KARYOTYPIC ORTHOSELECTION FOR ADDITIONS OF HETEROCHROMATIC SHORT ARMS IN GRASSHOPPER MICE (ONYCHOMYS: CRICETIDAE)

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ABSTRACT.—A comparison of the G- and C-banded chromosomes from Onychomys torridus torridus to those of other known karyotypic forms of peromyscine rodents results in a revision of the proposed primitive karyotype for the genus Onychomys. The primitive karyotype for Onychomys probably contained five pairs of armed autosomes with euchromatic short arms and 17 pairs of acrocentric autosomes. Morphology of one pair is uncertain; however, it was either biarmed with a heterochromatic short arm or acrocentric. If the primitive karyotype is as hypothesized and currently accepted systematic arrangements are correct, many convergent heterochromatic short arm additions or deletions must have occurred during the evolution of the cytotypes of Onychomys in order to explain their distribution in living taxa. Although the respective fundamental numbers of O. t. torridus and O. arenicola differ by two, G- and C-band data reveal that as many as 15 heterochromatic short arm differences may distinguish the two cytotypes.

All karyotypes reported for grasshopper mice of the genus Onychomys have a constant diploid number of 48, but variation in fundamental number ranges from 72 to 92 (Hsu and Benirschke, 1967, 1968; Hinesley, 1979). Baker et al. (1979) reported that all variation among the FN = 92, FN = 80, FN = 78 and FN = 72 karyotypic forms can be explained by the presence or absence of heterochromatic short arms with no alterations of the euchromatic segments. Those authors concluded that the primitive karyotype for Onychomys was biarmed in chromosomes 1, 2, 3, 9, 14, 19, 22, and 23 and that chromosomes 2, 3, and 14 had heterochromatic short arms. Baker et al. (1979) concluded that convergent additions of heterochromatic short arms must have occurred if the present distribution of heterochromatic short arms among these taxa is to be explained. A third cytotype of O. torridus (FN = 74) has been described from standard karyotypes (Hinesley, 1979). The number of arms in the autosomal complement (fundamental number) can be related most parsimoniously to the standard karyotype of O. arenicola (FN = 72) by a single addition or deletion of a heterochromatic short arm. However, G-band data presented in this paper indicate that explanation of the differences in these cytotypes requires several additional events which, if the currently accepted systematic arrangements are accurate, means an even greater amount of convergent addition of heterochromatic short arms has occurred in the genus. Data from the FN = 74 cytotype also result in a revision of the proposed primitive karyotype for the genus. Additionally, G-band data from this cytotype provide evidence in support of the proposed primitive peromyscine karyotypes (Greenbaum and Baker, 1979; Yates et al., 1979; Baker et al., 1979).

METHODS AND MATERIALS.—G- and C-banded karyotypes were prepared from tissue cultures of fibroblast from lung and ear biopsies as described by Greenbaum et al. (1978). Identification and numbering of G-banded chromosomes are according to the standardized karyotype for Peromyscus (Committee, 1977). The karyotype in Fig. 1a is from a single metaphase. Figure 1b is a composite
karyotype containing haploid complements of a single metaphase from Onychomys arenicola and Onychomys torridus torridus, respectively. Cell lines from both individuals studied were suspended by freezing in The Museum, Division of Living Tissues, Texas Tech University. Specimens examined were one male and one female from 14 mi. SE Portal on road to Rodeo, Cochise Co., Arizona. Both specimens are deposited in the Division of Mammals, Texas Tech University.

The direction and sequence of chromosomal evolution in the taxa involved in this discussion were determined by cladistic methods (Hennig, 1966) as employed by Mascarello and Hsu (1976), Greenbaum and Baker (1979), Yates et al. (1979), and Baker et al. (1979). In this study we have used data from G- and C-banded chromosomes of Peromyscus (Committee, 1978; Greenbaum and Baker, 1979), Baiomys (Yates et al., 1979) and Neotoma (Mascarello and Hsu, 1976) as outgroups.

RESULTS.—Both specimens of Onychomys torridus torridus examined had a diploid number of 48, with a fundamental number of 74. Chromosomes 1, 9, 19, 22, and 23 are biarmed with euchromatic short arms (Fig. 1a). Chromosomes 11, 12, 13, 14, 16, 17, 18, 20, and 21 are biarmed with heterochromatic short arms (Fig. 1a). The X chromosome is a large submetacentric chromosome and the Y chromosome is a small submetacentric which stains C-band positive.

Chromosomes 1, 9, 19, 22, and 23 of O. t. torridus are identical in G-band patterns to O. arenicola (Fig. 1b) and to the three other karyotypic forms of Onychomys examined in Baker et al. (1979). The euchromatic segments of all other autosomes also are identical in G-banding pattern to the proposed homologous segments of O. arenicola (Fig. 1b), as well as to those of the other three karyotypic forms (Baker et al., 1979) which have been studied.

DISCUSSION.—Hinesley (1979) found that all specimens of O. t. torridus examined from south-central New Mexico and extreme West Texas have 10 pairs of acrocentric chromosomes, as opposed to nine pairs in specimens from southern Arizona. Hinesley studied a contact zone in southwestern New Mexico and found no hybridization between these two karyotypic forms. As a result of karyotypic data and a morphological analysis, Hinesley (1979) raised the FN = 72 form to specific status, Onychomys arenicola.

G- and C-band data for Onychomys arenicola, and for three other cytotypes, O. leucogaster, O. t. longicaudus, and O. t. pulcher, were examined by Baker et al. (1979). In those cytotypes, five pairs (1, 9, 19, 22, and 23) of autosomes are biarmed with euchromatic short arms. All other autosomes which are biarmed have heterochromatic short arms. Onychomys leucogaster (FN = 92) was found to have a totally biarmed karyotype. The karyotype of representatives of O. t. pulcher (FN = 80) contains six pairs (5, 6, 7, 8, 10, and 11) of acrocentric elements, whereas representatives of O. t. longicaudus (FN = 78) have seven pairs (4, 5, 6, 7, 8, 10, and 11) of acrocentric elements. The karyotype of specimens of O. arenicola (FN = 72) has 10 pairs (8, 10, 12, 13, 15, 16, 17, 18, 20, and 21) of acrocentric elements. From these data, Baker et al. (1979) hypothesized a primitive karyotype for these karyotypic forms which was biarmed in chromosomes 1, 2, 3, 9, 14, 19, 22, and 23 and that the short arms of chromosomes 2, 3, and 14 were heterochromatic.

The data presented herein support those results, but indicate that a greater number of independent convergent changes in number of heterochromatic short arms must have occurred than was thought previously. In at least one of the five known karyotypic forms of Onychomys, each autosome except 1,
9, 14, 19, 22, and 23 is found in the acrocentric condition, which suggests that the primitive karyotype for *Onychomys* was biarmed in chromosomes 1, 9, 14, 19, 22, and 23, but not in chromosomes 2 and 3, as proposed by Baker et al. (1979). Of the six elements that are biarmed in all five cytotypes, only 14 has a heterochromatic short arm, and it should be noted that the short arm of 14 varies greatly in size and, therefore, this chromosome may also have been acrocentric in the primitive karyotype and the short arms of this chromosome in the present taxa may be the result of independent additions rather than a common event.

Our data are compatible with the conclusions of Greenbaum and Baker (1979) and Yates et al. (1979) which suggest that the karyotype of the ancestral *Peromyscus* lineage was essentially acrocentric and that the karyotype of the *Peromyscus-Baiomys* progenitor was essentially acrocentric. Since *Ony-
chomys has long been considered to be a close relative of Peromyscus and Baiomys (Hibbard, 1968; Hooper, 1968), evidence that Onychomys also may have had an essentially acrocentric primitive karyotype is compatible with those conclusions. Every chromosome in the proposed Peromyscus-Baiomys primitive karyotype can be found in an identical condition in Onychomys, with the exception of chromosome 14 (which may also have been acrocentric in Onychomys) and 19 (which differs from the primitive Peromyscus-Baiomys chromosome 19 by a pericentric inversion). If the primitive Peromyscus-Onychomys-Baiomys karyotype was essentially biarmed, similar to that which occurs in Peromyscus eremicus, then deletion of heterochromatic short arms must have occurred in several different lineages; respectively, the lineages of Baiomys taylori, Onychomys torridus, and Onychomys arenicola, and in several lineages of Peromyscus. Also, many cases of heterochromatic short arm deletion and subsequent pericentric inversion of a portion of the remaining euchromatic arm would be necessary to explain the euchromatic biarmed chromosomes which occur in Onychomys (pairs 9 and 19) and in many species of Peromyscus (Greenbaum and Baker, 1979; Yates et al., 1979). The hypothesis of an essentially acrocentric primitive karyotype for the progenitor of Baiomys, Onychomys, and Peromyscus requires only a single event (an inversion or an addition of a heterochromatic short arm) during the evolution of these chromosomes to explain their morphology in the taxa which have been studied. Additions of heterochromatin and pericentric inversions from an acrocentric to a biarmed condition offer the most parsimonious explanation of the presently available data for these genera.

Because of the compatibility of the proposed primitive karyotypes of Greenbaum and Baker (1979), Yates et al. (1979), and the present paper, we have proposed only additions of heterochromatin in the subsequent cladograms of Onychomys (Figs. 2 and 3). It should be kept in mind that deletions are also possible and, in some examples, a deletion after an addition may result in a more parsimonious explanation in that fewer events are required. However, we hypothesize that numerous convergent heterochromatic additions have occurred and we have constructed our cladogram based on additions only. From our data and those presented in Baker et al. (1979), a cladogram (Fig. 2) involving the least number of independent events can be drawn and even this most parsimonious arrangement of events requires seven additions which occurred independently twice and one addition which occurred independently three times. This arrangement implies that Onychomys leucogaster shared a common ancestor with one karyotypic form of O. torridus after divergence from the other forms of O. torridus and that O. leucogaster shared a common ancestor with O. torridus after the divergence of O. arenicola. This phylogeny is unlikely because O. arenicola has recently been regarded as being conspecific with O. torridus (Hinesley, 1979) and they probably are more closely related to each other than either is to O. leucogaster. If the currently recognized systematic relationships are correct, then the cladogram presented in Fig. 2 cannot be accurate. Another cladogram is shown (Fig. 3) which assumes that the three karyotypic forms of O. torridus shared an ancestor which was not shared with either O. arenicola or
O. leucogaster and that O. arenicola is more closely related to O. torridus than to O. leucogaster. This arrangement requires 11 events which occurred independently twice and four events which occurred independently three times. If the current systematic relationships in the genus Onychomys are accurate and if the proposed primitive karyotype is correct, then orthokaryoselection (White, 1973) for addition of heterochromatic short arms has occurred in these taxa. It is clear that the living forms of this genus cannot be arranged in such a manner that changes in heterochromatic short arms can be explained as a single event in each homologous pair.

The various cytotypes of Onychomys and Peromyscus vary extensively in amount and placement of C-band positive material, whereas other groups such as phyllostomatid bats show little C-band variation (Patton and Baker, 1978). Whether these differences in karyotypic evolutionary mechanisms are a result of external selective pressures or a result of unequal mutation rates between different groups is unclear. In some closely related groups, species such as Peromyscus maniculatus and Onychomys leucogaster which have
the greatest amount of heterochromatin also have the largest geographic distribution (Baker et al., 1979). This correlation and the hypothesis that heterochromatic short arms have been added to the karyotype in these groups raise many questions concerning possible advantages to an organism which undergoes this type of cytogenetic phenomenon. Clearly, taxa of peromyscine rodents which have the greatest amount of heterochromatin are not at any apparent disadvantage. Peromyscine rodents appear to offer a very fertile area in which to study the role of heterochromatin in evolution.

These data should serve as a caveat for systematists who might use heterochromatic additions or deletions to indicate phylogenetic relationships. For instance, Peromyscus eremicus and Onychomys leucogaster have what are thought to be highly derived karyotypes with many additions of heterochromatic short arms, but this similarity in heterochromatic short arms is best explained as convergent evolution, not a common ancestry after separation from other Peromyscus and Onychomys or a retained primitive condi-
tion (sympleismorphic). Heterochromatic additions appear to have limited value as a taxonomic tool.

Additionally, it should be noted that it has been shown that in areas of a chromosome which contain highly repetitive DNA sequences, repeated breaks may occur (Lee, 1975). Repeated breaks in the same segment could result in convergent inversions or translocations, which could lead to erroneous taxonomic conclusions in cytotaxonomy. Such possibilities lead to a need for several different events (inversions, translocations, etc.) to identify a clade, before a high level of credibility can be placed in the conclusion that the forms had a common ancestor, as implied by chromosomal synapomorphism.

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