Conspecificity of *Mastomys natalensis* (Rodentia : Muridae) from Senegal and South Africa : evidence from experimental crosses, karyology and biometry

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Summary. – On the basis of chromosomal analyses, *Mastomys natalensis* has recently been shown to occur in Senegal. To definitely prove the conspecificity of these animals with specimens from South Africa, where the species has been described, crossing experiments between individuals from both origins have been conducted. Karyological and biometrical comparisons between samples from these regions have also been performed. They have shown a very small chromosomal differentiation between individuals from Senegal and South Africa, and a morphometrical separation that can only be evidenced through multivariate discriminant analyses based on cranial and mandibular measurements. Crosses between animals from these two origins, as well as "backcrosses" and crosses between "hybrids" were all fertile, the latter having produced the largest litters on average. These results confirm the conspecificity of *M. natalensis* from Senegal and South Africa, while displaying some minor variations between these populations from the two extremes of the species' range.

Résumé. – Des populations référables à *Mastomys natalensis* sur la base d'analyses chromosomiques ont été récemment mises en évidence au Sénégal. Afin de prouver la conspécificité de ces individus avec ceux d'Afrique du Sud, pays où l'espèce a été décrite, des expériences de croisements en captivité entre des animaux des deux origines ont été menées. Des comparaisons caryologique et biométrique d'échantillons de ces régions ont été également réalisées. Ces dernières ont montré une très faible différenciation chromosomique entre les individus du Sénégal et d'Afrique du Sud, et une séparation morphométrique matérialisable uniquement par analyse factorielle discriminante sur un ensemble de mesures crâniennes et mandibulaires. Les croisements entre individus des deux origines, de même que les croisements en retour et les croisements entre « hybrides » ont tous été fertiles, les derniers nommés étant même ceux ayant produit les portées de taille moyenne la plus élevée. L'ensemble de ces résultats confirme la conspécificité des *M. natalensis* du Sénégal et d'Afrique du Sud, et met en évidence de petites différenciations entre ces populations situées aux extrémités de l'aire de distribution de l'espèce.

INTRODUCTION

A central tenet of the biological species concept (BSC) is that members of a species form a reproductive community, the integrity of which is maintained by iso-

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lating mechanisms against unsettling gene flow from other closely-related species (Dobzhansky 1937, Mayr 1942). Many criticisms of the BSC have focused on this problem of demonstrating the existence of reproductive isolation mechanisms (reviewed *in* King 1993, Sbordoni 1993, Mallet 1995, among others). This problem is more acute in the case of allopatric populations, which are unable to interbreed by virtue of their geographic separation. In such cases, captive-breeding provides a means of testing for the presence or absence of reproductive barriers between populations. However, even under these conditions, conclusions drawn are equivocal : an absence of reproductive success in captivity does not necessarily imply an inability to breed in nature ; and successful inter-breeding does not definitely prove conspecificity, as shown for mammals by the impressive list of interspecies hybrids produced in captivity (Gray 1971), and even by the known cases of hybrid fertility (Short 1976). Partly based on these criticisms, a number of alternative concepts integrating new methodological approaches have been developed (see for instance the multidimensional species concept of Sbordoni (1993) and the genotypic cluster definition of Mallet (1995)). In these new frameworks, reproductive data remain of value in the understanding and interpretation of evolution at the species level, but only as a complement to other data sets.

Our knowledge of the systematics and evolutionary history of the murid rodent genus *Mastomys* has improved considerably of late, thanks to the integration of different methodological approaches (see Britton-Davidian *et al.* 1995, for review). In this genus, the best tool for unambiguous species characterization remains chromosomal analysis, as each species seems to be characterized by a specific karyotype. As described in Britton-Davidian *et al.* (1995), the one of *M. natalensis* is typically composed of 32 chromosomes, with an autosomal fundamental number of 54. Nevertheless, intraspecific variation already identified in this species as well as in *M. coucha, M. erythroleucus* and *M. huberti* (Hallett 1977, Duplantier *et al.* 1990, Lavrenchenko *et al.* 1992, Britton-Davidian *et al.* 1995) raises the question of the potential polytypism in these species.

In this paper, we examine this question in *M. natalensis*. This species has recently been shown to occur in Senegal, where it is represented by exclusively synanthropic populations in the south-east of the country (Duplantier 1988, Duplantier and Granjon 1988, Granjon and Duplantier 1993). This suggests that its distribution probably covers almost all Africa south of the Sahelian zone. Cross-breeding experiments between specimens from the two extremes of this range (Senegal and South Africa) were conducted to evaluate inter-fertility between them. The results are compared with data from pairs between individuals from Senegal. Biometric and cytogenetic differences between samples from Senegal and South Africa are also presented, to quantify the level of differentiation reached by these geographically distant populations.

MATERIAL AND METHODS

Laboratory crosses

Reproductive data for *M. natalensis* from Senegal were generated using live-trapped individuals, which were paired and bred in captivity during three distinct periods : 1984-1986 (Dakar, N = 12 pairs), 1989 (Mbour, N = 4 pairs), 1990-1994 (Dakar, N = 20 pairs). Pairs were kept in cages with food (commercial pellets) and water provided *ad libitum,* and maintained at ambient conditions of temperature, humi-

dity and light. Data from pairs that were kept more than two months were considered (N = 36), this representing the average time between the pairing and the first litter.

The crosses between *M. natalensis* from Senegal and South Africa were performed using captive-born progeny of two pairs of each country. The wild-caught parents originated from Kedougou (Senegal) and Durban (South Africa). Eight of these inter-regional bidirectional pairs, each involving one individual from South Africa and the other from Senegal were considered, using the same criterion as for the Senegalese sample (minimum pairing time of two months). In 4 of these pairs, the male was from Senegal, in the 4 others from South Africa. These pairs produced litters hereafter referred to as F1 hybrids. These hybrids were either paired together to establish F2 crosses (N = 6 pairs), or backcrossed to individuals from Senegal (3 pairs) or South Africa (3 pairs). All inter-regional crosses were performed in Paris, at a temperature close to 25°C, with commercial pellets and apple supplied *ad libitum.*

Cages were checked every second day at the most. The sex-ratio of each litter was determined as soon as possible in most of the cases, and young were kept with their parents until weaning. In a few cases, testes were measured when adult males from these different crosses were sacrificed. This was done to obtain another indirect estimate of male fertility, as it has been shown that fertility impairment is generally associated with a significant decrease in testes size, even at the intraspecific level (Forejt and Ivanyi 1975).

Karyology

Two F1 male progeny of a cross between a female from Senegal and a male from South Africa were karyotyped. Chromosome spreads were prepared from bone marrow cells of yeast-stimulated individuals (Lee and Elder 1980) using the air-drying method (Evans *et al.* 1963) and stored at – 20°C in fixative. G-banding was performed following the method of Seabright (1971). Three to four G-banded metaphases were prepared for each animal using a Zeiss Axiophot microscope equipped with a Genevision analyser. Chromosome pairs were identified according to Britton-Davidian *et al.* (1995).

Biometry

Twenty two measurements (Fig. 1) were taken to the nearest 0.1 mm on 100 skulls of adult *M. natalensis* from Senegal (Kedougou and Bransan, 15 males, 20 females), South Africa (KwaZulu-Natal, 17 males, 13 females) and Tanzania (Chingulungulu and Morogoro, 16 males, 19 females). No attempt was made to account for age and sex effects, in order to maximise the variability in each of the samples. These measurements were subjected to principal component and discriminant analyses using the software programme SYSTAT (1992).

RESULTS

Laboratory crosses

In the course of these experiments, certain pairs did not reproduce within the two-month threshold defined above : only 22 out of 36 (61 %) pairs from Senegal and 6 out

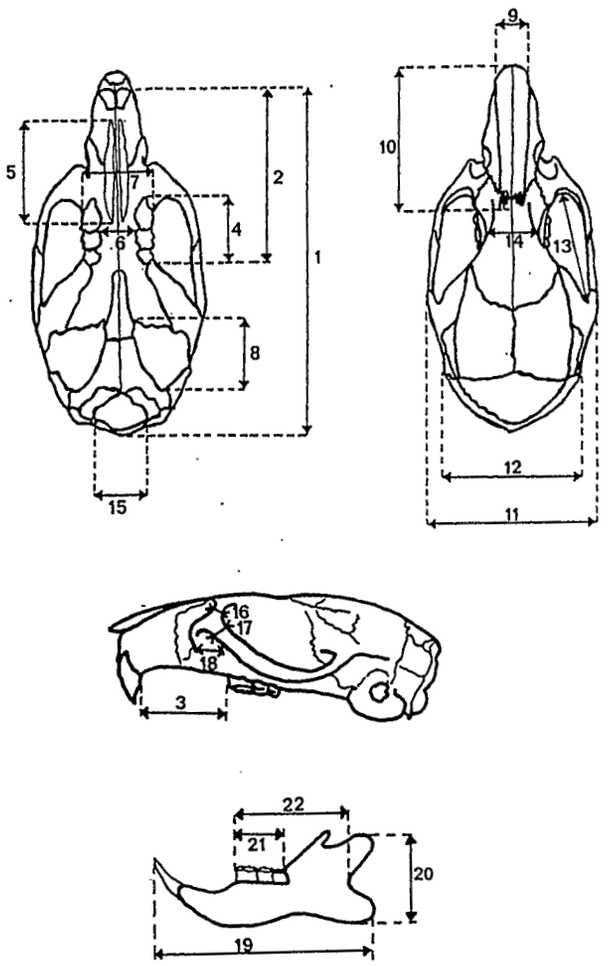

Fig. 1. – Measurements taken on skulls and mandibles of *M. natalensis*.

of 8 (75 %) F1 Senegal x South Africa pairs reproduced, whereas all backcrosses and F2 crosses were successful.

The results presented in Table 1 concern only those pairs that actually reproduced. No difference was found between the two types of backcrosses (F1 x Senegal or F1 x South Africa), so the results were pooled for statistical analyses. The same was done for the results of both combinations in bidirectional pairings. Backcrosses produced the smallest litters (5.35 young/litter), whereas F2 pairs gave birth to relatively large litters, with a mean size of 8.31 young/litter. Statistical comparisons between these series are provided in Tab. 2 (Wilcoxon-Mann Whitney test, Siegel and Castellan 1988). The size of litters produced by the F2 crosses was significantly larger than that of crosses between wild-caught individuals from Senegal or backcrosses.

Sex-ratios at birth were balanced in all cases (Tab. 1). As for intervals between litters, the mean values in our experiments ranged between 30.3 days (N = 10, SD = 8.8) for F1 crosses to 46.8 (N = 37, SD = 22.9) for intra-Senegal ones (Tab. 1). The difference between these two values was the only statistically significant one (t test, p = 0.034).

The testes of 17 males (4 from Senegal, 2 from South Africa, and 11 F1 hybrids) were measured following sacrifice. There was no difference (t-test) between the mean length of testes of parental males (N = 6, $\bar{x}$ = 15.25, SD = 1.84) and that of F1s (N = 11, $\bar{x}$ = 15.32, SD = 1.21).

TABLE 1. – Reproductive data in different samples of *M. natalensis* (sex-ratio has not been systematically recorded).

	Nb pairs	Nb litters	Nb young born	litter size ± SD	sex-ratio (m/f)	interval (d) between litters + SD (sample size)
South-East Senegal	22	58	348	6.0 ± 2.1	37/52	46.8 ± 22.9 (N=37)
Senegal x S.Africa	6	15	93	6.2 ± 3.1	44/49	30.3 ± 8.8 (N=10)
" hybrids " x " parents "	6	17	91	5.4 ± 2.7	44/38	37.1 ± 13.7 (N=11)
" hybrids " x " hybrids "	6	16	133	8.3 ± 3.4	75/54	38.2 ± 12.5 (N=10)

TABLE 2. – Results of pair-wise non parametric Wilcoxon-Mann Whitney tests between litter sizes in different types of crosses of *M. natalensis*.

	Senegal x South Africa	"hybrids" x "parents"	"hybrids" x "hybrids"
" hybrids " x " parents "	z = 0.724; p = 0.469		
" hybrids " x " hybrids "	z = 1.709; p = 0.087	z = 2.484; p = 0.013	
Senegal x Senegal	z = 0.165; p = 0.869	z = 1.123; p = 0.261	z = 2.744; p = 0.006

Chromosomal analysis

The karyotypes of the two male F1 hybrids (Fig. 2) showed the expected diploid number for *M. natalensis* (2n = 32 ; autosomal fundamental number = 54). Chromosome pairs were identified and showed a G-band pattern similar to that previously published in Britton-Davidian *et al.* (1995) for this species in Senegal. Two exceptions were noted in chromosome pairs 3 and 12 which were dimorphic, one member of each pair corresponding to the G-band pattern of Senegalese *M. natalensis*. In pair 3, the

other chromosome member showed an additional segment near the centromere and in pair 12, a more intense staining of the centromeric region was observed. Both of these differences are present in the karyotype of *M. natalensis* from Zimbabwe published by Lyons *et al.* (1980). These results suggest that the karyotypes of *M. natalensis* from Zimbabwe and from Durban in South Africa share similar G-banding patterns and show only slight differences with those of *M. natalensis* from Senegal.

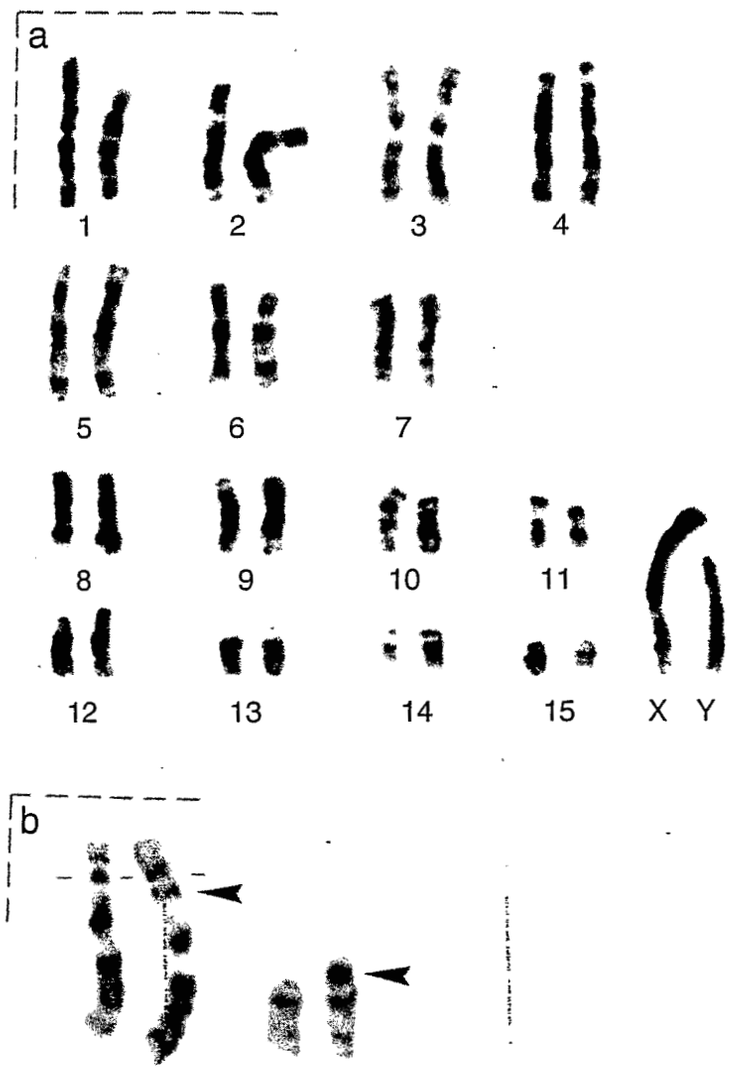

Fig. 2. – a) G-banded karyotype of a male F1 hybrid. b) Insert showing dimorphism in chromosome pairs 3 and 12. Dashes indicate the position of the centromere ; arrows higlight the additional segments corresponding to the G-band patterns of the karyotype of the South African parent.

Biometrical analysis

Principal component analysis (PCA) yielded no clear separation between the 3 samples studied (Senegal, South Africa, Tanzania). The use of discriminant analysis led to better separation between them (Fig. 3), but some overlap was still evident. The variables that participated the most to the overall discrimination between the three groups were n° 1, 4, 8, 9, 13, 14, 15, 16, 17, 21 and 22 (F tests, $p < 0.005$), indicating that both differences in size and shape were occurring. Three of the 35 individuals from Senegal were classified to the incorrect *a priori* groups (2 to the South African sample, 1 to the Tanzanian one), while 1 individual from Tanzania was classified into the South African sample.

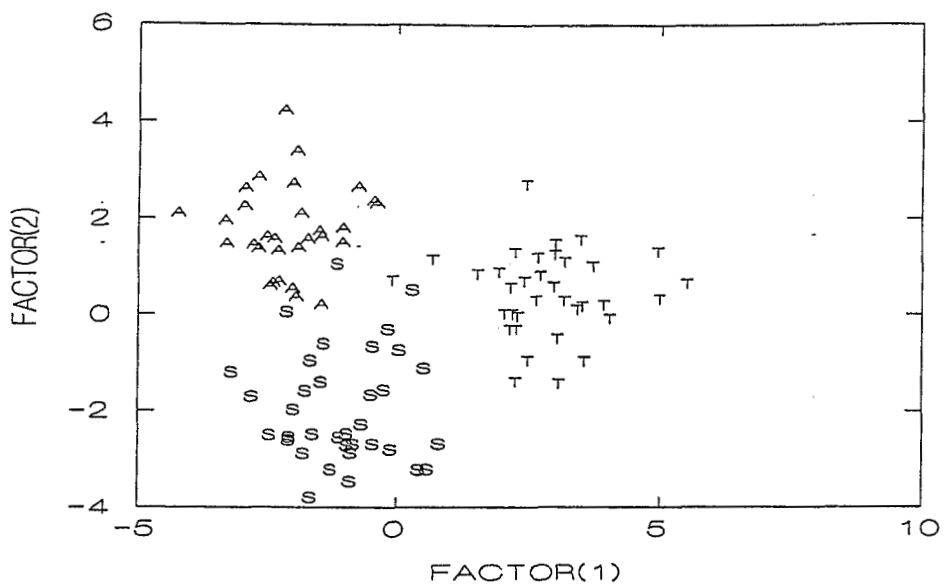

Fig. 3. – Scatterplot of the scores of *M. natalensis* individuals in the discriminant space defined by the first two factors ; Individuals from A : South Africa, S : Senegal and T : Tanzania.

DISCUSSION

General considerations

Oliff (1953) and Meester (1960) published results on the reproductive performances in captive colonies of what they called *M. natalensis*. However, the stocks used by these two authors were obtained from the vicinity of Johannesburgh and Pretoria respectively, where only *M. coucha* has been recorded (Hallett 1977, Green *et al.* 1980). The animals they studied were thus probably *M. coucha,* which precludes comparison with our study.

The rather low reproductive success observed in the case of intra-Senegal pairs (only 61 % of the pairs produced at least one litter) may be due to the fact that these

pairs were constituted from wild-caught individuals, whereas the Senegal x South Africa crosses were performed with captive-born animals. *N. natalensis* is known to be quite aggressive to conspecifics (Hallett 1977, Coetzee 1975, Granjon and Duplantier 1993), and this may be more the case in wild-caught than in captive-born individuals, thereby leading to a reduction in mating activity.

Conspecificity of M. natalensis *from Senegal and South Africa*

Intraspecific cross-breeding experiments between individuals from allopatric origins have seldom been conducted in African rodents. Petter *et al.* (1969) demonstrated complete interfertility between *Arvicanthis niloticus* from Ethiopia and Senegal, as well as between their progeny. Kaminski *et al.* (1984) described the same result between *A. niloticus* from Senegal and Egypt. Chromosomal studies on specimens from the latter two countries further demonstrated their karyotypic identity (Volobouev *et al.* 1988). The recent experiments of Pillay *et al.* (1992, 1995) concerned samples from different populations of *Otomys irroratus* that were not separated by a very large geographic distance, but had distinct karyotypes. This chromosomal differentiation resulted in a severe reduction of reproductive performances in interpopulation crosses, and of hybrid fitness.

In our case, only slight chromosomal differences have been evidenced by G-banding analyses between *M. natalensis* from Senegal and South Africa (see insert of Fig. 3). Similarly, our preliminary biometric results indicated no clearcut differentiation between samples of *M. natalensis* from Senegal and South Africa. While the lack of any separation in PCA may be understandable given the difficulties in distinguishing the different species of the genus by this type of analysis (Duplantier 1988, Dippenaar *et al.,* 1993), even the use of discriminant analysis (which maximises inter-group separation), did not lead to a complete discrimination among the samples.

The slight chromosomal and biometrical differences we observed are not *a priori* likely to induce any post-zygotic barrier to reproduction. However, such barriers may also originate from genic or behavioural incompatibilities. The results obtained clearly indicate that no such problems seem to exist. All types of crosses between Senegalese and South African *M. natalensis* were successful, particularly those involving F1 hybrids. Moreover, F1 hybrid males had testes of the same size as parental individuals, suggesting that their reproductive potential was not impaired. Crosses between F1 hybrids were the most successful in terms of mean litter size. Given the relatively small samples considered here, this result may be due to chance. The alternative hypothesis would involve "hybrid vigour", which may correspond to a lower rate of embryo resorption in these F2 crosses than in the other types of crosses. In preliminary support of this hypothesis, we observed upon necropsy that two F1 females involved in backcrosses had 11 and 13 embryos, whereas three F1 females sacrificed after several months of pairing with F1 partners had 7, 9 and 10 embryos. For comparison, Duplantier *et al.* (1996) found a mean number of embryos of 8.6 (SD = 2.6) in 30 females of *M. natalensis* from Senegal.

On the basis of these results, we conclude that *Mastomys* from Senegal and South Africa characterized by a diploid number of 32 chromosomes and an autosomal fundamental number of 54 chromosomal arms are conspecific. The small karyotypic and biometrical differences observed are fully compatible with the concept of genotypic or morphological clusters described in Mallett (1995). Local differentiation are likely in a species with such a large distribution area as *M. natalensis,* and may be promoted by differences in local ecological situations within its range, without involving the deve-

lopment of any reproductive barriers. This would be worth testing in such situations as the one described by Lavrenchenko *et al.* (1992) in Ethiopia, where 3 forms of *Mastomys* with apparently similar karyotypes but different hemoglobin patterns, are found.

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