BAC Library Proposal
Peromyscus maniculatus

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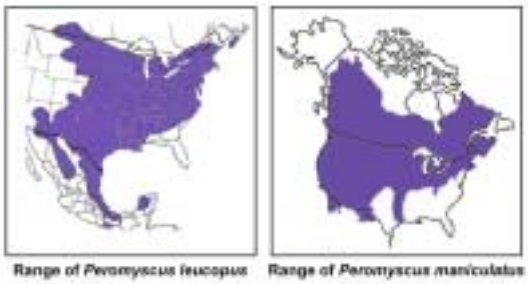
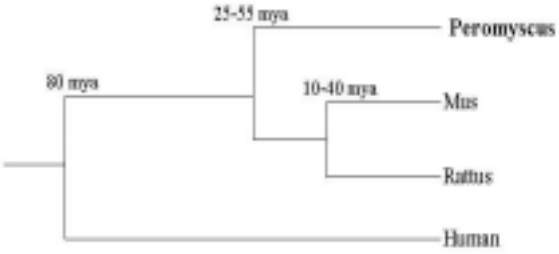
Thus far, no BAC library is available for any of the new world rodents. We propose the production of a BAC library for the most abundant of North American rodents, *Peromyscus sp*



Two peromyscines, *P maniculatus* (deer mice) and *P leucopus* (white footed mice), are collectively the most common and abundant native North American mammals, ranging from Alaska to Central America and from the Atlantic to the Pacific. They occur in a wide range of habitats including sea-level wetlands and beaches, forests, prairies, deserts, and mountains of elevation up to 14,000 ft.

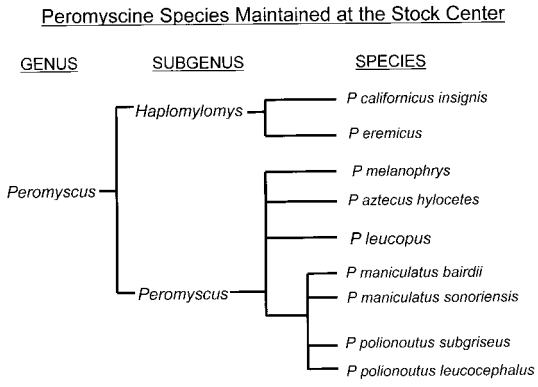
Though superficially similar to laboratory mice (*Mus domesticus*) and rats (*Rattus norvegicus*) deer mice are not closely related to these species. *Peromyscus* and *Mus*/*Rattus* diverged from a common ancestor considerably more ancient than the ancestor common to just *Mus* and *Rattus*. Phylogenetically it provides a species intermediate between the two major genetic models, humans and lab mouse, thereby providing an intermediate for studies of gene and genome evolution. A *Peromyscus* BAC library would fill an evolutionary gap in keeping with the goal of the NHGRI program to develop phylogenetically diverse resources.



It is also our goal to develop *Peromyscus* genomics to enable utilization of the species for studies for which it is uniquely suited, while simultaneously taking advantage of comparative information provided by the gene-dense Human and *Mus* genome projects and using these as reference species.

The *Peromyscus* Genetic Stock Center & the *Peromyscus* Community

One of the major advantages of research with *Peromyscus* is their ready adaptability to colony conditions. Mice manifesting traits of interest in the field are easily moved into the laboratory where the trait can be studied under controlled conditions (reviewed in (Joyner et al. 1998). In recognition of this fact, and to facilitate laboratory studies with *Peromyscus,* the *Peromyscus* Genetic Stock Center was established at the University of South Carolina in 1985 with funding originally derived from NSF, and later, with additional funding from NIH.



One reviewer described the Stock Center as the "Jackson Laboratory of *Peromyscus*." Its main missions are 1) to furnish disease free, genetically defined, variant and normal, living, pedigreed *Peromyscus* to investigators and educators for research and instruction, and 2) to improve *Peromyscus* as a laboratory animal resource.

Currently the Stock Center maintains about 3000 animals representing seven species and two subspecies of

Peromyscus along with 19 pure-breeding lines of single mutations affecting behavior, physiology, and coat color. In the last four years ~4000 live animals were provided to over 90 different laboratories engaged in *Peromyscus* research. In the last four years there has been a 50% increase in the number of labs annually utilizing resources of the Center. The Stock Center supplies biological materials including fresh, frozen, and preserved tissues and molecular probes and libraries. The Center also functions as a clearinghouse for information regarding this genus by sponsoring an internet database (*Pero*Base, http://wotan.sce.sc.edu/perobase/index.html), a Stock Center web page (http://stkctr.biol.sc.edu), and the semiannual *Peromyscus* Newsletter which includes a listing of new publications in the field (~100/yr). The Newsletter is mailed to about 850 individuals. Additionally, the Stock Center web page lists reprints of more than 3000 article-length papers on *Peromyscus* held by the Stock Center. Older literature is well represented among these and is not readily obtained elsewhere.

The Community. There is a large number of researchers involved in both field- and lab-based *Peromyscus* research. Approximately half of the animals provided by the Stock Center are to researchers involved in epidemiology, infectious disease and toxicology research. The other half includes a diversity of researchers studying various aspects of *Peromyscus* biology, especially including physiological and behavioral studies. Another large group of researchers is conducting studies on wild-caught *Peromyscus* and includes research in evolution, biogeography, ecology, and systematics. An increasing number of these researchers are utilizing molecular techniques as new genomic approaches and resources become available. Many of these researchers would benefit directly from the immediate availability of a BAC library. It is anticipated that *Peromyscus*, for its myriad research potentials, will continue to attract an increasingly larger community of molecular biologists with the development of genomic resources, including mouse geneticists interested in comparative genomic data.

Research Interest in *Peromyscus*.

Deer mice and allied species have significant present and potential value in biological and biomedical sciences. They have been aptly described as "The *Drosophila* of North American Mammology."(Musser and Carleton 1993). Such a view reflects the potential *Peromyscus* presents as a model for studying 1) the genes responsible for reproductive isolation and speciation, and 2) the genes enabling the physiological and behavioral adaptation to changing environmental conditions, adaptation to other species, adaptation to each other, and adaptation to microbial and other parasites (Dewey and Dawson 2001). Specific research areas which will benefit from the development of BAC libraries as part of our overall program to develop genomic resources for these species include the following.

A. Mouse Models for Human Disease and Aging. Although not intended to compete with *Mus* in this arena, several mutants maintained in the Stock Center affect development or behavior and are worthy of study; they include boggler (*bg/bg*), cataract-webbed (*cwb/cwb*), epilepsy (*ep/ep*), juvenile ataxia (*ja/ja*), and variable white (*Vw/+*). None of these have been mapped. Also, white-footed mice (*P leucopus)* are regarded as an interesting model for longevity and aging studies since they live three times longer than lab mice (Duffy et al. 1997; Anspach et al. 2001).

B. Public Health. Due to their broad geographic distribution and abundance in the United States, peromyscines, particularly *P maniculatus* and *P leucopus,* have attracted public health concerns for their potential as reservoirs of infectious disease organisms. This is the case for certain current pathogens as well as ones yet to emerge. Just in the past decade peromyscines were found to be primary reservoirs for two emerging infectious diseases, hantavirus pulmonary syndrome (HPS) and Lyme disease. *P maniculatus* is the primary carrier of the Sin Nombre strain of Hantavirus responsible for the alarming 1993, outbreak of HPS. Though cases of HPS are not numerous, the major concern is its high rate of mortality which is over 40%.

Peromyscus carries a number of other disease pathogens recognized as tick-borne diseases. Lyme disease was first recognized during the late 1970's in a cluster of cases in Lyme, Connecticut as a persistent malady characterized by fever and aches, which progresses to neuralgia and arthritic symptoms. Though seldom lethal, Lyme disease presents a significant public health concern; in 1999 over 16,000 cases were reported. Small mammals, particularly *P leucopus* constitute the major reservoir for the spirochete.

Other diseases for which deer mice are likely to play an epidemiological role include various forms of Ehrlichiosis caused by rickettsia, *Ehrlichia chaffeensis, E ewingii*, and one yet to be classified, and babesiosis, a malaria-like disease caused by *Babesia microti* a protozoan that infects erythrocytes.

There is some evidence suggesting that deer mice may also be a reservoir for vesicular stomatitis virus, an agent that substantially affects the cattle and equine industries. Currently studies are underway to determine whether *Peromyscus sp* may have a role in the epidemiology of West Nile Virus.

A BAC library would be of immediate use to investigators interested in developing reagents for evaluating the immune status of *Peromyscus* as they interact with and carry the above pathogens as well as their own parasites (see Hjelle and Schountz below).

C. Behavior. *Peromyscus* has been the subject of numerous behavioral studies, many of which beg to be further pursued genetically.

Peromyscus maniculatus is notoriously prone to the development of stereotypical behavior (chronic repetitive movements) and is being studied as a model for repetitive movement disorders in humans (Presti et al. 2002). To understand the genetics underlying this predisposition, lines of *P maniculatus* manifesting high vs low susceptibility to development of stereotypy are being developed (MH Lewis, University of Florida, personal communication).

With regard to partner fidelity, polygamy is the norm for most rodents, but among peromyscines two species, *P polionotus* and *P californicus,* provide models for monogamy (Gubernick and Teferi 2000; Ryan and Altmann 2001). Of particular interest to geneticists are the interfertile species *P polionotus* (monogamous) and *P maniculatus* (polygamous) which offer the opportunity to define the genes underlying differences in partner fidelity.

Another behavior is the fascinating interspecific variation in the structure of burrows between *P maniculatus,* which builds shallow nests, and *P polionotus* which digs deep tunnels. A genetic analysis of the difference showed tunneling to be dominant, and that the difference between species appears to be controlled by *as few as one or two genes* (Dawson et al. 1988).

D. Genomic Imprinting, Hybrid Dysgenesis, and Speciation. The genetic mechanisms underlying genomic imprinting, reproductive isolation and speciation are among the most exciting areas of research in *Peromyscus sp*. An especially fruitful model involves species seemingly at the initial stages of reproductive isolation such as *P maniculatus* and *P polionotus*. Offspring of interspecific crosses exhibit hybrid dysgenesis and this was found to be associated with a breakdown in genomic imprinting (Vrana et al. 1998; Vrana et al. 2000). Thus, speciation is likely driven by rapidly co-evolving, interacting loci (such as imprinted loci and their regulators). Hybrids between closely related species provides a powerful means of identifying such loci, due to the physiological anomalies resulting from negative interactions of diverged alleles from the two species.

E. Habitat Adaption: Coloration. The older literature documents a rich source of examples of intraspecific coat color variation enabling peromyscines to blend in with their environment. From more recent work in *Mus* the cell biology, biochemistry, and genetics of pigmentation have come to be well understood (Silvers 1979). This provides an excellent background to now determine which genes are *naturally* exploited, and what genetic changes result in the production of protective coloration.

F. Habitat Adaptation: Photoperiod Sensitivity. Like many species in temperate and boreal zones, *Peromyscus* do not breed the year around but are subject to seasonal breeding patterns. An adaptive manifestation of this is the gonadal regression stimulated by short photoperiods. Selective breeding resulted in the generation of photoperiod sensitive and resistant lines for both *P leucopus* (Heideman et al. 1999) and *P maniculatus* (Desjardins et al. 1986)

G. Habitat Adaptation: Altitude. Found in a wide range of elevations, *Peromyscus* has attracted considerable attention as model of genetic adaptation to altitude. Two *P maniculatus* subspecies held by the stock center can be used as comparative models for such studies, *P m bairdii* originated from near sea level *P m sonoriensis*, from 10,000 ft. In fact the now classic studies demonstrating the adaptive benefits of hemoglobin polymorphism were demonstrated in *P maniculatus* and since has been the subject of numerous other studies addressing the physiology and genetics of high altitude adaptation.

Immediate BAC Library Utilization

Below examples of ongoing projects that would immediately benefit from the production of a BAC library.

1. Infectious Disease: Development of Immunological Reagents. A BAC library for *Peromyscus maniculatus* (deer mouse) would be of great value to the community that works on zoonotic diseases such as HPS, Lyme disease, babesiosis, and Ehrlichiosis. Investigators in the biomedical community are greatly hindered in studying pathogen-reservoir interactions because there is a notable lack of reagents

to detect the important immune factors (cytokines, chemokines, cell surface markers, PCR primers, MHC locus genes) and intracellular signaling molecules in the deer mouse system. Antibodies for lab mice (*Mus musculus*) fail to detect such basic molecules as interferon, CD4, CD3 and CD8. The BAC library would be used by Brian Hjelle (U New Mexico; Botten et al. 2003) and Tony Schountz (Mesa State University; Herbst et al. 2002) to clone, express, and make antibodies against the important deer mouse immune system molecules and cell surface molecules.

2. Speciation: The Molecular Mechanism of Hybrid Dysgenesis in Reciprocal Hybrids between *P maniculatus* and *P polionotus*. The Paul Vrana group (UC Irvine) has defined linkage for an overgrowth condition found in *Peromyscus* hybrids to an imprinted region containing the zinc finger gene *Peg3* (Vrana et al. 1998; Vrana et al. 2000) . This region has recently been shown to contain a number of other overlapping imprinted loci in a fairly small (<200 kb) region. It is also clear that this region is diverging rapidly, as mouse and human have different gene expression profiles and different gene numbers in this region. They would like to investigate species differences in this region to uncover the source of the *Peromyscus* genetic incompatibility. Clearly a BAC of this region would greatly aid their efforts. A BAC library would enable them to clone regulatory regions (and test them via transgenesis), as well as more easily find loci and test their imprinting status in this complex region. They will also utilize *Peromyscus* BACs in a comparative genomics approach to understand other imprinted domains.

3. Speciation: The rapid evolution of reproductive proteins. Proteins involved in reproduction are among the most rapidly evolving proteins in a wide variety of taxa. Changes in reproductive proteins can cause reproductive incompatibilities and lead to divergence of conspecific populations and ultimately speciation. Hopi Hoekstra (UC San Diego) and members of her lab are identifying reproductive genes that are affected by natural selection in *Peromyscus*. *Peromyscus* is an ideal system in which to study reproductive protein evolution because of the variety of mating systems observed among *Peromyscus* species and the wealth of information about reproductive incompatibilities from laboratory crosses. A BAC library would serve two main purposes in this project: (1) to allow sequence determination of reproductive protein regulatory regions in cases where the proteins are differentially expressed, and (2) to characterize the effect of selection at linked sites (i.e., determine the extent of linkage disequilibrium) after we have identified reproductive proteins that are influenced by selection.

4. Genetics of Habitat Adaptation: Hemoglobin Variation Associated with Altitude Adaptation. The Nachman group (U of Arizona) is engaged in a study of nucleotide variation at the alpha globin loci in *Peromyscus maniculatus*. Their work is aimed at linking the known protein variation to underlying DNA sequence variation. *Peromyscus* is a classical model system for studies of alpha globin function and variation because of the well documented altitudinal clines in protein polymorphism. One of the long-term goals is to determine how selection at one site in the genome affects patterns of linked variation at different distances along the chromosome. BAC clones of the alpha globin complex will enable determination of the alpha globin organization followed by studies of linkage disequilibrium patterns throughout the alpha globin region correlated with altitude adaptation.

5. Genetics of Habitat Adaptation: Adaptive Coloration. *Peromyscus* is an ideal model for studying the molecular genetics of naturally occurring coat color variation, and the *Peromyscus* Stock Center houses several of these naturally occurring color mutants. Hopi Hoekstra (UC San Diego), having worked on adaptive coat color in pocket mice (Hoekstra and Nachman 2002; Hoekstra and Nachman 2003; Nachman et al. 2003) is initiating a large research program using *Peromyscus* as a model organism. The goal of this project is to identify genes and ultimately specific nucleotide mutations responsible for adaptive coloration in *Peromyscus polionotus* (closely related to *P maniculatus*). The availability of a BAC library is essential for cloning large pigmentation genes (e.g., mahogany locus is > 60kb in mouse) and those with extensive regulatory regions (e.g., agouti signaling protein regulatory region is >100kb in mouse, human and dog). Large contiguous sequences will allow for surveys of nucleotide diversity in coding regions and in non-coding regulatory regions, in which primer design based on conservation between mouse and human are often impossible. It is likely that many mutations involved in adaptive coloration (e.g. those at the agouti locus) occur in regulatory regions. Secondly, a BAC library is also vital for a long-term goal of this project to conduct functional tests of pigmentation

genes by generating transgenic house mice transfected with *Peromyscus* pigmentation genes contained in BAC clones. This work will be done in collaboration with Greg Barsh (Stanford).

6. Multigene Families: The Major Histocompatibility Complex. Adam Richman (Montana State University) is studying the maintenance and mechanism of MHC variation using *Peromyscus* as a model. His past studies have focussed on Class II variation (Richman et al. 2001; Richman et al. 2002; Richman et al. 2003). The development of a BAC library would have immediate impact on his studies, by providing important information on the structure of the MHC in this species. It would also permit ready extension of his studies to include Class I variation, which is responsible for presentation of endogenous antigens.

7. Multigene Families: Cytochrome P450. The cytochrome P450 2a-2b-2f cluster is an ideal model for studying the creation and evolution of large gene families. According to Susan Hoffman (Miami U of Ohio) the process is likely to involve a complex series of duplications, insertions, and gene conversions (Chen et al. 2002). She is mapping genes from the cytochrome P450 2a-2b-2f cluster in *Mus musculus* (using BAC clones) so that she can compare it to the corresponding map from humans. The next phase of the evolutionary study will include mapping the gene cluster in a third mammalian species, *Peromyscus,* and doing so will be possible only if a *Peromyscus* BAC library is available.

8. *Peromyscus* Genomics: Synteny Determination by FISH. The Stock Center, in collaboration with Paul Vrana (Vrana et al. 2001), Hopi Hoekstra, and others is the focus of an effort to develop an intermediate-density genetic map, relying heavily on PCR-based Type I (coding sequence) and Type II (microsatellite) markers. The immediate goal is the development and mapping of ~300 markers. These will be useful for QTL mapping for traits associated with the various aspects of *Peromyscus* biology outlined above. *Subsequent cross-referencing of identified QTLs to the marker-rich mouse and human genomes will provide significant clues as to candidate gene loci*. Systems for linkage analysis for map development will include genetic crosses of (*P maniculatus* X *P polionotus*)F_1 as well as a panel of 105 whole genome radiation hybrids (*P maniculatus* X Chinese Hamster Ovary) recently developed at the Stock Center. We and others have developed over 125 microsatellite loci (Prince et al. 2002), and are in the process of developing ESTs (Expressed Sequence Tags) for coding sequence markers, and soon will be doing segregation analysis to define linkage groups.

Supplementing this approach Fluorescent In Situ Hybridization (FISH) analysis has been fruitfully applied by investigators in the Stock Center to determine the extent of *Peromyscus*/*Mus* synteny (Wang et al. 1995; Dawson et al. 1999). *It will be important to more extensively define such syntenic regions using the BAC clones we and others select from the library.*

Peromyscus BAC Library

The primary strain proposed for BAC library construction is *Peromyscus maniculatus bairdii* which is available at the Stock Center. Of all the peromyscine species, *P maniculatus* has the broadest geographical distribution and inhabits the widest range of biomes. *P maniculatus* is the natural host for hantavirus, and a number of other pathogens. The *Peromyscus* linkage map is being developed in *P maniculatus*. The cross of *P m bairdii* with its sister species *P polionotus* is 1) the cross routinely used aforementioned hybrid dysgenesis studies, 2) the cross used for meiotic segregation development of the linkage grant, and 3) the cross used for tracking QTLs for a number of traits. The radiation hybrid panel was developed with *P maniculatus*. If additional funding for a second BAC library were available, *P leucopus* would be the next logical choice. This species is also available at the Stock Center.

We can provide tissues in the form of live animals, flash frozen tissues, splenic lymphocytes embedded in agarose plugs, or cultures of embryonic fibroblasts. High molecular weight DNA from tissue embedded in agarose plugs and size fractionated by pulsed field agarose electrophoresis would also be available.

The genome size is estimated as 3×10^9 bp/haploid genome for both species. A minimum of at least 5-fold coverage and average insert size of at least 120kb are sufficient for the projects above and most others that would make use of a Peromyscus BAC library. A second library from *P leucopus* would be of higher value than 10-fold coverage of *P m bairdii*.

Although it is possible for us to work with any of the BAC production facilities, our physical proximity to Clemson University's Genomic Institute (CUGI) and our interactions with researchers there

would make that our first choice. The library will be of use as soon as it is available and we urge high priority. No other support has been requested for *Peromyscus* BAC library construction.

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