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Biogeographical and karyological data of the *Microtus savii* group (Rodentia, Arvicolidae) in Italy

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Abstract. Two species in an early stage of speciation were found in Italy within the *Microtus savii* complex: *M. savii* in the described area of the species and *M. brachycercus* in Calabria. Karyological analyses of specimens from a wider geographic area confirmed the occurrence of the G-banded karyotype of *M. savii* in north-central Italy and Sicily. The G-banded karyotype of *M. brachycercus* was found in Calabria and a third chromosome form with an acrocentric X in the south-east of Italy. The C-banded karyotype confirms the homogeneity of autosomal C-bands, apart from a small variant in the third chromosomes pair, and C-band variations in the sex chromosomes.

Key words. Rodents, *Microtus savii* complex, chromosome banding, polymorphism.

Introduction

On the basis of slight differences in morphological traits three different subspecies of the Savi pine vole *Microtus savii* (De Sel.) were described in Italy. Comparisons among banded karyotypes of specimens from Rosarno (Calabria), where the subspecies *M. savii brachycercus* (von Lehmann, 1961) occurs, and from central Italy (Pisa and Viterbo), where the subspecies *M. savii savii* (De Sélys Longchamps, 1838) is present, revealed identical autosomal complements but marked differences in sex chromosomes (Galleni et al. 1992). Since male hybrid specimens from crosses between the two different karyomorphs were sterile, *M. savii brachycercus* and *M. savii savii* were considered different species in an early stage of speciation (Galleni et al. 1994). Krapp & Winking (1976) found a karyotype similar to that of *M. savii savii* in a third subspecies, *M. savii nebrodensis* (Mina-Palumbo, 1868), from Sicily.

A different karyological form with an acrocentric X was described in south-eastern Italy (Monte Gargano) (Niethammer 1981) and later classified as *M. savii savii* on the basis of tooth morphology (Brunet-Lecomte 1988). As no banded karyotype was reported, nothing can be said about the general level of autosomal similarity with the other karyotypes.

For a better knowledge of the level of chromosome polymorphism within the *Microtus savii* complex (according to Santini 1978), further investigations on C- and G-banded karyotypes of specimens from different Italian populations were carried out in this study.

Material and methods

Specimes were trapped at different localities (Fig. 1): Ficuzza (Palermo, Sicily; 1 female (F); Fiume Freddo (Cosenza, Calabria; 1 F); Metaponto (Potenza, Basilicata; 1 F), Monte Cimone (Modena, Emilia; 1 F) and Parco Lambro (Milano, Lombardia, 1 male (M)).

Fig. 1: Collecting sites of specimens reported in the present paper (open symbols) and in literature (filled symbols) (Krapp & Winking 1976; Niethammer 1981; Galleni et al. 1992). Square: *Microtus brachycercus*; circle: *Microtus savii* (X metacentric); triangle *Microtus savii* (X acrocentric).

Chromosomes were prepared from fibroblast cultures of short terminal tail biopsies (Stanyon & Galleni 1991) and C-, G-banded following previously reported techniques (Galleni et al. 1992).

Results

The G-banded karyotype of specimens from Ficuzza (PA) Monte Cimone (MO) and Parco Lambro (MI) matched that of *Microtus savii* from central Italy while the G-banded chromosomes of the female from Fiume Freddo (CS) agreed with those previously found in *Microtus brachycercus* (see Galleni et al. 1992).

The female from Metaponto exhibited the same autosomal pattern of the other specimens but an acrocentric medium size X chromosome which differed from the metacentric X of *Microtus savii* (Fig. 2).

Autosome C-banding pattern was the same in all the specimens surveyed except a larger amount of pericentromeric heterochromatin in the third chromosomes pairs of specimens with the *M. savii* X metacentric karyotype (Fig. 3).

A centromeric C-band, a band in the middle of the arm and a third band about half way between them were detected in the X chromosome of the female from Metaponto (Fig. 4). All the other specimens showed C-banded karyotypes similar to those described in Galleni et al. (1992).

Fig. 2: G-banded karyotype of a female *M. savii* from Metaponto (PZ).

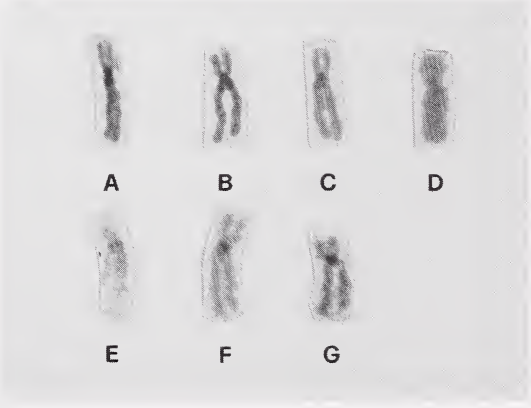

Fig. 3: C-bands of chromosome 3 (A = Milano; B = Viterbo; C = Pisa; D = Monte Cimone; E = Metaponto; F = Rosarno; G = Fiume Freddo).

Discussion

Morphological, karyological data and hybridological tests showed that the Savi pine vole of the Italian peninsula actually is a heterogeneous group including two different species in an early stage of speciation: *M. savii* in north and central Italy and *M. brachycercus* in the south-west (Galleni et al. 1994, Galleni 1995). The

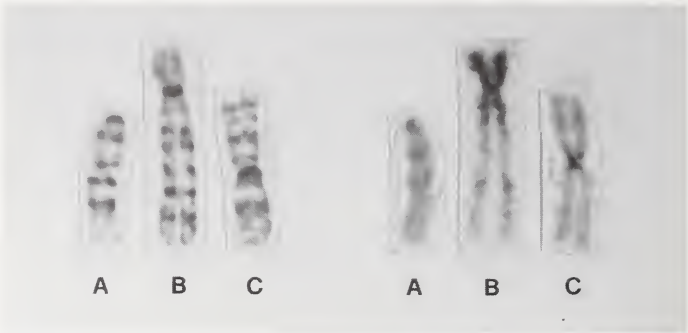

Fig. 4: G-banded (left) and C-banded (right) X chromosomes of *M. savii* (A: acrocentric, C: metacentric) and *M. brachycercus* (B).

question of the taxonomic state of populations from different localities of southern Italy and Sicily is still open however.

The agreement between karyotypes of specimens from northern and central Italy (Parco Lambro and Monte Cimone) and that of the specimen from Sicily supports the classification suggested by Krapp & Winking (1976) who considered these populations as all belonging to the subspecies *M. savii savii*. It appears, in this case, that allopatric distribution has not been followed by differentiation at specific level at least in respect of morphological and karyological traits.

The presence of specimens with the same karyotype as *M. brachycercus* at Fiume Freddo (Calabria) confirms the occurrence of this species in the Calabrian peninsula.

The X-acrocentric karyotype of the female from Metaponto (Potenza) is similar to that described by Niethammer (1981) for a specimen from Monte Gargano (Foggia). Because of the proximity of the two collecting sites (Fig. 1) it is likely that we are dealing with the same form.

Hypotheses on the relationships between the metacentric X chromosome of *M. savii* and the sub-metacentric X of *M. brachycercus* were discussed previously (Galleni et al. 1992). A similarity of G-banding pattern between the euchromatic part of X chromosome long arm of *M. brachycercus* and the whole metacentric X of *M. savii* was also confirmed in this study (Fig. 4). The acrocentric X chromosome is similar to the metacentric X but it is not possible to resolve the bands to ascertain if a pericentric inversion took place in the X of *M. savii* generating the acrocentric form.

According to the taxonomic revision based on tooth morphology (Brunet-Lecomte 1988) the forms of *M. savii* with different X chromosomes would belong to the same species. However no crosses have been carried out so far to confirm this view. Up to date a clear speciation process has been demonstrated only for karyomorphs (*M. savii* and *M. brachycercus*) with different heterochromatin composition of their sex chromosomes, although unequivocal evidence of an active role of this class of chromatin in the formation of reproductive barriers has not been demonstrated yet (Galleni et al. 1994).

Finally, heterochromatin polymorphism observed for the third chromosome will be a useful marker for studying phyletic relationships among the different forms of

this group if confirmed on a wider sample. However, this polymorphism does not exceed the level of polymorphism found in many other mammalian species and is not considered to affect fitness.

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Zusammenfassung

Innerhalb des *Microtus savii* Komplexes können 2 Arten unterschieden werden: *M. savii* im beschriebenen Areal und *M. brachycercus* in Kalabrien. Die beiden Arten befinden sich in einem frühen Stadium der Artbildung. Karyologische Analysen von Individuen eines weiteren geographischen Gebietes bestätigten das Vorkommen von G-gebänderten Karyotypen von *M. savii* in Nord-Zentral-Italien und auf Sizilien. G-gebänderte Karyotypen von *M. brachycercus* wurden in Kalabrien gefunden und eine dritte Chromosomenform mit einem akrozentrischen X in Süd-Ost-Italien. Die C-gebänderten Karyotypen bestätigten die Homogenität der autosomalen C-Bänderung, zum Teil von einer kleinen Variante im dritten Chromosomenpaar, und eine Variation der C-Bänderung in den Geschlechtschromosomen.

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