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Karyotypic Variation in Two Species of Jerboas *Jaculus jaculus* and *Jaculus orientalis* (Rodentia, Dipodidae) from Tunisia

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The karyotypes of the lesser Egyptian jerboa *Jaculus jaculus* and the greater Egyptian jerboa *Jaculus orientalis* from Tunisia are described and compared with available data particularly from Egypt. The species examined have a similar karyotype consisting of 2n = 48 chromosomes and a fundamental number of autosomes (NFa) varying from 88 to 90 in *J. jaculus* and from 84 to 88 in *J. orientalis*. The X chromosome is submetacentric in both species, while the Y is submetacentric in *J. orientalis* and acrocentric in *J. jaculus*. Most of the autosomes are meta/submetacentric but the small pairs 22 - 23 in *J. jaculus* and 20-23 in *J. orientalis* are frequently acrocentric, yielding considerable differences in the NFa within and among species. Morphological variation in these small pairs of autosomes and/or in the Y chromosome in *J. orientalis* may distinguish populations of the two species from Egypt and Tunisia. The differences observed either between Egypt and Tunisia or between the Tunisian *Jaculus* species are probably associated with chromosomal rearrangements such as pericentric inversions or heterochromatin variation. They appear of lesser magnitude than other changes (especially molecular) that have occurred during the evolution of this genus.

Key words: Karyotype, polymorphism, conservatism, rodents, Tunisia.

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Two species of jerboas of the genus *Jaculus* (Erxleben 1777) occur in Tunisia: the lesser Egyptian jerboa *Jaculus jaculus* and the greater Egyptian jerboa, *Jaculus orientalis* (VESMANIS 1984; GHARAIBEH 1997). Historically, the taxonomy of these species has been the subject of controversy. POCOCK (1922) placed *jaculus* in the genus *Jaculus*, and *orientalis* in the genus *Scirtopoda* due to differences in the external genitalia, but VINOGRADOV (1930), on the basis of cranial and dental characters, reclassified *orientalis* into *Jaculus*. Following these earlier works, several additional studies have been carried out to assess the relationships between these taxa. For instance, WASSIF (1960) examined the osteology of the Egyptian jerboas and found that *J. jaculus* and *J. orientalis* were very similar in this respect. OSBORN and HELMY (1980) also studied their morphology and osteology and confirmed their classification into the genus *Jaculus*. In addition, they recognized four subspecies of the lesser Egyptian jerboa, namely *J. j. butleri* (Thomas 1922), *J. j. flavillus* (Setzer 1955), *J. j. jaculus* (Linnaeus 1758) and *J. j. schlueteri* (Nehring 1901), based on morphological differences. More recently, similarity in sperm morphology (SHAHIN & IBRAHEEM 1998) as well as in molar and soft palate characters (SHAHIN 1999) supported the hypothesis that *J. jaculus* and *J. orientalis* represent congeneric species. Biochemical studies also demonstrated the close relatedness of *J. jaculus* and *J. orientalis* relative to *Allactaga tetradactyla*, the third dipodid species from Egypt (SHAHIN 2003). Chromosomal studies (ATA & SHAHIN 1999; SHAHIN & ATA 2001; ATA *et al.* 2001) also showed that these two species share the same diploid number (2n) of 48, and fundamental number (FN) of 95 in males and 96 in females, in Egypt. Only small differences in G-band distribution in four chromosome pairs were found that were related to variation in heterochromatin content between the Egyptian specimens of *J. j. jaculus* and *J. orientalis* studied (ATA & SHAHIN 1999). Additionally, GRANJON *et al.* (1992) found 2n = 50, and FN = 90 in a single female from Sene-

gal, DOBIGNY *et al*. (2002) 2n = 48 and NFa = 86 in two specimens from Niger, and AL SALEH and KHAN (1984) 2n = 48 and NFa = 92 in specimens from Saudi Arabia. The same characteristics (2n = 48; NFa = 92) were also found by VORONTSOV and MALYGINA (1973) in *J. turcmenicus* (syn. *J. blandfordi*). Cytogenetics has long been a useful tool in rodent taxonomy (MATTHEY 1953; PETTER 1971), and has proven especially discriminant in African rodents (TAYLOR 2000; ROBINSON 2001; GRANJON & DOBIGNY 2003). The Dipodidae family, and within it the genus *Jaculus*, has received relatively little attention in this respect, most of the data at hand having been collected in Egypt (see references above), and none are available for jerboas from Tunisia. This may be due to their ecological characteristics, as they are restricted to arid areas, and are often difficult to capture. In the context of a wide scale study of the genus *Jaculus* in Tunisia (BEN FALEH *et al*. 2010), we decided to undertake a comparison of the intraspecific and interspecific chromosomal characteristics of the two species occurring in this country. The major objectives of this study were: 1) to describe the karyotypes of the jerboa species present in Tunisia, 2) to compare these data with those collected elsewhere, especially in Egypt, and 3) to appraise the value of these chromosomal data relative to other sets of characters used in the systematics of the genus *Jaculus*.

Material and Methods

A total of 33 specimens of *J. jaculus* and 12 individuals of *J. orientalis* collected in 13 localities in Tunisia between June 2006 and August and 2007 was studied (Fig. 1, Table1). The jerboas captured were brought to the laboratory and karyotyped following a slightly modified version of the air-drying technique (EVANS *et al.* 1963). Animals were yeast-stimulated overnight, and injected with an anti-mitotic solution (vinblastin sulphate) 40 min before euthanasia. Bone marrow was extracted and incubated for 18 min at 37°C in 8 ml KCl 0.075 M. Fixation involved methanol and acetic acid 3:1 v/v. Metaphasic suspensions were deposited on slides, stained using 4% Giemsa and

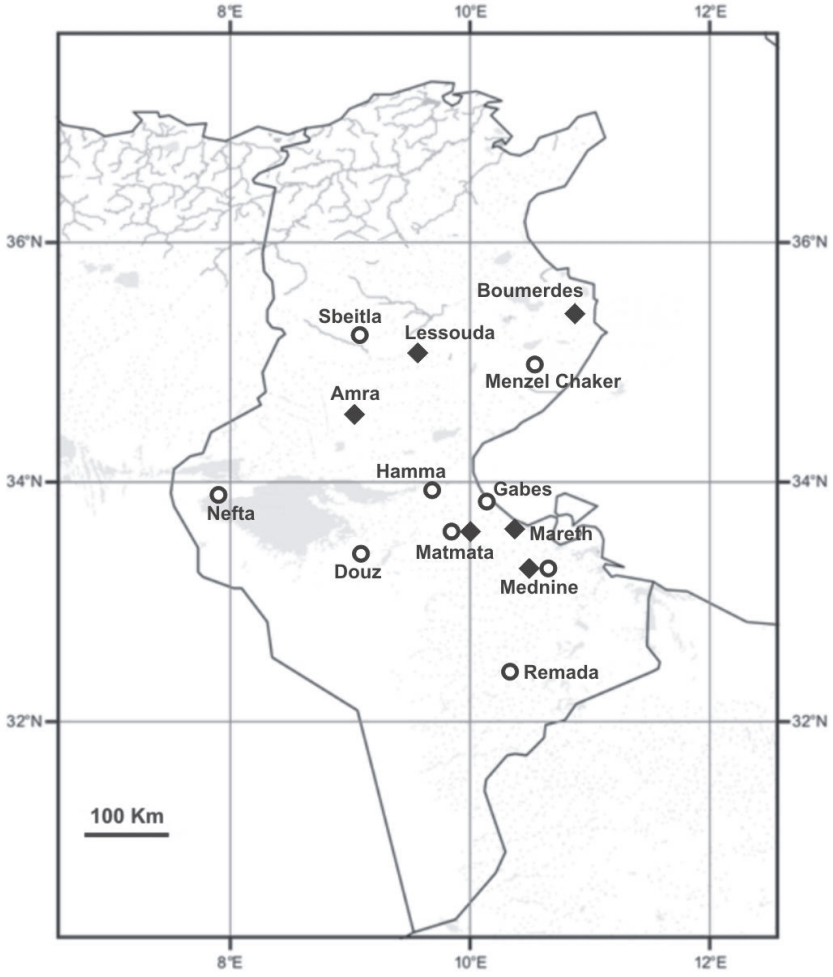

Fig. 1. The geographic localities from which samples of *Jaculus orientalis* and *Jaculus jaculus* were collected. Different symbols indicate samples belonging to the two different species (♦: *J. orientalis*; ❍: *J. jaculus*).

Table 1

List of the specimens of *Jaculus orientalis* and *J. jaculus* examined in the cytogenetic study with their main characteristics (a range in NFa indicates uncertainties in classifying small autosomes as metacentric or acrocentric)

Species	Samples locality	Latitude	Longitude	Samples codes	Sex	2n/NFa
J. orientalis	Boumerdes	35°30'11" N	11°03' 81" E	010	F	48/84
J. orientalis	Boumerdes			021	M	48/84
J. orientalis	Amra	34° 25'48" N	8°47' 56" E	034	M	48/85
J. orientalis	Amra			036	M	48/84
J. orientalis	Amra			037	F	48/84
J. orientalis	Amra			039	F	48/84
J. orientalis	Lessouda	35°02' 96 " N	9° 29'55" E	086	M	48/84
J. orientalis	Mareth	33°37'14" N	10°16'58"E	374	F	48/84
J. orientalis	Mareth			375	M	48/84
J. orientalis	Matmata	33°32' 97"N	9°57' 62 "E	033	M	48/87-88
J. orientalis	Mednine	33°20' 45"N	10°29' 91"E	385	F	48/84
J. orientalis	Mednine			388	M	48/84
J. jaculus	Sbeitla	35°13' 60" N	9°08'28"E	129	M	48/88-90
J. jaculus	Sbeitla			127	F	48/90
J. jaculus	Sbeitla			128	M	48/88
J. jaculus	Sbeitla			351	F	48/88
J. jaculus	Menzel Chaker	34°56'92 "N	10°21'96 "E	118	M	48/90
J. jaculus	Gabes	33°53 '12 "N	10°05'36 "E	325	M	48/88
J. jaculus	Gabes			336	F	48/88
J. jaculus	Gabes			337	M	48/88
J. jaculus	Gabes			333	M	48/88
J. jaculus	Nefta	33°52'52"N	7°52' 36"E	202	M	48/88
J. jaculus	Nefta			201	F	48/88
J. jaculus	Douz	33°27'95"N	9° 01' 11"E	220	F	48/88
J. jaculus	Douz			213	M	48/90
J. jaculus	Hamma	34°00'20"N	8° 09' 12"E	348	F	48/88
J. jaculus	Hamma			320	F	48/88
J. jaculus	Hamma			321	F	48/88
J. jaculus	Matmata	33°32' 97"N	9°57' 62 "E	396	F	48/88
J. jaculus	Matmata			322	F	48/88-89
J. jaculus	Matmata			539	F	48/89-90
J. jaculus	Matmata			391	M	48/89
J. jaculus	Matmata			263	F	48/89
J. jaculus	Matmata			264	F	48/89
J. jaculus	Matmata			265	F	48/89
J. jaculus	Matmata			266	F	48/89
J. jaculus	Matmata			256	F	48/88-90
J. jaculus	Matmata			254	F	48/88
J. jaculus	Matmata			323	M	48/88-90
J. jaculus	Matmata			399	F	48/89-90
J. jaculus	Mednine	33°20' 45"N	10°29' 91"E	331	F	48/89
J. jaculus	Remada	32°19' 84"N	10°24' 00"E	342	F	48/88
J. jaculus	Remada			364	M	48/88
J. jaculus	Remada			381	F	48/90
J. jaculus	Remada			332	M	48/88

observed under a Zeiss A1 microscope (Zeiss S.A.S., Pecq, France). The best-spread metaphases were recorded, and karyotypes were organized using the software Genus (Cytovision, Applied Imaging). Sex chromosomes were identified, and the diploid number of chromosomes (2n) as well as the autosomal fundamental number of arms (NFa) was systematically counted.

Results

All individuals of the species examined, as a rule, have similar karyotypes consisting of 2n = 48 chromosomes, with NFa varying from 88 to 90 in *J. jaculus* (Figs 2 & 3), and from 84 to 88 in *J. orientalis* (Figs 4 & 5). However, the morphology of the chromosomes of the two species displays several differences, leading to variation in the NFa even at the intra-population level (Table 1). The autosomes of each species vary in size, and were arranged according to length. In both species, the first pair of autosomes is composed of large near-metacentric chromosomes, the second pair of large submetacentric chromosomes with very short small arms, pairs 3 to 19 of meta/submetacentric chromosomes of decreasing size, the four last pairs (n°20 to 23) consisting of very small chromosomes, within which most of the observed variation is recorded (Figs 2-5). Given the reduced size of the latter chromosomes, the NFa was sometimes difficult to establish with certainty for some individuals (see Table 1).

Peculiarities of the karyotype of each species are as follows:

Jaculus jaculus

The most commonly found karyotype for this species has NFa = 88 and comprises 20 submetacentrics pairs, one metacentric pair (18) and two pairs (22 and 23) of usually small-sized acrocentric chromosomes (Fig. 2). In individuals with NFa = 89 or 90, one or two of these smallest chromosomes are meta/submetacentric (Fig. 3). The X chromosome is a medium-sized submetacentric, and the Y is a small acrocentric (Figs 2 & 3).

Jaculus orientalis

In this species, the karyotype always comprises 19 meta/submetacentric pairs and in most individuals four pairs of small acrocentric pairs (NFa = 84). Variation was however observed in the morphology of these small pairs in two specimens leading to NFa ranging between 84 and 88 (Figs 4 & 5). The X chromosome was a medium-sized submetacentric, and the Y was tentatively considered as a small submetacentric (Figs 4 & 5).

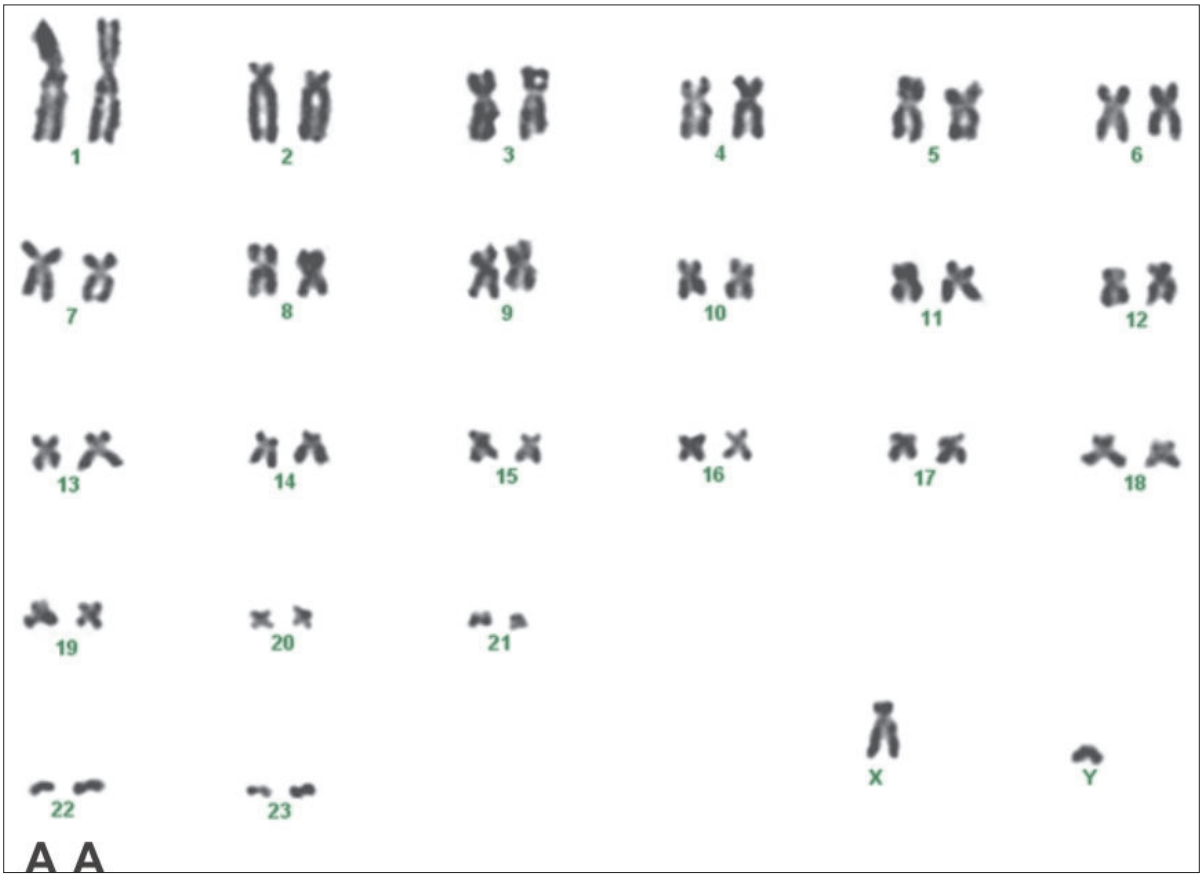

Fig. 2. Karyotype of male of *Jaculus jaculus*: 2n = 48 and NFa = 88 (A = acrocentric).

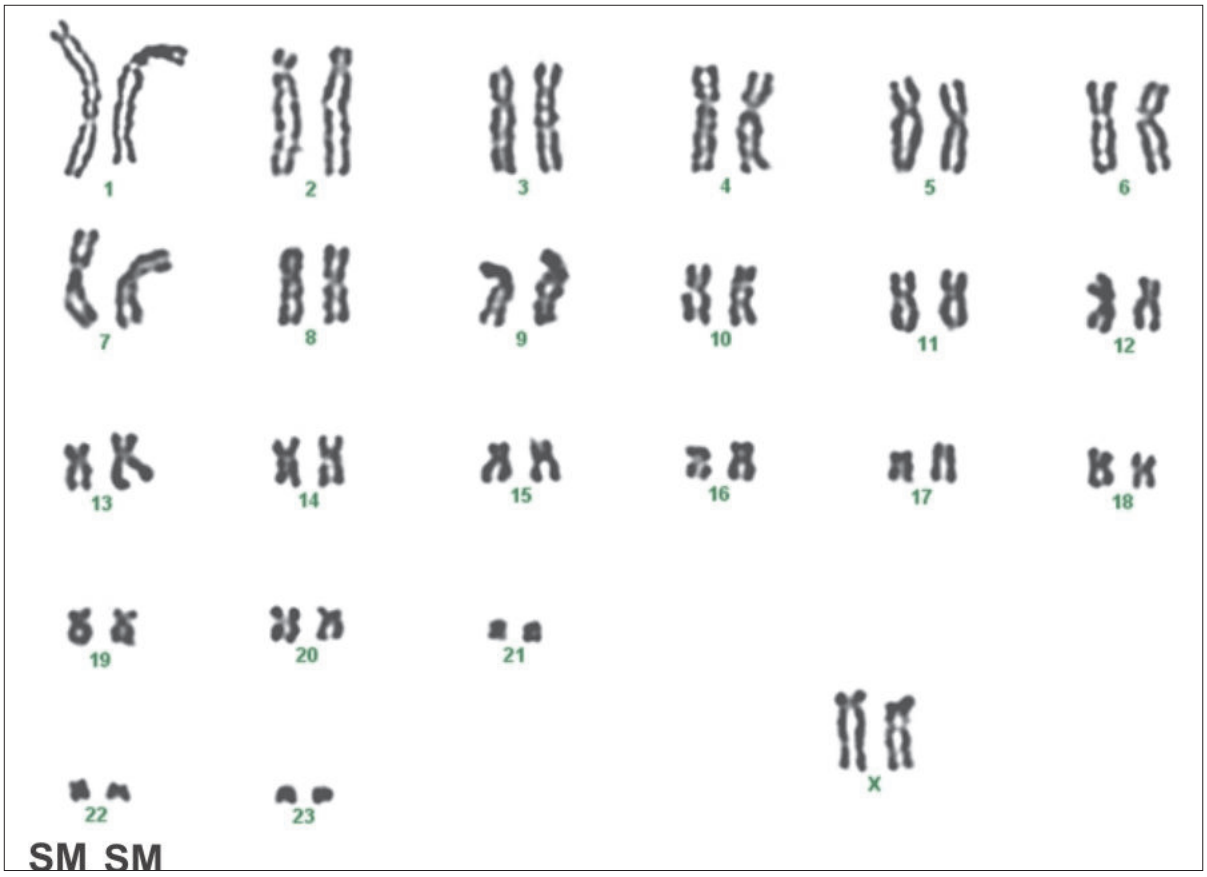

Fig. 3. Karyotype of female of *Jaculus jaculus*: 2n = 48 and NFa = 90 (SM = submetacentric).

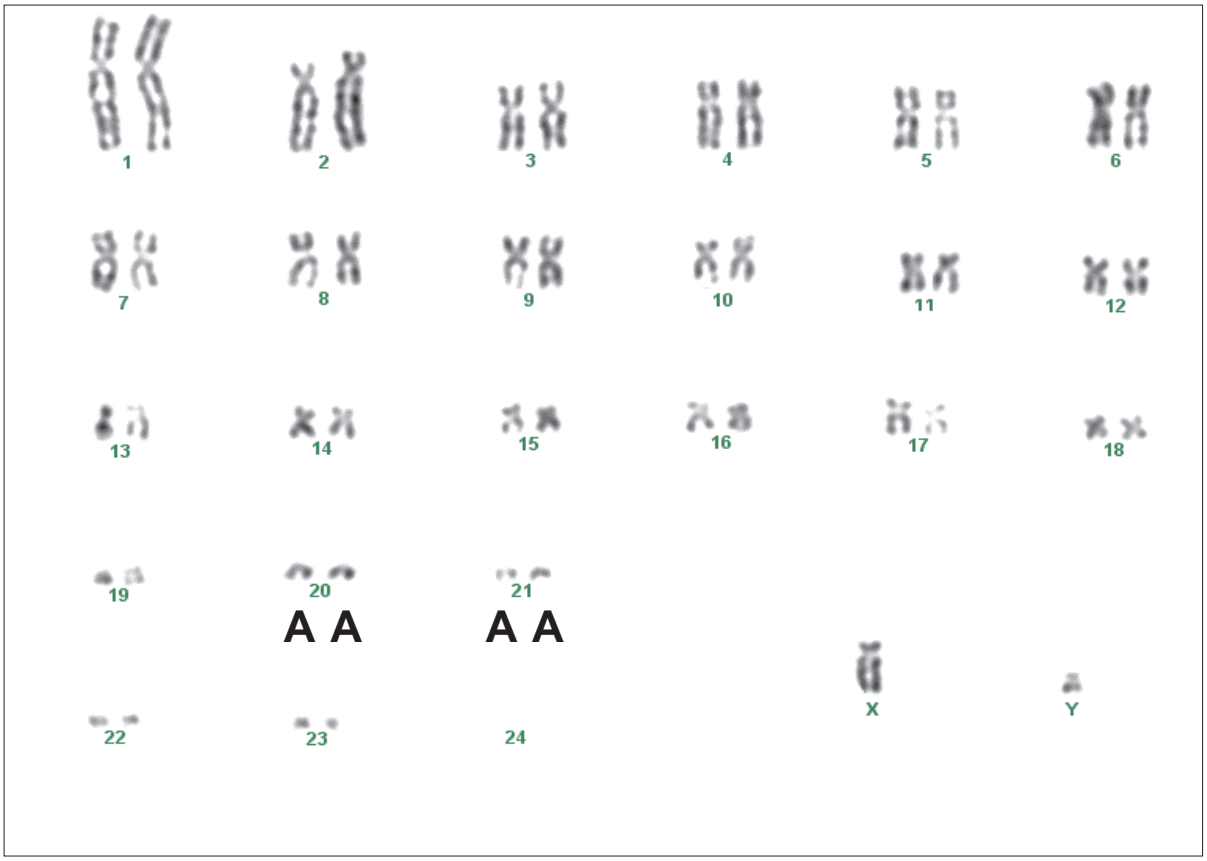

Fig.4. Karyotype of male of *Jaculus orientalis*: 2n = 48 and NFa = 84 (A = acrocentric).

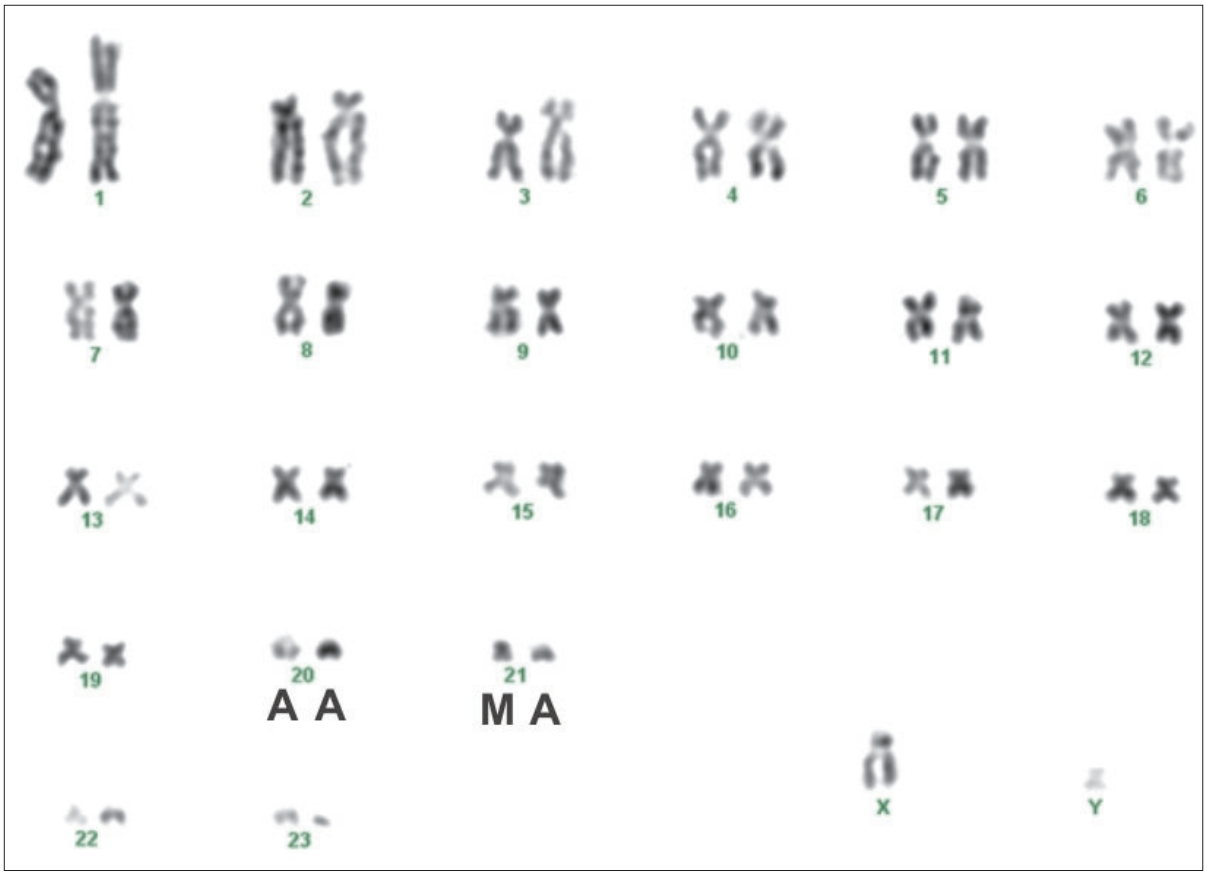

Fig.5. Karyotype of male of *Jaculus orientalis*: NFa = 85 (M = metacentric ; A = acrocentric).

Discussion

The karyotype (2n = 48) of the two congeneric species *J. jaculus* and *J. orientalis* from Tunisia exhibited obvious differences only in the autosomal pairs 20 and 21, as well as in the Y chromosome, yielding differences in the NFa. Pairs 20 and 21 were always submetacentric in *J. jaculus* whereas they were acrocentric in *J. orientalis* in almost all specimens, while pairs 22 (or 23) varied in morphology, especially in *J. jaculus*. In *J. jaculus* the Y chromosome was acrocentric, while it was submetacentric in *J. orientalis*.

AL SALEH and KHAN (1984) found 2n = 48 and NFa = 92 for *J. jaculus* in Saudi Arabia, the sex chromosomes X and Y being metacentric and acrocentric, respectively. SHAHIN and ATA (2001) showed the two species of Egyptian *Jaculus* to have 2n = 48 and a FN of 96 in females and 95 in males (corresponding to a NFa of 92). They considered the smallest pairs in both *J. jaculus* and *J. orientalis* as either subtelocentric or submetacentric (see their Table 1), thus biarmed, which is not clear from most of the karyotypes they published on various occasions (ATA & SHAHIN 1999; SHAHIN & ATA 2001; SHAHIN & ATA 2004). Moreover, they added that the two species displayed only variation in one pair (pair 20), metacentric in *J. jaculus* and submetacentric in *J. orientalis* (SHAHIN & ATA 2001). Our results, however, suggest that at least two of these four pairs of chromosomes are most of the time acrocentric in *J. jaculus* and *J. orientalis* in Tunisia. In addition, the Y chromosome was defined as acrocentric (actually telocentric; SHAHIN & ATA 2001) in both species in Egypt, while we tentatively recognized it as submetacentric in the specimens of *J. orientalis* examined herein. Furthermore, GRANJON *et al*. (1992), in an investigation carried out on rodents from Senegal, noted that the karyotype of one female of *J. jaculus* from the Djoudj National Park had a 2n = 50 and a NF of 90 (X chromosome not identified). Lastly, DOBIGNY *et al.* (2002) mentioned 2n = 48 and an NFa around 86 in low-quality preparations of two specimens of *J. jaculus* from Niger. All these data suggest that a large-scale polymorphism probably exists in natural populations of *Jaculus* species, especially in *J. jaculus* where it may even involve the diploid number. In this case, fusion/fission-type rearrangements or the presence of B-chromosomes (CAMACHO *et al*. 2000) may be responsible for the variation observed.

Intraspecific karyotypic variability was also found at the scale of Tunisia in the two species, mainly

associated with small differences in the morphology among the smallest chromosome pairs. This variation does not seem to be geographically structured, as different NFa were observed in the same locality in both species (see Table 1). These variations have been reported in several species of rodents in Africa such as *Arvicanthis ansorgei* (2n = 62; NFa = 74-76) and *Arvicanthis niloticus* (2n = 62; NFa = 62/64; VOLOBOUEV *et al*. 2002), *Mastomys erythroleucus* (2n = 38; NFa = 50-54; GRANJON *et al*. 1997; DOBIGNY *et al*. 2010). Such a polymorphism, which does not affect the diploid number, is often associated with rearrangements such as pericentric inversions (see DOBIGNY *et al.* 2010 for details), or heterochromatin addition (see VOLOBOUEV *et al.* 1988; MATSUBARA *et al*. 2004, among others). This kind of variation does not lead to major problems for the synapsis and segregation of the homologous chromosomes during meiosis in heterozygotes (KING 1993). Indeed, various wild rodent populations have shown high numbers of heterozygous individuals without any evidence of fertility reduction (GREENBAUM & REED 1984; HALE 1986; DOBIGNY *et al.* 2010).

As acknowledged in BEN FALEH *et al*. (2010), cytogenetic data in these jerboa species do not seem as discriminant as they often are in African rodent systematics, due to the apparently important chromosomal conservatism of the genus *Jaculus* (see references above and VORONTSOV and MALYGINA (1973) for *J. turcmenicus*). In this context, even though slight, the differences in chromosome morphology hypothesized here between *J. orientalis* populations from Tunisia and Egypt can be paralleled by the results of recent genetic investigations of the mitochondrial cytochrome b gene of samples collected from these two countries: two clades corresponding to populations of *J. orientalis* from Tunisia and Egypt were strongly supported by 100% bootstrap values in NJ, ML and Bayesian analyses (BEN FALEH *et al*. in preparation). At the same time, BEN FALEH *et al*. (2010), based on cytochrome b sequencing, craniodental and cytogenetic data of *J. jaculus* populations from Tunisia, evidenced two clades, which may likely correspond to the species *J. jaculus* and *J. deserti*. The specimens in each clade have a similar karyotype consisting of 2n = 48 chromosomes and NFa = 88-90, indicating that they apparently share the same chromosomal polymorphism.

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