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Meiotic studies of Robertsonian polymorphisms in the South American marsh rat, *Holichilus brasiliensis*

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Abstract. Meiosis was studied in male South American marsh rats (1) to help clarify the mechanisms that allow unusually high levels of Robertsonian (Rb) polymorphisms to be maintained in wild populations of these animals and (2) to test competing assumptions in two distinct models of chromosomal speciation. In both simple Rb heterozygotes and Rb heterozygotes with monobrachial homology, no univalency was observed in prophase I or metaphase I. Rates of nondisjunction were uniformly low (<10%) and did not differ significantly among any of the animals studied, regardless of karyotype and in contrast to the frequency of nondisjunction in other mammalian species. Robertsonian heterozygotes exhibited significantly more chiasmata than did homozygotes, largely owing to an increase in the number of terminally located chiasmata. There was a significant bias favoring the transmission of two acrocentrics over the single metacentric for some Rb rearrangements in the heterozygous state. In addition, the frequency of sex-chromosome univalency increased with increasing Rb heterozygosity, although the ratio of X- and Y-bearing secondary spermatocytes did not differ significantly from 1:1, and no secondary spermatocytes were observed that were nullisomic or disomic for an X or Y chromosome.

Understanding the role of chromosomes in evolution requires an understanding of how selection affects chromosomal change. Most discussions of selection on chromosomal rearrangements focus on meiotic events because (1) the proper regulation of meiosis is essential for the production of balanced gametes and (2) the complexity of the meiotic process provides many separate events (e.g., pairing, recombination, segregation) on which selection may act.

South American marsh rats, *Holichilus brasiliensis,* are unusual among mammals in possessing extensive within-population chromosomal variability (Nachman, 1992). Consequently, they provide a useful system for studying the meiotic consequences of several different chromosomal rearrangements. In particular, marsh rats exhibit both simple and monobrachial Robertsonian (Rb) variation within natural populations. This makes it possible to evaluate contrasting assumptions in two distinct models of chromosomal speciation. One model (White, 1978) assumes that simple Rb heterozygotes will lead to high rates of nondisjunction. The other model (Baker and Bickham, 1986) assumes that simple Rb heterozygotes will have little or no effect on meiosis, but that monobrachial heterozygotes, which require the formation of quadrivalents (or even more complex chains) at prophase I, will lead to high rates of nondisjunction. Among mammals, rates of nondisjunction for simple Rb heterozygotes have been measured in only a few species. Nondisjunction estimates for monobrachial heterozygotes have been measured only in house mice, one of the species for which the monobrachial speciation model was created. Understanding how Rb rearrangements behave during meiosis is important, not only for explaining their potential role in speciation but also for explaining how these rearrangements persist as polymorphisms, spread, or become fixed in natural populations.

In this paper, I address the effects of simple and monobrachial Rb heterozygotes on the pairing, recombination (as measured by the frequency of chiasmata), and segregation of Rb translocation products, other autosomes, and the sex chromosomes. The marsh rats included in this study also possess an interesting system of supernumerary (B) chromosomes; however, meiotic studies of these B chromosomes will be published elsewhere (Nachman and Brown, unpublished data).

Materials and methods

Animals and breeding

Animals were collected alive for this study from three populations in Paraguay (Nachman, 1992) and used to found breeding colonies. Lineages were established to preserve the different rearrangements, and crosses were set up to produce heterozygotes for each of the Rb rearrangements, both singly and in combination. The collecting localities, specimens, crosses, and karyotypes are given in the Appendix.

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Table I. Numbers of paired and unpaired chromosomes at diakinesis and metaphase I in specimens of *Holochilus brasiliensis* with differing amounts of Robertsonian (Rb) heterozygosity

Rb heterozygotes	Number of cells	Number of paired configurations at metaphase I (observed/expected)				Number of unpaired chromosomes at metaphase I		Specimens
		Quadri-valents	Tri-valents	Autosomal bivalents	X-Y bivalents	Auto-somes	X-Y	
Homozygous	57			1,311/1,311	57/57	0	0	MWN 561, MWN 222
3/4	73		73/73	1,606/1,606	73/73	0	0	MWN 426, MWN 108
6/7	11		11/11	242/242	11/11	0	0	MWN 351
3/4, 6/7	33		66/66	693/693	32/33	0	2	MWN 502
1/2, 2/18, 6/7	70	70/70	70/70	1,400/1,400	65/70	0	10	MWN 346, MWN 560, MWN 601
1/2, 2/18, 3/4, 6/7	33	33/33	66/66	627/627	28/33	0	10	MWN 600
Total	277	103/103	286/286	5,879/5,879	266/277			

Chromosome preparations

Mitotic karyotypes were determined from cell cultures of peripheral lymphocytes (Davisson and Akeson, 1987). Meiotic preparations were made from males only, following Evans et al. (1964) with minor modifications, and C-banded preparations (Leversha et al., 1980) were made to help clarify the orientation of some rearrangements in prophase I and metaphase I. Representative cells from all animals were photographed.

Analysis

Pairing was studied in 277 cells (representing 10 animals) at diakinesis or early metaphase. These cells were scored for the following parameters: (1) total number of configurations, (2) number of chain configurations (quadrivalents and trivalents), (3) number of metacentric autosomal bivalents, (4) number of acrocentric autosomal bivalents, (5) presence of a sex-chromosome bivalent, (6) number and identity of univalents, and (7) associations between sex chromosomes and other chromosomes.

Chiasma counts were made on a total of 135 cells (from seven animals) in late diplotene and early diakinesis. Only cells in which sister chromatids could be resolved and in which all configurations were present and unobscured were included. "Polyploid" spreads (which are thought to be artifacts [Beatty et al., 1975]) were excluded from the analysis of cells in both diakinesis and other stages of meiosis. Chiasmata were counted for all configurations, and among the eight largest autosomes, chiasmata were scored as terminal, interstitial, or proximal to the centromere. Chiasmata were scored as terminal or proximal if they occurred within one-fourth the length of the chromosome arm to the telomere or centromere, respectively, and all chiasmata occurring in the middle half of the chromosome arm were scored as interstitial. All associations between the X and Y chromosomes were scored as a single chiasma, although it is not clear whether the observed associations are truly chiasmatic (see below).

Segregation was studied by looking at the distribution of chromosomes in a total of 1,003 secondary spermatocytes at metaphase II, representing 12 animals. These cells were scored for the following parameters: (1) total number of chromosomes, (2) number of major chromosome arms, (3) number of large metacentrics (pairs 1/2, 2/18, 3/4, 5/8, and 6/7), (4) presence of the X or Y chromosome (where possible), (5) presence of alternative states (i.e., single metacentric vs. two acrocentrics) of translocation products, and (6) origin of disomics (where possible). Rates of nondisjunction for each rearrangement or group of rearrangements were calculated as twice the number of hyperploid cells, divided by the total number of cells scored (Cattanach and Moseley, 1973). This estimate makes the assumption that nullisomy and disomy occur at equal rates in secondary spermatocytes, but it avoids including cells that are missing a chromosome due to cell breakage. For animals possessing a single B chromosome, I calculated the rates of nondisjunction as four times the number of cells with more than the haploid + B-chromosome number of chromosome arms, divided by the total number of cells scored. This estimate assumes that (1) transmission of a single B chromosome to either daughter cell is equally likely and (2) the probability of a nondisjunction event is independent of B-chromosome segregation. The first assumption has been tested and appears to be valid (Nachman and Brown, unpublished data).

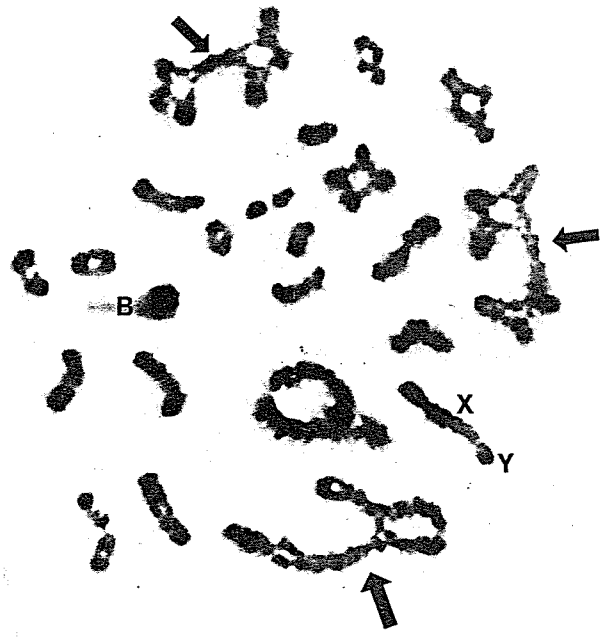

Fig. 1. Diakinesis cell of a male *Holochilus brasiliensis* (MWN 600) with a single B chromosome and heterozygous for four Rb translocations (Rb1/2, Rb2/18, Rb3/4, and Rb6/7), including two that share monobrachial homology. The trivalents (3-3/4-4 and 6-6/7-7) are indicated by small arrows, and the quadrivalent (1-1/2-2/18-18) is indicated by a large arrow.

Results

Pairing and rates of univalency

The frequency of chromosome pairing at metaphase I for animals with differing amounts of Rb heterozygosity is shown in Table I. In all cases (389 configurations), Rb translocation heterozygotes formed regular chain configurations (Fig. 1). The orientations of trivalents and quadrivalents varied considerably, depending on the number and position of the chiasmata present. Trivalents (Fig. 2) were observed in a "straight" orien-

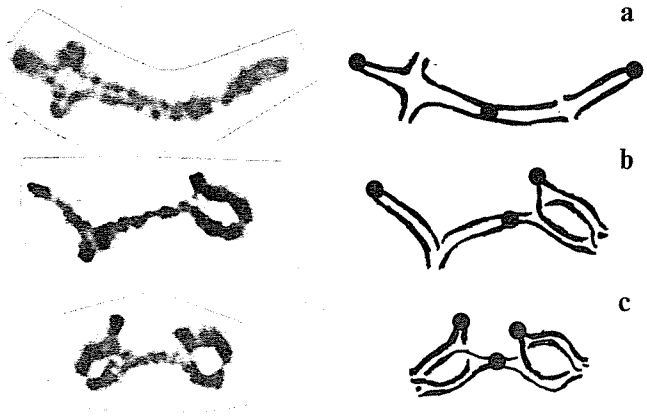

Fig. 2. Orientations of trivalents in diakinesis. Photographs shown at left and interpretative drawings shown at right. Black dots indicate the position of the centromeres. (a) Straight orientation of trivalent 3-3/4-4, with two terminal chiasmata. (b) Tail-and-loop orientation of trivalent 3-3/4-4 with two terminal and one proximal chiasmata. (c) Two-loop orientation of trivalent 6-6/7-7 with two terminal and two proximal chiasmata. Chromosome preparations from animal MWN 600.

tation with two terminal chiasmata (58.9%; Fig. 2a), a "tail and loop" orientation with two terminal and one proximal chiasmata (36.8%; Fig. 2b), or a "two loop" orientation with two terminal and two proximal chiasmata (4.3%; Fig. 2c). Quadrivalents (Fig. 3) were found in either a "straight" orientation with three terminal chiasmata (61.7%; Fig. 3a) or a "parallel" orientation with up to three terminal and two proximal chiasmata (38.3%; Fig. 3b). No associations were observed between chain configurations and other autosomes.

Pairing of the sex chromosomes was observed in 266 of the 277 cells examined. In these 266 cells, the X and Y chromosomes were in contact or nearly in contact, although typical chiasmata were never observed. In some cases, however, chromatin fibers could be seen that appeared to bridge the space between the two chromosomes. In most prophase I and metaphase I spreads, both the X and the Y were heteropycnotic. The frequency of X-Y univalency increased with increasing amounts of Rb heterozygosity. No univalency was observed in cells heterozygous for a single Rb translocation, 3.0% X-Y univalency was observed in double heterozygotes, 7.1% X-Y univalency

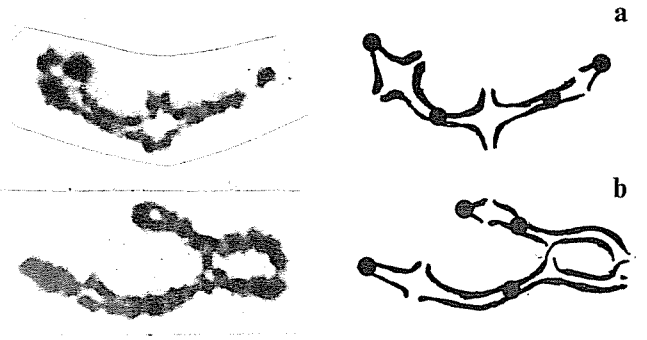

Fig. 3. Orientations of quadrivalents in diakinesis. (a) Straight orientation of quadrivalent 1-1/2-2/18-18 with three terminal chiasmata. (b) Parallel orientation of same quadrivalent with three terminal and one proximal chiasmata. Preparations from animal MWN 600.

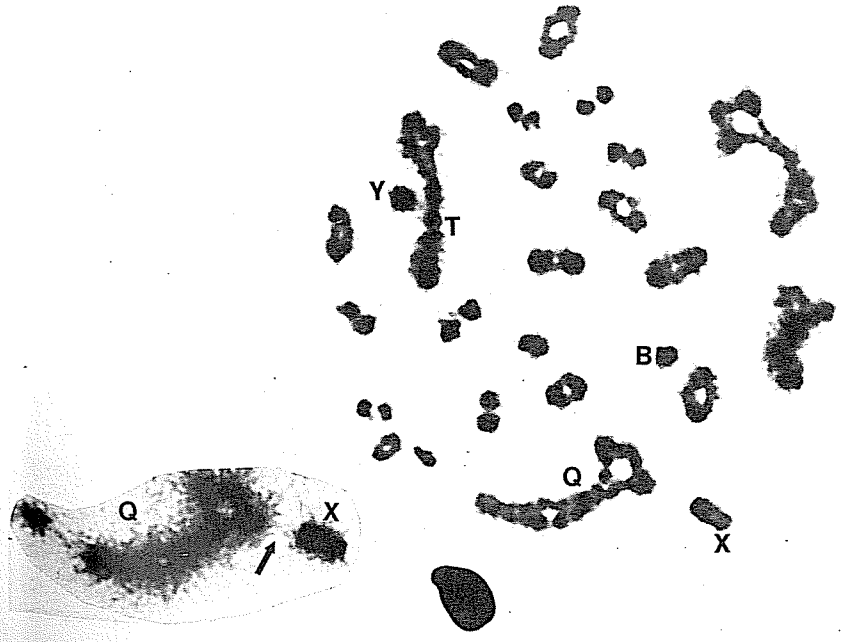

Fig. 4. Metaphase I in a male *Holochilus brasiliensis* (MWN 600) with a single B chromosome and heterozygous for Rb1/2, Rb2/18, Rb3/4, and Rb 6/7 with univalent X and Y chromosomes. The X is associated with the quadrivalent (Q), and the Y is associated with the trivalent (T). The B chromosome and the univalent Y are distinguishable through the microscope because the Y is more heteropycnotic (not clearly visible in photograph). Inset shows the same cell after over-staining with Giemsa. Chromatin fibers are visible between the X chromosome and the quadrivalent (arrow).

was observed in triple heterozygotes (with monobrachial homology), and 15.2% X-Y univalency was observed in quadruple heterozygotes (with monobrachial homology). While these differences form a clear trend, they were not significant (log-likelihood ratio, $G = 3.345$, $P > 0.1$). No associations were observed between the X-Y bivalent and any other chromosomes. In one cell in which the X and Y were unpaired, the X appeared to be in close association with a quadrivalent, and the Y appeared to be in close association with a trivalent, although no true or partial pairing was observed (Fig. 4).

Chiasma frequency and position

A total of 4,005 chiasmata were scored from 135 cells representing seven animals. The mean number of chiasmata per cell was 29.7 ± 1.264 (SD), with a range of 28 to 34 chiasmata per cell. This distribution is truncated and one-tailed due to the absence of fewer than 28 chiasmata per cell, and this distribution differs significantly from normal (Kolmogorov-Smirnov test, $P < 0.001$), as does the distribution of chiasmata for each animal considered separately (for all tests, $P < 0.005$). The minimum number of chiasmata observed per cell (28) is also the number required to maintain a single chiasma per major chromosome arm. There is a significant increase in the mean number of chiasmata per cell in Rb heterozygotes (mean = 29.815) as compared to homozygotes (mean = 29.074), and this difference is largely due to an increase in the number of terminal chiasmata (Table II). No significant differences were observed in the number of proximal or interstitial chiasmata between Rb heterozygotes and homozygotes (Table II).

Segregation ratios and rates of nondisjunction

The segregation of the X and Y chromosomes, as measured by their frequencies in 364 metaphase II cells from seven animals, did not differ significantly from a 1:1 ratio (goodness of fit, $\chi^2 = 0.176$, df = 1, $P > 0.5$, Table III). The segregation of a single metacentric versus two acrocentrics (scored at metaphase II) for Rb heterozygotes is shown in Table IV ($N = 938$ metaphase II cells from 11 animals). The numbers of metacentrics versus twin acrocentrics for the single heterozygote, Rb6/7, and for the double heterozygote, Rb3/4, Rb6/7, differ significantly from those expected under Mendelian segregation. In both cases, the deviation is for a greater-than-expected number of acrocentrics and fewer-than-expected number of metacentrics. This same bias against metacentrics is also seen in Rb3/4, although it is not statistically significant. Thus, in two and possi-

Table II. Comparison of the mean number of total, terminal, interstitial, and proximal chiasmata per cell between homozygous cells and cells with Robertsonian (Rb) heterozygotes[a]

Chiasma type[b]	Mean number of chiasmata/cell (± SD)		Adjusted *P* value[d]
	Homozygotes (27 cells)	Rb heterozygotes[c] (108 cells)	
Total	29.074±0.874	29.815±1.305	0.028
Terminal	6.074±1.412	6.796±1.150	0.048
Interstitial	1.667±1.441	1.204±1.244	0.384
Proximal	1.333±1.074	1.815±1.320	0.472

[a] Specimens: homozygotes, MWN 561; Rb heterozygotes, MWN 346, MWN 426, MWN 502, MWN 560, MWN 600, and MWN 601.
[b] Chiasmata were scored as terminal, interstitial, or proximal only for the eight largest autosomes (see text).
[c] Rb heterozygotes include a pooled sample of individuals with one, two, three, and four Rb heterozygotes.
[d] *P* values obtained from individual Mann-Whitney U-tests were multiplied by 4 to adjust for the number of tests performed.

Table III. Comparison of observed and expected numbers of X-bearing and Y-bearing metaphase II cells (364 cells, seven animals), using a χ^2 test[a]

	X-bearing	Y-bearing	Total	χ^2	*P* value
Observed	186	178	364	0.176	>0.5
Expected	182	182			

[a] Specimens: MWN 125, MWN 346, MWN 408, MWN 426, MWN 502, MWN 560, and MWN 561.

Table IV. Segregation of single metacentric versus two acrocentrics in Robertsonian (Rb) heterozygotes[a]

Rb heterozygotes	Number of cells/animals	Number of metaphase II cells (observed/expected)				χ^2
		Two metacentrics	Three metacentrics	Four metacentrics	Five metacentrics	
Homozygous	265/3	0/0	1/0	263/265	1/0	
3/4	188/2	0/0	105/94	83/94	0/0	2.574
6/7	260/4	0/0	155/130	105/130	0/0	9.615 ($P < 0.005$)
3/4, 6/7	225/2	70/56.25	113/112.5	42/56.25	0/0	6.988 ($P < 0.05$)

[a] Homozygous animals (row 1) contain eight metacentrics at metaphase I, single Rb heterozygotes (rows 2 and 3) contain seven metacentrics at metaphase I, and double Rb heterozygotes (row 4) contain six metacentrics at metaphase I. Expected values, based on random segregation, are compared to observed values using chi-square tests. Specimens: homozygotes, MWN 125, MWN 222, and MWN 561; single heterozygotes (Rb3/4): MWN 135 and MWN 426; single heterozygotes (Rb6/7): MWN 351, MWN 408, MWN 601, and MWN 560; double heterozygotes (Rb3/4, Rb6/7): MWN 502, and MWN 600.

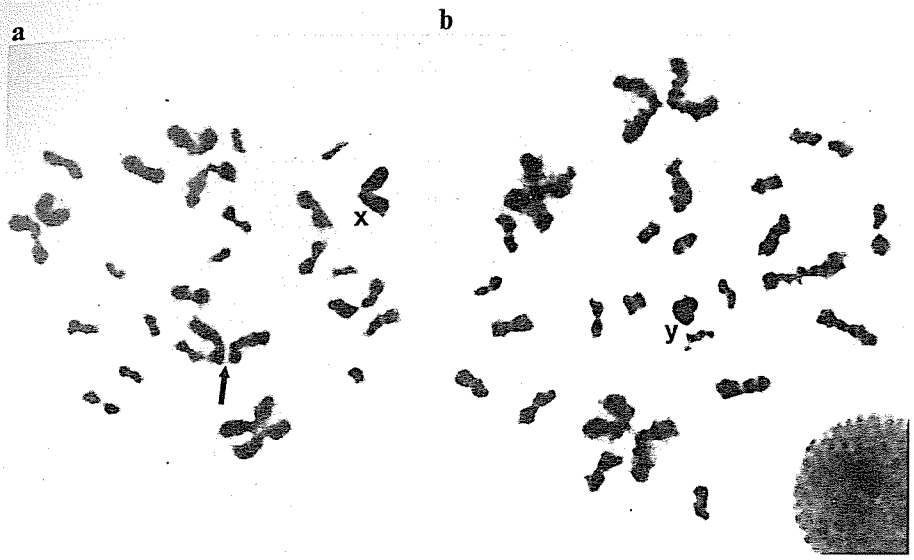

Fig. 5. Metaphase II cells from a *Holochilus brasiliensis* male (MWN 560) heterozygous for Rb1/2, Rb2/18, and Rb6/7. (a) This cell is X-bearing and euploid, with 28 major chromosome arms. The X stains darkly (heteropycnosis) in metaphase II. This cell contains four large metacentrics, one of which (2/18) can be identified based on morphology (arrow). (b) This cell is Y-bearing and euploid, with 28 major chromosome arms; it contains three large metacentric chromosomes. The Y chromosome is heteropycnotic in metaphase II preparations.

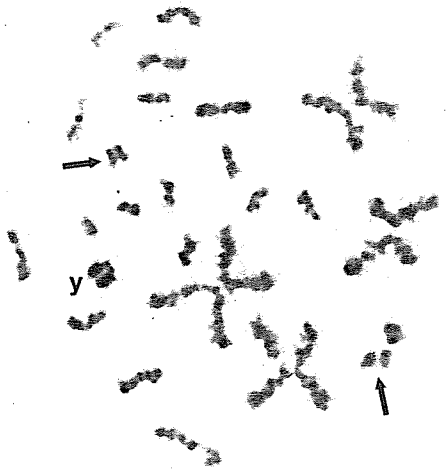

Fig. 6. Metaphase II cell from a *Holochilus brasiliensis* male (MWN 426) heterozygous for Rb3/4. This cell is Y-bearing and aneuploid, with 29 major chromosome arms. It appears to be disomic for autosome 23 (arrows), the only small metacentric chromosome in the karyotype.

Table V. Rates of nondisjunction estimated from metaphase II counts for homozygous animals and animals heterozygous for one to four Robertsonian (Rb) translocations, including both simple and monobrachial heterozygotes[a]

Number of Rb heterozygotes	Arm combination	Number of cells/animals	MII cells $\leq n_a$[b]	MII cells $> n_a$	Rate of nondisjunction[c]
0		267/3	263	4	3.0%
1	6/7	100/2	99	1[d]	4.0%
1	3/4	200/2	197	3	3.0%
2	3/4, 6/7	80/1	79	1	2.5%
3	1/2, 2/18, 6/7	206/3	202	4	3.9%
4	1/2, 2/18, 3/4, 6/7	150/1	148	2[d]	5.3%
Total		1003/12	989	14	

[a] Specimens: homozygotes: MWN 125, MWN 222, and MWN 561; single Rb heterozygotes (Rb6/7): MWN 351 and MWN 408; single Rb heterozygotes (Rb3/4): MWN 135 and MWN 426; double Rb heterozygotes: MWN 502; triple Rb heterozygotes: MWN 346, MWN 560, and MWN 601; quadruple Rb heterozygotes: MWN 600.

[b] Number of metaphase II (MII) cells with less than or the exact haploid number of chromosome arms ($\leq n_a$).

[c] Rates of nondisjunction do not differ significantly among the five groups tested (G log-likelihood ratio = 2.066, $P > 0.5$). See text for calculation of rates.

[d] Karyotype contains a single B chromosome.

bly three cases, some form of meiotic drive appears to be operating to favor the transmission of acrocentrics over metacentrics in Rb heterozygotes.

Rates of nondisjunction estimated from 1,003 metaphase II cells, representing 12 animals, are shown in Table V. Euploid and aneuploid secondary spermatocytes are shown in Figs. 5 and 6. No significant differences were found in the rates of nondisjunction among homozygous animals and animals heterozygous for one to four Rb translocations, including rearrangements with monobrachial homology ($G = 2.066$, df = 4, $P > 0.5$). In this analysis, cells from different individuals with the same karyotype have been pooled and treated as a single sample. The underlying assumption of this approach is that interindividual differences in rates of nondisjunction (within any given "rearrangement category") are not significant. This assumption was tested by comparing rates of nondisjunction among individuals within the three karyotype groups where sample size permitted statistical analysis (the control; Rb3/4; and Rb1/2, Rb2/18, and Rb6/7). No significant differences in rates were found between individuals in these categories (G log-likelihood test, $P > 0.5$ for all cases), suggesting that no large "hidden" differences between individuals are obscured by pooling samples.

There was no apparent trend for higher rates of nondisjunction with increasing structural heterozygosity. The lowest estimated rate was found in the sample with two Rb heterozygotes (2.5%), and the highest rate was found in the sample with four Rb heterozygotes including a monobrachial homology (5.3%). These rates should be interpreted as rough estimates only, as accurate measurements of any event occurring at such low frequencies would require very large samples. The sample sizes in this analysis are sufficient to detect frequency differences of about 6% at a 0.05 level of significance (based on a sample size of 200, and a mean rate of nondisjunction in homozygotes of 3.0%). Thus, these data indicate that rates of nondisjunction for all groups sampled are probably less than 10%.

Table VI. Anaphase I nondisjunction rates for Robertsonian (Rb) heterozygotes in different mammalian species

Genus and species	Type and origin of Rb heterozygote	Genetic background[a]	Sex	Anaphase I nondisjunction rate (%)	Reference
Mus domesticus[b]	1 Simple, wild	Laboratory	M	2–28	Gropp and Winking (1981)
	1 Simple, wild	Laboratory	F	33–61	Gropp et al. (1982)
	1 Simple, wild	Laboratory	M	2–40	Cattanach and Moseley (1973)
	1 Simple, wild	Wild	M, F	10–16	Harris et al. (1986)
	1 Simple, laboratory	Laboratory*	M	3–6	Gropp and Winking (1981)
	2–3 Simple, laboratory	Laboratory	M, F	16–24	White et al. (1972, 1978); Tettenborn and Gropp (1970)
	7 Simple, wild	Laboratory	M	50	Winking and Gropp (1976)
	7–9 Simple, wild	Laboratory	M	51–52	Winking and Gropp (1976)
	7–9 Simple, wild	Laboratory	F	68–77	Gropp et al. (1975)
	2 monobrachial, wild	Laboratory	M	26–44	Redi and Capanna (1978)
	16 Monobrachial, wild	?	M	60–70	Merani et al. (1980)
Akodon molinae	1 Simple, wild	Wild	M	20	Logue and Harvey (1978)
Bos taurus	1 Simple, domestic	Domestic	M	6	Stewart-Scott and Bruere (1987); Chapman and Bruere (1975); Long (1978)
Ovis aries	1–3 Simple, domestic	Domestic	M	4–18	Searle (1986)
Sorex araneus	1 Simple, wild	Wild	M	1–4	Nachman (this study)
Holochilus brasiliensis	1–2 Simple, wild	Wild	M	2–4	Nachman (this study)
	1–2 Simple, 2 monobrachial, wild	Wild	M	4–5	

[a] Studies in which a Rb heterozygote was introduced into a genetic background different from that in which the rearrangement originated are indicated by "Laboratory" except where denoted by an asterisk (*).

[b] There is disagreement in the literature on the correct designation for this taxon, which is also referred to as *Mus musculus domesticus*.

Discussion

Pairing, univalency, and chiasma frequency

The data from diplotene/diakinesis and metaphase I preparations indicate that Rb heterozygotes consistently pair, even when quadrivalents are involved. White (1973) suggested that proper disjunction of the chromosomes involved in a multivalent will be facilitated if a single distal chiasma is formed per chromosome arm and if the chromosome arms are similar in size. He argued further that chiasmata proximal to the centromere will typically interfere with orientation of the homologs on the spindle. In this study, 58.9% of trivalents and 61.7% of quadrivalents exhibited a single distal chiasma per chromosome arm. Most of the remaining chain configurations had at least one chiasma proximal to the centromere. Rb1/2, Rb3/4, and Rb6/7 all have arms of very similar size; however, the arms of Rb2/18 are very different in length. Although White's (1973) criteria are not met, pairing and segregation proceed normally.

In both mice (Forejt et al., 1981) and humans (Rosenmann et al., 1985; Chandley, 1988), spermatogenesis can be impaired either by failure of the X and Y to pair or by associations between the sex vesicle in pachytene or the sex bivalent in diakinesis and unpaired autosomal regions (such as unpaired regions of trivalents or quadrivalents). Forejt (1982) has suggested that the normal inactivation of the X chromosome occurring during male meiosis in mammals is a basic control mechanism that is required for spermatogenesis. He postulates that the association of autosomal sequences with unpaired regions of the X may interfere with normal X-chromosome inactivation, causing transcription of "nonpermissible" genes during spermatogenesis. Although the rate of X-Y univalency is approximately 15% in the quadruple Rb heterozygote group from this study, only 1 of 33 cells revealed an association between the X and a chain configuration. Furthermore, no such associations were found in the other 213 cells examined (with fewer Rb heterozygotes). It seems unlikely that Rb heterozygosity is causing spermatogenic impairment in *H. brasiliensis,* since the testes appeared to be normal in all males and since simple, double, and monobrachial heterozygotes appear to be fully fertile in laboratory crosses (unpublished data). It is interesting that despite the increase in X-Y univalency in highly heterozygous individuals, the ratio of X to Y secondary spermatocytes in these same individuals was 1:1 and no sex-chromosome nondisjunction was observed.

Several previous studies (e.g., Cattanach, 1978) have shown that crossing-over is suppressed near the centromere in some Rb heterozygotes. This observation has led to the hypothesis that Rb heterozygosity may increase fitness by preserving coadapted gene complexes intact near the centromeres. The results presented here do not support this hypothesis. Rb heterozygosity had no effect on the number of chiasmata near the centromeres (and was, in fact, associated with an increase in the number of terminal chiasmata).

Robertsonian variation, nondisjunction, and speciation

The data from metaphase II preparations indicate no association between rates of nondisjunction and amount of Rb heterozygosity, even when monobrachial heterozygotes are involved. The rates of nondisjunction reported in this study (2–5%) are generally low, compared to those reported in previous studies (Table VI). Of particular interest are the rates of nondis-

junction estimated for *H. brasiliensis* monobrachial heterozygotes (2–4%). Rates of nondisjunction for this type of rearrangement have not been measured for any species other than the house mouse, which shows uniformly high rates (> 26%, Gropp et al., 1975).

Comparisons of rates of nondisjunction among the studies in Table VI are instructive but should be interpreted with caution, for several reasons. In general, these studies only provide estimates of rates of nondisjunction but do not analyze these estimates statistically. This is a consequence of the small samples usually available (due to the difficulty of obtaining MII cells) and the often low frequency of the events being measured. There is also a clear bias in the literature toward studies on *Mus*, although there is no evidence that *Mus* is either atypical or representative of other mammalian species in this regard. In addition, most nondisjunction estimates are obtained from males, although there is some evidence (Gropp et al., 1982) that rates can differ between males and females. Finally, genetic effects may have a strong influence on rates of nondisjunction (Cattanach and Moseley, 1973). Most estimates in *Mus* have been obtained by crossing wild Rb mice with inbred laboratory strains of non-Rb mice, thus introducing the Rb chromosome into a genetic background different from that in which it arose. These laboratory strains are typically a mixture of *M. musculus* and *M. domesticus* genomes, although in the wild, Rb rearrangements are known only from *M. domesticus* populations. While the "compatibility" between Rb chromosomes and other portions of the genome appear to affect rates of nondisjunction (Gropp and Winking, 1981), there is currently no clear pattern to this interaction. In the present study, all marsh rats came from direct lineages of single wild populations (i.e., no interpopulation crosses were made), and, therefore, the potentially confounding influence of a foreign genome may be excluded. It is possible that the low rates of nondisjunction observed for *H. brasiliensis* derive from some unique aspect of meiosis in this species. It is also possible that, at least in part, the low rates simply reflect the "compatibility" between the Rb chromosomes and other portions of the genome.

The low rates of nondisjunction estimated in this study violate the assumptions in traditional (White, 1978) and monobrachial (Baker and Bickham, 1986) models of chromosomal speciation. While it is not expected that such models will apply to all organisms, the lack of data (and conflicting results) on meiotic consequences of chromosomal heterozygosity from different species suggests that generalizations are still premature.

Maintenance of Rb variation in natural populations

The factors maintaining Rb variation in populations of *H. brasiliensis* remain obscure. Although low rates of nondisjunction suggest that there is not strong selection against heterozygotes, there is also no evidence at present for heterosis. Furthermore, in the absence of strong balancing selection, high mutation rates, or heterosis, the meiotic drive documented in males would quickly drive populations toward fixation for the acrocentric state. The observation of a segregation bias favoring transmission of acrocentrics over metacentrics, as shown here, has previously been reported in sheep (Stewart-Scott and Bruere, 1987), cattle (Logue and Harvey, 1978), and mice (Gropp et al., 1982). The observation of high levels of Rb polymorphism in all populations of *H. brasiliensis* (Nachman, 1992) suggests that some force is necessary to offset the meiotic drive found in males. It is possible that meiotic drive operates in females to favor metacentrics or that selection in either or both sexes favors metacentrics.

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Appendix

Specimens included in this study are listed by locality. All specimens used in meiotic studies were males, and numbers refer to field catalog numbers. Specimens and the field catalog are deposited in the collections of the Museum of Zoology, University of Michigan. Crosses showing the ancestry of all laboratory-born animals are given. In all cases, these crosses begin with wild-caught animals. Homozygous animals have four metacentric Rb translocation chromosomes: Rb1/2, Rb3/4, Rb5/8, and Rb6/7 (as well as a small pair, 23, whose arms are not assigned different numbers, following convention); all other chromosomes are acrocentric. Heterozygous animals differ from homozygous animals only by the rearrangements shown. All rearrangements listed below occur in the heterozygous state.

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:

- MWN 346; wild-caught; Rb1/2, Rb2/18, Rb6/7
- MWN 351; wild-caught; Rb6/7, 1B
- MWN 560; laboratory born to (MWN 352F × MWN 346M); Rb1/2, Rb2/18, Rb6/7
- MWN 561; laboratory born to (MWN 352F × MWN 346M); homozygous
- MWN 600; laboratory born to (MWN 344F × MWN 346M); Rb1/2, Rb2/18, Rb3/4, Rb6/7, 1B
- MWN 601; laboratory born to (MWN 344F × MWN 346M); RB1/2, Rb2/18, Rb6/7

Paraguay, Dept. Presidente Hayes, west bank of Rio Paraguay, 4 km NW of Puerto Fonciere, 22°25′ S, 57°52′ W:

- MWN 408; wild-caught; Rb6/7
- MWN 426; wild-caught; Rb3/4
- MWN 502; laboratory born to (MWN 359F × MWN 361M); Rb3/4, Rb6/7

Paraguay, Dept. Presidente Hayes, 24 km (by air) NW of Villa Hayes, Estancia La Golondrina, 25°05′ S, 57°45′ W:

- MWN 108; wild-caught; Rb3/4, 1B
- MWN 125; wild-caught; homozygous
- MWN 135; wild-caught; Rb3/4
- MWN 221; laboratory born to (MWN 180F × MWN 147M); MWN 180F born to (MWN 104F × MWN 105M); MWN 147M born to (MWN 116F × MWN 109M); 2 Bs
- MWN 222; laboratory born to (MWN 180F × MWN 147M), MWN 180F born to (MWN 104F × MWN 105M); MWN 147M born to (MWN 116F × MWN 109M); 2 Bs

References

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

Beatty RA, Lim M-C, Coulter VJ: A quantitative study of the second meiotic metaphase in male mice *(Mus musculus)*. Cytogenet Cell Genet 15:256–275 (1975).

Cattanach BM: Crossover suppression in mice heterozygous for tobacco mouse metacentrics. Cytogenet Cell Genet 20:264–281 (1978).

Cattanach BM, Moseley H: Nondisjunction and reduced fertility caused by the tobacco mouse metacentric chromosomes. Cytogenet Cell Genet 12:264–287 (1973).

Chandley AC: Meiotic studies and fertility in human translocation carriers, in Daniel A (ed): The Cytogenetics of Mammalian Autosomal Rearrangements, pp 361–382 (Alan R Liss Inc, New York 1988).

Chapman HM, Bruere AN: The frequency of aneuploidy in the secondary spermatocytes of normal and Robertsonian translocation-carrying rams. J Reprod Fertil 45:333–342 (1975).

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

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

Forejt J: X-Y involvement in male sterility caused by autosome translocations—a hypothesis, in Crosignani PG, Rubin BL, Fraccaro M (eds): Genetic Control of Gamete Production and Function, pp 135–151 (Academic Press, New York 1982).

Forejt J, Gregorova S, Goetz P: XY pair associates with the synaptonemal complex of autosomal male-sterile translocations in pachytene spermatocytes of the mouse *(Mus musculus)*. Chromosoma 82:41 (1981).

Gropp A, Kolbus U, Giers D: Systematic approach to the study of trisomy in the mouse. II. Cytogenet Cell Genet 14:42–62 (1975).

Gropp A, Winking H: Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. Symp zool Soc Lond 47:141–181 (1981).

Gropp A, Winking H, Redi CA: Consequences of Robertsonian heterozygosity: segregation impairment of fertility versus male-limited sterility, in Crosignani PG, Rubin BL (eds): Genetic Control of Gamete Production and Function, pp 115–135 (Grune and Stratton, New York 1982).

Harris MJ, Wallace ME, Evans EP: Aneuploidy in the embryonic progeny of females heterozygous for the Robertsonian chromosome (9.12) in genetically wild Peru-Coppock mice *(Mus musculus)*. J Reprod Fertil 76:193–203 (1986).

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).

Logue DN, Harvey MJA: Meiosis and spermatogenesis in bulls heterozygous for a presumptive 1/29 Robertsonian translocation. J Reprod Fertil 54:159–165 (1978).

Long SE: Chiasma counts and non-disjunction frequencies in a normal ram and in rams carrying the Massey I (t1) Robertsonian translocation. J Reprod Fertil 53: 353–356 (1978).

Merani MS, Capanna E, Bianchi NO: Cytogenetics of South American akodont rodents. VI. Segregation of the polymorphic chromosomes 1 in the testicular meiosis of *Akodon molinae*. Nucleus 23:226–233 (1980).

Nachman MW: Geographic patterns of chromosomal variation in South American marsh rats, *Holochilus brasiliensis* and *H. vulpinus*. Cytogenet Cell Genet 61:10–16 (1992).

Redi CA, Capanna E: DNA-content variation in mouse spermatozoa arising from irregular meiotic segregation. Boll Zool 45:315 (1978).

Rosenmann A, Wahrman J, Richler C, Voss R, Persitz, Goldman B: Meiotic associations between the XY chromosomes and unpaired autosomal elements as a cause of human male sterility. Cytogenet Cell Genet 39:19 (1985).

Searle JB: Meiotic studies of Robertsonian heterozygotes from natural populations of the common shrew, *Sorex araneus*. Cytogenet Cell Genet 41:154–162 (1986).

Stewart-Scott IA, Bruere AN: Distribution of heterozygous translocations and aneuploid spermatocyte frequency in domestic sheep. J Hered 78:37–40 (1987).

Tettenborn U, Gropp A: Meiotic non-disjunction in mice and mouse hybrids. Cytogenetics 9:272–283 (1970).

White BJ, Crandall C, Raveche ES, Tjio JH: Laboratory mice carrying three pairs of Robertsonian translocations: establishment of a strain and analysis of meiotic segregation. Cytogenet Cell Genet 21:113–138 (1978).

White BJ, Tjio JH, Van de Water LC, Crandall C: Studies of mice with a balanced complement of 36 chromosomes derived from F_1 hybrids of T1Wh and T1Ald translocation homozygotes. Proc natl Acad Sci, USA 69:2757–2761 (1972).

White MJD: Animal Cytology and Evolution (Cambridge University Press, London 1973).

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

Winking H, Gropp A: Meiotic non-disjunction of metacentric heterozygotes in oocytes versus spermatocytes, in Crosignani PG, Mishell, DR (eds): Ovulation in the Human, pp 47–56 (Academic Press, London 1976).