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 tDNA 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

Received 15 May 1992; revision received 3 September 1992

Introduction

The white-footed mouse Peromyscus leucopus is a widely distributed and abundant species that occurs from southeastern 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 (Hail 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 (Avise 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 cistrone, 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 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× 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× 10⁶ c.p.m./ml probe. The K-18 probe was nick translated to specific activities greater than 10⁶ c.p.m./µg. Following hybridization, blots were washed twice for 15 min at 22°C in 2× SSC, 0.1% SDS, and twice for 15 min at 50°C in 0.1× 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 PstI 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).
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).

Acknowledgments

We thank K.L. Bowers, L.L. Janecek and M. S. Powell for reviewing the manuscript. R.D. Bradley, N.C. Brown, M.D. Engstrom, M.J. Hamilton, M. Maltbie, C.A. Porter, R.A. Van Den Bussche, and S.M. Witte assisted in collecting the specimens and in laboratory work. This study was funded by grants from NSF and NIH to RJB and HAW, respectively.

References


Longmire J, Ambrose RE, Brown NC et al. (1991) Use of sex-linked minisatellite fragments to investigate genetic differentiation and migration of North

This paper is a result of collaborative research between the laboratories of R. J. Baker (Texas Tech University), J. L. Longmire (Los Alamos National Laboratory), and H. A. Wichman (University of Idaho at Moscow) to develop the use of DNA markers to identify populations of widely distributed species. The satellite repeat was cloned and characterized by H. A. Wichman and T. W. Reeder with the support of NIH. Population analyses were performed by A. D. Simmons, J. L. Longmire, and R. J. Baker at Texas Tech University and Los Alamos National Laboratory with support from NSF and the US DOE.