HYBRID BREAKDOWN AND CELLULAR-DNA CONTENT IN A
CONTACT ZONE BETWEEN TWO SPECIES OF
POCKET GOPHERS (GEOMYS)

ROBERT D. BRADLEY, SCOTT K. DAVIS, SAMUEL F. LOCKWOOD,
JOHN W. BICKHAM, AND ROBERT J. BAKER

Department of Biological Sciences and The Museum,
Texas Tech University, Lubbock, TX 79409 (RDB and RJB)
Department of Animal Sciences, Texas A&M University, College Station, TX 77843 (SKD)
Department of Wildlife and Fisheries Sciences,
Texas A&M University, College Station, TX 77843 (SFL and JWB)

ABSTRACT.—DNA content and coefficients of variation surrounding mean values were examined
in 35 individuals from a contact zone between Geomys bursarius major and G. knoxjonesi in
New Mexico. Three classes of individuals (parental types and hybrids) from the contact zone were
compared to reference samples from outside the contact zone. No significant differences were
found in either DNA content or in individual coefficients of variation among any of the five classes
examined. Comparison of data from Peromyscus leucopus and pocket gophers documents that
hybrid breakdown in cellular-DNA content is not correlated with magnitude of chromosomal,
allozymic, and mitochondrial-DNA, or ribosomal-DNA differentiation that distinguishes parental
types. Mechanisms that maintain the integrity of the respective gene pools of G. b. major and G.
knnoxjonesi are not associated with differences in mean DNA content or with variation in quantity
of DNA among cells of an individual.

Natural hybridization between two genetically different populations often results in negative
effects associated with the hybrid progeny. Two terms have been used to define these events.
The first, hybrid breakdown, is indicative of some type of genetic incompatibilities (Burton, 1990)
and has been shown to be involved in elevated chromosomal and genic mutations and
reduction in fertility (Dawson, 1965; Hagele, 1984; Naveira and Fontdevila, 1985; Peters, 1982;
Shaw et al., 1983). The second, hybrid dysgenesis, involves a syndrome of aberrant genetic traits
(Kidwell and Kidwell, 1976) and is restricted to examples involving transposable elements (Kid-
well, 1990). In most cases, it is impossible to ascertain from earlier papers if the phenomenon
described as hybrid breakdown is in fact an example of hybrid dysgenesis. Recently, in a study
of DNA content and coefficients of variation associated with individual cells, Baker et al. (1991)
documented that hybridization of two chromosomal races of Peromyscus leucopus resulted in
the increase of the coefficients of variation. They concluded, because there is yet no evidence
to indicate that this increase is a result of transposable elements, that hybrid breakdown is the
most appropriate term to describe this phenomenon. We follow Baker et al. (1991) in using the
more general term of hybrid breakdown.

Additionally, it is well documented that the genome of mammals consists of far greater amounts
of DNA than is needed to code for the required structural and regulatory genes (Doolittle and
Sapienza, 1980; Orgel and Crick, 1980; Patton and Sherwood, 1982; Sherwood and Patton, 1982).
This relationship of excessive DNA content, called the C-value paradox (Patton and Sherwood,
1982; Sherwood and Patton, 1982), is based on an overview generated from studies of samples
from isolated taxa representative of living forms of animals. However, before the significance
of such excessive amounts of DNA can be appreciated, there is a need to understand within-
group as well as among-group variation (Burton et al., 1989; Sherwood and Patton, 1982) in
DNA content.

Chromosomally characterized taxa of pocket gophers (Geomys bursarius major and G. knox-
jonesi) hybridize at a contact zone studied in detail by Pembleton and Baker (1978) and Baker
et al. (1989). Although these two chromosomal races are nearly identical in diploid number (G.
b. major, 2n = 72; G. knoxjonesi, 2n = 70), as many as 23 pairs of autosomes may be altered

J. Mamm., 72(4):697–705, 1991 697
by heterochromatic additions or other chromosomal rearrangements (Qumsiyeh et al., 1988). In the study by Baker et al. (1989), 54% (41 of 75) of the individuals in the zone appeared to be of hybrid origin. Parental types exhibited fixed differences in diploid number, ribosomal DNA, mitochondrial DNA, and three allozymes (Baker et al., 1989). Each individual was scored unequivocally as either a parental type or as a hybrid. Baker et al. (1989) proposed that F₃s and highly heterozygous individuals were less fertile than parental types. They also suggested that both pre mating and postmating isolating mechanisms were operative, and the G. b. major and G. knoxjonesi were distinct species with effectively isolated gene pools. This type of contact zone, where hybridization between the two parental types is common, but where parental types are genetically quite divergent, offers an ideal situation to test for hybrid breakdown. Recently, flow cytometry has been used to estimate genome size and relative DNA quantities per cell (Bickham et al., 1988; Burton and Bickham, 1989; Burton et al., 1989; McBee and Bickham, 1988; Sherwood and Patton, 1982) and has been used to document hybrid breakdown in natural populations (Baker et al., 1991). Flow cytometry allows large numbers of cells to be analyzed for DNA content precisely and quickly (Bickham, 1990), and it is sensitive enough to distinguish between cells that differ by as little as 2–3% in DNA content (Deavan, 1982; Steen and Lindmo, 1979). Herein we examine the magnitude of variation in cellular DNA that has accompanied speciation between G. bursarius and G. knoxjonesi. Secondly, we determine if genome size and coefficients of variation of DNA content provide a measure of hybrid breakdown in hybrid individuals in Geomys as reported for P. leucopus (Baker et al., 1991). Thirdly, we test the hypotheses that greater individual variation in cellular-DNA content is a function of either magnitude of genome differentiation or chromosomal differentiation that distinguishes parental types.

Materials and Methods

Specimens from natural populations were livetrapped (Baker and Williams, 1972) along a 6-km north-south transect along the DeBaca–Roosevelt county line, 25.5 km S and 4.9 km E Taiban, New Mexico. Animals were trapped on 9 days during July–November 1988. Ten G. b. major were collected along the right-of-way of U.S. Highway 84 southeast of Lubbock, Lubbock Co., Texas, and 12 G. knoxjonesi were collected along the right-of-way of U.S. Highway 380 west of Brownfield, Terry Co., Texas. These were used as genotypic and flow-cytometric reference samples. Chromosomal analyses were conducted at Texas Tech University, allozymic, ribosomal-DNA, and mitochondrial-DNA analyses were conducted in Davis’ laboratory at Texas A&M University, and flow-cytometric analyses were conducted in Bickham’s laboratory at Texas A&M University. Results of each system under study were scored for each animal without the knowledge of the scores from the other systems.

Specimens were karyotyped by using the yeast-stress method (Baker et al., 1981; Lee and Elder, 1980), and five metaphase cells were counted to determine the diploid number for each individual. Additional cells were photographed and counted if determination of the diploid number was inconclusive. Samples of heart, kidney, liver, muscle, and spleen were collected and frozen at −80°C immediately after sacrificing each specimen. Muscle tissue was prepared for DNA analyses using total DNA isolation (Hillis and Davis, 1986). Both ribosomal DNA and mitochondrial DNA were visualized from Southern blots (Southern, 1975) by using the pGb28S (ribosomal DNA) and Ortho4 (mitochondrial DNA) probes described in Davis (1986) and Baker et al. (1989). Three allozyme systems, fixed for alternative alleles between parental types (Baker et al., 1989), including alcohol dehydrogenase (Enzyme Commission No. 1.1.1.1; ADH), lactate dehydrogenase (1.1.1.27; LDH1), and peptidase (3.4.13.11; PEPB1), were prepared from heart, kidney, and liver generally following the techniques of Selander et al. (1971) and Harris and Hopkinson (1977). Preparations for flow-cytometric analyses generally followed that of Burton and Bickham (1989) and Burton et al. (1989) except that spleen tissues were utilized and were homogenized using a “Tissue Tearor” (Biospec Products, Bartlesville, OK). These preparations were treated as unknowns in a blind test and were compared to an internal standard of erythrocytes of domestic chicken (Gallus domesticus). Erythrocytes for the standard were obtained from one individual of a highly inbred strain obtained from the College of Veterinary Medicine, Texas A&M University. This individual was identical to the standard used in similar studies in Bickham’s laboratory (Burton and Bickham, 1989; Burton et al., 1989). G. domesticus has a reported genome size of 2.54 pg, as determined by direct biochemical analysis (Rasch et al., 1971). Coefficients of variation were determined for the Gₐ peaks of all individuals by placing the peak of Gₐ cell populations of the gopher spleen cells at,
or as near as possible to, channel 100. Subsequently, chicken erythrocytes were mixed with the gopher spleen cells. The chicken erythrocytes were used as an internal standard; the energy to the photomultiplier was adjusted so that the chicken erythrocytes appeared at, or as near as possible to, channel 50. Genome size was determined for each individual as a ratio of the mean channel where chicken red blood cells equal 2.54 pg to the mean channel of gopher spleen cells equals X.

Individuals were grouped into five classes for statistical analyses: individuals from the G. b. major reference population, individuals from the G. knoxjonesi reference population; G. b. major individuals from the contact zone; G. knoxjonesi individuals from the contact zone; and hybrid individuals from the contact zone. Differences in mean DNA content and mean coefficient of variation of each class were tested for significance by analysis of variance, with sex used as a blocking factor to remove variation due to heteromorphic sex chromosomes.

**RESULTS**

All individuals from the reference population of G. knoxjonesi had 2n = 70. Individuals from the reference population of G. b. major had 2n = 70, 71, or 72. The chromosomal polymorphism in G. b. major is not present in the New Mexico contact zone and involves centric fusion-fission rearrangements (described in Baker et al., 1983a) not related to the variation that distinguishes the two taxa in the contact zone. In the contact zone, G. b. major is monomorphic with 2n = 72, and G. knoxjonesi is monomorphic with 2n = 70 (Baker et al., 1989). Within the contact zone, 13 individuals had 2n = 70, 9 had 2n = 71, and 13 had 2n = 72.

The ribosomal DNAs of the two reference populations were identical to those described by Davis (1986) and Baker et al. (1989) for G. b. major and G. knoxjonesi, respectively. Of the 35 individuals from the contact zone, 14 had only the 3.4-kb BamH I fragment characteristic of G. b. major, 13 had the 1.0- and 2.4-kb BamH I fragments characteristic of G. knoxjonesi, and 8 had the 1.0-, 2.4-, and 3.4-kb BamH I fragments characteristic of hybrid individuals.

The mitochondrial DNAs of the reference populations of G. b. major and G. knoxjonesi were typical of those taxa as described by Davis (1986) and Baker et al. (1989). In the contact zone, 17 had the mitochondrial DNA type of G. b. major, and 18 had the mitochondrial DNA type of G. knoxjonesi. No hybrid class existed as mitochondrial DNA is uniparentally inherited.

Reference populations were fixed for alternative alleles for the LDH1, PEPB1, and ADH loci. In the contact zone for the LDH1 locus, 12 individuals were homozygous for the allele characteristic of G. b. major, 12 were homozygous for the allele characteristic of G. knoxjonesi, and 10 were heterozygous. For the PEPB1 locus, 14 individuals were homozygous for the allele characteristic of G. b. major, 14 were homozygous for the allele characteristic of G. knoxjonesi, and 7 were heterozygous. For the ADH locus, a more complex situation exists as a third allele was present in the contact zone that was not found in either reference population. Although the rare allele may have arisen as a product of hybridization, Baker et al. (1989) found this allele present in five individuals that ultimately classified (based on other genetic markers) as parental G. b. major; no individuals classified as parental G. knoxjonesi carried this allele. For these reasons, Baker et al. (1989) concluded that the rare allele probably has its origin with G. b. major. Assuming this is correct, 12 individuals were homozygous for alleles characteristic of G. b. major (including those heterozygous for the rare G. b. major allele and the more common G. b. major allele), 14 were homozygous for the allele characteristic of G. knoxjonesi, and 9 were heterozygous for G. b. major and G. knoxjonesi alleles.

Genome size of each individual was estimated by comparing the ratio of the channel numbers of the G1 peaks to those of the internal standard, domestic chicken (Fig. 1). The analysis of variance revealed no significant differences among any of the five samples examined for either mean DNA content or mean coefficients of variation (Table 1). However, significant differences between males and females were found across all individuals (F = 8.64, P = 0.005), revealing that females (X = 7.30 pg) had a greater DNA content per cell than males (X = 7.11 pg). Small samples within study groups precluded statistical comparisons by sex among groups.

In addition to 10 specimens of G. b. major and 12 of G. knoxjonesi collected ca. 200 km from the contact zone, we obtained 35 individuals from the contact zone. Using the combination of
the six genetic markers (diploid number, ribosomal DNA, mitochondrial DNA, LDH1, PEPB1, and ADH), 8 individuals from the contact zone were classified as G. b. major, 11 were classified as G. knoxjonesi, and 16 as hybrids. The local geographic pattern of genotypic variation was like that described by Baker et al. (1989), with G. b. major distributed in the more endurate soils to the north, G. knoxjonesi distributed in the sandy soils to the south and hybrids distributed in the center (Fig. 2). Based on a chi-square goodness-of-fit contingency table, the number of hybrid individuals (16 of 35, 46%) did not differ significantly ($\chi^2 = 0.77, d.f. = 1, P < 0.05$) from that reported by Baker et al. (1989) who found 54% hybrids. The similarity of these data, to those of Baker et al. (1989) is interpreted to indicate that the composition and dynamics of the contact zone did not change between 1986 and 1988. This observation suggests that appropriate genotypic classes were sampled from the contact zone to provide adequate testing of hybrid breakdown and variation within and between the two species.

Table 1.—Mean DNA content and coefficients of variation for G. b. major, G. knoxjonesi, and their hybrids. The parental G. b. major and G. knoxjonesi reference samples were taken from populations distant from the hybrid zone. Analysis of variance indicated that differences in DNA content were not significant ($F = 1.20, P = 0.32$) and differences in coefficients of variation ($F = 1.16, P = 0.34$) were not significant. Values for DNA content are in picograms.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>DNA content</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{X}$</td>
<td>Range</td>
</tr>
<tr>
<td>G. b. major (reference)</td>
<td>10</td>
<td>7.34</td>
<td>6.76–7.62</td>
</tr>
<tr>
<td>G. b. major (hybrid zone)</td>
<td>8</td>
<td>7.22</td>
<td>6.82–7.67</td>
</tr>
<tr>
<td>G. knoxjonesi (reference)</td>
<td>12</td>
<td>7.27</td>
<td>6.72–7.67</td>
</tr>
<tr>
<td>G. knoxjonesi (hybrid zone)</td>
<td>11</td>
<td>7.13</td>
<td>6.84–7.47</td>
</tr>
<tr>
<td>Hybrids</td>
<td>16</td>
<td>7.24</td>
<td>6.89–7.67</td>
</tr>
</tbody>
</table>
Fig. 2.—Geographic distribution of 35 individuals of Geomys bursarius major, G. knoxjonesi, and hybrids from the contact zone, in New Mexico along the DeBaca–Roosevelt county line. Filled circles denote G. b. major, open circles, G. knoxjonesi, and squares, hybrid individuals. Stippled regions indicate areas of deep, sandy soil; nonstippled regions indicate harder, endurate soils. Black lines represent roads or fences used as reference points for plotting individual collecting sites.

**Discussion**

*Variation in DNA content between sexes and species.*—Differences were found in the mean DNA content between males and females across all individuals. Females possessed higher DNA contents than males ($\bar{X} = 7.30$ pg compared to $7.11$ pg, $P = 0.005$). This is consistent with karyotypic data (Baker and Genoways, 1975; Baker et al., 1973) from Geomys that show the X chromosome to be considerably larger than the Y chromosome. Considering the differences in DNA content between males and females, it would be most appropriate to compare males of *G. b. major* with males of *G. knoxjonesi* and females of *G. b. major* with females of *G. knoxjonesi*. Because our samples are inadequate to permit such statistical comparisons, males and females of *G. b. major* were combined as were the males and females of *G. knoxjonesi*. Although this may reduce the sensitivity of the technique and cause the loss of some resolution, the finding of significant differences between males and females documents that the methods used in this study are sufficiently sensitive to reveal differences as small as those that distinguish the X and Y chromosomes in Geomys.

After combining the males and females of each respective species, the mean DNA content was 7.22 pg for *G. b. major* and 7.13 pg for *G. knoxjonesi*. These values suggest that *G. b. major* and *G. knoxjonesi* have larger genomes than known for some mammals, including Thomomys monticola and *T. talpoides* (Sherwood and Patton, 1982), *G. attwateri* and *G. breviceps* (Burton...
and Bickham, 1989), and bats (Burton and Bickham, 1989; Capanna and Manfredi-Romanini, 1971; Kato et al., 1980). However, the values for mean DNA content reported here (Table 1) are smaller than those reported for *T. bottae*, *T. umbrinus*, and *T. townsendii* (Sherwood and Patton, 1982).

No significant differences in the DNA content between species were detected, although parental types exhibited fixed differences at three allozymes, ribosomal DNA, mitochondrial DNA, and diploid number. This study is consistent with the results of Burton and Bickham (1989) wherein DNA content was examined in individuals from a contact zone of three chromosomal races of pocket gophers representing two species, *G. attwateri* (races F and G) and *G. breviceps* (race E). They detected no significant differences in DNA content among the three chromosomal races that possessed markedly different patterns of heterochromatin; however, they detected a significant difference between the mean DNA content of race E and race F × G hybrids. However, Burton and Bickham (1989) designed their study to test only for mean DNA contents and not for coefficients of variation associated with mean DNA content; nor did they examine samples at a single calibration as was done in this study. Analyses performed with subsequent calibrations are subject to additional experimental error. Whereas the study of Burton and Bickham (1989) involved multiple calibrations, in our study results from all individuals were based on a single calibration. The data for *Geomys* (Burton and Bickham, 1989; this study) are contrasted by the data from *Thomomys* where some congeneric species differed by as much as 230% (Sherwood and Patton, 1982).

Additionally, given the differences in heterochromatic additions between *G. b. major* and *G. knoxjonesi* (Qumsiyeh et al., 1988), it might be expected that genome size would vary as found by Deavan et al. (1977). Just as in *Thomomys*, in which heterochromatin (as reflected in cellular-DNA content) has played no direct role in speciation (Patton and Sherwood, 1982, 1983; Sherwood and Patton, 1982), within our sample no significant differences in genome sizes were found between species of *Geomys*. Sherwood and Patton (1982) suggested that additional data concerning intraspecific and interspecific variation in DNA content were necessary to resolve the C-value paradox. Our study provides such empirical data.

*Test for breakdown in cellular-DNA content in hybrid individuals.*—There is evidence in *P. leucopus* that some aspect of hybridity per se causes an increase in variation of the amount of DNA among cells within an individual (Baker et al., 1991). Such variation can be detected by elevated coefficients of variation in hybrid individuals using a flow cytometer (Baker et al., 1991). One possible explanation of such an elevation of coefficient of variation in hybrids is a lack of compatibility resulting from genome differentiation. If this is the causative factor, then hybrids between highly differentiated parental types might be expected to have higher coefficients of variation than hybrids between poorly differentiated parental types. Relative to our study, the magnitude of differentiation that distinguishes *G. b. major* and *G. knoxjonesi* is far greater than that which distinguishes the parental types in *P. leucopus* studied by Baker et al. (1991). *G. b. major* and *G. knoxjonesi* are distinguished by fixed differences in chromosomes, three allozymes, mitochondrial DNA, and ribosomal DNA, whereas the races of *P. leucopus* show differences in chromosomes, but not in the other markers. Given the large amount of genetic divergence between these two species of pocket gophers, we initially expected that a significant increase in coefficients of variation would be found in hybrids. However, results revealed no significant differences between samples of *G. b. major*, *G. knoxjonesi*, or the hybrid classes. This observation provides a critical piece to the puzzle in determining factors that cause increased variation among cells in hybrid individuals. Magnitude of genetic differentiation, per se, as the causative factor of an elevated coefficient of variation in cellular DNA can be rejected.

Another potential explanation for hybrid breakdown in DNA content pertains to numbers and types of chromosomal rearrangements that distinguish parental types. At the contact zone of *P. leucopus*, the parental types, and one parental type in comparison with the hybrid class, had significantly different coefficients of variation associated with DNA content. The contact zone of *P. leucopus* is defined by only three chromosomal rearrangements (Baker et al., 1985b; Stangl,
1986; Stangl and Baker, 1984), whereas in the Geomys contact zone as many as 23 pairs of chromosomes may have been changed in some aspect (Qumsiyeh et al., 1988). However, in Geomys no significant differences were found in the coefficients of variation of DNA content, whereas in P. leucopus statistical differences were found between one parental type and the hybrid class. It thus appears that it is not the magnitude of chromosomal rearrangements, at least not the type found in G. b. major and G. knoxjonesi, that produces differences in the coefficients of variation of DNA content at hybrid zones.

We can conclude only that other genetic differences, account for the hybrid breakdown in cellular-DNA content in P. leucopus and for its absence in the Geomys contact zone. Perhaps other mechanisms such as cytonuclear incompatibilities documented in hyllid tree frogs (Asmussen et al., 1987; Lamb and Avis, 1986), X- and Y-chromosome incompatibilities such as reported from Drosophila pseudoobscura and D. persimilis hybrids (Orr, 1989), or accumulation of transposable elements (Engels, 1979, 1983; Kidwell, 1983; Kidwell and Kidwell, 1976; Kidwell et al., 1977; Lai and Mackay, 1990; Mackay, 1989) are responsible for hybrid breakdown in fertility observed in the Geomys contact zone (Baker et al., 1989; Bradley et al., 1991).

Baker et al. (1991) noted that the level of hybrid breakdown in the P. leucopus contact zone fits the description of the hybrid-bounded model of Moore (1977) or the hybrid-equilibrium model of Endler (1977), whereas the contact zone of pocket gophers was described by Baker et al. (1989) as being most compatible with the dynamic-equilibrium model of Bigelow (1965). To these differences in maintenance of contact zones can be added elevation of coefficients of variation associated with DNA contents in the P. leucopus contact zone and stability of coefficients of variation in the Geomys contact zone.

Acknowledgments

We thank Mr. and Mrs. F. Jewell and Mr. and Mrs. B. Hall for granting permission to collect specimens on their property and for generous hospitality. Additionally, we thank L. C. Bradley and C. A. Porter for providing critical reviews of an earlier draft of this manuscript, and M. J. Hamilton, S. Pletscher, P. Sudman, and R. A. Van Den Bussche for assistance in the laboratory and in specimen collection. M. Smolen and B. Hanks kindly provided computer programs for data analysis. This study was funded by an American Society of Mammalogists Grants-in-Aid of Research to RDB, Society of the Sigma Xi Grant-in-Aid of Research to RDB, Helen Hodges Educational Charitable Trust Scholarship to RDB, Texas Tech University Graduate School Summer Research Assistantship to RDB, National Science Foundation grants BSR 86-00646 to RJB and BSR 88-22751 to SKD, and by Expanded Research Area funds from the Texas Agricultural Experiment Station to JWB.

Literature Cited


ology in South America (M. A. Mares and H. H. Genoways, eds.). Special Publication Series, Py- maturing Laboratory of Ecology, University of Pittsburgh, 6:1–539.


NAVEIRA, H., and A. FONTDEVILA. 1985. The evolu- tional history of Drosophila buzzati. IX. High frequencies of new chromosome rearrangements
induced by introgressive hybridization. Chromosoma, 91:87-94.


