Female Meiosis Drives Karyotypic Evolution in Mammals

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ABSTRACT

Speciation is often accompanied by changes in chromosomal number or form even though such changes significantly reduce the fertility of hybrid intermediates. We have addressed this evolutionary paradox by expanding the principle that nonrandom segregation of chromosomes takes place whenever human or mouse females are heterozygous carriers of Robertsonian translocations, a common form of chromosome rearrangement in mammals. Our analysis of 1170 mammalian karyotypes provides strong evidence that karyotypic evolution is driven by nonrandom segregation during female meiosis. The pertinent variable in this form of meiotic drive is the presence of differing numbers of centromeres on paired homologous chromosomes. This situation is encountered in all heterozygous carriers of Robertsonian translocations. Whenever paired chromosomes have different numbers of centromeres, the inherent asymmetry of female meiosis and the polarity of the meiotic spindle dictate that the partner with the greater number of centromeres will attach preferentially to the pole that is most efficient at capturing centromeres. This mechanism explains how chromosomal variants become fixed in populations, as well as why closely related species often appear to have evolved by directional adjustment of the karyotype toward or away from a particular chromosome form. If differences in the ability of particular DNA sequences or chromosomal regions to function as centromeres are also considered, nonrandom segregation is likely to affect karyotype evolution across a very broad phylogenetic range.

THERE is broad agreement that the establishment of a new species is often accompanied by changes in chromosome number or morphology (White 1978). However, there is little agreement on the mechanism by which such changes become fixed in populations (Hedrick 1981). Because chromosomal hybrids generally have reduced fertility due to increases in meiotic errors (White 1978; Searle 1988), there is a basic paradox: Changes in karyotype are frequently associated with speciation but the fixation of any particular karyotypic change in a population appears unlikely. White (1978) suggested that four factors could influence the probability of fixation of a new chromosomal variant: genetic drift, selection in favor of individuals that are homozygous for the new variant, inbreeding, and meiotic drive. Mathematical models (Hedrick 1981) have indicated that meiotic drive is the factor with the greatest potential to establish a new chromosomal variant.

"Meiotic drive" is a term that is most often associated with systems of male gamete dysfunction, such as *Segregation distorter* in Drosophila and the *t*-haplotype in the mouse (reviewed in Lyttle 1991 and Pardo-Manuel de Villena and Sapienza 2001a), but the original definition dictated that the term be used to describe unequal representation of alleles among the gametes as "a consequence of the mechanics of meiotic divisions" (Sandler and Novitski 1957). In this context, the process of oogenesis provides a nearly universal opportunity for the occurrence of meiotic drive. In most multicellular organisms, each of the two meiotic divisions in females results in an oocyte and a polar body, each of which has a distinct morphology and developmental fate. Any bias in the segregation of one or the other of a pair of homologous chromosomes or chromatids between the oocyte and the polar body can have a dramatic effect on the genetic makeup of a population (Buckler *et al.* 1999).

If meiotic drive is to occur via nonrandom segregation of chromosomes, three conditions must be fulfilled (reviewed in Pardo-Manuel de Villena and Sapienza 2001a): (1) asymmetry in the meiotic division, with respect to cell fate (*i.e.*, not all products of meiosis can become functional gametes; Sturtevant and Beadle 1936); (2) functional polarity of the meiotic spindle (*i.e.*, there must be a functional distinction between the egg side of the spindle and the polar body side; Rhoades 1942; Catcheside 1945; Novitski 1951, 1955, 1967; Hewitt 1976; LeMaire-Adkins and Hunt 2000); and (3) functional heterozygosity at a locus that mediates attachment of a chromosome to the spindle (*i.e.*, there must be a way to distinguish which chromosome is to

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be passed to which pole; Rhoades 1942; Catcheside 1945; Novitski 1967; Zwick *et al.* 1999).

All three conditions are apparently fulfilled during female meiosis in many phylogenetic groups when unusual chromosome morphology, chromosome rearrangements, or other karyotypic abnormalities are present (reviewed in Pardo-Manuel de Villena and Sapienza 2001a). Some of the best documented examples of nonrandom segregation include the supernumerary chromosomes of insects (Hewitt 1976; Jones and Rees 1982; Cano and Santos 1989) and plants (Kayano 1957), "knob"-containing chromosomes in maize (Rhoades 1942, 1952; Rhoades and Dempsey 1966), some chromosome rearrangements in Drosophila (Novitski 1951, 1967), a mouse chromosome 1 containing a "heterogeneously staining region" (Agulnik *et al.* 1990), a mouse chromosome 11 from the DDK strain (Pardo-Manuel de Villena *et al.* 2000), the unpaired X chromosome of XO female mice (Cattanach 1962; Kaufman 1972; Sakurada *et al.* 1994; LeMaire-Adkins and Hunt 2000), chromosome fissions in the chicken (Dinkel *et al.* 1979), and nonhomologous Robertsonian translocations in both the human (Pardo-Manuel de Villena and Sapienza 2001b) and the mouse (see Table 1 and references therein).

The unequal centromere number rule: A common feature of most of the above examples is that nonrandom segregation is observed when different numbers of centromeres or structures that can act as centromeres ["neocentromeres" (Rhoades and Vilkomerson 1942; Peacock *et al.* 1981)] are found on opposite sides of the meiotic spindle at either meiosis I or meiosis II. In the case of the nonrandom segregation of knob-containing chromosomes in maize, for example, one homologue contains two potential centromeres (the true centromere and the knob) while the other homologue contains only one. The generality of the "unequal centromere number rule" for nonrandom segregation becomes more apparent when the segregation of chromosome rearrangements and aneuploidy are also considered. Among the chromosomally balanced offspring of balanced carriers of nonhomologous Robertsonian translocations, one side of the metaphase contains the Robertsonian translocation [with one active centromere (Earnshaw *et al.* 1989; Page *et al.* 1995; Sullivan and Schwartz 1995)] while the other side of the metaphase contains the two acrocentric chromosomes (with a total of two active centromeres) that are homologous to the Robertsonian. This situation leads to nonrandom segregation at meiosis I in both the mouse and the human (Gropp and Winking 1981; Ruvinsky *et al.* 1987; Tease and Fisher 1991; Aranha and Martin-DeLeon 1994; Pacchierotti *et al.* 1995; Pardo-Manuel de Villena and Sapienza 2001b; Figure 1). Nonrandom segregation does not appear to depend on the particular chromosomes involved in the rearrangement in either species (see Table 1 for summary of the mouse data and Pardo-Manuel de Villena and Sapienza 2001b for summary of the human data), reinforcing the fact that centromeres, themselves, are the relevant chromosomal entity in nonrandom segregation. Almost all instances in which two acrocentric chromosomes (with two centromeres) combine to form a bi-armed chromosome (with one centromere) result in nonrandom segregation in balanced female carriers in both species (Figure 1; Table 1; Pardo-Manuel de Villena and Sapienza 2001b). In the chicken, the conceptually identical segregation of chromosome fissions in heterozygous carriers leads to a similar disparity (Dinkel *et al.* 1979); one side of the metaphase has two chromosomes containing two centromeres paired with a single chromosome with only

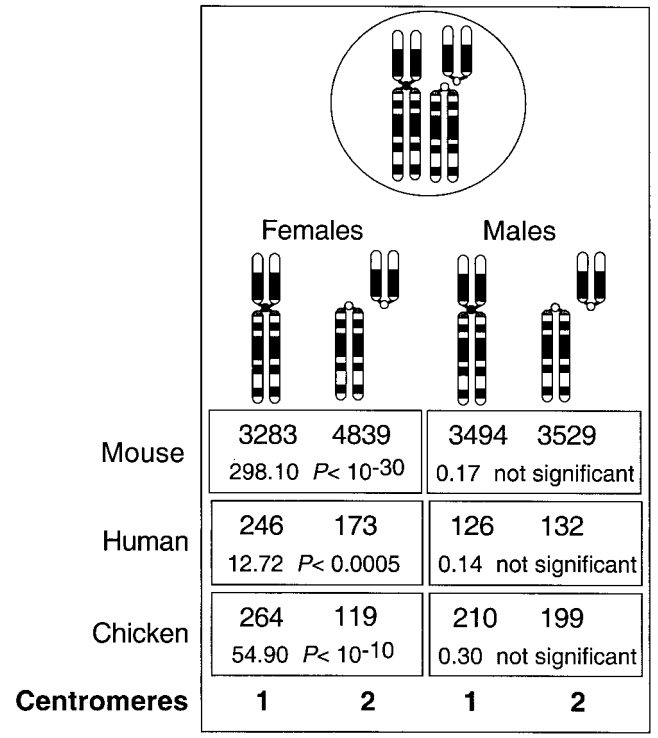


Figure 1.—Meiotic segregation in balanced products from balanced carriers of chromosome rearrangements. Male and female meiotic segregation in balanced carriers of Robertsonian translocations in mouse and human and in balanced carriers of chromosome fissions in the chicken is shown. Data include only chromosomally balanced products of meiosis. Data for the mouse have been compiled from 33 different Robertsonian translocations (Table 1), involving 18 of the 19 autosomes, as well as the X chromosome. Data for the human have been reported previously (Pardo-Manuel de Villena and Sapienza 2001b) and data for the chicken have been taken from Dinkel *et al.* (1979). Top row of numbers in each box is the number of balanced meiotic products containing either the Robertsonian translocation (with one centromere) or the acrocentric homologues (with two centromeres). Bottom row of numbers in each box is the χ^2, followed by the *P* value for H_0:random segregation. Note that nonrandom segregation, resulting in meiotic drive, occurs only during female meiosis (Pardo-Manuel de Villena and Sapienza 2001a).

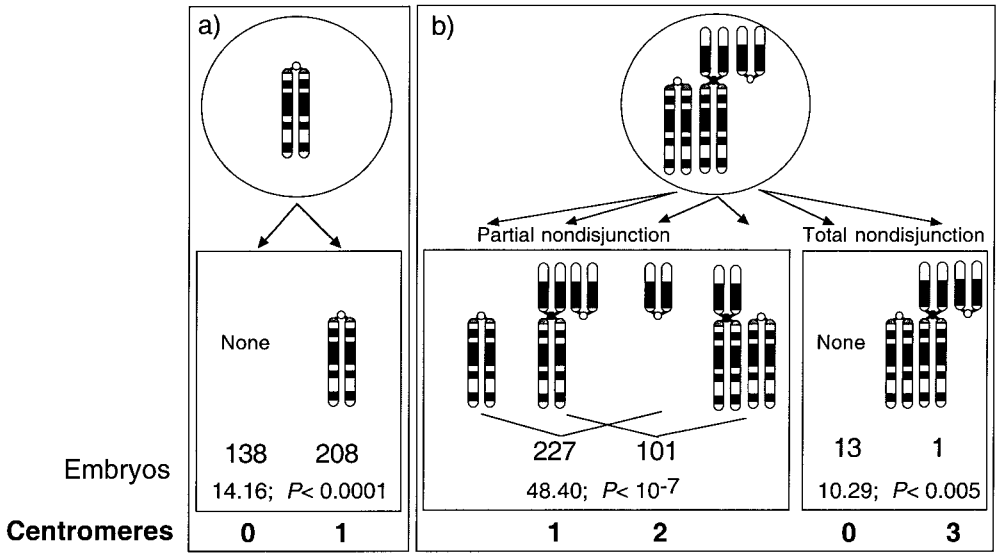


FIGURE 2.—Chromosome segregation in unbalanced products of female meiosis. Segregation in XO female mice (a) and segregation in chickens that are balanced carriers of chromosome fissions (b) are depicted. Top row of numbers in each box is the number of meiotic products containing the chromosome depicted above. In b, the top row of numbers represents the sum of all unbalanced products with the indicated number of centromeres. Bottom row of numbers in each box is the χ^2, followed by the *P* value for H_0: random segregation.

one centromere on the other side of the metaphase (Figure 1).

The data in Figure 1 include only balanced offspring (so that the segregation bias cannot be explained by postfertilization selection against aneuploid offspring) but unbalanced products of meiosis also adhere to the unequal centromere number rule (Figure 2). In the case of nonrandom segregation of the unpaired X chromosome in XO female mice (Figure 2a; LEMAIRE-ADKINS and HUNT 2000), one side of the metaphase contains an X chromosome (with one centromere) while the other contains no X chromosome (and no centromere). It is noteworthy that the egg side of the metaphase division preferentially retains the greater number of centromeres in this instance (one *vs.* zero) as also occurs in the preferential segregation of the acrocentric homologues over the Robertsonian translocations in the mouse (*i.e.*, two centromeres *vs.* one; Figure 1).

In the chicken, the polar body side of the spindle is the side to which the greater number of centromeres is segregated preferentially, regardless of whether this leads to a balanced (Figure 1) or an unbalanced (Figure 2b) meiotic product. The side of the metaphase with the larger number of centromeres is segregated preferentially to the polar body, regardless of which chromosome is involved in creating the aneuploidy or whether the disparity in centromere number on opposite sides of the metaphase is one *vs.* two or zero *vs.* three (DINKEL *et al.* 1979; see also Figure 2b). The bias appears to be particularly strong in those few instances in which there is total nondisjunction (*i.e.*, 13 instances of three centromeres segregating to the polar body and no centromeres to the egg *vs.* 1 instance of three centromeres segregating to the egg and no centromeres segregating to the polar body; Figure 2b).

The observed characteristics of nonrandom segregation are consistent on three levels: (1) Nonrandom segregation is observed for many different examples of the same type of chromosome abnormality; (2) the direction of nonrandom segregation of each type of abnormality is constant, within the species; and (3) the direction of nonrandom segregation observed for different types of abnormalities can be predicted on the basis of the unequal centromere number rule.

The consistency with which segregation bias is associated with differences in paired centromere number indicates that meiotic spindle polarity reflects differences in the ability of the two poles to "capture" centromeres. This point has been raised by a number of investigators in discussions of nonrandom segregation (CATCHESIDE 1945; HEWITT 1976; PARDO-MANUEL DE VILLENA and SAPIENZA 2001a); one pole of the spindle is thought to be more efficient or faster at capturing centromeres and the other less efficient or slower. The biochemical basis for this difference is unknown, but cytologically visible manifestations of spindle polarity are seen in some organisms (HEWITT 1976; KUBAI 1982; FUGE 1994) as differences in the length or number of microtubules emanating from each pole.

We noted that the direction of nonrandom segregation in human and mouse is in the same direction as the major chromosome form in the karyotype of each species. The mouse karyotype contains exclusively acrocentric chromosomes while the human karyotype contains 18 metacentric of submetacentric chromosomes and only five acrocentric chromosomes. If meiotic spindle polarity is a general feature of female meiosis, then nonrandom segregation is predicted to play a major role in karyotypic evolution. Whenever a chromosomal variant occurs (and Robertsonian translocations are the most common chromosome rearrangement in many mammals, including humans, where they occur with a frequency of 0.1% of meioses; HAMERTON *et al.* 1975),

the metacentric or bi-armed chromosomes should be favored in those species, such as humans (Pardo-Manuel de Villena and Sapienza 2001b), in which the most efficient pole of the meiotic spindle is on the polar body side of a meiotic division. Conversely, acrocentric chromosomes should be favored in those species, such as mouse, in which the most efficient pole of the spindle is on the egg side of a meiotic division (Table 1). Over the course of evolution, nonrandom segregation is predicted to result in many species with predominantly acrocentric karyotypes and many species with predominantly bi-armed chromosomes. Few species, on the other hand, would be expected to have karyotypes in which there are equal numbers of bi-armed and acrocentric chromosomes.

The class Mammalia provides a unique opportunity to test this hypothesis because karyotypic evolution in mammals is thought to occur principally via Robertsonian translocation intermediates (Qumsiyeh 1994); *i.e.*, mammalian karyotypes have changed predominantly through the creation of metacentric or bi-armed chromosomes from two acrocentrics or the creation of two acrocentrics from a metacentric or bi-armed chromosome. We examined the karyotypes of 1170 species of mammals and characterized the chromosome complement of each species according to the fraction of its karyotype that is composed of acrocentric chromosomes as a test of this hypothesis.

MATERIALS AND METHODS

Definition of chromosome form: The diploid number of chromosomes, the number of acrocentric chromosomes, and the number of bi-armed (metacentric or submetacentric) chromosomes in each species' karyotype was taken from published reports. These data are summarized in Supplemental Appendix 1 and the references from which the data have been compiled are given in Supplemental Appendix 2 (http://www.genetics.org/supplemental). Only autosomes were used in the analysis to avoid introducing bias due to morphological differences in the sex chromosomes and the existence of XY1Y2 sex determination systems in some species (*e.g.*, Fredga 1972; Gardner 1977; Benirschke *et al.* 1980; Vassart *et al.* 1995). Note that nonrandom segregation may occur only in asymmetric meioses, *i.e.*, females, in mammals (reviewed in Pardo-Manuel de Villena and Sapienza 2001a). We calculated the fraction of acrocentric chromosomes among each karyotype by defining autosomes that were described as "acrocentric" or "telocentric" as acrocentric and all submetacentric or metacentric autosomes as "bi-armed." In addition, we examined the published figures (photographic reproductions, in general) in which the karyotypes were reproduced to ensure that the use of terms was consistent between reports and between authors.

Selection of species: To ensure fair representation of species from each order, species were selected in approximate proportion to the "species richness" (Purvis and Hector 2000) of each order among the entire mammalian class (Figure 3). In addition, we examined the karyotypes of 64 marsupials and three species of monotremes [neither group was included in the published distribution of species richness (Purvis and Hector 2000)].

Statistical analysis: The null hypothesis of random segregation of homologous chromosomes was tested for data summarized in Figures 1 and 2 using the χ^2 test statistic. To determine whether the distribution of acrocentric chromosomes among the karyotypes of mammalian species was random, the binomial distribution was used to calculate the expected distribution of the 1170 species as a function of percentage of acrocentric autosomes in the karyotype. Expected values of percentage of acrocentric chromosomes were calculated for 18 equal intervals and the distribution curve (see Figure 5) was fit to the expected values by eye. The number of intervals used to plot the observed values of percentage of acrocentric autosomes in the karyotype (nine in Figure 5 and three in Figure 6) was chosen to accommodate the number of independent variables (haploid number of autosomes) and the number of species being compared within each group and to allow graphical representation of the distribution such that the expected mean of the distribution would be contained in a single interval.

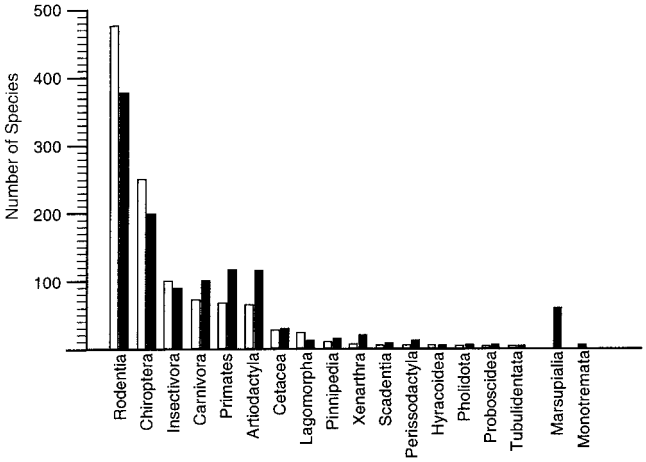


Figure 3.—Species richness in mammalian orders. Solid bars represent the number of species from 16 eutherian orders, as well as marsupials and monotremes, analyzed in this report. Open bars reflect the predicted number of species in each order if the 1103 eutherian species were distributed according to the observed species richness of the entire class (Purvis and Hector 2000).

RESULTS

General characteristics of the sample of mammalian karyotypes: The 1170 mammalian species (20–25% of extant mammalian species) that we examined (see materials and methods) have a mean diploid number of 43.3, ranging from 2n = 6 to 102 (Figure 4a). Among the entire collection of 48,375 autosomes, bi-armed and acrocentric chromosomes are nearly equally represented (49.4% are bi-armed and 50.6% are acrocentric). The distribution of species with respect to diploid number (Figure 4a) and fundamental number (diploid number of chromosome arms, Figure 4b) also indicates that there is no strong preference for bi-armed or acrocentric (uni-armed) chromosomes, overall.

Distribution of species as a function of chromosome form: If acrocentric and bi-armed chromosomes are distributed randomly among the karyotypes of all species examined, then the distribution of species, as a function of the fraction of acrocentric chromosomes in

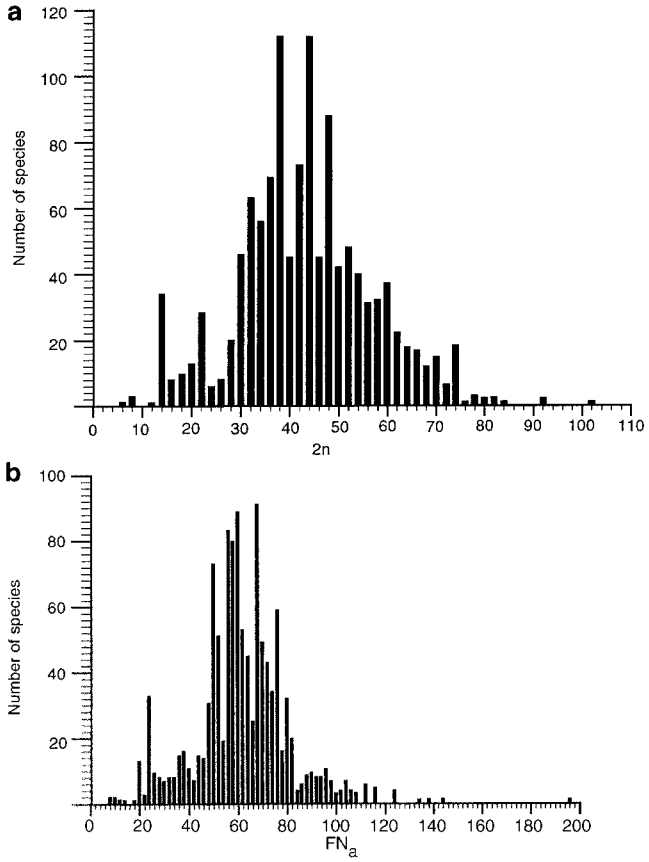


FIGURE 4.—Diploid number and fundamental number of mammalian species. (a) Diploid number (2n) distribution among the 1170 mammalian species analyzed. (b) Fundamental number of autosomes (FN_a) among the 1170 species analyzed. FN_a is equal to the number of autosome arms. Acrocentric chromosomes have a single arm while metacentric and submetacentric chromosomes have two arms.

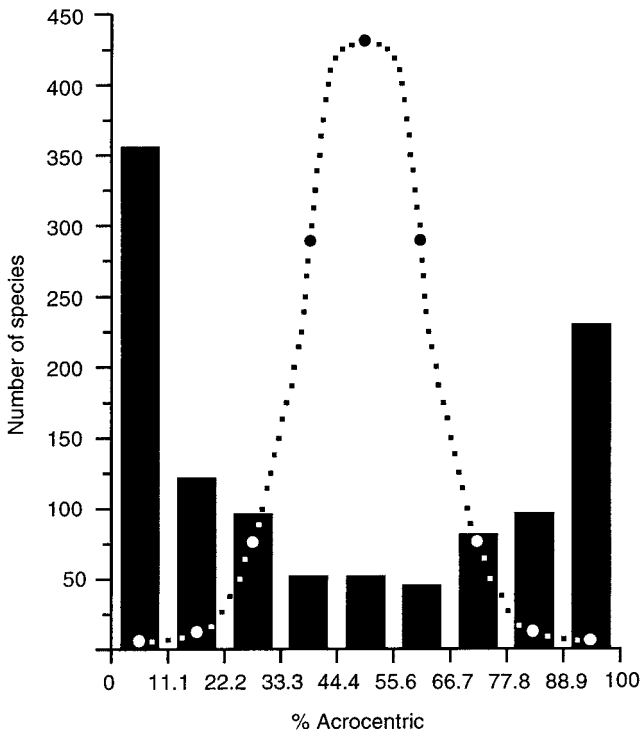


FIGURE 5.—Distribution of acrocentric chromosomes among the karyotypes of mammalian species. Each species has been assigned to one of nine equal categories with respect to the fraction of its chromosomes that are acrocentric (N.B.: only autosomes have been considered). Nine intervals, rather than deciles, were selected so that the expected mean value would be contained in a single central interval. Bars represent the number of species in each category. The dotted line represents the expected number of species in each category under a binomial distribution centered on the mean (50.6% acrocentrics).

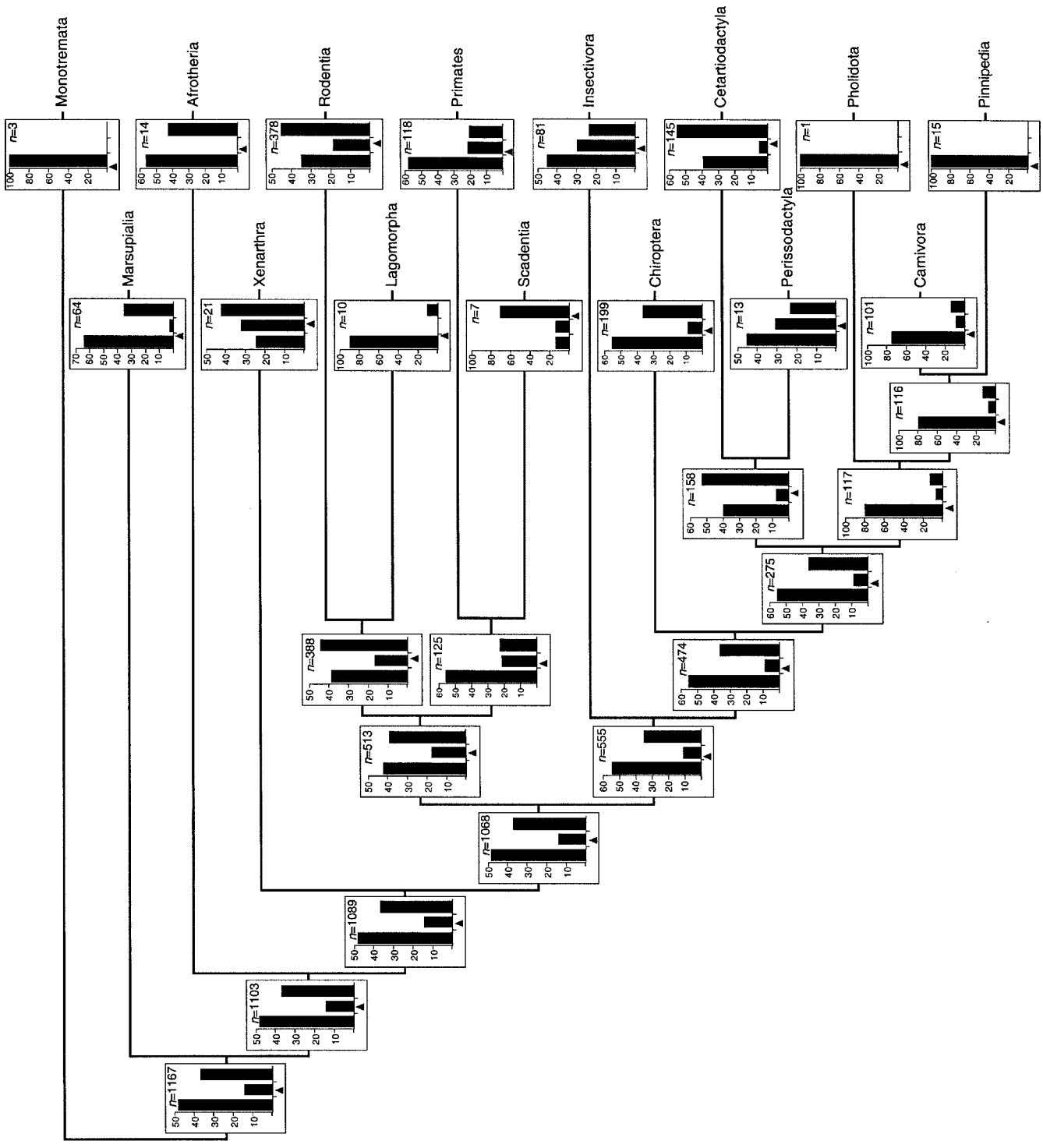
the karyotype, is expected to follow a binomial distribution, with a mean of 50.6% acrocentric chromosomes. If, on the other hand, karyotypic evolution is driven by nonrandom segregation of either bi-armed or acrocentric chromosomes to the ovum, then the majority of species are predicted to have karyotypes that contain predominantly bi-armed chromosomes or predominantly acrocentric chromosomes and few species are predicted to cluster near the population mean. The observed distribution of mammalian species, as a function of the fraction of acrocentric chromosomes in the karyotype, is shown in Figure 5. In contrast to the expectations for a binomial distribution (dashed line in Figure 5), >50% of the species have karyotypes that are in the two extremes of the distribution (<11.1% acrocentric or >88.9% acrocentric) while <14% of species fall in the three central intervals of the distribution (33.4–66.7% acrocentric).

Importantly, the clustering of species in the extremes and relative scarcity in the center of the distribution is not a result that is driven by any single phylogenetic group. Figure 6 shows a phylogenetic tree (MURPHY *et al.* 2001) and the distribution of percentage of acrocentric chromosomes in each group is also represented. This analysis demonstrates that: (1) There is no group in which the central interval contains the largest number of species; (2) there is no group in which the species appear normally distributed; and (3) a very skewed unimodal distribution, suggestive of selection for a particular chromosome form, appears only in three groups, each containing relatively few species (Monotremata, Pholidota, Pinnipedia).

Overall, we may reject the hypothesis that there is a random distribution of chromosome morphology among mammalian species. On the other hand the data in Figures 5 and 6 are consistent with the predictions of the nonrandom segregation hypothesis.

DISCUSSION

Factors that may explain mammalian karyotypic evolution: Our analysis indicates that the distribution of acrocentric and bi-armed (metacentric and submetacentric) chromosomes among the karyotypes of mammalian species is not random. The karyotype of individ-


Monotremata
n=3
Afrotheria
n=14
Rodentia
n=378
Primates
n=118
Insectivora
n=81
Cetartiodactyla
n=145
Pholidota
n=1
Pinnipedia
n=15
Marsupialia
n=64
Xenarthra
n=21
Lagomorpha
n=10
Scadentia
n=7
Chiroptera
n=199
Perissodactyla
n=13
Carnivora
n=101
n=116
n=158
n=117
n=275
n=474
n=388
n=125
n=513
n=555
n=1068
n=1089
n=1103
n=1167

ual species appears to be driven toward the accumulation of either acrocentric chromosomes or bi-armed chromosomes (Figure 5). This result cannot be explained by a major role for either genetic drift or inbreeding in the evolution of mammalian karyotypes. Both of these mechanisms predict a random distribution of chromosome morphology among species as the result of chance fixation of chromosome variants in small, isolated populations. However, nonrandom segregation of one chromosome morphology at the expense of the other (meiotic drive) or adaptive selection of one chromosome morphology over the other can explain the observed distribution of acrocentric and bi-armed chromosomes among mammalian species.

Direct evidence in support of the nonrandom segregation hypothesis has been obtained from chromosomally hybrid individuals (carrying an odd number of chromosomes and active centromeres) in mouse and human (Ruvinsky *et al.* 1987; Tease and Fisher 1991; Aranha and Martin-DeLeon 1994; Pacchierotti *et al.* 1995; Pardo-Manuel de Villena and Sapienza 2001b; see also Figure 1), as well as in nonmammalian species (Dinkel *et al.* 1979; see also Figure 1). Two additional points argue that nonrandom segregation has played a major role in mammalian karyotype evolution: (1) Nonrandom segregation is the only one of the four mechanisms discussed by White (1978) that predicts the clustering of species in the two extremes of the distribution (see Introduction and Pardo-Manuel de Villena and Sapienza 2001a; Figures 5 and 6) and (2) nonrandom segregation is an expected and mechanistic consequence of the presence of different numbers of centromeres on paired homologous chromosomes (reviewed in Pardo-Manuel de Villena and Sapienza 2001a).

In contrast, there is little experimental support for the hypothesis that a particular chromosome morphology provides a selective advantage within any particular lineage. We are unaware of any mechanism by which chromosome morphology has been demonstrated to provide an adaptive advantage. Even the proposed effect of chromosome morphology on recombination (Qumsiyeh 1994) is unlikely to explain much of the observed variation in recombination among different mammalian species (Pardo-Manuel de Villena and Sapienza 2001c). In addition, comparisons of mice with the standard *Mus musculus* karyotype of 2n = 40 with chromosomal races where 2n = 22 have failed to find any support for the hypothesis of "metacentric superiority" (Nachman and Searle 1995) despite a near reversal in autosome form.

It is also difficult to rationalize the adaptive value of a particular chromosome morphology in a particular lineage with the fact that an excess of species with *both* extremes of karyotype (from predominantly acrocentric chromosomes to predominantly bi-armed chromosomes) are found within mammalian orders (Figure 6), within some families, *i.e.*, Cricetidae (24/10/63; numbers in parentheses represent the number of species in each of the three intervals shown in Figure 6), Arvicolidae (5/1/19), Canidae (3/2/9), Vespertilionidae (18/11/38), Geomydae (4/1/3), and even within some genera, i.e., Muntiacus (4/0/2), Lepilemur (4/0/2), Reithrodontomys (4/0/6), Gerbillus (5/0/4) (see Supplemental Appendix 1 at http://www.genetics.org/supplemental for documentation).

Given the theoretical and experimental difficulties in explaining these observations by adaptive selection, we argue that nonrandom segregation stands as the only one of the four mechanisms for which there is experimental evidence as well as a mechanistic explanation for the role of chromosome morphology in the evolution of mammalian karyotypes. In this regard, it is important to remember that although we used the nonrandom segregation hypothesis to predict the distribution of chromosome morphology among species, the mechanism of nonrandom segregation is not based on differences in chromosome morphology *per se*. Nonrandom segregation results from functional differences between the meiotic spindle poles in their ability to capture centromeres and the fact that different numbers of centromeres are found on paired chromosomes whenever Robertsonian translocations (bi-armed chromosomes with a single active centromere) are paired with acrocentric homologues (two uni-armed chromosomes with a total of two centromeres). Because nonrandom segregation in heterozygous carriers of Robertsonian translocations is based on the unequal centromere number rule, we were able to simplify our analysis (Figure 5; Supplemental Appendix 1 at http://www.genetics.org/supplemental) to consideration of only uni-armed and bi-armed chromosomes. We acknowledge that some chromosome rearrangements that can change chromosome morphology (pericentric inversions, centromere "switches," *etc.*) are not predicted to result in nonrandom segregation and will appear as background noise in the analysis. On the

Figure 6.—Phylogenetic distribution of acrocentric chromosomes within the class Mammalia. The branching order is based on a molecular phylogenetic analysis published recently (Murphy *et al.* 2001). Branch lengths were chosen to accommodate all nodes and do not reflect phylogenetic distance. The distribution of acrocentric chromosomes among the karyotypes of species in each branch is shown. Each species has been assigned to one of three equal categories with respect to the fraction of its chromosomes that are acrocentric. The number of categories was reduced from nine in Figure 5 to three in this analysis because very few species are represented in some orders. Bars represent the percentage of species in each category. Arrowheads shown on the horizontal axes denote the mean percentage of acrocentric chromosomes in each group. The number of species analyzed (*n*) in each group is provided in the top right-hand corner of each graph.

TABLE 1

Nonrandom segregation of Robertsonian chromosomes in female mice

	Females				Males				
Rob type	39	40	*n*	χ^2	39	40	*n*	χ^2	Reference
Rb(1.2)18Lub	36	89	125	22.47	119	103	222	1.15	12
Rb(1.3)1Bnr	127	243	370	36.37	216	245	461	1.82	2, 12
Rb(1.3)1Ei	36	68	104	9.85	38	39	77	0.01	12
Rb(1.7)1Rma	45	51	96	0.38	36	35	71	0.01	12
Rb(1.10)10Bnr	70	72	142	0.03					2
Rb(2.17)11Rma	70	134	204	20.08	139	165	304	2.22	3, 14
Rb(2.18)6Rma	45	78	123	8.85	76	126	202	12.38	14
Rb(3.8)2Rma	33	66	99	11.00	111	103	214	0.30	14
Rb(4.12)	83	86	169	0.05	59	64	123	0.20	11
Rb(4.12)9Bnr	49	58	107	0.76	61	64	125	0.07	2
Rb(4.15)4Rma	24	43	67	5.39	60	64	124	0.13	3
Rb(4.17)13Lub	69	112	181	10.22	188	26	214	122.64	3
Rb(5.15)4Lub	15	21	36	1.00	62	64	126	0.03	12
Rb(5.15)3Bnr	78	98	176	2.72	80	74	154	0.23	3, 12
Rb(6.7)13Rma	43	96	139	20.21	128	127	255	0.00	12
Rb(6.13)3Rma	16	46	62	14.52	41	52	93	1.30	2
Rb(6.15)1Ald	40	63	103	5.14	45	58	103	1.64	3
Rb(6.16)24Lub	41	85	126	15.37	113	155	268	6.58	10, 13
Rb(6.18)2Dn	38	93	131	23.09	107	106	213	0.00	14
Rb(7.18)9Lub	73	118	191	10.60	46	49	95	0.09	12
Rb(8.12)5Bnr	82	78	160	0.10	80	74	154	0.23	2
Rb(8.17)1lem	487	701	1188	38.55	49	65	114	2.25	2, 6
Rb(8.17)6Sic	41	61	102	3.92	42	54	96	1.50	3
Rb(9.12)	61	29	90	11.38					4
Rb(9.19)	31	43	74	1.95	300	327	627	1.16	1
Rb(10.11)5Rma	42	84	126	14.00	90	101	191	0.63	3
Rb(10.11)8Bnr	48	72	120	4.80	59	51	110	0.58	2
Rb(11.13)4Bnr	53	55	108	0.04	69	63	132	0.27	3
Rb(16.17)7Bnr	835	1231	2066	75.90	217	180	397	3.45	2, 5, 6, 15
Rb(16.17)	50	52	102	0.04	32	37	69	0.36	8
Rb(X.2)2Ad	175	259	434	16.26	585	583	1168	0.00	7, 9
Rb(X.9)6H	210	252	462	3.82	246	275	521	1.61	9
Rb(X.12)7H	137	202	339	12.46					9
Total	3283	4839	8122	298.10	3494	3529	7023	0.17	

39, no. of balanced offspring that inherit the Robertsonian translocation. 40, no. of balanced offspring with the normal karyotype. *n*, number of samples analyzed. χ^2, chi square under the expectation of the null hypothesis of random segregation. Numbers underlined represent significant departures from the expectations. References: 1, Evans *et al.* (1967); 2, Gropp and Winking (1981); 3, Boue *et al.* (1985); 4, Harris *et al.* (1986); 5, Sanchez and Erickson (1986); 6, Ruvinsky *et al.* (1987); 7, Adler *et al.* (1989); 8, Britton-Davidian *et al.* (1990); 9, Tease and Fisher (1991); 10, Chayko and Martin-DeLeon (1992); 11, Viroux and Bauchau (1992); 12, Davisson and Akeson (1993); 13, Aranha and Martin-DeLeon (1994); 14, Pacchierotti *et al.* (1995); and 15, Everett and Searle (1995).

other hand, other types of rearrangements that change the diploid number by one but need not change chromosome morphology, such as tandem fusions, should also be subject to nonrandom segregation (Pardo-Manuel de Villena and Sapienza 2001a). Overall, we assumed that the prevalent role of Robertsonian translocations in mammalian evolution would overcome these smaller effects and that the analysis of a large number of species would reveal the unusual distribution predicted by nonrandom segregation (Figure 5).

On what phylogenetic and time scale does reversal of spindle polarity occur? The simultaneous presence of both extremes of karyotype within orders, families, and genera and even within different races of the same species indicates that the mechanism leading to the accumulation of one chromosome morphology within a species has been present throughout mammalian evolution and that reversal of the direction of nonrandom segregation has occurred many times. In fact, data gathered from studies of wild populations of *M. musculus* indicate that nearly complete reversal of the prevalent chromosome form can occur both within a species as well as rapidly, in evolutionary time. Populations that have been separated by as few as 500 years [on the island

of Madeira (Britton-Davidian *et al.* 2000)], as well as populations separated by 5–10,000 years [in the Alps (Britton-Davidian *et al.* 1989; Nachman *et al.* 1994)] are observed to have undergone such "karyotypic reversal."

These comparative studies are also supported by more direct experimental evidence from both field studies and laboratory studies that indicate that reversal of spindle polarity has occurred within races of the same species. Some wild populations of *M. musculus* show transmission ratio distortion in favor of bi-armed (Robertsonian) chromosomes rather than acrocentrics (Harris *et al.* 1986; Scriven 1992). Harris *et al.* (1986) have suggested that the preferential loss of the Robertsonian translocation to the first polar body observed in previous studies (Gropp and Winking 1981) was an effect of the transfer of the wild-occurring (*M. musculus domesticus*) Robertsonian translocation chromosomes to a laboratory mouse of mixed *M. musculus musculus*/*M. musculus domesticus* genetic background.

Such "reversals" of spindle polarity within phylogenetic groups can explain why closely related species sometimes have dramatically different karyotypes (Supplemental Appendix 1 at http://www.genetics.org/supplemental) but it can also explain why karyotype evolution appears to have taken place by directional adjustment [karyotype orthoselection (White 1978)] toward or away from a particular chromosome form in some lineages. The level of transmission bias observed with Robertsonian translocations in both human and mouse (Pardo-Manuel de Villena and Sapienza 2001b; Table 1) is predicted to be sufficient to fix a new chromosome variant in a population because the segregation bias in favor of one chromosome can overcome the loss of fitness resulting from the observed levels of aneuploid gametes in heterozygotes (Hedrick 1981). The general operation of this process during female meiosis would, therefore, solve the paradox of how new chromosome variants are established and also confirm the prediction (Hedrick 1981) that meiotic drive has the strongest potential impact of the four factors that influence the fixation of chromosome variants.

General evolutionary implications of nonrandom segregation: Although karyotypic changes have occurred frequently during evolution and are associated with speciation, the fixation of such changes has been assumed to occur by chance, in small populations, or through natural selection operating at the level of organismal phenotype. Our analysis indicates that nonrandom segregation of chromosomes, as a general facet of female meiosis, represents an important selective force in the evolution of genomes. This mechanism has great potential to affect the number of linkage groups within a species.

While most nucleotide sequence diversity appears to be generated in the male germline (Crow 2000), the prevalent chromosome form within the karyotype of a species appears to be determined by natural selection acting directly on centromere function and indirectly on chromosome morphology during female meiosis. An important practical implication of this conclusion is that phylogenetic relationships may be rapidly obscured by the operation of nonrandom segregation. Species that are closely related may differ dramatically in karyotype if a reversal of spindle polarity has occurred between two lineages, such as we propose to explain the karyotype reversal that has occurred in some mice.

Although the evidence on which this discussion is based has been derived from mammalian species, the fundamental asymmetry of female meiosis and functional asymmetry of the meiotic spindle is likely to be nearly universal. Therefore, nonrandom segregation based on differences in centromere number, differences in the ability of particular regions of chromatin to function as centromeres (Novitski 1951, 1967; Zwick *et al.* 1999; reviewed in Karpen and Allshire 1997 and Pardo-Manuel de Villena and Sapienza 2001a), as well as DNA sequence polymorphisms associated with centromere function (Malik and Henikoff 2001) are likely to affect karyotype evolution across a very broad phylogenetic range.

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