

SHORT COMMUNICATION

Restriction fragment length polymorphisms in satellite DNA distinguish chromosomal races of the white-footed mouse *Peromyscus leucopus*

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Abstract

We describe a polymorphism revealed by a high-copy-number tandem repeat which serves to distinguish most individuals sampled (96%) from two chromosomal races of *Peromyscus leucopus*. Classical morphology, allozymes, mtDNA, and rDNA have all failed to provide fixed markers which separate these two chromosomal races. Data from *P. leucopus* further documents the utility of DNA polymorphisms to establish the natal origin (DNA 'zipcodes') of populations or individuals.

Keywords: DNA polymorphisms, DNA zipcodes, molecular cloning, *Peromyscus leucopus*, population genetics, satellite DNA

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Introduction

The white-footed mouse *Peromyscus leucopus* is a widely distributed and abundant species that occurs from south-eastern Canada to south-eastern Mexico. From an overview, this is among the most intensively studied non-game North American species (Lackey, Huckaby & Ormiston 1985). Such a species provides an excellent opportunity to document and understand how aspects of the genome are distributed and evolve in natural populations.

Peromyscus leucopus is divided into two chromosomal races that are distinguished by three presumed pericentric inversions (Baker *et al.* 1983; Stangl 1986). The two races hybridize along a contact zone in central Oklahoma (Stangl 1986). The magnitude of morphological evolution that has accompanied the establishment of these two chromosomal races has been minimal. For example, the variation based on skin and skull characteristics and measurements is no greater than is typically characteristic of conspecific populations (Hall 1981).

One current focus in population biology is to identify

molecular markers that correspond to subdivisions within populations and species (Brown 1980; Baker *et al.* 1989; Longmire *et al.* 1991). Several attempts have been made to find molecular markers which correlate with the occurrence of the chromosomal races in *P. leucopus* (Avisé *et al.* 1983; Nelson, Baker & Honeycutt 1987).

Examination of allozymes by starch gel electrophoresis (38 presumptive loci) and mitochondrial DNA (mtDNA) by restriction-site analysis failed to document a single locus or restriction site that reliably distinguished individuals of the two chromosomal races (Nelson *et al.* 1987). However, both mtDNA and allozymes did document that the center of the hybrid zone is identical to that calculated from chromosomal frequencies (Nelson *et al.* 1987). Restriction analysis of the ribosomal cistron, from individuals across the hybrid zone, also failed to identify any sites that define the chromosomal races (R. J. Baker unpublished data).

Here we examine the geographic distribution of a restriction enzyme polymorphism in a tandemly repeated portion of the *P. leucopus* genome in order to document the effectiveness of this polymorphism in identifying the geographic origin of individuals.

Materials and methods

All specimens ($n = 54$) examined were collected from natural populations throughout the geographic range of the species (Fig. 1). Thirty-two mice were collected from four sites along a transect of the hybrid zone in Oklahoma as described elsewhere (Stangl 1986; Nelson *et al.* 1987). Our sample of hybrids came from an interbreeding population at the center of the contact zone. The hybrid status of specimens from Oklahoma was determined by chromosomal markers. All specimens are deposited as voucher skins and skeletons in The Museum, Texas Tech University.

High-molecular-weight DNA was isolated from liver, muscle, or heart and kidney using previously described methods (Longmire *et al.* 1988). DNA was digested using

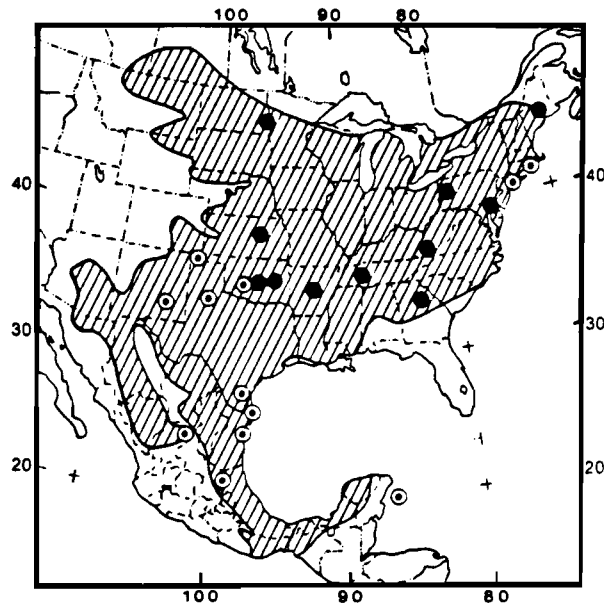


Fig. 1 Geographic distribution of *P. leucopus* (shaded area). The solid circles represent localities where individuals that have the 1.4-kb fragment were collected. Open circles with dots represent localities where individuals that do not show the 1.4-kb band were collected. The three circles in central Oklahoma indicate localities along the hybrid zone. In Oklahoma, all of the individuals examined with hybrid ($n = 14$) and north-eastern ($n = 12$) karyotypes display the 1.4-kb band, whereas all of the individuals with south-western cytotypes ($n = 6$) did not show the 1.4-kb band. One individual each was collected from: Saline Co., Ark.; Clarke Co., Ga.; Riley Co., Kan.; Penobscot Co., Me.; Allegany Co., Md.; Lincoln Co., N.M.; Rockland Co., N.Y.; Cass Co., N.D.; Allegheny Co., Pa.; Jim Wells Co., R.I.; Haywood Co., Tenn.; Alice Co., Tex.; Garza Co., Tex.; Kenedy Co., Tex.; Giles Co., Va.; Ontario, Canada; San Luis Potosi, Mexico; Quintana Roo, Mexico; Tamulipus, Mexico; and Zacatecas, Mexico. Localities (all in Oklahoma) where $n > 1$ were: Cimmaron Co. ($n = 2$); Hughes Co. ($n = 2$); Kiowa Co. ($n = 6$); McIntosh Co. ($n = 12$); and Potawattomie Co. ($n = 12$).

reaction conditions recommended by the supplier (New England BioLabs).

Digested DNAs were separated on 1.5% agarose gels at 35–40 V for periods of 30–40 h. DNA in the agarose gels was denatured and transferred to Boehringer Mannheim positively charged nylon membranes using capillary transfer under alkaline conditions. Membranes were prehybridized for 2 h at 42°C in 35% formamide, $6\times$ SSC, 5-mM EDTA (pH 8.0), and 0.25% w/v powdered milk (Vassart *et al.* 1987). Hybridization was performed overnight at 42°C in the same solution containing 1×10^6 c.p.m./ml probe. The K-18 probe was nick translated to specific activities greater than 10^8 c.p.m./ μ g. Following hybridization, blots were washed twice for 15 min at 22°C in $2\times$ SSC, 0.1% SDS, and twice for 15 min at 50°C in $0.1\times$ SSC, 0.1% SDS. Washed blots were autoradiographed at -70°C in cassettes containing intensifying screens.

The satellite DNA clone K-18 was isolated from a genomic library of *Sau3A1* partially digested *P. leucopus* DNA (individual collected from Connecticut) ligated into pBR322 (Wichman *et al.* 1985, 1990). The restriction map of this clone has been described elsewhere (Hamilton, Hong & Wichman 1992).

Results

Digestion of *P. leucopus* genomic DNA with *Sau3A1* or *PvuII* followed by electrophoresis and Southern blot analysis using K-18 as a probe, revealed a tandem-repeat ladder that was present in all individuals. In addition, a 1.4-kb band was observed that was characteristic of the north-eastern cytotype (Fig. 2). A 1.3-kb band was also visible but it was not as diagnostic for individuals of the north-eastern cytotype. To determine the distribution of the 1.4-kb marker, representative individuals were screened from throughout the range of the species (Fig. 1). The 1.4-kb band was present in nine of the 11 individuals examined from localities within the range of the north-eastern chromosomal race and absent in all 11 of the individuals from the south-western chromosomal race. We also examined 32 individuals from Oklahoma using G-bands to document that the individuals were of north-eastern ($n = 12$), south-western ($n = 6$), and hybrid ($n = 14$) origins. In the Oklahoma specimens, the 1.4-kb band was present in all of the north-eastern and hybrid individuals tested, and was absent from individuals with the south-western karyotypes.

Discussion

Although the clone K-18 was originally isolated from *P. leucopus*, it hybridizes to DNA and chromosomes of all species ($n = 9$) of *Peromyscus* examined (Baker & Wichman 1990; Wichman *et al.* 1990; Hamilton *et al.* 1992).

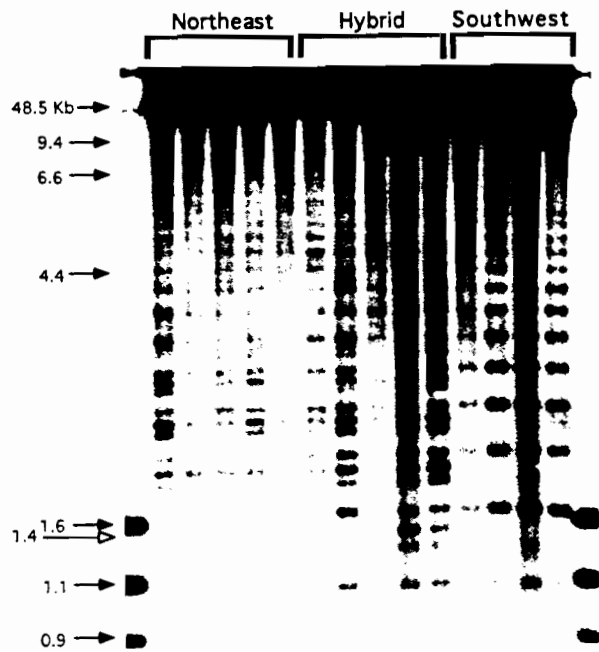


Fig. 2. Genomic DNA from individuals with north-eastern, hybrid, and south-western cytotypes digested with *Pvu*II, electrophoresed, Southern blotted, and hybridized to K-18. Open arrow indicates the diagnostic 1.4-kb band.

Fluorescent *in situ* hybridization has shown that this tandemly repeated element is distributed in the centromeric region of all 48 chromosomes, as well as in other heterochromatic short arms which are sometimes present in the *Peromyscus* karyotype. Southern blot analysis has documented restriction length variants that differ between species (Wichman *et al.* 1990). In the present study, we demonstrate that K-18 also detects variation within a single species, *P. leucopus*. The 1.4-kb band is present in 21 of 23 individuals from the north-eastern chromosomal race and in 12 individuals of hybrid origin and was absent in 11 individuals from the south-western chromosomal race (Fig. 2). No other marker thus far described so strongly correlates with the chromosomal data. The two individuals that are not properly classified to the correct chromosomal race by the 1.4-kb polymorphism were collected from New York and Rhode Island. At least three possibilities exist to explain this phenomenon:

1. it is possible that these individuals were physically transplanted from a region within the range of the south-western cytotype;
2. concerted evolution may have resulted in the loss of this site from these individuals;
3. the diagnostic site may have arisen in parts of the north-eastern race but never became established in the New York and Rhode Island populations.

These two individuals, notwithstanding the observation that a single marker found in satellite DNA is accurate in identifying the origin of 96% of the individuals, is significant, especially in light of the fact that such diagnostic markers were not revealed by studies of allozymes, mtDNA, and rDNA. These results further document the utility of satellite DNA markers as geographic zipcodes that can be used in conservation and management of natural populations and to better document basic population genetics (Longmire *et al.* 1988; Baker *et al.* 1989).

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