*Heredity* **84** (2000) 570–577 *Received 21 June 1999, accepted 16 November 1999*

# A comparison of spermatogenesis in homozygotes, simple Robertsonian heterozygotes and complex heterozygotes of the common shrew (*Sorex araneus L.*)

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Spermatogenesis was studied in 56 shrews (*Sorex araneus* L.) from two chromosomal hybrid zones in Poland. The hybrid zones were formed between chromosome races that differed in Robertsonian metacentrics. Shrews were compared in four classes: homozygotes, simple Robertsonian heterozygotes, complex heterozygotes forming four-element rings in meiosis I, and complex heterozygotes forming four- or five-element chains. There was a significant effect of karyotype on the level of germ-cell death and chain-forming complex heterozygotes suffered the greatest germ-cell loss. However, the estimated level of germ-cell death is probably insufficient to influence the fertility of these males.

**Keywords:** chromosome races, common shrew, complex heterozygotes, hybrid zone, *Sorex araneus*, spermatogenesis.

## Introduction

The common shrew (*Sorex araneus* L. 1758) shows exceptional chromosome variation at both the intra- and interpopulation level. The variability of the common shrew karyotype is based on the occurrence of Robertsonian (centric) fusions and whole-arm reciprocal translocations (see Searle, 1993). Interpopulation chromosome variation manifests itself as the presence of chromosome races, that differ either in the chromosome arm combination of the metacentrics, or the number of these metacentrics relative to the ancestral acrocentric complement (for the list of races see Zima *et al*., 1996). Metacentrics specific to neighbouring races may show monobrachial homologies relative to one another (i.e. metacentrics have one arm in common). In consequence, interracial hybridization gives rise to hybrids that form chromosome multivalents at prophase I of meiosis. Chromosome multivalents consisting of meta- and acrocentrics form chain (CH) configurations, whereas ring (R) configurations are formed by the metacentrics only.

It is commonly thought that a balance between dispersal and selection against hybrids maintains chromosomal hybrid zones. Selection against hybrids results from their lowered fertility caused by the presence of atypical configurations at meiosis I. Meiotic disorders, such as a failure of homologous chromosomes to pair accurately at prophase I and an increased level of nondisjunction at anaphase I, may lead to the greater germ-cell death and postzygotic loss of aneuploid embryos. Pairing irregularities may lead to germ-cell death as a result of inappropriate expression of genes at unpaired regions of autosomes (Miklos, 1974; Burgoyne & Baker, 1984) or as a consequence of interactions between the sex chromosomes in males and unsynapsed regions of autosomes (Forejt, 1984).

Although the lower fitness of hybrids may be inferred from the structure of many chromosomal hybrid zones (see Searle, 1993; Searle & Wójcik, 1998), direct data on the fertility of hybrids of the common shrew are still scarce.

This paper presents results of studies on spermatogenesis in male common shrews aimed to establish the relationship between level of germ-cell death and karyotype. The males come from two hybrid zones: one between the Drnholec (Dn) and Łęgucki Młyn (Łg) chromosome races and another between the Guzowy Młyn (Gu) and Łg races. The Dn/Łg hybrid zone was described incorrectly as the Stobnica/Łg hybrid zone

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(Fedyk *et al*., 1991; Fedyk, 1995). The change in race classification is caused by a mistake in chromosome arm identification and the incorrect description of chromosome *nr* specific for the Dn race as chromosome *np* (Fedyk *et al*. unpubl. data). The Gu/Łg hybrid zone was initially described as the Drużno/Łg hybrid zone (Banaszek, 1994). The analysis of a larger data set showed that pure populations of the Drużno race were absent from the hybrid zone and that they were replaced by populations of the Gu race, a karyotypic form of hybrid origin (Banaszek, 1997).

Both hybrid zones are different sections of the same contact zone between the races belonging to the West European karyotypic group (WEKG) and the East European karyotypic group (EEKG) that come into contact in north-eastern Poland (Fedyk, 1995).

## Materials and methods

### *The animals*

Samples were collected between June and July from 1986 to 1995 in the Dn/Łg hybrid zone, and in 1992–95 in the Gu/Łg hybrid zone. Fifty-six males were used for detailed analysis of spermatogenesis: 29 from the Dn/Łg hybrid zone, and 27 from the Gu/Łg hybrid zone. In addition, as an indicator of spermatogenic performance, the combined testis weight was analysed in 78 karyotyped males (33 from the Dn/Łg and 45 from the Gu/Łg hybrid zone). The correlation between testis weight and body mass was analysed for a bigger sample of 164 karyotyped and unkaryotyped shrews from these hybrid zones. The samples used for spermatogenesis and for testis weight analyses overlap only in the case of 27 males from the Gu/Łg hybrid zone.

The animals were killed and dissected directly after capture. Both testes were weighed to 0.001 g accuracy and the left testis was fixed in Bouin's solution. As there were no differences in the mean weights of the left and right testes ($t_{137} = 0.463$, $P = 0.644$), in some cases, where only one testis was weighed, its weight was doubled for the statistical analysis. The animals were weighed to 0.1 g accuracy. The karyotypes of all individuals were determined from routine spleen preparations (Fedyk, 1980), stained by a G-banding method (Seabright, 1971).

The animals in both samples were subdivided into four categories on the basis of chromosome pairing at meiosis I: homozygotes (bivalents only, except for the sex chromosome trivalent formed by multiple sex chromosomes $XY_1Y_2$), simple Robertsonian (Rb) heterozygotes (one, two or three autosomal trivalents each formed by a metacentric and two homologous acrocentrics), RIV-forming hybrids (ring configuration of four metacentrics) and CHV-forming and CHIV-forming hybrids (chain configuration of three meta- and two acrocentrics and a chain configuration of two meta- and two acrocentrics, respectively). In the common shrew's karyotype the chromosome arms are described by letters according to their length from *a* (the longest) to *u* (the shortest) (Searle *et al*., 1991). Polymorphic chromosome pairs are labelled with a slash (e.g. *m*/*n*) following the nomenclature given by Searle *et al*. (1991). Homozygotes and simple Rb heterozygotes belong to the Dn race (specific metacentrics: *hi, ko, g*/*m, n*/*r*), Łg race (*hk, io, g*/*r, m*/*n*) and Gu race (*hi, k*/*o, g*/*r, m*/*n*). Individuals with the Gu race karyotypes are also present in the Dn/Łg hybrid zone; however, they do not form pure populations there and are classified as recombinants. Recombinants are individuals of mixed origin with the metacentrics *hi, ko* derived from the Dn race and *gr, mn* from the Łg race (Fedyk *et al*., 1991). All the possible types of chromosome complexes are given in Appendix 1. The chromosome composition of complexes in shrews used in this study is the following. The ring (RIV) configuration consists of the same four metacentrics (*kh*/*hi*/*io*/*ok* — $C_1$ complex) in both hybrid zones. The chain configurations consist of four (CHIV: *r*/*rg*/*gm*/*m* or *g*/*gm*/*mn*/*n* or *m*/*mn*/*nr*/*r* — $C_2$ complex) and five elements (CHV: *r*/*rg*/*gm*/*mn*/*n* — $C_2$ complex or *o*/*ok*/*kh*/*hi*/*i* — $C_1$ complex) in the Dn/Łg hybrid zone. In the Gu/Łg hybrid zone, chain configurations consist of five elements (CHV: *k*/*kh*/*hi*/*io*/*o* or *o*/*ok*/*kh*/*hi*/*i* — $C_1$ complex).

### *Histology and cell counting*

A standard histological protocol was adopted to obtain 10 $\mu$m paraffin sections. The preparations were stained with haematoxylin and eosin or by the periodic acid–Schiff reaction and counterstained with haematoxylin. The H + E stained preparations were used to determine the presence or absence of spermatids or spermatozoa in seminiferous tubules. Two perpendicular diameters were measured in 10 seminiferous tubule cross-sections on H + E preparations and used for the calculation of mean tubular cross-sectional area for each individual. A video-computer system with frame grabber and CSS software were used for the measurements. The preparations stained by the PAS technique were used for cell scoring at three stages of the seminiferous epithelium cycle.

Following Mercer *et al*. (1992) for detailed analysis, the most easily recognized and very characteristic stages II, VI and VIII of the 10-stage seminiferous epithelium cycle (Garagna *et al*., 1989a) were chosen. Primary pachytene spermatocytes (sc) and round spermatids (sd) were scored at stages II and VI. Only primary spermatocytes were scored at stage VIII, because spermatids

are elongated and difficult to count at this stage. The cells were scored in five cross-sections of each stage and the Abercrombie (1946) correction was applied. One person made all measurements and counts.

For each individual a mean sc:sd ratio was calculated. If spermatogenesis is not distorted, one would expect a sc:sd ratio of 1:4. Therefore, the sc:sd ratio provides information on germ-cell death during the first meiotic division. The sc:sd ratio was also calculated for separate tubule cross-sections of each individual and the proportion of tubules with substantial germ-cell death was estimated. After Garagna *et al.* (1989b) the arbitrary limit of the sc:sd ratio less than 1:3 was accepted for 'defective' tubules.

### *Statistics*

The effects of karyotype, date (months of the breeding season) and place of capture (the hybrid zones Gu/Łg and Dn/Łg) were determined by single analysis of variance (ANOVA) and covariance (ANCOVA). Interaction terms (between factors and between factors and covariate) were also tested. As these proved to be statistically nonsignificant, the models without interactions were used. *Post-hoc* comparisons within sets of means were performed by the protected least-significant difference test (LSD). The means are given with standard errors (±SE). Prior to the analysis of variance the data sets were tested for normality with the Kolmogorov–Smirnov test for goodness of fit. All the data sets fitted a normal distribution. The homogeneity of variances was checked by Bartlett's test.

## Results

The effects of three factors on the combined testis weight were tested: karyotype, the place of capture (the Dn/Łg and Gu/Łg hybrid zones) and month of capture (June and July) by three-way ANCOVA with body mass as covariate ($n = 78$). Testis weight was correlated with body mass ($r = 0.296$, d.f. $= 163$, $P < 0.001$). There were no significant effects of any factor on testis weight. As the effect of karyotype on testis weight was of primary interest, it was additionally tested by single ANCOVA with body mass as covariate; no significant effect of karyotype on testis weight of individuals belonging to different karyotypic groups was found ($F_{3,73} = 0.517$, $P = 0.672$). Testis weight did not decrease with increasing karyotype complexity (Fig. 1). Also testis weight was not correlated with the sc:sd ratio (in males from the Gu/Łg hybrid zone, $r = 0.229$, d.f. $= 26$, $P = 0.251$).

General examination of seminiferous tubules indicated that in all males spermatogenesis proceeded without major disruption and spermatozoa (stages I–V of seminiferous epithelium cycle) or spermatids (stages VI–X) were present in all tubules.

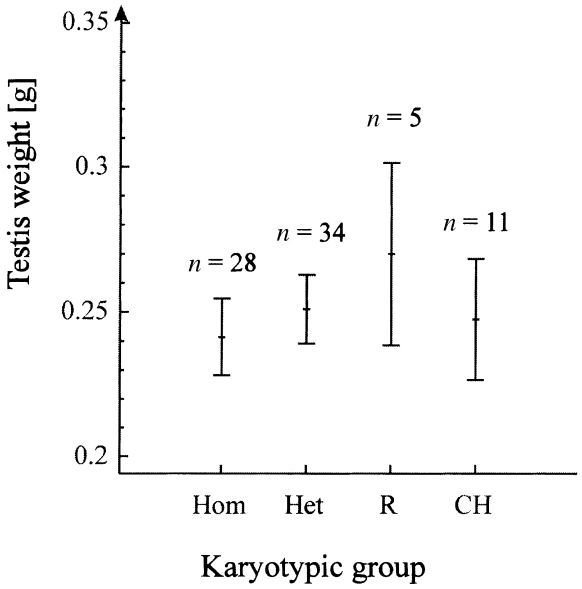


**Fig. 1** Testis weight (±SE) in homozygous (Hom), simple Rb heterozygous (Het), ring-forming (R) and chain-forming (CH) complex heterozygous male *Sorex araneus* from the Dn/Łg and Gu/Łg hybrid zones.

There were no significant differences in the mean area of tubule cross-section of individuals belonging to different karyotypic groups and caught in different months of the breeding season (ANOVA; $F_{3,50} = 0.640$, $P = 0.593$; $F_{1,50} = 0.183$, $P = 0.676$, respectively). However, the mean area of tubule cross-section in males from the Dn/Łg hybrid zone ($1.096 \pm 0.031$ mm$^2$) was significantly greater than in males from the Gu/Łg hybrid zone ($0.960 \pm 0.037$ mm$^2$) (ANOVA; $F_{1,50} = 7.407$, $P = 0.009$). To compare individuals from different hybrid zones the numbers of primary spermatocytes and spermatids were recalculated per 100 $\mu$m$^2$ area (Table 1) and the sc:sd ratio was determined.

The effect of karyotype on the sc:sd ratio was tested by two-way ANOVA in 56 males collected in both hybrid zones in June and July; in these months the sc:sd ratio did not vary significantly (ANOVA; $F_{1,54} = 2.028$, $P = 0.160$). There was a significant effect of karyotype and place of capture on the sc:sd ratio (ANOVA; $F_{3,51} = 7.262$, $P < 0.001$; $F_{1,51} = 17.978$, $P < 0.001$, respectively). Chain-forming hybrids had a sc:sd ratio lower than homozygotes and simple Rb heterozygotes (Fig. 2); this was significant with the LSD test. The sc:sd ratio of chain-forming hybrids was also lower than that of ring-forming hybrids, but this difference was not statistically significant. Ring-forming hybrids had a sc:sd ratio significantly lower than simple Rb heterozygotes. In

**Table 1** Mean numbers of primary spermatocytes (sc) and spermatids (sd) per 100 $\mu m^2$ of cross-section of seminiferous tubule and proportion of 'defective' tubules (±SE) in male *Sorex araneus* belonging to different karyotypic groups from the Dn/Łg and Gu/Łg hybrid zones

| | Dn/Łg hybrid zone | | | | Gu/Łg hybrid zone | | | |
|---|---|---|---|---|---|---|---|---|
| Karyotypic group | *n* | sc number* | sd number* | 'Defective' tubules [%] | *n* | sc number* | sd number* | 'Defective' tubules [%] |
| Hom | 7 | 39.36 ± 4.75 | 132.51 ± 13.80 | 8.6 ± 7.2 | 5 | 51.16 ± 5.62 | 167.71 ± 16.32 | 22.0 ± 8.5 |
| Het | 12 | 40.10 ± 3.62 | 142.83 ± 10.54 | 11.7 ± 5.5 | 12 | 54.06 ± 3.63 | 168.99 ± 10.54 | 32.5 ± 5.5 |
| RIV | 6 | 39.10 ± 5.13 | 127.71 ± 14.90 | 11.7 ± 7.8 | 4 | 52.93 ± 6.28 | 160.74 ± 18.25 | 45.0 ± 9.5 |
| CHIV and CHV | 4 | 39.18 ± 6.28 | 119.96 ± 18.25 | 37.5 ± 9.5 | 6 | 53.58 ± 5.13 | 152.26 ± 14.90 | 61.7 ± 7.8 |

*The difference in mean numbers of primary spermatocytes and spermatids between the males coming from different hybrid zones results from the recalculation of the germ cell numbers per 100 $\mu m^2$ area of cross-section of seminiferous tubule.

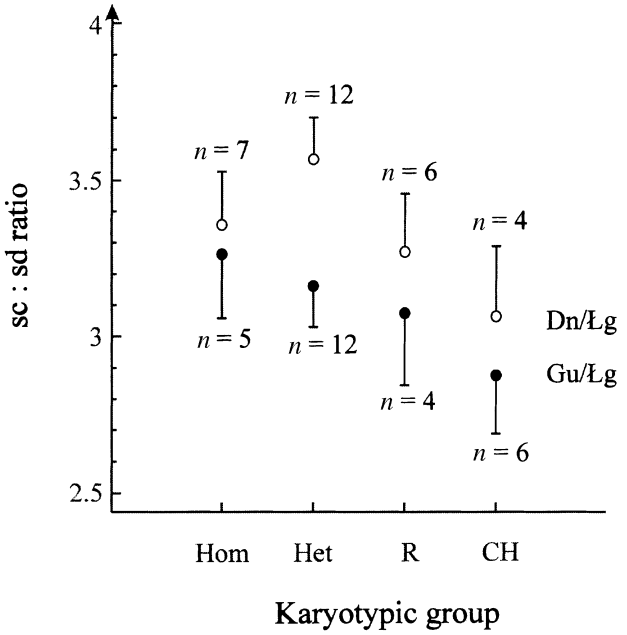


**Fig. 2** A comparison of the mean sc:sd ratio (±SE) in male *Sorex araneus* of the same karyotypic group — homozygotes (Hom), simple Rb heterozygotes (Het), ring-forming hybrids (R) and chain-forming hybrids (CH) — from the Dn/Łg and Gu/Łg hybrid zones.

general, all males from the Dn/Łg hybrid zone had higher sc:sd ratios (3.31 ± 0.04) than males caught in the Gu/Łg hybrid zone (3.06 ± 0.05). Nevertheless, the tendencies were similar in both zones; the sc:sd ratio decreased from values characteristic for individuals of pure races (homozygotes and simple Rb heterozygotes) through ring-forming hybrids to the lowest values in chain-forming hybrids (Fig. 2). The only clear difference between the hybrid zones was that simple Rb heterozygotes had a higher sc:sd ratio than homozygotes in the Dn/Łg hybrid zone, whereas in the Gu/Łg hybrid zone homozygotes had the highest sc:sd ratio.

The proportion of 'defective' tubules was closely correlated with the sc:sd ratio ($r = 0.823$, d.f. = 55, $P < 0.001$). Likewise the proportion of 'defective' tubules varied significantly with karyotype and the place of capture (ANOVA; $F_{3,51} = 6.806$, $P < 0.001$; $F_{1,51} = 19.028$, $P < 0.001$, respectively). Chain-forming hybrids had the highest proportion of 'defective' tubules (Table 1) and differed significantly from individuals of other karyotypic groups. The differences between homozygotes, simple Rb heterozygotes and ring-forming hybrids were not statistically significant. However, all males from the Gu/Łg hybrid zone had more 'defective' tubules (39.8%) than males from the Dn/Łg hybrid zone (17.8%).

There was no difference in the sc:sd ratio between the shrews of the Dn, Łg races and recombinants in the Dn/Łg hybrid zone (ANOVA; $F_{2,16} = 0.009$, $P = 0.991$) and between shrews of the Gu and Łg races in the Gu/Łg hybrid zone (ANOVA; $F_{1,15} = 0.346$, $P = 0.571$).

## Discussion

Amongst the shrews from the Dn/Łg and Gu/Łg hybrid zones, there was no evidence that simple Rb heterozygotes had reduced fertility relative to homozygotes. Homozygotes and simple Rb heterozygotes did not differ significantly in their sc:sd ratios (Fig. 2) and proportion of 'defective' tubules (Table 1). The mean sc:sd ratio for simple Rb heterozygotes tended to be even higher, especially in the Dn/Łg hybrid zone, than for homozygotes (Fig. 2). Garagna *et al.* (1989b) also did not find significant differences in the sc:sd ratio between homozygotes and simple Rb heterozygotes collected from the vicinity of the hybrid zone between the Oxford and Hermitage chromosome races in Britain, although simple Rb heterozygotes tended to have lower values of the sc:sd ratio. The lack of clear differences in the level of germ-cell death between homozygotes and simple Rb heterozygotes is not surprising, as in meiosis

of the common shrew trivalents pair very regularly and only 8% of cells show unpairing around the centromere at pachytene (Wallace & Searle, 1990). Moreover, there is no substantial increase in the degree of contact between autosomes and sex chromosome trivalents in heterozygotes over that in homozygotes (Wallace & Searle, 1990).

With regard to sc:sd ratio and proportion of 'defective' tubules, males with chain configurations suffered from the highest germ-cell loss, whereas ring-forming hybrids had an intermediate position between chain-forming hybrids and individuals of pure races (homozygotes and simple Rb heterozygotes) (Table 1, Fig. 2). In general, ring configurations clearly caused less meiotic disturbance than chain configurations. Likewise, Narain & Fredga (1997) failed to find significant differences in the incidence of germ-cell death between ring-forming hybrids (RIV) and homozygotes collected in the hybrid zone between the Uppsala and Hallefors races in Sweden. Also Garagna *et al.* (1989b) found that CHIV-forming hybrids from the Oxford/Hermitage hybrid zone tended to have higher germ-cell loss than homozygotes and simple Rb heterozygotes, although the differences between those karyotypic groups were not statistically significant.

The most important question is, whether the poorer spermatogenic performance of hybrid individuals compared with homozygotes and simple Rb heterozygotes may cause a reduction of their fertility. Garagna *et al.* (1989b), on the basis of sperm-count values, stated that all the males they studied were fully fertile. Although we did not carry out sperm counts, it seems that the level of spermatogenesis disruption was also minimal; in chain-forming hybrids the sc:sd ratio was 2.97 and in ring-forming hybrids it was higher (3.16). This assumption is supported by the lack of clear differences in testis weight between males belonging to different karyotypic groups (Fig. 1). A substantially increased level of germ-cell death should be reflected in lower testis weights, as was found in heterozygous mice *Mus musculus domesticus* (Searle & Beechey, 1974) and common shrews with numerical chromosome aberrations (Searle, 1984; Searle & Wilkinson, 1986). Garagna *et al.* (1989b) found that CHIV-forming hybrids had a significantly lower testis weight than simple Rb heterozygotes. Moreover, the sc:sd ratio and testis weights in hybrids were correlated (Garagna *et al.*, 1989b), although the estimation was based on four individuals and therefore potentially subject to error. We did not find such relationships in males from the Dn/Łg and Gu/Łg hybrid zones. Testis weight did not depend on karyotype (Fig. 1) and was not correlated with the sc:sd ratio. A higher level of germ-cell loss could also influence the cross-sectional area of seminiferous tubules. However, there was no relationship between the area of tubule cross-sections and karyotype. The area of tubule cross-sections was lower in males from the Gu/Łg than from Dn/Łg hybrid zone. This indicates general differences in the spermatogenic performance of males from different areas. All males from the Dn/Łg hybrid zone had significantly higher sc:sd ratios than males caught in the Gu/Łg hybrid zone (Fig. 2). We do not know the reason for this difference. We have no independent evidence of greater genetic differences between the Gu and Łg than between the Dn and Łg chromosome races (see Chętnicki *et al.*, 1996). In this study we found no clear differences in spermatogenic performance between individuals of pure races.

The substantial individual variation of the sc:sd ratio observed by Garagna *et al.* (1989b) prompted them to assume that chain-forming hybrids in the common shrew could have a greater predisposition to sterility, similar to heterozygotes for Rb metacentric t(14;21) in man, where individuals may range from fully fertile to sterile (Rosenmann *et al.*, 1985). Garagna *et al.* (1989b) stated that even a marginally greater proportion of sterile males among hybrids than among individuals of pure races would be sufficient to influence the gene flow between hybridizing races. However, this assumption is weakened by the lack of severe disruption of spermatogenesis in males studied to date.

Thus, the estimated level of germ-cell loss is probably not sufficient to reduce the fertility of hybrids. So it is rather surprising that the presence of chromosomal multivalents in meiosis causes only minor disorders in spermatogenesis of the common shrew. As spermatogenic disorders result from errors in chromosomal pairing (Miklos, 1974; Burgoyne & Baker, 1984; Forejt, 1984), it has to be assumed that chromosome pairing in heterozygous complexes is very regular in the common shrew. Indeed, a direct analysis of chromosome pairing at pachytene and diakinesis supports this assumption. For example, the frequency of univalence scored at diakinesis in complex heterozygotes was very low and there were no univalents from chromosomal complexes, either in CHIV-forming hybrids from the Oxford/Hermitage hybrid zone (Searle, 1986; Mercer *et al.*, 1991), or in CHV-forming hybrids from the Gu/Łg hybrid zone (Banaszek, 1997). Furthermore, the frequency of associations between heterozygous complexes and the sex chromosome trivalent was low (Searle, 1986; Banaszek, unpubl. data). In CHVII-forming hybrids obtained from laboratory crosses of shrews belonging to the Oxford and Aberdeen races, there were only 33% of cells with fully synapsed chain complexes at pachytene whereas at diakinesis just 1.6% of chain configurations showed incomplete synapsis (Mercer *et al.*, 1992). Mercer *et al.* (1992) were not able to substage pachytene spreads accurately, but they suggested that the majority

of cells showing asynapsis became fully synapsed in late pachytene. Moreover, at pachytene and at diakinesis there were only infrequent associations between the sex trivalent and chain VII configurations (13% and 6% of cells, respectively; Mercer *et al*., 1992). In RIV-forming hybrids from the Uppsala/Hallefors hybrid zone, 58% of cells in pachytene showed a broken ring configuration, but as the hybrids did not suffer from any substantial germ cell death, the defective cells must produce completely viable spermatozoa (Narain & Fredga, 1997). Interactions between the ring configuration and sex trivalent were extremely rare and occurred in only 2% of cells (Narain & Fredga, 1997).

Even if chain-forming hybrids finish their breeding season earlier than individuals of pure races, because of the higher level of germ-cell death, it does not have to impair their reproductive success. The majority of births (usually three litters) occur between May and July in Britain (Searle & Stockley, 1994). Under Polish climatic conditions the breeding season may be longer, with more litters (up to five) in some years. However, the reproductive potential of females clearly reduces with age. The numbers of nonreproducing females increase in August, and those that are pregnant have greater foetal loss (Tarkowski, 1957). Thus the reproductive success of an individual probably does not depend on its ability to reproduce at the end of the breeding season.

Therefore, selection against hybrids, especially chain-forming ones, as can be inferred from the chromosomal structure of many hybrid zones in the common shrew (see Searle, 1993; Searle & Wójcik, 1998), is unlikely to be the result of a lowered number of gametes. Likewise the hypothesis of selective advantage of ring-forming hybrids over chain-forming hybrids (Fedyk *et al*., 1991) cannot be supported on the basis of the present paper. Germ-cell loss caused by the presence of chain configurations at meiosis was greater than by ring ones. However, it probably did not influence the fertility of hybrids. It seems that selection against those hybrids can be caused only by the lower quality, not by the smaller numbers, of gametes produced by hybrids. Lower fertility of those males and also possible differences between chain-forming and ring-forming hybrids may result from a higher level of nondisjunction and, as a consequence, a loss of aneuploid embryos.

## Acknowledgements

We are grateful to Dr A. Krasowska, Dr M. Ratkiewicz, A. Busłowska, M. Romanowska and M. Kozioł for their help in field and laboratory work. We thank Dr Marek Konarzewski and Dr Jeremy B. Searle for valuable comments and revising the English style of the manuscript. This work was supported by grants 6P204 034 05 and 6P04C 035 15 of the Polish State Committee of Scientific Research.

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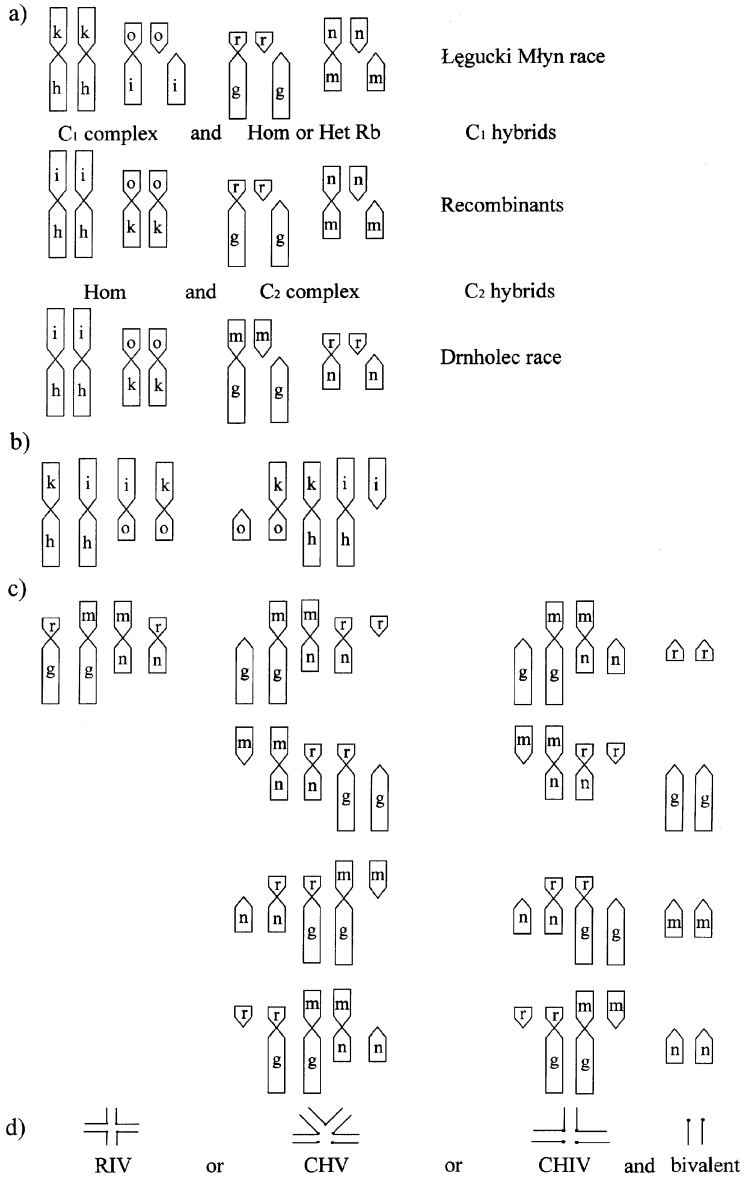
## Appendix 1




The types of hybrids present in the Dn/Łg and Gu/Łg hybrid zones of *Sorex araneus*. (a) Schematic representation of the most common crosses giving rise to hybrids in the Dn/Łg hybrid zone. The individuals of the Dn, Łg race and recombinants are shown as simple Rb heterozygotes with respect to polymorphic pairs of chromosomes. The recombinants are the most common category of individuals in the centre of the hybrid zone. The crosses between recombinants and shrews of the Łg race give rise to hybrids with $C_1$ meiotic complex. $C_1$ hybrids are homozygotes or simple Rb heterozygotes with respect to *gr* and *mn* pairs of chromosomes that are specific for both recombinants and shrews of the Łg race. The crosses between recombinants and shrews of the Dn race give rise to hybrids with $C_2$ meiotic complex. $C_2$ hybrids are homozygotes with respect to *hi* and *ko* chromosome pairs. The crosses between shrews of the Łg and Dn races give rise to hybrids with two independent meiotic complexes $C_1$ and $C_2$. These hybrids are extremely rare in the hybrid zone due to high frequencies of recombinants (Fedyk *et al*., 1991). (b) chromosome composition of $C_1$ complex. (c) chromosome composition of $C_2$ complex. The $C_2$ chromosome complex is highly variable due to polymorphism of all chromosome pairs forming this complex. In the case of CHV and CHIV configurations of $C_2$ complex different chromosome arms may occur as acrocentrics. If the $C_2$ complex is of CHIV configuration, one pair of acrocentrics is excluded from the complex and forms independent bivalent in meiosis I. (d) meiotic configurations of $C_1$ and $C_2$ complexes at pachytene stage: RIV — four-element ring configuration or CHV — five-element chain configuration or CHIV — four-element chain configuration. The chromosomal structure of the Gu/Łg hybrid zone generally corresponds to the part of the Dn/Łg hybrid zone, e.g. the crosses between the recombinants (the Gu race) and shrews of the Łg race. The only difference is the polymorphism of *ko* chromosome pair which results in the presence of one more type of $C_1$ hybrid with CHV configuration — *k*/*kh*/*hi*/*io*/*o* (Banaszek, 1994).

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