THE ROYAL SOCIETY

Microgeographical distribution of two chromosomal races of house mice in Tunisia: pattern and origin of habitat partitioning

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Two chromosomal races of the house mouse occur in Tunisia, a standard morph (40St) found all over the country, and a derived morph (22Rb) occurring only in central Tunisia. In this region, habitat partitioning between the two morphs was investigated by a microgeographical analysis of their distribution, assessing habitat characteristics and demographic parameters. Results showed that the 22Rb mice always occurred in the oldest sections of towns (medinas), often extending to more recent surrounding neighbourhoods where the 40St morph was most abundant. The latter was never trapped within the medinas. The transition between the two morphs was located within cities in the more recent areas, the hybrid zone being estimated at less than 0.5 km in width by a clinal analysis of chromosomal data. Although differences between habitats exist, almost no demographic differences were found between populations of the two morphs when they occurred in the same or in different habitats. Two hypotheses are discussed to account for the origin of habitat partitioning. The first relies on competitive exclusion of the 40St mice from the medinas by the derived 22Rb mice; the second is based on stochastic processes related to historical evolution of Tunisian urban communities.

Keywords: chromosomal races; cline; habitat partitioning; house mice; hybrid; Robertsonian fusions

1. INTRODUCTION

Chromosomal divergence is observed in many species and the role of such a process in speciation is regularly debated (White 1968; King 1993; Searle 1993). The main hypotheses of speciation involving chromosomal rearrangements are based on the observation that when two taxa with rearranged karyotypes meet parapatrically and hybridize, the chromosomal heterozygosity of hybrids may lead to a reduction in fertility and even to sterility, owing to malsegregation and germ-cell death during gametogenesis. Chromosomal rearrangements could thus contribute to reproductive isolation and promote genic changes leading to speciation (Sites & Reed 1994).

Chromosomal variation in the house mouse (*Mus musculus domesticus*) occurs in Europe and North Africa within the range of the standard type ($2n = 40$ acrocentric chromosomes). Chromosomal differences among populations are due to centric translocations (Robertsonian fusions, Rb) leading to a reduced chromosomal number ($2n = 39$–22). Contacts between chromosomal races yield hybrid zones varying in width and structure, depending on the degree and type of chromosomal underdominance (Searle 1993). In Tunisia, only two karyotypic races are present, one of which has accumulated nine pairs of fusions ($2n = 22$ chromosomes; 22Rb race), and is restricted to the central region of the country where it is sympatric with the standard morph (40St race), the latter occurring all over the country (Saïd *et al*. 1999). Saïd & Britton-Davidian (1991) reported that the 22Rb race is characterized by a patchy distribution as it is present only in urban centres surrounded by the standard morph, which occupies peripheral zones as well as rural villages, whereas outside the area of sympatry the 40St race occurs in both types of habitat. Contact between these two chromosomal races leads to sparsely distributed hybrid populations. Although the fertility of hybrids between the two morphs was found to be partly reduced, experimental matings between parental genotypes were successful (Saïd *et al*. 1993). Hence, the scarcity of hybrid populations was not predicted by the fitness level of chromosomal heterozygotes, nor was it related to topographical barriers. This contrasts with other chromosomal hybrid zones in house mice, where the structure is largely thought to be determined by such factors (Hauffe & Searle 1993; Searle *et al*. 1993). From their preliminary survey, Saïd & Britton-Davidian (1991) hypothesized that the two races were adapted to different types of commensal habitat. These habitats can be very variable depending on availability of resources and shelter and their stability through time (Boursot *et al*. 1993). These factors can affect demographic

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parameters such as density and reproduction (Laurie 1946; Bronson 1979; Singleton 1989) as well as rates of extinction. Thus, although both chromosomal morphs in Tunisia inhabit anthropogenic structures, variation in commensal habitat structure may influence their dynamics and evolution.

The objectives of the present study were to: (i) characterize the distribution of the chromosomal races and localize the contact zone at a microgeographical level by intensive sampling; (ii) determine habitat characteristics and their distribution between the karyotypic morphs; and (iii) compare demographic parameters (density, sex ratio, proportion of breeding females and body mass) between the chromosomal races in the different microhabitats. The relationship between habitat partitioning and distribution of the chromosomal races as well as of their hybrids is discussed. In addition, adaptive and stochastic patterns that could account for the patchy distribution of the 22Rb race are presented to assess the origin of habitat partitioning.

2. MATERIAL AND METHODS

(a) *Trapping procedure*

Mice were caught using traps placed in houses, shops, farm buildings, gardens and cultivated fields in different localities (towns and villages) of central Tunisia (figure 1). Traps were set in a total of 303 sites consisting of 281 indoor sites and 22 gardens or agricultural fields between 1994 and 1996 (April–May 1994, October 1995, May–July 1996). Of these sites, 168 produced mice. Single- (Firobind, Longworth, Sherman) and multiple-capture (a wire mesh hemispherical trap) traps were baited with apple or sardine paste. The number of traps depended on room size but a minimum of two traps (or one multiple-capture trap) was set at each site. Traps were set during two to seven nights depending on trapping success. Data on the macrogeographical distribution of mice were complemented with 35 mice collected between 1983 and 1989 from villages and towns throughout Tunisia (Saïd & Britton-Davidian 1991).

(b) *Demographic data*

Analysis of demographic data was performed on mice trapped during the 1995–1996 field sessions, which yielded a total of 552 mice for 1805 trap-nights. Demographic characteristics consisted of parameters that could be collected by using the trapping-with-removal method: sex ratio, population age structure and indices of abundance and fertility. At capture, each mouse was sexed and weighed to the nearest 0.5 g and its tail and head + body length were measured to the closest millimetre. Age structure was estimated by assigning mice to one of two categories: juvenile when head + body length was less than 6.6 cm; adult when the length was equal to or higher than 6.6 cm. This limit was determined by the smallest size at which a female was found to be pregnant (see also Chambers *et al.* 1996), because no other rigorous criterion was available. The same value was used for males, on the basis of the almost synchronous timing of sexual maturity between sexes (Pelikan 1981). However, because sexual maturity depends on environmental conditions (food availability, temperature, etc.; see Bellamy 1981; Pelikan 1981), such a procedure necessarily hypothesizes similar conditions for all mice. Females were considered to be breeding if lactating or pregnant, and the ratio of the number of breeding females to the total number of adult females was calculated. Data on embryo litter size were collected but were too limited to be presented here (range 1–6; $n = 6$). Male reproductive state based on the position of testes was unreliable and therefore disregarded. Population size was expressed as the number of mice per 100 trap-nights (when only single-capture traps were used), and the number of mice per night (when different types of trap were used). The sex ratio was calculated as the number of males:females.

(c) *Chromosome preparations*

After capture, mice were kept under standard conditions (temperature 20–25 °C, photoperiod 12 L : 12 D) until karyotyped. Chromosomes were prepared from a suspension of yeast-stimulated bone marrow cells (Lee & Elder 1980) by the air-drying method. Diploid number was determined by examining five to ten well-spread preparations under a Zeiss Axiophot photomicroscope. All mice from hybrid populations and 85% of those from homozygous sites were karyotyped.

(d) *Habitat description*

Two criteria based on direct observations were determined to describe house mouse habitats. The first was related to the architectural style, age and use of human dwellings; the second referred to the distance between potential sites within each type of habitat. Four categories were distinguished for the first criterion, as follows.

Category A corresponded to habitations, shops and industrial buildings when they occurred inside the typical old Arab town (medina), which consisted of very close and often single-storey buildings in narrow and usually dead-end streets. Although the oldest parts of the medinas date from the Arab period (mainly the eighth to the tenth century AD), in most cases extensions were added contiguously following the same architectural style (Lowy 1986).

Category B corresponded to the same type of sites when located in neighbourhoods other than the medinas; the oldest areas were built during the 16th century, but most date from the past 50 years.

Category C was assigned to sites in industrial zones, and category D to agricultural fields, gardens or greenhouses. These two categories of habitat were often located at the periphery of towns. In the D type of habitat, mice were sometimes trapped with wild mice (*Mus spretus*), whereas in categories A, B and C rats (*Rattus rattus*) were the only other rodent present.

The second criterion was an index of site dispersion within the four habitat categories, and was determined by reference to the available information on average migration distance of mice (about 50 m per generation) (Berry & Jakobson 1974; Cassaing & Croset 1985; Baker & Petras 1986). Habitat was considered as continuous when sites were contiguous with other potential ones (i.e. there was no distance between them) and was then assigned an index value of 0. The two other indices described a discrete distribution with two levels of site dispersion: index 1 when sites were separated by less than 50 m, and index 2 when they were more than 50 m apart. Although the two criteria resulted in 12 possible combinations, only eight were observed in the field (A0, B0, B1, C0, C1, C2, D1, D2).

(e) *Data analyses*

Distribution of karyomorphs between habitat categories A–D was investigated for 174 sites from 18 towns throughout Tunisia (figure 1), including data collected from 1983 to 1996.

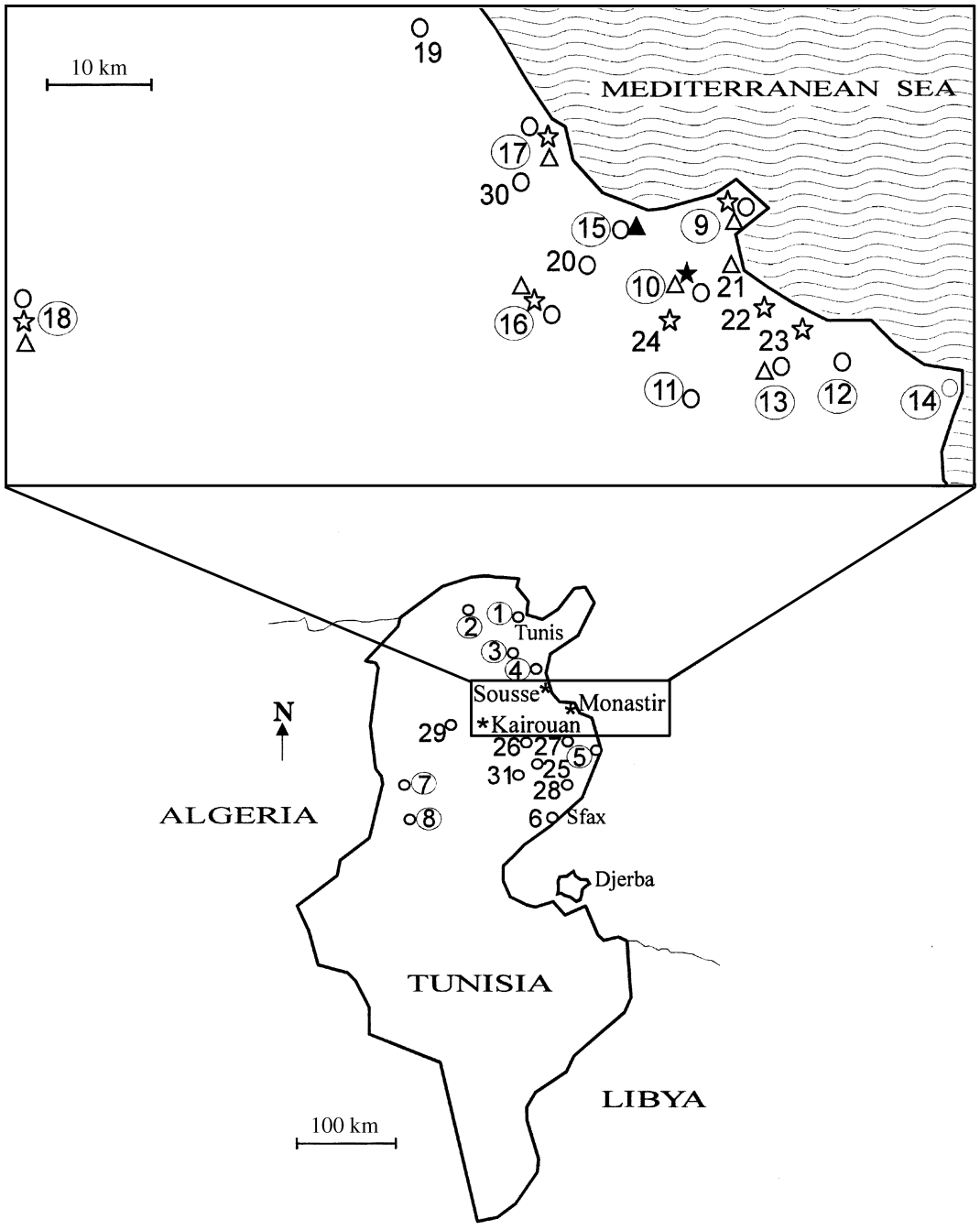

Figure 1. Distribution of localities where mice were sampled with reference to their chromosomal type. The map includes data collected by Saïd & Britton-Davidian (1991). Empty circles, standard populations; empty triangles, 22Rb populations; empty stars, hybrid populations. Filled symbols indicate that the chromosomal morph observed in a previous sampling period was missing in 1995 and 1996. Circled localities are those included in the present study. Localities: 1, Tunis; 2, Tebourba; 3, Grombalia; 4, Enfida; 5, Mahdia; 6, Sfax; 7, Sbeitla; 8, Sidi Bouzid; 9, Monastir; 10, Bembla; 11, Jemmal; 12, Moknine; 13, KsarHellal; 14, Teboulba; 15, Sahline; 16, M'saken; 17, Sousse; 18, Kairouan; 19, Sidi Bou Ali; 20, Maatmer; 21, Khenis; 22, Ksibet; 23, Lamta; 24, Menzel Ennour; 25, Smiret; 26, Zeramdine; 27, Benni-Hassen; 28, Jebiniana; 29, Chebika; 30, Zaouet Sousse; 31, Bou-Merdes.

Each site was characterized first by its habitat and second by the karyotypes of its mice. Sites bearing mice with different karyotypes ($22 \leqslant 2n \leqslant 40$) were considered as hybrid, and those where only one of the parental karyotypes was present were considered as belonging to one or the other of the chromosomal races. The distribution of the two chromosomal morphs among categories of habitat and indices of dispersion was analysed by using the *G*-test of independence (Sokal & Rohlf 1995). This analysis was performed for the seven towns in central Tunisia where either all three morphs or at least the 22Rb one were present (Monastir, Kairouan, M'saken, Sousse, Bembla, Jemmal and Ksar Hellal). Detailed mapping of karyomorph and habitat distribution was established for the first four of these towns.

Clinal variation in diploid number was investigated following a tension-zone model with the Monastir data. Geographic distance was measured by the radial distance between each site and the approximate centre of the medina (see figure 2). The

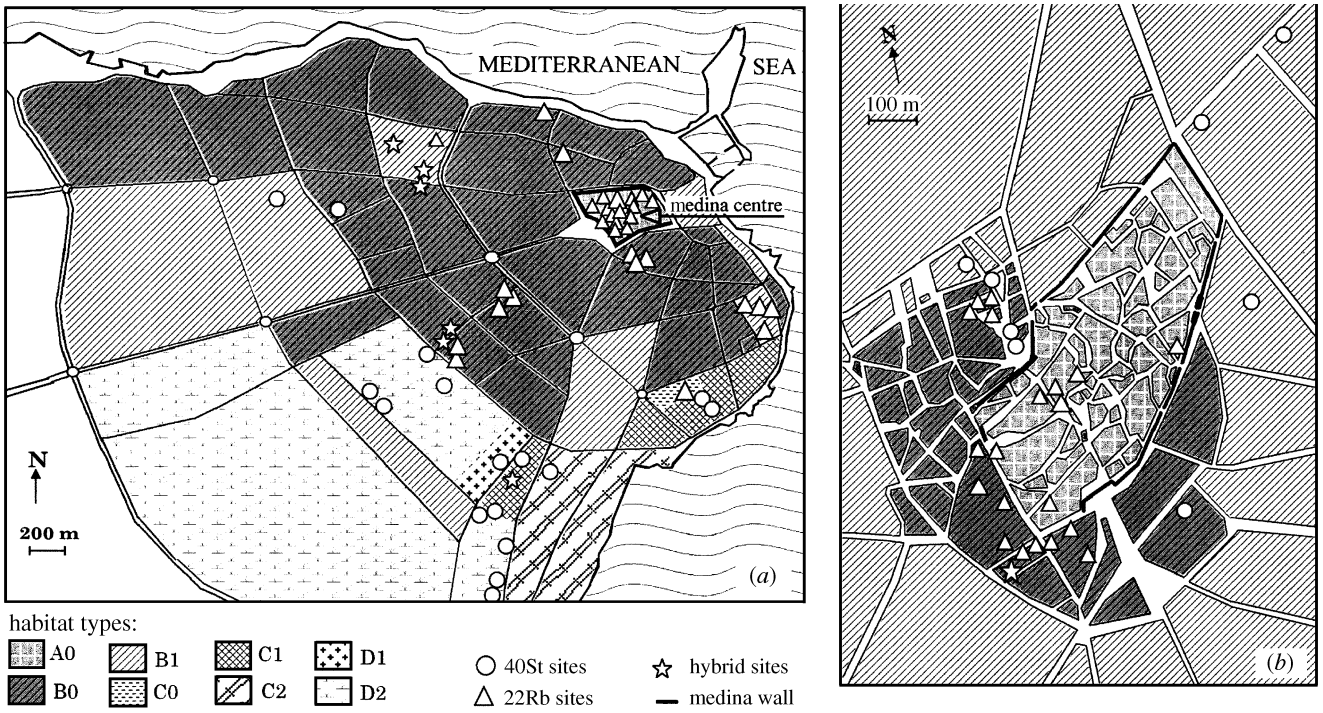

Figure 2. Microgeographical distribution of mice according to habitat and chromosomal types in (*a*) Monastir and (*b*) Kairouan. See text for description of habitat characteristics. *In situ* observations allowed us to extend habitat indices, determined for all sites where traps were set, to whole neighbourhoods.

frequency of fusions was estimated from the diploid numbers and fitted to a scaled logit function:

$$f(x) = \frac{e^{w(x-c)}}{1 + e^{w(x-c)}},$$

where x is the radial distance, w the width and c the centre of the cline (Barton & Gale 1993). The fit of the model was tested and estimates of these parameters were computed by using the GLIM package. Overdispersion was corrected for according to Crawley (1993).

The symmetry in the distribution of diploid numbers within hybrid populations was tested by comparing the number of mice carrying many fusions ($2n = 23$–30) with those with few ($2n = 39$–32) by means of Wilcoxon's signed-rank test.

Demographic parameters were analysed by using a non-parametric analysis of variance (Kruskal–Wallis ANOVA). The decision to reject the null hypothesis was based on probabilities lower than or equal to a level adjusted to the number of tests performed, according to the sequential Bonferroni method (Rice 1989). We first compared karyomorphs and habitat structure and checked for a locality effect. As the latter was not significant, we focused on habitat characteristics by grouping localities according to karyotype and habitat structure. Gamma correlation tests were used to further analyse effects of factors yielding a level of significance lower or equal to 0.05.

Numbers of juveniles and adults were compared (Wilcoxon's signed-rank test), and the effects of trapping session, habitat and karyotype on the distribution of their ratios were analysed by using the Kruskal–Wallis ANOVA. The relationship between size and mass and effects of different parameters on this relationship were investigated by using covariance analyses, and the mean ratio of males to females was compared to 1 using a Student's t-test. Unless otherwise stated, all analyses concerned data presented in table 4. For a given test the sample size is provided by the number of rows in the column.

3. RESULTS

(a) *Habitat partitioning*

Analysis of habitat distribution among the karyotypic morphs showed a significantly non-random pattern in central Tunisia (table 1), whether the number of individuals ($G = 228.6$; $p < 10^{-6}$) or of sites was considered ($G = 66.2$; $p < 10^{-13}$). In this region, the 22Rb morph was common in both habitats A and B, whereas the 40St race was absent from habitat A and most abundant in habitat B. In habitats C and D, the 22Rb race was rarer than the 40St one. Hybrid populations were found in all habitats except D, but were most frequent in habitat B. Elsewhere in Tunisia, the 40St morph occurred in habitat A as well as B and D (habitat C was not sampled). These results clearly indicated that in central Tunisia no 40St mice were present in the type A habitat, which was exclusively occupied by the 22Rb morph and a few hybrid populations. This habitat corresponded to the medinas, which in most cases were surrounded by the type B habitat where all morphs were frequent. The local distribution of habitat and chromosomal types is exemplified by the detailed mapping of four of the oldest towns, two of which are presented in figure 2.

In Monastir, traps were set in 94 sites, 53 of which yielded 196 mice. Mice of the 22Rb morph were found in the medina and contiguous areas (A and B habitats), whereas standard mice occurred in all habitat categories except A. Hybrid populations were found in six sites dispersed along the contact zone within the B and C habi-

Table 1. *Habitat distribution of individuals carrying different karyotypes*

(The central region includes the seven towns where at least 22Rb mice were trapped; other regions represent localities where only $2n = 40$ mice were trapped. The corresponding number of sites is indicated in parentheses.)

		habitat structure				
region	$2n$	A	B	C	D	total
central	22	148 (44)	145 (43)	6 (3)	3 (1)	302 (91)
	40	0 (0)	86 (23)	29 (10)	41 (12)	156 (45)
	hybrid	7 (3)	42 (10)	8 (2)	0 (0)	57 (15)
other	40	19 (2)	99 (20)	—	7 (3)	125 (25)

Table 2. *Distribution of individuals carrying different karyotypes according to site dispersion indices within habitat B*

(Samples refer to the seven towns in central Tunisia where at least 22Rb mice were trapped. The corresponding number of sites is indicated in parentheses.)

	habitat dispersion		
$2n$	B0	Bl	total
22	124 (36)	21 (7)	145 (43)
40	33 (6)	53 (17)	86 (23)
hybrid	17 (4)	25 (6)	42 (10)

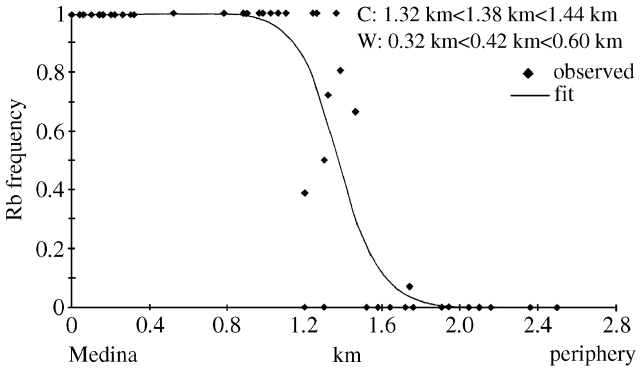

Figure 3. Clinal distribution of the frequency of Rb fusions along a radial medina-to-periphery axis in Monastir. The estimates of the centre (C) and width (W) of the cline with their respective 95% confidence limits are indicated.

tats (figure 2*a*). In Kairouan, out of the 57 trapping sites, 31 produced a total of 112 mice (figure 2*b*). Mice of the 22Rb race were found in the medina and the western and southern parts of the city, which corresponded, respectively, to the A and B types of habitat. Mice with a standard karyotype occurred in B habitats surrounding the medina. No hybrid populations were found in the north-western part of the city where the closest sites bearing the two karyomorphs were less than 10 m apart. Only one hybrid sample was recorded in the south-western part of the city (habitat B) in an area mainly inhabited by the 22Rb morph. Out of the 36 sites sampled in Sousse, 21 produced 59 mice. Only 22Rb mice were found in the old town defined by habitat A. Mice of the latter morph also occurred in surrounding areas characterized by habitats B and D to the north, and C towards the south. In the latter habitat, standard mice were also found and there was also one site with hybrid individuals. In M'saken, 45 sites were sampled along a south–north transect within the town, 21 of which produced 52 mice. Standard mice were found in the southern (B and D habitats) and eastern (B habitat) parts of the town, while the centre and northern part, respectively characterized by A and B habitats, harboured 22Rb mice, but also hybrids, which were trapped at five sites.

(b) *Within-habitat site dispersion*

The overall and detailed analyses of habitat distribution clearly indicated that both morphs were abundant in habitat B, suggesting that the transition from one karyotype to the other occurred within this habitat category. This is supported by the presence in this habitat of 74% of the hybrid individuals (table 1). Habitat B also represents the transition between site dispersion indices: habitat A carries only index 0, and C and D are almost exclusively defined by indices 1 and 2 (one site in habitat C was ascribed to index 0; figure 2*a*), whereas both B0 and Bl are present in habitat B. To determine whether the distribution of the two karyomorphs was related to within-habitat site dispersion, the chromosomal data were compared between the two dispersion indices (0 and 1) within habitat B (table 2). The results showed significant differences in their distribution (number of mice: $G = 55.2$; $p < 10^{-12}$; number of sites: $G = 21.9$; $p < 10^{-5}$). The 22Rb were more abundant in the B0 habitat, whereas the 40St mice were more common in Bl sites. Hybrid individuals were slightly more frequent in habitat Bl than in B0.

(c) *Clinal and hybrid distribution*

Distribution of diploid numbers in Monastir along a radial axis fitted the clinal model tested ($F_{1,51} = 273.8$; $p < 10^{-4}$), which provided estimates of the cline centre and width (figure 3). The clinal transition from one karyomorph to the other was very steep, the width being estimated at less than 0.5 km. The chromosomal analysis of the 15 hybrid populations (table 3; figure 4) indicated that individuals with intermediate diploid numbers ($23 < 2n < 39$) were not frequent; this low frequency may be related to their higher selective disadvantage. The only exception to this pattern was the relatively large number of $2n = 31$ individuals, which most probably represented a mixture of recombinant and first-generation hybrids; this observation suggests that direct contact between both

Table 3. *Distribution of diploid numbers and habitat indices among the 15 hybrid populations in five Tunisian cities*

locality	2*n*	habitat
Monastir	3 × 23, 2 × 24, 2 × 25, 2 × 26, 2 × 28, 31	B0
	27	B0
	2 × 31	B0
	31, 35	B1
	26, 27, 31	B1
	31, 6 × 40	C1
Kairouan	38, 39	B0
Sousse	24	C1
Bembla	3 × 31, 40	B1
	2 × 22, 23	B1
M'saken	31	A0
	31	A0
	4 × 22, 23	A0
	24, 2 × 25, 2 × 26, 28	B1
	4 × 22, 3 × 23	B1

karyomorphs might not be uncommon. In addition, the shape of the chromosomal distribution within the hybrid populations suggested that an excess of hybrids carrying many fusions might be present. As this skewness could be related to the position of the hybrid sites within the cline, the data were analysed separately among the two dispersion indices, 0 characterizing the 22Rb side of the cline and (1 + 2) the 40St site (figure 4). The symmetrically paired distribution of hybrid diploid numbers within each of these groups was compared ($31 < 2n \leqslant 39$ compared with $31 > 2n \geqslant 23$), omitting mice with 22 and 40 chromosomes, the status of which (parental or recombinant) could not be established. Both tests indicated that individuals with many fusions were more frequent than those with few in both types of habitats (Wilcoxon's signed-rank test: dispersion index 0: $z = 2.2$, $p = 0.03$; dispersion index (1 + 2): $z = 2.02$, $p = 0.04$).

(d) *Demographic characteristics*

Whether trapping sites were grouped according to locality and habitat characteristics or according to the latter only (table 4), sex ratio, number of mice per night, and proportion of breeding females were not found to be significantly different between the two trapping sessions or karyotypes (Kruskal–Wallis test: $p > 0.1$). Abundance (number of mice per 100 trap nights) was significantly different only between trapping sessions (Kruskal–Wallis test: $H = 9.02$, $p = 0.003$). Indeed, the latter was found to be significantly higher during the second trapping session (summer 1996, 23.6 ± 2.1 mice per 100 trap-nights) than during the first (autumn 1995, 10.7 ± 3.8).

The mean sex ratio (SR) (1.41 + 0.24) did not differ significantly from unity ($t_{20} = 1.71$, $p > 0.05$). However, sex ratio tended to differ between habitat categories (Kruskal–Wallis test: $p = 0.04$, Bonferroni rejection region $p > 0.01$), the number of males being higher than that of females in the industrial and agricultural zones (categories C and D, respectively SR = 3.1 ± 2.0 and 3.7 ± 0.3), whereas in the old and more recent town neighbourhoods (categories A, B) the sex ratio was balanced (respectively 0.87 ± 0.1; 0.97 ± 0.2). Habitat characteristics correlated significantly with sex ratio (γ-correlation: habitat structure $z = 2.57$, $p = 0.01$; site dispersion $z = 2.91$, $p = 0.003$).

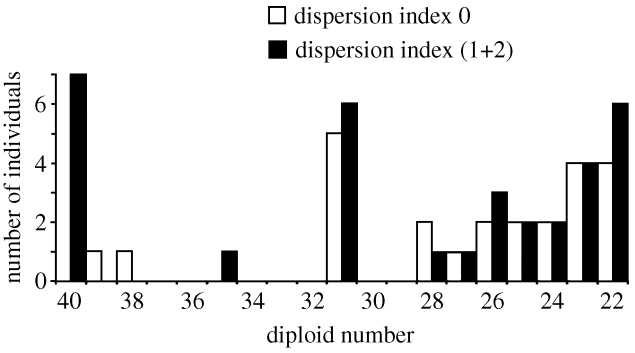

Figure 4. Pooled distribution of diploid numbers within 15 hybrid populations according to two site dispersion indices (0, 1 + 2).

The general trend across habitats, karyotypes and sessions showed a larger number of adults than of juveniles (Wilcoxon's signed-rank test: $z = 4.72$, $p < 0.001$), but the ratio of adults to juveniles did not differ significantly between sessions, karyotypes or habitats. Size and mass of mice were highly and positively correlated ($n = 515$, $r = 0.85$), but were not different between sessions, sexes, karyotypes or habitats (ANCOVA: $p > 0.05$).

4. DISCUSSION

(a) *Habitat partitioning*

Our study confirmed habitat partitioning between the two chromosomal races in central Tunisia. Standard mice occurred in all types of habitat except in the area of sympatry with the 22Rb morph. Of the two races, the 22Rb morph exclusively occupied the oldest sections of towns (medinas), often extending to more recent adjacent neighbourhoods, but only rarely to industrial zones and gardens. In general, each patch of 22Rb mice was surrounded by standard populations, which were present in the recent neighbourhoods as well as the industrial and agricultural zones. Contact between the two morphs always occurred within towns in the more recent areas, and did not always yield detectable hybrids. The transition from one morph to the other was very sharp and the width of the hybrid zone estimated for Monastir was extremely narrow, much more so than that observed for Italian mice differing by the same number of Rb fusions (14 km) (Spirito *et al.* 1980). These results agree well with the low fertility measured in laboratory-reared hybrids between the two races (Saïd *et al.* 1993). A recent study on developmental stability, which was lower in hybrid than in parental wild mice, revealed the existence of genetic incompatibilities between the two races. Such a differentiation may also be contributing to decreased hybrid fitness (Chatti *et al.* 1999).

Habitats occupied by the house mouse were found to present different structure and site-dispersion features, which may involve differences in the availability of food and shelter as well as in extinction and recolonization potential. A measure of these differences can be approximated by the occupancy rate, defined as the percentage of sites that yielded mice compared with the total number of sites where traps were set. Habitats A and

Table 4. *Demographic parameters calculated for sites pooled according to their habitat characteristics and the karyotype of their mice* (*/M, */F, sites yielding only one sex; —, impossible to compute.)

locality[a]	trapping session	2*n*	habitat indices: structure	habitat indices: dispersion	*n*	SR	juvenile/adult	mice per 100 trap-nights	mice per night	females (%)
9	1995	22	A	0	50	0.7	0.43	8.7	1.2	4.8
9,11,13	1995	22	B	0	52	0.7	0.43	15.8	1.5	23.8
9,10,11	1995	22	B	1	27	1.5	0.42	5.3	1.0	12.5
9,10,12,14,15	1995	40	B	0	49	0.6	0.36	11.4	1.8	25.0
9,10,12,13,14,15	1995	40	B	1	53	1.0	0.11	30.0	1.7	34.8
9	1995	40	C	0	1	*/M	0.00	9.1	1.0	—
9	1995	40	C	1	2	*/F	0.00	5.6	0.5	0.0
9	1995	40	C	2	8	7	0.33	5.9	0.5	0.0
9	1995	40	D	1	8	3.0	1.00	4.4	0.6	0.0
9,10	1995	40	D	2	5	4.0	0.00	12.5	0.6	0.0
9	1995	hybrid	B	0	15	0.7	0.25	16.7	3.0	50.0
9	1995	hybrid	B	1	4	0.3	1.00	7.0	0.4	0.0
16,17,18	1996	22	A	0	81	0.9	0.23	36.6	1.3	21.2
9,16,17,18	1996	22	B	0	43	1.0	0.24	37.7	1.0	36.8
16,17	1996	22	B	1	15	2.0	0.67	25.0	1.5	0.0
17	1996	22	C	1	5	1.5	0.00	20.8	1.7	100.0
17	1996	22	D	1	3	*/M	0.50	10.3	1.5	—
14,18	1996	40	B	0	47	0.6	0.38	28.9	2.8	31.8
14,16,18	1996	40	B	1	22	1.2	0.10	13.0	1.0	44.4
17	1996	40	C	1	10	0.7	0.43	50.0	5.0	25.0
16	1996	40	D	1	5	4.0	0.25	14.3	1.3	0.0
16	1996	hybrid	A	0	2	1.0	0.00	22.2	0.4	0.0
16,18	1996	hybrid	B	0	11	0.6	0.09	14.7	1.8	28.6
16	1996	hybrid	B	1	13	0.3	0.30	—	2.2	57.1
17	1996	hybrid	C	1	1	*/F	—	10.0	1.0	—

[a]Refers to legend of figure 1.

B had relatively high occupancy rates (both 52%), which decreased slightly in habitat C (42%), and more so in habitat D (30%). Our results showed almost no differences between population characteristics in the different habitats, nor did we find demographic differences between the two morphs when they occurred in the same type of habitat. However, our sampling method did not provide detailed information on the actual density and population turnover in these habitats. The only difference observed between habitat types concerned the sex ratio, which was balanced in all areas except the industrial and agricultural zones, where an excess of males was recorded. A similar unbalanced sex ratio was observed in a low-density Rb population from northern Scotland (Ganem *et al.* 1996), and is commonly reported for wild-living populations of mice (Cassaing & Croset 1985; Ritte *et al.* 1992) where habitat conditions fluctuate. Such data would support the assumption that differences in demographic characteristics may exist between populations of urban sites (A and B) and those in industrial and agricultural zones where resources may be more dispersed in time and space.

If differences in population density were not observed between habitats, the distribution of the two morphs followed a site dispersion gradient, which provides a rough measure of site density change. The 22Rb race was found to occupy areas of higher site density (0 m between sites: A0, B0, C0) whereas the 40St mice were more common in less densely populated zones (from a few metres to more than 50 m between sites: B1, C1, C2, D1, D2), the transition between the karyotypes matching that of the density change. All other factors being equal, such a distribution would lead to a higher density per unit area of the 22Rb mice than of the 40St race. This difference in density would be expected to affect the structure and dynamics of the contact zone, leading to a relative overflow of 22Rb mice into habitats occupied by the 40St ones. This density gradient would tend to shift the centre of the cline towards the peripheries of towns (Barton 1979). Such a feature may be responsible for the biased chromosomal structure of some of the hybrid populations. If an excess of hybrids carrying many fusions is expected in sites close to the 22Rb-inhabited zones, this is not predicted for those located in areas predominantly occupied by the 40St mice. Although other factors, such as a selective advantage of fusion-carrying hybrids, transmission distortion or insufficient sampling, could account for this distribution, a site density gradient would be expected to produce such a pattern if an equilibrium has not yet been reached.

(b) *Origin of habitat partitioning*

Because standard mice occur in all types of habitat outside the area of sympatry, habitat partitioning between the two morphs in central Tunisia could result from two features, one of which is competitive exclusion, i.e. a selective advantage of the 22Rb morph in the old sections of towns. Because the standard race is the ancestral morph, it was probably widespread throughout North Africa when the derived 22Rb morph evolved

(Auffray 1993). Moreover, allozyme analyses of Tunisian mice from different localities indicated a monophyletic origin of the 22Rb morph (Saïd *et al* 1999), which suggests that the latter race most likely originated in one area, from which it spread to different parts of central Tunisia. The extant pattern of distribution thus suggests that the 22Rb morph most likely migrated into resident 40St populations. However, experimental introductions of colonizers into established commensal populations of mice (Lidicker 1976; Van Zegeren 1980) indicate that effective immigration is often limited by social behaviour, and is most successful in empty or disputed areas. Thus, settlement of 22Rb immigrants into habitats occupied by 40St mice would be difficult, unless a selective advantage allowed them to displace the standard mice. This in turn raises the question of the limited distribution of the 22Rb morph. If the absence of the 40St morph in habitat A is related to competitive exclusion by the 22Rb morph, the latter would be expected to have colonized the medinas in other towns throughout Tunisia, given the importance of trade within the central region and between the latter and the north of the country.

An alternative hypothesis accounting for both the absence of the standard morph in the oldest sections of several towns in central Tunisia, and the limited distribution of the 22Rb morph, relies on the age and initial chance of colonization of these habitats. The oldest human habitat in Tunisia is the medinas, which date back to the first Arab conquest (AD 670–1000), during which most towns and villages were destroyed and many medinas built, particularly in central Tunisia (Despois 1955). Subsequently, development slowed down until the 16th century or so, and it is only recently, i.e. in the past 50 years, that urban development has once again become extensive (Lowy 1986). These historical data suggest that between the seventh and the tenth century opportunities for local reductions of mouse populations may have occurred. Construction of new habitats combined with development of trade in central Tunisia may have favoured dispersal of the 22Rb race through passive migration to vacant sites, from its point of origin to the other towns as they developed. Such a pattern of colonization requires that the 22Rb morph was present in central Tunisia at that time, and that migration occurred very rapidly before recolonization by the standard morph. Once saturation was achieved, the probability of immigration by other mice would be very low, owing to the social structure of house mice (Lidicker 1976). Secondary recolonization of this area by the standard morph would have occurred from commensal habitats in which the standard morph was the first to settle, and/or through low-density wild-living populations (Bernard 1969). The occurrence of the 22Rb morph in some recent towns and villages and its absence from other regions of Tunisia may be due to random processes: geographical distance, intensity of passive transport, distance to main trade routes, and/or density of the other morph. That migration does not always lead to successful establishment is evidenced by changes in the karyotype configuration of mice between sessions (i.e. in originally hybrid or $2n = 22$ sites, subsequent sampling yielded either no mice or individuals with $2n = 40$; see figure 1). Thus, historical evolution of Tunisian urban communities may have provided the stochastic conditions leading to a non-random distribution of the 22Rb race.

(c) *Reproductive isolation*

The two karyotypic races present in Tunisia show a very sharp transition, which maps to a site density gradient. This habitat partitioning, whether related to adaptive traits or not, would be expected to favour the spread of 22Rb mice from the centre to the periphery of towns at a rate dependent on the intensity of the density gradient and of urbanization. Contact between the two races leads to a very narrow hybrid zone, indicating a low degree of hybrid fitness. Thus, postzygotic isolation between the Tunisian chromosomal races is quite effective in limiting genetic introgression. However, whereas the number of potential contacts between the two morphs is high, hybrid populations are fewer than would be expected from laboratory assays of inter-racial matings (Saïd *et al*. 1993). This is particularly noteworthy in Kairouan, where no hybrid populations were observed at a contact between the two morphs (figure 2).

The scarcity of hybrid populations may have several causes. Chromosomal and genetic incompatibilities and eventually differences in adaptive traits between individuals of the two races may be sufficiently important to result in a high rate of hybrid population turnover and extinction. An additional contributing factor may be related to a prezygotic barrier. In this case, infrequent matings between the two morphs would lead to a low occurrence of hybrid populations. Such premating isolating mechanisms may involve behavioural divergence in mating-signal systems, which are known to make a major contribution to genetic isolation between species (Butlin & Ritchie 1994). In the house mouse, discriminatory aggressiveness has been observed between chromosomal morphs in Scotland (Ganem & Searle 1996), and a case of reproductive isolation that may involve ethological barriers has been documented between Rb races in Italy (Capanna & Corti 1982; Fraguedakis-Tsolis *et al*. 1997). Unravelling the respective contributions of these different traits to reproductive isolation between the two chromosomal races in Tunisia requires further genetic, ecological and behavioural studies.

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