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ORIGINAL ARTICLE

Preferential segregation of metacentric chromosomes in simple Robertsonian heterozygotes of *Sorex araneus*

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One of the hypotheses explaining preferential transmission of metacentrics among simple Robertsonian (Rb) heterozygotes of the common shrew (*Sorex araneus* L.) invokes the existence of meiotic drive. Thus far, evidence that metacentrics are favoured at meiosis has been obtained indirectly, on the basis of crosses made under controlled conditions. The aim of the present work was to test the hypothesis in a direct study. We analysed products of chromosome segregation among 12 simple heterozygote male subjects from a wild population, with regard to *jl*, *io*, *nr* and *mn* Rb fusions. We were able to demonstrate significant segregation distortion in favour of all four metacentrics. The level of preferential segregation was independent either of the composition of chromosome arms or the dimensions of metacentrics. We also found that X chromosomes were favoured over Y1Y2 chromosomes during segregation. We discuss the role of meiotic drive in the evolutionary success of metacentric chromosomes in *S. araneus*, as well as in the emergence of post-hybridization modifications in the zones of contact between races.
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Introduction

There is a widely accepted view that the ancestral karyotype of the common shrew (*Sorex araneus*) was entirely composed of acrocentric chromosomes, being similar to that found in the Iberian species *Sorex granarius* (Searle, 1993). Moreover, most cytogeneticists agree that metacentrics of contemporary chromosomal races of *S. araneus*, characterized by different combinations of chromosomal arms, emerged by centric fusions or whole arm reciprocal translocations (see Searle, 1993 for the schemes of these structural mutations). On first consideration, the evolutionary success of metacentric chromosomes seems paradoxical, since their rise would lead to a gradual increase in chromosomal heterozygosity, and thus a reduction in fertility (cf. King, 1993). One of the theoretical models attempting to resolve the paradox is based on the assumption that, at the population level, standard karyotypes can be eliminated by meiotic drive among heterozygotes. On this basis, it has been suggested that a distortion of segregation could account for the increased frequencies, and consequently the spread of new chromosomal mutations (Bengtsson, 1980).

These theoretical considerations have received empirical support from studies of the karyotypes of foetuses or newborns and their mothers. Searle (1986a) reported preferential transmission of metacentrics between parents and offspring. While the reports of multiple paternity in *S. araneus* (Tegelström *et al*., 1991) cast doubt on the reliability of the data presented by Searle (1986a), mating of heterozygotes with homozygotes under laboratory controlled conditions (Wyttenbach and Hausser, 1996; Wyttenbach *et al*., 1998) provided results strongly supporting preferential transmission of metacentrics on both the maternal and (to even a greater extent) paternal sides. Wyttenbach and Hausser (1996) advanced speculative hypotheses to explain the unequal transmission, suggesting that either (1) oocytes may preferentially accept sperms with metacentric chromosomes, or (2) the excess of transmitted metacentrics arises through the distortion of meiotic segregation. In relation to the second hypothesis, Wyttenbach *et al*. (1998) assumed that nonhomologous acrocentrics of the trivalent could form a 'side-arm configuration' in pachytene of prophase I, which facilitates recombination within the pericentric area of the acrocentrics. Such a trivalent could form a ring configuration during diakinesis, and consequently could produce a half of unbalanced gametes. To date, these alternative hypotheses have not been verified in direct studies on the meiotic segregation of chromosomes.

Studies of meiosis in *S. araneus* have so far focused on the conjugation or the lack of pairing and non-disjunction of chromosomes in the context of impairment of fertility in heterozygotes (see Searle, 1986b; Banaszek *et al*., 2002). Wyttenbach *et al*. (1998) considered the mechanisms of unequal transmission of metacentrics and concluded that any verification of their hypotheses would require a detailed analysis of the products of meiosis in heterozygotes. To date, however, only segre-

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gation of the sex chromosomes in *S. araneus* has been studied in detail, initially on very limited material (Fedyk, 1980; Searle, 1986b), and more recently (following modification of the meiotic preparation technique) on large numbers of metaphase II (MII) cells (Fedyk *et al*., 2005). In the present paper, we report the results of analysis of products of chromosome segregation at meiosis among simple Robertsonian (Rb) heterozygous male subjects. We then use these results to test the hypotheses put forward by Wyttenbach *et al*. (1998).

Materials and methods

Handling of animals

We analysed the products of chromosome segregation in 12 sexually mature male subjects of the common shrew. Ten individuals were captured at seven different locations in eastern Poland (Table 1). One individual from Wiartel was trapped in May 2004, and the shrews from the Rokitnia I, Plebanka VI and Zgniłocha were caught in May and June 2005. These were sexually mature male subjects, and their meioses were studied immediately after capture. In contrast, the shrews from Barczewko, Łyna and Klonowo were captured in September 2003 as sexually immature male subjects born in the same year. In order for them to reach sexual maturity, they were maintained for the following 4 months in the laboratory under conditions of 16 h light:8 h dark photoperiod, imitating long days. The remaining two male subjects (nos. 8 and 9; Table 1) were born in the laboratory in February 2004. For the two first months of life, they were maintained under short days (8 h light:16 h dark) and then, for the next 2 months exposed to long daylengths (16 h of light), to accelerate their physical and sexual maturity (Mercer and Searle, 1994).

Karyotypes

Chromosomal preparations were obtained from the spleen using the *in vivo* method of Fedyk (1980). Race identification—according to commonly accepted nomenclature (Searle *et al*., 1991)—was carried out on the basis of G-banding after Seabright (1971). The shrews represented three chromosomal races (Table 1). Three individuals from Barczewko belonged to the Łęgucki Młyn (Łg) race, two of them having 24 chromosomes and being Rb heterozygotes for the *io* chromosomes. The third had 26 chromosomes. It was a heterozygote for the *jl* arm combination and had *i* and *o* in an acrocentric state. The shrew captured in the Zgniłocha population was the Łęgucki Młyn/Drnholec (Dn) inter-racial recombinant carrying the *kh* and *io* metacentrics specific to the Łg race, as well as the *gm* and *nr* chromosomes characteristic for the Dn race. It had 24 chromosomes and was a heterozygote for the *io* combination. The above four shrews form a sub-class with meiotic trivalents of medium-sized autosomes. The remaining eight male subjects are Rb heterozygotes for small metacentrics: seven male subjects of the Dn race had $2N = 24$ and were *nr* heterozygotes. One individual of the Popielno race had 26 chromosomes and was an *mn* heterozygote (Table 1). We deliberately selected individuals heterozygous for arm combinations differing in size, because we wanted to test whether meiotic segregation favours metacentrics of larger sizes over smaller ones, as hypothesized by Wyttenbach *et al*. (1998). Four male subjects (no. 4, 10, 11 and 12) came from the hybrid populations; the remainder was caught outside hybrid zones.

Preparation of meiotic chromosomes

The right testes of all the male subjects were fixed in Bouin solution and preserved for histological study of spermatogenesis. The left testes were placed in an isotonic solution of sodium citrate. The seminiferous tubules released into the isotonic fluid were divided into two parts, one being used in the preparation of surface spreads for the study of synaptonemal complexes under an electron microscope, the other for the analysis of MII cells. Meiotic chromosomes were prepared using the method of Evans *et al*. (1964), as modified by Searle (1986b). Each male subject yielded 15–20 conventionally Giemsa-stained preparations, giving a large number (>100) of MII cells suitable for analysis. To generate the large number of MII cells, *in vivo* exposure to colchicine was prolonged to 4, or even 6 h (Fedyk *et al*., 2005).

Analysis of the segregation of chromosomes

The analysed shrews had 24 chromosomes with two pairs of acrocentrics (*p* and *q*), or 26 chromosomes with

Table 1 Details of individual shrews used in the study

Size of heterozygous chromosomes	*No. of shrew*	2N	*Variable part of karyotype*[a]	*Race*	*Population*	*Geographic coordinates*
Medium	1	24	*jl, hk, i/io/o, gr, mn, p, q*	Łg	Barczewko	20°45′E; 53°51′N
	2	24	*jl, hk, i/io/o, gr, mn, p, q*	Łg	Barczewko	
	3	26	*j/jl/l, hk, gr, mn, i, o, p, q*	Łg	Barczewko	
	4	24	*jl, hk, i/io/o, gm, nr, p, q*	Łg/Dn recombinant	Zgniłocha	20°34′E; 53°33′N
Small	5	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Łyna	20°27′E; 53°27′N
	6	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Klonowo	19°47′E; 53°15′N
	7	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Klonowo	
	8	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Laboratory reared	
	9	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Laboratory reared	
	10	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Rokitnia I	21°48′E; 51°36′N
	11	24	*jl, hi, ko, gm, n/nr/r, p, q*	Dn	Plebanka VI	21°49′E; 51°36′N
	12	26	*jl, ik, gr, m/mn/n, h, o, p, q*	Po	Wiartel	21°40′E; 53°36′N

Abbreviations: Dn, Drnholec; Łg, Łęgucki Młyn; Po, Popielno.
[a]Chromosome arms labelled with small letters according to standard nomenclature (Searle *et al*., 1991); combination *ab*—a homozygous pair of metacentrics; combination *a/ab/b*—a heterozygous pair; *a*, *b*—two homozygous pairs of acrocentric chromosomes.

two additional pairs of acrocentrics (*i* and *o* or *h* and *o*). In addition, twin acrocentrics were present in polymorphic pairs of autosomes (Table 1). Irrespective of the proportion of metacentric to acrocentric autosomes, somatic cells of common shrews contain a constant number of chromosome arms (NF = 40), whereas normal MII cells should have NF reduced to 20. Accordingly, independent segregation of the sex chromosome trivalent and the autosomal trivalent should result in four types of MII cells with the same frequency (0.25:0.25:0.25:0.25) but differing in their numbers of chromosomes. For example, shrew no. 1 with 24 chromosomes and the full karyotype X/Y1/Y2, *bc*, *af*, *jl*, *hk*, *i*/*io*/*o*, *gr*, *mn*, *p*, *q* and *tu* should produce four types of MII cells, with chromosome numbers 11, 12 or 13, that is:

Type A: X, *bc*, *af*, *jl*, *hk*, *io*, *gr*, *mn*, *p*, *q*, *tu* ($N = 11$)
Type B: Y1, Y2, *bc*, *af*, *jl*, *hk*, *io*, *gr*, *mn*, *p*, *q*, *tu* ($N = 12$)
Type C: X, *bc*, *af*, *jl*, *hk*, *i*, *o*, *gr*, *mn*, *p*, *q*, *tu* ($N = 12$)
Type D: Y1, Y2, *bc*, *af*, *jl*, *hk*, *i*, *o*, *gr*, *mn*, *p*, *q*, *tu* ($N = 13$)
(Figure 1).

Types B and C have 12 chromosomes each, but can be distinguished easily from one another on the basis of the numbers of acrocentric chromosomes: in the type B, there are three small acrocentrics (*p*, *q* and Y1) and the subtelocentric Y2, and in the type C—four acrocentrics (*p*, *q*, *i* and *o*), and the metacentric X of large size (comparable with the chromosomes *bc* and *af*). Obviously, male subjects with $2N = 26$ should produce MII cells with 12, 13 or 14 chromosomes (Figure 2).

It needs to be emphasized that, among the four products of segregation from male subject meiosis, there are two pairs of complementary types of MII cells: A + D and B + C, whose chromosome numbers add up to $2N = 24$ (or 26). In other words, there are two possibilities for the correct segregation of the diploid set of chromosomes (A and D versus B and C). A and D cells represent the extreme types—with five acrocentrics (type D) or two (type A). In contrast, type B and C cells—as has been mentioned—differ in the presence of one acrocentric. Likewise, in male subjects with $2N = 26$, cells of types A and D differ in three acrocentrics, those of types B and C in one only. The χ^2 test was used to assess the deviations from the expected proportions of MII cells.

MII spreads with incorrect numbers of chromosomes ($NF \neq 20$) can readily be identified. Such spreads may arise through the action of two factors. In the first place, some part of MII cells recorded with counts of less than 20 chromosome arms arise as a result of cell damages. Artefacts of this kind may at times come to represent a considerable fraction of the material studied. In other instances, they can be attributed to non-disjunction, the effect of which is for half of cells to have $NF < 20$, the other half $NF > 20$. As the cases of chromosome deficits arising through non-disjunction cannot be distinguished from artefacts, the estimation of non-disjunction frequency was made using hyperploid MII spreads only, their numbers then being doubled to compensate for the ignored hypoploid spreads.

Results

The segregation of chromosomes

The four types of MII cells arise with different frequencies. Metacentric chromosomes are favoured in the segregation of meiotic trivalents, and this is true for both autosomes (frequency of metacentrics = 0.64), and sex chromosomes (frequency of X chromosomes = 0.61). In each case, there are highly significant deviations from a frequency of 0.5, although the excess of X chromosomes in four individual male shrews did not achieve statistical significance (Table 3). In consequence, all 12 shrews show the frequencies of type A MII cells that far exceed the theoretical value of 0.25 (mean frequency is 0.42). The frequencies of cells of types B and C are rather similar and close to the expected values (0.23 and 0.20, respectively). Type D cells were noted most rarely (with the mean frequency of 0.15). In only three cases was the frequency of type D cells higher than that of type C (Table 2).

The level of non-disjunction (the vast majority of hyperploid MII spreads had one acrocentric chromosome extra; Figure 3a) is rather higher among the *i*/*o* and *j*/*l* heterozygotes than among the male subjects heterozygous for small-sized chromosomes (*nr*, *mn*) (Table 3). However, the size of a metacentric does not influence the degree of segregation distortion. The mean frequencies of the four types of MII cells are homogeneously distributed among medium-sized (*io*, *jl*) and small-sized (*nr*, *mn*) heterozygous autosomes ($\chi^2_{(3)} = 0.60$; $P > 0.9$). On the other hand, the segregation distortion in favour of metacentrics is higher (0.68) for the male subjects coming from hybrid

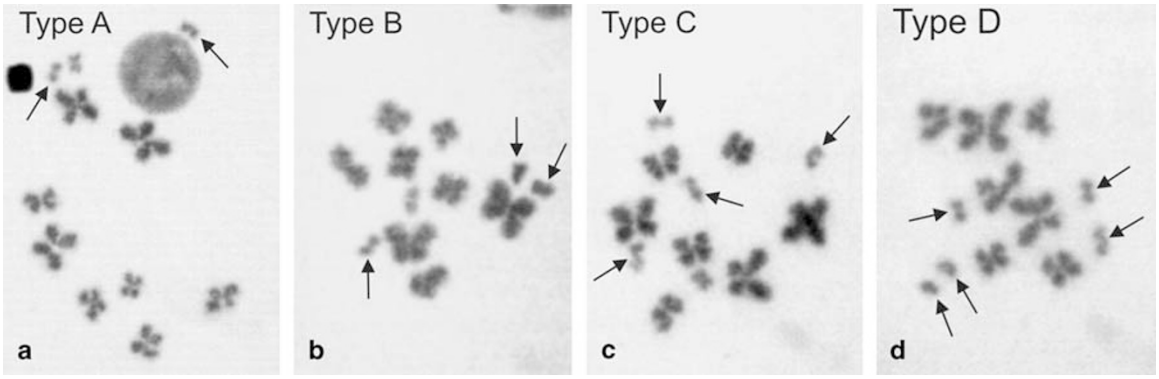

Figure 1 MII spreads of an individual, heterozygous for *nr* chromosome, with 24 chromosomes in somatic cells. (**a**) The MII cell with 11 chromosomes, arrows indicate *p* and *q* acrocentrics. (**b**) The MII cell with 12 chromosomes, arrows indicate *p*, *q* and Y_1 acrocentric chromosomes. (**c**) The MII cell with 12 chromosomes, arrows indicate four acrocentrics *n*, *p*, *q* and *r*. (**d**) The MII cell with 13 chromosomes, arrows indicate *n*, *p*, *q*, *r* and Y_1 chromosomes. MII, metaphase II.

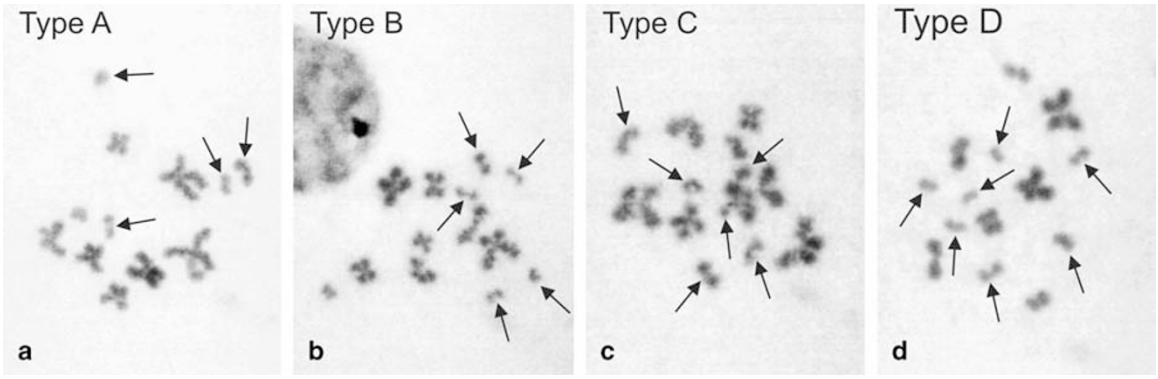

Figure 2 MII spreads of an individual, heterozygous for *jl* arm combination, with 26 chromosomes in somatic cells. (**a**) The MII cell with 12 chromosomes, arrows indicate *i*, *o*, *p* and *q* acrocentrics. (**b**) The MII cell with 13 chromosomes, arrows indicate *i*, *o*, *p*, *q* and Y_1 chromosomes. (**c**) The MII cell with 13 chromosomes, arrows indicate six acrocentrics (*j*, *l*, *i*, *o*, *p* and *q*). (**d**) The MII cell with 14 chromosomes, arrows indicate seven acrocentric chromosomes (*j*, *l*, *i*, *o*, *p*, *q* and Y_1). MII, metaphase II.

Table 2 Counts of the four products of segregation (frequencies given in parentheses)

No. of shrew	*Hpa*	*Products of segregation*				*Total scored MII cells*	*Departure from 1:1:1:1 proportion*
		A	*B*	*C*	*D*		$\chi^2_{(3)}$
1	*io*	81 (0.37)	50 (0.23)	44 (0.20)	42 (0.19)	217	18.226; $P<0.0005$
2	*io*	70 (0.37)	55 (0.29)	28 (0.15)	38 (0.20)	191	21.628; $P<0.0005$
3	*jl*	87 (0.48)	33 (0.18)	40 (0.22)	22 (0.12)	182	54.088; $P<0.0005$
4	*io*	77 (0.50)	33 (0.21)	30 (0.19)	15 (0.10)	155	55.142; $P<0.0005$
Subtotal		315 (0.42)	171 (0.23)	142 (0.19)	117 (0.16)	745	126.511; $P\ll0.0005$
5	*nr*	63 (0.39)	35 (0.21)	41 (0.25)	22 (0.14)	161	21.832; $P<0.0005$
6	*nr*	61 (0.40)	36 (0.21)	29 (0.19)	26 (0.17)	152	19.947; $P<0.0005$
7	*nr*	49 (0.39)	35 (0.28)	21 (0.17)	21 (0.17)	126	17.111; $P<0.001$
8	*nr*	63 (0.38)	39 (0.24)	34 (0.21)	28 (0.17)	164	17.219; $P<0.001$
9	*nr*	51 (0.39)	35 (0.27)	21 (0.16)	22 (0.17)	129	18.318; $P<0.0005$
10	*nr*	81 (0.45)	34 (0.19)	45 (0.25)	21 (0.12)	181	44.039; $P<0.0005$
11	*nr*	80 (0.43)	36 (0.20)	45 (0.24)	23 (0.13)	184	38.826; $P<0.0005$
12	*mn*	70 (0.43)	39 (0.24)	21 (0.13)	31 (0.19)	161	33.360; $P<0.0005$
Subtotal		518 (0.41)	289 (0.23)	257 (0.20)	194 (0.15)	1258	190.426; $P\ll0.0005$
Total		833 (0.42)	460 (0.23)	399 (0.20)	311 (0.15)	2003	316.426; $P\ll0.0005$

Abbreviations: Hpa, heterozygous pair of autosomes; MII, metaphase II.

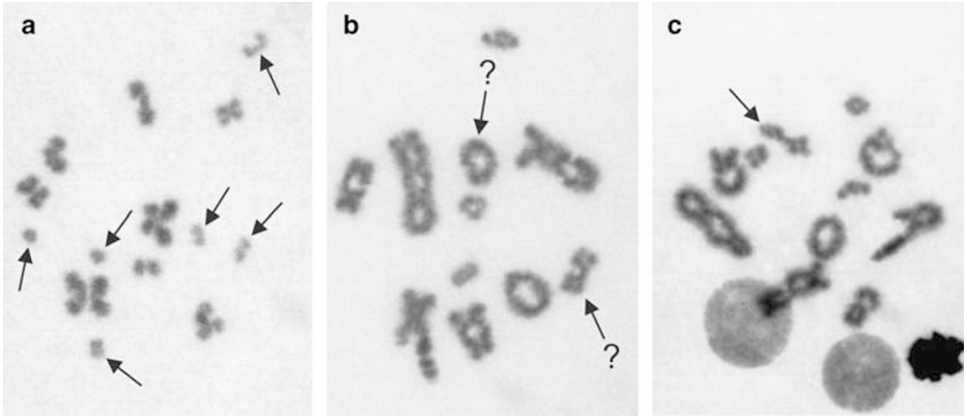

Figure 3 (**a**) Hyperploid MII spread of the individual heterozygous for *jl* chromosome arm combination; the type B cell with 14 chromosomes and one acrocentric extra (arrows indicate six acrocentric chromosomes). (**b**) The only case of diakinesis spread with probable ring configuration of *nr* trivalent. It is not clear which one, out of the two elements indicated by arrows, really represents *nr* arm combination. (**c**) Arrow indicates chain configuration of *nr* trivalent. MII, metaphase II.

populations (shrews no. 4, 10, 11 and 12) than for the remaining individuals (0.62); the difference is statistically significant ($\chi^2_{(1)} = 7.81$; $P < 0.01$).

The analysis of the complementary pairs of MII cells confirms the segregation distortion in favour of the metacentric chromosomes. The cells of types B and C occur in similar proportions, although when scores from all shrews are totalled, B is slightly but significantly higher than C ($P < 0.05$; mean B:C = 1.1:1, Table 4). In contrast, within the second pair of complementary cells an excess of type A over type D was found in all 12 male subjects studied (mean A:D = 2.7:1; $P < 0.005$–0.001, Table 4).

These results might implicate preferential loss of acrocentric chromosomes during preparation as the main reason for the deficit of type D euploid spreads, as compared with type A. This is not the case. Spreads with losses of single acrocentric chromosomes (NF = 19) are homogeneously distributed among the four types of segregation products (type A—27.4% of spreads, type B—26.3% of spreads, type C—19.8% and type D—26.5% of spreads; $P > 0.5$). Thus, even a considerable number of chromosome losses, arising during preparation, did not affect the proportion of scored euploid products of segregation.

Configurations of trivalents in diakinesis

According to the model accounting for the unequal transmission of meta- and acrocentric chromosomes through recombination in the pericentric area of twin acrocentrics forming a trivalent, at the diakinesis stage of simple Rb heterozygotes, a ring configuration of trivalents ought to be observed. To check for this, 187 diakinesis spreads coming from four heterozygous male subjects were analysed. However, in this material, it was

Table 3 The meiotic segregation of chromosomes (frequencies given in parentheses)

No. of shrew	*Hpa*	*Total scored MII cells*	*Segregation of autosomes*			*Segregation of sex chromosomes*			*Non-disjunction (%)*
			Meta	*Acro*	$\chi^2_{(1)}$	*X*	Y_1Y_2	$\chi^2_{(1)}$	
1	*io*	217	131 (0.60)	86 (0.40)	9.33; $P < 0.005$	125 (0.58)	92 (0.42)	5.02; $P < 0.05$	7.0
2	*io*	191	125 (0.65)	66 (0.35)	18.22; $P \ll 0.001$	98 (0.51)	93 (0.49)	0.13; $P > 0.7$	5.9
3	*jl*	182	120 (0.66)	62 (0.34)	18.48; $P \ll 0.001$	127 (0.70)	55 (0.30)	28.48; $P \ll 0.001$	6.4
4	*io*	155	110 (0.71)	45 (0.29)	27.26; $P \ll 0.001$	107 (0.69)	48 (0.31)	22.45; $P \ll 0.001$	3.8
Subtotal		745	486 (0.65)	259 (0.35)	69.17; $P \ll 0.001$	457 (0.61)	288 (0.39)	38.34; $P \ll 0.001$	5.9
5	*nr*	161	98 (0.61)	63 (0.39)	7.61; $P < 0.01$	104 (0.65)	57 (0.35)	13.72; $P \ll 0.001$	3.3
6	*nr*	152	97 (0.64)	55 (0.36)	11.60; $P < 0.001$	90 (0.59)	62 (0.41)	5.16; $P < 0.025$	1.2
7	*nr*	126	84 (0.67)	42 (0.33)	14.00; $P \ll 0.001$	70 (0.56)	56 (0.44)	1.55; $P > 0.2$	1.6
8	*nr*	164	102 (0.62)	62 (0.38)	7.90; $P < 0.005$	97 (0.59)	67 (0.41)	5.49; $P < 0.025$	4.8
9	*nr*	129	86 (0.67)	43 (0.33)	14.33; $P \ll 0.001$	72 (0.56)	57 (0.44)	1.74; $P > 0.2$	3.0
10	*nr*	181	115 (0.64)	66 (0.36)	13.26; $P \ll 0.001$	126 (0.70)	55 (0.30)	27.85; $P \ll 0.001$	7.4
11	*nr*	184	116 (0.63)	68 (0.37)	12.07; $P < 0.001$	125 (0.68)	59 (0.32)	23.09; $P \ll 0.001$	4.3
12	*mn*	161	109 (0.68)	52 (0.32)	20.18; $P \ll 0.001$	91 (0.56)	70 (0.44)	2.74; $P > 0.05$	3.5
Subtotal		1258	807 (0.64)	451 (0.36)	100.74; $P \ll 0.001$	775 (0.62)	483 (0.38)	67.78; $P \ll 0.001$	3.1
Total		2003	1293 (0.64)	710 (0.36)	169.69; $P \ll 0.001$	1232 (0.61)	771 (0.39)	106.10; $P \ll 0.001$	4.1

Abbreviation: Hpa, heterozygous pair of autosomes.

Table 4 Segregation of complementary MII cells

No. of shrew	*Hpa*	*A*, n *(fr.)*	*D*, n *(fr.)*	*Proportion A:D*	$\chi^2_{(1)}$	*B*, n *(fr.)*	*C*, n *(fr.)*	*Proportion B:C*	$\chi^2_{(1)}$
1	*io*	81 (0.66)	42 (0.34)	1.9:1	12.37; $P < 0.001$	50 (0.53)	44 (0.47)	1.1:1	0.38; $P > 0.5$
2	*io*	70 (0.65)	38 (0.35)	1.9:1	9.48; $P < 0.005$	55 (0.66)	28 (0.34)	2:1	8.78; $P < 0.005$
3	*jl*	87 (0.80)	22 (0.20)	3.9:1	38.76; $P \ll 0.001$	33 (0.45)	40 (0.55)	0.8:1	0.67; $P > 0.4$
4	*io*	77 (0.84)	15 (0.18)	5.1:1	41.78; $P \ll 0.001$	33 (0.52)	30 (0.48)	1.1:1	0.14; $P > 0.7$
$\sum$		315 (0.73)	117 (0.27)	2.7:1	90.75; $P \ll 0.001$	171 (0.55)	142 (0.45)	1.2:1	2.69; $P > 0.1$
5	*nr*	63 (0.74)	22 (0.26)	2.9:1	19.78; $P \ll 0.001$	35 (0.46)	41 (0.54)	0.8:1	0.47; $P > 0.5$
6	*nr*	61 (0.70)	26 (0.30)	2.3:1	14.08; $P \ll 0.001$	36 (0.55)	29 (0.45)	1.2:1	0.75; $P > 0.3$
7	*nr*	49 (0.63)	21 (0.30)	2.3:1	11.20; $P < 0.001$	35 (0.62)	21 (0.37)	1.7:1	3.50; $P > 0.05$
8	*nr*	63 (0.69)	28 (0.31)	2.2:1	13.46; $P \ll 0.001$	39 (0.53)	34 (0.47)	1.1:1	0.34; $P > 0.5$
9	*nr*	51 (0.70)	22 (0.30)	2.3:1	11.52; $P < 0.001$	35 (0.62)	21 (0.38)	1.7:1	3.50; $P > 0.05$
10	*nr*	81 (0.79)	21 (0.21)	3.9:1	35.29; $P \ll 0.001$	34 (0.43)	45 (0.57)	0.7:1	1.53; $P > 0.2$
11	*nr*	80 (0.78)	23 (0.22)	3.5:1	31.54; $P \ll 0.001$	36 (0.44)	45 (0.56)	0.8:1	1.00; $P > 0.3$
12	*mn*	70 (0.69)	31 (0.31)	2.3:1	15.06; $P \ll 0.001$	39 (0.65)	21 (0.35)	1.9:1	5.40; $P < 0.025$
$\sum$		518 (0.72)	194 (0.28)	2.7:1	147.44; $P \ll 0.001$	289 (0.53)	257 (0.47)	1.1:1	1.87; $P > 0.2$
$\sum\sum$		833 (0.73)	311 (0.27)	2.7:1	119.09; $P \ll 0.001$	460 (0.53)	399 (0.47)	1.1:1	4.33; $P < 0.05$

Abbreviation: Hpa, heterozygous pair of autosomes.

only possible to note one probable case of a ring (Figure 3b). It was not clear whether the ring configuration in question comprised the *n*/*nr*/*r* trivalent, or one of the small-sized bivalents. In remaining cases, trivalents formed chain configurations (Figure 3c).

Discussion

The first data on the preferential transmission of metacentrics in the common shrew were obtained in studies of the chromosomes of heterozygous pregnant female subjects from the field and their offspring (Searle, 1986a). However, multiple paternity in common shrews has been demonstrated (Tegelström *et al*., 1991), which complicates the interpretation of data from natural pregnancies. Crosses of Rb heterozygotes with homozygotes under controlled laboratory conditions (Wyttenbach and Hausser, 1996; Wyttenbach *et al*., 1998) have provided definitive proof that transmission of metacentrics—above all those on the paternal side—to offspring is significantly more frequent than of their acrocentric homologues. Concerning the mechanism of preferential transmission, Wyttenbach and Hausser (1996) suggested that either (a) there is a distortion of the segregation in favour of metacentric chromosomes or (b) the oocytes more often accept sperm with metacentrics than with acrocentrics (the 'choice effect' hypothesis).

Our study shows that segregation distortion in simple Rb heterozygote male subjects does favour metacentric chromosomes at the level of 0.60–0.71 (Table 3). These data agree with the 0.64–0.65 level of transmission of metacentrics reported by Searle (1986a), and with the level of transmission from the male side (of 0.64–0.68) noted in crosses obtained in controlled conditions (Wyttenbach *et al*., 1998). Our data thus suggest segregation distortion, rather than 'choice effect', as the cause of excess of metacentric chromosomes in the offspring of Rb heterozygotes. The higher level of transmission of metacentrics on the male subject as opposed to the female side inclined Wyttenbach and Hausser (1996) to consider possible mechanisms of segregation disturbances involving both sexes. They paid attention to the earlier suggestion by Fedyk (1987), who pointed out the fact that the centromeres of the acrocentrics that form (together with the metacentrics) meiotic complexes are in close proximity to one another during prophase I, could increase the chances of fusions between acrocentrics. Likewise, the model described by Wyttenbach *et al*. (1998) proposes that metacentrics arise as a result of crossing over between the twin acrocentrics forming a trivalent. The consequence of the crossing over between non-homologous chromatids in a trivalent should be the emergence of ring configurations at the diakinesis. However, such ring configurations were not observed in our study, which corroborates the earlier observations by Searle (1986b). Therefore, the excess of metacentric chromosomes cannot result from recombination among twin acrocentrics. On the other hand, formation of a short non-homologously paired side arm in the centromere region of twin acrocentrics seems to play a significant role. Wallace (2003) demonstrated that in heterozygous mice over 84% of trivalents have paired side arm, whereas in common shrews only around 28%. Unlike in shrews, preferential segregation in favour of acrocentric chromosomes was observed in mice (Gropp and Winking, 1981). Further detailed analyses of pachytene stage in shrews are therefore needed to elucidate the causality between formation of paired side arm in trivalents and preferential segregation of chromosomes. Another conclusion is that the meiotic drive system in *S. araneus* can be regarded rather as a chromosomal, than a genic drive, since the segregation distortion affects different (*gm*, *hi*, *jl*) Rb fusions (Wyttenbach *et al*., 1998). This conclusion gains further confirmation in our study. We have demonstrated that, not only large metacentrics (*jl*, *io*) but also small ones (*nr*, *mn*) are favoured significantly during meiosis. The level of meiotic drive does not depend on either the size of metacentrics or the composition of chromosome arms in MII cells, the frequency of *nr* reached 0.64, that of *io* 0.65, that of *jl* 0.66 and that of *mn* 0.68, the differences between these figures did not reach statistical significance ($\chi^2_{(3)} = 1.46$; $P > 0.7$).

The consequences of segregation distortion ought to be a steady rise in the frequency of metacentrics, until their fixation. The effects of this were observed in site no. 93 at Białowieża (NE Poland) (Fedyk, 1980). Taking frequencies of metacentrics in the sample from 1968 as a basis for calculation, it was possible to show—for the 80-shrew sample from 1971—a statistically significant deficit of *g*/*r* and *j*/*l* heterozygotes. In contrast, the deficits observed in 1972 and 1974 were not significant (Table 5), perhaps, as a result of a declining influence of preferential transmission of metacentrics. Maybe, the marked decrease in frequency of acrocentrics in the period 1968–1971 resulted from a rarity of heterozygotes in the population, reducing the effect of segregation distortion on chromosome frequency.

It seems natural to connect the evolutionary success of the Rb fusion in *S. araneus* with segregation distortion in favour of metacentrics, as emphasized by Bentgsson (1980) Wyttenbach and Hausser (1996) and Wyttenbach *et al*. (1998). Mathematical models suggest that even a

Table 5 Deficit of heterozygous shrews in locality no. 93 at the Białowieża Primeval Forest (data compiled from Tables 11 and 12 in the paper by Fedyk, 1980)

Year	1968	1971	1972	1974
Sample size	40	80	32	19
Fraction of heterozygotes	0.275	0.125	0.094	0.000
Heterozygote deficits[a]				
gr	—	7.340 ($P < 0.01$)	3.023 ($0.10 > P > 0.05$)	
jl	—	4.292 ($P < 0.05$)	2.987 ($0.10 > P > 0.05$)	

[a]The $\chi^2_{(1)}$ values were calculated in four independent goodness-of-fit tests between the observed and expected numbers of hetero- and homokaryotypes estimated on the basis of frequencies of *gr* and *jl* chromosomes in 1968.

limited level of meiotic drive favouring a new chromosomal variant should act like directional selection, thereby increasing the likelihood of fixation of the mutation (Hedrick, 1981). On this basis, the critical period of the increase in heterozygosity, relating to the lower fitness of heterozygotes (Gropp and Winking, 1981), may be shortened markedly under the pressure of meiotic drive. It can be suggested that selection against Rb heterozygotes, supported by meiotic drive in favour of metacentric chromosomes, should effectively eliminate heterozygotes and consequently fix metacentrics in population of *S. araneus*. However, throughout the whole range of the species, Rb polymorphism has been found (Fedyk, 1995), and what is more, the frequencies of polymorphic chromosomes in some populations would seem to be stable (Searle, 1986c). Wyttenbach *et al*. (1998) explained the presence of balanced polymorphism—according to their own model—in terms of the possibility of recombination frequencies changing under the pressure of natural selection. The same was also suggested by Felsenstein and Yokoyama (1976) in their model. It is possible, however, that the stabilization of Rb polymorphism in shrew populations does not result solely from such an intrapopulation mechanism. In most cases, the areas of Rb polymorphism are located between monomorphic areas with fixed metacentrics and acrocentrics. This point is well illustrated by the zone of hybridization, about 100 km wide, between the Dn race (*hi*, *ko*, *gm*, *nr*) and the Ulm race (*hi*, *gm*, *k*, *o*, *n*, *r*). It is obvious that this contact zone must be characterized by regular frequency clines of metacentrics *ko* and *nr* (Lukáčova *et al*., 1994). Migration of individuals between adjacent populations will effectively counteract the reduction of heterozygosity caused by meiotic drive. Such exchanges between populations may, however, be limited at the local level by various environmental barriers (Fedyk *et al*., 1993; Narain and Fredga, 1996; Szalaj *et al*., 1996; Moska, 2003).

The present study demonstrates stronger distortion of segregation among the male subjects coming from hybrid zones than those from pure race populations. This is quite surprising, because biased segregation in favour of metacentrics would seem to be inconsistent with the acrocentric peak described by Searle (1986c) in the contact zone between the Oxford and Hermitage races, and also present in several other hybrid zones (Fedyk, 1986; Banaszek, 1994; Szalaj *et al*., 1996; Fredga and Narain, 2000; Moska, 2003). The presence of acrocentrics in a hybrid zone is 'desirable' because, the higher the frequency of acrocentrics in the contact zone, the rarer the appearance of complex heterozygotes, which are characterized by impaired fertility (Searle, 1993). Simulations show that, if selection against the heterozygotes is strong enough, a maximization of the frequency of acrocentrics may occur (Hatfield *et al*., 1992). However, hybrid zones are also known in which acrocentrics are entirely lacking (Fedyk *et al*., 1993; Narain and Fredga, 1996). Accordingly, it may be concluded that the mechanisms favouring acrocentrics and metacentrics in hybrid populations are not at equilibrium. There are—apparently—certain hybrid zones in which selection acts more powerfully against complex heterozygotes, thereby limiting the influence of meiotic drive, as well as others in which the more moderate selection pressure against the heterozygotes allows meiotic drive to prevail over any trend towards acrocentrics being favoured. In contrast, at the edge of contact zones, preferential segregation may be effective in eliminating of acrocentric chromosomes from the populations, as could be observed around the Oxford-Hermitage hybrid zone (Searle, 1986a).

Preferential segregation of X chromosomes has been reported in complex heterozygotes forming pentavalent chain configurations (CV) during meiosis (Fedyk *et al*., 2005). In contrast, both homozygous and heterozygous shrews with meiotic quadrivalents (CIV and RIV + CIV) display segregation of sex chromosomes that do not differ significantly from 1:1 (Fedyk *et al*., 2005). Searle (1986b) also reported equal segregation of sex chromosomes among simple Rb heterozygotes. These data suggested to Fedyk *et al*. (2005) that the chromosomes forming longer (at least CV) meiotic chains interact with chromosome Y1, which most often occurs as the univalent (Searle 1986b; Banaszek *et al*., 2002; Fedyk *et al*., 2005), giving greater mortality of MII cells with Y1Y2 chromosomes. Therefore, the preferential segregation of X chromosomes in eight out of 12 of the studied simple Rb heterozygote male subjects (Table 3) is a surprise. When this new finding is taken into account, we are led to the paradoxical conclusion that, among the male subjects that form complexes of even numbers of autosomes conjugating in common (meiotic bivalents and quadrivalents), the normal (1:1) segregation of sex chromosomes occurs, while disturbed segregation in favour of X chromosomes takes place in males whose meiotic complexes are formed by odd numbers of autosomes (trivalents and pentavalents). Nonetheless, this conclusion seems improbable, so it is perhaps better to presume that in the material for the previous study (Fedyk *et al*., 2005)—comprising two CIV hybrids and one RIV + CIV—sex chromosome segregation was, by pure chance, close to 1:1, as in the case of several CV hybrids (Fedyk *et al*., 2005) and simple heterozygous individuals (the present study). If not, we should suppose that, irrespective of their size, odd-numbered complexes of chromosomes exert a disruptive influence on the segregation of sex chromosomes. This issue will only be resolved on the basis of re-examination of a large sample of CIV and/or RIV hybrids.

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