Reassessment of the range of the Drnholec race: studies on meiosis in *Sorex araneus* hybrids

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The Stobnica race (gm, hi, ko, np) and Drnholec race (gm, hi, ko, nr) are difficult to distinguish and so studies were carried out to determine which is prevalent in eastern Poland. Meiotic studies on hybrids showed that it is the Drnholec race rather than the Stobnica race that makes contact with the Białowieża, Guzowy Młyn and Popielno races.

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Introduction

Cytogeneticists have assumed that centric fusions and whole arm reciprocal translocations are the basis of the phenomenal differentiation of *Sorex araneus* karyotypes. Over 50 chromosomal races have been described up to now (Zima et al. 1996). In order to properly establish the chromosomal races of *Sorex araneus*, a method for accurate identification of chromosome arms has been essential. Once differential staining techniques (Q- and G-banding) were applied to common shrew chromosomes (Halkka et al. 1974, Fredga and Nawrin 1977), identification of all the chromosome arms became possible. In fact, it is easy to discriminate large and medium size (a-l) chromosome arms (see Searle et al. 1991). However, the smallest arms (m-r) can be misidentified.

The chromosome arms p and r are the most difficult to discriminate. Both have one dark band close to the centromere, and in the acrocentric state, they can additionally be mistaken for the Y1 chromosome (Searle et al. 1991). Theoretically, arm r should be smaller than arm p, but this difference in size can only be visible in the best preparations.

Particular precaution is necessary when two very similar metacentrics, that differ in p and r chromosome arms, occupy neighbouring territories. Two contiguous chromosomal races, differing only in nr and np metacentrics, have been described
in Poland (Fig. 1). One of them, the Stobnica race \((gm, hi, ko, np)\), was described from western Poland (Wójcik 1986) and the shrews with the same specific metacentrics were described as race II from north-eastern Poland (Fedyk 1986, Fedyk and Leniec 1987). The Drnholec race \((gm, hi, ko, nr)\) was described in southern Moravia (Fig. 1), and its range was originally limited to Bohemia and Moravia (Zima and Kral 1985). Zima et al. (1988) suggested that the range of Drnholec race extended northwards, to southern Poland, but Wójcik (1993) considered it limited to the very southernmost part of the country. Wójcik (1993) described the Stobnica race as having a more northern and eastern distribution within Poland. Towards the east, Wójcik (1993) considered that it made contact with the Białowieża, Popielno, Guzowy Młyn and Łęgucki Młyn races, all of which belong to the East European Karyotypic Group (EEKG) as opposed to the Stobnica race which is a member of the West European Karyotypic Group (WEKG) (see Fig. 1). The hybrid zones between the Stobnica and Białowieża races (Fedyk et al. 1993) and the Stobnica and Łęgucki Młyn races have been described (Fedyk et al. 1991, Szalaj et al. 1995, Fedyk 1995). However, subsequent to Wójcik’s distributional

Fig. 1. Distribution of chromosomal races of *Sorex araneus* in Poland (according to Wójcik 1993 and Fedyk 1995), Czech Republic and Slovakia (according to Lukášová et al. 1994). Type localities for the Stobnica and Drnholec races (triangle and square, respectively), and populations studied (circles) are shown. Codes for races: Ul – Ulm, No – Nogat, Dr – Drużno, Gu – Guzowy Młyn, Lg – Łęgucki Młyn, Go – Goldap, La – Laska, Po – Popielno, Bi – Białowieża, St – Stobnica, Dn – Drnholec. Populations studied: 1 – Plebanka, 2 – Wykno, 3 – Czarny Piec, 4 – Mycyny. Shaded area: isolated population of the Laska race in Świętokrzyskie Mts (Wójcik 1993).
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study, it was shown that shrews of the Drnholec race were found to hybridize with
the Białowieża race in south-eastern Poland, hence the range of the Drnholec race
was extended to the east (Szalaj et al. 1996). Therefore, at least in some parts of
contact zone between the races of WEKG and EEKG in Poland, the Drnholec race,
not the Stobnica, is the representative of the WEKG races. Given the difficulty in
distinguishing nr and np, the occurrence of the Stobnica race in other sections of
the contact zone between the WEKG and EEKG races has become doubtful and the
aim of the present study is to establish whether it is the Drnholec or Stobnica race
that occurs in north-eastern Poland.

Material and methods

We conducted meiotic studies on hybrids collected from four sites in north-eastern Poland from
three different hybrid zones between the EEKG and WEKG races. The races involved were known to
be the Drnholec (Dn) and Białowieża (Bi) races at Plebanka (site 1 in Fig. 1) (Szalaj et al. 1996). In the
other sites we were aiming to determine whether the WEKG race was the Stobnica (St) or Dn race.
The EEKG races were the Guzowy Młyn (Gu) race at Wykno and Mycyny (sites 2 and 4 in Fig. 1) and
the Popielno (Po) race at Czarny Piec (site 3 in Fig. 1). The karyotypes of the F₁ hybrids expected in
these hybrid zones are shown in Table 1. It can be seen that the meiosis I configurations would differ
between Po × Dn and Po × St F₁ hybrids and between Gu × Dn and Gu × St hybrids.

In an area where there is Robertsonian polymorphism, identification of hybrids with nr or np is
more difficult. But the individuals with acrocentrics, instead of one of the metacentrics forming the
meiotic complex, can be useful. In such a case a chain-of-five (CHV) arises instead of a ring-of-four (R₄)
configuration when the hybrids possess chromosome nr, whereas in hybrids with the np metacentric,
polymorphism of gm or mn divides the CHV₁ configuration into two complexes (CHV₀ and CHV₂), and
polymorphism of gr or np chromosomes shortens the CHV₁ complex to CH₁ with an acrocentric
bivalent excluded from the chain (Table 1).

Meiotic and mitotic preparations were made according to standard techniques (Verma and Babu
1989). At least 20 good diakineses were analysed from each of four hybrids studied.

Table 1. Karyotypes expected in F₁ hybrids between the EEKG races and the Drnholec or Stobnica
races (WEKG) in eastern Poland. Boldface indicates the position of chromosomes in meiotic complexes
in relation to the presence of nr or np combination.

<table>
<thead>
<tr>
<th>Gametes of WEKG races</th>
<th>Drnholec (Dn)</th>
<th>Stobnica (St)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gametes of EEKG races</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Białowieża (Bi)</td>
<td>hi, ko, gm, nr, p, q</td>
<td>hi, ko, gm, np, r, q</td>
</tr>
<tr>
<td></td>
<td>o/ok/ki/ik/hn/hi/rg/gm/mp/p,</td>
<td>o/ok/ki/ik/hn/hi/np/pm/mg/gr/r,</td>
</tr>
<tr>
<td></td>
<td>q, q</td>
<td>q, q</td>
</tr>
<tr>
<td>Popielno (Po)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>o/ok/ki/ik/hq/q,</td>
<td>o/ok/ki/ih/hq/q,</td>
</tr>
<tr>
<td></td>
<td>r/n/m/mg/gr,</td>
<td>r/rg/gm/mn/mp/p</td>
</tr>
<tr>
<td></td>
<td>pp</td>
<td>pp</td>
</tr>
<tr>
<td>Guzowy Młyn (Gu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hi hi, ko ko,</td>
<td>hi hi, ko ko,</td>
</tr>
<tr>
<td></td>
<td>r/n/m/mg/gr,</td>
<td>r/rg/gm/mn/mp/p,</td>
</tr>
<tr>
<td></td>
<td>pp, q</td>
<td>q, q</td>
</tr>
</tbody>
</table>
Results

The karyotypes of the shrews studied and the expected configurations at meiosis I are shown in Table 2. Four pairs of autosomes (af, bc, jl, tu), as well as the sex chromosomes, were invariant in the karyotypes of all hybrids studied. One pair of acrocentrics (qq) also occurred in all material studied (Table 2).

On the basis of the karyotype of shrew No. 2680, Szałaj et al. (1996) decided that the Drnholec race instead of the Stobnica race occurred in the hybrid zone at Plebanka (Fig. 1). Here we wanted to give the precise analysis of this shrew karyotype. This shrew had 24 chromosomes and, among others, chromosomes g and r were acrocentric. The meiotic configurations expected differed clearly according to whether the karyotype included nr or np (Table 2). Analysis of diakinesis figures showed that the first possibility was correct (Fig. 2a). There was no doubt that shrew No. 2680 was a hybrid between the Białowieża and Drnholec races.

A hybrid between the Guzowy Młyn and either the Stobnica or Drnholec races from Wykno (No. 3739) had 23 chromosomes. Again the meiotic configuration revealed that the Drnholec race was involved (Table 2, Fig. 2b, c).

Shrew No. 3758 from the Czarny Piec population had 25 chromosomes and two ik metacentrics diagnostic of the Popielno race. This feature and the presence of only one meiotic complex (Table 1 and 2) suggested that this shrew was a backcross hybrid. The four element ring configuration found at diakinesis of this shrew (Fig. 2d) confirmed that it was a Po × Dn hybrid.

Shrew No. 2541 from the Mycyny population was a hybrid of the Gu × St or Gu × Dn type, but had acrocentrics m and n instead of the mn metacentric, and in

Table 2. Partial karyotypes and expected meiotic figures in the hybrids studied. See text for further explanation. Abbreviations: str – sex chromosomes trivalent, bi – bivalent, CH – chain configuration, R – ring configuration, Roman numerals indicate number of elements in complexes.

<table>
<thead>
<tr>
<th>No. of shrew, Population</th>
<th>Two variants (nr versus np)</th>
<th>Expected configurations at meiosis I</th>
</tr>
</thead>
<tbody>
<tr>
<td>2680 Plebanka</td>
<td>olok/ki/ih/hn/nr, gm/mp/p, qq</td>
<td>str, 5 bi, CH_{VII}, CH_{IV}</td>
</tr>
<tr>
<td></td>
<td>olok/ki/ih/hn/mp/pm/gm/g, rr, qq</td>
<td>str, 6 bi, CH_{IX}</td>
</tr>
<tr>
<td>3739 Wykno</td>
<td>hihi, koko, rg/gm/mn/nr, pp, qq</td>
<td>str, 8 bi, R_{IV}</td>
</tr>
<tr>
<td></td>
<td>hihi, koko, rir/gm/mn/mp/p, qq</td>
<td>str, 7 bi, CH_{VI}</td>
</tr>
<tr>
<td>3758 Czarny Piec</td>
<td>hh, kiki, rg/gm/mn/nr, oo, pp, qq</td>
<td>str, 9 bi, R_{IV}</td>
</tr>
<tr>
<td></td>
<td>hh, kiki, rir/gm/mn/mp/p, oo, qq</td>
<td>str, 8 bi, CH_{VI}</td>
</tr>
<tr>
<td>2541 Mycyny</td>
<td>hihi, koko, mig/sgir/mn, pp, qq</td>
<td>str, 8 bi, CH_{VII}</td>
</tr>
<tr>
<td></td>
<td>hihi, koko, mig/sgir, mnp/p, qq</td>
<td>str, 7 bi, CH_{IV}, CH_{III}</td>
</tr>
</tbody>
</table>
Fig. 2. a – diakinesis of shrew No. 2680, 8 elements, two chains configurations (CHvII and CHiv) are present; b and c – diakinesis of shrew No. 3739, 10 elements, ring of four chromosomes can be seen; d – diakinesis of shrew No. 3758, 11 elements, ring of four chromosomes is present; e – diakinesis of shrew No. 2541, 10 elements; chain of five chromosomes is present.

consequence 24 chromosomes. Two complexes (CHIV + CHIII) and 7 bivalents would be expected at meiosis if np occurred, and only one (CHv) complex accompanied by 8 bivalents would indicate the presence of nr (Table 2). A chain-of- five chromosomes was found at diakinesis (Fig. 2e) confirming that this was a Gu × Dn hybrid.
Table 2. Partial karyotypes and expected meiotic figures in the hybrids studied. See text for further explanation. Abbreviations: str - sex chromosomes trivalent, bi - bivalent, CH - chain configuration, R - ring configuration, Roman numerals indicate number of elements in complexes.

<table>
<thead>
<tr>
<th>No. of shrew, Population</th>
<th>Two variants (nr versus np) of variable part of karyotype</th>
<th>Expected configurations at meiosis I</th>
</tr>
</thead>
<tbody>
<tr>
<td>2680 Plebanka</td>
<td>olok/ki/hi/ha/nr/r, g/gm/mp/ip, qq</td>
<td>str, 5 bi, CH_{IV}, CH_{IV}</td>
</tr>
<tr>
<td></td>
<td>olok/ki/hi/ha/mp/pm/mgig, rr, qq</td>
<td>str, 6 bi, CH_{IX}</td>
</tr>
<tr>
<td>3739 Wykno</td>
<td>hihi, koko, rr/gg/mn/nr, pp, qq</td>
<td>str, 8 bi, R_{IV}</td>
</tr>
<tr>
<td></td>
<td>hihi, koko, rr/gg/mn/mp/p, qq</td>
<td>str, 7 bi, CH_{IV}</td>
</tr>
<tr>
<td>3758 Czarny Piec</td>
<td>hh, kiki, rr/gg/mn/nr, oo, pp, qq</td>
<td>str, 9 bi, R_{IV}</td>
</tr>
<tr>
<td></td>
<td>hh, kiki, rr/gg/mn/mp/p, oo, qq</td>
<td>str, 8 bi, CH_{IV}</td>
</tr>
<tr>
<td>2541 Mycyny</td>
<td>hihi, koko, m/mgigr/nn/n, pp, qq</td>
<td>str, 8 bi, CH_{IV}</td>
</tr>
<tr>
<td></td>
<td>hihi, koko, m/mgigr/nn/p, qq</td>
<td>str, 7 bi, CH_{IV}, CH_{III}</td>
</tr>
</tbody>
</table>

Discussion

Halkka et al. (1987) warned of the possibility of mistakes in the identification of chromosomes in the common shrew karyotype, particularly those of small size. Other cytogeneticists have expressed a similar opinion (cf Zima et al. 1988). In fact, Halkka et al. (1974) and Fredga and Nawrin (1977) labelled chromosome arms m and o conversely, although some features should make identification of these unequivocal when the chromosome preparations are of good quality.

As chromosome arms p and r are also difficult to distinguish from each other we suppose that there are more cases of incorrect identification. Two races described from the vicinity of Moscow are such a case. The first of these had kp and qr metacentrics and was described from Zvenigorod (Ivanitskaya 1985), and the second one, with combinations kr and pq was found in Chernogolovka (Aniskin and Lukianova 1989). Only the kr and pq metacentrics have recently been confirmed from the region of Moscow (Bulatova et al. 2000, Kozlovsky et al. 2000). Fusions of p and/or r with arms k, m, n, o and q are characteristic for some other races distributed throughout the whole range of the species (see Zima et al. 1996). At least some of these chromosome descriptions could be doubtful.

The present study shows that the Drnholec race has a wider distribution than was described by Wójcik (1993) and Szalaj et al. (1996). Although only four individuals have been examined, there is no doubt that in the contact zone between the WEKG and EEKG races in Poland only chromosome nr occurs, both in the southernmost part (Plebanka – the hybrid zone with the Bi race), as well as in the northernmost part (Mycyny – the hybrid zone with the Łg race) (Fig. 3). On the
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Fig. 3. Proposed distribution of the Drnholec race. Presence of the Stobnica race is doubtful (St?).

other hand, it cannot be excluded that the metacentric \( np \) occurs in western Poland. If metacentric \( np \) is actually present in Poland, it must have a very small range, limited only to the periphery of the wide range of the metacentric \( nr \) (Fig. 3). But, taking the present study into account, occurrence of chromosome \( np \) in western Poland needs additional proof.

Establishing that the metacentric \( nr \), instead of \( np \), is present in the hybrid zones is important in the context of our knowledge of the structure of the hybrid zones. Material used for the description of the structure of the hybrid zone near Olsztynek (Fedyk et al. 1991, Fedyk 1995, Szalaj et al. 1995) needs reexamination. The precise description will be given elsewhere and here we want to correct the basic information about the meiotic complexes that characterize hybrids in the contact zone. In the Dn/Lg hybrid zone (near Olsztynek) two meiotic complexes designated \( C_1 \) and \( C_2 \) were found in hybrids. The \( C_1 \) complex occurs mostly as a ring configuration \( kh/hi/io/ok \) (Fedyk et al. 1991). Only one pair of chromosomes involved in the \( C_1 \) complex is polymorphic (\( io \)) and with a low frequency of acrocentrics, hence \( CH_V \)-forming hybrids \( o/ok/kh/hi/ii \) are extremely rare (Fedyk et al. 1991). The second meiotic complex in the Dn/Lg hybrid zone, designated \( C_2 \), is also a four element ring configuration of \( rg/gm/mn.nr \) (Table 1) and not a six element chain, as was described earlier (Fedyk et al. 1991). But all the chromosome
pairs forming the complex are polymorphic and the frequency of acrocentrics is moderate (Fedyk et al. 1991). Hence, the $C_2$ complex usually occurs as a CH$_V$ or CH$_{IV}$ configuration in hybrids.

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