CHROMOSOMAL VARIATIONS IN PEROMYSCUS

BOYLEI GLASSELLI, P. BOYLEI ROWLEYI, AND P. STEPHANI

by

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This thesis has been approved on the date shown below:

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Lecturer
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>6</td>
</tr>
<tr>
<td>Field Work</td>
<td>6</td>
</tr>
<tr>
<td>Standard Karyotypes</td>
<td>6</td>
</tr>
<tr>
<td>Chromosomal Banding</td>
<td>7</td>
</tr>
<tr>
<td>RESULTS</td>
<td>9</td>
</tr>
<tr>
<td>Standard Karyotypes</td>
<td>9</td>
</tr>
<tr>
<td>Chromosomal Banding</td>
<td>14</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>20</td>
</tr>
<tr>
<td>APPENDIX A: COLLECTION RECORDS</td>
<td>29</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>30</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>1.</td>
<td>Collection sites of Peromyscus in southern Arizona and the Gulf of California</td>
</tr>
<tr>
<td>2.</td>
<td>Standard karyotypes of (a) <em>P. stephani</em>, (b) <em>P. b. rowleyi</em>, and (c) <em>P. b. glasselli</em></td>
</tr>
<tr>
<td>3.</td>
<td>C-banded karyotypes of (a) <em>P. stephani</em>, (b) <em>P. b. rowleyi</em> and (c) <em>P. b. glasselli</em></td>
</tr>
<tr>
<td>4.</td>
<td>Pericentric inversion in <em>P. b. glasselli</em>, and heteromorph in <em>P. b. rowleyi</em> (a), C band distinction in no. 1, 22 (homozygous C+ standard:heterozygotes:homozygous C-), X, and Y (b), and composite G banding of <em>P. stephani</em>, <em>P. b. glasselli</em>, and <em>P. b. rowleyi</em></td>
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# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Centromeric index (CI = p/p+q) of chromosomes 1, X, and Y, and length ratio (L = X/Y) for males of <em>P. b. glasselli</em>, <em>P. b. rowleyi</em>, and <em>P. stephani</em></td>
<td>13</td>
</tr>
<tr>
<td>2. C band variation in chromosome 22</td>
<td>18</td>
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</table>
ABSTRACT

This investigation employs the cytological techniques of G and C banding to identify homologues and the location of inversions and heterochromatin in *Peromyscus*. Centromeric indices and lengths of sex chromosomes are also used to measure chromosomal variation. The species studied were two subspecies of *P. boylei*, *rowleyi* from the Sonoran Desert and *glasselli* from the Gulf of California, and *P. stephani* also from the Gulf. All species of *Peromyscus* have a diploid number of 48, but the fundamental number (NF) ranges from 56-96. In this study the NF ranges from 56-58 as a result of pericentric inversions and heterochromatin. G and C band patterns are similar. The increased size of the Y chromosome in *P. b. rowleyi* and of the X chromosome short arm in *P. b. glasselli* result from increases in heterochromatin. Length ratio comparisons (X/Y) between the two *boylei* subspecies show a significant difference, as do comparisons between *P. b. glasselli* and *P. stephani*. This cytological study lends support to suggestions that the affinities of *P. stephani* lie with the *boylei* species group.
INTRODUCTION

Peromyscus (Rodentia, Cricetidae) is a small rodent that ranges over much of the New World, from the arctic of Canada at the north to the tropics of Colombia in the south (Hooper, 1968). At least 57 species occur (Hooper, 1968), many of which are polytypic in both genotype and karyotype. Unlike many mammals whose chromosomes vary in diploid number, all species of Peromyscus have a diploid number of 48; however, the fundamental number (NF) has been observed to range from 56-96 (Hsu and Arrighi, 1966).

This study describes the chromosomes (1) of two species of Peromyscus endemic to islands in the Gulf of California, and (2) of a related mainland species. Members of the genus studied are P. stephani (Townsend) from Isla San Esteban, P. boylei glasselli (Burt) from Isla San Pedro Nolasco, and P. boylei rowleyi (Allen) from two locations in Arizona. At present, P. b. glasselli and P. b. rowleyi, of the subgenus Peromyscus, are considered to be subspecies on the basis of distribution (Hooper, 1968) and morphology (Burt, 1932). Mating studies have not been undertaken to establish their true relationship. P. stephani has been classified in a separate subgenus (Hall and Kelson, 1959), Haplomylosomys, but phenotypically,
genotypically (Avise et al., 1974) and karyotypically (Lawlor, 1971) it resembles the boylei species. The purpose of the present investigation is (1) to produce definitive karyotypes of the species using modern methods of cytogenetics, i.e., chromosome banding, (2) to determine the karyological relationships between the species in terms of these karyotypes, and (3) to assess whether the chromosomal phylogeny thus obtained is compatible with the phylogeny as suggested by other methods (morphology and electrophoresis).

Robertsonian fusion and the process of fission are common mechanisms for chromosomal change in mammals (Hsu and Arrighi, 1966). In the first instance, two acrocentric chromosomes appear to fuse at their centromeres to yield a metacentric chromosome. In the second case, a metacentric chromosome splits at the centromere forming two acrocentrics. Each of these mechanisms produces changes in the diploid number, but does not change the fundamental number. Peromyscus, which maintains a constant diploid number but varying fundamental numbers, must employ other techniques of karyotype evolution. Three mechanisms could alter arm number without affecting the diploid number: (1) heterochromatinization, (2) pericentric inversions, and (3) reciprocal translocations. Up to the present time, only the
first two have been found to occur in *Peromyscus* (Hsu and Arrighi, 1966; Ohno et al., 1966; Singh and McMillan, 1966; Sparkes and Arakaki, 1966; Hsu and Arrighi, 1968; Arakaki, Veomett, and Sparkes, 1970; Te and Dawson, 1971; Bradshaw and Hsu, 1972; Duffey, 1972; Bowers, Baker and Smith, 1973; Schmidly and Schroeter, 1974). Pathak, Hsu and Arrighi (1973) found that heterochromatinization played a role in chromosomal variation in a study which compared the distribution of constitutive heterochromatin (C+ material) in *P. eremicus* (NF = 96) and *P. crinitus* (NF = 56). They found the short arms of both *P. eremicus* and *P. crinitus* chromosomes to be C band positive or heterochromatic. They suggested the increase in genome size of *P. eremicus* could be due solely to late replicating DNA. Heterochromatinization is not the only mechanism at work. Arrighi, Stock and Pathak (1976) and Murray and Kitchin (1976) have described pericentric inversions in *P. leucopus* and *P. maniculatus* populations respectively. Variation in karyotype can thus occur without altering genome size or diploid number, and only the position of the genes need be affected.

Such subtle changes as inversions or translocations generally undetectable by conventional cytological analysis, can be readily observed by chromosomal banding techniques. While the mechanism of G banding is not yet well understood (Hsu, 1973), the patterns produced along the length of the
chromosomes are both repeatable and unique for each pair of homologues. By comparing the G bands of two species of *Peromyscus*, Pathak, Hsu, and Arrighi (1973) found that the G bands are conserved within these species. Other reports are similar for several genera and species of rodents (Mascarello, Stock and Pathak, 1974; Nesbitt, 1974; Pathak, Hsu, and Shirley, 1973), and for primates as well (Stock and Hsu, 1973). The banding techniques allow the localization of chromosomal changes to specific chromosomal regions. Karyotypic polymorphism in *Peromyscus* has been reported within the boylei subspecies by Schmidly and Schroeter (1974). Arm numbers in the boylei subspecies range from NF = 56, in *P. b. rowleyi*, to NF = 86, in *P. b. simulus*. Since the boylei subspecies are polymorphic, it should not be surprising that there is also diversity within boylei populations. Schmidly and Schroeter (1974) found polymorphism in the subspecies beatus, levipes, and spicilegus, but failed to find any chromosomal variation in populations of rowleyi.

Lawlor (1971) found the standard karyotypes of *P. b. rowleyi*, *P. b. glasselli*, and *P. stephani* were identical except for the Y chromosome, which was smaller in *P. stephani* than that in either boylei subspecies. Ward (1976), however, found a different biarm number in *P. b. glasselli* resulting from a pericentric inversion. Avise
et al. (1974) suggested, in an interpretation of their electrophoretic data, that P. stephani, although tentatively placed in the subgenus Haplomyomys, resembled the boylei group of the subgenus Peromyscus. In addition, Lawlor (1971) came to the same conclusion using karyotypic and phenotypic determinations. Because of confusion remaining in the classification of this species, studies involving more critical cytological techniques would seem to be in order.
MATERIALS AND METHODS

Field Work

Five specimens of *P. b. glasselli* were live-trapped on Isla San Pedro Nolasco in the Gulf of California by Ward in March and November of 1975, and November of 1976. Three *P. stephani* were collected in November of 1976 by Ward from Isla San Esteban, also in the Gulf. Nine specimens of *P. b. rowleyi* were live-trapped by Roth at Helvetia, Arizona in May of 1976 and one by Elder near Oracle, Arizona in December of 1976. Field numbers and additional collection data are described in the appendix. The specimens have been deposited in the mammal collection at The University of Arizona for preservation as skins and skulls.

Standard Karyotypes

Chromosome spreads were prepared from bone marrow, lung, heart, and ear tissues. Cultures of lung, ear, and heart were grown for approximately two weeks in McCoy's 5A modified media (Gibco 166-20) with neomycin (0.25 mg/ml final concentration) at 37°C. The cultures were arrested at metaphase with Colcemid (0.1 mcg/ml final concentration) 1-2 hours before harvesting. The monolayer of cells was removed from the surface of the culture flask with 2 ml of
0.25% (w/v) trypsin at 37°C for 5 minutes. These cells were then treated by a modified Patton (1967) method as were bone marrow cells. The cells were placed in a hypotonic solution of 0.075 M KCl at 37°C for 10 minutes and then fixed a 3:1 solution of methanol:glacial acetic acid. Slides were prepared by both flame and air dried techniques.

A minimum of 50 spreads from each animal were scored for the number of metacentric-submetacentric and acrocentric chromosomes. The chromosomes were arranged and numbered according to decreasing length with the exception of the sex chromosomes and the two smallest biarmed pairs, which were placed after the smallest acrocentric autosomes. The centromeric index, defined as the ratio of the short arm to the total chromosomal length \( p/p+q \), where \( p = \text{length of short arm} \) and \( q = \text{length of long arm} \), was determined for the no. 1 and sex chromosomes. The length ratio of the X to the Y chromosome was also scored for males. All measurements were made on a graphics calculator (Numonics Corp., Lansdale, PA).

**Chromosomal Banding**

Various modifications of both C and G banding were tried. The following procedures gave the best results. The C band technique was modified from Arrighi and Hsu (1971). The modifications were as follows: the slides
were treated with 0.2 N HCl for 12 minutes, rinsed in cold distilled water, and again in water at room temperature (23°C). They were then placed in 5% (w/v) Ba(OH)$_2$ at 50°C for 8-12 minutes, rinsed in distilled water, and placed in 2X SSC (0.30 M NaCl + 0.03 M trisodium citrate) at 60°C for 2-3 hours. The cells were then dehydrated in 70% and 95% ethanol baths before staining in 4% (v/v) Giemsa in Gurr's phosphate buffer (pH 6.8) for 20-30 minutes. After drying, the preparations were made permanent with Permount.

The G banding technique varied more with respect to quality than the C banding technique. The most successful technique was one modified from that of Seabright (1971). The modifications were as follows: after drying overnight at 65°C, the slides were placed in 0.01% or 0.005% (w/v) trypsin (1:250 Difco or 3X recrystallized Worthington) solution in Hanks' Balanced Salt Solution (Ca$^{++}$ and Mg$^{++}$ free) for 8-15 seconds, rinsed in Hanks' solution, and then dehydrated in 70% and 95% ethanol baths. The cells were stained 5-10 minutes in 4% (v/v) Giemsa solution in 0.01 M potassium monophosphate buffer. Preparations were permanentized as described previously.
RESULTS

Standard Karyotypes

The study involves two Peromyscus species, P. boylei subspecies rowleyi from Oracle and Helvetia, Arizona and glasselli from Isla San Pedro Nolasco in the Gulf of California and P. stephani from Isla San Esteban in the Gulf (Fig. 1). At least 50 cells of each of 18 animals were counted to assess fundamental and diploid numbers. All of the animals had a diploid number of 48 with a basic karyotype consisting of 20 pairs of acrocentrics, one pair of large submetacentrics, two pairs of small metacentric-submetacentrics, a large submetacentric X and a small metacentric Y (Fig. 2).

All P. b. glasselli were collected from the same ravine on Isla San Pedro Nolasco. Of the five examined, four were found to have the standard arm number of 56, as Lawlor described in 1971, and one was heterozygous for a pericentric inversion in chromosome no. 9 (Fig. 4a). Ward (1976) has found the polymorphism to occur in the proportion 2:3:1 (homozygous standard:heterozygote:homozygous inversion) in a sample of size 6. My data changes the distribution to 5:3:1. P. b. rowleyi was collected from two locations, Helvetia and Oracle, near Tucson, Arizona.
Figure 1. Collection sites of *Peromyscus* in southern Arizona and the Gulf of California.
Figure 2. Standard karyotypes of (a) *P. stephani*, (b) *P. b. rowleyi*, and (c) *P. b. glasselli*. 
All nine specimens from Helvetia had the standard karyotype described by Lawlor (1971). The homologues of the no. 14 chromosome pair were found to be heteromorphic in the one animal from Oracle (Field No. 50). No karyotypic variation was found in the three specimens of *P. stephani*.

The centromeric indices for the X chromosome were significantly different both within and among all species. More variation was found in the X chromosome within the *rowleyi* specimens than in the X chromosome of either *glasselli* or *stephani* (Table 1). The X of *P. b. rowleyi* is significantly more metacentric than that of either of the other species.

The variation in the Y chromosome was also determined by centromeric index analysis. Data from *P. b. glasselli* and *P. b. rowleyi* show that while in each subspecies the largest mean of the Y chromosome is significantly different from the smallest mean, neither is different from the intermediate mean (Table 1). When the data are pooled there is a significant difference between the centromeric indices of *P. b. glasselli* and *P. b. rowleyi* Y chromosomes. The centromeric index of the Y chromosome of *P. stephani* is not significantly different from either *boylei* subspecies. A centromeric index was also determined for the no. 1 chromosome of the same mice. However, because of extensive within species variation in this
Table 1. Centromeric index (CI = p/p+q) of chromosomes 1, X, and Y, and length ratio (L = X/Y) for males of *P. b. glasselli*, *P. b. rowleyi*, and *P. stephani*.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Specimens</th>
<th>Number of Each Chromosome Counted</th>
<th>Mean L</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td><em>P. b. glasselli</em></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 54</td>
<td>12</td>
<td>.538&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.256</td>
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<tr>
<td>No. 16</td>
<td>12</td>
<td>.537</td>
<td>.223</td>
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<tr>
<td>No. 4</td>
<td>18</td>
<td>.475&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.232&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>.527</td>
<td>.237&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. b. rowleyi</em></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 64</td>
<td>18</td>
<td>.578</td>
<td>.325</td>
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<td>.288&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td></td>
<td>33</td>
<td></td>
<td>.304&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. stephani</em></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 25</td>
<td>18</td>
<td>.552</td>
<td>.279&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

a. The high mean is significantly different from the low, but neither are significantly different from the intermediate mean.

b. The mean of each specimen is different from all others within a species.

c. The pooled mean of each species is significantly different from the other species except in the case of L 1.70 and L 1.80.
chromosome, no further comparisons were made. The length ratios of the X:Y chromosomes indicate that the Y chromosomes of *P. b. glasselli* and *P. b. rowleyi* are different, and suggest that the Y of *P. stephani* is very similar to that of *P. b. rowleyi*. The ratios are 2.02, 1.70, and 1.80, respectively. The mean of the Y chromosome in *P. b. glasselli* is about half the size of the X, whereas in *P. b. rowleyi* the Y is approximately two-thirds that of the X. The Y chromosome of *P. stephani* appears only slightly smaller than that of *P. b. rowleyi*. As only one *P. stephani* male was analyzed, no within species variation could be determined. Thus, comparisons with this species should be made with reservation. Lawlor (1971) reported no difference between the karyotypes of *P. b. rowleyi* and *P. b. glasselli* and that those karyotypes varied from that of *P. stephani* only in the size of the Y chromosomes. Lawlor's report was based on a small sample and utilized no chromosomal measurements.

**Chromosomal Banding**

The C band pattern between the species is generally similar (Fig. 3). The short arm of chromosome no. 1 is C positive in all species. In all species the entire arm can be completely heterochromatic or, the heterochromatin can appear as a dark band within the arm. The short arm
Figure 3. C banded karyotypes of (a) P. stephani, (b) P. b. rowleyi and (c) P. b. glasselli.
of the X, the entire Y, and the terminal end of both homologues of a long acrocentric pair (no. 4) are C positive in all specimens. Without fluorescence banding using quinacrine or other fluorescence agents, the C positive material in the long acrocentric cannot be localized to a specific chromosome. Variation in C-band material on the short arm of chromosome 22 was observed in all karyotypes (see Fig. 3 and Fig. 4b). In *P. b. rowleyi* the majority of spreads showed only one homologue banding (Table 2). This is as opposed to the data in *P. b. glasselli* and *P. stephani* where equal frequencies of each category are found. As chromosome 22 is small, many spreads could not be scored definitively.

The G bands are quite similar in all three *Peromyscus* (Fig. 4c). There are discrepancies in the small chromosomes. The most obvious of these irregularities are found in chromosome pairs 10, 16, 18, 19, and 20. The banding of chromosome nos. 18, 19, and 20 in *P. b. rowleyi* often show one wide band in the place of two thinner ones. This difference probably is due to unequal condensation rather than actual structural change. The irregularity in nos. 10 and 16 could be due to treatment or mispairing during construction of the karyotype. No other obviously mispaired homologues seem to be present, however. The small chromosomes are quite hard to match and occasionally
Figure 4. Pericentric inversion in *P. b. glasselli*, and heteromorph in *P. b. rowleyi* (a), C band distinction in no. 1, 22 (homozygous C+ standard:heterozygotes:homozygous C-), X, and Y (b), and composite G banding of *P. stephani* *P. b. glasselli*, and *P. b. rowleyi* (c).
Table 2. C band variation in chromosome 22.

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<tr>
<td></td>
<td>C Positive</td>
<td>7</td>
<td>10</td>
<td>4</td>
<td>21</td>
<td></td>
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</tr>
<tr>
<td>P. b. glasselli</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>One Homologue</td>
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<tr>
<td></td>
<td>C Positive</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>P. b. rowleyi</td>
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<td>Neither Homologue</td>
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one chromosome is left without a good match. The biarmed form of chromosome 14 of *P. b. rowleyi* is probably due to heterochromatinization rather than to an inversion. The G banding shows that there is extra chromatin in the submetacentric homologue (Fig. 4a).
DISCUSSION

Within the boylei species there are 13 subspecies (Hall and Kelson 1959), many of which are chromosomally polymorphic (Lee, Schmidly, and Huheey, 1972; Schmidly and Schroeter, 1974). The majority of the changes appear to be due to pericentric inversions, although banding studies were not performed. Many of the subspecies are polymorphic both between and within populations.

Until recently no chromosomal polymorphisms had been found in P. b. glasselli or P. b. rowleyi (Hsu and Arrighi, 1968; Lawlor, 1971; Lee et al., 1972; Schmidly and Schroeter, 1974). A total of 144 specimens from 14 populations were examined in the four studies cited, showing no polymorphism in P. b. rowleyi. The present study represents the first report of a chromosomal rearrangement in P. b. rowleyi. The only specimen from Oracle that was examined had NF = 57 and was consequently heteromorphic for a short arm in chromosome 14 (Fig. 4a). There is an increase in chromatin in the short arm as shown in Fig. 4a. The change may be due to heterochromatinization. C banding was carried out on four P. b. rowleyi from Helvetia, but not on the specimen from Oracle. There is a possibility that this animal represented a subspecies hybrid, but no other
P. boylei subspecies have been reported to occur in the location. C band studies on the Oracle population should be undertaken to determine the actual mechanism and the extent of the polymorphism. If heterochromatinization is involved, it could have occurred by saltatory replication as described by Murray and Kitchin (1976).

The inversion in P. b. glasselli was found by Ward (1976). All of the glasselli specimens that have been examined were collected from the same ravine on Isla San Pedro Nolasco. The fact that Lawlor (1971) examined only one male could account for his report of no difference between the karyotypes of P. b. rowleyi and P. b. glasselli. The ravine population is polymorphic for an inversion in chromosome 9. Ward's study described animals that were homozygous for the standard karyotype and for the inversion, as well as animals heterozygous for the inversion (see Fig. 4a). The G banding pattern clearly shows that the chromosomal change in P. b. glasselli is the result of a pericentric inversion. Thus, the data from P. b. glasselli confirms reports of others (Arrighi, Stock, and Pathak, 1976; Murray and Kitchin, 1976) that pericentric inversions are an important mechanism in karyotypic evolution in the genus Peromyscus.

Lawlor (1971) stated that P. b. rowleyi and P. b. glasselli were karyotypically the same, and that both
karyotypes were identical to that of *P. stephani* except for length of the Y chromosomes. He reported that the Y chromosome was smaller in *P. stephani* and in the *boylei* subspecies.

Analysis of the centromeric indices show that the Y chromosomes of all specimens are metacentric (Table 1). When the data are pooled there is a statistical difference between the centromeric indices of *rowleyi* and *glasselli* Y chromosomes, the Y of *rowleyi* being more metacentric. The centromeric index describes the relationship of the centromere's position to the rest of the chromosome and does not distinguish between a large and small chromosome. In order to compare the length of the Y chromosome from one species to another, a comparison was made between the ratios of length of the X to the Y chromosome in each (Table 1). The significant difference in this ratio between *rowleyi* (1.70) and *glasselli* (2.02) is probably due to the increased length of the Y in *rowleyi*, although variation could result from an increased length of the X in *glasselli*. The finding that there is a statistical difference in length between the Y chromosome of *P. stephani* and that of *P. b. glasselli*, and none between the Y chromosome of *P. stephani* and that of *P. b. rowleyi* conflicts with Lawlor's report.
In the present study computer analysis shows that there is considerable variation both among and within the boylei subspecies with respect to the centromeric index of the X chromosome (Table 1). The X in *P. b. glasselli* is slightly more submetacentric than that of *P. b. rowleyi* and *P. stephani*. The short arm of the X in all three mice is C positive and no G-band changes were observed. This X variation may be due to either unequal amounts of or condensation differences in constitutive heterochromatin. The centromeric indice, of the *P. stephani* X is intermediate between that of *P. b. rowleyi* and *P. b. glasselli* (Table 1). The results for *P. stephani* should be interpreted only as a trend because of the sample size of one. The definitive conclusion permitted is that the X chromosomes of the two boylei subspecies are significantly different.

The proper method to measure relative lengths of specific chromosomes is to compare their lengths to that of the entire haploid genome. This method could not be employed since the large number of similar acrocentrics make distinction of a haploid set very difficult without G banding. Of the G banded spreads, many did not show complete complements. A modified test of Duffey (1972) was attempted which assumes that the number one chromosome, which is easily identified, is constant in length and therefore a possible indicator for the constant length of the
entire genome. Upon measuring the no. 1 chromosome, however, it was found to be as variable as the X, and therefore was of little value as a standard. The variability of the length of the Y and the short arm of both the X and the no. 1 chromosome is not surprising since all are C positive.

Murray and Kitchin (1976) suggest that increases in constitutive heterochromatin in _Peromyscus_ can occur by saltatory replication. Differential amounts could easily have accumulated in _boylei_ chromosomes since the subspecies were separated probably in early Pleistocene (Lawlor, 1971). Most of the time the short arm of no. 1 appeared entirely C positive, yet never as densely so as the X short arm. Occasionally only a single band occurred in the center of the short arm. The other source of C band polymorphism was chromosome 22. Pattern variability in this chromosome, like that described for chromosome no. 1 was found in all specimens of this study. Since chromosome no. 22 is small, the banding pattern is difficult to determine. The region of centromeric heterochromatin could be very large on this chromosome and the short arm, which is extremely small, might often have appeared to be stained when in reality it was not.

The overall G band pattern has been conserved in the karyotypes of the mice examined, extending similar
observations in other rodents (Arrighi et al., 1976; Mascarell o et al., 1974; Nesbitt, 1974; Pathak, Hsu and Shirley, 1973). A few discrepancies, found in the minor bands of nos. 18, 19, and 20, are probably due to irregular condensation of the chromosomes. Often two close bands appear as one large band. The smaller chromosomes (nos. 10, 16, 18, 19 and 20) are hard to match due to variation in the technique applied and the tissues used. Commonly when bone marrow is used, as was the case for P. b. rowleyi, only the major bands are detectable.

The two boylei subspecies, glasselli and rowleyi appear morphologically (Burt, 1932; Hall and Kelson, 1959) and karyotypically similar (Lawlor, 1971). They are morphologically distinct in skull width and pelage color (Burt, 1932). Karyologically they are distinct in size of the X and Y chromosomes, a heterochromatic short arm, and a centric inversion (this study). Because these mice have been geographically isolated since early in the Pleistocene epoch (Lawlor, 1971), it is not difficult to explain the diversity. A million years is probably sufficient time for natural selection to have effected these changes. The differences in pelage color and skull structure could have occurred by genetic drift or founder effect. As for the chromosomal differences, the increase of length in C
positive chromosome arms could have occurred by saltatory replication (Murray and Kitchin, 1976).

The occurrence of inversions within a species is particularly hard to evaluate. The production of chromosomal races does not seem to be occurring in the manner predicted by some (White, 1973; Wilson et al., 1975; Wilson, Sarich and Maxson, 1974) who suggest that chromosomal heterozygote disadvantage is a primary cause of genetic isolation in small mammals. Because of the relatively high frequency of heterozygotes in the P. b. glasselli population observed in the current study, heterozygote disadvantage does not seem to be significant. All of the specimens are presumed to be from the same breeding population as they were collected from the same ravine. Although no mating studies have been carried out to determine the breeding structure, the number of inversion heterozygotes (3 out of 9 individuals) found in the P. b. glasselli population, would indicate a relatively high level of chromosomal polymorphism can be tolerated. Inversions allow an organism to change its phenotype and adaptability without changing its genotype, by creating new gene interactions and regulation. Heterozygotes fix the genes included in the inversion in a specific combination because recombination is inhibited. Selection can then act on a specific gene arrangement. The high frequency of
heterozygotes in *P. b. glasselli* may be accounted for by a selective advantage of the new gene arrangement.

The extra arm (no. 14) in *P. b. rowleyi* should cause no problem in the heterozygous state. Crossing over is reduced in heterochromatic regions so no meiotic aberrations should occur. The heterochromatin may play a role in regulation and thereby change the adaptability of the organism.

*P. stephani* was tentatively placed in subgenus *Haploomyomys* by Hall and Kelson (1959) and Hooper (1968). Since that time two studies have questioned its placement and have suggested it be included in the *boylei* group of subgenus *Peromyscus* (Avise et al., 1974; Lawlor, 1971). In Townsend's (Hall and Kelson, 1959) original description he compared *P. stephani* to *P. eremicus*, a mainland *Haploomyomys* species, and found them similar for dentition and color alone. Avise et al. (1974) compared *P. stephani* with many other *Haploomyomys* species by electrophoretic analysis of serum proteins. They found that the serum proteins of *P. stephani* were not similar to those of any of other *Haploomyomys* species tested. The karyotype of *P. stephani* and *P. eremicus* represent the two extremes of variation within the genus; that of *eremicus* consists of 48 biarmed chromosomes (NF = 96), while that of *stephani* comprises mostly acrocentrics (NF = 56). My cytological
study, in which *P. stephani* shows homology with respect to G and C banding with two subspecies of *P. boylei* supports the proposal of Lawlor (1971) and Avise et al. (1974) that *P. stephani* should be classified with the *boylei* species group. Additional studies will be needed to determine the final taxonomic status of *P. stephani*. For example, the species should be compared morphologically and electrophoretically with other species of *Haplomyomys* such as *P. crinitus* that have a karyotype similar to *P. stephani*.

There are significant studies that have yet to be done with *P. stephani*, *P. b. rowleyi*, and *P. b. glasselli*. These involve studies concerning the location and amounts of satellite DNA, which could lead to another distinction among their karyotypes. Also studies of matings between *P. b. glasselli* and *P. b. rowleyi* should be included, with particular emphasis on the analysis of meiosis in hybrids and heterozygotes.

*P. b. glasselli* and *P. b. rowleyi* are karyotypically distinct and *P. stephani* is quite similar to *P. b. rowleyi*, contrary to Lawlor (1971). Computer analysis has permitted the detection of chromosomal variation at a level not possible by conventional cytological studies. As a result of chromosomal banding and computer graphics, differences that could not be seen six years ago, are now detectable.
APPENDIX A

COLLECTION RECORDS

**Peromyscus boylei glasselli.** Mexico: Isla San Pedro Nolasco, 1 ♀ (no. 55), 4 ♀♂ (nos. 4, 16, 46, and 54), collected by Dr. O. G. Ward in 1975 and 1976.

**Peromyscus boylei rowleyi.** Arizona: 2 mi. SE Oracle, 1 ♂ (no. 50), collected by Mr. F. Elder in 1976; 2 mi. E Helvetia, 5 ♀♀ (nos. 57-61), 3 ♀♂ (nos. 62-64), collected by Dr. E. Roth in 1976.

**Peromyscus stephani.** Mexico: Isla San Esteban, 2 ♀♀ (nos. 18 and 56), 1 ♂ (no. 25), collected by Dr. O. G. Ward in 1976.
REFERENCES


