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A cytogenetical analysis of reproduction in common shrews *(Sorex araneus)* from a karyotypic hybrid zone

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Sixteen pregnant female common shrews were collected near Oxford (U.K.) from a hybrid zone between two karyotypic races which differ by Robertsonian rearrangements. Some females were homozygotes and others were 'simple' or 'complex' Robertsonian heterozygotes. The females and their fetuses were karyotyped and number of ovulations and regressing implants scored. Prenatal losses were noted in female simple Robertsonian heterozygotes. Some losses, including two trisomics, were likely to be due to anaphase I nondisjunction of the maternal autosomal trivalent. However, numbers of ovulations were found to be higher in these heterozygotes than in the homozygotes, and this could compensate for prenatal losses due to nondisjunction. Overall, fertility of females from the hybrid zone did not differ substantially from that of females from other areas of karyotypic polymorphism or monomorphism. There was no evidence for assortative mating or assortative fertilisation within the hybrid zone. On chromosomal grounds, the hybrid zone is unlikely to be a major barrier to gene flow.

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Cytogenetic studies have revealed that many species of wild animal are subdivided into geographically localised forms which differ in karyotype (WHITE 1978). These 'karyotypic races' have a parapatric distribution, with narrow hybrid zones in which karyotypic heterozygotes are found. Such heterozygotes are expected to be less fit than karyotypic homozygotes from either of the hybridizing races as a result of meiotic irregularities and the consequent reduced reproductive performance. The extent of such unfitness is important because it could contribute to the interruption of gene flow between karyotypic races and, perhaps, lead to speciation by creating a selection pressure for assortative mating or assortative fertilisation. However, despite the recent interest in hybrid zones in general (BARTON and HEWITT 1985) and karyotypic hybrid zones in particular (WHITE 1978; CAPANNA 1982; PATTON and SHERWOOD 1983; BAKER and BICKHAM 1986), we are still largely ignorant of the degree of infertility in karyotypic heterozygotes in nature.

For several years I have studied the hybrid zone in the common shrew (*Sorex araneus*) between the Oxford and Hermitage karyotypic races in southern Britain (SEARLE 1986a, 1988a, b), particularly that section of zone within the vicinity of the city of Oxford (Fig. 1). The karyotypes of these hybridizing races are characterised by different sets of metacentrics formed by independent Robertsonian fusions of the ancestral acrocentric chromosomes (WÓJCIK and SEARLE 1988). The karyotype of the Oxford race possesses metacentrics *kq* and *no* while that of the Hermitage race includes metacentric *ko* and acrocentrics *n* and *q* (each chromosome arm in the karyotype is labeled by a letter of the alphabet, with *a* the largest arm and *u* the smallest; see Fig. 2 and 3). Enzyme and morphological studies suggest an absence of major genic differences between the races (SEARLE 1985; SEARLE and THORPE 1987).

Within the vicinity of the Oxford-Hermitage hybrid zone, chromosome arms *k* and *o* also occur as acrocentrics. So, in addition to the pure race karyotypes (Oxford: *kq, no, kq, no* and Hermitage: *ko, n, q, ko, n, q*) and F_1 hybrids between pure race individuals (with karyotype *kq, no, ko, n, q;* see Fig. 3), one would expect recombinant individuals which show Oxford or Hermitage race characteristics to a greater or lesser degree. I classify those recombinant individuals with only Oxford race-specific metacentrics as being of the Oxford race (their karyotypes: (1) *kq, no, kq, n, o;* (2) *kq, no, k, q, no;*

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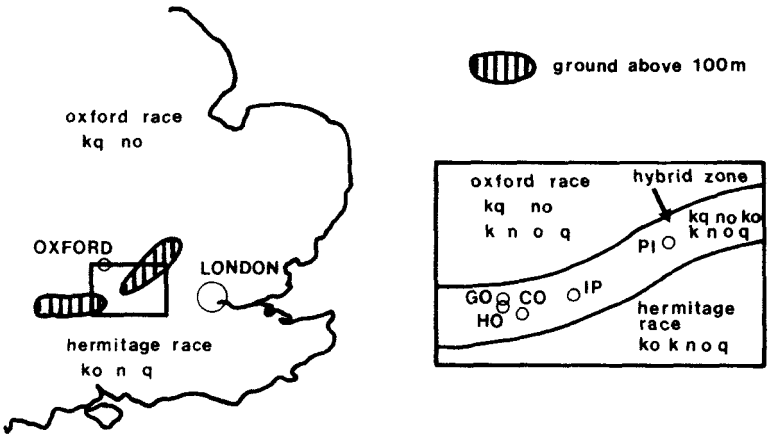

Fig. 1. Map showing collection sites within the 10 km wide hybrid zone between the Oxford and Hermitage karyotypic races (GO: Gore Hill, HO: Hodcott Down, CO: Compton, IP: Ipsden, PI: Piddington). At all collection sites both Oxford race metacentrics (*kq*, *no*) and the Hermitage metacentric (*ko*) occur. Acrocentrics *k*, *n*, *o*, and *q* occur within the hybrid zone and to a distance of at least 60 km to the north of the zone and at least 30 km south. The Oxford race occupies central England while the Hermitage race occurs in southeastern England, to the south of the Berkshire Downs and Chiltern Hills (high ground shown on map) and London.

(3) *kq*, *n*, *o*, *kq*, *n*, *o*; (4) *k*, *q*, *no*, *k*, *q*, *no* (see Fig. 2); (5) *kq*, *no*, *k*, *q*, *n*, *o*; (6) *kq*, *n*, *o*, *k*, *q*, *n*, *o*; (7) *k*, *q*, *no*, *k*, *q*, *n*, *o*); those with only the Hermitage race-specific metacentric as belonging to the Hermitage race (their karyotype: *ko*, *n*, *q*, *k*, *o*, *n*, *q*); while those with both Oxford and Hermitage race-specific metacentrics are classified as interracial hybrids (their karyotypes: *kq*, *n*, *o*, *ko*, *n*, *q* and *k*, *q*, *no*, *ko*, *n*, *q*). Individuals with no race-specific metacentrics (*k*, *q*, *n*, *o*, *k*, *q*, *n*, *o*) also occur. These acrocentric-dominated individuals are at particularly high frequency wherever interracial hybrids are found; this is thought to result from selection against the interracial hybrids because of a lower fertility relative to individuals of other karyotypes (SEARLE 1986a). By the presence of chain IV or chain V configurations at prophase I and metaphase I of meiosis, the hybrids should be predisposed to high frequencies of anaphase I nondisjunction and germ cell death (SEARLE 1988a). Interracial hybrids are limited to a region of about 10 km in width in my primary study area (the 'hybrid zone' proper: Fig. 1) but there is a wider area of karyotypic polymorphism flanking this. The area of occurrence of Oxford race recombinant individuals extends at least 60 km north of this hybrid zone, while the area of occurrence of the Hermitage race recombinant individuals extends at least 30 km south.

It should be noted that while 'complex' Robertsonian heterozygotes (which form long chain configurations at meiosis I) are limited to the hybrid zone, 'simple' Robertsonian heterozygotes (which form trivalents at meiosis I) for arm combinations *kq*, *no* and *ko* are found both in the hybrid zone and the flanking regions of polymorphism. Simple heterozygotes for arm combinations *pr* and *jl* are additionally found over this whole area. Simple heterozygotes would be expected to suffer reduced fertility relative to homozygotes, because of higher frequencies of anaphase I nondisjunction, although to a lesser extent than complex heterozygotes (SEARLE 1988a). Thus several factors will determine the width and structure of the Oxford-Hermitage hybrid zone and surrounding areas of polymorphism and the extent of gene flow through this region. These factors include the degree of infertility of simple and complex Robertsonian heterozygotes, the extent of selective differences between the different categories of homozygotes, and the vagility of common shrews.

I have previously examined reproduction in common shrews from the region of Robertsonian polymorphism around the Oxford-Hermitage hybrid zone by studies of wild-caught pregnant females (SEARLE 1984). This paper will extend the study of pregnant females to the hybrid zone itself. From

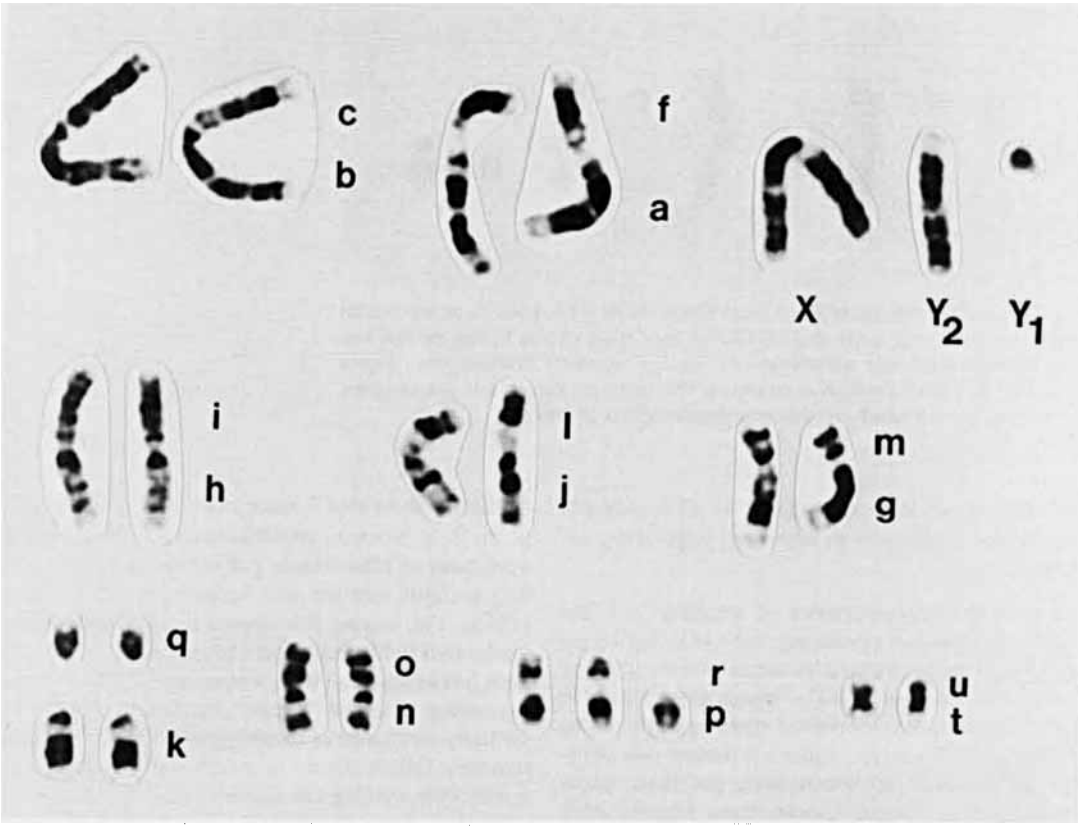

Fig. 2. Karyotype of male fetus from shrew 1676, trisomic for chromosome arm *p*. Note that the sex chromosomes and autosomes *bc, af, hi, gm,* and *tu* are invariant in Britain. The most crucial karyotypic variation in the vicinity of the Oxford-Hermitage hybrid zone involves chromosome arms *k, n, o, p, q,* and *r*. This individual is homozygous metacentric for *no* and is thus classified as belonging to the Oxford race (see text).

details of prenatal losses and maternal and fetal karyotypes, I will assess the overall reproductive output of shrews from the hybrid zone relative to other areas and, in particular, the fertility reduction in Robertsonian heterozygotes that results from anaphase I nondisjunction. Also, I will examine the extent of assortative mating and assortative fertilisation and will investigate differences in viability between individuals of different karyotypes.

Material and methods

Animals. — Pregnant common shrews were collected during April and May, 1987, at Piddington (map reference: SU 8194), Gore Hill (SU 4883), Compton (SU 5280), Hodcott Down (SU 4882) and Ipsden (SU 6284). These sites were all located within the hybrid zone between the Oxford and Hermitage races, within 30 km south or south-west of the city of Oxford, U.K. (Fig. 1). All animals were believed to be in their first pregnancy, on the basis of the criteria in SEARLE (1984). They were maintained for up to five days indoors, but with natural illumination, and were killed when judged to be at a suitable stage of pregnancy for analysis (SEARLE 1990).

Karyotypes. — Chromosome spreads were made from bone marrow cells of the pregnant females by the method of FORD (1966) and from fetal membranes of individual implants by the method of EVANS et al. (1972). Contrary to previous expectation (SEARLE 1984), good G-banded preparations were obtained from fetal material, after staining by the method of EVANS (1987). At least five G-banded spreads were scored per adult or fetal preparation.

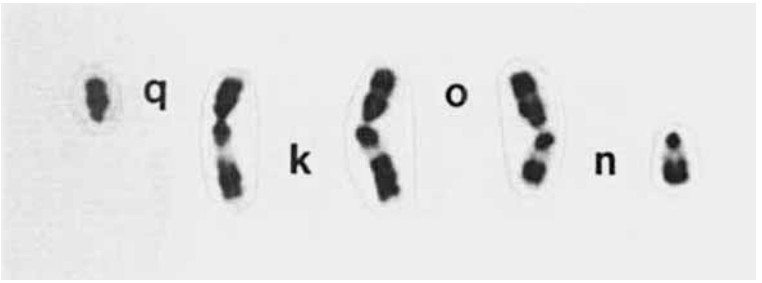

Fig. 3. Partial karyotype of fetus from shrew 1676. This fetus is an interracial hybrid which has one copy each of Oxford race metacentrics *kq* and *no* and one copy of Hermitage race metacentric *ko*. Such a 'complex heterozygote' should form a chain V configuration at meiosis I. The fetus was female and homozygous metacentric for the other variable arm combinations (*jl* and *pr*).

Satisfactory results were obtained for all individuals except four implants at an advanced stage of regression.

Number and characteristics of implants. — The number of implants (including those undergoing regression) was determined by inspection through the uterine wall (SEARLE 1984). Fetuses were allocated to one of seven developmental stages recognized by STERBA (1977). (Sterba defines a further two developmental stages in insectivores, but these occur postnatally in *Sorex*.) Crown-rump lengths were measured with an eyepiece graticule in a dissection microscope. All successful preparations were made on fetuses of developmental stages 3–6, i.e., fetuses which had formed limb buds but which had not yet formed eyelids. Based on the growth regression equation of STERBA (1977), the crown-rump lengths of these fetuses indicated an age range of 12–19 d. The gestation period is 20 days in the common shrew (DEHNEL 1952).

Counts of corpora lutea. — Previous analyses showed that counts of corpora lutea based on inspection of a fresh ovary under a dissection microscope are, in general, as accurate as counts made from histological sections and that number of corpora lutea truly corresponds to number of ovulations (SEARLE 1984). Therefore, fresh ovary counts were made and histological sections were examined only when there was any difficulty in making this count or if the luteinised structures appeared peculiar.

Although corpora lutea counts provide an accurate estimate of number of ovulations, some ova may be in excess to requirements when there are a large number of ovulations, because of a ceiling to the number of fetuses tolerated by the uterus. Among 47 pregnancies that I have examined over several years (J. B. SEARLE, unpublished data), there was a maximum of nine fetuses per clutch. A similar ceiling to fetus number was recorded by BRAMBELL (1935). Yet, among the corpora lutea counts I have made, two individuals had 10, and one had 13 corpora lutea (SEARLE 1984). I consider that ovulations exceeding nine are 'excess' ovulations that have virtually no chance of resulting in viable fetuses and that their failure should be ascribed to physiological rather than cytological causes (although it is possible that there may be cytogenetic abnormalities among 'excess' embryos).

Results

Characteristics of the pregnant females and their fetuses

Sixteen pregnant females and their fetuses were analysed (Table 1). Six females were simple Robertsonian heterozygotes for one or both of the Oxford race metacentrics (*kq*, *no*), two of these were also heterozygous for arm combination *pr* and an additional individual was heterozygous for *pr* only. Two females were simple Robertsonian heterozygotes for the Hermitage race metacentric *ko*. One female (1699) was an interracial hybrid and as such was a complex Robertsonian heterozygote which was expected to form a quadrivalent (*q-qk-ko-o*) and trivalent (*p-pr-r*) at meiosis I. The remaining six females were homozygous acrocentric for chromosomes *k*, *n*, *o*, *p*, *q*, and *r* ('all acrocentric' individuals). Arm combination *jl* occurred in a homozygous metacentric state in all individuals examined and will not be considered further.

Table 1. Fertility and karyotypic data for 16 pregnant females from the hybrid zone between the Oxford and Hermitage karyotypic races

1. Heterozygous arm combinations[a]
2. Number of corpora lutea
3. Prenatal losses[b]
4. Developmental stage fetuses

Shrew	Site of capture	1	2	3	4
1657	Piddington		6		4
1658	Piddington		8	1 R	3
1662	Piddington	*kq*	9		3
1663	Piddington	*ko*	6	1 R, 1 U	3
1664	Piddington		8[c]		4
1667	Piddington		6	1 S	6
1669	Piddington	*kq*	8[d]	1 N, 2 R	5
1670	Piddington		7		6
1673	Gore Hill	*pr*	7		6
1676	Gore Hill	*kq, no, pr*	9	1 N, 1 U	5
1682	Compton	*no, pr*	7		5
1679	Hodcott Down		8		5
1685	Hodcott Down	*no*	9		3
1686	Hodcott Down	*no*	7		6
1694	Hodcott Down	*ko*	7	1 N	6
1699	Ipsden	*kq, ko, pr*	8		5

[a] For each individual, the elements among variable chromosome arms *k, n, o, p, q* and *r* that are not tabulated are present in a homozygous acrocentric state

[b] Number of ovulations accounted for by no visible implants (N), regressing implants (R), karyotypically unbalanced fetuses (U), or small, karyotypically-balanced fetuses (S)

[c] Additionally, two luteinised follicles and further small regions of luteinised tissue

[d] Also, one luteinised follicle surrounded by a small area of luteinised tissue

Altogether 120 corpora lutea were scored in the 16 females (mean: 7.5 per female). However, in two individuals, additional luteinised structures were observed and were examined in histological sections (Table 1). In particular, there were three luteinised follicles, i.e., structures in which remains of the oocyte were still present. There were also additional regions of luteinised tissue considerably smaller than normal corpora lutea. The occurrence of these peculiar luteinised structures is unlikely to have been an artifact of maintenance conditions as neither female was maintained in captivity for longer than one day. BRAMBELL (1935) in a study of snap-trapped, wild-caught females, recorded single luteinised follicles in five individuals out of 97 examined.

Of the 120 ovulations recorded, 117 could be accounted for by an implant (Table 1). The three ova unaccounted for presumably died before fertilisation or as preimplantation or early postimplantation zygotes. From the developmental stage of the surviving fetuses, the females in which these deaths occurred could be said to be 16 days *post coitum* (1669, 1676) or 18 days *pc* (1694). Presumably, an early postimplantation death could have completely regressed (leaving no embryonic remnants), by the time these females were killed. Regressing implants were actually observed in three females, accounting for a further four ovulations. In all four regressions, remnants of fetal membranes were visible and in two instances, remains of the fetus were discernible. Therefore, these represent late deaths that occurred after a substantial period of postimplantation development.

There were two apparently healthy fetuses, which were obviously smaller than other fetuses in their clutch. The small fetus of shrew 1667 had a crown-rump length of 8.9 mm as compared with 9.7 mm for the other five fetuses. All these fetuses were at a late stage of development (stage 6: toes separated, approx 18 days *pc*). The karyotypes of all fetuses in the clutch were balanced. The small fetus of shrew 1663, although distinctly thinner, was, with a crown-rump length of 2.6 mm, not obviously shorter than the other four fetuses in the clutch (range 2.7–3.1 mm). These fetuses were at developmental stage 3 (limb buds present, approx. 12 days *pc*). The thin fetus, but not the others in this clutch, had an unbalanced karyotype. It was male, trisomic for chromosome arm *o*, heterozygous for *ko*, and homozygous acrocentric for chromosome arms *n*, *p*, *q*, and *r*. All nine spreads examined had an extra copy of chromosome arm *o*.

A second fetus with an unbalanced karyotype was found in the clutch of female 1676. It was male, trisomic for chromosome arm *p*, homozygous metacentric for arm combinations *no* and *pr* and homozygous acrocentric for chromosome arms *k* and *q* (Fig. 2). Two spreads out of 30 scored definitely lacked the acrocentric *p*, so there was a possibility of a diploid cell line (although these spreads may also have been the product of cell breakage). The trisomic fetus was of similar size to other fetuses in the clutch, despite the late developmental stage (5: handplate indented, approx. 17 days *pc*).

Every other ovulation could be accounted for by a karyotypically-balanced, developmentally-normal fetus. Altogether, 24 different autosomal karyotypes were recorded among the fetuses as a result of Robertsonian variation. One of the seven complex heterozygous fetuses recorded was a female heterozygous for Oxford race-specific metacentrics *kq* and *no* and Hermitage race-specific metacentric *ko* (Fig. 3), recorded for the first time in studies on the Oxford-Hermitage hybrid zone.

Table 2. Chromosomes transmitted from males in relation to the karyotype of their mates, as deduced from the karyotypes of pregnant females (collected from the Oxford-Hermitage hybrid zone) and their fetuses

Maternal karyotype[a]	Site of capture	Number of		Minimum number of fertilizing sperm with				
		pregnant females	fetuses	metacentrics				no metacentrics
				kq	*no*	*pr*	*ko*	
Oxford race								
kq, no, pr	Gore Hill	1	8	1	1	5	2	0
no, pr	Compton	1	7	0	0	1	3	0
kq	Piddington	2	14	0	4	0	0	5
no	Hodcott Down	2	16	0	3	1	3	4
Hermitage race								
ko	Piddington	1	5	0	0	2	0	0
ko	Hodcott Down	1	6	0	2	2	0	0
Interracial hybrid								
kq, ko, pr	Ipsden	1	8	2[b]	0	1	1[b]	2
Other karyotypes								
pr	Gore Hill	1	7	2	4	2	0	0
'all acro'[c]	Piddington	5	34	0	14	6	0	16
'all acro'[c]	Hodcott Down	1	8	0	4	2	1	3

[a] Including heterozygous arm combination. See legend to Table 1

[b] Alternatively, one *kq* and two *ko* metacentrics

[c] 'All acrocentric' individuals, homozygous acrocentric for chromosomes *k, n, o, p, q, r*

Prenatal losses and estimates of nondisjunction

From the data available (Table 1), prenatal losses (i.e., wasted ovulations) among shrews in their first pregnancy from the Oxford-Hermitage hybrid zone are estimated to be 8.3 %. I have included both trisomic fetuses in this estimate (although the fetus that was trisomic for arm *p* was not obviously retarded developmentally and may have survived at least a short period post-natally) and also the small but karyotypically balanced fetus in female 1667 (given its retarded growth it is reasonable to consider that this fetus would not have survived much longer). This estimate for prenatal losses does not take into account deaths from physiological causes which may occur later in pregnancy than the time at which the females were killed.

Most prenatal losses were recorded in pregnant females that were simple Robertsonian heterozygotes. Of the ten 'wasted ovulations', none was found in the complex Robertsonian heterozygote and only one regressing and one small implant were recorded in homozygous, 'all acrocentric' individuals (Table 1). Some of the prenatal losses in simple heterozygotes could result from anaphase I nondisjunction of the autosomal trivalent configurations. Unfortunately, due to the occurrence of multiple paternity in common shrews (SEARLE 1990), it is not possible to establish whether the male parents were homozygotes or heterozygotes. However, anaphase I nondisjunction frequencies in male simple Robertsonian heterozygous common shrews from the vicinity of the Oxford-Hermitage hybrid zone appear to be low (about 1 %: SEARLE 1986b; MERCER and SEARLE, in preparation). Therefore, as an approximation, it may be assumed that all prenatal losses attributable to Robertsonian heterozygosity were due to anaphase I nondisjunction of autosomal trivalents in the female; this fits with data from both man and house mouse which indicate a substantially higher frequency of nondisjunction in female Robertsonian heterozygotes than in males (review: SEARLE 1988a). On this assumption, the estimates of anaphase I nondisjunction frequency in simple heterozygotes from the Oxford-Hermitage hybrid zone are 5.8–11.6 % per heterozygous female or 4.3–8.5 % per heterozygous arm combination (see SEARLE 1984, for method of calculation; female 1699, which is both a simple and complex heterozygote, is excluded). For the maximum estimates, all prenatal losses in heterozygotes were assumed to result from anaphase I nondisjunction in the female. For the minimum estimates, both trisomics were assumed to arise by anaphase I nondisjunction of a maternal autosomal trivalent (an assumption which is compatible with maternal karyotype in each instance) and it is assumed that two other early prenatal losses were due to autosomal monosomy (autosomal monosomics, which die at around the time of implantation, would be expected at the same frequency as autosomal trisomics: SEARLE 1984).

Table 3. A comparison of metacentric and karyotype frequencies between adults (A) and fetuses (F) from sites in the Oxford-Hermitage hybrid zone

Site of capture	Life stage	N	Frequency of metacentrics *kq*	*no*	*pr*	*ko*	'all acrocentric' individuals[a]	complex heterozygotes[b]
Piddington	A	14	0.07	0	0	0.04	0.79	0
	F[c]	53	0.08	0.17	0.08	0.04	0.49	0
Hodcott Down	A	25	0	0.20	0.12	0.18	0.40	0.08
	F	30	0	0.32	0.08	0.12	0.27	0.03
Gore Hill	A	6	0.08	0.25	0.25	0.08	0.33	0
	F	15	0.27	0.37	0.67	0.07	0.27	0.13
Ipsden	A	11	0.27	0.05	0.27	0.23	0.09	0.09
	F	8	0.38	0	0.31	0.31	0	0.13
Compton	A	10	0.05	0.10	0.20	0.20	0.20	0
	F	7	0	0.36	0.36	0.21	0.29	0.43

[a] Individuals homozygous acrocentric for chromosome arms *k, n, o, p, q, r*

[b] Individuals with Hermitage race metacentric *ko* and Oxford race metacentrics *no* and/or *kq* in their karyotypes

[c] The fetuses derive from 8, 4, 2, 1, and 1 pregnant females, respectively

It should be noted that while there were more prenatal losses in simple Robertsonian heterozygotes than in homozygous, 'all acrocentric' individuals, there were also more corpora lutea (7.67±1.12 versus 7.17±0.98, respectively, mean ± standard deviation), although not significantly so. This reduces the discrepancy in numbers of viable fetuses between the two karyotypic categories (all acrocentric individuals: 6.83±1.17, simple Robertsonian heterozygotes: 6.78±1.64).

Chromosome transmission from the male side

Although it is not possible to deduce the complete karyotypes of the sires of the fetuses examined in this study because of multiple paternity (SEARLE 1990), some inferences can be made about the chromosomes transmitted on the male side (Table 2). In particular, there is no evidence of assortative mating; females do not necessarily mate with individuals of the same karyotype. Thus, Oxford race females (heterozygotes for arm combinations *kq* and/or *no*) mated with individuals with the Hermitage metacentric (*ko*) in their karyotypes; Hermitage race females mated with individuals that had Oxford race metacentrics in their karyotype; 'all acrocentric' females (homozygous acrocentric for *k, n, o, p, q* and *r*) mated both with individuals with Oxford race metacentrics and with individuals with Hermitage race metacentrics in their karyotypes; and interracial hybrids mated with non-hybrid individuals (viz. individuals with sperm that carried no metacentrics).

Considering the Oxford race metacentrics *kq* and *no*, it is possible to test whether there was a tendency for gametes that carried either of these metacentrics to 'avoid' being fertilised by gametes that carried the Hermitage race metacentric *ko* (assortative fertilisation). When one or both parents are Robertsonian heterozygotes, avoidance could be achieved if gametes that carry a race-specific metacentric preferentially fertilise gametes without a race-specific metacentric (when the partner is a simple heterozygote of the other race) or fertilise gametes that carry a metacentric specific to the same race (when the partner is a complex heterozygote). From data for pregnancies 1682, 1685, 1686, 1694, and 1699 (where the female was a complex heterozygote or heterozygous for a single race-specific metacentric) there were six instances when a sperm carrying a race-specific metacentric 'avoided' fertilising an oocyte carrying a metacentric of the other race, and five instances when the sperm 'preferred' fertilising such an oocyte. Thus, there is no evidence for assortative fertilisation.

Differential viability in relation to karyotype

A simple way to test whether individuals of different karyotypes have different viabilities is to compare the frequencies of the different karyotypes be-

Table 4. The fertility of common shrews in a karyotypic hybrid zone and regions of karyotypic polymorphism and monomorphism

Geographic location (karyotypic characteristics)	Number of pregnant females	Mean number of corpora lutea per female	Mean number of normal embryos per female[a]	Prenatal losses (%)[b]
Southern England (hybrid zone)[c]	23	7.7	7.0	7.4
Southern England (polymorphism)[d]	24	7.9	7.4	3.3
Northern Wales (monomorphism)[e]	22	7.2	6.8	5.7

[a] Excluding any known karyotypically-unbalanced fetuses and the growth-retarded fetus recorded in the present study

[b] For any individual, when there are more than 9 ovulations, those that exceed 9 are considered 'excess ovulations' and are excluded from this estimate

[c] Data from present study and J. B. SEARLE (unpublished data)

[d] Data from SEARLE (1984) and J. B. SEARLE (unpublished data)

[e] Data of BRAMBELL (1935) for females collected during May

tween age classes. Unfortunately, there are still too few data for a rigorous test (Table 3). However, there are interesting trends. In particular, the frequency of all acrocentric individuals tended to be higher among adults than among fetuses and there was a concomitant higher frequency of race-specific metacentrics among fetuses. Also, there was a slight tendency for complex heterozygotes (i.e., interracial hybrids) to be more frequent among fetuses than among adults.

Discussion

The trisomic fetuses

The single fetus recorded here that was trisomic for chromosomes arm *o* displayed growth retardation already by 12 days of development. By 14 days, the retardation associated with this trisomy can be substantial (SEARLE 1984), but at neither 12 days nor 14 days of development have externally obvious malformations been observed. Compared with the autosomal trisomies in the house mouse, this trisomy can be graded moderately severe. While trisomics for chormosomes 5 and 15 in the mouse likely would die earlier than shrews trisomic for chromosome arm *o*, trisomics for mouse chromosomes 16 and 19 would probably not appear abnormal at this development stage (GROPP 1982; EPSTEIN 1985). The other shrew trisomy recorded here, that for chromosome arm *p*, had much greater similarity to these relatively mild trisomies in the mouse. There was no external sign of retardation after 17 days of development. (It should be realised that the common shrew is relatively undeveloped at birth in comparison with other mammals, including the mouse: STERBA 1977.)

Fetal karyotypes were determined from membrane preparations (EVANS et al. 1972); so, it is possible that the apparent trisomic for chromosome arm *p* had predominantly trisomic fetal membranes but a completely, or predominantly, diploid fetus. Recent results from chorion villus samples in women highlight this possibility (CRANE and CHEUNG 1988). The karyotype data indicate that there may be a diploid cell line in the membranes of the fetus in question. However, chromosome arm *p* is a small chromosome (2–3 % of the haploid female genome of shrews: SEARLE 1983) and it is possible that trisomy for this element is compatible with survival late into development. In mammals, those autosomal trisomies that survive for a long period postnatally tend to involve small chromosomes (SEARLE 1989).

Fertility of common shrews in relation to karyotype

The limited evidence available suggests that the fertility of common shrews from the Oxford-Hermitage hybrid zone is, on average, similar to that elsewhere in Britain (Table 4). Thus, the data show no substantial difference in number of ovulations, number of healthy embryos or prenatal losses between pregnant females from North Wales (BRAM-

Table 5. Anaphase I nondisjunction frequencies in common shrews, homozygous or heterozygous for Robertsonian rearrangements, from the Oxford-Hermitage hybrid zone and associated area of polymorphism, as determined from prenatal losses[a]

Maternal karyotype	Number of ♀♀	corpora lutea	ovulations accounted for by: no implant	regressing implant	trisomic fetus	diploid fetus	Nondisjunction (%)
Homozygote	10	74	1	3	0	70	0–5.4
Simple heterozygote	17	138	9	4	3	122	4.5–8.3[b]
Complex heterozygote	1	8	0	0	0	8	–

[a] Combines data from the present study and SEARLE (1984)

[b] 'Excess' ovulations are excluded from this estimate (see Table 4)

BELL 1935), an area of karyotypic monomorphism (SEARLE 1988b), and pregnant females from the Oxford-Hermitage hybrid zone (present study and unpublished data) and surrounding area of polymorphism (SEARLE 1984 and unpublished data) in southern England. On average, there were greater prenatal losses in females from the hybrid zone compared with the areas of polymorphism and monomorphism, but this may reflect that the females analysed from the hybrid zone were, generally, at a later stage of pregnancy than those from regions of polymorphism and monomorphism (BRAMBELL 1935; J. B. SEARLE, unpublished data).

Thus, in the Oxford-Hermitage hybrid zone, despite the occurrence of complex Robertsonian heterozygotes (albeit at a low frequency) and simple Robertsonian heterozygotes (at a much higher frequency: Table 1; SEARLE 1986a), the average fecundity of common shrews, as measured by number of healthy embryos per pregnant female, is not obviously lower than in areas of polymorphism (which also have a high frequency of simple heterozygotes: SEARLE 1986a) and monomorphism (where there are only homozygotes). This requires some explanation, given that Robertsonian heterozygotes are expected to suffer higher frequencies of anaphase I nondisjunction than homozygotes.

The previous estimates of anaphase I nondisjunction, deduced by analysis of pregnant females from the region of polymorphism around the Oxford-Hermitage hybrid zone (SEARLE 1984), were made on the assumption of a single male parent per litter. This assumption is now known to be erroneous (SEARLE 1990). Following the same methods as used in this paper (including the assumption that all anaphase I nondisjunction is on the female side and disregarding 'excess' ovulations), anaphase I nondisjunction frequencies are recalculated to be 3.1–4.7 % per heterozygous female or 2.1–3.2 % per heterozygous arm combination for shrews from the region of polymorphism near the Oxford-Hermitage hybrid zone. These are lower estimates than those made on females from the Oxford-Hermitage hybrid zone itself (5.8–11.6 % per heterozygous female and 4.3–8.5 % per heterozygous arm combination). Further sampling is needed to establish whether this difference is real. It could be a reflection of small sample sizes, particularly as there are frequently multiple wasted ovulations within a particular female (Table 1; SEARLE 1984).

The occurrence of substantial prenatal losses in common shrews from areas where Robertsonian polymorphism is absent (Table 4), suggest it is unrealistic to attribute all wasted ovulations to anaphase I nondisjunction resulting from Robertsonian heterozygosity. This is further substantiated by a comparison of prenatal losses between *all* homozygotes and simple Robertsonian heterozygotes from the vicinity of the Oxford-Hermitage hybrid zone (i.e., including the areas of polymorphism: Table 5). Prenatal losses in the homozygotes (5.4 %) are unlikely to be entirely the result of nondisjunction due to Robertsonian heterozygosity on the male side. Anaphase I nondisjunction in male Robertsonian heterozygous common shrews is low (about 1 %: SEARLE 1986b; MERCER and SEARLE, in preparation) and not all offspring will have been sired by Robertsonian heterozygotes. Some prenatal loss in the female homozygotes may be due to anaphase I nondisjunction of bivalents in the female. Other studies in small mammals indicate anaphase I nondisjunction frequencies of 0–5 % in females of standard homozygote karyotype (NIJHOFF and DE BOER 1981). Prenatal losses in homozygous females

could also be due to factors such as maternal physiology, environmental effects, and genotype. The importance of these factors has been well-documented in other species (JACOBS 1982). As illustrated by the clutch of female 1667, growth retardation (and presumably death) may occur in a common shrew fetus that is karyotypically balanced.

Therefore, one would expect a proportion of prenatal losses in female simple heterozygotes to be the result of physiological, environmental, or genic factors. So, the maximum nondisjunction estimate of 8.3 % per heterozygous female (5.8 % per heterozygous arm combination) for females from the vicinity of the Oxford-Hermitage hybrid zone (Table 5) are undoubtedly overestimates. However, a minimum estimate of anaphase I nondisjunction in female single Robertsonian heterozygotes of 4.5 % per heterozygous female (3.2 % per heterozygous arm combination) is more plausible. Altogether, three trisomic fetuses have been found in simple Robertsonian heterozygous females, which can reasonably be ascribed to nondisjunction of a Robertsonian trivalent in the female. On the basis of other fetal karyotypes from the same litters, the trisomic for chromosome arm *p* (in the clutch of shrew 1676) could also plausibly be the product of anaphase I nondisjunction in a male simple Robertsonian heterozygote, the other trisomics, less likely so. The only trisomic that could be the product of nondisjunction of a bivalent (in the male) is that trisomic for chromosome arm *o* in female 1663, but that would be an unlikely coincidence. For each trisomic that results from nondisjunction of a Robertsonian trivalent, one would expect an equal number of monosomics which die early in gestation. Other trisomics may die before fetal analysis.

From these arguments, it is reasonable to suggest an average increased nondisjunction frequency in Robertsonian heterozygote females over homozygotes of 3–5 % per heterozygote female and 2–4 % per heterozygous arm combination, in common shrews from the vicinity of the Oxford-Hermitage hybrid zone. This does not mean that fecundity was lowered by that extent in heterozygous individuals. In fact, there were more corpora lutea in heterozygotes and the number of healthy diploid fetuses produced by homozygotes (mean: 6.9, excluding the small karyotypically-balanced fetus) was very similar to simple heterozygotes (mean: 7.2). This tendency for a greater number of ovulations in female simple Robertsonian heterozygotes than in homozygotes has an analogy in the male, in which heterozygotes tend to have larger testes than homozygotes, which may compensate for greater losses of germ cells at meiotic and postmeiotic stages (GARAGNA et al. 1989). These possible examples of reproductive compensation are of interest as they may come about in response to natural selection and may reflect greater genic heterozygosity in simple Robertsonian heterozygotes relative to homozygotes (SEARLE 1988a).

Towards an understanding of the Oxford-Hermitage hybrid zone

The relative fertility of simple Robertsonian heterozygotes and homozygotes must have a major influence on the width of the area of Robertsonian polymorphism around the Oxford-Hermitage hybrid zone (SEARLE 1986a). The less fertile the heterozygotes, the narrower this region is expected to be. Taking the data gathered here together with estimates of vagility in the common shrew (J. B. SEARLE and A. J. REILLY, unpublished data) it will be possible to compare the observed features of the region around the Oxford-Hermitage hybrid zone with the expectations from theoretical models (BARTON and HEWITT 1985; BARTON and BENGTSSON 1986).

Information on the fertility of complex Robertsonian heterozygotes is also crucial to our understanding of the Oxford-Hermitage hybrid zone, but unfortunately such information is lacking. The single pregnant female examined that was a complex Robertsonian heterozygote (1699) had a normal litter. However, germ cell losses tend to be greater and testis weights lower in complex Robertsonian heterozygote males (GARAGNA et al. 1989). Where complex Robertsonian heterozygotes occur there are high frequencies of individuals homozygous acrocentric for chromosome arms *k, n, o, q* (SEARLE 1986a). These high frequencies may reflect selection against complex Robertsonian heterozygotes because of reduced fertility (SEARLE 1986a). However, my results tentatively suggest that they may also partly reflect greater viability of 'all-acrocentric' individuals compared to that of other karyotypic categories. In further support of this idea, the frequency of all acrocentric individuals among immatures from the hybrid zone site of Eling was noticeably (but not significantly) higher than that of the Hardy-Weinberg expectation (SEARLE 1986a). Clearly, further sampling of shrews from the vicinity of the Oxford-Hermitage hybrid zone is required for clarification.

There is a wide range of karyotypes in the vicinity of the Oxford-Hermitage hybrid zone (Table 1;

SEARLE 1986a). To date, this has been considered to reflect approximately random union of gametes within sites in a region where several chromosomes have variant forms. The data presented here on the lack of assortative mating and fertilisation, support this interpretation of panmixis within sites.

In conclusion, the Oxford-Hermitage hybrid zone and associated area of polymorphism is dominated by individuals which are homozygotes and simple Robertsonian heterozygotes. The fertility reduction due to Robertsonian heterozygosity is small, and there is likely to be little interruption of gene flow between the Oxford and Hermitage races in our primary study area.

Relevance to other organisms

The present study together with those of SEARLE (1984, 1986a) and GARAGNA et al. (1989) show that, compared to homozygotes, common shrews that are simple Robertsonian heterozygotes have normal fertility. This is in sharp contrast to the high nondisjunction frequencies and significant germ cell losses often recorded in laboratory-reared Robertsonian heterozygote house mice (reviewed in GROPP and WINKING 1981; REDI and CAPANNA 1988; HANSMANN et al. 1988). Such mice usually have a predominantly laboratory mouse genetic background, and are, to an extent, hybrids between feral mice (from whom the Robertsonian metacentric derives) and laboratory mice; they have not been subject to selection pressures, which may be expected to minimise fertility problems. In contrast, wild-caught mice which are Robertsonian heterozygotes have very low frequencies of anaphase I nondisjunction (WINKING 1986; WINKING et al. 1988; J. B. SEARLE, unpublished data). Wild lizards, heterozygous for Robertsonian rearrangements, also exhibit low frequencies of anaphase I nondisjunction (PORTER and SITES 1985). Therefore, if races of other species differ by Robertsonian rearrangements, such that simple Robertsonian heterozygotes are formed, there may be a rather small reduction in fertility associated with the Robertsonian heterozygosity.

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