POPULATION GENETIC STUDY OF THE CHEWING LOUSE *GEOMYDOECUS EWINGI*

A Thesis

by

CAITLIN ELIZABETH NESSNER

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Chair of Committee,	Jessica Light
Committee Members,	Gary Voelker
	Gregory Sword
Head of Department,	Michael Masser

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ABSTRACT

Geomydoecus ewingi is a relatively well-known chewing louse that parasitizes the pocket gopher *Geomys breviceps* in the rodent family Geomyidae. Pocket gophers have been documented to exhibit long-term associations with their parasites, specifically lice. The flightless and obligate nature of the lice coupled with few opportunities to colonize new hosts has helped to make them model organisms for cospeciation studies. A main objective of my research was to determine the microevolutionary processes driving macroevolutionary patterns, such as cospeciation, in gopher-louse assemblages. Through the use microsatellite data, a series of population genetic analyses were conducted on lice parasitizing *G. breviceps* to better understand the population structure of lice among host individuals and across localities. With no previous microsatellite data available, I report 17 novel microsatellite loci in the parasitic chewing louse *G. ewingi*. Population genetic analyses infer significant structure among infrapopulations and potential inbreeding occurring within and among infrapopulations, possibly contributing to heterozygote deficiency and deviations from Hardy-Weinberg equilibrium. The microsatellite markers characterized in this study will be useful in future studies exploring the population dynamics in host-parasite systems, potentially yielding a better understanding of the processes underlying symbiotic associations.

DEDICATION

To adventurers, scientists, inquirers and seekers everywhere. “The true sign of intelligence is not knowledge but imagination.” –Albert Einstein

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CHAPTER I

INTRODUCTION

Much of earth's biodiversity is composed of parasitic organisms (Poulin and Morand 2004). Parasites display a huge diversity of not only life cycles but also lifestyles, varying in population sizes, dispersal abilities, modes of reproduction, and disease transmittance (Criscione et al. 2005). With this diversity, parasites offer the opportunity to investigate various questions about evolution. For example, because parasites depend upon their hosts for survival and often have complex ecological interactions with their hosts that persist over long evolutionary timescales (Hafner and Nadler 1990; Hafner and Page 1995), understanding how parasites and their hosts interact can yield highly significant information about these evolutionary interactions. These long-term interactions led to the hypothesis of Fahrenholz's Rule (Eichler 1948), which states that phylogenies of parasites generally will mirror that of their hosts. In the past, systematists often used Fahrenholz's rule as rationale for classifying parasites by reference to host associations and phylogenies (Brooks 1977; Brooks and Overstreet 1978; Demastes and Hafner 1993; Hafner et al. 2003). However, mirrored relationships between parasites and their host are not always true. Incidences of host switching, parasite speciation, or parasite extinction can occur within a lineage (Light and Hafner 2007; Garamszegi 2009; Johnson et al. 2011).

Lice (Insecta: Phthiraptera) are model organisms for testing Fahrenholz's rule and examining host-parasite relationships (Lyal 1986; Johnson and Clayton 2003a;

Taylor and Purvis 2003). Lice are small, wingless insects that are obligate and permanent parasites of birds and mammals. These parasites often are considered pests and are known to cause irritation, inflammation, pruritis, and dermatitis on their living hosts by feeding and crawling about the skin (Price and Graham 1997). Lice tend to exhibit high host specificity, often specialized and restricted to a particular host species (Price et al. 2003; Johnson et al. 2004). Furthermore, lice may show specificity regarding the body region of the host they prefer to infest (Johnson and Clayton 2003b). Formerly, two separate suborders, Mallophaga (chewing lice) and Anoplura (sucking lice), within the order Phthiraptera were recognized. These two groups are still informally recognized, but now lice are classified in four suborders: chewing louse suborders Amblycera, Ischnocera and Rhynchophthirina and the sucking louse suborder Anoplura.

Named for their mandibulate mouthparts, chewing lice feed mainly on feathers, dead skin, blood, or secretions. Chewing lice are hemimetabolous, exhibiting three nymphal stages prior to adult maturation (Marshall 1981). Females produce approximately 12-20 eggs (1 egg a day) in their reproductive lifetime (Price et al. 2003). Eggs require 4-10 days of incubation depending on species and some species produce eggs that are heavily sculptured or equipped with projections that facilitate attachment to the host (Marshall 1981). Each of the three nymphal stages requires 3-12 days for completion and each is successively larger than the previous stage (Marshall 1981). Adult chewing lice range in size from 0.8 to 11 mm with the tendency of females to be larger than males (Price et al. 2003). Utilizing sensory organs in their mouths as well as on their antennae, lice are attracted to the warmth and odor of their host by means of

chemosensory rather than visual cues. A few species of chewing lice have small eyes, which are probably little more than light sensors (Price et al. 2003) and the antennal sense organs of Ischnocera are more specialized than those of Amblycera (Clay 1970).

The chewing louse suborder Ischnocera includes two families: Philopteridae and Trichodectidae, with the latter parasitizing solely mammals and comprised of 19 genera and over 360 species (Price et al. 2003). Lice within Trichodectidae are morphologically adapted to live on their mammal hosts, with their body and head shape conducive to lying flat on the host. These chewing lice often exhibit a skewed sex ratio, with more females than males on a host (Price et al. 2003). The family Trichodectidae has been shown to be a monophyletic group, and the sister group to ishnocerans parasitizing birds (Blagoveshtchensky 1956; Smith 2001; Price et al. 2003). Relationships within Trichodectidae have been examined using morphological and molecular data. For example, Lyal (1985) analyzed 187 morphological characters for 351 species and subspecies of Trichodectidae. From these data, Lyal was able to construct the classification of species within Trichodectidae down to subgenus (Price et al. 2003). Molecular data, such as the elongation factor one alpha gene, support the monophyly of Trichodectidae as well as the sister relationship of the subfamily Bovicolinae to other Trichodectinae (Cruickshank et al. 2001). Chewing lice are often highly prevalent and abundant on their hosts (Nadler et al. 1990) and tend to exhibit high host-specificity and serve as model organisms in host-parasite studies. However, in some cases, chewing lice illustrate aggregated populations patterns that often conform to a negative binomial distribution (Clayton and Tompkins 1995; Lee and Clayton 1995; Rozsa et al. 1996;

Clayton et al. 1999; Johnson and Clayton 2003a) where few hosts have many lice and many hosts have few lice.

The trichodectid genera *Geomydoecus* and *Thomomydoecus* are relatively well-known lice that parasitize pocket gophers in the rodent family Geomyidae. Pocket gophers are highly modified morphologically for a fossorial, underground lifestyle (Sulentich et al. 1991). Morphological adaptations include being tubular shaped, having shortened forelimbs, increased muscle mass, and large, long claws at the anterior end of their bodies to loosen and break up the soil (Stein 2000) all resulting in their inability to move well outside of the burrow system. By building new tunnels and sealing those no longer in use, pocket gophers spend the majority of their lives underground (Sulentich et al. 1991). Due to the solitary, fossorial nature of pocket gophers, it is expected that their reduced dispersal capabilities can result in isolated populations with limited gene flow among populations especially across large distances (Hafner et al. 1983; Burt and Dowler 1999). In fact, asocial geomyid pocket gophers have been shown to have low effective rates of dispersal (Daly and Patton 1990)**.** As a result of low dispersal, the majority of pocket gopher species rarely encounter individuals of another species and often are parasitized by one species of chewing louse (Demastes and Hafner 1993), thus affording chewing lice few opportunities to colonize more than one species of pocket gopher host.

The Baird's pocket gopher (*Geomys breviceps*) is distributed across parts of Texas, Arkansas, Oklahoma, and Louisiana (Sulentich et al. 1991; Schmidly 2004). *G. breviceps* is parasitized by the louse species *Geomydoecus ewingi*, which is also found

on westernmost populations of *G. attwateri* (Demastes and Hafner 1993). Most previous studies examining *Geomys* and their lice have focused on louse distribution and alpha-level taxonomy (Price and Hellenthal 1980; Timm and Price 1980; Hellenthal and Price 1984; Nadler et al. 1990). At the louse species level, phylogenies have been constructed for *Geomydoecus* based on allozymes (Demastes and Hafner 1993; Hafner and Nadler 1990), mitochondrial cytochrome oxidase *c* subunit one sequences (Hafner et al. 1994), and morphology (Timm 1983).

Chewing lice complete their entire life cycle on the host and move between hosts primarily through direct host-to-host contact. Therefore, lice are confined to their hosts both in ecological and evolutionary time. By having limited intrinsic vagility, lice depend on interhost contact for dispersal (Timm 1983). Dispersal of chewing lice across multiple hosts is thought to occur only during direct contact between host individuals, as in mating encounters (horizontal transfer) or while rearing young (vertical transfer, Hafner and Nadler 1990). Thus, the behavior and population structure of pocket gophers should be major determinants of the genetic structure of their louse populations (Nadler et al. 1990). The patchy distribution of pocket gopher populations (Patton and Feder 1981) and the asocial nature of individuals within a population can potentially restrict opportunities for louse transfer between hosts (Nadler et al. 1990). Therefore, the biology of both pocket gophers and chewing lice has helped to make them model organisms for cospeciation studies and they can act as valuable informants of their host's evolutionary history.

While pocket gophers and their chewing lice have been examined extensively to try to better understand the evolution of host-parasite associations on a grand scale (i.e., broad macroevolutionary processes such as cospeciation, host switching, parasite extinction, or parasite speciation; Hafner and Nadler 1988; Demastes and Hafner 1993; Hafner et al. 2003; Light and Hafner 2008), there have been few attempts to explore the small scale (microevolutionary) processes that shape these associations (Nadler and Hafner 1989). Microevolutionary processes occurring within and among populations likely play a crucial role in parasite speciation and the establishment and maintenance of host-parasite associations (Criscione et al. 2005; Huyse et al. 2005). With the recent investigation of population genetics of the Baird's pocket gopher (*Geomys breviceps;* Welborn 2012; Welborn and Light in review), I have an unique opportunity to expand upon previous macroevolutionary research. For my thesis research, I will investigate population genetics of *Geomydoecus ewingi* (the chewing louse parasitizing *Geomys breviceps*) and the microevolutionary processes acting in this host-parasite assemblage.

CHAPTER II

CHARACTERIZATION OF 17 NOVEL POLYMORPHIC MICROSATELLITE LOCI IN THE MAMMAL CHEWING LOUSE *GEOMYDOECUS EWINGI* (INSECTA: PHTHIRAPTERA) FOR POPULATION GENETIC ANALYSES*

Lice (Insecta: Phthiraptera) are wingless insects that are obligate and permanent parasites of birds and mammals. Chewing lice belonging to the genus *Geomydoecus* (Ischnocera: Trichodectidae) are relatively well-known lice that parasitize only mammals, specifically pocket gophers in the rodent family Geomyidae. Pocket gophers are fossorial, spending the majority of their lives underground in elaborate burrow systems, rarely coming above ground (Sulentich et al. 1991). These rodents are highly modified morphologically for this fossorial lifestyle, having shortened, muscular forelimbs and large incisors and claws for digging (Stein 2000). Because of their conservative morphology and resulting limited dispersal ability, pocket gophers have been documented as having long-term associations with co-existing organisms, specifically lice (Hafner et al. 2003).

*Reprinted with permission from "Characterization of 17 novel polymorphic microsatellite loci in the mammal chewing louse *Geomydoecus ewingi* (Insecta: Phthripatera) for population genetic analyses" by C. E. Nessner, J. J. Andersen, M. A. Renshaw, M. M. Giresi, and J. E. Light, 2014. The Journal of Parasitology, Forthcoming, Copyright [2014] by Allen Press Publishing Services

Chewing lice can act as valuable informants of their host's evolutionary history because they complete their entire life cycle on the host and move between hosts primarily through direct host-to-host contact (Whiteman and Parker 2005; Nieberding and Olivieri 2007). Lice are therefore confined to their hosts both in ecological and evolutionary time and this, in addition to the biology of pocket gophers, has helped make lice and their pocket gopher hosts model organisms for cospeciation studies[2] (Hafner and Nadler 1988; Demastes and Hafner 1993; Hafner et al. 1994; Hafner et al. 2003; Light and Hafner 2008; Demastes et al. 2012). Despite extensive research, however, there have been few attempts to explore the microevolutionary processes occurring within and among parasite populations which likely play a crucial role in parasite speciation and the establishment and maintenance of host-parasite associations (Criscione et al. 2005; Huyse et al. 2005).

A main objective of this research was to identify useful genetic markers and examine population structure in the chewing louse *G. ewingi*. Although several mitochondrial genes have successfully been used to examine cospeciation, and are commonly used markers for many louse molecular studies, mitochondrial genes do not evolve quickly enough in lice to be informative at the population level (Ascunce et al. 2013; Nessner unpubl. data). Rapidly evolving markers such as microsatellites are more appropriate to address population level questions such as estimation of inbreeding, migration, relatedness, parentage, effective population size, and population assignment, among others (Criscione et al. 2007; Ascunce et al. 2013). Although there have been

several recent studies identifying microsatellite markers in lice (Leo et al. 2005; McMeniman and Barker 2006; Peters et al. 2009a; Peters et al. 2009b; Scholl et al. 2012; Ascunce et al. 2013), there are no known reports identifying variable microsatellite loci from mammalian chewing lice. Thus, designing microsatellite loci for pocket gopher chewing lice may provide markers that can be used to gain a better understanding of population dynamics in these host-parasite assemblages. Herein, we describe microsatellite loci for the chewing louse *Geomydoecus ewingi*, a parasite of the Baird's pocket gopher (*Geomys breviceps*), and examine these loci for their utility in a population genetic context.

The protocol outlined in Welborn et al. (2012) was used to develop the enriched genomic microsatellite library for *Geomydoecus ewingi*. Genomic DNA was isolated from a pooled sample of 50 individuals of *G. ewingi* using the DNeasy Blood and Tissue Kit (QIAGEN Inc.; Valencia, California). DNA fragments were hybridized with biotin-modified di-, tri- and tetra-oligonucleotides, incubated with streptavin-coated magnetic M-280 Dynabeads (Invitrogen), and rinsed. The quantity of this enriched DNA was increased via polymerase chain reaction (PCR) amplification and cleaned with a PCR purification kit (QIAGEN Inc.; Valencia, California). Cleaned products were ligated into PCR 2.1 TOPO vectors (Invitrogen) and transformed in *Escherichia coli* (One Shot TOP10 Chemically Competent Cells, Invitrogen). Cells were dispersed onto X-Gal/LB/Agar plates treated with ampicillin and incubated overnight at 37°C. Positive clones (white) were sent to the University of Florida Interdisciplinary Center for

Biotechnology Research Genomics Division (Gainesville, Florida) for sequencing with the M13 forward primer.

Sequences were edited using SEQUENCHER 4.1 (Gene Codes) and screened for microsatellites. Primer sequences for unique microsatellite loci initially identified by the software package PHOBOS (Mayer 2006, www.rub.de/spezzoo/cm/cm_phobos.htm) were developed using Primer3 (http://frodo.wi.mit.edu/primer3/), and these loci were tested for amplification and polymorphisms across 16 louse individuals. Genomic DNA was extracted from individual lice using the DNAeasy Tissue Kit (QIAGEN Inc.; Valencia, California). The abdomen of each louse was punctured and lacerated with a sterile insect pin prior to DNA extraction. The cleaved bodies were individually incubated in an ATL lysis buffer overnight with extraction processes continuing the following day, after which the exoskeletons of the lice were removed for slide mounting as voucher specimens (Cruickshank et al. 2001). Manufacturer's recommendations were followed for the remainder of the extraction process except that the total DNA elution volume was 60 µL. PCR amplifications of microsatellites followed Karlsson et al. (2008) and were performed in 10 µL reactions containing 3.7 µL Emerald Master Mix (Takara Bio Inc.), 4.25 µL water, 0.05 µL forward primer (10 µM) with an additional 0.5 µL fluorescently-labeled tail primer (6-FAM; 5'-GCCTCGTTTATCAGATGTGGA-3'; 10 µM), 0.5uL reverse primer (10 µM), and 1 µL DNA. Amplified PCR products were multiplexed when possible (loci grouped according to allele size), combined with 400 HD Rox size-standard DNA ladder (Applied Biosystems), loaded on a polyacrylamide gel, and electrophoresed on an ABI PRISM 377 DNA Sequencher (Applied

Biosystems). Sizes of microsatellite fragments were visualized in GENESCAN v. 3.1.2 (Applied Biosystems) and assessed using GENOTYPER v. 2.5 (Applied Biosystems). In total, 17 loci amplified successfully and were polymorphic (Table 1).

To assess variability of the 17 polymorphic loci, five *Geomys breviceps* pocket gophers (where each pocket gopher is an infrapopulation) and their lice were collected from within 0.5 km of each other from one locality in Brazos County, Texas (Texas A&M University's Riverside Campus, a 1,900-acre campus adjacent to State Highway 47 and Highway 21, west of Bryan, Texas). Pocket gophers collected for this study were

Table 1. Microsatellite marker information and measures of genetic diversity for 36 genotyped louse individuals, *Geomydoecus ewingi*, from one geographic locality in Brazos County, Texas.

Locus	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Repeat motif	N	NA	Range	H_E	H_O	HWE	Average FIS
Gew35	TTCACGCTTTTGCATCACAT	GGAATGGAAGTTACGACTACGC	$(CA)_{18}$	36	10	161-181	0.782	0.694	0.026	0.113
Gew39†	GGGAGGAGTGAAAAATAGAAAGC	TTCCGAAGGAACGTTACAGG	$(CA)_{11}$	36	10	203-225	0.822	0.556	0.002*	0.327
Gew40¥	GGTTTATGACACCGGTCACG	TCGACGACTTACTGGGTTGG	$(GA)_7$	36	11	256-300	0.847	0.778	0.116	0.083
Gew41¥†	TGGGCATTGCTAAGAAGTCC	TCAGTTCATTTGATGTTTTGTCG	$(AG)_{11}$	36	6	178-190	0.719	0.250	0.000*	0.656
Gew43†	TTCGATTCTTTCGCGTTTCT	GCAATTCGATCGTTTATTTCG	$(CAT)_6$	36	4	238-247	0.583	0.250	0.000*	0.575
Gew44	TTCTCACTCGAAAAATTTAATGC	TGTTGTTTTGCCAACGGTTA	$(TC)_{13}$	36	8	200-222	0.813	0.694	0.001*	0.147
Gew47†	ACCACAAGGGGATTTTCTGG	TCACAGCCTCATTTTCTACGG	$(GA)_{10}$	36	8	256-274	0.802	0.500	0.000*	0.380
Gew51†	AGCCAAACCCAGATTTACCG	TTTAAATTCCCCTCCCTAACG	$(CA)_{10}$	36	8	169-185	0.819	0.639	0.000*	0.223
Gew52	GTTTGCTGTTGCCATTTCG	AAAGGAAGCAGAGACTGAATGC	$(CTT)_5$	36	3	193-199	0.505	0.389	0.054	0.233
Gew54†	GGTCGAAGGAATTTAAACATAAGC	GCGTCTGAAGTGAAGATTTACG	$(CT)_7$	36	14	244-284	0.900	0.722	0.007	0.200
Gew55	AAGCGGCAGATAAATTAAAGACC	CATTCCCGTTTAACCATTTCC	$(GAAT)_5$	34	5	163-183	0.633	0.500	0.443	0.213
Gew56	GGAACCGATTGTAATGAGACC	GTTTTCGCTAACAGGACTCG	$(ATTT)_4$	36	4	240-252	0.563	0.583	0.881	-0.037
Gew57	AATTCGCCTCAGGTTGAGC	TCGGCAAAGATGGTAAAACC	$(TCTT)_5$	35	4	224-236	0.674	0.556	0.173	0.178
Gew58†	CAATTTTTCCTCGCCTCTCC	GACAGGAAAAGATGCGAAGC	$(TCA)_6$	36	5	201-213	0.622	0.278	0.000*	0.557
Gew59	CGATTCTCTTTTTCTTTTTACTTCTGG	AAAAAGCCGAGAAAAACTGG	$(ATC)_9$	35	6	259-277	0.709	0.629	0.844	0.115
Gew60	AGTTCCGTGCAACTCATGG	GGACAAATTCCGCAAAAAGG	$(ATC)_{10}$	34	6	207-228	0.758	0.629	0.283	0.173
Gew62†	CCGGGATGATGTTTAACTCC	TTCAAGCCTTCATTTTCACG	$(CAT)_{17}$	36	9	172-208	0.830	0.389	0.000*	0.535

*significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction (Rice 1989)
¥ indicates linkage between loci
†indicates potential null alleles at loci as indicated by Micro-Checker
N Number of individuals with data for each locus
NA Number of alleles expressed for each locus
H_E expected heterozygosity
H_O observed heterozygosity
HWE probability of conforming to Hardy-Weinberg equilibrium

treated humanely according to the guidelines of the Texas A&M University Animal Care and Use Committee and the American Society of Mammalogists (Sikes et al. 2011). Specimen voucher information for hosts and lice is available from Texas A&M University's Biodiversity Research and Teaching Collections. PCRs for the 17 polymorphic loci were performed on a total of 36 *Geomydoecus ewingi* individuals (1-11 lice per pocket gopher host) as described above. Arlequin v. 3.5 welborn(Excoffier and Lischer 2010) was used to calculate the expected heterozygosity (H_E), observed heterozygosity (H_O), and probability of conformance to Hardy-Weinberg equilibrium (HWE) at each locus. The 17 microsatellite loci were highly variable, with the number of alleles per locus ranging from 3 to 14 and H_E ranging from 0.25 to 0.78 (Table 1). The 36 louse individuals showed significant deviation from HWE. Of the 17 variable loci, eight fell outside of Hardy-Weinberg expectations after Bonferroni correction: Gew39, Gew41, Gew43, Gew44, Gew47, Gew51, Gew58, and Gew62 (Table 1). When lice were analyzed separately by infrapopulation (3 sets of 11 lice each from a different pocket gopher host; the remaining 3 lice were distributed over 2 pocket gophers and were not analyzed due to small sample size), number of alleles per locus, H_E, and H_O were similar, ranging from 2 to 11, 0.32 to 0.94, and 0.0 to 0.91, respectively (values per host are available upon request). Importantly, the number of loci deviating from HWE was significantly reduced (1-3 loci per infrapopulation), indicating the possibility that the global data set of 36 lice consisted of genetically distinct groups of lice that were artificially grouped together, resulting in a Wahlund effect (Nadler et al. 1990; Selkoe and Toonen 2006).

Micro-Checker (Van Oosterhout et al. 2004) was used to test for null alleles. The presence of null alleles is indicated if the combined probability test shows there is an overall significant excess of homozygotes, and when this excess is evenly distributed across the homozygote-classes (Van Oosterhout et al. 2004). Possible null alleles were detected at eight loci: Gew39, Gew41, Gew43, Gew47, Gew51, Gew54, Gew58, and Gew62 (Table 1). Five percent of the PCRs were repeated, and no inconsistent results were found. When lice were analyzed separately by infrapopulation (3 sets of 11 lice each from a different pocket gopher host), the number of possible null alleles dropped drastically to four loci. We believe that null alleles are unlikely due to the fact that all of these microsatellite loci successfully amplified and were polymorphic in *Geomydoecus subgeomydis*, a close relative of *G. ewingi* and ca. 12% genetically divergent (uncorrected *p* distance for the mitochondrial cytochrome oxidase *c* subunit I gene; Nessner, unpub. data). Rather, it is likely that loci deviating from HWE are exhibiting heterozygote deficiencies, corresponding to the biological reality of this population (and possibly the species) and Micro-Checker results are detecting existing population characteristics. Such results would be consistent with the extremely low dispersal abilities of *G. ewingi*. In fact, several recent population genetics studies of lice and other ectoparasites have reported similar findings (Dharmarajan et al. 2011; Veracx et al. 2012; Ascunce et al. 2013), supporting the commonness of heterozygote deficiencies in these organisms.

Genotypic linkage disequilibrium (LD) was measured between all pairs of loci using GENEPOP v. 2.5 (Rousset 2008; Markov chain parameters: 1,000 dememoriations;

1,000 batches; 1,000 iterations) and a sequential Bonferroni method to correct for multiple tests (Rice 1989). LD was detected between loci Gew40 and Gew41 after Bonferroni corrections (Table 1). The p-value for these loci, which was less than 0.0001, denotes the possibility of linkage.

An Analysis of Molecular Variance (AMOVA) was performed in Arlequin v. 3.5 (Excoffier and Lischer. 2010) to evaluate overall population structure. Results from the AMOVA analysis across the geographic locality including all sampled lice (from all pocket gopher hosts) and loci indicate significant variation among individuals (F_{IS} = 0.273; $P < 0.001$) and that the greatest source of genetic differentiation was observed within individuals (F_{IT}) at 72.7%. The reported positive values of F_{IS}, with an average of 0.239 across loci (Table 1), are consistent with the homozygote excess observed in HWE analyses. With the exception of Gew56, all loci are characterized by homozygote excess, and 10 of these comparisons were statistically significant (Table 1).

When AMOVA analyses were performed defining the lice from each pocket gopher host as a separate infrapopulation (11 lice from each of 3 pocket gophers, see above), results showed significant variation among individuals (F_{IS} = 0.24; $P < 0.001$), as well as among infrapopulations (F_{ST} = 0.0672; $P < 0.001$). Similar to the results of the global results presented above, all loci except Gew56 showed positive F_{IS} values (homozygote excess) and 10 were significant. Only six loci (Gew40, Gew41, Gew54, Gew59, Gew 60, and Gew62) showed significant population structure (F_{ST} P values < 0.05). These results indicate not only significant homozygote excess and some level of inbreeding, but also significant structure among infrapopulations.

Internal relatedness (IR) and individual homozygosity weighted by locus (HL) were calculated with the IR macro (Amos et al. 2001) to determine level of louse inbreeding, where high positive values indicate inbreeding and negative values indicate a highly outbred ancestry (Amos et al. 2001; Aparicio et al. 2006). IR and HL were calculated across the entire geographic sample to allow comparisons of individuals from different hosts or infrapopulations. IR and HL values were relatively high, ranging from 0.075 to 0.659 (IR) and 0.212 to 0.770 (HL), suggesting some level of inbreeding. One louse individual presented a negative IR value, which could be due to it being a single louse sampled from one pocket gopher host. Additionally, IR and HL were calculated defining the lice from each pocket gopher host as a separate population (11 lice from each of 3 pocket gophers). IR and HL were again positive, ranging from 0.009 to 0.678 (IR) and 0.291 to 0.768 (HL). These results indicate that there are high levels of inbreeding among pocket gopher lice from this geographic locality.

Biological aspects of a parasite may lead to deviations from Hardy-Weinberg expectations (Criscione et al. 2005; Criscione 2008; Dharmarajan et al. 2011). A variety of studies have shown heterozygote deficiencies attributing to deviations of genotypic frequencies are not uncommon for parasite populations (Nadler et al. 1990; Plantard and Porte 2004; Leo et al. 2005; Criscione et al. 2007; Guzinski et al. 2009; Dharmarajan et al. 2010; Kempf et al. 2010; Dharmarajan et al. 2011; Veracx et al. 2012; Ascunce et al. 2013). The majority of the analyses reported here further support the commonality of heterozygote deficiency in lice, which may be the result of high levels of louse inbreeding.

Heterozygote deficiency could also occur in louse populations that were recently founded by a small number of lice (Nadler et al. 1990; Nadler 1995; Leo et al. 2005). Given the low probability of opportunities for lice to colonize new hosts, the homozygote excess observed in this study is likely the result of nonrandom mating within hosts, inbreeding, and/or subdivision between parasites from different hosts (i.e., Wahlund effect). Based upon the combined biological characteristics of pocket gophers (solitary lifestyle) and chewing lice (low vagility), it may be expected that the potential for louse colonization of new hosts is limited (Nadler et al. 1990; Hafner and Page 1995; Demastes et al. 2012) and non-random mating may be occurring within this louse species increasing the probability of inbreeding. Further investigation is needed to evaluate genetic processes, such as inbreeding and non-random mating, occurring within and among *Geomydoecus ewingi* in this geographic locality.

CHAPTER III

POPULATION GENETICS OF *GEOMYDOECUS EWINGI* ACROSS POCKET GOPHER HOSTS AND GEOGRAPHIC LOCALITIES

Introduction

In a recent population genetics study, Welborn (2012; Welborn and Light in review) illustrated a general relationship between geographic distance and level of gene flow in the Baird's pocket gopher (*Geomys breviceps*) in eastern Texas. She found that as the distance among localities increased, levels of gene flow among pocket gophers decreased. However, for both mitochondrial and microsatellite data, there were high levels of gene flow among three geographic localities located within 2 km of each other (Highway 47 North at 30°38.132 N 96°26.859 W, Highway 47 South at 30°38.092 N 96°26.885 W and Riverside Campus at 30°38.453 N 96°27.722 W), indicating that these localities act as one functioning *G. breviceps* population, or genetic cluster (Welborn 2012; Welborn and Light in review). The high levels of pocket gopher gene flow suggest that the highways dividing these localities do not hinder pocket gopher movement and that these fossorial rodents must be dispersing above ground for at least small distances (Welborn 2012; Welborn and Light in review).

The pocket gopher species examined by Welborn (2012), *Geomys breviceps*, is the host for the chewing louse *Geomydoecus ewingi*, the parasite under investigation in the current research. Thus, Welborn's (2012; Welborn and Light in review) findings serve as a frame a reference for expectations of parasite population genetics. Nessner et al. (accepted manuscript) described microsatellite loci for *Geomydoecus ewingi*, and examined these loci for their utility in a population genetic context (see Chapter II). Samples for this study came from one of Welborn's (2012; Welborn and Light in review) localities, Riverside Campus. With these microsatellite loci characterized, chewing louse population genetics across a larger geographic region can now be examined. With the presence of host gene flow observed among hosts across the Highway 47 North, Highway 47 South, and Riverside Campus localities (2012; Welborn and Light in review), further investigation of louse population genetics at all three of these localities will potentially yield a better understanding of the microevolutionary processes acting in this symbiotic association.

Materials and Methods

Sampling— Host specimens, *G. breviceps*, were collected from three localities in the Brazos Valley, Texas, as part of a previous study (Welborn 2012; Welborn and Light in review; Figure 1). These three localities (Highway 47 North, Highway 47 South, and Riverside Campus) were located within two kilometers of each other (Highway 47 North and Highway 47 South are separated by ~0.750km; Highway 47 South and Riverside Campus, ~1.6km; Highway 47 North and Riverside Campus, ~1.850km), facilitating a reasonable geographic area to assess louse (*Geomydoecus ewingi*) population genetics among host individuals and geographic localities. Ten pocket gophers and their associated ectoparasites were collected according to procedures approved by the Texas A&M University Animal Care and Use Committee and the American Society of Mammalogists (Sikes et al. 2011) and deposited in the Biodiversity Research and Teaching Collections (BRTC) at Texas A&M University. In total, 19 pocket gophers (seven from Highway 47 North, seven from Highway 47 South, and five from Riverside Campus) and 110 lice (1-11 per host, see Appendix I) were sampled.

Figure 1. Sampling localities of *Geomys breviceps*, indicated by symbols, within Brazos County, Texas. The Highway 47 localities are separated by a small section of highway. Exact locality information is available in Appendix 1.

Laboratory Methods—Genomic DNA was isolated from each louse using the DNeasy Blood and Tissue Kit (Qiagen) and louse-specific protocols (Cruickshank et al. 2001; Johnson and Clayton 2003b). Lice were screened for each of the 17 polymorphic loci identified in Chapter II and Nessner et al. (accepted) using polymerase chain reactions (PCR). PCR amplifications followed Karlsson et al. (2008) and contained a forward primer with an attached 16-bp tail sequence (5‘CAGTCGGGCGTCATCA-3‘), a 6-FAM, 6-HEX, or 6-NED (Dye Set D, Applied Biosystems) labeled tail sequence (defined above), and a reverse primer (Table 1 in Chapter II). Amplified DNA from each PCR reaction was combined with a 400 HD Rox size-standard DNA ladder (Applied Biosystems) loaded on a polyacrylamide gel, and electrophoresed on an ABI PRISM 377

DNA Sequencer (Applied Biosystems; Biosystematics Center, College Station, Texas). PCR products were electrophoresed on a polyacrylamide gel for 2.5 hours to separate and visualize amplification products. Sizes of microsatellite fragments were visualized and allelic identity values of the microsatellites were scored in GENESCAN v. 3.1.2 (Applied Biosystems) and assessed using GENOTYPER v. 2.5 (Applied Biosystems). Microsatellite allele scores also were confirmed by eye.

Data Analysis—Genotype data were organized by louse specimen and host number for each geographic locality, and data input files were generated with the program Convert v. 1.31 (Glaubitz 2004). Each microsatellite locus was tested for conformance to Hardy-Weinberg equilibrium, using Arlequin v. 3.5 (Excoffier and Lischer 2010). Arlequin also was used to calculate the expected heterozygosity (H_E) and observed heterozygosity (H_O) for all individuals within a geographic locality, all infrapopulations (i.e., host individuals) within a geographic locality, and all infrapopulations across geographic localities. These scales of analyses were repeated throughout this study. For analyses involving infrapopulations, only hosts with large louse sample sizes of at least 10 individuals were examined. Therefore, infrapopulation analyses included three pocket gophers, each with 10-11 lice, per geographic locality (Appendix I).

Micro-Checker (Van Oosterhout et al. 2004) was used to detect the presence of any possible null alleles due to homozygote excess or allele dropout and stutter (Van Oosterhout et al. 2004). The program indicates the possible presence of null alleles if the combined probability test shows an overall significant excess of homozygotes and if this

excess is evenly distributed across the homozygote-classes (Van Oosterhout et al. 2004). Loci were analyzed by geographic locality as well as by infrapopulations within geographic localities.

Genotypic linkage disequilibrium (LD) was measured between all pairs of loci within each geographic locality using GENEPOP v. 4.0 (Raymond and Rousset 1995; Rousset 2008), Markov chain parameters of 1,000 dememoriations, 1,000 batches, and 1,000 iterations and a sequential Bonferroni method to correct for multiple tests (Rice 1989).

F statistics were calculated for each geographic locality as well as for infrapopulations within and across geographic localities using Arlequin v. 3.5 (Excoffier and Lischer 2010) with 10,000 Markov-chain steps. Population structure was also assessed by an analysis of molecular variance (AMOVA; Excoffier and Lischer 2010). An initial AMOVA analysis was run with an *a priori* viewpoint of either nine populations (when a population is defined as an infrapopulation across all three geographic localities; recall that there are three infrapopulations per geographic locality with sufficient sampling) or as three populations (when a population is defined as a geographic locality). AMOVA analyses also were run across the geographic localities, including all sampled lice (from all pocket gopher hosts). For all analyses, significance was determined using 10,000 randomization replicates.

The patterns of population clustering among *G. ewingi* individuals was obtained using the Bayesian-inference based program STRUCTURE (Pritchard et al. 2000) to detect separate clusters of genotypic variation of *G. ewingi* across each geographic locality. All

lice within each geographic locality were assessed together with the population admixture model and with 10 runs from $K = 1$ to $K = 5$ where K is a user-defined number of clusters. Each run consisted of a burn-in of 10,000 Markov chain-Monte Carlo repetitions followed by 100,000 additional repetitions (Evanno et al. 2005). An additional analysis was performed within and across geographic localities with three infrapopulations from each locality (for a total of nine infrapopulations and 10-11 louse individuals per infrapopulation). The population admixture model was used with 10 runs from $K = 1$ to $K = 10$, and each run consisted of a burn-in of 10,000 Markov chain-Monte Carlo repetitions followed by 100,000 additional repetitions. STRUCTURE HARVESTER v 0.6 (Earl and VonHoldt 2012) was used to determine the ΔK, mean ln Prob(Data) (Evanno et al. 2005), and the most likely number of clusters (K) for each separate analysis.

GENELAND v3.3.0 (Guillot et al. 2005) was used to detect population spatial structure using a Bayesian Monte Carlo Markov Chains method within each geographic locality. GENELAND uses individual multilocus genetic data of geographically referenced individuals for assessment at each geographic locality. All lice were evaluated together to estimate the number of distinct clusters and delineate their spatial organization. GENELAND was run as a GUI add-on in R (R Development Core Team 2011) using 1,000,000 iterations with a thinning of 100 on a true spatial model and the population minimum set to one and the maximum to 19. Burnin was set at 20% for 2,000 iterations and a map of posterior probabilities was obtained.

Internal relatedness (IR) and individual homozygosity weighted by locus (HL) were calculated with the IR macro (Amos et al. 2001) to determine level of louse inbreeding, where high positive values indicate inbreeding and negative values indicate a highly outbred ancestry (Amos et al. 2001; Aparicio et al. 2006). IR and HL were calculated within each geographic locality including all individuals within each locality to allow comparisons of individuals from different hosts or infrapopulations. Additionally, IR and HL were calculated for each infrapopulation within a geographic locality.

COLONY v2.0 (Wang 2004; Wang and Santure 2009) was used to reconstruct individual louse relationships within an infrapopulation. COLONY implements a maximum likelihood method to provide a posterior probability for each relationship assignment. COLONY allows for two generation sampling to infer any possible parentage relationships as well as sib-ships. Parameters were set with a polygamous mating system, diploid species, full likelihood analysis, possible inbreeding, and no sibling prior. Marker type and error rate for loci, offspring genotypes, and candidate male and female data for individuals were evaluated by infrapopulation for relationships within geographic localities. The probability of any relationship greater than 50% was plotted via the sibling dyad output.

Results

Arlequin results indicate the 17 microsatellite loci were highly variable, with the number of alleles per locus ranging from 3 to 15 (Highway 47 North) and 3 to 14 (Highway 47 South), H_E ranging from 0.39 to 0.91 (Highway 47 North) and 0.39 to 0.90 (Highway 47 South), and H_O ranging from 0.25 to 0.78 (Highway 47 North) and 0.32 to 0.79 (Highway 47 South). Louse individuals showed significant deviation from Hardy-Weinberg expectations (HWE) at both geographic localities. Eleven (Gew35, Gew39, Gew41, Gew43, Gew44, Gew47, Gew51, Gew54, Gew57, Gew58, and Gew62) and five loci (Gew39, Gew41, Gew54, Gew56, and Gew58) deviated from HWE after Bonferroni corrections at the Highway 47 North and Highway 47 South localities, respectively. These results are similar to those for Riverside Campus (Chapter II; Nessner et al. accepted) with the number of alleles per locus ranging from 3 to 14, H_O ranging from 0.25 to 0.78, H_E ranging from 0.51 to 0.90, and eight loci (Gew39, Gew41, Gew43, Gew44, Gew47, Gew51, Gew58, and Gew62) falling outside of HWE after Bonferroni correction.

When lice were analyzed separately by infrapopulation, number of alleles per locus, H_E, and H_O were similar, ranging from 2 to 11, 0.32 to 0.94, and 0.0 to 0.91 respectively, for both Highway 47 North and Highway 47 South. Additionally, the number of loci deviating from HWE was significantly reduced (1-2 loci per infrapopulation). Again, these values are similar to those reported in Chapter II and Nessner et al. (accepted) for the third geographic locality (Riverside Campus) with the number of alleles per locus, H_E, and H_O ranging from 2 to 11, 0.32 to 0.94, and 0.0 to

0.91 respectively. The number of loci deviating from HWE was also significantly reduced (1-3 loci per infrapopulation) at Riverside Campus.

Micro-Checker detected possible null alleles at 12 and 11 loci from Highway 47 North and Highway 47 South, respectively (Table 2). Five percent of the PCRs were repeated, and no inconsistent results were found. When Micro-Checker was used to analyze loci by infrapopulation, the number of loci with possible null alleles was significantly reduced, varying from 2 to 4 per infrapopulation (Table 2).

Although GENEPOP detected genotypic linkage disequilibrium (LD) between loci Gew40 and Gew41 after Bonferroni correction at the Riverside Campus locality ($P < 0.0001$; Chapter II), LD was not detected among loci at either the Highway 47 North or Highway 47 South localities.

Results of the AMOVA analyses for each geographic locality were similar to those reported for Riverside Campus (Chapter II; Nessner et al. accepted). Results from the Highway 47 North AMOVA analysis across the geographic locality including all sampled lice and loci indicated significant variation among individuals ($F_{IS} = 0.28$; $P < 0.001$) and that the greatest source of genetic differentiation was observed within individuals (F_{IT}) at 71.9% (Table 3). All loci were characterized by homozygote excess and 12 of these comparisons were statistically significant (Gew40, Gew41, Gew43, Gew44, Gew 47, Gew51, Gew54, Gew56, Gew57, Gew58, Gew60, and Gew62). Highway 47 South AMOVA results also indicated significant variation among individuals ($F_{IS} = 0.21$; $P < 0.001$) and that the greatest source of genetic differentiation was observed within individuals (F_{IT}) at 78.5% (Table 3). All loci were characterized by homozygote excess

and 11 of these comparisons were statistically significant (Gew39, Gew40, Gew41, Gew43, Gew47, Gew51, Gew52, Gew54, Gew58, Gew60 and Gew62).

AMOVA analyses defining lice from each pocket gopher host as a separate population (i.e, infrapopulation) showed significant variation among individuals as well as among infrapopualtions for Highway 47 North and Highway 47 South, respectively (Table 4). For Highway 47 North, all loci showed positive F_{IS} values (homozygote excess) and 12 were significant (Gew40, Gew41, Gew43, Gew44, Gew47, Gew51, Gew54, Gew56, Gew57, Gew58, Gew60 and Gew62). Three loci showed significant population structure (Gew35, Gew55, Gew62, F_{ST} *P* values < 0.05), and four loci showed negative F_{ST} values (Gew41, Gew56, Gew57 and Gew58 where negative values are a function of sampling bias correction in AMOVA calculations). For Highway 47 South, all loci except Gew35 showed positive F_{IS} values and eight were significant (Gew39, Gew40, Gew41, Gew43, Gew47, Gew52, Gew58 and Gew62). Only one locus showed significant population structure (Gew51, F_{ST} *P* values < 0.05), and four loci showed negative F_{ST} values (Gew41, Gew58, Gew59 and Gew62). For both Highway 47 North and Highway 47 South, the greatest source of genetic differentiation was observed within individuals (F_{IT}) at 68.6% and 77.4%, respectively (Table 4). Results were again similar to the findings for Riverside Campus (Chapter II; Nessner et al. accepted; Table 4

Table 2. Micro-Checker results indicating null alleles across loci. See Appendix 1 for infrapopulation definitions. Riverside Campus results are also reported in Chapter II and Nessner et al (accepted).

Geographic Locality	Infrapopulation	Null Alleles Indicated at Loci
Highway 47 North	Gew40, Gew41, Gew43, Gew44, Gew47, Gew51, Gew54, Gew56, Gew57, Gew58, Gew60, Gew62	
	9	Gew51, Gew56, Gew58, Gew62
	10	Gew44, Gew47, Gew58
	1547	Gew39, Gew41
Highway 47 South	Gew39, Gew40, Gew41, Gew43, Gew47, Gew51, Gew52, Gew54, Gew56, Gew58, Gew62	
	5	Gew39, Gew41
	1550	Gew40, Gew47, Gew62
	297	Gew44, Gew47, Gew58
Riverside Campus	Gew39, Gew41, Gew43, Gew47, Gew51, Gew54, Gew58, Gew62	
	7	Gew41, Gew43, Gew47, Gew62
	8	Gew41, Gew43, Gew47, Gew62
	13	Gew41, Gew43, Gew47, Gew62

Table 3. AMOVA results for three *Geomydoecus ewingi* geographic localities (Highway 47 North, Highway 47 South, and Riverside Campus) using microsatellite data (see text for explanation of locality assignment). Significance of variance component (*P*) was tested by permutation according to Excoffier and Lischer 2010. Results for Riverside Campus were obtained from Chapter II and Nessner et al. (accepted).

Source of Variation	Variance Components	% of Variation	Fixation Index	*P* value
Highway 47 North				
Within Individuals	4.056	71.85		
Among Individuals	1.589	28.15	$F_{IS} = 0.282$	$P < 0.0001$
Highway 47 South				
Within Individuals	4.821	78.529		
Among Individuals	1.318	21.470	$F_{IS} = 0.215$	$P < 0.0001$
Riverside Campus				
Within Individuals	4.517	72.683		
Among Individuals	1.698	27.317	$F_{IS} = 0.273$	$P < 0.0001$

Table 4. AMOVA results for *Geomydoecus ewingi* infrapopulations at each geographic locality (Highway 47 North, Highway 47 South, and Riverside Campus) using microsatellite data (see text for explanation of locality assignment). Significance of variance component (*P*) was tested by permutation according to Excoffier and Lischer 2010. Results for Riverside Campus were obtained from Chapter II and Nessner et al. (accepted).

Source of Variation	Variance Components	% of Variation	Fixation Index	*P* value
Highway 47 North				
Within Individuals	4.173	68.581	$F_{IT} = 0.314$	$P < 0.0001$
Among Individuals within Populations	1.591	26.147	$F_{IS} = 0.276$	$P < 0.0001$
Among Populations	0.321	5.271	$F_{ST} = 0.053$	$P < 0.0001$
Highway 47 South				
Within Individuals	4.805	77.407	$F_{IT} = 0.226$	$P < 0.0001$
Among Individuals within Populations	1.186	19.105	$F_{IS} = 0.198$	$P < 0.0001$
Among Populations	0.216	3.488	$F_{ST} = 0.035$	$P < 0.0001$
Riverside Campus				
Within Individuals	4.466	70.890	$F_{IT} = 0.291$	$P < 0.0001$
Among Individuals within Populations	1.412	22.392	$F_{IS} = 0.240$	$P < 0.0001$
Among Populations	0.423	6.718	$F_{ST} = 0.067$	$P < 0.0001$

A *K* of 2 was determined to be the most likely set of genetic clusters for both the assessments across the three geographic localities and the nine infrapopulations in STRUCTURE and STRUCTURE HARVESTER with a Δ ln Prob(Data) = 336.266 and Δ ln Prob(Data) = 258.164, respectively. Highway 47 North and Highway 47 South were clustered together to form one defined group (Figures 2 and 3) with Riverside Campus being a separate clustered group. A *K* of 3 was also likely (Figures 4 and 5) for both the geographic locality and infrapopulation assessment, but did not score as highly as 2 clusters with Δ ln Prob(Data) = 1.575 and 2.339.

GENELAND detected the presence of two moderately differentiated clusters for a $K = 2$ (Figure 6). When the probability of $K = 2$ is mapped with the GPS coordinates for each locality, Highway 47 North and Highway 47 South are grouped together as one genetically distinct unit to the exclusion of Riverside Campus separate. The clusters show moderate to high genetic differentiation across the three geographic localities for both F_{IS} (Highway 47 North $F_{IS} = 0.303$; Highway 47 South $F_{IS} = 0.282$; Riverside Campus $F_{IS} = 0.242$) and F_{ST} (Highway 47 North and South $F_{ST} = 0.085$; Highway 47 North and Riverside Campus $F_{ST} = 0.013$; Highway 47 South to Riverside Campus $F_{ST} = 0.0578$).

Figure 2. Bar plot produced by STRUCTURE utilizing microsatellite data from all three *Geomydoecus ewingi* geographic localities with an *a priori* hypothesis of defining louse population by geographic locality (Highway 47 North, Highway 47 South, and Riverside Campus). The Highway 47 North and Highway 47 South localities are grouped together and Riverside Campus is separated suggesting 2 genetic population clusters. *K*=2.

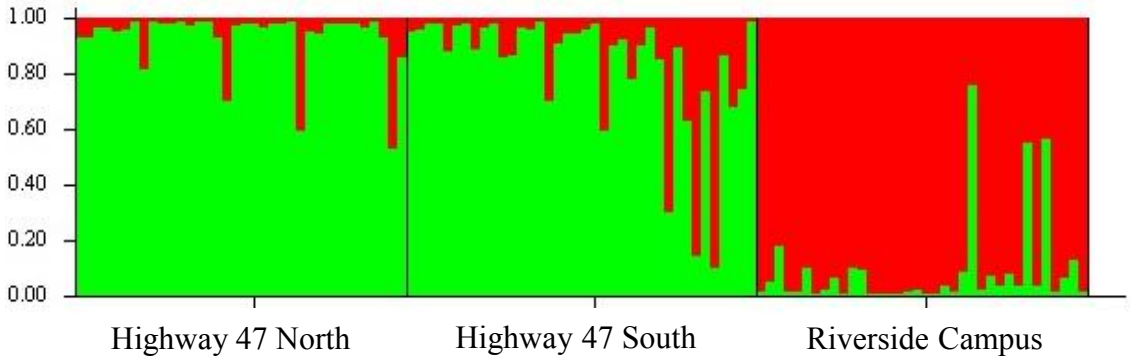


Figure 3. Bar plot produced by STRUCTURE utilizing microsatellite data from all three *Geomydoecus ewingi* geographic localities with an a priori hypothesis of defining louse populations by infrapopulation (with 10-11 lice per pocket gopher host). The infrapopulations from Highway 47 North and Highway 47 South are grouped together separate from the Riverside Campus infrapopulations suggesting 2 genetic population clusters. *K*=2.

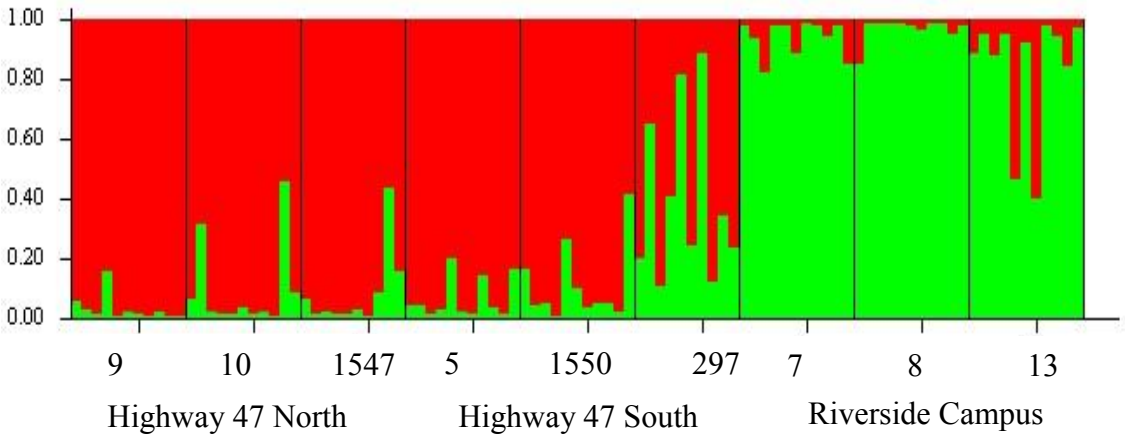


Figure 4. Bar plot produced by STRUCTURE utilizing microsatellite data from all three *Geomydoecus ewingi* localities with an a priori hypothesis of defining louse population by geographic locality (Highway 47 North, Highway 47 South, and Riverside Campus). The Highway 47 North and Highway 47 South localities are more grouped together than Riverside Campus, but are still splitting into separate genetic clusters suggesting 3 genetic population clusters. *K*=3.

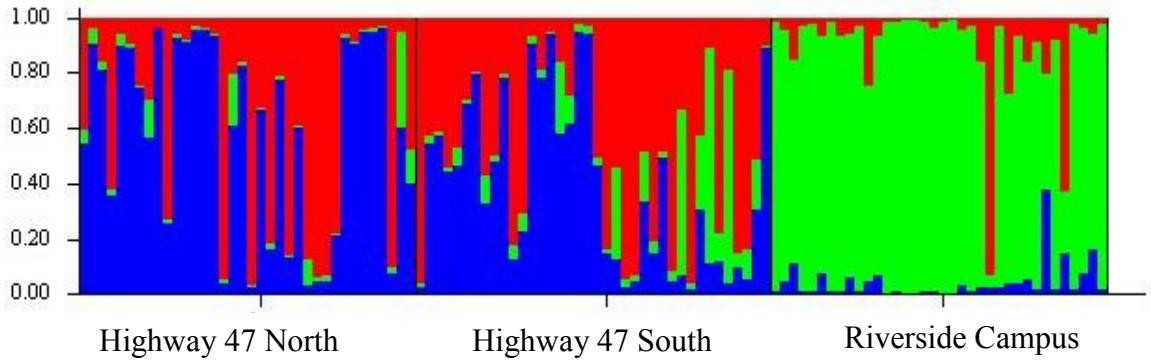


Figure 5. Bar plot produced by STRUCTURE utilizing microsatellite data from all three *Geomydoecus ewingi* localities with an a priori hypothesis of defining louse populations by infrapopulations of ~10-11 lice per host. The Highway 47 North and Highway 47 South localities are more grouped together than Riverside Campus, but are still splitting into separate genetic clusters suggesting 3 genetic population clusters. *K*=3.

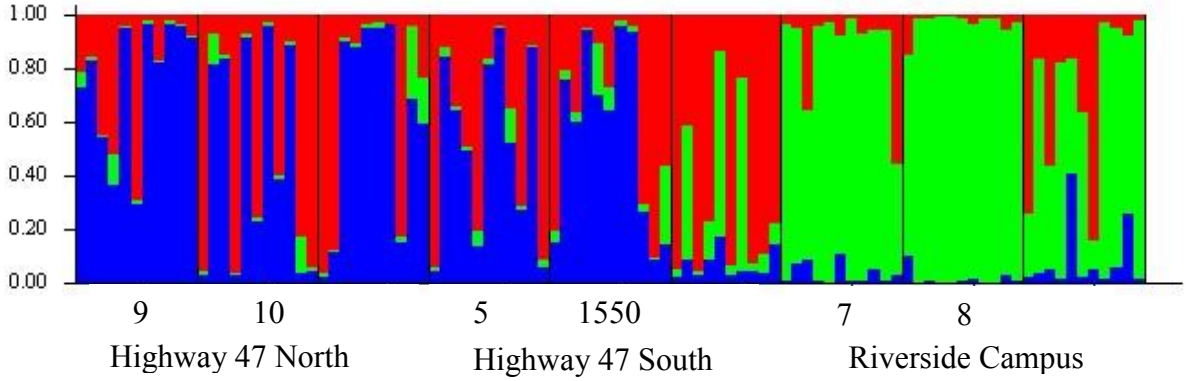
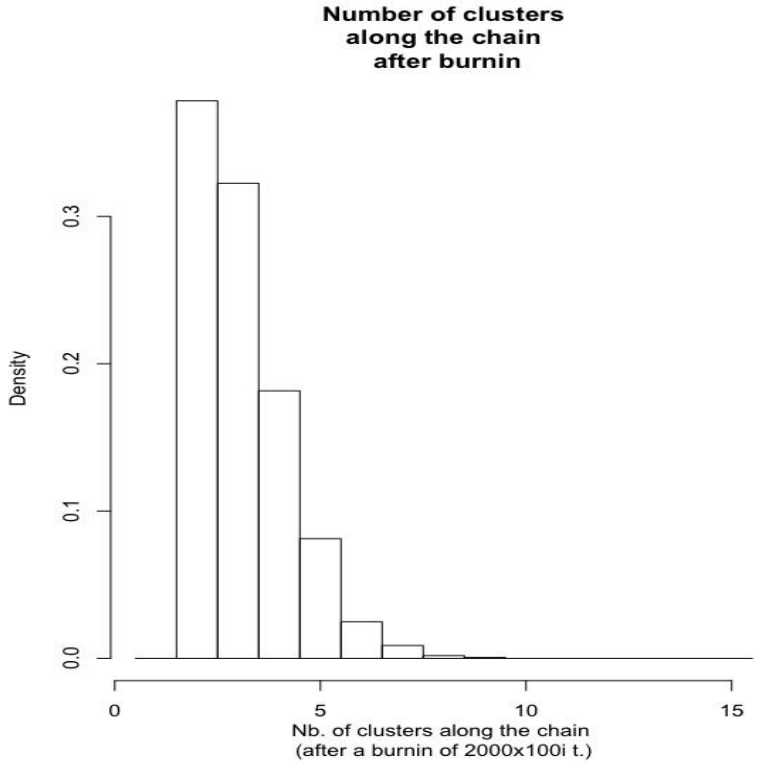


Figure 6. Results of the Bayesian assignment performed by GENELAND showing the posterior probability of the number of clusters (*K*, from 1 to 19) in the run with the highest probability of *K*=2.



IR and HL values were relatively high across geographic localities (Table 5). Similar to the findings from Riverside Campus (Chapter II and Nessner et al. accepted), the Highway 47 North values ranged from 0.283 to 0.812 (IR) and 0.249 to 0.843 (HL), and the Highway 47 South values ranged from -0.129 to 0.516 (IR) and 0.169 to 0.669 (HL). Three individuals from Highway 47 South presented negative IR values (1550.1, 297.5, and 5.6). When IR and HL were calculated defining the lice from each pocket gopher host as a separate population, similar patterns to the geographic analysis were exhibited. Two individuals from Highway 47 North and Highway 47 south had negative IR values (9.1 and 9.11 and 5.6 and 297.5, respectively).

COLONY produced very similar results across localities when reconstructing individual louse relationships within infrapopulations. Each infrapopulation was found to have high amounts of relatedness, with the number of instances of full siblings ranging from 0 to 2 and the number of half sibling instances ranging from 7 to 25 (Table 6; Figure 7 portrays sibling relationships for infrapopulation 13 in Riverside Campus). In all analyses, no instances of either paternity or maternity were detected.

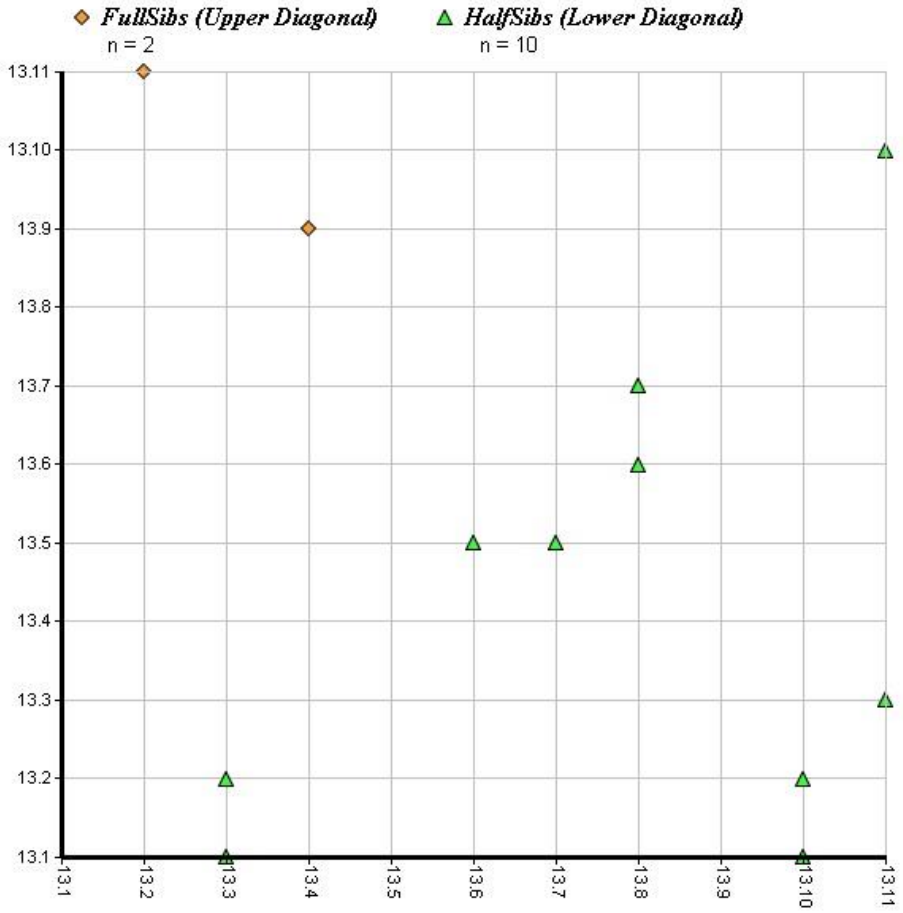
Table 5. IR results for *Geomydoecus ewingi* geographic localities and infrapopulations where IR = internal relatedness and HL = homozygosity weighted by locus. See Appendix 1 for infrapopulation definitions. Results for Riverside Campus were obtained from Chapter II and Nessner et al. (accepted).

Geographic Locality	Infrapopulation	IR	HL
Highway 47 North		0.283 – 0.812	0.249 – 0.843
	9	-0.060 – 0.378	0.273 – 0.572
	10	0.410 – 0.646	0.416 – 0.605
	1547	0.282 – 0.8129	0.289 – 0.845
Highway 47 South		-0.129 – 0.516	0.169 – 0.669
	5	-0.044 – 0.347	0.223 – 0.479
	1550	-0.203 – -0.123	0
	297	-0.124 – 0.426	0.239 – 0.559
Riverside Campus		0.075 – 0.659	0.212 – 0.770
	7	0.009 – 0.631	0.657 – 0.770
	8	0.139 – 0.658	0.313 – 0.768
	13	0.133 – 0.678	0.316 – 0.756

Table 6. COLONY results reconstructing louse relationships from each infrapopulation within each geographic locality. See Appendix 1 for infrapopulation definitions. Results for Riverside Campus were obtained from Chapter II and Nessner et al. (accepted).

Geographic Locality	Infrapopulation	Full Sibling Relationships	Half Sibling Relationships	ML Estimate of
Highway 47 North				
	9	2	13	0.1688
	10	2	14	0.2210
	1547	2	12	0.2840
Highway 47 South				
	5	2	12	0.1080
	1550	0	14	0.1733
	297	0	14	0.1229
Riverside Campus				
	7	2	7	0.2221
	8	1	25	0.0972
	13	2	10	0.2220

Figure 7. COLONY sibling relationship plot for infrapopulation 13. See Appendix 1 for infrapopulation definition. Additional infrapopulation figures available upon request.



Discussion

Microsatellites are useful markers for molecular population genetic studies in that they can be used to investigate gene flow, inbreeding, relatedness, parentage, and effective population sizes. To date, studies utilizing microsatellite markers to address questions in mammalian chewing lice and other parasites are uncommon (Criscione et al. 2007; Ascunce et al. 2013). This study is novel in identifying and analyzing microsatellite loci in the chewing louse *Geomydoecus ewingi* and comparing the findings to their pocket gopher hosts (*Geomys breviceps*; Welborn 2012; Welborn and Light in review). Results from this study are informative for trying to better understand population processes of parasites coevolving with their hosts over long evolutionary timescales.

Similar to previous results (Chapter II; Nessner et al. accepted), when analyzing lice by geographic locality many of the microsatellite loci showed significant deviations from HWE. Loci deviating from HWE seems to be somewhat common in parasites, and likely reflects the effects of real biological processes such as the presence of undetected hierarchical structure, life history characteristics, and kin structure (Dharmarajan et al. 2011; see below). In the current study, these deviations are possibly the result of heterozygote deficiencies, which is supported by H_E almost always being lower than H_O. Coinciding with the reduced heterozygosity, Micro-Checker consistently detected possible null alleles by the general excess of homozygotes for most allele size classes. While these possible null alleles may in fact really be null alleles and interpreted as a technical error, Micro-Checker may also be detecting biological factors such as the

heterozygote deficiency due to inbreeding or nonrandom mating. Positive F_{IS} values across geographic localities also support the homozygote excess. Furthermore, positive F_{IS} values and results from the IR, HL, COLONY, and GENELAND analyses also indicate that there are heterozygote deficiencies within lice, likely due to high levels of inbreeding within each geographic locality, which is consistent with the homozygote deficiencies observed in the HWE analyses. All in all, the majority of analyses performed in this study support the commonality of heterozygote deficiency in lice, which appears to be common in other parasite species (Nadler et al. 1990; Plantard and Porte 2004; Leo et al. 2005; Criscione et al. 2007; Guzinski et al. 2009; Dharmarajan et al. 2010; Kempf et al. 2010; Dharmarajan et al. 2011; Veracx et al. 2012; Ascunce et al. 2013) and also consistent with the extremely low dispersal abilities of *G. ewingi* .

When lice were analyzed by infrapopulation within each geographic locality, the number of loci deviating from HWE and the possibility of null alleles was significantly reduced at each of the three localities. Similar to global results, positive F_{IS} values were reported at all infrapopulation analyses with the exception of one locus (Gew35, Highway 47 South). These positive F_{IS} values and results from IR, HL, and COLONY analyses indicate that homozygote excess may still be an issue within infrapopulations, likely due to inbreeding. COLONY results further supports inbreeding with high levels of internal relatedness among louse individuals within each infrapopulation (Table 6). All in all, analyses by infrapopulation indicate that each geographic locality consists of genetically distinct groups of lice and that there is significant structure among infrapopulations. This means that louse habitat is not spatially continuous and consists of

'islands' of hosts (infrapopulations), where infrapopulations are discrete subsets of the entire populace which is invariably fragmented into subgroups (Poulin 2006). Analyses by geographic locality therefore artificially grouped distinct genetic lineages together, resulting in a Wahlund effect (Nadler et al. 1990; Selkoe and Toonen 2006). These results support the hypothesis that each individual host harbors a distinct louse population (Nadler et al. 1990).

Given their proximity, the STRUCTURE and GENELAND findings presented here indicate that Highway 47 North and Highway 47 South are acting as a genetic cluster and Riverside Campus as a separate genetic cluster (Figures 2, 3, and 7), regardless of if analyzed by geographic population or infrapopulation. These findings differ with the genetic clustering found with the pocket gopher hosts among five localities (Welborn 2012; Welborn and Light in review) but are in agreement when localities Highway 47 North, Highway 47 South, and Riverside Campus are analyzed together (Welborn unpubl. data) and suggest that lice are tracking their hosts at the level of the population. However, the STRUCTURE and GENELAND findings do seem to conflict with the results presented above where each distinct louse population is confined to one host individual. Understanding broad ecological patterns relies on focusing on smaller (microevolutionary) processes within a population. Therefore, it should be noted that STRUCTURE attempts to identify groups of individuals corresponding to the uppermost hierarchical level (Evanno et al. 2005), which would not take into account the population sub-structuring of this parasite system. This means that STRUCTURE may be underestimating the number of genetic clusters thereby explaining the apparent conflict

between the various results. Furthermore, it is also unknown if STRUCTURE can efficiently detect the true number of clusters within hierarchical systems that have uneven migration between populations (Evanno et al. 2005), which may be the case for pocket gopher chewing lice. It is currently unknown if GENELAND behaves similarly, although it is possible that our use of the same geographic coordinates for all louse individuals within a geographic locality may be affecting the analyses.

CHAPTER IV

CONCLUSION

Although parasites form an interconnected network among hosts, parasite exchange is mediated solely by host migration (Poulin 2006). Based upon the combined biological characteristics of pocket gophers (solitary lifestyle; low dispersal rates) and chewing lice (low vagility), it may be expected that opportunities for a louse to colonize a new host are highly limited (Nadler et al. 1990; Hafner and Page 1995; Demastes et al. 2012) resulting in non-random mating which increases the probability of inbreeding within a population. My results support increased inbreeding within this parasite species and contribute to previous findings that a parasite's biology may lead to deviations from Hardy-Weinberg expectations (Criscione et al. 2005; Criscione 2008; Dharmarajan et al. 2011). Notably, my data indicate that a louse population can be defined as all individuals distributed among one host, thus supporting Nadler's hypothesis based on allozyme data (Nadler and Hafner 1989; Nadler et al. 1990). Microevolutionary processes occurring within and among louse populations are almost certainly playing a crucial role in the establishment and maintenance of host-parasite associations (Criscione et al. 2005; Huyse et al. 2005). Due to parasites being effectively stranded on the host lineage, codivergence can lead to cospeciation (Light and Hafner 2007) yielding congruent phylogenies (Demastes et al. 2012). By chewing lice being so closely tied to their hosts in evolutionary time (Demastes and Hafner 1993; Hafner et al. 2003; Demastes et al.

2012), the patterns of inbreeding, homozygote excess and high levels of internal relatedness coincide with the biology of this chewing louse-pocket gopher system.

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APPENDIX I

Appendix I. List of louse specimens examined organized by geographic locality (Highway 47 North, Highway 47 South, and Riverside Campus) and host number (TCWC specimen number at Texas A&M University's Biodiversity Research and Teaching Collections). Each louse specimen is indicated with a unique identification number. Infrapopulation number and louse specimen number correspond to collector number (Preparation number abbreviations are Jessica E. Light (JEL), Ben D. Marks (BDM), and Sarah R. Welborn (SRW). Gender is given when known ("N" denotes nymphal individuals of uncertain gender).

Locality	**Host Preparation Number**	**Infrapopulation Number**	**Louse Specimen Number**	**Sex**
Highway 47 North	TCWC 60719		JEL283.4	F
30°38.132 N 96°26.859 W	TCWC 60720		JEL286.4	M
	TCWC 60721		JEL287.4	M
	TCWC 60722		JEL288.2	M
	TCWC 61029	9	SRW9.1	F
			SRW9.2	M
			SRW9.3	F
			SRW9.4	M
			SRW9.5	F
			SRW9.6	F
			SRW9.7	N

			SRW9.8	F
			SRW9.9	F
			SRW9.10	N
			SRW9.11	F
	TCWC 61030	10	SRW10.1	F
			SRW10.2	F
			SRW10.3	F
			SRW10.4	M
			SRW10.5	M
			SRW10.6	M
			SRW10.7	F
			SRW10.8	F
			SRW10.9	F
			SRW10.10	F
			SRW10.11	N
	TCWC 60723	1547	BDM1547.2	F
			BDM1547.3	M
			BDM1547.4	M
			BDM1547.5	F
			BDM1547.6	F
			BDM1547.7	F

			BDM1547.8	F
			BDM1547.9	F
			BDM1547.10	F
			BDM1547.11	F
Highway 47 South	TCWC 60718	1550	BDM1550.1	F
30°38.092 N 96°26.885 W			BDM1550.2	M
			BDM1550.3	F
			BDM1550.4	M
			BDM1550.5	M
			BDM1550.6	M
			BDM1550.7	M
			BDM1550.8	M
			BDM1550.9	M
			BDM1550.10	N
			BDM1550.11	M
	TCWC 60756	297	JEL297.1	M
			JEL297.2	M
			JEL297.3	M
			JEL297.4	F

			JEL297.5	F
			JEL297.6	M
			JEL297.7	M
			JEL297.8	M
			JEL297.9	M
			JEL297.10	M
			JEL297.11	F
	TCWC 60757		JEL298.4	M
	TCWC 62455		SRW3.4	F
	TCWC 62456		SRW4.1	M
			SRW4.2	F
			SRW4.3	N
	TCWC 61028	5	SRW5.1	F
			SRW5.2	F
			SRW5.3	F
			SRW5.4	M
			SRW5.5	F
			SRW5.6	M
			SRW5.7	F
			SRW5.8	F
			SRW5.9	F

			SRW5.10	F
	TCWC 61193		SRW6.1	F
Riverside Campus	TCWC 61193	7	SRW7.1	F
30°38.453 N 96°27.722 W			SRW7.2	F
			SRW7.3	F
			SRW7.4	F
			SRW7.5	M
			SRW7.6	M
			SRW7.7	M
			SRW7.8	M
			SRW7.9	M
			SRW7.10	F
			SRW7.11	F
	TCWC 61192	8	SRW8.1	M
			SRW8.2	M
			SRW8.3	M
			SRW8.4	F
			SRW8.5	F
			SRW8.6	F

			SRW8.7	F
			SRW8.8	F
			SRW8.9	N
			SRW8.10	F
			SRW8.11	F
	TCWC 61924		SRW11.4	F
	TCWC 61913		SRW12.3	F
			SRW12.4	F
	TCWC 61925	13	SRW13.1	M
			SRW13.2	F
			SRW13.3	M
			SRW13.4	M
			SRW13.5	M
			SRW13.6	M
			SRW13.7	M
			SRW13.8	F
			SRW13.9	F
			SRW13.10	F
			SRW13.11	F