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Invalidation of Stobnica chromosome race of the common shrew *Sorex araneus*

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In Central Poland, two similar chromosome races of the common shrew *Sorex araneus* Linnaeus, 1758 were earlier described: Drnholec race (arm combinations *gm, hi*, *ko*, *nr*) and Stobnica race (*gm*, *hi*, *ko*, *np*). Great similarity in size and G-banding patterns between the *nr* and *np* metacentrics leave open to doubt the actual existence of both races in Poland. The present study, which is based on good quality karyotypes of common shrews from 18 sites, showed the presence of the *nr* arm combination. There is therefore strong evidence that the *np* arm combination was wrongly described and thus the Stobnica race should not be considered valid.

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Introduction

Great polytypic variation of chromosomes exists in the common shrew *Sorex araneus* Linnaeus, 1758 and eleven out of 68 chromosome races have been found in Poland (Wójcik *et al*. 2003). The autosomes show brachial homologies within the whole range of the species, and consequently G-banding patterns permit discrimination between each chromosomal arm in different races (Searle *et al*. 1991). The bigger arms (labeled by letters *a* – *l*) can be easily distinguished, contrary to the series of shorter arms (*m* – *s*), which were erroneously identified in some races. For instance, in Finland the arms *m* and *o* were confused (Halkka *et al*. 1974, 1994). Likewise near Moscow, two chromosomal races of the common shrew were described: Zvenigorod race (arm combinations *gm*, *hi*, *kp*, *no*, *qr* – Ivanitskaya 1986) and Moscow race (*gm*, *hi*, *kr*, *no*, *pq* – Aniskin and Lukianova 1989). Re-examination of the shrews from Zvenigorod showed the presence of the Moscow race (Kozlovsky *et al*. 2000).

Two similar races of *S*. *araneus* were found in central Poland. Shrews caught in Stobnica were the basis for description of fusions *gm*, *hi*, *ko*, *np* (Wójcik 1986). This arm composition – known as the Stobnica race or race II – was found in some sites in Poland: in Lidzbark and Marózek (Fedyk 1986, Fedyk and Leniec 1987) in several points of contact between the West European (WEKG) and the East European (EEKG) karyotypic groups (Fedyk *et al*. 1991, 1993, Fedyk 1995, Szałaj *et al*. 1995), as well as in east edge of WEKG range in Dziekanów Leśny, Pułtusk, Kołodziąż, Wilga and Wilczyska (Wójcik 1993). The Drnholec race, first described in Moravia (Czech Republic), is characterised by arm combination *gm*, *hi*, *ko*, *nr* (Zima and Král 1985). The presence of the Drnholec race was confirmed in five populations in southern Poland (Wójcik 1993). The arms *p*, *r* and *s* (= Y1) have the main dark band close to the centromere and can be most difficult to distinguish (Searle *et al*. 1991). Meiotic study of hybrids that occurred in four places in eastern Poland along a 200 km of contact zone showed beyond any doubt that the Drnholec and not the Stobnica race come into contact with the races of EEKG (Fedyk *et al*. 2000). However, that study was insufficient to nullify the Stobnica race in Poland. Some earlier published karyotypes suggested the presence of the *np* fusion (see, for example, Wójcik 1986, 1993). So, the provisional range of the Stobnica race, limited to the area close to the type locality, was maintained (Fedyk *et al*. 2000). On the other hand, lack of *np* fusion out of Polish/German border-line, and presence of *nr* combination in the vicinity of Dresden (Brünner *et al*. 2002) are further arguments against the presence of Stobnica race in Poland.

The aim of the present paper is to examine chromosomes of the common shrew trapped at Stobnica village, as well as those caught from several populations within the provisional range of the Stobnica race. We also re-examined the material earlier gathered from populations formerly attributed to the Stobnica race.

Table 1. Data on karyotypes of the common shrew examined (and re-examined) from different localities in Poland. ? indicates uncertain attribution. * Shrews with *n, p, r* acrocentrics only were not taken into account. ** Material from the papers by: a – Wójcik 1986, b – Wójcik 1993, c – Fedyk 1986, d – Fedyk and Leniec 1987, e – Fedyk *et al*. 1991, f – Szałaj *et al*. 1995, g – Fedyk 1995, h – Fedyk *et al*. 1993.

Sites	Geographic coordinates		Sample size*		Number of spreads classified as		
			Homoz.	Heteroz.	*nr*	*np*	?
1. Stobnica	52°42'N	16°36'E	2	3	16	0	5
2. Piotrowo	52°43'N	16°29'E	–	3	11	0	1
3. Popławy	52°41'N	21°06'E	6	–	19	2	7
4. Ruda Tarnowska	51°41'N	21°27'E	2	–	7	0	0
5. Klonowo	53°14'N	19°45'E	1	5	13	0	3
6. Wlecz	53°15'N	19°43'E	1	1	5	1	1
7. Czarny Bryńsk	53°12'N	19°44'E	–	3	9	0	4
8. Łyna	53°26'N	20°24'E	–	1	6	0	0
9. Selwa	53°31'N	20°26'E	–	1	4	1	1
Subtotals			12	17	90	4	22
Re-examined karyotypes**							
1. Stobnica[a, b]	52°42'N	16°36'E	1	1	3	1	1
10. Dziekanów Leśny[b]	52°20'N	20°50'E	–	1	1	1	1
11. Lidzbark[c, d]	53°16'N	19°49'E	–	1	4	0	2
12. Mańki[e, f, g]	53°39'N	20°15'E	–	5	17	3	5
13. Mycyny[e, f, g]	53°38'N	20°16'E	–	2	7	2	3
14. Jagiełek[e, f, g]	53°36'N	20°13'E	–	2	5	0	1
15. Sokołówek[h]	52°28'N	21°11'E	3	1	9	2	3
16. Dręszew[h]	51°31'N	21°17'E	7	–	12	0	3
17. Słopsk[h]	52°31'N	21°20'E	4	–	9	1	1
18. Niegów[h]	52°30'N	21°23'E	1	–	3	0	0
Subtotals			16	13	70	10	20
Totals			28	30	160	14	42

Material and methods

Fifty-eight individuals of the common shrew coming from 18 sites in Poland were used for chromosome analysis. Among these the *np* arm combination was earlier found in 10 sites (see re-examined karyotypes in Table 1). Five shrews were trapped again at Stobnica. These specimens, together with 3 shrews from Piotrowo (8 km from Stobnica village) were the basic material for decision whether *np* arm combination actually exists in western and central Poland.

Direct chromosome preparations were made from spleen cells using the G-banding method after Seabright (1971). Discrimination between *nr* and *np* metacentrics was performed on the basis of two criteria: (a) – chromosome arm *p* is larger than arm *r*, and (b) – main dark band on *p* is stronger, wider, and closer to the centromere than that found on *r* (Searle *et al*. 1991). These patterns are not always clearly expressed, particularly in spreads with shortened chromosomes. On the other hand, acrocentrics *p* and *r* may have different appearance than arms *p* and *r* in metacentric chromosomes. Accordingly, we used comparative karyotypes of the shrews from northern Moravia (Czech Republic) and from Valöy (Norway), which represent the Drnholec and Sidensjö races, respectively (*nr* fusion), as well as karyotypes of shrews from Arendal (Norway) (Arendal race *np* fusion).

Results and discussion

Thirty-three metaphase spreads with chromosomes of good quality and clear banding pattern coming from eight specimens caught in Stobnica and Piotrowo were selected for analyses. In 27 cases, we found unequivocal *nr* combinations, that is, the arm *r* was shorter than the acrocentric *p*, and *q* chromosome with the dark band actually on the centromere point, which easily permitted discrimination from *r* and *p* (Fig. 1a). However, on other chromosome

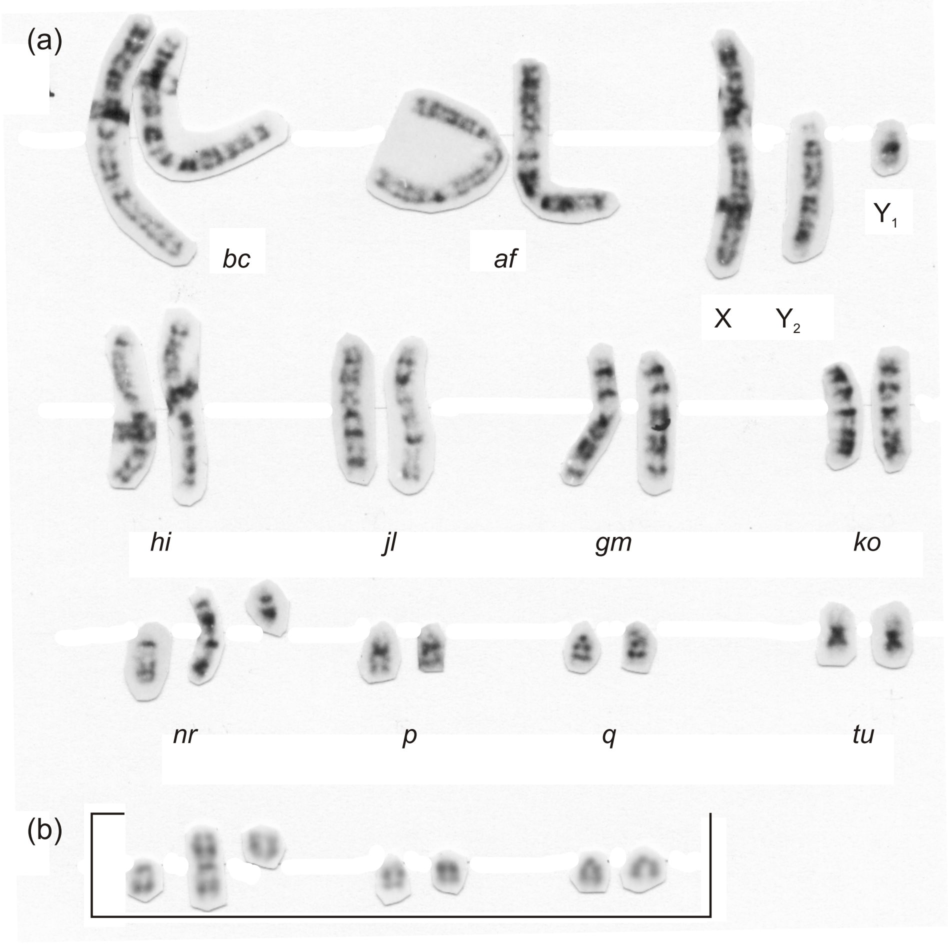

Fig. 1. G-banded karyotype of the common shrew from Stobnica (a), and partial karyotype of the same specimen (b), demonstrating the difficulties to identify arms *p* and *r*.

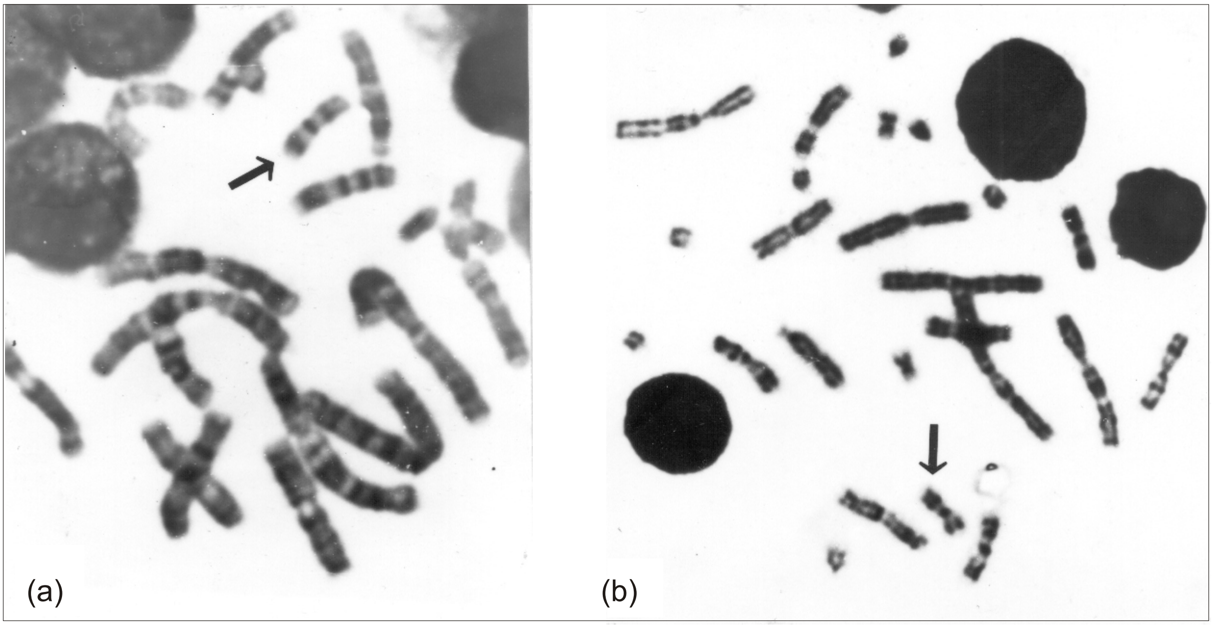

Fig. 2. (a) Metaphase plate of a common shrew from Mycyny; arrow shows the chromosome with banding pattern suggesting *np* combination. (b) Metaphase plate with the chromosomes too shortly exposed to trypsin digestion; arrow indicates the chromosome of uncertain attribution.

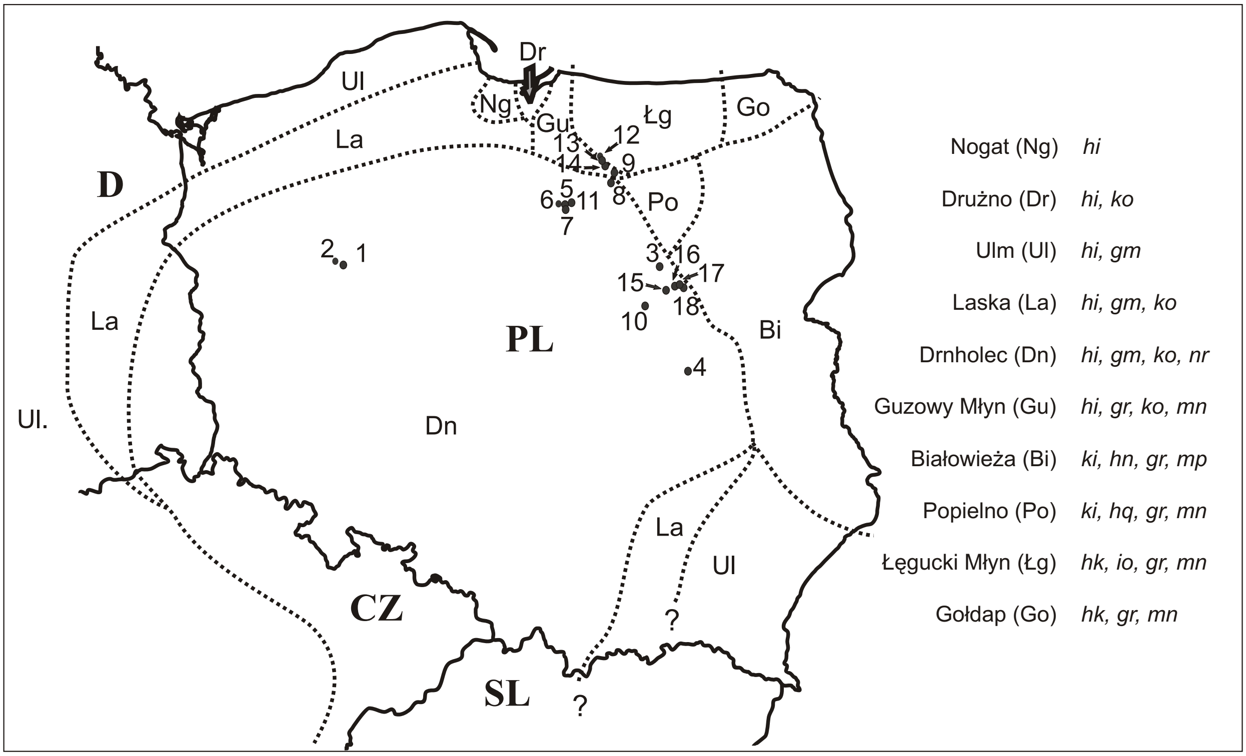

Fig. 3. Updated ranges of the ten chromosome races of the common shrew present in Poland (PL) and neighbouring countries: Germany (D) – after Brünner *et al*. (2002), Czech Republic (CZ) and Slovakia (SL) – after Zima and Král (1985) and Zima *et al*. (2003). Numbers refer to localities as in Table 1.

spreads coming from even the same individual (mainly in the case of more contracted chromosomes) acrocentric *p* appeared to be smaller than the arm *r* (Fig. 1b). Such chromosome spreads were classified as uncertain attribution (?). However, in material from Stobnica and Piotrowo, cells with unequivocal attribution to *nr* dominated over those of questionable attribution (Table 1). Analysis of five chromosome spreads coming from two individuals from Stobnica, which were used to describe the Stobnica race (Wójcik 1986), showed *nr* combination in three cases. The two remaining cells were classified as *np* and ? (Table 1). There are, therefore, *nr* instead of *np* metacentrics in the type locality for the shrews of Stobnica race.

In the remaining populations further east from Stobnica, *nr* metacentrics were found most frequently too (Table 1). Only 13, out of 178 cells analysed were scored as *np*. Such a case represents the metaphase spread of a complex heterozygote collected from Mycyny (Fig. 2a). The banding pattern on the chromosome (see Fig. 2a) suggests the *np* combination (compare the karyotype of Arendal race in Fredga 2003). However, we found *nr* combination in four other metaphase spreads of the same specimen (two remaining cells were of uncertain attribution). Likewise, the meiotic study showed beyond any doubt that *nr* instead of *np* combination occurs in Mycyny population (Fedyk *et al*. 2000).

In 42 out of 216 cells studied, analysis was not conclusive. This mainly refers to spreads with more contracted chromosomes, but in some cases applies to chromosomes which were underexposed to trypsin digestion (Fig. 2b). Again, in all studied populations, spreads of uncertain attribution appeared rarer than those of unequivocal *nr* (Table 1). So, our analyses led us to conclude that only *nr* combination exist in Poland. Consequently, the Stobnica race (*gm*, *hi*, *ko*, *np*) should not be considered valid.

The outcomes of chromosome studies of shrews in eastern Germany are concordant with our findings. In Eberswalde (Brandenburg) the Laska race (*gm*, *hi*, *ko*) was found, and Elbe River is the limit between the Laska and Drnholec races (Brünner *et al*. 2002). Occurrence of *nr* combination in the contact zone between WEKG and EEKG races in eastern Poland (Fedyk *et al*. 2000) provides additional confirmation for our conclusion.

Former erroneous assumptions of *np* combination were based mainly on the karyotypes of poor quality. On the other hand, taking the law of parsimony into account, all doubtful cases were attributed to *np* combination. As a result of the meiotic study of hybrids between WEKG and EEKG races in eastern Poland the provisional range of Stobnica race was limited to a slender area close to the type locality in western Poland (Fedyk *et al*. 2000). Ratkiewicz *et al*. (2002) – without any proof – erroneously included the provisional area of the Stobnica race into the range of the Laska race. Figure 3 shows adjusted ranges of all ten chromosome races of *S*. *araneus* in Poland. Extension of the ranges outside of the Polish border is based on the data by Brünner *et al*. (2002) and Zima *et al*. (2003).

Results of this study are important to improve our understanding of post-glacial history and the evolution of chromosomes of the common shrew in central Europe. Now it is obvious that the shrews which populated Poland from southwest (or west) display less chromosome variation than those which colonized northeastern Poland from east.

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