CHROMOSOME ANALYSIS OF THREE SPECIES OF MYOXIDAE

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ABSTRACT – Karyotype analysis was carried out on three species of dormice: *Myoxus glis,* 4 populations from Northern and Southern Italy and from Turkey; *Dryomys nitedula* , 4 populations from Northern and Southern Italy, from Israel and from Turkey; *Myomimus roachi,* 1 specimen from Turkey. *Myoxus glis* shows 2n=62; comparison of our specimens from different localities shows complete correspondence between karyotypes, both for the autosomes and the heterochromosomes. *Dryomys nitedula* shows 2n=48. **All** populations we studied, show the same karyotypic pattern, except for the NOR-bearing chromosomes. *Myomimus roachi,* here studied for the first time, shows 2n=44. **All** the autosomes are biarmed of decreasing size. The X-chromosome is a medium size metacentric, while the Y-chromosome is the smallest one. **All** the three species we studied, show one pair of NOR-bearing chromosomes, Ag-NORs always correspond to the secondary constriction. Differences in the fundamental number and in heterochromosome morphology, have been observed by other authors, in different European populations. This variability is analysed and discussed.

Key words: Myoxidae, Karyotype, Variability.

RIASSUNTO – *Analisi cromosomica in tre specie di Myoxidae* – L'analisi cromosomica e stata condotta su popolazioni europee di tre specie di Myoxidae: *Myoxus glis,* 4 popolazioni provenienti dal Nord e Sud Italia, e dalla Turchia; *Dryomys nitedula,* 4 popolazioni provenienti dal Nord e Sud Italia, da Israele e dalla Turchia; *Myomimus roachi,* 1 esemplare, proveniente dalla Turchia. *Myoxus glis* presenta 2n=62. Gli esemplari, provenienti dalle diverse popolazioni, mostrano corrispondenza nella morfologia sia degli autosomi che degli eterocromosomi. *Dryomys nitedula* presenta 2n=48. La morfologia dei cromosomi nei cariotipi appare corrispondente mentre diversa e la localizzazione degli Ag-NOR. *Myomimus roachi,* del quale viene analizzato per la prima volta il cariotipo, presenta 2n=44. Tutti i cromosomi sono metacentrici o submetacentrici di dimensioni decrescenti; il cromosoma X è un metacentrico di medie dimensioni, mentre l'Y e il piu piccolo elemento dell'assetto. Le tre specie da noi studiate mostrano tutte una sola coppia di cromosomi postatori di NOR, gli Ag-NOR sono sempre localizzati in corrispondenza di una regione eterocromatica. Vengono analizzate e discusse le differenze osservate, da altri autori, in altre popolazioni europee, riguardo al numero fondamentale e alla morfologia degli eterocromosomi.

Parole chiave: Myoxidae, Cariotipo, Variabilita.

INTRODUCTION

Among European representatives of the family Myoxidae, the genus *Eliomys* is the most studied from a karyological point of view because of its karyotypic variability. The karyological structure of *Eliomys* has been extensively studied in the last twenty years and five different karyotypes, corresponding to five different diploid numbers, have been described (2n = 46, 48, 50, 52, and 54). These are originated mostly by recurrent Robertsonian fissions of biarmed chromosomes in circum-Mediterranean populations (Leonard et al., 1970; Cristaldi & Canipari, 1976; Tranier & Petter, 1978; Delibes et al., 1980; Arroyo-Nombela et al., 1982: Murariu et al., 1985; Filippucci et al., 1988 a, 1988 b; Filippucci et al., 1990; Vujošević et al., 1993).

In contrast, information about other dormouse genera is relatively scarce. The only study in which the karyotypes of *Myoxus, Eliomys, Dryomys* and *Muscardinus* were analysed and compared was carried out by Renaud in 1938. More recently Dulic et al. (1971) studied the karyotype of *Myoxus glis,* Savić & Soldatovic (1972) studied the kayotype of *Muscardinus avellanarius* from ex-Yugoslavia and Raicu et al. (1972) that of *Dryomys nitedula* from Romania. Diaz de la Guardia et al. (1980) analysed the karyotype of Spanish populations of *Glis glis.* Zima (1987) studied the karyotype of *Glis glis, Dryomys nitedula* and *Muscardinus avellanarius* from Czechoslovakia. The karyotype of *Dryomys nitedula* was studied in Southern Italy by Filippucci et al. (1985). in Turkey by Dograinaci & Kefelioglu (1990) and in Bulgaria by Peshev & Delov (1995).

In the present paper cytogenetic data are presented on the karyotype of Italian, Turkish and Israeli populations belonging to three species of *Myoxidae (Myoxus glis, Dryomys nitedula* and *Myomimus roachi).* The karyotype of *Myomimus roachi* has been studied for the first time.

MATERIAL AND METHODS

Karyotype analysis was carried out on 21 animals of three species of Myoxidae, *Myoxux glis, Dryomys nitedula* and *Myomimus roachi.*

The specimens were collected in Northern and Southern Italy, in Turkish Thrace and in Upper Galilee (Israel). The number of specimens, their sex, sample designations and collecting sites for each species are specified in Table 1.

Karyotype analyses were performed on somatic metaphase plates obtained from bone marrow preparations following the standard air-drying procedure suggested by Hsu & Patton (1969). Vinblastine sulphide was used as the cytostatic drug; 0.075 M KCI hypotonic medium was used for mitotic preparations. A standard staining was carried out with 4% Giemsa. Meiotic diakinesis from testicles were prepared as indicated by Evans et al. (1964). G-banding was obtained by digestion with trypsin according to Seabright's method (I 971). Nucleolus organizer regions (NOR's) were detected by the $AgNO_3$ following Howell & Black (1980).

RESULTS

Myoxus glis.

The diploid number of *Myoxus glis* was established as 62 and the autosomal

fundamental number (NFA) was established as 120 . All the chromosomes are biarmed except the Y-chromosome.

The karyotype displays (Fig. 1 a and b) 28 pairs of metacentric or submetacentric chromosomes of decreasing size and two pairs of clearly distinguishable subtelocentric chromosomes. In the Turkish population (IST), a medium-sized metacentric pair (No. 16) shows an evident heterochromatic region and NORs correspond to the secondary constriction on this chromosomal pair. The X-chromosome is a large sized metacentric element, while the Y-chromosome is dot-like.

Tab. 1 – Collecting site. number of specimens exarnincd and sample designation of each dorrnousc population analyscd.

SPECIES	LOCALITIES	CODE	No.
Myoxus glis	Tarvisio, Friuli (Italy)	TAR	2
	Cerenzia, Sila Mts., Calabria (Italy)	SIL	3
	Gambarie, Aspromonte Mts., Calabria (Italy)	**ASP**	1
	Viggianello, Pollino Massif, Basilicata (Italy)	POL	2
	Yenicekoy, Istranca Mts., Thrace (Turkey)	IST	2
Dryomys nitedula	Tarvisio, Friuli (Italy)	TAR	2
	Piani di Ruggio, Pollino Massif, Basilicata (Italy)	POL	1
	Hurfesh, Upper Galilee (Israel)	ISR	1
	Edirne, Thrace (Turkey)	EDT	6
Myomimus roachi	Sütlüce, Gelibolu, Thrace (Turkey)	GEL	1

Dryomys nitedula.

The diploid number of *Dryomys nitedula* was established as 48.

The karyotype displays (Fig. 2 a and 3 a) 14 pairs of metacentric or submetacentric chromosomes (pairs No. 1 - **8** and 13 - 18) and 9 pairs of telocentric or subtelocentric chromosomes (pairs No. 9 - 12 and 19 - 23). The X-chromosome is a medium-sized metacentric element, while the Y-chromosome is punctiform. NORs correspond to the secondary constriction on two small subtelocentric chromosomes, but the NOR-bearing chromosomes are not the same in the various populations investigated. In the Pollino population (POL) NORs are located in the 19th pair (Filippucci et al., 1985), while in the Israeli population (ISR), the NOR-bearing chromosomes are the 21st (Fig. 2 a). The same pair, in the Turkish population (EDI), shows a secondary constriction, near to the centromere (Fig.3 b). G-bands analysis was carried out on three of the populations studied (POL, EDI, ISR) and reveals the complete correspondence of the karyotype in the different populations (Fig. 2 b and ***3*** b).

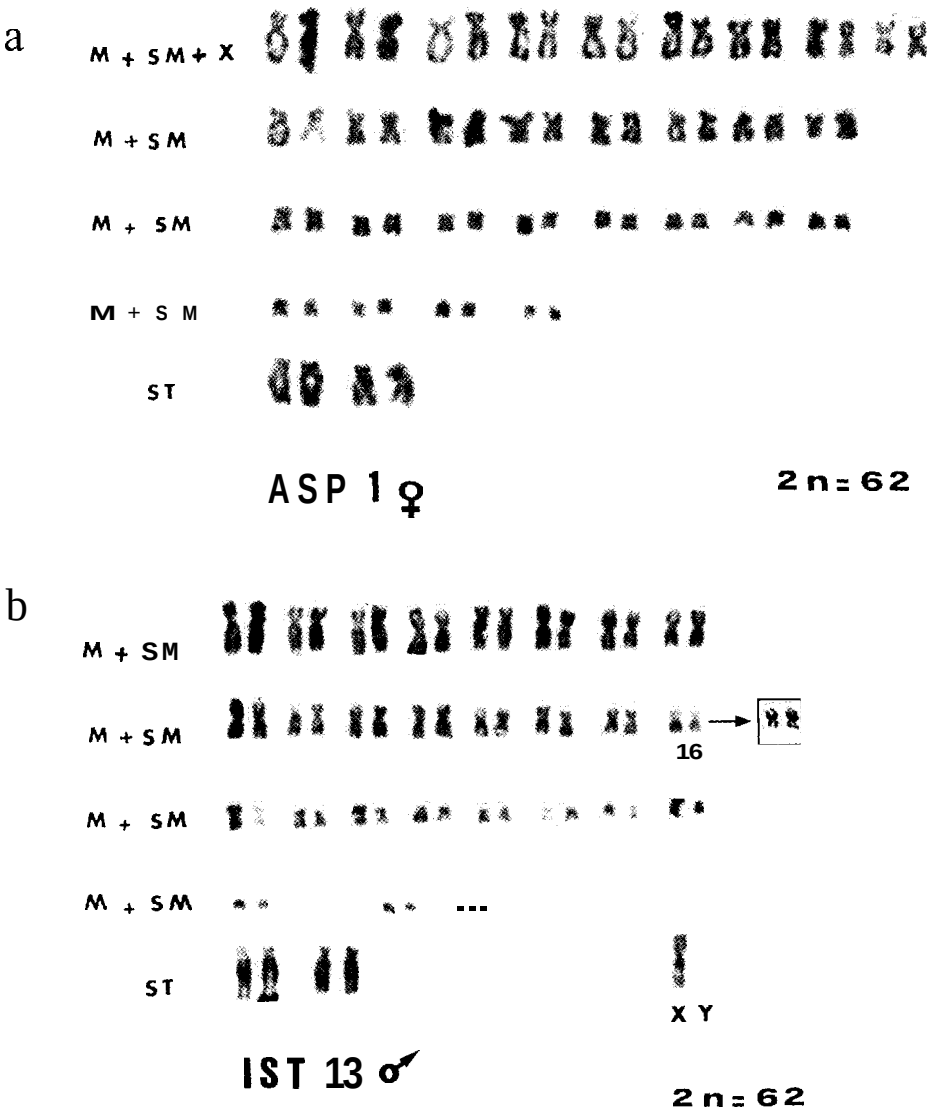


Fig. 1 – *Myoxus glis.* a) Karyotype of a female from the Aspromonte Mts. (Italy). b) Karyotype of a male from Turkey. The 16th chromosome pair bear the nucleolus organizer regions (NORs). The silver stained chromosomes are boxed at the right side.

Myomimus roachi.

The diploid number of *Myomimus roachi* was established as 44 and the **NFA** was established as 84 (Fig. 4 a and b). The karyotype of this species was studied for the first time. **All** the autosomes are biarmed of decreasing size. The X-chromosome is a medium-sized metacentric, while the Y-chromosome is the smallest one. NORs are located in one small pair of autosomes (Fig 4 c).

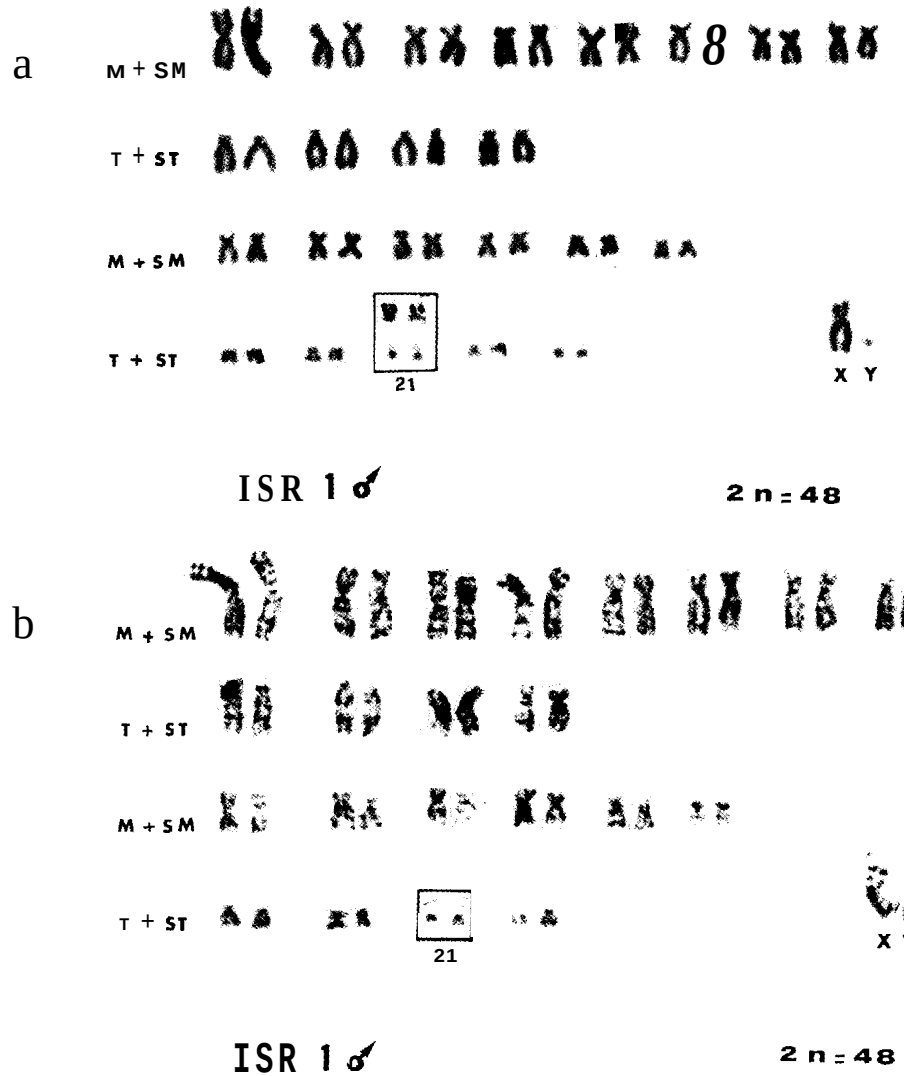


Fig. 2 - *Dryomys nitedula*. a) Karyotype of a male from Israel. The 21st chromosome pair bear the nuclcolus organizer regions (NORs). The nucleolar chromosomes are boxed and the silver stained chromosomes included in the upper row of same box. b) G-banded karyotype from same specimen as (a). The boxed chromosomes are the nucleolar chromosomes. showing a secondary constriction near to the centromere.

DISCUSSION AND CONCLUSIONS

Among the species of Myoxidae studied we observed a large karyotype variability in the diploid number and the autosomic fundamental number. Nevertheless, all species investigated do not show differences in the morphology of heterochromosomes; and NORs correspond to the secondary constrictions of two among the smallest chromosomes in the karyotype.

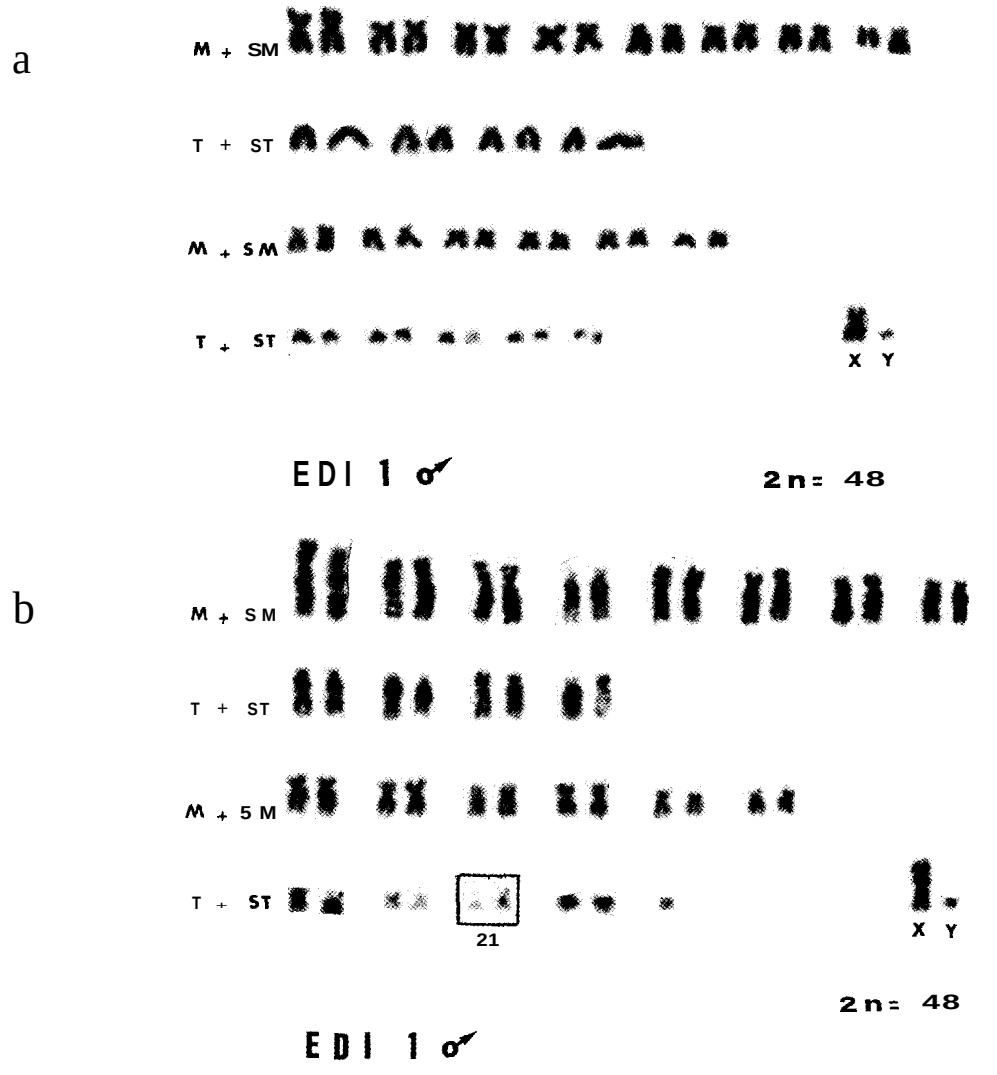
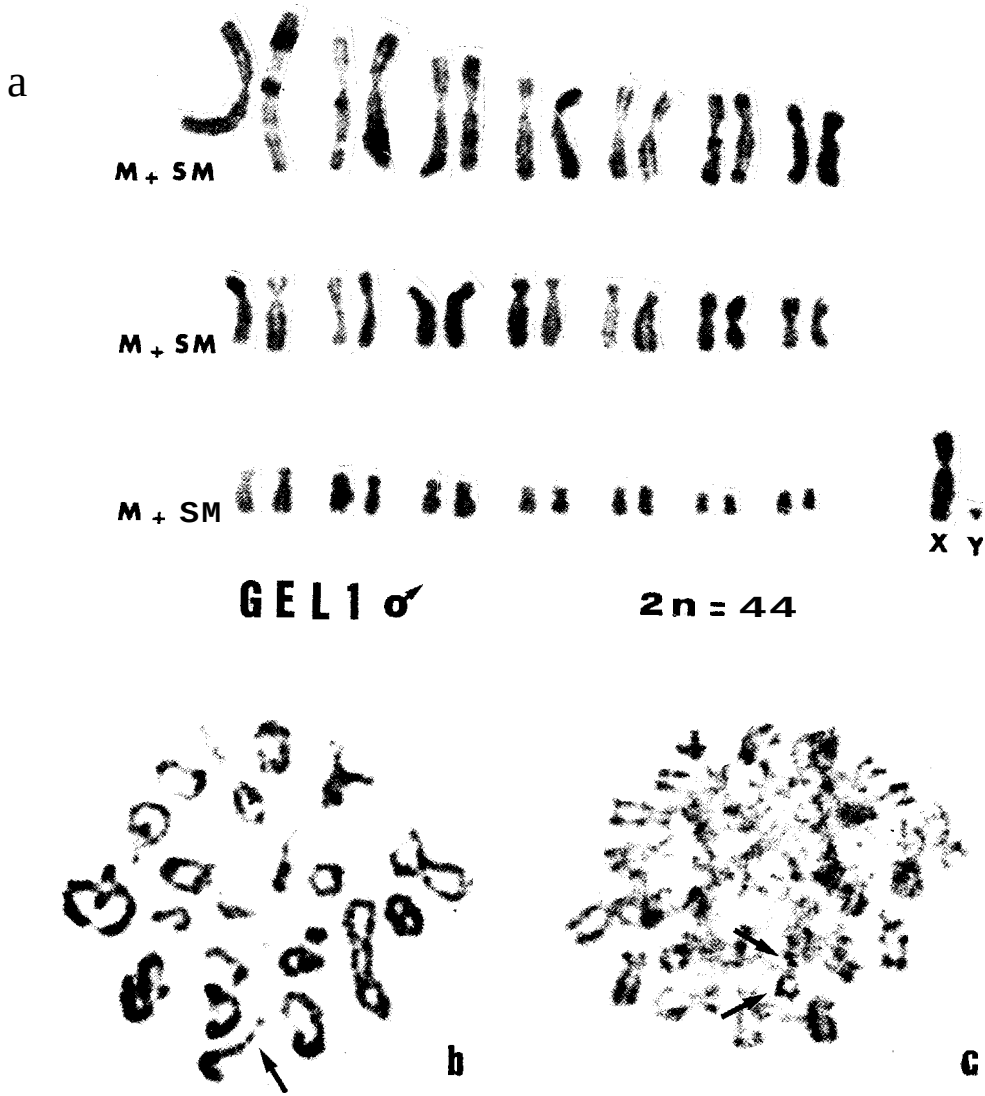


Fig. 3 – *Dryomys nitedula.* a) Karyotype of a male from Turkey. b) G-banded karyotype from the same specimen as (a). The boxed chromosomes show a secondary constriction near to the centromere.

The karyotype of *Myomimus roachi* shows the same karyological homologies, regarding the sex-chromosome morphology and the number of the NOR-bearing chromosomes. The diploid number of this species is the lowest for Palaearctic Myoxid genera. Only in *Graphiurus hueti* from Ivory Coast (Tranier & Dosso, 1979) has a lower diploid number (2n=40) been observed.

In *Myoxus glis* comparison of our specimens from different sites shows complete correspondence between karyotypes, whereas some differences are found in comparison with other European populations previously analysed by other authors. These differences particularly concern sex chromosome morphology,

while differences regarding autosomes are less important. In fact, the Y-chromosome morphology, which is always the smallest of the set and dot like in the populations we studied, sometimes appears metacentric in Serbian and Dalmatian populations (Dulić et al., 1971), as well as in those from Spain (Diaz de la Guardia et al., 1980), but in males from Czechoslovakia (Zima, 1987) it was acrocentric.



Pig. 4 – *Myomimus roachi.* a) Karyotype of a male from Turkey. b) Meiotic diakinesis showing 22 bivalents. obtained from the testis. Arrow indicates the scx-bivalent. c) Metaphase plate stained with $AgNO_3$. Arrows indicate the nucleolus organizer regions (NORs).

The X-chromosome is a large metacentric chromosome in our population, whereas it is a medium size metacentric in populations studied by Dulić et al. (1971) and by Zima (1987). It was also classified as submetacentric by Diaz de la Guardia et al. (1980) in Spain.

Only one pair of NOR bearing chromosomes were seen in in our population, coming from Istranca Mountains. These chromosomes show an evident heterochromatic region on which NOR are located. In the Serbian and Dalmatian populations, investigated by Dulić et al. (1971), heterochromatic regions are present on two pairs of small chromosomes.

As far as autosome morphology is concerned, in our populations we found two pairs of clearly identifiable subtelocentric chromosomes, as already described by Dulić et al. (1971) in Serbian and Dalmatic populations. By contrast, Zima (1987) found all chromosomes metacentric or submetacentric in populations from northern Moravia, central Bohemia and Slovakia, and Peshev & Delov (1995) described one acrocentric pair in the edible dormouse from Bulgaria.

In *Dryomys nitedula,* except for Ag-NOR chromosome identification, all populations we studied show the same karyotypic pattern, even though they are geographically widely separate. Nevertheless, populations from Turkish Thrace (EDI) and the Middle East (ISR), compared to that of southern Italy (POL; Filippucci et al., 1985), show a different location of the Ag-NORs, always on heterochromatic regions. Zima (1987), did not study Ag-NOR in specimens from Moravia, nevertheless he observed an heterocroinatic region on a smallish pair. probably the 19th, as we found in our Pollino population.

A large variability in the fundamental number contrasts with the other European populations, in which different results have been found (Dogramaci & Kefelioglu, 1990 and Zima, 1987). In fact in Turkish and Czechoslovakian populations the number of metacentric chromosome pairs appears four or six units higher than in our populations.

As far as lieterochromosome morphology is concerned, differences were only observed in the Bulgarian population, in which the Y-chromosome is metacentric (Peshev & Delov, 1995).

We think the observed differences in the fundamental number values, within the populations of the Edible dormouse and Forest dormouse, could be due to technical reasons, i.e. different chromosome condensation states, rather than to real morphological diversity.

The variation in the location of the Ag-NORs in different populations of *Dryomys nitedula* could be easily explained as the Ag-method reveals only the NORs simultaneously active in the previous interphase.

On the contrary the variability in shape and size of the heterochromosomes could be due to a real morphological diversity. Such variability is well known in various species of rodents, in conspecific populations, coming from different geographic regions. Variability, in size and in centromere position of the sex-chromosomes, has been already observed in the common hamster from eastern Austria by Vistorin et al. (1976). This phenomenon has been explained as a consequence of the changes in the amount of the constitutive heterochromatin, in the pericentroineric region of the sex-chromosomes.

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