thomomys bottae* and the outgroup, *T. umbrinus*, based on parsimony analysis of restriction-site data. Numbers refer to haplotypes (Table 1) and letters refer to zone regions on the map (Fig. 1).

(Patton *et al.* 1984) may have resulted from only one (or very few) nonmaternal transmission events. Falsification of the strict maternal-transmission hypothesis leaves two alternate hypotheses to explain louse transmission: lice may be transmitted predominantly, but not exclusively, from mother to offspring, or louse transmission may be altogether independent of pocket gopher genealogy. These mutually exclusive hypotheses are being tested in an ongoing microspatial analysis of pocket gopher genealogy in relation to resident populations of lice.

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This work is a portion of the doctoral research conducted by J. W. Demastes under the supervision of M. S. Hafner. Hafner's lab has been studying host–parasite coevolution since 1984. Current research includes parasite transmission and an in-depth study of cospeciation in the Mexican genera of pocket gophers, *Pappogeomys* and *Cratogeomys*, and their chewing lice.
