

# Increased Variation in Cellular DNA Content at a Hybrid Zone: Hybrid Breakdown in *Peromyscus leucopus*

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**A natural hybrid zone of the white-footed mouse (*Peromyscus leucopus*) was studied to determine variation in cellular DNA content. The parental populations are distinguished by pericentric inversions in three chromosome pairs. Fifty-seven individuals were examined from localities in Oklahoma and Texas. Localities represented the two parental populations and a location from the center of the hybrid zone. Samples of spleen tissue were examined by flow cytometry, and the mean coefficient of variation of the northeastern sample was significantly lower than that of either the hybrid or the southwestern sample. We found no significant difference between hybrid and southwestern populations. In some individuals, we found evidence of mosaic cell populations indicated by overlapping G<sub>1</sub> peaks. We conclude that hybrid breakdown can result in natural hybrids as an increased variation in cellular DNA content that is detectable by flow cytometry. In the *P. leucopus* hybrid zone, the effect is not symmetrical in both directions.**

Hybridization may result in the breakdown of homeostatic mechanisms that normally operate in parental populations (Kidwell et al. 1977). Empirical observations that document the negative effects of hybridization include elevated chromosomal and genic mutation rates, sterility, and morphological variation in hybrids (Bregliano and Kidwell 1983; Dawson 1965; Hagele 1984; Kidwell et al. 1977; Naveira and Fontdevila 1985; Peters 1982; Shaw et al. 1983; Sved 1976). Shaw et al. (1983) proposed that hybridization is a significant source of genetic and chromosomal variation for evolutionary change and speciation. The term hybrid dysgenesis was defined as a "syndrome of aberrant genetic traits induced in hybrids between certain interacting strains, usually in one direction only" (Kidwell and Kidwell 1976). Recently, Kidwell (1990) has restricted hybrid dysgenesis to examples involving transposable elements. Transposable elements may or may not be involved in the *Peromyscus leucopus* hybrid breakdown described below. Until transposable elements are shown to be involved, the term "hybrid breakdown" is most appropriate.

In this study a natural hybrid zone of the white-footed mouse (*P. leucopus*) was examined to test the hypothesis that hybrid breakdown increases genome size variation by a mechanism that directly alters DNA. Previous studies concluded that

factors that increase chromosomal mutation rates also increase variation in cellular DNA content (Bickham et al. 1988; McBee and Bickham 1988). The significance of this observation is that flow cytometry, which allows hundreds of thousands of cells per individual to be examined for DNA content, can be used to increase the capability of statistically detecting the effects of hybrid breakdown.

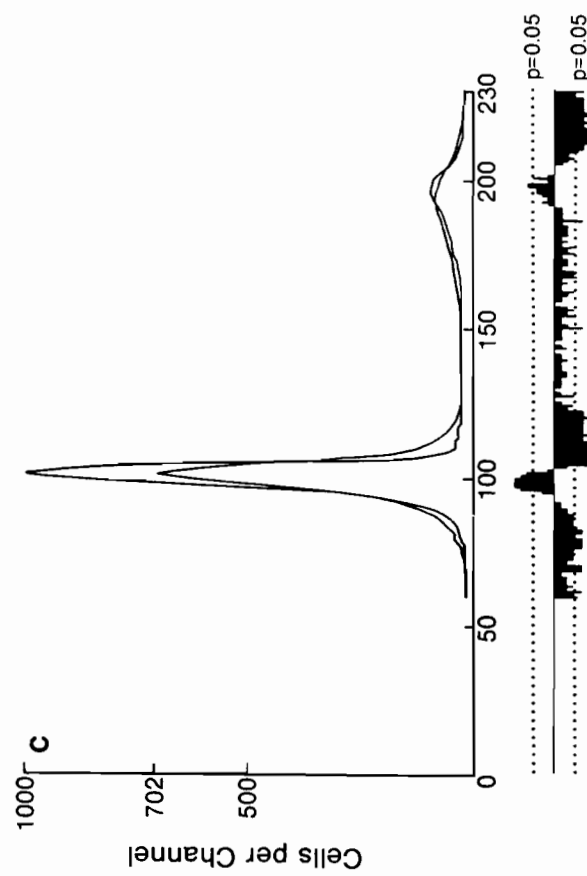
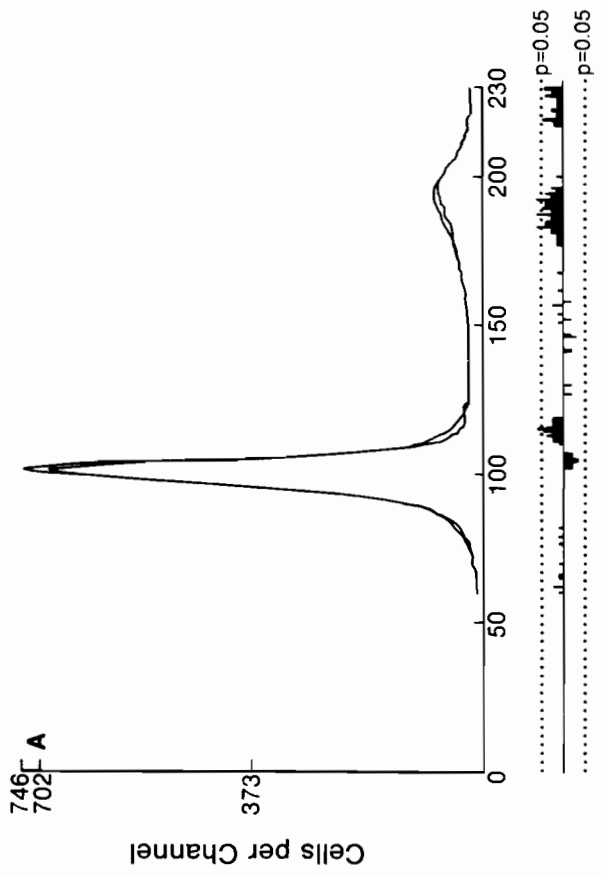
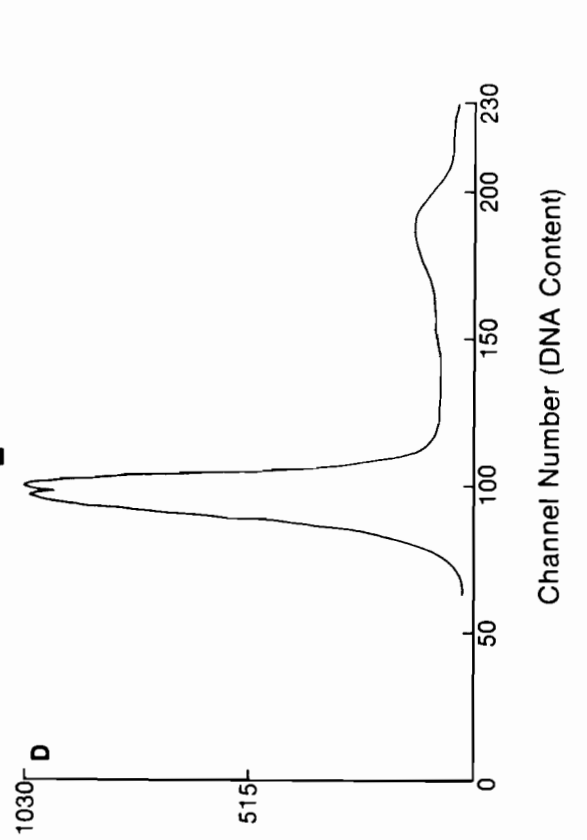
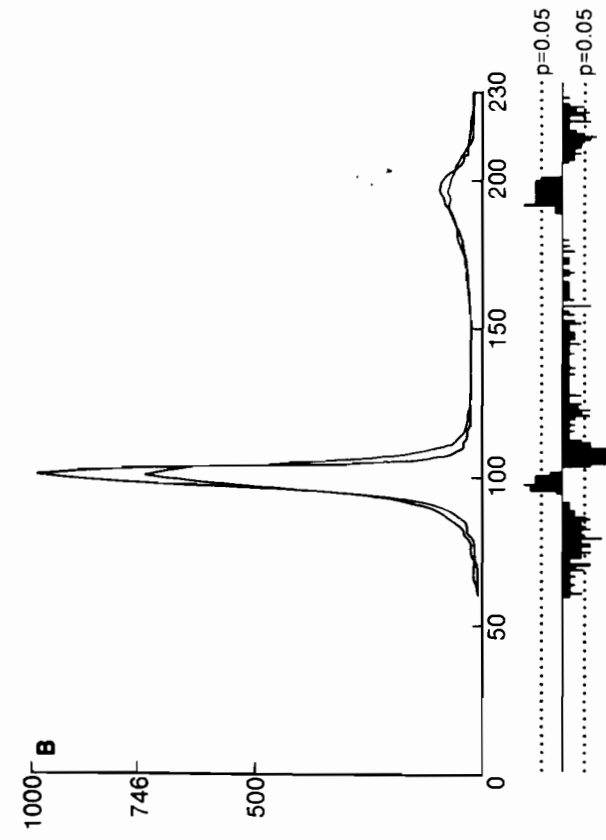
The hybrid zone studied had a 20/80 zone width (the distance over which the frequency of one parental type changes from 0.2 to 0.8) of 30.6 km and occurred in central Oklahoma between two populations of *P. leucopus* (Baker et al. 1983; Stangl and Baker 1986). The parental populations are fixed for the alternate condition in three pericentric inversions (Baker et al. 1983; Stangl 1986). Based on chromosomal, allozymic, and mtDNA markers, the zone has been shown to be asymmetrical with more extensive introgression of the northeastern markers into the southwestern population (Nelson et al. 1985; Stangl 1986).

## Materials and Methods

We initially collected from three localities in Oklahoma mice that previously had been chromosomally characterized to represent the northeastern ( $n = 11$ ), hybrid

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**Table 1. Coefficients of variation (CV) for 35 individuals from the initial analysis of the G<sub>1</sub> cell population**

Southwestern population		Hybrid population		Northeastern population	
Specimen no. <sup>a</sup>	CV	Specimen no. <sup>a</sup>	CV	Specimen no. <sup>a</sup>	CV
TK 31507	3.43	TK 30493	4.91	TK 31522*	5.17
TK 31509*	7.36	TK 30494*	5.29	TK 31523	4.30
TK 31510	6.46	TK 30495	5.34	TK 31524	2.97
TK 31513*	4.66	TK 30496	3.72	TK 31525	3.43
TK 31514	5.11	TK 30498	8.24	TK 31526	3.18
TK 31516	4.41	TK 30499*	5.93	TK 31527	4.74
TK 31517	5.87	TK 30500*	8.53	TK 31528	3.90
TK 31518	3.82	TK 31501*	4.67	TK 31529	3.16
TK 31519	5.62	TK 31502	5.99	TK 31530	4.12
TK 31520	5.67	TK 31503	3.68	TK 31531	5.10
TK 31521	4.68	TK 31504	9.25	TK 31532	3.77
		TK 31505	4.06		
		TK 31506	7.49		
Mean	5.19		5.93		3.99

<sup>a</sup> Asterisks indicate individuals that showed evidence in the G<sub>1</sub> peak of mosaic cell populations.

**Table 2. Coefficients of variation (CV) for nine additional individuals from the central (hybrid) population and 13 individuals from Wichita County, Texas**

Hybrid population (additions)		Texas population	
Specimen no.	CV	Specimen no.	CV
TK 31597	9.80	TK 31612	4.92
TK 31598	3.84	TK 31613	4.77
TK 31599	3.84	TK 31615	6.49
TK 31600	7.56	TK 31616	2.92
TK 31601	5.53	TK 31617	5.57
TK 31602	5.58	TK 31619	3.17
TK 31604	4.21	TK 31620	4.75
TK 31605	5.40	TK 31621	8.84
TK 31606	4.04	TK 31622	9.06
		TK 31623	8.12
		TK 31624	3.55
		TK 31626	5.12
		TK 31627	3.52
Mean	5.53		5.45

(*n* = 13), and southwestern (*n* = 11) populations. The three localities are 2.2 miles east of Raiford, McIntosh County; 5.9 miles east and 2.5 miles north of Tecumseh, Pottawatomie County; and 0.8 miles west and 1.5 miles south of Mountain View, Kiowa County, respectively. A subsequent analysis included nine more individuals from the hybrid locality compared to 13 individuals of the southwestern cytotype collected from Wichita County, Texas.

We returned the animals to the lab, sacrificed them, and removed their spleens for flow cytometric analysis. We produced cell suspensions and stained cells in DAPI, as described by Burton et al. (1989). Each sample was assigned a code number and assayed in a blind analysis, with the operator of the flow cytometer not knowing the identity of the sample. A total of 18,000–21,000 cells were analyzed in the G<sub>1</sub> peak for each individual on a Leitz MPV flow cytometer. The mice initially collected from the three Oklahoma populations were prepared and analyzed together and are thus directly comparable. Likewise, the two samples used in the subsequent analysis were processed together. Because of technical variation resulting from differences

in fixation, staining, and instrumental drift, samples processed and analyzed several days or weeks apart might not be directly comparable.

## Results

Within-individual variation in cellular DNA content was measured as the coefficient of variation (CV) of the G<sub>1</sub> cell population. CVs are reported for each of the 35 individuals examined in the initial analysis (Table 1). A Mann-Whitney *U* test showed no significant difference between mean CVs of the hybrid and southwestern populations (*P* > .10). However, the mean CV of the northeastern sample was significantly lower than the mean CVs of the hybrid (*P* < .005) and southwestern (*P* < .01) populations (Figure 1A–C).

Because the southwestern sample was taken from a population known to be chromosomally polymorphic (Stangl 1986), nine additional individuals from the hybrid population were compared with 13 mice collected from a Texas population of the southwestern cytotype, which was known to be chromosomally monomorphic (Stangl FB, personal communication). A Mann-Whitney *U* test showed no significant difference between the mean CVs of the hybrid population and the Texas sample (Table 2).

Evidence of mosaicism, in which the presence of two distinct cell populations was indicated by separate or partially overlapping G<sub>1</sub> peaks (Figure 1D), was observed in one of 11 individuals of the northeastern population, four of 23 individuals of the hybrid population, and two of 26 individuals of the southwestern and Texas populations (Tables 1 and 2).

## Discussion

Effects of hybrid breakdown have been demonstrated by the occurrence of chromosomal and genic mutations or by decreased fertility in hybrid offspring. Consistent with this is the observation of significantly higher CVs in cell populations from hybrid-zone individuals relative to those of a sample of northeastern parental types. The asymmetry of the zone (Nelson et al. 1985; Stangl 1986) would result in hybrid breakdown being more evident in southwestern populations than in northeastern populations if it is caused by genic introgression. Results presented in this paper are consistent with this expectation.

One mechanism that could alter DNA content is chromosomal mutation. Naveira and Fontdevila (1985) found that the progeny of certain hybrid crosses of *Drosophila* had mutation frequencies 30 times higher than other hybrid crosses. Shaw et al. (1983) found an increased chromosomal mutation rate in the progeny of F<sub>1</sub> hybrid grasshoppers. The range of variation in traits affected by hybrid breakdown might be greater in natural hybrid zones because progeny from many different crosses could be encountered. The ranges of CV values were 2.97–5.17 in the northeastern population, 3.68–9.25 in the hybrid population, and 3.43–7.36 in the southwestern population. The significance of this observation is that the populations affected by hybrid breakdown might have a greater range of variation in addition to an increased mean value.

The results of this study show a significant increase in mean CVs, not only from the hybrid population but also for individ-

**Figure 1.** Overlays of mean DNA histograms among the Oklahoma samples: (A) southwestern (CV = 5.19%) and hybrid (CV = 5.93%) populations; (B) northeastern (CV = 3.99%) and southwestern populations; (C) northeastern and hybrid samples. Bars that extend above the upper or below the lower dotted lines below the histograms correspond to channels that exhibit different numbers of cells among the overlaid histograms (*P* < .05, Student's *t* test). (D) An example of a DNA histogram from a mosaic individual (TK 31509) that exhibited two overlapping G<sub>1</sub> cell populations.

uals of the southwestern cytotype well removed from the center of the hybrid zone. Based on the fact that low CVs, comparable to those found in the northeastern cytotype, were observed in *P. leucopus* from College Station, Texas (McBee and Bickham 1988), it is concluded that high CVs are not characteristic of the southwestern cytotype but result from the effects of hybridization. Thus, the hybrid zone as defined by three chromosomal inversions is narrower than the same zone as defined by effects of hybrid breakdown (increased CVs).

It is not certain that visible chromosome aberrations are the sole cause of the increased CVs in *P. leucopus*. Transposable elements also might be a source of DNA content variation. Such an element (*mys*) was isolated from *P. leucopus* (Wichman et al. 1985). These authors concluded that 500–1,000 copies of the transposable element were present in the haploid genome. Results from in situ hybridization have shown that the *mys* element is distributed on all chromosomes in individuals from within and outside the contact zone (Baker RJ and Wichman HA, unpublished data). Such an element might be operating by altering the DNA content in hybrids by producing breaks (resulting in the translocations, inversions, and duplications mentioned above) or by radically altering the number of copies in the genome.

Although the CVs are elevated in hybrid individuals there is little evidence that this elevated DNA content variation is translated into an overall reduced fitness of hybrids (Baker et al. 1987). According to Baker et al. (1987), the width of the zone could be maintained by hybrids with a reduced fitness equal to  $1.7 \times 10^{-5}$ . It is interesting to note that the observed level of hybrid breakdown is associated with a

hybrid zone that closely fits the description of the hybrid-bounded model of Moore (1977). This model explains the maintenance and stability of a hybrid zone as a result of the hybrids being best suited to the intermediate habitat rather than to a high degree of reduced fitness as predicted by the dynamic equilibrium model (Moore 1977). As in *Drosophila* (Kidwell 1990), hybrid breakdown in *P. leucopus* does not appear to be an adequate mechanism to provide maintenance of the genetic integrity of populations across the hybrid zone.

In sum, the present study demonstrates the capability of flow cytometry to detect an as yet unreported effect of hybrid breakdown—increased variation in cellular DNA content. Hybridization is now added to the list of factors, including low level radiation and chemical mutagens (McBee and Bickham 1988), that result in increased coefficients of variation of DNA content of animals taken from natural populations.

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