PEROMYSCUS NEWSLETTER

Number Forty-Three

Fall 2008

Cover:

Anastasia Island Beach Mouse, *Peromyscus polionotus phasma*, in a patch of *Spartina patens*. Photograph by J.B. Miller, Senior Land Resource Planner, St. Johns River Water Management District.

***Peromyscus Newsletter* Number 43**

Hello, All!

In this latest issue of *Peromyscus Newsletter* there are several exciting bits of news for you. There is the announcement of a newly created list serve for *Peromyscus* researchers, and an update on the ESTs and genome sequence which are almost ready to be posted to GenBank, and some really great research in the in the Contributed Accounts section, to name a few. I hope you enjoy them.

If you are planning to purchase mice from the Stock Center, please note that there is a new list of user fees and some stocks are no longer available. Also, if you need to place a large order let Janet know as soon as possible by emailing her at crosslan@biol.sc.edu. And you should definitely check out the article on Janet on page 16. Those of you who don't know her will be amazed at all she does and those of you who do know her will nod your head in agreement!

As I mentioned in the last issue, many people are still not receiving emails from the peromyscusnewsletter@biol.sc.edu account. This, I believe, is caused by people's spam filters, so if you know of anyone having this problem please have them check their filters and specify this address as legitimate. I am limited in what I can do from this end. This will be especially important in the next few weeks as I invite people to join the new Google Groups list serve.

And as always, all suggestions to improve the Newsletter are appreciated! Everything related to *PN* should be sent to peromyscusnewsletter@biol.sc.edu. Take care and enjoy!

Julie

PEROMYSCUS NEWSLETTER is produced by the

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CONTENTS

News, Comments, and Announcements

RECENTLY PUBLISHED!!!!

"The relationships of major lineages within peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal" by Jacqueline R. Miller and Mark D. Engstrom was just published in Journal of Mammalogy, vol 89 (5):1279-1295.

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OBITUARY

We are sad to report the death of Dr. Jerry O. Wolff of St. Cloud State University. He was last seen getting off a shuttle in Canyonlands National Park, Utah, on May 10, 2008. Letters sent to friends and family before his disappearance have led authorities to believe he may have gone to the park to die. Dr. Wolff was well respected and he will be missed.

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PEROMYSCUS GOOGLE GROUP

In an effort to improve communication within the *Peromyscus* community I have created a GoogleGroup. For those of you who are not familiar, this is a list serve complete with searchable archives. So members may post questions to other *Peromyscus* researchers instantly. In order to maintain your privacy I have restricted the group to people who have been invited to join. Only members can post messages, read the archives, view the members list, create pages and upload files. This group and its archives does not appear in public Google search results or the directory. Over the next several weeks I will be sending invitations to everyone who currently receives *Peromyscus Newsletter*. This may take awhile because I will only be able to send invitations to a subset of recipients at a time, so please be patient. Many of the email addresses I have may be out of date, so if you know of anyone who would like to subscribe please have them send me an email at peromyscusnewsletter@biol.sc.edu

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PROGRESS IN *PEROMYSCUS* EMBRYOLOGY!

Recently Amanda Duselis and Paul Vrana at the University of California, Irvine, published two accounts of their techniques for artificial insemination and retrieval of oocytes in *Peromyscus*. The reports are in the Journal of Visualized Experiments so there are wonderful videos to watch as well (doi:10.3791/184 and doi:10.3791/185)! They developed these methods using techniques they were taught by the former director of the PGSC, Mike Dewey. See the "Recent Publications" section at the end of the Newsletter for the full citations!

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UPDATE ON THE PERDIDO KEY BEACH MICE

As many of you may remember, the *Peromyscus* Genetic Stock Center helped rescue 10 endangered Perdido Key beach mice (*Peromyscus polionotus trissyllepsis*) from Hurrican Ivan in September 2004. Those mice and their descendants, totaling 52 individuals, were then transferred to Santa Fe Community College Teaching Zoo in Gainesville, Florida in June 2007. Since then, other institutions have gotten involved with preserving these mice. Santa Fe sent 12 mice to the Brevard Zoo in Melbourne, Florida, 12 went to The Florida Aquarium, 10 went to Palm Beach Zoo at Dreher Park, and Santa Fe kept the rest. Palm Beach Zoo is putting together a studbook to help guide future breeding plans. So far, the mice are doing well—Santa Fe Zoo has produced three offspring and Brevard Zoo has produced ten!

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The 88th annual meeting of the **American Society of Mammalogists** took place Sunday June 22 to Wednesday June 25, 2008 at South Dakota State University, Brookings, SD. There were 11 presentations relating to *Peromyscus* research, some of which are included in the contributions section.

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The 2009 annual meeting of the **American Society of Mammalogists** will take place Wednesday June 24 to Sunday June 28, 2009 at the University of Alaska Fairbanks.

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THE *PEROMYSCUS* GENETIC STOCK CENTER

General

The University of South Carolina has maintained a genetic stock center for *Peromyscus* (deer mice and congeneric species) since 1985. The center was established under a grant from the Living Stocks Collection Program of the National Science Foundation and continues to be supported by NSF and the NIH Biological Models and Materials Research Program. It also receives support from the University and from user fees.

The major function of the Stock Center is to provide genetically characterized types of *Peromyscus* to scientific investigators and educators. Continuation of the center is dependent upon significant external utilization, therefore potential **users are encouraged to take advantage of this resource**.

Policies and Procedures

The Stock Center maintains several categories of stocks of living animals: 1) Closed colony random-bred[1] "wild-type" stocks of seven species of *Peromyscus*. 2) Two highly inbred[2] stocks of "wild-type" *P. leucopus*. 3) Stocks of fifeen coat color mutations, mostly in *P. maniculatus*. 4) Stocks of eight other monogenic traits. The Stock Center operates in strict compliance with the Animal Welfare Act and is located in an AAALAC approved facility. All animal care is performed by certified technicians. Stocks are monitored regularly for presence of disease and parasites and are free of hantavirus and 15 murine viruses.

The Stock Center also provides blood, organs, tissues, fetuses, skins and other biological materials from *Peromyscus.* The Stock Center operates a Molecular Bank where selected genomic libraries and probes are available. Other resources include a reference collection of more than 2,500 reprints of articles on peromyscine rodents, copies of which may be provided. The Stock Center is the primary sponsor of ***Pero*Base**, an on-line database dedicated to information regarding *Peromyscus* and closely related species.

Sufficient animals of the mutant types generally can be provided to initiate a breeding stock. Somewhat larger numbers, up to about 50 animals, can be provided from the wild-type stocks. Animals requested in greater numbers frequently require a "breed-up" charge and some delay in shipment.

Orders and Pricing

A user fee is charged for animals or materials provided by the Stock Center. A schedule of fees is shown on the next page. Fees vary with species and type of service provided. User assumes the cost of all shipment. Animals lost in transit are replaced without charge. Tissues, blood, skins, *etc*. are supplied at a modest fee that includes technician time. Arrangements for special orders will be negotiated. Billing will be submitted upon satisfactory delivery. **Write or call for details or special requirements.**

SCHEDULE OF USER FEES

Item	**Academic and Government**	**Commercial**
MATURE ANIMALS (each)		
Wild-type Stocks		
Smaller species (*P. maniculatus, P. polionotus, P. leucopus, P. eremicus*)	**$ 25.00**	**$40.00**
Larger species (*P. californicus, P. melanophrys, P. aztecus*)	**33.00**	**45.00**
Mutant and Inbred Stocks	**33.00**	**45.00**
Pregnant females (Smaller species)	**45.00**	**55.00**
(Larger species)	**60.00**	**70.00**
Special Attention (Diet, *etc*.)	**45.00**	**55.00**
F_1 Species Hybrids	**35.00**	**45.00**
TISSUE SAMPLES (Per sample)	Cost of the animal + lab fee + materials	
Solid		
Fluid (Blood, urine, saliva, etc.) per ml		
Flat skins (each)		
MOLECULAR MATERIALS		
Extracted DNA, 10 µg	**20.00**	
PCR Primers (500 µl @ 10 µM)	**10.00**	
Genomic & cDNA libraries	**300.00**	

OTHER CHARGES

Shipping costs = actual shipper's charges plus cost of mouse containers, packaging.

Lab fee for sample preparation ($35.00/hr).

Breed-up fees (for orders exceeding 50 animals) = *per diem* cage charges X cages required.

STOCKS AVAILABLE

WILD TYPE STOCKS	**ORIGIN**
P. maniculatus bairdii (BW Stock) Deer Mouse	Closed colony bred in captivity since 1948. Descended from 40 ancestors wild-caught near Ann Arbor MI.
P. maniculatus sonoriensis (SM2 Stock) Sonoran Deer Mouse	Derived from about 50 animals wild-caught by Jack Hayes in 1995 near White Mountain Research Station CA.
P. polionotus subgriseus (PO Stock) Oldfield Mouse	Closed colony since 1952. Derived from 21 ancestors wild-caught in Ocala Nat'l. Forest FL. High inbreeding coefficient.
P. leucopus (LL Stock) White-footed Mouse	Derived from 38 wild ancestors captured between 1982 and 1985 near Linville NC.
P. californicus insignis (IS Stock) California Mouse	Derived from about 60 ancestors collected between 1979 and 1987 in Santa Monica Mts. CA.
P. aztecus (AM Stock) Aztec Mouse	Derived from animals collected on Sierra Chincua Michoacan, Mexico in 1986.
P. melanophrys (XZ Stock) Plateau Mouse	Derived from animals collected between 1970 and 1978 from Zacatecas, Mexico and bred by R. Hill.
P. eremicus (EP Stock) Cactus Mouse	Originated from 10-12 animals collected at Tucson AZ in 1993.

INTERSPECIFIC HYBRIDS

P. maniculatus X P. polionotus F_1 Hybrids	Bred by special order.
P. leucopus X P. gossypinus F_1 Hybrids	Sometimes available by special arrangement.

[3]**COAT COLORS**	**ORGINAL SOURCE**
Blonde *bln/bln*	Mich. State U. colony (Pratt and Robbins, 1982)
Albino *c/c*	Sumner's albino deer mice (Sumner, 1922)
Ashy *ahy/ahy*	Wild-caught in Oregon ~ 1960 (Teed et al., 1990)
[4]Brown *b/b*	Huestis stocks (Huestis and Barto, 1934)
California blonde *cfb/cfb*	Santa Cruz I., Calif., stock (Roth and Dawson, 1996)
Dominant spotting *S*/+	Wild caught in Illinois (Feldman, 1936)
Golden nugget b^{gn}/b^{gn}	Wild caught *P. leucopus* (Horner and Dawson, 1993)
Ivory *i/i*	Wild caught in Oregon (Huestis, 1938)
Platinum *plt/plt*	Barto stock at U. Mich. (Dodson et al., 1987)
[4]Silver *sil/sil*	Huestis stock (Huestis and Barto, 1934)
Tan streak *tns/tns*	Clemson U. stock from NC (Wang et al., 1993)
Variable white *Vw*/+	Mich. State U. colony (Cowling et al., 1994)
Wide-band agouti A^{Nb}/a	Natural polymorphism U. Mich. (McIntosh, 1954)

OTHER MUTATIONS AND VARIANTS

Alcohol dehydrogenase negative	Adh^{0}/Adh^{0}	South Carolina BW stock (Felder, 1975)
Alcohol dehydrogenase positive	Adh^{f}/Adh^{f}	South Carolina BW stock (Felder, 1975)
Boggler *bgl/bgl*		Blair's *P. m. blandus* stock (Barto, 1955)
Cataract-webbed *cwb/cwb*		From Huestis stocks (Anderson and Burns, 1979)
Epilepsy *epl/epl*		U. Michigan *P. m. artemisiae* stock (Dice, 1935)
Hairless-1 *hr-1/hr-1*		Sumner's hairless mutant (Sumner, 1924)
Hairless-2 *hr-2/hr-2*		Egoscue's hairless mutant (Egoscue, 1962)
Juvenile ataxia *ja/ja*		U. Michigan stock (Van Ooteghem, 1983)
Enzyme variants		Wild type stocks provide a reservoir of variants (Dawson, 1983)

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[1] "Random bred" without deliberate selection, sib-sib matings avoided. [2] Inbred lines bred by sib-sib and/or parent-offspring mating for 21 generations or more. [3]Unless otherwise noted, mutations are in *P. maniculatus*. [4]Available only as silver/brown double recessive.

Other Resources of the *Peromyscus* Stock Center

Highly inbred *P. leucopus* (I_{30+}) are available as live animals or as frozen tissues. Two lines developed by George Smith (UCLA) are currently maintained by the Stock Center.

Limited numbers of other stocks are on hand, but not currently available. Inquire.

Preserved or frozen specimens of types given in the above tables.

Flat skins of mutant or wild-type coat colors of any of the stocks listed above.

Reference library of more than 2500 reprints of research papers, articles and reports on *Peromyscus.* Single copies of individual articles can be photocopied and mailed. Please limit requests to not more than five articles at any given time. There will be a charge of 10 cents per photocopied page after the initial 20 pages.

Photocopies of back issues of *Peromyscus Newsletter* ($5 ea.) or single original back copies, when still available, without charge.

Materials are available through the *Peromyscus* Molecular Bank of the Stock Center. Allow two weeks for delivery. Included is purified DNA or frozen tissues of any of the stocks listed above. Several genomic libraries and a variety of molecular probes are available. (Inquire for more information)

For additional information or details about any of these mutants, stocks or other materials contact: Janet Crossland, Colony Manager,* Peromyscus *Stock Center, (803) 777-3107, e-mail crosslan@biol.sc.edu

PLEASE CALL WITH INQUIRIES

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Update On Molecular Resources For *Peromyscus*

100,000 Expressed Sequence Tags

The Department of Energy's Joint Genome Institute has sequenced about 50,000 clones in both directions to generate about 100,000 expressed sequence tags from the following 6 normalized libraries:

1. Brain and testes from *P. maniculatus bairdii* (BW) – 10,000 clones
2. Brain and testes from *P. polionotus subgriseus* (PO) – 10,000 clones
3. E12-14 embryos from *P. maniculatus bairdii* (BW) – 7,500 clones
4. Newborn *P. maniculatus bairdii* (BW) – 7,500 clones
5. Liver, kidney, and skin from *P. maniculatus bairdii* (BW) – 7,500 clones
6. Spleen from *P. maniculatus bairdii* (BW) – 7,500 clones

ESTs from all libraries were Sanger sequenced. The brain and testes libraries for BW and PO (libraries 1 and 2) were also sequenced using a 454 FLX sequencer, yielding about 200,000 additional sequences per library. The data is not yet publicly available but is expected to be uploaded to GenBank soon.

Full Genome Sequence

The *National Human Genome Research Institute* (*NHGRI*) - a division of the National Institutes of Health - has granted our request to determine the complete DNA sequence of four *Peromyscus* species. The actual work is being done at the Baylor College of Medicine and anticipated to be complete in the summer of 2009. There is just a short list of mammals whose DNA sequence is decoded. The genomic information of the four *Peromyscus* species will answer many questions in speciation and evolution along with clues to longevity and diseases. The *P. maniculatus bairdii* (BW) genome will receive 6x coverage while the other three species, *P. polionotus subgriseus* (PO), *P. leucopus* (LL), and *P. californicus insignis* (IS), will receive 2x coverage.

In an effort to preserve as much information about the individuals whose DNA is actually being sequenced, the Stock Center sent siblings of the sequenced mice to the Smithsonian for use as voucher specimens. This is a unique situation, as for most mammals there is very little information about the animal that was used for sequencing. Photographs of the animals sent to Smithsonian can be found at: http://stkctr.biol.sc.edu/Smithsonian_photos/

The catalog numbers for the voucher specimens are:

Peromyscus californicus insignis	USNM 585467	F
Peromyscus californicus insignis	USNM 585468	M
Peromyscus leucopus	USNM 585469	F
Peromyscus leucopus	USNM 585470	M
Peromyscus maniculatus bairdii	USNM 585471	F
Peromyscus maniculatus bairdii	USNM 585472	M
Peromyscus polionotus subgriseus	USNM 585473	M
Peromyscus polionotus subgriseus	USNM 585474	F

The acronym USNM stands for the United States National Museum, but citations should be to the Smithsonian Institution.

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Meet Janet Crossland, Colony Manager

Shortly after Dr. Wally Dawson founded the *Peromyscus* Genetic Stock Center he realized he would need a full-time colony manager. He was impressed with her prior experience with dairy goats, but perhaps what tipped the scales in her favor was the fact that she had already scooped up one loose mouse before the interview even started. That's when Wally knew he had found the right woman for the job. That was 1985 and Janet has been there ever since.

In her role as colony manager Janet manages approximately 3000 mice. She sets up matings, weans litters, marks each animal, and keeps records of everything that happens to every individual. She takes care of all orders from start to finish, making sure there are enough breeding pairs to produce the needed mice at the required time, then getting them boxed and shipped. If there are tissue orders, Janet is unsurpassed in her ability to quickly collect those as well, whether it's blood samples, study skins, or organ collection. Janet monitors the health of the mice, and watches closely for signs of inbreeding depression among the mutant stocks. If inbreeding depression is noted she out-crosses the stock and then re-derives the mutation. Her desire to be exceptional at her job drove her to sit in on classes at the University of South Carolina such as chemistry, genetics, parasitology, human physiology, medical terminology, and animal nutrition. She has also gained certification from the American Association for Laboratory Science (AALAS) as a Laboratory Animal Technologist, the highest technician certification AALAS offers. She frequently attends AALAS meetings with posters about *Peromyscus* and promotes their use as well.

Although her official title is colony manager, Janet does so much more than simply deal with mice. She handles nearly all communications with the research community, handles invoicing, maintains the Stock Center website, hires and trains undergraduate helpers, coordinates visitor research, and gives tours to VIPs. She knows the names, school affiliations, and area of specialty for an unimaginable number of *Peromyscus* researchers making her an even greater asset to the Stock Center. And as if that wasn't enough, Janet's knowledge of

Peromyscus biology exceeds that of many well-known researchers in the field. Perhaps this is in part due to her additional efforts maintaining the Stock Center's large repository of reprints and her monthly monitoring of the new *Peromyscus* literature. In fact, the literature list at the end of every *Peromyscus Newsletter* is thanks solely to Janet.

Born and raised in South Carolina, Janet has the outgoing charm many people associate with southern ladies. She comes to work smiling every day, and even at the most stressful of times manages to keep a sense of humor and laugh. Janet is always pleasant to be around, and because she is frequently the first point of contact for external users she projects a wonderfully positive impression that reflects well on herself and the Stock Center. This is proven again and again at conferences when users inquire how Janet is doing before they ever think to mention the mice.

Suffice it to say the Stock Center couldn't function without Janet. She is the oil that keeps all the wheels running smoothly. She has been a stable fixture through three directors, easing their transition into their new role and providing a continuity that was invaluable. Nothing happens at the Stock Center that doesn't have Janet's touch on it and for that we are thankful. We are lucky to have her.

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The Stock Center Prints Its First Advertisement!

The Peromyscus Genetic Stock Center printed its first advertisement in the October 2008 issue of Lab Animal (vol. 37, issue 10, page 463). Let's hope more people see the value in using our favorite mice!

Peromyscus Genetic Stock Center

http://stkctr.biol.sc.edu

Deer Mice: "The Drosophila of North American Mammology"

The Peromyscus Genetic Stock Center, established in 1985, provides a reliable source of laboratory stocks of both wild-type and genetically variant Peromyscus animals to the scientific and educational communities.

These animals are random-bred avoiding sib-mating, thus they retain some genetic heterogeneity, representing a natural population.

Available are:
- live animals
- tissues
- blood products
- molecular materials
- literature and other support
- genomic resources (BAC libraries EST sequences, genetic map)

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NOTICE

PEROMYSCUS NEWSLETTER **IS NOT A FORMAL SCIENTIFIC PUBLICATION.**

THEREFORE…

INFORMATION AND DATA IN THE CONTRIBUTIONS SECTION SHOULD NOT BE CITED OR USED WITHOUT PERMISSION OF THE CONTRIBUTOR.

THANK YOU!

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Unusual Observation and a Question About *Peromyscus leucopus*

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In other locations where I have studied small mammals, I have always found *Peromyscus leucopus* to be an early colonizer of oldfields. But not in extreme eastern Virginia where I have been doing monthly Capture-Mark-Recapture trapping for six years in two oldfield sites owned by The Nature Conservancy. These former farm fields quickly revert to grass/forb communities in which populations of cotton rats, eastern harvest mice, marsh rice rats, meadow voles, and house mice become established within a year. Despite proximity to nearby forests with white-footed mice, these oldfields are never colonized by *P. leucopus,* which is puzzling. On both sites, marsh rice rats were present early but disappeared in 6-12 months, after populations of cotton rats and voles came to dominance. Thus, it seems that in my location, rice rats have taken up the role of early colonizer from white-footed mice. Has anyone else observed a similar phenomenon? Please send comments to brose@odu.edu.

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Apparent Absence of Deer Mice at Various Localities in the Labrador Region of Eastern Canada

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The type locality of the deer mouse *Peromyscus maniculatus* is "the Moravian settlements in Labrador" (Hall 1981), in the province of Newfoundland and Labrador, Canada. By 1845, when the description of "*Hesperomys maniculatus*" was published (Hall 1981, and references therein), such settlements had been established at several coastal locations in the Labrador region, including Nain, Hebron, and Hopedale (www.heritage.nf.ca/society/moravian.html). Marginal records for *P. m. maniculatus* in the eastern portion of the Labrador Peninsula are the coastal towns Gready, North West River, and Port Burwell (Hall 1981, and references therein; Harper 1961); Dragoo et al. (2006) reported additional specimens from Nain and Hebron.

As part of a long-term project to collect deer mice (*Peromyscus maniculatus*) throughout their North American range, live-trapping activities were conducted at various Labrador localities, so as to include the extreme northeastern portion of the species' range (Fig. 1). These trapping localities were selected based on past records of *P. maniculatus* occurrence and on accessibility by automobile (from the Trans-Labrador Highway). The six localities comprised an interior site (between Goose Bay and Churchill Falls—Fig. 2) as well as several coastal localities in the vicinity of a marginal record (North West River—Fig. 3) described for *P. m. maniculatus* (Harper 1961).

Extensive trapping in favorable habitat and weather at the five localities in the vicinity of North West River and Goose Bay and at the single interior locality along the Trans-Labrador Highway failed to yield any *P. maniculatus*. General trapping success was moderate, however, as numerous *Myodes gapperi* (red and gray phases) and *Zapus hudsonius* were captured. On the same extended collecting trip, many *P. maniculatus* were trapped along the north shore of the Gulf of Saint Lawrence (*P. m. plumbeus*), at the west end of Anticosti Island (*P. m. anticostiensis*), and at various localities in Maine (*P. m. abietorum*) and Vermont (*P. m. gracilis*).

The failure to capture *P. maniculatus* from a variety of promising habitats suggests that deer mice are presently rare or nonexistent in the vicinity of North West River and Goose Bay. This seemingly face-saving suggestion is substantiated by Inuit unfamiliarity with these rodents. While examining photos in a field guide, several Native individuals in North West River confirmed those rodents that were trapped and observed in the area, but indicated that they had never seen deer mice.

An open question is whether the reported occurrences of *P. maniculatus* at coastal localities in the Labrador region represent sampling-site bias (i.e., vicinity of established settlements) or introductions. The sites of early successful collections might simply reflect accessibility, rather than the absence of deer mice at localities away from human habitation. The difficulty of trapping at any appreciable distance from the coastal towns might have compelled earlier workers to concentrate their efforts more locally. Alternatively, *P. maniculatus* may not be native to the region, and the deer mice historically reported from these settlements and towns may have represented small transitory populations established from animals introduced by cargo vessels or fishing boats. In his work in Labrador, Outram Bangs (1913) noted a preferential association of deer mice with human habitations, and explicitly indicated that "[t]he Labrador deer-mouse...is apt to take up its abode in buildings and huts like the house mouse, and in Labrador seems to be much more abundant in such places than in the woods and among rocks." The occurrence of deer mice in seemingly inhospitable habitats such as Port Burwell, Quebec (Harper 1961; see aerial and ground photographs at www.pinetreeline.org/other/other8/other8cf.html) is also consistent with introductions.

The nature of the occurrence of deer mice in the Labrador region has considerable systematic relevance. *If* introductions have occurred at coastal settlements, *P. maniculatus* may not be indigenous to the type locality. More extensive collecting over a broader geographic area is needed to ascertain the distribution and indigenity of *P. maniculatus* in the Labrador region.

Figure 1.—Map of the Labrador region (Map by Duleepa Wijayawardhana. ©1999 Newfoundland and Labrador Heritage Web Site Project).

Figure 2.—Interior trapping locality along the Trans-Labrador Highway, between Goose Bay and Churchill Falls.

Figure 3.—Trapping locality near North West River.

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www.pinetreeline.org/other/other8/other8cf.html Port Burwell, Labrador: Assorted Detail and Photos 1940 to Present Day.

Acknowledgements

I thank Ira Greenbaum (Texas A&M University) and Al Lackey (Oswego State University) for informative and enjoyable discussions on deer mice in the Labrador region. Vince Walsh of the Newfoundland and Labrador Heritage Web Site (www.heritage.nf.ca) granted permission to use the Labrador map in Figure 1. This fieldwork was supported by grants from the National Geographic Society (5012-93) and the National Science Foundation (DEB-9201509).

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Tick Burdens Of *Peromyscus leucopus* and *Borrelia* Infection Rates In Ixodid Ticks In a Fragmented Virginia Landscape

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The etiologic bacterial agent of Lyme disease, *Borrelia burgdorferi*, is transmitted to hosts in the eastern United States by *Ixodes scapularis*, the black-legged tick (Ostfeld 1997). Approximately 95% of reported cases occur in the northeastern and northcentral United States (CDC 2004) where *Peromyscus leucopus*, the white-footed mouse, is considered the primary reservoir for *B. burgdorferi* (Ostfeld 1997). Complicating the diagnosis of the disease in the southern United States is an emerging illness called Southern Tick-Associated Rash Illness (STARI), the symptoms of which are indistinguishable from those of Lyme disease. STARI is caused by the bacterium *B. lonestari* and transmitted by *Amblyomma americanum*, the lone star tick. *A. americanum* currently ranges throughout the southern United States (CDC 2005) where it is sympatric in some areas with *I. scapularis*. This raises questions about the diagnoses of Lyme disease patients in the South, given the identical symptoms and the lack of a reliable, standard clinical test for either disease.

Ixodes scapularis, its major hosts, and *B. burgdorferi* thrive in deciduous forests, but controversy exists over the effect of forest-patch size on the abundance of *I. scapularis*, its hosts, and the prevalence of *B. burgdorferi* (Allan et al. 2002; Wilder and Meikle 2004). Laboratory studies have shown 20-100% of *I. scapularis* to be infected with *B. burgdorferi* in focal portions of the country compared to 4% in Virginia (VDH 1997). The average infection rate of *B. lonestari* in *A. americanum* throughout its range is approximately 2% (Bacon et al. 2003). Currently, no other vectors of *B. lonestari* are recognized. *Dermacentor variabilis*, the American dog tick, is capable of acquiring but not maintaining *B. burgdorferi* (Johns et al. 2000). To our knowledge neither *D. variabilis* nor *I. scapularis* has been tested for the ability to acquire *B. lonestari*.

The main objectives of our research (Tanner 2007) were to 1) examine the relationship between forest-patch size and *I. scapularis*, *A. americanum*, and *D. variabilis* burdens on *P. leucopus*, 2) examine the relationship of tick densities to forest-patch size and season, 3) compare minimum infection rates (MIRs) of *B. burgdorferi* and *B. lonestari* between small (<3 ha) and large (>3 ha) forest patches, and 4) test 2 species (*D. variabilis* and *I. scapularis*) not yet examined as possible vectors of STARI to determine their abilities to acquire *B. lonestari*.

We examined 3 small (<3-ha) and 3 large (>3-ha) forest patches in Fredericksburg and Spotsylvania County Memorial National Military Park (FRSP) in Virginia. Sherman live traps were used from April-October 2005 to capture *P. leucopus*. We removed ticks from mouse pinnae (Ostfeld et al. 1993 – Fig. 1) to estimate burdens (number of larvae and nymphs combined per individual). Ticks were also collected by drag sampling (Falco and Fish 1992) to estimate densities. Tick DNA was extracted from all samples and amplified by nested polymerase chain reaction (PCR) to test for the presence of *B. burgdorferi and B. lonestari* DNA (Tanner 2007).

Mean tick densities were analyzed using repeated-measures analysis of variance (rmANOVA – Zar 1999) to test for differences between patch sizes and seasons. We used 1-way ANOVA to test for patch-size differences in mouse densities, mean infestation burdens (number of ticks per infested mouse), and infestation rates (percentages of mice infested). Simple linear regression was used to test for functional dependence of both tick density and mean tick burdens (average burden) on mouse density.

Average density of *P. leucopus* was greater in small forest patches (Fig. 2). Drags yielded no *D. variabilis* larvae or nymphs or *I. scapularis* larvae or adults. The densities of adult *D. variabilis*, nymphal *I. scapularis*, and adult and nymphal *A. americanum* did not differ between patch sizes or among seasons. We found no functional dependence of *A. americanum*, *I. scapularis* nymphs, or *D. variabilis* adults on mouse density, and no *A. americanum* were found on mice. Mean burdens, infestation burdens, and infestation rates by *I. scapularis* and *D. variabilis* did not differ by patch size. We found no functional dependence of *I. scapularis* or *D. variabilis* burdens on mouse density. All samples were negative for *B. burgdorferi* infection (0% MIR). MIRs of *A. americanum* for *B. lonestari* were 5.9% (1 of 17) for adult ticks and 3.4% (8 of 237) for nymphs.

Because mouse density was greater in small forest patches but tick burdens and bacterial prevalence were not, deer movement and factors that affect it may exert a greater influence than mouse density by directly influencing tick distribution and densities. Variables that affect deer movement, such as mast production (McShea and Schwede 1993), have a significant effect on distribution and density of ticks. Thus, many other habitat characteristics may be as important as patch size in determining Lyme disease risk. Although *P. leucopus* is more abundant in small forest patches (Nupp and Swihart 1996), habitat characteristics other than forest-patch size determine deer and mouse activity and tick density and survival.

That *D. variabilis* and *I. scapularis* were negative for both *B. lonestari* and *B. burgdorferi* suggests their incompetencies as STARI vectors. Our laboratory results further support the distribution and incidence of *B. lonestari* in *A. americanum* throughout the South. This, combined with the failure to isolate *B. burgdorferi* from *I. scapularis*, support our claim that most Lyme-like symptoms throughout the South are caused by *B. lonestari* from *A. americanum* and not by *B. burgdorferi* from *I. scapularis*. Although infection rates by *B. lonestari* are relatively low in the South, infection rates by *B. burgdorferi* appear even lower, suggesting that most Lyme disease cases reported in the southern United States are misdiagnosed cases of STARI.

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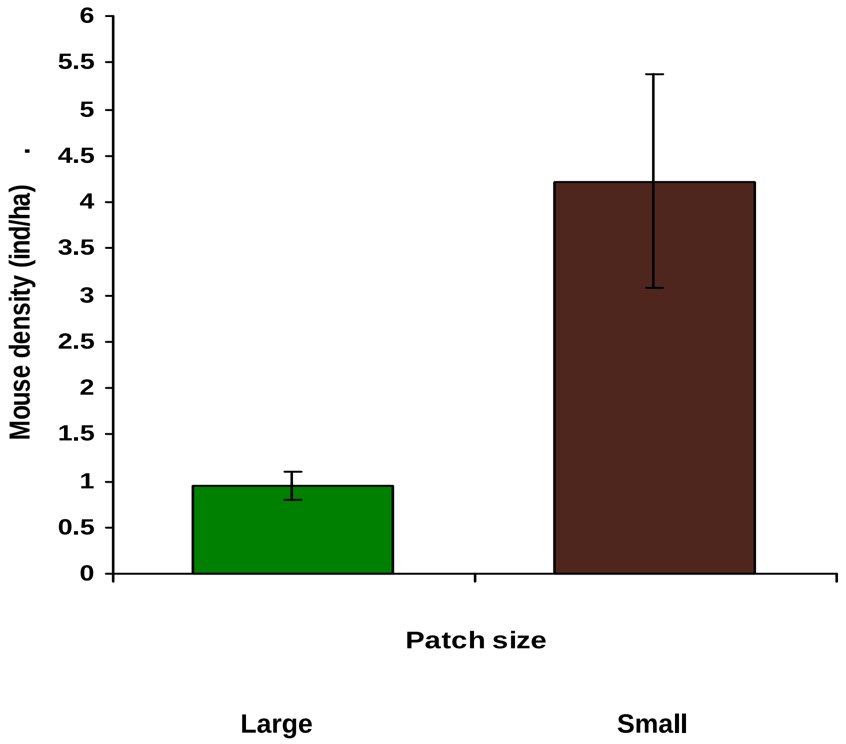
Figure 1.—*P. leucopus* with 2 larval ticks attached to pinna. Photo by Cheryl Tanner.

Figure 2.—Density of *P. leucopus* in small (< 3-ha) and large (> 3-ha) forest patches.



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High Density Housing in the Rodent Community: New Questions About Hantavirus Hosts

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Host density has been a reliable predictor of pathogen prevalence in a number of zoonotic disease systems, including hanatviruses. For example, prevalence of Limestone Canyon virus was positively associated with the density of brush mice (*Peromyscus boylii*) in southern Arizona (Kuenzi et al. 2007). In Panama, increased prevalence of Choclo and Calabazo hantaviruses are correlated with higher densities of the viral hosts, pygmy rice rats (*Oligoryzomys fulvescens*) and short-tailed cane mice (*Zygodontomys brevicauda*; Suzan et al. 2008). This density-dependent relationship has also been documented in the deer mouse - Sin Nombre virus (SNV) system (Glass et al. 2002; Madhav 2007; Parmenter et al. 1999).

Although the primary reservoir species for SNV is the deer mouse (*Peromyscus maniculatus*), SNV has been observed in other secondary reservoir species including pinyon mice (*Peromyscus trueii;* Otteson et al. 1996) and desert woodrats (*Neotoma lepida;* Dearing et al. 1998). The mechanism for this inter-specific transmission of SNV is unknown, as there is little spatial overlap among these species in terms of foraging behavior and nest locations. Whereas deer mice tend to forage on the leaves and seeds of herbaceous vegetation and nest at the base of shrubs, pinyon mice and woodrats nest in or near the trunks of pinyon and juniper trees (Dial 1988; Ruffner et al. 1978). Pinyon and juniper leaves also serve as the primary dietary components of pinyon mice and woodrats (Dial 1988; Ruffner et al. 1978). Although these species rarely occupy the same microhabitat, woodrat middens provide an exception to this lack of spatial overlap, as they provide nesting sites for all three SNV hosts. Woodrat middens are complex nesting structures constructed by woodrats out of twigs, vegetation, rock and other scavenged materials (Figure 1). Because middens are relatively large in size and provide ample protection from weather, they are often favored as nesting sites by a number of small mammal species, including deer mice and pinyon mice (McGill et al. 2005). Therefore, compared to other nesting sites, woodrat middens represent a high-density, high diversity habitat with frequent opportunities for contact among the SNV host species. In a number of host-pathogen systems, pathogen prevalence is higher in populations with greater species diversity (Ostfeld et al. 2008). This phenomenon, referred to as the Amplification Effect, occurs when separate host species act as independent reservoirs having, in essence, a combined effect on pathogen prevalence. This, in turn, allows for greater persistence and abundance of a pathogen than would otherwise be possible if the pathogen existed in a low diversity community with fewer potential host species (Ostfeld et al. 2008).

The objective of our study was to examine whether woodrat middens serve as sites of inter-specific transmission of SNV. Our primary questions were: 1) are population density and species diversity higher in woodrat middens compared to non-midden habitats and 2) is SNV prevalence higher among SNV host populations that reside in woodrat middens, compared to SNV hosts that reside outside of middens. We predicted that woodrat middens would have both higher population density and species diversity compared to comparable non-midden habitats. Because woodrat middens provide high-density habitat with frequent SNV transmission opportunities, we also predicted that prevalence in all potential SNV host species would be higher among populations captured within middens than in populations captured outside of middens.

Figure 1.—Photograph of a woodrat midden. Photo was taken at one of our 9 study sites, located in the Great Basin Desert of central Utah.

Methods

Study Sites.—Small mammals were non-destructively sampled from 9 sites (3.14 hectares each) near the West Tintic Mountains in the Great Basin Desert of central Utah (Juab County) on lands administered by the U.S. Department of Agriculture. Vegetative communities of both sites were similar and dominated by big sagebrush (Artemisia tridentata) and Utah juniper (Juniperus osteosperma). To ensure independence, study sites were separated by approximately 3 km.

Small Mammal Sampling.—Sites were sampled for small mammals in October of 2004 during a 3-night period that coincided with the new moon. To sample small mammals that would be considered non-midden residents, we live-trapped (H.B. Sherman Traps, Inc.) animals using 148 traps distributed in a web configuration across the site (Mills et al. 1999). All animals captured on the trapping webs were considered to be non-midden residents. To sample small mammals that would be considered midden residents, we selected 6 middens within each of the 9 sites (54 middens total). In this process, 15 traps were placed around the entrance to the midden. After capture, animals were verified to be midden residents by watching their return into the midden upon release; animals that did not immediately return to the midden after release were considered non-midden residents, whereas animals that returned immediately to the midden were considered midden residents.

Blood Collection.—After capture, animals were identified to species and sex. Blood (0.3 ml) was collected from the retro-orbital sinus of all deer mice, woodrats and pinyon mice upon initial capture within the 3-day sampling period. Blood was immediately stored on dry ice until transfer to a –80^{o}C freezer. Following processing, all animals were released at their location of capture. Personnel involved in handling rodents took precautions for working with animals potentially infected with hantavirus (Centers for Disease Control and Prevention 1995). General techniques for capturing and processing animals were approved by the Institutional Animal Care and Use Committee at the University of Utah.

SNV Antibody Detection.—We performed enzyme linked immunosorbent assays (ELISA) for antibodies (IgG) against SNV in blood samples, as described previously by Otteson et al. (1996) and Feldman et al. (1993). Briefly, 96 well plates were coated with recombinant SNV nucleocapsid (N) antigen at 1:400 in phosphate buffered saline (PBS) and stored overnight at 4^{o}C. After washing the wells in PBS with 0.5% Tween-20 (PBS-T), serum samples were diluted 1:50 in PBS with 0.5% Tween-20 and 5% skim milk (milk diluent) and plated in duplicate for 60 -120 minutes at 37^{o}C. Goat anti-P. leucopus HRP secondary antibody was diluted to 1:1000 in milk diluent and incubated for 60 minutes at 37^{o}C. 2-2'-asino-di-(3-ethyl-benzthiozaline-sulfonate) (ABTS) peroxidase substrate (Kirkegaard and Perry Laboratories) was incubated for 30 minutes at 37^{o}C to develop the assay. Plates were read using Gene 5 software on a Biotek plate reader at

405nm. Samples were considered positive if the optical density reading was three times higher than that of the known negative serum controls on a plate.

Analyses.—Density of small mammals on trapping webs was calculated as the number of non-midden residents captured per hectare; density of midden residents was calculated as the number of animals captured per midden. Species diversity of middens and non-midden sites were calculated using Simpson's Reciprocal Index ($D = 1 / [\Sigma\ n(n-1)] / n(n-1)$). SNV prevalence was calculated as the total number of SNV seropositive individuals divided by the total number of individuals captured in a particular habitat (i.e. webs and middens). Prevalence was calculated separately for deer mice, pinyon mice and woodrats.

Results

Over the 3-night sampling period, we captured 444 unique rodents classified as non-midden residents and 225 rodents that were classified as midden residents (Table 1). Density of small mammals within trapping webs (e.g. non-midden residents) was 15.7 animals per hectare, whereas density of small mammals within middens was 4.2 animals per midden. Although the absolute value of density was higher within trapping webs, raw estimates of density are somewhat misleading, as individual woodrat middens are contained within an area that is much smaller than 1 ha (e.g. contained within a single tree). Thus, density was considerably higher in middens compared to non-midden trapping webs. Likewise, species diversity was also higher in woodrat middens compared to trapping webs (midden D = 3.47, non-midden D = 3.19).

Across species, SNV prevalence among non-midden residents was more than double that of midden residents (non-midden residents = 16.3%, midden residents = 7.2%; Table 1). Similar patterns were observed within individual host species, as SNV prevalence was higher in both non-midden deer mice (non-midden = 22.3%, midden = 12.4%) and non-midden pinyon mice (non-midden = 6.7%, midden = 0.00%). An exception to this pattern was observed in woodrats, as SNV prevalence was slightly higher in woodrats considered midden residents, compared to woodrats considered non-midden residents (non-midden = 2.90%, midden = 4.17%).

Table 1.—Summary of small mammal captures of animals considered to be residents of woodrat middens, as well as those considered to be non-midden residents. Non-midden residents were captured on 9, 3.14 ha sites using a web trapping design. Midden residents were captured from 1 of 54 middens contained within the 9 larger trapping sites.

	Non-Midden Residents		Midden Residents	
	Total Captured	Number Infected	Total Captured	Number Infected
Deer mice (*Peromyscus maniculatus*)	220	49	97	12
Pinyon mice (*Peromyscus trueii*)	89	6	60	0
Desert woodrats (*Neotoma lepida*)	35	1	24	1
Great Basin pocket mice (*Perognathus parvus*)	33	N/A	7	N/A
Ord's kangaroo rats (*Dipodomys ordii*)	59	N/A	33	N/A
Harvest mice (*Reithrodontomys megalotis*)	8	N/A	4	N/A

Discussion

Contrary to our predictions, SNV prevalence was lower among host populations captured within woodrat middens than among hosts captured outside of middens. These patterns were especially intriguing, considering that woodrat middens had higher densities of small mammals, as well as higher species diversity than non-midden sites.

Although increased species diversity is associated with increased disease prevalence in a number of host-pathogen systems (Ostfeld et al. 2008), species diversity has also been shown to decrease the prevalence of infectious disease, a phenomenon referred to at the Dilution Effect (Ostfeld and Keesing 2000). This inverse relationship between diversity and prevalence may result from individual animals reducing their number of intraspecific encounters at high levels of species diversity, resulting in fewer opportunities for transmission within the population (Keesing et al. 2006). Alternatively, species diversity may reduce transmission probability if the presence of other species reduces the duration of intraspecific encounters between hosts or by reducing host survival because of increased competition for resources (Keesing et al. 2006). Previous studies have documented that SNV dynamics follow predictions of the Dilution Effect hypothesis (Clay 2007; Mills 2005). These studies proposed that in habitats high in species richness, deer mice may shift microhabitat use to avoid encounters with other rodent species to a greater extent that they would in comparable habitat with lower species richness (Clay 2007; Falkenberg and Clarke 1998).

Although results of our study indicate that neither species diversity nor population density is related to higher SNV prevalence, our results do not explain the mechanism for interspecific transmission of SNV. Based on our findings, it seems that SNV hosts avoid contact with each other to a greater extent under high density, high diversity conditions, similar to conditions that exist within a woodrat midden. The inverse of this prediction would be that deer mice, pinyon mice and woodrats have greater contact with each other when not residing in woodrat middens. To elucidate the validity of these predictions, future research should more directly examine rates of contact and interaction among the potential SNV host species in both middens and non-midden habitats.

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Protein and *Peromyscus* Life Histories: A Proposal

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Bioenergetic considerations have been fundamental to studies of mammalian life histories but cannot fully explain observed patterns because metabolism is primarily related to thermoregulation in endotherms. Nagy (1987) found the daily energy expenditure (DEE) of endotherms to average 17X that of ectotherms, indicating that well over 90% of DEE is required to maintain an elevated body temperature. Recently, we examined DEE of small mammals in the field and confirmed that energy expenditure within species is primarily related to thermoregulation. Our first study examined DEE in *Peromyscus californicus* and *Peromyscus boylii* in California (Kalcounis-Rueppell 2000) and showed that DEE was related to ground temperature but not gender or reproductive status in either species. Our second study examined the energetics of seasonal breeding in *Peromyscus leucopus* in southern Ontario (Havelka 2002) and showed that DEE varied only with body size and ambient temperature. The contributions of gender, age, and reproductive status were not significant. A third study examined metabolism in *Peromyscus maniculatus* at Kananaskis, Alberta (Herdman 2005). DEE varied with body size and weakly with ground temperature, but not with gender or reproductive condition. Thus, both intra- and inter-specific variation in DEE is dominated by thermoregulatory requirements and the costs of reproduction involve a very small proportion of the overall energy budget. Finally, lactation further confounds the energetics of mammalian reproduction because milk is generally derived directly from diet, especially in income breeders (individuals that provision offspring using energy gained concurrently). Diet is converted to milk by mothers but not metabolized by them, so that the metabolism of mothers does not reflect the full energetic costs of reproduction. It should not be surprising that metabolism and aspects of reproduction appear to be only weakly related (see Kalcounis-Rueppell 2007 and references within) or that reproductive trade-offs are not always apparent (see Trebatická et al. 2007 and references within).

Further advances in our understanding of the evolution of small mammal life histories may require considerations of factors other than energetics. One such factor is dietary protein because protein is known to constrain small mammal reproduction both in the laboratory and in the wild (see Derrickson and Lowas 2007 and references therein). However, the sources of dietary protein in small mammal populations are not well known.

Proposal

We propose to profile the sources of dietary protein in *Peromyscus* using stable isotopes of nitrogen. *Peromyscus* provide a model system for such a study because there are many species and their life history (particularly reproductive) characteristics are relatively well documented. Some species are widespread and exhibit geographic trends in reproduction. While their specific diets are difficult to monitor in the wild, they are generally known to be omnivorous, consuming both plant and animal matter. Those feeding primarily on plant matter must contend with plant secondary compounds, a constraint that is minimized for those feeding primarily on animal matter. Stable isotopes of nitrogen can be used to identify the source of dietary protein. They do not indicate the total amount of protein in the diet, but reflect the relative amounts of plant and animal protein consumed. $\delta^{15}N$ values increase with trophic levels and are higher in carnivores than in herbivores. Nitrogen isotopes have frequently been used to identify the relative proportions of plant and animal protein in the diets of omnivores (Kelly 2000) and there is no reason to think that the same will not apply to *Peromyscus*.

Our plan is to profile nitrogen isotopes in as many species of *Peromyscus* as possible to determine the extent to which their life histories (body size, litter size, and other reproductive characteristics) can be explained by source of dietary protein. We also plan to profile as many populations of widespread species as possible to determine the extent to which geographic gradients in life history characteristics can be explained by source of dietary protein.

To conduct such a study it will be necessary to develop a sampling protocol based on the dynamics of stable isotopes in small mammals. Toward this end we have been conducting baseline studies on isotope fractionation among tissues (Sare et al. 2005a; Miller 2006), isotope fractionation between mothers and offspring (Sare et al. 2005b; Miller 2006), the effects of digestive processes on isotope fractionation in diets and feces (Hwang et al. 2007), and habitat-specific differences in isotope fractionation (Ansell 2007). We are currently examining isotope fractionation in relation to rates of metabolism (Smith in progress) and seasonal variation in isotope fractionation (Tabacaru in progress). Hair might be the tissue of choice because sampling is non-invasive, so we are documenting molting patterns as well (Sare et al. 2005c; Tabacaru in progress). Isotope fractionation among years will be examined in 2009.

We anticipate being in a position to develop a sampling protocol next winter. At that time we will be seeking collaborators who are conducting field studies on *Peromyscus.* Anyone wishing to have a stable isotope profile of their population (for free!) is welcome to join the group. If interested, please contact me at jsmillar@uwo.ca and I will add you to our mailing list. Potential graduate students interested in this type of research can inquire at the same address.

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Using Stable Carbon and Nitrogen Isotopes to Infer Seasonal Dietary Changes and Niche Separation Between Deer Mice and White-Footed Mice in Southeastern South Dakota

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Two species of *Peromyscus* mice, the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*), occur sympatrically throughout much of eastern North America. Both closely related species are nocturnal, omnivorous and exhibit significant overlap in morphological characteristics. Theoretically, two morphologically and ecologically similar species cannot coexist over time using the same limited resources in identical ways. Niche separation is thought to occur along three primary axes: (1) spatial segregation (including differential use of habitat); (2) temporal avoidance; and/or (3) dietary differences. Several studies have investigated spatial separation and temporal avoidance as possible key mechanisms for niche differentiation between the white footed mouse and deer mice. But because of the limitations associated with using traditional dietary analysis techniques, few studies have been able to detect seasonal dietary shifts between these two species or identify differing microhabitat sources of similar food items. Recently, this work has been facilitated by stable isotope analysis (SIA) of carbon and nitrogen. In this study, we sought to determine how deer mice and white-footed mice use and partition food resources within the agriculturally dominated landscape of southeastern South Dakota. Our results indicate that deer mice obtain most of their food directly from agricultural fields while white-footed mice feed primarily in woodland and grassland areas surrounding agricultural fields. These data were strengthened with trapping data indicating microhabitat preference for cropland in deer mice versus woodlands for white-footed mice. This study demonstrates how SIA can be a useful tool for investigating niche separation between sympatric species as well as providing detailed information on seasonal changes in food habits over time.

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