Cytogenetics and Cell Genetics
Editor-in-Chief: H.P. Klinger, Bronx, N.Y.

Separatum
Publisher: S. Karger AG, Basel
Printed in Switzerland

Cytogenet Cell Genet 61:10–16 (1992)

Geographic patterns of chromosomal variation in South American marsh rats, *Holochilus brasiliensis* and *H. vulpinus*

M.W. Nachman

Museum of Zoology, University of Michigan, Ann Arbor, MI (USA)

Abstract. Karyotypes were prepared from 146 individuals, representing nine populations evenly spaced along a 2,000-km north-south transect in Paraguay and Argentina, to determine the nature, extent, and pattern of chromosomal variation in *Holochilus brasiliensis chacarius* and *H. vulpinus.* Two distinct patterns of chromosomal variation characterized these two species. In *H. brasiliensis,* the diploid number (2n) ranged from 48 to 56 and the *nombre fondamental* (NF) from 57 to 63. Four classes of chromosomal variation were found in populations of *H. brasiliensis:* whole-arm Robertsonian (Rb) translocations, including Rb changes with monobrachial homology, variation in the number and kind of supernumerary (B) chromosomes, centromeric rearrangements (putative pericentric inversions), and variation in the amount of euchromatin. The amount of structural variation was uniformly high in all populations of *H. brasiliensis* sampled, and all rearrangements appeared to be in Hardy-Weinberg proportions, corroborating the hypothesis that chromosomal rearrangements are not strongly underdominant in this species. In *H. vulpinus,* 2n ranged from 35 to 39 and NF from 57 to 61. Two classes of variation were found in this species: variation in the number, but not the kind, of supernumerary chromosomes and variation in the amount of euchromatin.

The South American marsh rat (genus *Holochilus*) provides an unusual opportunity for studying mammalian chromosomal evolution. Previous work on this genus has documented surprisingly extensive chromosomal variation within populations (Yonenaga-Yassuda et al., 1987; Sangines and Aguilera, 1991), between populations (Riva et al., 1977), and between species (Freitas et al., 1983). Variation within and between populations is due primarily to Robertsonian differences and the presence or absence of supernumerary chromosomes, although polymorphisms due to pericentric inversions and changes in the amount of euchromatin are also known.

In the present study, I seek to make inferences about chromosomal evolution by looking at patterns of geographic variation. I document intrapopulation, interpopulation, and interspecific chromosomal variation in *H. brasiliensis chacarius* and *H. vulpinus* among 146 animals collected along a 2,000-km north-south transect involving nine populations from northern Paraguay to central Argentina. These data are used to address three main questions:

1. Do all populations of *H. brasiliensis* exhibit high levels of chromosomal polymorphisms? Previous work on this group in Paraguay demonstrated an exceptionally high level of chromosomal variation for a single mammalian population and suggested that heterozygous animals suffer little or no reduction in fitness (Nachman and Myers, 1989). If chromosomal variation in *H. brasiliensis* is underdominant, then extensive chromosomal polymorphisms should be rare.

2. What geographic pattern of chromosomal variation exists among populations of *H. brasiliensis?* An all acrocentric karyotype with 2n = 56 chromosomes has been reported in specimens of *H. brasiliensis* from central Brazil (Freitas et al., 1983). Freitas et al. (1983) have suggested that this 2n = 56 karyotype is ancestral for the genus and that derived, metacentric karyotypes (2n < 56) are found to the north and south. This study focuses on populations connecting the high-diploid-number animals of central Brazil with the low-diploid-number animals of central Argentina to see if a gradual reduction in diploid number is observed.

3. What chromosomal differences exist between *H. vulpinus* and *H. b. chacarius?* These two taxa are morphologically distinct (Hershkovitz, 1955; Massoia, 1971) and have parapatric distributions: *H. vulpinus* is found primarily in central Argentina, whereas *H. b. chacarius* is found in northern Argentina, Paraguay, and adjacent areas in Brazil. There is disagreement on

Supported by the Rackham Graduate School and the Museum of Zoology at The University of Michigan, Sigma Xi, The American Museum of Natural History, and grant GM30144 from the National Institutes of Health to Dr. W.M. Brown.

Received 8 April 1991; revision accepted 21 January 1992.

Request reprints from Dr. Michael W. Nachman, Section of Genetics and Development, 403 Biotechnology Building, Cornell University, Ithaca, NY 14853-2703 (USA).

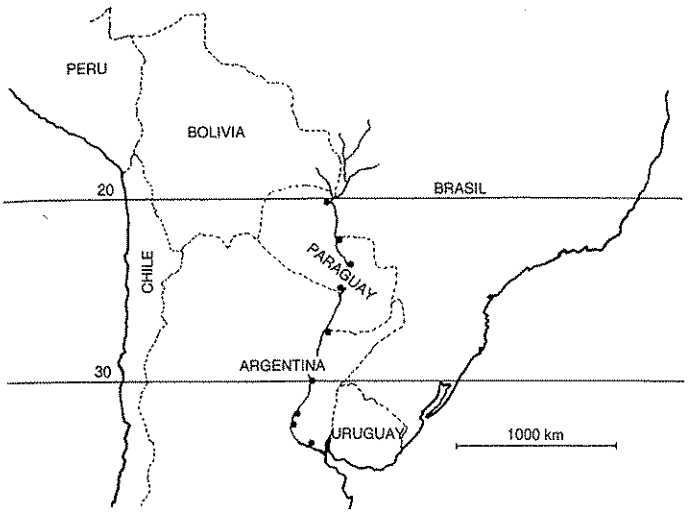

Fig. 1. Map of southern South America showing the nine localities (solid circles) in Paraguay and Argentina where *Holochilus brasiliensis* (seven northern localities) and *H. vulpinus* (two southern localities) were collected. The populations, from north to south, are Bahia Negra, Fonciere, Rosario, Golondrina, Itati, Esquina, Santa Fe, Las Cuevas, and Puerto Ibicuy.

whether these taxa represent one or two biological species. The present transect was also chosen to locate a potential zone of contact between these taxa and to see if there is any chromosomal evidence of hybridization.

Materials and methods

Animals

One hundred forty-six marsh rats from nine localities (Fig. 1 and Appendix) along a single river drainage in Paraguay and Argentina are included in this study. The seven northern populations, amounting to 122 animals, contain only *H. brasiliensis,* whereas the two southern populations (24 animals) contain only *H. vulpinus,* based on morphological criteria.

Marsh rats are semiaquatic rodents, and thus the river acts as a natural corridor for dispersal. Animals were trapped alive in river islands and marshes, or were born to pregnant females shortly after capture, in September and October of 1986, 1988, and 1989. Karyotypic data from the Golondrina population have been published previously (Nachman and Myers, 1989).

Cell cultures and chromosome banding

Animals from Bahia Negra, Fonciere, Rosario, and Ibicuy were returned alive to the University of Michigan and used in breeding and meiotic studies (Nachman, 1992). Karyotypes of these animals were obtained from cell cultures of peripheral lymphocytes following the techniques of Davisson and Akeson (1987), with minor modifications. Animals from Itati, Esquina, Santa Fe, and Las Cuevas were sacrificed in the field, and karyotypes were obtained from bone marrow following the technique of Patton (1967).

G- and C-banded karyotypes were prepared by the method of Leversha et al. (1980) from 90 animals representing all nine populations. Replication (RBA) banding with 5-bromo-2′-deoxyuridine (BrdU) and acridine orange was done on preparations from 30 animals, representing the four Paraguayan populations, using the technique of Verma (1982) with modifications. Karyotypes from conventionally stained preparations were also prepared for all animals.

An average of 10 cells with a balanced diploid complement were analyzed per animal (total cells analyzed = 1,470), and 3–10 cells were photographed for each animal. To confirm the identity of some of the rearrangements as deduced from banded, mitotic chromosomes, meiotic preparations were made from 30 males following the technique of Evans et al. (1964).

Results

Holochilus brasiliensis

The most common karyotype, with 2n = 48 chromosomes and a *nombre fondamental* (NF) of 58, was found in 19 of the 122 animals sampled (1 from Bahia Negra, 10 from Fonciere, and 8 from Golondrina). A G-banded figure of this karyotype has been published previously (Nachman and Myers, 1989). The karyotype contains four pairs of large metacentric chromosomes (1/2, 3/4, 5/8, and 6/7, the nomenclature corresponding to the major autosome arms, which are numbered according to decreasing length) and a very small metacentric pair (23, whose individual arms are not assigned different numbers [after Vidal et al., 1976]). The remaining autosomes are acrocentric and form a series of gradually decreasing length.

C-banding revealed that centromeric heterochromatin is present on all chromosomes except the large metacentric pair 3/4, as previously reported (Nachman and Myers, 1989). There are no additional blocks of heterochromatin, except on the Y, which is almost entirely C-band positive. RBA-banding revealed that most G-band-positive regions are late replicating, as previously demonstrated for other species (see, e.g., Holmquist et al., 1982).

Variation within and between populations (Table I) results from four different kinds of chromosomal rearrangements, which are described below. Fifty-one different karyotypes were present among the 122 animals sampled. The diploid number varied from 48 to 56, with the lower diploid numbers appearing in the northern four populations and the higher diploid numbers in the southern three populations. The NF varied from 57 to 63, with no apparent geographic pattern.

Robertsonian (Rb) translocations (Fig. 2) involve nine autosomal arms in seven different arm combinations: 1/2, 1/5, 2/5, 2/18, 3/4, 5/8, and 6/7. Each of these arms is unambiguously identifiable except one, which is provisionally labeled as 18 based on its size and morphology. Fifty-nine animals (48%) were heterozygous for a single Rb translocation, 16 animals (13%) were heterozygous for two Rb translocations, 2 animals (2%) were heterozygous for three Rb translocations, and the remaining 45 animals (37%) showed no heterozygosity for Rb translocations. One of the triple Rb heterozygotes (MWN 346 from Bahia Negra) contained monobrachial homologies (arm combinations: 1-1/2-2/18-18), based on G-banding; this finding was confirmed by an analysis of 100 meiotic metaphase cells.

Supernumerary (B) chromosomes (Fig. 3) have been divided into two categories on the basis of their size, shape, and staining characteristics (Nachman and Myers, 1989). BI chromosomes are small to large, metacentric or submetacentric chromosomes that contain only centromeric heterochromatin (based on C-banding) and therefore appear much like the autosomes. There is considerable variation in the size of BI chromosomes: the smallest are the size of autosome 23, and the largest are 40% larger than autosome 1, or approximately 10% of the length of the haploid genome. In contrast, BII chromosomes are small, acrocentric chromosomes that are largely heterochromatic (i.e., C-band positive). RBA-banding provides an unambiguous method of identifying B chromosomes in the karyotype: B chromosomes replicate only after replication of all other autosomes

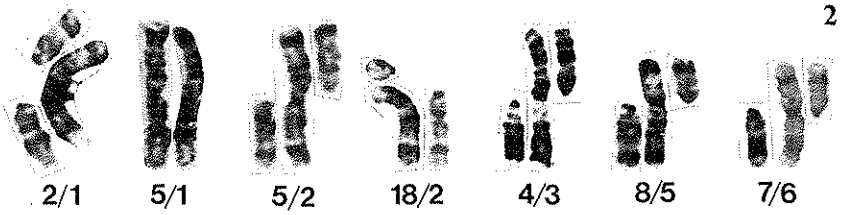

Fig. 2. Robertsonian (Rb) polymorphisms present in *H. brasiliensis,* shown in the heterozygous state in G-banded preparations. Rb5/1 is shown in the homozygous metacentric condition because no heterozygous animals for this translocation were found, although both homozygous forms (metacentric and twin acrocentric) were found in the same population (see Table I).

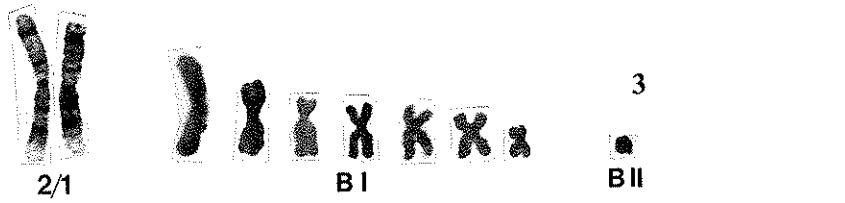

Fig. 3. B chromosomes present in *H. brasiliensis,* from conventionally stained preparations. BI elements vary considerably in size and are metacentric and largely euchromatic. BII elements are small, acrocentric, and largely heterochromatic. The largest metacentric from the *H. brasiliensis* karyotype, 2/1, is included for size comparison.

Table I. Chromosomal polymorphisms among seven populations of *Holochilus brasiliensis*[a]

N	2n	NF	Rb 1/2	Rb 3/4	Rb 5/8	Rb 6/7	B	Other[b]
Bahia Negra								
1	48	58	++	++	++	++	0	
1	48	59	++	++	++	++	0	inv11
1	49	58	+–	++	++	+–	0	Rb2/18(+–)
2	49	58	++	++	++	+–	0	
1	49	59	++	++	++	+–	0	inv11
2	49	60	++	++	++	++	1I	
2	50	60	++	++	++	+–	1I	
1	51	58	++	––	++	+–	0	
1	51	60	++	+–	++	+–	1I	
1	51	62	++	++	++	+–	2I	
Fonciere								
10	48	58	++	++	++	++	0	
1	48	59	++	++	++	++	0	inv11
5	49	58	++	+–	++	++	0	
5	49	58	++	++	++	+–	0	
1	49	59	++	++	++	+–	0	eaX
1	49	59	++	++	++	++	1II	
2	49	60	++	++	++	++	1I	
2	50	58	++	++	++	––	0	
2	50	60	++	++	++	+–	1I	
1	50	62	++	++	++	++	2I	
Rosario								
1	48	57	++	+–	++	++	0	XO
5	49	58	++	+–	++	++	0	
1	49	59	++	+–	++	++	0	inv11
4	49	60	++	++	++	++	1I	
1	50	59	++	++	++	+–	1II	
1	50	62	++	++	++	++	2I	
1	51	58	++	––	++	+–	0	
1	51	60	++	+–	++	+–	1I	
1	51	62	++	++	++	+–	2I	
Golondrina								
8	48	58	++	++	++	++	0	
1	48	59	++	++	++	++	0	invX
3	49	58	++	++	++	+–	0	
3	49	58	++	+–	++	++	0	
1	49	58	++	+–	++	++	0	ea17
1	49	59	++	++	++	+–	0	eaX
1	49	59	++	+–	++	++	0	eaX
Golondrina (cont.)								
1	49	59	++	++	++	+–	0	inv11
3	49	60	++	++	++	++	1I	
1	49	60	++	++	++	++	1II	inv11
1	50	58	++	+–	++	+–	0	
1	50	59	++	+–	++	++	1II	
2	50	60	++	+–	++	++	1I	
2	50	60	++	++	++	+–	1I	
1	50	60	++	++	++	+–	1I	ea17
1	51	59	++	+–	++	+–	1II	
Itati								
2	51	58	++	++	+–	––	0	
1	51	59	++	++	––	+–	0	eaX
1	52	58	++	++	––	––	0	
3	52	58	+–	++	––	+–	0	
1	52	59	++	++	+–	––	1II	
1	53	58	++	––	––	+–	0	
1	53	58	––	+–	+–	––	0	
1	53	59	+–	+–	+–	––	0	inv11
1	53	59	––	++	+–	––	0	eaX
1	53	60	++	++	––	––	1I	
1	54	58	+–	+–	––	––	0	
1	54	58	+–	––	+–	––	0	
2	54	59	––	+–	+–	––	0	eaX
1	54	59	++	––	+–	––	1II	
1	54	60	+–	++	––	––	1I	
1	54	63	++	––	++	––	2I	eaX
1	56	59	+–	––	––	––	1II	
Esquina								
1	51	58	++	+–	––	+–	0	
1	51	59	++	++	––	+–	0	eaX
1	52	58	++	++	––	––	0	
1	52	58	––	++	––	++	0	
1	52	58	+–	++	––	+–	0	
1	52	59	++	++	––	––	0	inv11
Santa Fe								
1	52	58	––	++	––	––	0	Rb1/5(++)
1	54	58	––	+–	––	––	0	Rb2/5(+–)
3	54	60	––	+–	––	––	1I	Rb1/5(++)

[a] Within each population, each row depicts a unique karyotype. Specimens corresponding to each karyotype are listed in the Appendix. *N* = sample size, 2n = diploid number, NF = *nombre fondamental,* Rb = Robertsonian translocation, + = metacentric, – = acrocentric. B chromosomes are type I or type II, as indicated.

[b] Rearrangements listed under "Other" occur as heterozygotes, except where indicated. inv = Pericentric inversion on designated chromosome; ea = euchromatic addition on designated chromosome; XO = female with single X.

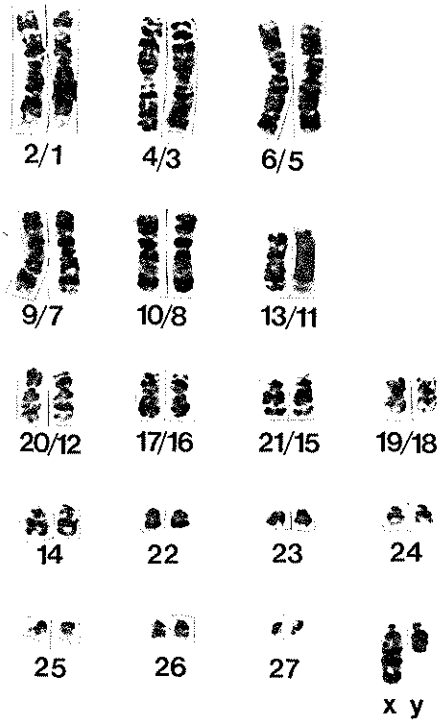

Fig. 4. G-banded preparation of the most common karyotype among *H. vulpinus* (MWN 401). Autosome arms are numbered according to their homology with those possessed by *H. brasiliensis*.

and both sex chromosomes is complete (Nachman and Brown, unpublished data). Twenty-seven animals (22%) had a single BI chromosome, 5 (4%) had two BI chromosomes, 8 (7%) had a single BII chromosome, and the remaining 82 (67%) had no B chromosomes. B chromosomes were found in six of the seven populations studied.

The centromeric rearrangements (putative pericentric inversions) and the differences in amount of euchromatin found in this study appear to be identical to those already described for the Golondrina population (Nachman and Myers, 1989). Because of the small size of the putative inversions, banding data provided little resolution, and the possibility that these rearrangements are due to either centromeric shifts or activation/inactivation of existing centromeres cannot be ruled out. Pericentric inversions have previously been described in *Holochilus* by Vidal and Riva (1978) and by Sangines and Aguilera (1991). A pericentric inversion on one copy of autosome 11 (inv11) was found in eight animals (7%), representing six of the seven populations. A pericentric inversion on a single X (invX) chromosome was found in one female (1%) from the Golondrina population. The differences in amount of euchromatin can be described as additions relative to the most common karyotype, although there is no basis at present for identifying the ancestral condition and thereby determining whether chromatin has been lost or gained. Nine animals (7%), representing four populations, had terminal euchromatic additions (C-band negative, G-band negative) to the short arm of the X chromosome (eaX), and two animals (2%) from the Golondrina population had euchromatic additions (C-band negative, G-band positive) adjacent to the centromere on the major arm of autosome 17 (ea17).

One female (MWN 327) from the Rosario population was unusual in that she had only one X chromosome. This female produced two litters of normal size (one with three and the other with five offspring) in captivity.

The frequencies of heterozygotes and homozygotes within each population were compared to those expected under Hardy-Weinberg equilibrium, using χ^2 goodness-of-fit tests. Tests were performed on all rearrangements where sample sizes justified statistical analysis (Rb3/4 and Rb6/7 from Bahia Negra, Fonciere, Rosario, Golondrina, and Itati; Rb1/2, Rb5/8, and eaX from Itati). To increase the power of the test, both homozygous classes were pooled and compared against heterozygotes (Porter and Sites, 1985). No significant differences were found in any of the populations tested ($P > 0.05$) except for Itati, where there is a very slight excess over expected values of homozygotes for Rb3/4 ($P = 0.05$).

To see if levels of chromosomal variation were similar among the five northern populations (where sample size justified the analyses), several comparisons were made. G tests (log-likelihood ratios) of independence indicate that the frequencies of homozygotes and heterozygotes for Rb3/4 and for Rb6/7 do not differ significantly among these five populations (for Rb3/4, $G = 9.1$ and $P > 0.05$; for Rb6/7, $G = 9.0$ and $P > 0.05$). G tests were also calculated on (1) the frequencies of individuals with and without B chromosomes among the five populations and (2) the frequencies of B chromosomes among populations without regard to their distribution among individuals within a population; neither of these tests revealed significant differences among populations ($G = 5.8$, $P > 0.1$ and $G = 6.26$, $P > 0.05$).

To assess whether there is any geographic pattern to the Rb variation, the frequencies of acrocentrics and metacentrics for Rb3/4 and Rb6/7 were calculated and compared among the five northern populations. The predicted pattern is a north-to-south reduction in the frequency of acrocentrics (Freitas et al., 1983; Aguilera and Perez-Zapata, 1989). Results show that the frequencies of acrocentrics differed significantly for Rb3/4 ($G = 15.9$, $P < 0.005$) and for Rb6/7 ($G = 76.8$, $P < 0.001$) among these populations, but not in the predicted manner. Contrary to expectations, there may be a slight overall trend toward a more acrocentric karyotype in the southern populations (Table I); however, this can be accounted for by the Itati population, which displays a high frequency of acrocentrics for both rearrangements. If the Itati animals are eliminated from the analysis, then the frequencies of acrocentrics and metacentrics for both Rb3/4 and Rb6/7 do not differ significantly among the remaining four populations (for Rb3/4, $G = 7.94$ and $P = 0.05$; for Rb6/7, $G = 4.76$ and $P > 0.05$).

Holochilus vulpinus

The most common karyotype of this species, with 2n = 36 chromosomes and an NF = 58, was present in three animals from Las Cuevas and eight animals from Ibicuy. The major autosome arms of this karyotype have been numbered according to their homologies with the karyotype of *H. brasiliensis*, as determined from G-banded metaphase preparations (Fig. 4). The karyotype contains six pairs of large metacentric or subme-

Table II. Chromosomal polymorphisms among two populations of *Holochilus vulpinus*[a]

N	2n	NF	BII	Other
Las Cuevas				
1	35	57	0	XO
3	36	58	0	
5	37	59	1	
1	37	60	1	eaX
3	38	60	2	
1	39	61	3	
Puerto Ibicuy				
8	36	58	0	
1	37	59	1	
1	39	61	3	

[a] See footnotes to Table I.

tacentric chromosomes, whose arms are readily identifiable with G-banding (1/2, 3/4, 5/6, 7/9, 8/10, and 11/13), four pairs of medium-sized metacentric or submetacentric chromosomes, whose arms are *not* easily identified with G-banding, a single small, metacentric pair which appears to be homologous with autosome 23 in *H. brasiliensis*, six pairs of small acrocentric autosomes, and acrocentric X and Y chromosomes. The acrocentric short arms are frequently visible on the X chromosome, but not on the Y. As in *H. brasiliensis,* the X is approximately the size of autosome 6, and the Y is approximately the size of autosome 16. C-banding reveals that the autosomes and X chromosome possess only centromeric heterochromatin, but that the Y chromosome is largely heterochromatic, as previously reported for this taxon (Freitas et al., 1983).

Variation within and between the two populations of *H. vulpinus* (Table II) results from two kinds of changes: variation in the number of BII chromosomes and variation in the amount of euchromatin. Six karyotypes were present among the 24 animals (2n = 35–39, NF = 57–61). All B chromosomes were small, acrocentric, and largely heterochromatic. Seven animals (29%) had a single BII chromosome, 3 (13%) had two BII chromosomes, 2 (8%) had three BII chromosomes, and the remaining 12 (50%) had no B chromosomes. An addition of euchromatin (relative to the most common condition) to the short arm of one X chromosome was detected in a single female from Las Cuevas. One female (MWN 824 from Las Cuevas) was found with only one X chromosome; no data exist on the fertility of this female.

Discussion

Karyotypic variability in H. brasiliensis

The findings presented here demonstrate an unusually high level of chromosomal polymorphism in all populations of *H. brasiliensis* sampled. Most of the highly frequent rearrangements within populations were found in most or all of the populations sampled (e.g., Rb3/4, Rb6/7, BI chromosomes). In contrast, rearrangements present in low frequencies within populations (e.g., eaX, BII chromosomes) were present in fewer of the populations sampled, and thus their absence from some populations may be due to sampling bias.

The finding of extensive structural heterozygosity in every population of *H. brasiliensis* argues against underdominance for these rearrangements. If chromosomal rearrangements are underdominant, then chromosomal heterozygotes should be rare in natural, nonhybrid populations. This holds true regardless of whether the effective population size (N_e) is small or large. In situations with a small N_e, new rearrangements will tend to be either rapidly fixed or eliminated, whereas in situations with a large N_e, new rearrangements will tend to be eliminated (Lande, 1979). Although the finding of extensive heterozygosity falsifies the hypothesis of strong underdominance, the finding of chromosomally monomorphic populations (as was found for *H. vulpinus* with respect to Rb mutations) neither corroborates nor falsifies the same hypothesis. This is because if chromosomal mutations are selectively neutral, then the degree of polymorphism in a population will depend on the mutation rate and N_e. Thus, in a situation with a low mutation rate and small N_e, a neutral model also predicts that structural heterozygotes will be rare.

Additional evidence against strong underdominance comes from comparisons of the observed frequencies of heterozygotes and homozygotes to those expected under Hardy-Weinberg equilibrium. However, these statistical results should be interpreted with at least two caveats. First, a few of the animals in the Bahia Negra and Fonciere populations are known to come from the same litter (born to wild-caught pregnant females) and thus do not represent independent samples. Second, sample sizes are not sufficiently large to detect small deviations (e.g., $< 10\%$) from Hardy-Weinberg equilibrium values, even though small deviations may be very important biologically.

The lack of obvious underdominance reported here is corroborated by meiotic studies on rates of nondisjunction (Nachman, 1992) and is inconsistent with assumptions in classical (White, 1978) and monobrachial (Baker and Bickham, 1986) models of chromosomal speciation. Of particular interest in this regard are the monobrachial homologies found among populations (e.g., Santa Fe and Esquina), within populations (Santa Fe, Bahia Negra), and within an individual (MWN 346 from Bahia Negra).

Although chromosomal rearrangements in *H. brasiliensis* are probably not underdominant, this does not explain why such polymorphisms persist. If heterozygotes are not selected against, they may be either neutral or at a selective advantage. Distinguishing between these alternatives is difficult, especially if selection coefficients are small. If chromosomal rearrangements are sufficiently heterotic, then the frequencies of heterozygotes may exceed those expected under Hardy-Weinberg equilibrium, as demonstrated by Baker et al. (1983). If rearrangements are neutral, then the frequencies of polymorphisms are determined by the mutation rate and genetic drift. In a neutral model, the high level of polymorphism observed in *H. brasiliensis* could be explained by a high mutation rate. Alternatively, it could be explained by population dynamics that permit the random fixation of alternative homozygous forms in small isolated demes for brief periods, followed by periods of expansion, during which these demes interbreed. It is possible that this type of

population structure exists in some parts of the range of *H. brasiliensis*. Marsh rats are strictly limited to aquatic habitats. In the Chaco region of Argentina and Paraguay, the seasonal environment results in extensive expansion and contraction of marshes. During wet periods, many of these marshes interconnect, but during dry periods, many small marshes are isolated from each other by large expanses of extremely arid land, which is unsuitable for *H. brasiliensis*.

Geographic pattern of variation in H. brasiliensis

Previous investigators (Freitas et al., 1983; Aguilera and Perez-Zapata, 1989) have noted that karyotypes of *H. brasiliensis* from central Brazil are entirely acrocentric (2n = 56) and that karyotypes to the north and south are more metacentric. They have proposed that the 2n = 56 karyotypes of central Brazil are ancestral and that a gradual reduction in diploid number links the central Brazilian populations with the lower-diploid-number populations known from Argentina. However, the results from this study indicate that such a pattern is not observed and that, on the contrary, the diploid numbers of the northern four populations are lower (2n = 48–51) than the diploid numbers of the southern three populations (2n = 51–56). This suggests a more complicated picture of chromosomal evolution than previously envisioned. The populations of *H. brasiliensis* sampled here range from 20° S to 31° S latitude and cover a wide transitional area between different ecological zones. The northern population lies within the tropics, is near the Mato Grosso of Brazil, and is associated with a fauna and flora that are similar to those found in the Amazon basin. The southern population, in contrast, shares little of the tropical fauna and flora and is in a temperate-subtropical region. Dobzhansky (1970) and Nevo et al. (1988) have argued that correlations between general features of the abiotic environment (e.g., temperature, rainfall, elevation) and features of the karyotype may indicate adaptations of particular chromosomal combinations to particular environments. Despite the substantial chromosomal variation found, there is no evidence for such correlations among populations of *H. brasiliensis*.

Evidence for distinct species

The relationship between *H. vulpinus* and *H. brasiliensis* has been debated since these taxa were first described; there is disagreement on whether these taxa represent one or two biological species. The taxonomic problem is complicated by the fact that all comparisons have been made between populations separated by hundreds of kilometers (see, e.g., Massoia, 1971). Comparisons of populations in allopatry cannot distinguish between geographic variation and differences due to speciation events. The present study was designed to narrow the known geographic distance between these two forms. The Santa Fe population, which consists entirely of *H. brasiliensis*, is 50 km from the Las Cuevas population, which consists entirely of *H. vulpinus*. Both populations were collected in a similar habitat from islands in the middle of the Parana river.

The karyotypic data further support the view that these taxa are biological species. The diploid numbers of *H. brasiliensis* (2n = 48–56) are considerably higher than those of *H. vulpinus* (2n = 35–39), and there is no overlap in diploid number between these taxa. While certain fusions are shared between these taxa (e.g., Rb1/2, Rb3/4), most chromosome-arm combinations are unique to either *H. brasiliensis* (Rb1/5, Rb2/5, Rb2/18, Rb5/8, Rb6/7) or *H. vulpinus* (Rb5/6, Rb7/9, Rb8/10, Rb11/13, and four additional small Rb rearrangements). Furthermore, the nature of chromosomal variation is distinct in these taxa. *Holochilus brasiliensis* is polymorphic for Rb mutations, BI and BII chromosomes, pericentric inversions, and euchromatic additions, whereas *H. vulpinus* is polymorphic only for BII chromosomes and euchromatic additions.

The chromosomal differences observed between these taxa could serve as genetic markers for studies of hybridization. The finding of "*vulpinus* Rb fusions" in "*brasiliensis* karyotypes," for example, would provide evidence of chromosomal introgression. At present, there is no karyological evidence for hybridization, although such hybridization could occur in a narrow zone between the Santa Fe and Las Cuevas populations.

The monobrachial differences observed between *H. brasiliensis* and *H. vulpinus* fit the pattern expected from models of chromosomal speciation by monobrachial centric fusion. However, the observation of monobrachial polymorphisms within populations of *H. brasiliensis* suggests that these rearrangements do not restrict gene flow. Consequently, the chromosomal differences observed between these two species are unlikely to have fostered speciation. An additional test of the effects of these rearrangements on fitness is provided by estimates of rates of nondisjunction in heterozygous carriers (Nachman, 1992).

Acknowledgements

For their help and hospitality in Paraguay, I thank Mr. and Mrs. Anthony Espinoza, Mr. and Mrs. Philip Myers Jr., Steven Goodman, Laurie Russman, and the Paraguayan Ministerio de Agricultura y Ganaderia. For their help and hospitality in Argentina, I thank Dr. Osvaldo Reig and the members of his laboratory, as well as Maria Alicia Barros, Amir Dyzenchauz, Jane Emley, and Patricia Kandus. For helpful discussions and comments on the manuscript, I thank Drs. W.M. Brown, E. Chu, C. Moritz, P. Myers, J.B. Searle, P. Smouse, P.K. Tucker, and two anonymous reviewers.

Appendix

Within each locality, specimens are listed by karyotype in the order in which they are listed in Tables I and II. When two or more specimens have the same karyotype, they are grouped within parentheses. Specimens and the field catalog are deposited in the collections of the Museum of Zoology, University of Michigan.

Holochilus brasiliensis

Bahia Negra (N = 13): Paraguay, Dept. Alto Paraguay, west bank of Rio Paraguay along Riacho Ramos, 6 km SE (by air) of Bahia Negra, 20°16′ S, 58°07′ W; specimens MWN 385, 350, 346, (347, 352), 351, (345, 386), (349, 354), 348, 344, 387. Fonciere (N = 30): Paraguay, Dept. Presidente Hayes, west bank of Rio Paraguay, 4 km NW of Puerto Fonciere, 22°25′ S, 57°52′ W; specimens MWN (363, 364, 413, 415, 416, 417, 419, 422, 424, 425), 355, (359, 414, 418, 420, 426), (361, 408, 410, 421, 423), 411, 412, (357, 409), (362, 405), (360, 406), 356. Rosario (N = 16): Paraguay, Dept. San Pedro, island in middle of Rio Paraguay, 10 km (by air) NW of Rosario, 24°19′ S, 57°10′ W; specimens MWN 327, (318, 320, 325, 328, 335), 319, (322, 326,

329, 331), 330, 324, 333, 334, 332. Golondrina ($N = 31$): Paraguay, Dept. Presidente Hayes, 24 km by air NW of Villa Hayes, Estancia La Golondrina, 25°05′ S, 57°45′ W; specimens MWN (110, 111, 113, 114, 115, 123, 125, 127), 124, (107, 117, 122), (112, 119, 133), 135, 129, 130, 134, (106, 109, 116), 118, 132, 120, (108, 126), (104, 121), 105, 131. Itati ($N = 21$): Argentina, Prov. Corrientes, 0.5 km N of Itati, island in Rio Parana, 27°15′ S, 58°20′ W; specimens MWN (710, 718), 715, 703, (711, 712, 714), 708, 719, 721, 705, 722, 701, 706, 713, (704, 707), 717, 709, 716, 702. Esquina ($N = 6$): Argentina, Prov. Corrientes, 0.5 km W of Esquina, island in Rio Parana, 30°00′ S, 59°35′ W; specimens MWN 734, 732, 730, 738, 739, 731. Santa Fe ($N = 5$): Argentina, Prov. Santa Fe, 12 km (by road) E of Santa Fe on island in Rio Parana, 31°40′ S, 60°35′ W; specimens MWN 752, 748, (749, 751, 757).

Holochilus vulpinus

Las Cuevas ($N = 14$): Argentina, Prov. Entre Rios, 35 km SSE of Diamante on island in floodplain of Rio Parana, Las Cuevas, 32°22′ S, 60°30′ W; specimens MWN 824, (816, 819, 827), (815, 817, 818, 821, 822), 823, (814, 820, 831), 813. Ibicuy ($N = 10$): Argentina, Prov. Entre Rios, 6 km S (by road) of Puerto Ibicuy, 33°47′ S, 59°10′ W; specimens MWN (392, 393, 394, 396, 397, 400, 401, 402), 395, 398.

References

Aguilera M, Perez-Zapata A: Cariologia de *Holochilus venezuelae* (Rodentia, Cricetidae). Acta Cie Venez 40:198–207 (1989).

Baker RJ, Bickham JW: Speciation by monobrachial centric fusions. Proc natl Acad Sci, USA 83:8245–8248 (1986).

Baker RJ, Chesser RK, Koop BF, Hoyt RA: Adaptive nature of chromosomal rearrangement: differential fitness in pocket gophers. Genetica 61:161–164 (1983).

Davisson MT, Akeson EC: An improved method for preparing G-banded chromosomes from mouse peripheral blood. Cytogenet Cell Genet 45:70–74 (1987).

Dobzhansky T: Genetics of the Evolutionary Process (Columbia University Press, New York 1970).

Evans EP, Breckon G, Ford CE: An air-drying method for meiotic preparations from mammalian testes. Cytogenetics 3:289–294 (1964).

Freitas TRO, Mattevi MS, Oliveira LFB, Souza MJ, Yonenaga-Yassuda Y, Salzano FM: Chromosome relationships in three representatives of the genus *Holochilus* (Rodentia, Cricetidae) from Brazil. Genetica 61:13–20 (1983).

Hershkovitz P: South American marsh rats, genus *Holochilus*, with a summary of sigmodont rodents. Fieldiana Zool 37:639–673 (1955).

Holmquist G, Gray M, Porter T, Jordan J: Characterization of Giemsa dark- and light-band DNA. Cell 31:121–129 (1982).

Lande R: Effective deme sizes during long-term evolution estimated from rates of chromosomal rearrangement. Evolution 33:234–251 (1979).

Leversha M, Sinfield C, Webb G: Rapid and reliable methods for the G-and C- banding of human and other mammalian chromosomes. Aust J med Lab Sci 1:139–143 (1980).

Massoia E: Caracteres y rasgos bioecologicos de *Holochilus brasiliensis chacarius* Thomas ("rata nutria") de la provincia de Formosa y comparaciones con *Holochilus brasiliensis vulpinus* (Brants). Rev Invest Agropecuarias, INTA, B.A. Argentina. Biol Prod Anim 8:13–40 (1971).

Nachman MW: Meiotic studies of Robertsonian polymorphisms in the South American marsh rat, *Holochilus brasiliensis*. Cytogenet Cell Genet 61:17–24 (1992).

Nachman MW, Myers P: Exceptional chromosomal mutations in a rodent population are not strongly underdominant. Proc natl Acad Sci, USA 86:6666–6670 (1989).

Nevo E, Corti M, Heth G, Beiles A, Simson S: Chromosomal polymorphisms in subterranean mole rats: origins and evolutionary significance. Biol J Linnean Soc 33: 309–322 (1988).

Patton JL: Chromosome studies of certain pocket mice, genus *Perognathus* (Rodentia, Heteromyidae). J Mammal 48:27–37 (1967).

Porter CA, Sites JW: Normal disjunction in Robertsonian heterozygotes from a highly polymorphic lizard population. Cytogenet Cell Genet 39:250–257 (1985).

Riva R, Vidal OR, Baro NI: Los chromasomas del genero *Holochilus*. II. El cariotipo de *H. brasiliensis vulpinus*. Physis Sec C 36:215–218 (1977).

Sangines N, Aguilera M: Chromosome polymorphism in *Holochilus venezuelae* (Rodentia: Cricetidae): G- and C-bands. Genome 34:13–18 (1991).

Verma RS: The varieties of R-banding—their methodology and application. Karyogram 8:72–73 (1982).

Vidal OR, Riva R: Los cromasomas del genero *Holochilus*. III. Inversion, fusion y chromosomas B, nuevos para *H. chacarius* Balnearum. Physis Sec C 38:1–5 (1978).

Vidal OR, Riva R, Baro NI: Los cromosomas del genero *Holochilus*. I. Polimorfismo en *H. chacarius* Thomas (1906). Physis Sec C 35:75–85 (1976).

White MJD: Modes of Speciation (WH Freeman, San Francisco 1978).

Yonenaga-Yassuda Y, do Prado RC, Mello DA: Supernumerary chromosomes in *Holochilus brasiliensis* and comparative cytogenetic analysis with *Nectomys squamipes* (Cricetidae, Rodentia). Rev Bras Genet 10:209–220 (1987).