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Mus and *Peromyscus* chromosome homology established by FISH with three mouse paint probes

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Abstract. Fluorescence-labeled DNA probes constructed from three whole house mouse (*Mus domesticus*) chromosomes were hybridized to metaphase spreads from deer mouse (*Peromyscus maniculatus*) to identify homologies between the species. *Mus* Chr 7 probe hybridized strongly to the *ad*-centromeric two-thirds of *Peromyscus* Chr 1q. Most of *Mus* 3 probe hybridized principally to two disjunct segments of *Peromyscus* Chr 3. *Mus* Chr 9 probe hybridized entirely to the whole *Peromyscus* Chr 7. Three *Peromyscus* linkage groups were assigned to chromosomes, based on linkage homology with *Mus.* The data also are useful in interpretation of chromosomal evolutionary history in myomorphic rodents.

Introduction

The development of fluorescently labeled chromosome paint probes from one species that can be hybridized to metaphase cell preparations from a different species provides a useful means for identifying genomic homologies (Hayes 1995; Kurtz and Zimmer 1995; Chaudhary et al. 1998). Prior to this development, whole-chromosome homology was based on matching G-banded karyotypes, with the inherent problem of misassignment of segments with similar patterns (Sawyer and Hozier 1986).

Among myomorphic rodents, matching G-band patterns exhibit similarities (Koop et al. 1984), but some species, notably house mouse (*Mus domesticus*), depart significantly from the consensus karyotype. In the case of *Mus*, this is a particular problem, since this species has been extensively mapped by recombination genetics. Thus, it has been difficult to establish chromosome assignments for species with gene loci known solely from formal genetic analysis. Tumor suppressor protein-53 (*Tp53*) and thymidine kinase (*Tk1*) are the only genes, thus far, assigned to a chromosome (Chr 13) in *Peromyscus* (Wang et al. 1995).

In the deer mouse (*Peromyscus maniculatus*), as in *Mus*, *Rattus*, *Mesocricetus*, and *Meriones*, the albino coat color (tyrosinase) (*c = Tyr1*) and pink-eye dilution (*p*) loci are linked by approximately 15–18 cM. The beta-globin (*Hbb*) locus and *c* locus are linked by about 4–8 cM in *Peromyscus*, *Mus*, and *Rattus.* In all three species the order is *p–c–Hbb.* Additional shared loci have been assigned to this linkage in the three species. These loci are within Linkage Group (LG) I of *Peromyscus* (Dawson and Rogers 1993). Other linkages, for example, amylase (*Amy1*)–alcohol dehydrogenase (*Adh1*) and leucine aminopeptidase (*Lap1*)–transferrin (*Trf*), occur in both *Mus* and *Peromyscus.* In *Peromyscus* these are located in LGs V and VI, respectively. Here we report high-confidence assignment of these three linkage groups to deer mouse chromosomes by homology, using whole *Mus* chromosome fluorescence-labeled probes (Liechty et al. 1995) hybridized to deer mouse metaphase chromosomes.

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Materials and methods

Sample preparation. Deer mouse (*P. m. bairdii*) neonates from the *Peromyscus* Genetic Stock Center at the University of South Carolina were used as a source of spleen tissue. This tissue was processed according to conventional cell culture and cytogenetic methods to obtain metaphase spreads for analysis (Schwarzacher and Wolf 1974; Sharma and Sharma 1980). Briefly, samples were minced with scissors until the tissue slurry could be passed into a Pasteur pipette. One ml of collagenase IA solution (250 units/ml; Sigma C-2674) was added to each sample, and enzymatic digestion was allowed to proceed at 37°C for 4–16 h. After digestion, the cells were washed twice with a phosphate-buffered saline or Hank's solution, treated with hypotonic solution (0.075 M KCl) for 20 min, and fixed with methanol:glacial acetic acid (3:1) for several hours. One drop of each cell suspension was placed on a separate slide and stored at −20°C. We noted that blowing gently on the freshly dropped slides improved interphase and metaphase spreading.

Fluorescence in situ hybridization (FISH). Slides were denatured in 70% formamide, 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate), pH 7.0 at 75°C for 3 min, followed by dehydration in a series of ice-cold 70%, 80%, 90%, and 100% ethanol washes of 2 min each. Slides were air dried until use. Whole-chromosome probes from microdissected mouse (*Mus*) chromosomes (Liechty et al. 1995) were obtained from Applied Genetics Laboratories, Inc. (Melbourne, Fla.). These probes were in a concentrated (70 ng/μl water) state. One μl of probe and 9 μl of Mouse Hybridization Buffer (Oncor, Inc., Gaithersburg, Md.) were used for each slide. The probe mix was denatured at 80°C for 5–10 min and cooled on ice. Ten μl of probe mix was placed in the center of a specimen slide and covered with a 22 × 22 mm glass cover slip. The cover slip was sealed by running a line of rubber cement along its edge, after which the slides were placed in a plastic box containing a damp paper towel. The box was then placed in a 37°C incubator for 72–96 h.

After incubation, slides were washed three times in 65% formamide, 2 × SSC, pH 7.0 at 43°C for 5 min each and then twice in 2 × SSC, pH 7.0, at 37°C for 5 min each. Biotinylated probe was detected using Oncor, Inc. reagents according to manufacturer's instructions. Slides were counterstained with either DAPI (4′,6′-diamino-2-phenylindole dihydrochloride) or propidium iodide, and viewed with a Zeiss Axioskop fluorescence microscope with either dual- or triple-excitation/emission bandpass filters (Chroma Technology, Battleboro, Vt.), Macintosh computer, and IPLab Spectrum (Signal Analytics, Fairfax, Va.) scientific imaging software. Locations of analyzed cells were carefully noted and coordinates recorded.

G-banding. Following FISH, chromosome identification was conducted with Giemsa staining (G-banding). Slides were washed in 65% formamide, 2 × SSC, pH 7.0, once at 50°C for 30 min and twice at 37°C for 5 min. Slides were then immersed in 10% formalin for 10 min at room tempera-

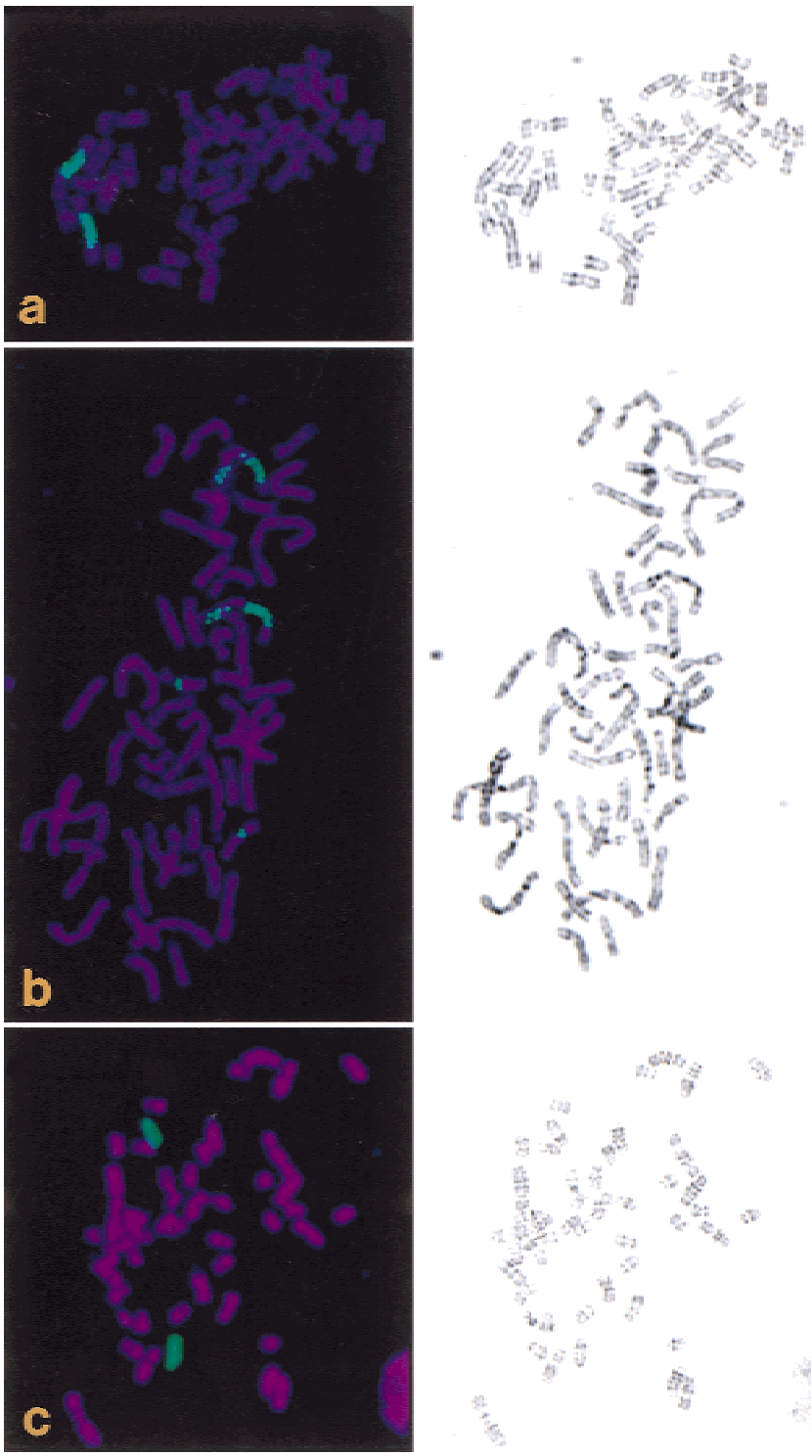

Fig. 1. Comparison of *Peromyscus* metaphases that were hybridized to *Mus* whole-chromosome paint probes and then G-banded. Labeling is indicated by fluorescence. **a.** *Mus* Chr 7 probe hybridized to the proximal region of *Peromyscus* Chr 1q. **b.** *Mus* Chr 3 probe hybridized primarily (~90%) to the disjunct segments of *Peromyscus* Chr 2q, and minimally to the centromeric region of a small bi-armed pair. **c.** *Mus* Chr 9 probe hybridized completely to the entire *Peromyscus* Chr 7.

ture, rinsed twice in 2 × SSC for 5 min, and dried on a 45°C slide warmer for 5 min.

Identification. For each individual probe, four to eight *Peromyscus* metaphase preparations were imaged after hybridization. These replicate images were compared with G-banded preparations of the same spread and scored for hybridization to *Peromyscus* chromosomes. Images were compared with the deer mouse standardized karyotype (Greenbaum et al. 1994). Chromosomal assignments were confirmed by three or more of the authors and a naive independent observer.

Results

Mus Chr 7 probe hybridized entirely to *Peromyscus* Chr 1q at the B and C zones of the karyotype (Figs. 1a, 2a). *Mus* Chr 3 probe hybridized predominantly to *Peromyscus* Chr 2qB and D, with an intervening non-hybridized gap at 2qC (Fig. 2b). A small amount, less than 10%, of *Mus* 3 probe hybridized to the *ad*-centromeric region of one of the small to medium-sized bi-armed *Peromyscus* chromosomes (Fig. 1b). The entire *Mus* 9 probe hybridized to the entire *Peromyscus* Chr 7 (Fig. 1c, 2c).

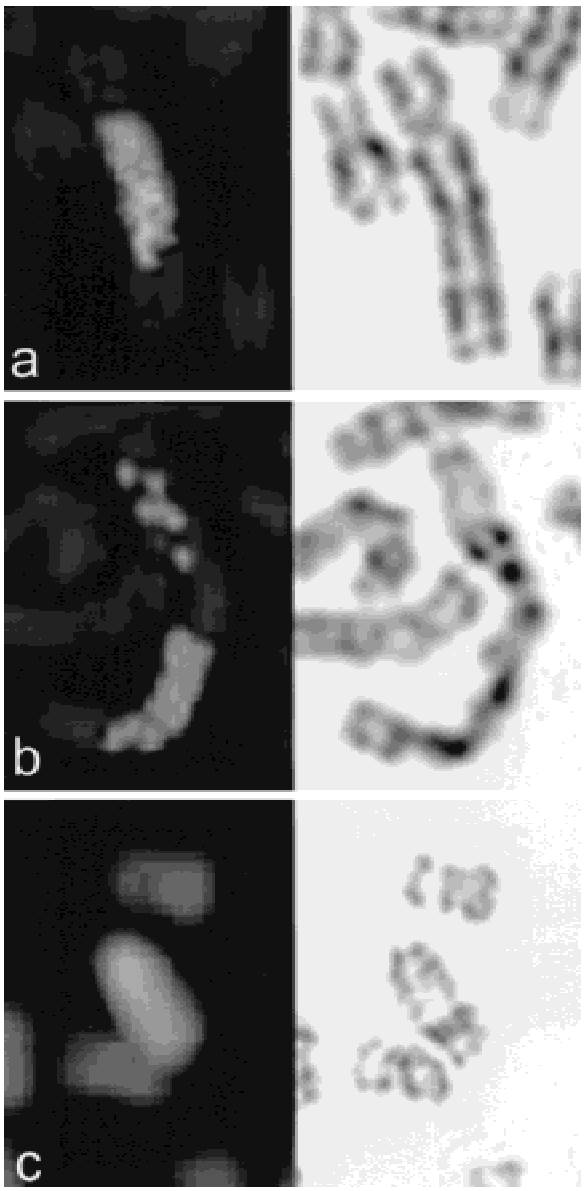

Fig. 2. Fluoresced chromosomes from Fig. 1, isolated and enlarged. **a.** *Mus* Chr 7 probe hybridized to a portion of *Peromyscus* Chr 1. **b.** The part of *Mus* Chr 3 hybridized to *Peromyscus* Chr 2. **c.** *Mus* Chr 9 hybridized to *Peromyscus* Chr 7.

By syntenic homology with *Mus*, *Peromyscus* LGs I and V can be assigned to Chrs 1 and 7, respectively, of *Peromyscus*. *Peromyscus* LG VI is very probably located on Chr 2.

Discussion

The efficacy of transpecific hybridization of whole chromosome probes to reveal genomic homologies between mammals of different taxa is demonstrated. *Peromyscus* and *Mus* probably share a common ancestry no more recently than 17 my ago (Catzeflis et al. 1993), and possibly as long as 25–30 my ago (Sarich 1985). From DNA/DNA hybridization, Brownell (1983) calculated the Wagner genetic distance between these two genera at 28.5 compared with 10.5 between *Mus* and *Rattus*.

It is noteworthy that no translocations have disrupted the linkage shared between *Peromyscus* and *Mus* on Chrs 7 and 9, respectively, since all of mouse Chr 9 hybridized entirely and only to deer mouse Chr 7. Furthermore, all of *Mus* Chr 7 has remained contiguous within the q arm of *Peromyscus* Chr 1. All of *Mus* 7 probe would likely hybridize to *Rattus* 1q based on comparative linkage maps and similarity of G-band patterns between *Rattus* and *Peromyscus*. If so, *Mus* Chr 7 apparently evolved by excision from a more basal murid chromosome homologous to that of *Rattus* Chr 1, *Peromyscus* Chr 1, and comparable long chromosomes of other rodent species (Koop et al. 1984).

The disjunct hybridization of most of *Mus* 3 probe to *Peromyscus* Chr 2 suggests that the intervening non-hybridized region of *Peromyscus* 2 arose by a translocation from elsewhere in the genome into a more basal contiguous linkage, or alternatively, the intervening non-hybridized region was lost from the homolog in the *Mus* ancestry by translocation to another chromosome.

The *Adh1* and *Amy1* loci are linked in both *Mus* and *Peromyscus* by ~12 cM. In *Peromyscus*, but not in *Mus*, the albumin (*Alb*) locus is also linked in this group. The map distance between *Amy1* and *Alb* is ~30 cM (Dawson and Rogers 1993). This observation would be consistent with the likelihood that the intervening non-hybridized segment on *Peromyscus* 2 includes a portion homologous with *Mus* Chr 5 containing the *Alb* locus.

All *Mus domesticus* chromosomes are telocentric. *Mus* 3 probe hybridized to two segments of the long arm of *P. maniculatus* Chr 2 and partly to a smaller bi-armed chromosome, thus the *Mus* 3 linkage was disrupted. But in the case of *Mus* 7 and 9 probes, only uninterrupted lengths of single arms of deer mouse chromosomes were painted. From comparisons of ideograms of the two species, it is not clear whether linkage arrangement remains intact or whether fixed inversions have disrupted the linear order since a common ancestry of *Mus* and *Peromyscus*.

In *Mus domesticus* N = 20, whereas in *Peromyscus* N = 24. From additional studies with FISH comparing the *Mus* genome with that of *Peromyscus*, *Rattus*, and other myomorphic species, a clear pattern of chromosomal evolution in these rodents should emerge.

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