Demonstration of a WART in a hybrid zone of the common shrew (*Sorex araneus* Linnaeus, 1758)

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Abstract. Among common shrews from the hybrid zone between the Moscow and Seliger chromosomal races, we found an adult male characterised by two metacentrics not found in either race (*hm, nq*). These metacentrics apparently arose by a whole-arm reciprocal translocation (WART) of two of the Seliger metacentrics (*hn, mq*). Although such rearrangements have been postulated previously to explain racial differences, this is the first direct evidence of a WART in the common shrew. Apart from the unusual metacentrics, the karyotype of the male shrew was that of an F1 hybrid, i.e. this individual was a complex heterozygote that formed a chain-of-11 configuration at meiosis. Regular synapsis was demonstrated at pachytene, complete configurations were scored at diakinesis, and mature sperm were observed within the meiotic preparations.

Key words: WART (whole arm reciprocal translocation), Robertsonian rearrangements, meiosis, common shrew, chromosome races, hybrid zone, *Sorex araneus*, synaptonemal complex.

Introduction

The common shrew (*Sorex araneus* Linnaeus, 1758) is characterised by over 68 chromosome races that hybridise on contact (Wójcik et al., 2003). Chromosomal hybrid zones are important for studying the role of chromosomes in reproductive isolation and race-formation, and the common shrew has become an important model for such studies (Searle, 1993; Searle, Wójcik, 1998).

In order to properly characterise the chromosome complement of the races of the common shrew and their hybrids, nomenclatures have been developed (Searle et al., 1991; Hausser et al., 1994). The variation in the common shrew involves a constant set of chromosome arms, which can either be in an unfused (acrocentric) state or in a fused (metacentric) state. The chromosome arms are labelled by italicised letters of the alphabet, with *a* largest and *v* smallest, and metacentrics are labelled by two letters. The common shrew has invariant sex chromosomes (XX/XY_1Y_2; or *de*, *de/de*, *dv*, *s* in terms of arm labels), and the metacentric autosomes *af*, *bc*, *jl* and *tu* also do not vary or vary little between races. The racial variation that is observed in the common shrew is determined by the chromosome arms *g − i*, *k* and *m − r* which can occur as acrocentrics or fused as 37 different metacentrics (Wójcik et al., 2003).

In this paper, we analyse the chromo-

somal complement of a hybrid between two of the chromosomal races of common shrew, the Moscow and Seliger, which occur in European Russia (Bulatova et al., 2000). It has been well demonstrated that the metacentric condition in the common shrew arises through Robertsonian fusions, i.e. the fusion of two acrocentrics at their centromeres to form a single metacentric (Searle, Wójcik, 1998). However, what this hybrid shows is that such metacentrics, once formed, may evolve into different metacentrics by whole-arm reciprocal translocations (WARTs), i.e. the swapping of arms between two metacentrics or between a metacentric and an acrocentric (Searle, 1993). This has been postulated before to explain karyotypic differences between chromosomal races in the common shrew (Searle, Wójcik, 1998), but the Moscow-Seliger hybrid that we describe provides the first direct evidence for such a rearrangement in this species.

Material and methods

Adult male common shrews were collected from the Moscow-Seliger hybrid zone (Bulatova et al., 2007) at the beginning of the breeding season, in April 2006. Of 16 specimens analysed cytogenetically, the chromosomes of two hybrids have been described previously (Pavlova et al., 2007); here we characterise a third hybrid.

Mitotic G-band chromosome preparations were generated as in Bulatova et al. (2007). One testis was used to make meiotic chromosome preparations in the field by a modified technique without centrifugation (Williams et al., 1971). Both the mitotic and meiotic chromosome preparations were scored with light microscopy. The second testis was taken to the laboratory and prepared by surface-spreading for electron microscopy. Although affected by the time-delay, synaptonemal complexes (SCs) spreads were thus obtained according to

a protocol described by Navarro et al. (1981), stained with a 50% solution of AgNO$_3$ and examined under a JEM100B electron microscope.

Results

Three-hundred-and-two out of 303 common shrews karyotyped since 1997 from the hybrid zone between the Moscow and Seliger chromosomal races have had karyotypes that are pure Moscow, pure Seliger or a mix of these two (Bulatova et al., 2007; Pavlova, 2007). Five metacentrics are diagnostic for the Moscow race (*gm, hi, kr, no, pq*) and four metacentrics (*hn, ik, mq, pr*) and two acrocentrics (*g, o*) characterise the Seliger race (Bulatova et al., 2000); these differences involve the maximum number of chromosome arms for the common shrew.

The one adult male with divergent chromosomes is described below.

Mitotic karyotype. The adult male had 2N=22 and a karyotype with all species-specific chromosome features of the common shrew including the XY1Y2 sex chromosomes (Fig. 1). The shrew was homozygous for four autosomal metacentrics (*af, bc, jl, tu*), and 11 other autosomal elements were singletons (*g – gm – hm – hi – ik – kr – pr – pq – nq – no – o*). Due to the monobrachial homology, this individual was a complex heterozygote (Searle, Wójcik, 1998) expected to form a chain-of-eleven configuration (CXI) at meiosis. The singleton chromosomes included a full set of Moscow race chromosomes (*gm, hi, kr, no, pq*), some of the Seliger race chromosomes (*g, ik, pr, o*; but not *hn, mq*) and two metacentrics previously unseen within the Moscow-Seliger hybrid zone (*hm, nq*).

Meiotic configurations. Electron microscopic examination (Fig. 2, a) revealed the regular formation of SCs along the whole length of the autosomal bivalents (*bc, af, tu,*

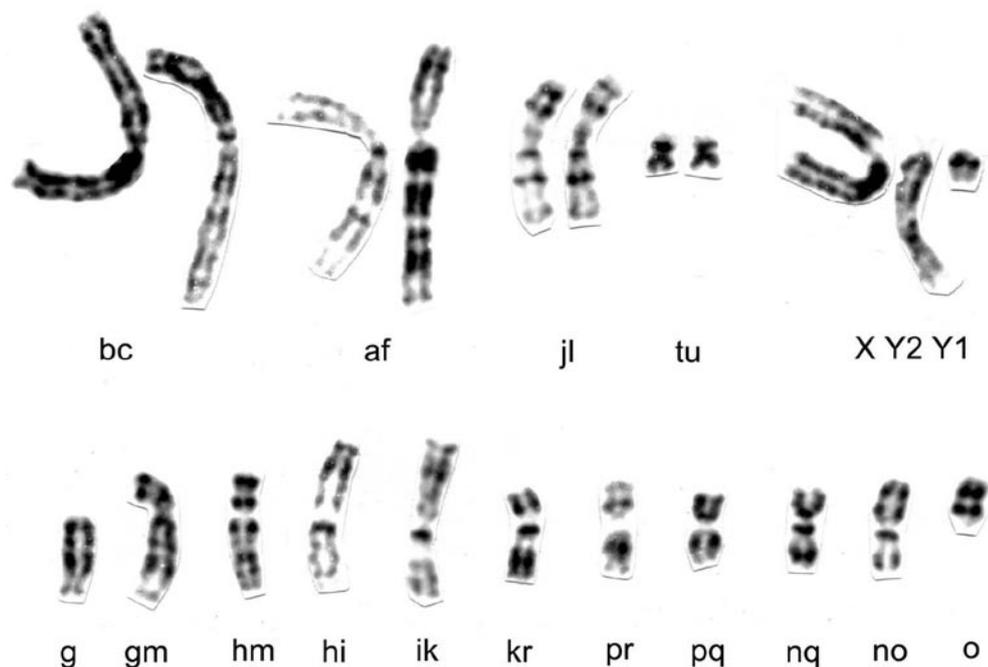**Fig. 1.** G-banded karyotype of the male common shrew under study from the Moscow – Seliger hybrid zone (see text for nomenclature).

jl) and sex trivalent (XY1Y2) without visible regions of asynapsis (Fig. 2, b). The full chain configuration composed of 11 elements (CXI), as predicted from the mitotic karyotype, can be seen in Fig. 2, c (schematically in Fig. 2, d).

The diakinesis/metaphase I spreads of the adult male also displayed a long chain configuration together with four autosomal bivalents and the sex trivalent (Fig. 3, a), as expected from the mitotic karyotype. A meiotic chain was observed in all 57 diakinesis/metaphase I spreads scored, none of which showed autosomal or sex chromosome abnormalities. MII spreads were only rarely observed in the preparations, but mature sperm were frequently seen (Fig. 3, b).

Discussion

The common shrew *S. araneus* is a widely distributed Eurasian small mammal with phenomenal chromosomal variation involving the chromosome arms *g − i*, *k* and *m − r*. Altogether 37 different metacentrics comprising these chromosome arms have been recorded hitherto; these characterise 68 different chromosomal races (Wójcik et al., 2003). Here, a previously unknown metacentric, *hm*, has been found in a single individual. This adds to five other metacentrics involving the chromosome arm *h*: *hi*, *hk*, *hn*, *ho*, *hq* (Wójcik et al., 2003).

The chromosomal complement of the specimen studied included a full set of Moscow race metacentrics (*gm*, *hi*, *kr*, *no*, *pq*) and some of the Seliger race chromosomes (*ik*, *pr*, *g*, *o*) and two extra metacentrics (*hm*, *nq*). As described above, the *hm* composition is unique, and *nq* is only known from two geographically distant chromosomal races of the common shrew: Åkarp (Sweden) and Ilga (Siberia) (Wójcik et al., 2003). Thus, there are no grounds to believe that the *hm* and *nq* meta-
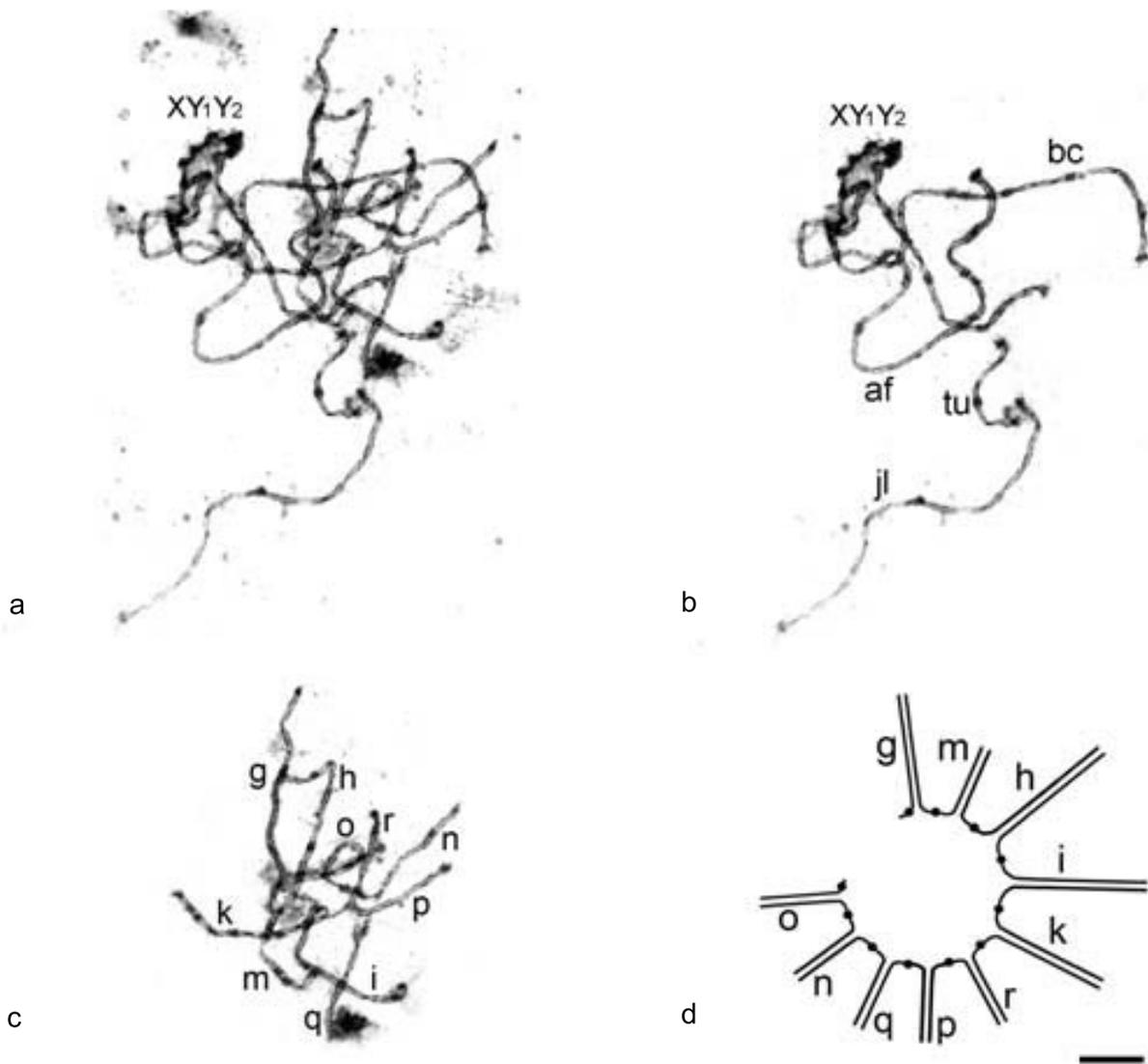
Fig. 2, a-d. Surface-spread synaptonemal complexes from a pachytene spermatocyte of the complex heterozygous common shrew under study. **a** - total complement. **b** - chromosomes not involved in the chain-of-eleven configuration. **c** - chain-of-eleven configuration with best-estimate labelling of chromosome arms. **d** - schematic representation of chain-of-eleven configuration. Images **b** and **c** were generated by computer manipulation. Bar = 1 μm.

centrics derive from another race. Instead they are most likely to have arisen from a WART between the metacentrics *hn* and *mq* of the Seliger race. Interestingly, WARTs have also been described from hybrid zones of the western house mouse *Mus musculus domesticus* (Capanna, Redi, 1995; Castiglia, Capanna, 1999), and from laboratory-reared hybrids (Catalan et al., 2000) and it has been noted there may be a predisposition for such mutations where there is hybridisation (Castiglia, Capanna, 1999; Catalan et al., 2000).
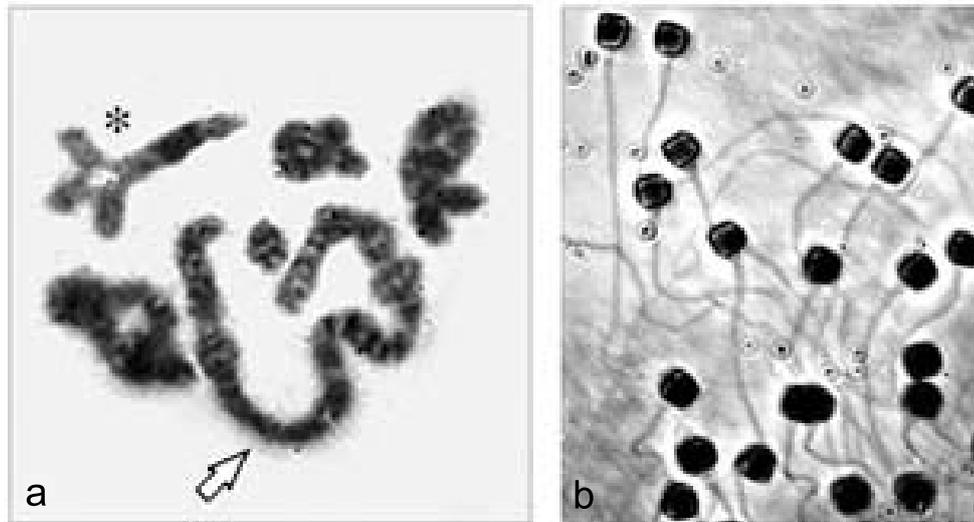
Fig. 3, a, b. a - Diakinesis/metaphase I spread and **b -** group of sperm from meiotic slides of the complex heterozygous common shrew under study. Arrow indicates the chain-of-eleven configuration. Asterisk indicates sex trivalent.

The specimen characterised by the WART-derived chromosomes was also a complex heterozygote with the maximum length of chain configuration for the common shrew (chain-of-eleven). This individual thus had similarities with true F1 hybrids between the Moscow and Seliger races, which likewise are expected to produce a CXI configuration at meiosis. As with these true F1 hybrids (Bulatova et al., 2007; Pavlova et al., 2007), chromosome pairing and chiasma formation appear to progress adequately such that long chain configurations without univalency are observed at diakinesis/metaphase I, and mature sperm are produced (see Searle, 1993). Data for male complex heterozygous shrews forming CIV/CV configurations (Fedyk et al., 2000, 2005; Narain, Fredga, 1998), CVII configurations (Mercer et al., 1992) and CX configurations (Szałaj et al., 1996; Jadwiszczak, Banaszek, 2006) also indicate that chain configurations in the common shrew are not associated with complete meiotic arrest. This contrasts with the house mouse where such arrest appears much more likely in the presence of moderate or long meiotic chain configurations.

In conclusion, a single adult male from the Moscow-Seliger chromosomal hybrid zone has provided an important demonstration of a WART in the common shrew and further confirmation that long meiotic configurations in this species do not have as severe an effect on meiotic progression as might have been expected.

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