DETERMINATION OF THE PRIMITIVE KARYOTYPE FOR
PEROMYSCUS

IRA F. GREENBAUM AND ROBERT J. BAKER

ABSTRACT.—In order to determine the most probable primitive karyotype for the
*Peromyscus maniculatus* complex and for the genus *Peromyscus*, G- and C-band data
for *P. floridanus* and *P. gossypinus* are analyzed and compared to data available for
other species. It is concluded that the primitive karyotype for the genus is characterized
by chromosomes with heterochromatin restricted to centromeric regions. Heterochro-
matic short arms appear to have been added independently to several *Peromyscus*
lineages, suggesting that heterochromatin provides some selective advantage in this
genus. When autosomes are identified according to the standardized karyotype for
*Peromyscus* (Committee, 1977), the primitive karyotype is biarmed for pairs 1, 22, and
23; pairs 2, 3, 6, and 9 may or may not be biarmed, and all other pairs are acrocentric.
Based on the trend in the *P. maniculatus* group for orthoselection, which increases the
number of biarmed elements, it is hypothesized that chromosomes 2, 3, 6, and possibly
9 are acrocentric in the primitive karyotype and that *P. crinitus*, with only chromosomes
1, 22, and 23 biarmed, has retained an essentially primitive karyotype for the genus.
The euchromatic long arm of the X chromosome has remained stable throughout all
species examined, although heterochromatic short arms on the X are quite variable. Y
chromosomes are biarmed and C-band positive in all species examined, but position of
the centromere varies. Chromosome banding data support the hypothesis of Bowers
et al. (1973) that the primitive karyotype for the *P. maniculatus* group is like that of *P.
melanotis* with 30 acrocentric elements. C-bands reveal that heterochromatin in the
primitive karyotype of the *P. maniculatus* group was confined to the centromeric re-
geons. The primitive karyotype for this group has pairs 1, 2, 3, 6, 9, 20, 22, and 23
biarmed and all other pairs acrocentric. *Peromyscus floridanus* (subgenus *Podomys*)
shares what are believed to be derived chromosomal inversions with members of the
subgenus *Peromyscus*, suggesting evolutionary affinities between these subgenera.
Chromosomal banding data appear to have considerable promise as a tool for deter-
mining clades within *Peromyscus*.

Determination of the primitive versus derived karyotypic condition for groups of
organisms is of considerable value in cytogenetic and evolutionary studies. Knowl-
dge of what constitutes derived chromosomal configurations is essential in deter-
mining the types of chromosomal events that are favored by survival as opposed to
those which are selected against. Such a determination enables an evaluation of the
evolutionary value of specific types of chromosomal events. For example, in the genus
*Peromyscus* it is well documented that a large amount of chromosomal variation is
accounted for by the presence or absence of heterochromatin. If karyotypes with large
amounts of heterochromatin are primitive, then there is selection for the deletion of
heterochromatin. Conversely, if the primitive condition was characterized by minimal
amounts of heterochromatin, then obviously there has been selection for additions of
heterochromatin, which in some way must confer selective advantage to the organism.
In the former case, heterochromatin might be envisioned as "junk DNA," whereas in
the latter, heterochromatin would be envisioned as genetically valuable. Conclusions
concerning the selective value of heterochromatin are strongly affected by conclusions
concerning primitive versus derived chromosomal conditions.

From a systematic point of view, determination of primitive versus derived kary-
yotypic conditions can be of great value in evaluating evolutionary affinities between
species. Presence of the same derived (synapomorphic; Hennig, 1966) karyotypic
arrangements in species or species groups is indicative of common ancestry for these
taxa and may serve as a basis for their systematic arrangement. It is essential for this


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type of determination to understand, which arrangement of the chromosomes is primitive (plesiomorphic) and which constitute rearranged (apomorphic) conditions.

In this paper we evaluate the G- and C-band data available for the genus Peromyscus in an attempt to determine the most probable condition for the primitive karyotype of the P. maniculatus group and for the common ancestor of the genus. We also present a dendrogram of chromosomal change from our hypothesized primitive condition to the karyotypes characteristic of living species.

A determination of primitive versus derived karyotypic conditions is dependent initially upon a priori knowledge of the relationships of the organisms being studied. Species, which may be considered primitive by morphological criteria, may or may not possess a primitive karyotypic condition. It is essential, therefore, that a determination of primitive versus derived karyotypic conditions be based (at least initially) on closely related species for which the evolutionary relationships are reasonably well understood. If the evolutionary affinities of a group of closely related species are known, then by determining the degree of chromosomal rearrangement between the species, it should be possible to determine, which chromosomal arrangements are primitive and which are derived. An example of this approach using the maniculatus group of Peromyscus is described below.

Deer mice of the genus Peromyscus are the most widely distributed and intensively studied native wild mammals in North America. Peromyscus maniculatus is probably the best known species of the genus (King, 1968). According to Hooper (1968) the abundant information on the biology of P. maniculatus group species is in harmony with the view that P. melanotis and P. polionotus are species, which have evolved independently from a pre-maniculatus parental stock as a result of peripheral isolation.

Bowers et al. (1973) hypothesized that the primitive karyotype for the maniculatus group of the subgenus Peromyscus was composed of a large number of acrocentric elements (probably near 30). Based on G- and C-band data for three species (P. maniculatus, P. polionotus, and P. melanotis) of the maniculatus complex, Greenbaum et al. (1978b) added to this hypothesis the condition that the primitive maniculatus group karyotype contained little autosomal heterochromatin and the G-banded arrangement characteristic of P. melanotis. In the euchromatic autosomal complement, maniculatus differs from melanotis and polionotus by four inversions (chromosome pairs 10, 11, 13, and 15). Additionally, maniculatus has heterochromatic short arms on chromosome pairs 17 and 21, whereas heterochromatin was not associated with these pairs in melanotis or polionotus. The karyotypes of maniculatus and polionotus share inversion rearrangements for two pairs of chromosomes (5 and 14) and heterochromatic associations for three pairs of chromosomes (16, 18, and 19). Because polionotus and melanotis are not believed to have shared ancestry after diverging from the maniculatus lineage, the most logical explanation for the common acrocentric condition of chromosome pairs 10, 11, 13, 15, 17, and 21 (all of which are biarmed in maniculatus), is that the acrocentric condition of these six pairs was present in the karyotype of the maniculatus group evolutionary stock prior to the divergence of polionotus and melanotis. The common biarmed condition of pairs 5 and 14 and heterochromatic associations of pairs 16, 18, and 19 in maniculatus and polionotus were suggested to be the result of pericentric inversions and heterochromatic additions in a common ancestral lineage between polionotus and maniculatus after the isolation of the stock which is now melanotis. The above assumptions are summarized in Fig. 9.

Peromyscus floridanus is the sole representative of the subgenus Podomys. This species is well differentiated from, and is not thought to be closely related to, any of the other species of Peromyscus (Hooper, 1968). The gross karyotype of P. floridanus
(Hsu and Arrighi, 1968) is very similar to that of *P. melanotis* (Bowers et al., 1973). A determination of the G- and C-banding patterns of *P. floridanus* should provide a critical outgroup test of the hypothesis proposed by Bowers et al. (1973) and Greenbaum et al. (1978b) that the primitive karyotype for the *maniculatus* group is essentially that characteristic of *P. melanotis*. If the similarity in numbers of biarmed and acrocentric chromosomes between *melanotis* and the distantly related *floridanus* is reflected by common arrangement of the G-bands, particularly for chromosome pairs 5, 10, 11, 13, 14, and 15, and if autosomal heterochromatin is essentially restricted to the centromeric regions, then this would be strong evidence in support of the 30 acrocentric primitive karyotype of the *maniculatus* group. A G- and C-banded analysis for *Peromyscus floridanus* is presented below. Additionally, we present G- and C-banding data for *P. gossypinus*. *Peromyscus gossypinus* (subgenus *Peromyscus, leucopus* group) also is characterized by 30 acrocentric autosomes. Further analysis of chromosomal evolution in *Peromyscus* can be provided by a determination of the homologies of this species to both the *maniculatus* group species and to *P. floridanus*.

**Materials and Methods**

G- and C-band preparations were obtained from fibroblast tissue-cultured cells initiated from ear biopsies as described in Greenbaum et al. (1978a). The G-banded chromosomes in our figures are numbered and arranged according to the standardized karyotype for *Peromyscus* (Committee, 1977).

**Results**

Specimens of *P. floridanus* studied had a diploid number of 48. Chromosome morphology is identical to that reported by Hsu and Arrighi (1968), with eight pairs of biarmed and 15 pairs of acrocentric autosomes. The X is a large subtelocentric and the Y is a small metacentric. No intraspecific chromosomal polymorphism was detected.

G- and C-band karyotypes of *P. floridanus* are presented in Fig. 1a and 1b, respectively. C-banding reveals that heterochromatin is restricted to the centromeric regions. None of the arms of the biarmed chromosomes (including the X) appear heterochromatic. The Y chromosome stains C-band positive.

G- and C-band data for *P. gossypinus* (Fig. 2a and 2b) reveal that the karyotype of this species is identical to that shown for *P. floridanus* (Fig. 1a and 1b) except that pair 11 is acrocentric and differs by a pericentric inversion. Pair 11 in *P. gossypinus* is identical in morphology to that shown for *P. melanotis* and *P. polionotus* (Fig. 8). C-banding of *P. gossypinus* reveals telomeric heterochromatin on pairs 21, 22, and 23. The short arm of the X does not stain C-band positive, and the nature of the Y is not known.

Composite G-banded karyotypes of the species *maniculatus, polionotus, melanotis*, and *floridanus* are presented in Figs. 3–8. An entire haploid complement from a single cell for each species is shown in Figs. 3–8. Due to the overall similarity between the G-banded patterns of *P. floridanus* and *P. gossypinus* and, for simplicity in discussion, only *P. floridanus* is shown in the composite karyotypes (Figs. 3–8). Ten pairs of autosomes appear unchanged in G-band patterns in all four species (Fig. 3). Additionally, the sex chromosomes appear to have undergone little change. The Y chromosome and the short arm of the X chromosome in *maniculatus, polionotus*, and *melanotis* are known to be C-band positive (Greenbaum et al., 1978a, 1978b), whereas the short arm of the X is euchromatic in *floridanus* and *gossypinus*.

Five pairs of chromosomes (16, 17, 18, 19, and 21; Fig. 4) differ between species by the presence or absence of heterochromatic short arms. Heterochromatin may be present on all five pairs in *maniculatus*, and pairs 16, 18, and 19 in *polionotus*, but het-
Fig. 1.—(a) G-banded and (b) C-banded karyotypes of *Peromyscus floridanus*. Homologous pairs in this and subsequent figures are numbered according to the standard *Peromyscus* karyotype (Committee, 1977).
Fig. 2.—(a) G-banded and (b) C-banded karyotypes of *Peromyscus gossypinus*.
erochromatic short arms have not been found on *gossypinus*, or on any autosomal pairs in *melanotis* and *floridanus*.

Figs. 5–8 include chromosome pairs, which have been altered between the species by pericentric inversion. C-banding reveals that heterochromatin is confined to the centromeric regions of these chromosomes.

Pairs 10, 13, and 15 (Fig. 5) are biarmed in *maniculatus* but are acrocentric (with the G-band pattern for the short arm inverted) in *polionotus*, *melanotis*, *gossypinus*, and *floridanus*. Pair 20 (Fig. 6) displays the identical biarmed condition in the three *maniculatus* group species studied and an acrocentric condition in *floridanus* and *gossypinus*. Pairs 5 and 14 (Fig. 7) are biarmed in *maniculatus* and *polionotus*, whereas in *melanotis*, *floridanus*, and *gossypinus* these chromosomes are acrocentric. Chromosome pair 6 (Fig. 8) is biarmed in *melanotis*, *gossypinus*, and *floridanus* and acrocentric in *polionotus*. In *maniculatus*, this pair is biarmed but appears slightly different from that of *melanotis* and *floridanus* (note the two bands proximal to the centromere in the long arm of chromosome 6 in *maniculatus*, as opposed to only one such band in both *melanotis* and *floridanus*). Pair 6 has been reported to be poly-
FIG. 4.—Composite partial G-banded karyotype comparing homologous elements of *Peromyscus maniculatus*, *P. polionotus*, *P. melanotis*, and *P. floridanus*, showing those elements, which differ due to the involvement of heterochromatic segments.

FIG. 5.—Composite partial G-banded karyotype comparing homologous elements of *Peromyscus maniculatus*, *P. polionotus*, *P. melanotis*, and *P. floridanus*, showing those pairs which differ in *P. maniculatus* by a pericentric inversion.

FIG. 6.—G-banded chromosomes comparing homologous elements of pair 20 of *Peromyscus maniculatus*, *P. polionotus*, *P. melanotis*, and *P. floridanus*, which is different in *P. floridanus* by a pericentric inversion.

FIG. 7.—G-banded chromosomes comparing homologous elements of pairs 5 and 11 of *Peromyscus maniculatus*, *P. polionotus*, *P. melanotis*, and *P. floridanus*. *P. maniculatus* and *P. polionotus* have the same banding pattern but differ from *P. melanotis* and *P. floridanus* by a pericentric inversion.

FIG. 8.—Composite partial G-banded karyotype comparing homologous elements of *Peromyscus maniculatus*, *P. polionotus*, *P. melanotis*, and *P. floridanus*. For pair 6, *P. polionotus* displays
morphic in *P. maniculatus* (possibly for a small inversion) and may be acrocentric or biarmed (Greenbaum et al., 1978a). Pair 11 is acrocentric in *polionotus*, *melanotis* (Fig. 8), and *gossypinus* (Fig. 2a). *Peromyscus maniculatus* and *floridanus* are biarmed for pair 11, but these conditions are not identical. The short arm in the *floridanus* pair 11 is longer than is the short arm for this pair in *maniculatus*.

**Discussion**

**Primitive Autosomal Karyotype for the P. maniculatus Group**

Greenbaum et al. (1978a, Fig. 8) presented a phylogenetic arrangement for the *maniculatus* group species *maniculatus*, *polionotus*, and *melanotis* based on G- and C-band chromosome data. The occurrence of shared chromosomal arrangements between the species in association with the well-documented evolutionary affinities of the *maniculatus* species group (see Hooper, 1968) were interpreted as supporting Bowers et al. (1973) hypothesis that the primitive karyotype for this group was composed of a large number of acrocentric elements (probably near 30). Additionally, the primitive karyotype was proposed to have little autosomal heterochromatin (Greenbaum et al., 1978b).

The autosomal banding configuration of *P. floridanus* differs from that of *melanotis* by only two chromosomal alterations (pairs 11 and 20, Figs. 6 and 8) and that of *gossypinus* differs only by the acrocentric condition of pair 20 in this species. Chromosome pair 20 is biarmed in all of the *maniculatus* group species thus far studied, whereas pair 11 displays both acrocentric and biarmed morphs in this group. The unaltered acrocentric conditions of chromosome pairs 5 and 14, and lack of heterochromatic short arms on acrocentric pairs 16, 18, and 19 between *melanotis*, *floridanus*, and *gossypinus*, indicate that the events, which resulted in biarmed chromosomes in *maniculatus* and *polionotus* for these pairs, occurred in the *maniculatus* group stock after the isolation of the line which is now *melanotis*. The overall extent of unaltered chromosome homology between *melanotis*, *gossypinus*, and *floridanus* supports the hypothesis that the primitive autosomal karyotype for the *maniculatus* group was essentially that of *P. melanotis*, and that karyotypic evolution in the *maniculatus* group has involved an increase in the number of biarmed chromosomes by additions of heterochromatin and pericentric inversions.

If *P. melanotis* has retained the primitive condition for the *maniculatus* group, then some interesting comments about the evolutionary significance of heterochromatin may be in order. *Peromyscus maniculatus* has the greatest amount of intraspecific chromosomal variability and C-band positive material of any of the species of the *maniculatus* group thus far examined (Greenbaum et al., 1978a, 1978b). Additionally, *P. maniculatus* is the most widely distributed, ecologically adapted, and successful member of this species group and of any small mammal native to North America. *Peromyscus melanotis* has a more restricted geographic and ecological range and the least amount of autosomal C-band positive material. If the *maniculatus* karyotype has evolved from a *melanotis*-like karyotype, then the heterochromatic short arms have been added to the genetic complement of the former rather than deleted from the latter. The fact that the most successful species of this complex has the greatest amount

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an acrocentric condition, whereas the other species are biarmed. For pair 11 *P. polionotus* and *P. melanotis* are acrocentric. *Peromyscus maniculatus* and *P. floridanus* are biarmed but the size of the second arms and distances between the centromere and G-positive band of the long arm in each case suggest that these conditions resulted from independent pericentric inversions in the two groups.
of C-band positive material and the fact that this condition is derived rather than primitive suggest positive evolutionary value for C-band positive material. How heterochromatin is valuable to this species is presently unknown.

**Primitive Autosomal Karyotype for the Genus Peromyscus**

There has been little agreement on what constitutes the primitive karyotype for the genus *Peromyscus*. In a consideration of the karyotypes of cricetine rodents, Baker and Mascarelo (1969) suggested that the primitive condition of this subfamily was largely composed of acrocentric elements.

Lawlor (1974), however, argued that acrocentric chromosomes represent a derived condition and not a primitive one. The subgenus *Haplomyomys* is generally considered to possess characters primitive for the genus (Hooper, 1968; Lawlor, 1971). Lawlor’s contention that a biarmed karyotype is primitive for *Peromyscus* is based upon biarmed chromosome predominance in this group. Additionally, Lawlor states that "the evidence suggest strongly that changes in the relative numbers of acrocentric and biarmed chromosomes occur in different directions, and that such changes are adaptive and dependent on the particular environmental circumstances to which the organisms are exposed."

G- and C-banding data for *P. maniculatus*, *P. melanotis*, and *P. polionotus* support the hypothesis that *P. melanotis* (with 30 acrocentric elements) has the least derived
karyotype of these three species. Lawlor's (1974) contentions that changes in the relative numbers of acrocentrics and biars occur in different directions and that such changes are adaptive and dependent upon the particular environment are not supported by G- and C-band data from species in the *maniculatus* group. Conversely, karyotypic orthoselection appears as an increase in the number of biarmed elements. The data suggest that in *Peromyscus* chromosomal characters evolve less rapidly than do general morphological, physiological, and behavioral features and, therefore, are better indicators of evolutionary history of members of this genus.

Bandung data have been reported for nine species of *Peromyscus* (Table 1). Fig. 9 diagrammatically shows our interpretation of chromosomal evolution for eight of these species. Based on the evolutionary trend in the *maniculatus* group to increase the number of biarmed elements and amount of heterochromatin, we believe that *P. crinitus* has retained the most primitive euchromatic karyotype for the genus. The karyotype of *P. crinitus* has been reported to have a heterochromatic short arm on chromosome number 1 (Arrighi et al., 1976). With the exception of numbers 1, 22, and 23, all autosomal elements are acrocentric. Our C-band preparations did not reveal C+ short arms on the number 1 chromosomes of the *Peromyscus* species *maniculatus*, *melanotis*, *polionotus*, *gossypinus*, and *floridanus*. Two problems complicate a determination of the evolutionary process regarding chromosome 1. Heterochromatic regions on different chromosomes often display differential responses to C-band treatment. For example, heterochromatic Y chromosomes in *Peromyscus* rarely stain with intensity equal to autosomal C+ short arms. The reported C+ associations to chromosome 1 in *P. crinitus* and *P. eremicus* (Arrighi et al., 1976; Fig. 1a and 1b) are considerably lighter stained than are all other heterochromatic segments shown. It is difficult from these figures to determine whether or not the short arms of chromosome 1 in these species are truly heterochromatic. The second complication involves the difficulty in comparing G-banding results between species from independent reports. It is possible that only the euchromatic long arm of chromosome 1 in *P. crinitus* and *P. eremicus* is homologous to the biarmed condition of the species in the right hand lineage of Fig. 9. If the biarmed conditions of both lineages in Fig. 9 are completely homologous, then there are inconsistencies in either C-band staining or heterochromatin between the lineages. If, however, *P. eremicus*, *P. crinitus*, and *P. boylilii* do have C+ short arms on chromosome 1 (A+ condition, Table 1) and chromosome 1 in the other species examined is homologous to the euchromatic long arm, then pericentric inversion and subsequent heterochromatic addition (or deletion) distinguish the evolution of this chromosomal pair in the two main branches of Fig. 9. Further banding analyses incorporating more species should ultimately resolve this problem. In the scheme presented in Fig. 9 we have assumed that the chromosome number 1 is identical between the species examined. The alternatives discussed above should, however, be considered when interpreting phylogenetic relationships for additional species.

If the karyotype of *P. crinitus* represents the primitive karyotype for the genus, then the pericentric inversions for pairs 2, 3, 6, and 9 (Fig. 9) occurred at the base of the right hand lineage of the cladogram. However, the presence of a biarmed condition for chromosome 9 in *P. boylilii*, and in all species in the right hand lineage (Fig. 9), suggests that this chromosome probably was biarmed in the primitive karyotype. *Peromyscus boylilii glasellii* (Committee, 1977) appears identical in G-banding pattern to *P. crinitus* (Pathak et al., 1973; Arrighi et al., 1976) except for a polymorphism for a pericentric inversion in chromosome number 9 (O. W. Ward, personal communication). The biarmed morph of 9 in *P. boylilii* is indistinguishable from the biarmed condition of 9 in *floridanus*, *gossypinus*, and the *maniculatus* group species.

Pathak et al. (1973) compared the G-bands of *P. crinitus* and *P. eremicus* and found that the bands in the euchromatic long arms are identical. The karyotype of *eremicus*,
| Species                  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | Source                  |
|--------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--------------------------|
| Peromyscus leucopus       | B  | B  | B  | A  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | B  | Greenbaum et al., 1977  |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | Greenbaum et al., 1977  |
| Peromyscus maniculatus    | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | Greenbaum et al., 1977  |
| Peromyscus brevicaudus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |
| Peromyscus polionotus     | B  | B  | B  | B  | B  | B  | B  | B  | B  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | A  | This study               |

**Table 1**—Summary of chromosomal G-banding data for nine species of Peromyscus. + = acrocentric; B = biarmed heterochromatic; A = acrocentric, short arm heterochromatic; ‡ = morphology of same designations not identical.
then, could have been derived from our proposed ancestral karyotype by addition of heterochromatic short arms to all elements except numbers 1 and 23.

Chromosome pair 11 is acrocentric in all species examined except floridanus and maniculatus. These data suggest that the biarmed condition of pair 11 in floridanus resulted from an inversion in floridanus lineage after it was isolated from the maniculatus group stock (Fig. 9). The biarmed condition of pair 11 in maniculatus is apparently the result of an independent inversion in this species. As mentioned above, the biarmed conditions in these two species are not identical (Fig. 8).

All of the maniculatus group species thus far examined are biarmed for chromosome pair 20. This lineage seems to be differentiated chromosomally from the P. floridanus and P. gossypinus lineages by an inversion in this chromosome. Based on its unique cranial and penal morphology (which serve as a basis for its placement in a distinct subgenus and its position in Fig. 8), Peromyscus floridanus was quite probably an early isolate off of the right-hand lineage of the cladogram. The inversion for pair 20 in the maniculatus group ancestral stock apparently occurred after the isolation of this stock from the other species in this clade.

Chromosome pair 6 is acrocentric in P. polionotus (and in crinitus and boyliti) but is biarmed in maniculatus, melanotis, and floridanus. The situation is further complicated by the fact that a chromosomal polymorphism (involving an acrocentric and biarmed condition) for chromosome 6 exists in P. maniculatus (Greenbaum et al., 1978a). It is possible that chromosome number 6 has undergone a second pericentric inversion in the pre-polionotus-maniculatus stock, resulting in the recreation of an acrocentric chromosome similar to that in crinitus and boyliti (and to the euchromatic arm of eremicus). It is not possible, from the data presently available, to definitely establish the evolutionary trend for this chromosomal pair in these species; however, multiple inversions must be involved.

G- and C-bands have been published for P. leucopus (Arrighi et al., 1976). Interstitial heterochromatin and polymorphism for heterochromatic short arms in P. leucopus were reported by these authors. From the figures presented by Arrighi et al. (1976), we were able to determine homologs for only the nine largest chromosomes of P. leucopus (Table 1). None of these appear to have heterochromatic short arms. Peromyscus leucopus is biarmed for chromosome pairs 2, 3, 6, and 9, which suggest association of P. leucopus with the lineage on the right side of Fig. 9. Additionally, pair 5 is biarmed in P. leucopus. The only other species thus far examined, which are biarmed for pair 5, are maniculatus and polionotus. Although this may suggest a close affinity between leucopus and maniculatus and polionotus, it will not be possible to place P. leucopus in the scheme presented in Fig. 9 until the remainder of the chromosomal arrangements (particularly that of pair 20) of this species are determined.

Peromyscus gossypinus is considered to be most closely related to P. leucopus. G-banding data for P. gossypinus (Fig. 2a) reveals that this species is biarmed for pairs 2, 3, 6, and 9 and that the banding patterns are indistinguishable from those for these pairs in the maniculatus group species and P. floridanus. Peromyscus gossypinus is acrocentric for the eight pairs, which have undergone inversions in the floridanus and maniculatus group lineages. The data suggest that P. gossypinus shared a common ancestor with the clade on the right hand side of Fig. 9. The karyotype of this species has apparently remained unchanged since the divergences of the maniculatus group and P. floridanus except for heterochromatic additions to pairs 21, 22, and 23. Peromyscus gossypinus is acrocentric for pair 5, and if this species and P. leucopus truly represent a common lineage, then the evolution of chromosome pair 5 in these and the maniculatus-group species will require at least two independent pericentric inversions. Further banding analyses on P. leucopus may resolve this problem and enable placement of the leucopus group species in the arrangement presented in Fig. 9.
Evolution of Heterochromatin in Peromyscus

If our hypotheses about the primitive chromosomal conditions for Peromyscus are correct, then independent heterochromatic additions appear to be characteristic of three of the lineages thus far studied (Fig. 9). Peromyscus eremicus has added the most heterochromatin and is apparently stable for C+ short arms associated with nearly all of the autosomal elements. Several close relatives of P. eremicus (P. merriami, P. interparietalis, and P. eva) have entirely biarmed chromosomal complements and P. guardia has only a single acrocentric pair. Although C-banding data are not yet available for these species, it seems likely that many, if not all, short arms (with the exception of pairs 1, 22, and 23) will prove to be heterochromatic.

Peromyscus leucopus has been reported to display a great deal of intraspecific karyologic polymorphism, at least some of which can be attributed to heterochromatic differences (Arrighi et al., 1976). The C-band karyotype presented by these authors has heterochromatic short arm associations to seven pairs of autosomes. Two of these pairs were polymorphic for the C+ short arms. Three of the homozygous pairs with heterochromatic short arms appear to be the three pairs (21, 22, and 23), which show C+ additions in P. gossypinus.

The third lineage to display heterochromatic additions is the P. maniculatus-P. polionotus stock of the maniculatus group. Heterochromatic short arm associations and karyotypic polymorphism in this group are well documented (for a review, see Bowers et al., 1973, and Greenbaum et al., 1978a). The independent addition of heterochromatin to several lineages within the genus is consistent with our hypothesis that C+ material has positive evolutionary value and that this group of cricetine rodents is in some way preadapted for the addition of heterochromatin.

Evolution of the Sex Chromosomes

The X chromosomes of the species of Peromyscus thus far examined are very stable with respect to the euchromatic long arm, displaying the same G-banded pattern in all species. The short arm is entirely or partially heterochromatic in all these species except floridanus, gossypinus, and leucopus in which the second arm is quite short. In P. polionotus the length of the heterochromatic short arm of the X was found to vary even with the same individual (Greenbaum et al., 1978b). The G-banding pattern of the euchromatic long arm of X chromosomes has been found to be stable among 77 species of mammals (Pathak and Stock, 1974). The euchromatic long arm of Peromyscus apparently has remained relatively unchanged over the course of evolution of the genus.

The Y chromosome is extremely variable in Peromyscus (Hsu and Arrighi, 1968). In those species thus far analyzed for heterochromatin, the Y stains C-band positive. From the available data we are not able to determine a primitive condition for the Y sex element.

Systematic Implications

G-band chromosomal data have some important systematic implications. For instance, based on classical studies (Hooper, 1968) there has been no agreement as to the evolutionary affinities of P. floridanus (the sole member of the subgenus Podomys). However, G-banding data suggest that this subgenus has a common evolutionary history with the subgenus Peromyscus.

The arrangement of species in Fig. 9 suggests a basic dichotomy in the genus Peromyscus and disagrees with the current view of interspecific evolutionary affinities (Hooper, 1968, Table 2) with reference to certain species. The species on the left of Fig. 9 seem to be characterized by very little rearrangement of the assumed primitive euchromatic autosomal complement and, therefore, may or may not be closely related.
However, the lineage on the right of Fig. 9 is characterized by numerous derived pericentric inversions, which would require a common evolutionary ancestor for the *maniculatus* and *leucopus* groups of the subgenus *Peromyscus* and *P. floridanus* (subgenus *Podomys*).

In summary, G-band chromosomal data appear to be an excellent tool for evolutionary and systematic studies in the genus *Peromyscus*. The primitive karyotype for the genus appears to have been composed primarily of acrocentric elements and heterochromatin has been added rather than deleted. As hypothesized by Bowers et al. (1973), the primitive karyotype for the *P. maniculatus* complex appears to be essentially that of *P. melanotis*. G-banding data do not support Lawlor's (1974) conclusion that changes in numbers of acrocentric and biarmed chromosomes occur in different directions, and are adaptive and dependent on the particular environmental circumstances to which the organisms are exposed.

**Specimens Examined**

Specimens of *Peromyscus floridanus* examined in this study were as follows: (2♂, 1♀) Florida: Orange Co.; 2.8 mi N, 2.2 mi E Union Park. Specimens of *P. polionotus* were reported by Greenbaum et al. (1978b), *P. melanotis* and *P. maniculatus* by Greenbaum et al. (1978a), *P. crinitus*, *P. eremicus* and *P. leucopus* by Arrighi et al. (1976), *P. boylitt by Committee (1977). The specimen of *P. gossypinus* (♀) was from the same locality reported above for *P. floridanus*. Voucher specimens are deposited in the Division of Mammals, The Museum, Texas Tech University. Frozen cell lines are maintained in the Division of Living Tissues, The Museum, Texas Tech University.

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**Literature Cited**


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*Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409 (present address of Greenbaum: Department of Biology, Texas A&M University, College Station, Texas 77843). Submitted 14 March 1978. Accepted 10 May 1978.*