Chromosome Variation in Peromyscus: A New Mechanism

Abstract. *Differences in total chromosome lengths between two karyotypically divergent groups of* Peromyscus maniculatus *are taken as evidence for an addition-deletion mechanism of chromosomal variation in the species. The differences may be due in part to variation in the amount of constitutive heterochromatin present in the two karyotypes.*

Karyotypic variation in the genus *Peromyscus* is characterized by a constant diploid number ($2n = 48$), and by a wide variation in the respective numbers of acrocentric, and metacentric or submetacentric, chromosomes (*1*). Most investigators have assumed that pericentric inversion is the chief mechanism by which this type of variation arises (*1*); however, there has been no evidence demonstrating that this kind of inversion occurs in *Peromyscus*. I have gathered data that suggest that a second mechanism, that of additions or deletions of material in the short arms of some chromosomes, account for some of the karyotypic variations observed in the genus.

Standard cytological preparations from bone marrow were made by the method of Lee (*2*) from five female mice from each of two isolated montane populations of *P. maniculatus* in Arizona. Both groups of mice are currently assigned to the subspecies *P. m. rufinus*. The karyotype from one population that was collected on Mount Lemmon in southern Arizona is characterized by a high number of acrocentric chromosomes (30), while the other population, collected on Mingus Mountain in the central part of the state, has a karyotype with relatively low numbers of acrocentric chromosomes (12–16).

Five metaphase spreads from each of the ten female mice were photographed. The negatives were projected at the same magnification, and were traced on large sheets of paper from which straight line measurements of short and long arms were made with a dial caliper. The short- and long-arm lengths were added to obtain data on total chromosome length. To compare chromosome lengths between cells, the absolute length data were normalized with respect to the two longest autosomes of each cell. This pair of autosomes was common to both populations, as judged by similarities between their absolute total lengths and their centromeric indices (Table 1). The arm-length data were normalized by setting the absolute length of the standard longest pair in each cell equal to an arbitrary value of 100 units. The sum of the measurements of the short arms, of the long arms, or of the total lengths of the diploid set in each cell was then converted to a value relative to the longest pair by the following formula:

$$\sum_{1}^{48} {}_{\mathrm{RP}}X_i = \frac{100\left(\sum_{1}^{48} {}_{\mathrm{AL}}X_i\right)}{{}_{\mathrm{AL}}T_1 + {}_{\mathrm{AL}}T_2}$$

where $\Sigma_{\mathrm{RP}}X_i$ = lengths of the short arms, of the long arms, or of the total chromosome length in a cell relative to the standard pair; $\Sigma_{\mathrm{AL}}X_i$ = sum of the corresponding absolute lengths per cell; and ${}_{\mathrm{AL}}T_1 + {}_{\mathrm{AL}}T_2$ = absolute total length of the standard pair in the cell.

An analysis of variance [a completely randomized design with subsampling, Model I (*3*)] was carried out on each set of the three normalized measurements, as well as on the absolute total lengths and centromeric indices of the standard pair. The results are presented in Table 1. These data indicate that the Mingus population has significantly more material in the short arms of its chromosomes as compared to that in chromosomes of the Lemmon population. This is expected regardless of the mechanism by which the karyotypic differences arose. However, the Mingus population also exhibits significantly more total chromosomal material per cell as compared to that in cells of the Lemmon population, and the two populations have equal amounts of long-arm chromosomal material per cell. These results would be expected only if additions or deletions of material in the short arms of chromosomes contributed to the observed karyotypic variation. Exactly the opposite results, with respect to the long arms and total lengths, would be expected if inversion was the sole mechanism responsible for the karyotypic variation observed in *Peromyscus*.

To determine the type of chromosomal material that might account for an addition-deletion mechanism for chromosomal variation, standard cytological preparations from two male mice from each population were tested for the presence and localization of constitutive heterochromatin by the method described by Arrighi and Hsu (*4*). This technique is a slight modification of the hybridization method of Pardue and Gall (*5*). Constitutive heterochromatin is observed at the centromeres of most chromosomes in both populations. In addition, six or eight submetacentric chromosomes in the Mingus karyotype have short arms composed almost entirely of constitutive heterochromatin (Fig. 1). A quantitative study of differences in the amount of heterochromatin in the two populations was not attempted because animals were unavailable at the time these cytochemical studies were undertaken. These initial observations do suggest that some of the differences in length

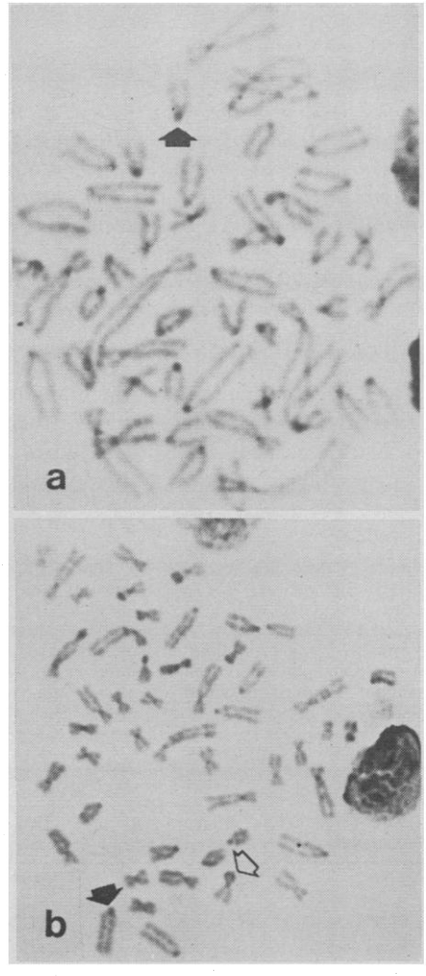

Fig. 1. (a) Metaphase spread from cell of Mount Lemmon male showing darkened areas of constitutive heterochromatin localized at the centromeres (closed arrows). (b) Metaphase spread from cell of Mingus male showing constitutive heterochomatin at the centromeres and on the short arms of some metacentric and submetacentric chromosomes (open arrow).

Table 1. Mean length data from chromosome arm measurements. AL, mean absolute lengths of standard pair; CI, centromeric indices of the standard pair. The short arms, long arms, and the total are the normalized mean lengths based upon the standard pair.

Test	Chromosome measurements		F
	Lemmon	Mingus	
AL standard	62.4	63.0	0.01
CI standard	0.25	0.25	0.2
Short arms	231.4	323.9	180.7*
Long arms	929.4	922.8	0.5
Total	1160.8	1246.6	56.6*

* Means differ at $P < .01$.

as well as some of the differences in karyotype between the two populations may be due to variations in the amount of constitutive heterochromatin present in the two karyotypes.

These results do not exclude the possibility of pericentric inversions contributing to chromosome variations in *Peromyscus*. They do indicate that another mechanism, addition or deletion of chromosomal material, is an important factor contributing to chromosomal variation in these organisms. If the differences in length and the differences in karyotype are due to differences in the amount of constitutive heterochromatin, then rather large amounts of karyotypic variations of the type reported in *Peromyscus* could occur with relatively few rearrangements within euchromatic portions of the genome.

PATRICIA A. DUFFEY

Zoology Department, Arizona State University, Tempe 85281

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Microbial Uptake of Lead

Abstract. Micrococcus luteus *and* Azotobacter *sp. cells grown in broth in contact with a dialysis membrane containing lead bromide were found to immobilize 4.9 and 3.1 × 10^2 milligrams of lead per gram of whole cells, on a dry weight basis, respectively. Culture turbidity and cell count measurements on these and other cell cultures show that lead bromide, lead iodide, and lead bromochloride in concentrations approaching solubility limits have no detectable effect on overall growth rate and cell viability. Analyses of cellular subfractions reveal that fractions of cell wall plus membrane contain 99.3 and 99.1 percent of the lead found associated with* Micrococcus luteus *and* Azotobacter *sp., respectively. The remainder is found associated with the cytoplasmic fractions.*

We have studied effects of several inorganic lead salts on a number of microorganisms as part of an investigation of the possibility that there is a lead cycle in nature, for which animals are the focal point. Previous studies have indicated that lead causes a marked increase in the resistance of red cells to hypotonic saline solutions (*1*), is of questionable toxicity to *Azotobacter* (*2*), and does not affect specific cell functions (*3*). While it has been reported that lead can affect respiration in cell-free extracts of corn mitochondria (*4*), the effect of inorganic lead on whole cells might be quite different if lead must first penetrate the cell membrane. This report concerns the immobilization of inorganic lead by microbial cells and the fraction of immobilized lead that passes through the cell envelope into the cytoplasm.

Microbial isolates were obtained from samples of fresh lake water at Fort Collins Park and from various soil samples by standard techniques (*5*) for the following: photosynthetic bacteria; aerobic, nonsymbiotic, and nitrogen-fixing bacteria; sulfur-reducing bacteria; and selected fungi. Selected Gram-positive and Gram-negative bacteria in pure cultures were also studied. Each culture isolate was grown in broth in the presence of PbI_2, $PbBr_2$, and PbBrCl in concentrations of the salt ranging from 0 to 0.7 mg per milliliter of solution. *Azotobacter* and *Micrococcus* were also grown in media containing 2.5 mg of $PbBr_2$ per milliliter. Precautions were taken in inoculating the growing cultures to prevent their being killed by sudden increases in lead concentration. Both measurement of culture turbidity with a Beckman spectrophotometer and the viable cell count show that the three lead salts in the concentrations employed had no measurable effect on overall growth rate and cell viability. One can immediately raise the question of whether lead is passing through the membrane or cell wall.

The distribution of lead among cellular subfractions for *Azotobacter* sp. (soil isolate) and *Micrococcus luteus* FD 533 (*6*) was determined. *Azotobacter* sp. was grown in either 100-ml or 1000-ml quantities of Burk's nitrogen-free medium (*7*). *Micrococcus luteus* was grown in Trypticase soy broth (Baltimore Biological Laboratories). Lead salts were introduced through suspended dialysis membranes to keep salt crystals from direct contact with cells and thus prevent shock due to sudden localized increases in lead concentration. The preparations were sterilized in a steam autoclave and inoculated with biologically active cultures (5 ml per 100 ml of the medium); the inoculated preparations were incubated at 25°C with agitation in a rotary shaker. In the early stationary phase of growth, cells were harvested by centrifugation and

Table 1. Distribution of lead in bacterial cultures. Values are averages for four separate culture preparations for each species. Approximately 300 mg of $PbBr_2$ was placed in dialysis bags and submerged in the culture. Each culture was inoculated with 5 ml of biologically active culture per 100 ml of medium. Cells were harvested by centrifugation. Approximately 242 mg [0.12 mg total lipid phosphorus (*12*)] and 115 mg [0.93 mg total lipid phosphorus (*12*)] of cells, on a dry weight basis, were obtained for *Micrococcus luteus* and *Azotobacter* sp., respectively. The culture medium is the supernatant solution obtained after cell removal. Uninoculated preparations were used as controls.

Substrate	Total lead recovered			
	Micrococcus luteus		*Azotobacter* sp.	
	Micrograms	Percentage	Micrograms	Percentage
Whole cells	1,180	80.2	35,650	99.94
Culture medium	290	19.7	13	0.04
Whole cell wash	2	0.1	8	0.02
Control	520		1,700	