Odor preference in house mice: influences of habitat heterogeneity and chromosomal incompatibility

Ana Claudia Nunes, Maria da Luz Mathias, and Guila Ganem

Centro de Biologia Ambiental, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Edifício C 2, 3º Piso, Campo Grande, 1749-016 Lisboa, Portugal and Institut des Sciences de l’Évolution de Montpellier (UM2, CNRS), Laboratoire Génétique et Environnement, C.C. 065, Université Montpellier II, 34095 Montpellier cedex 5, France

Theory predicts that when maladaptive hybridization occurs assortative mating preference should evolve. Moreover, habitat characteristics can influence quality of mates that is an important criterion in mate choice. Here we ask how chromosomal compatibility and differences in habitat quality might shape preference for odors of the opposite sex in the house mouse. Our study model is composed of 2 chromosomal races and their narrow hybrid zone that occur in habitats of different qualities. We performed 2-way choice tests during which opposite sex urine mixtures of each race were presented to mice from the 2 races and the hybrid zone. Differential investigation of the odor sources indicated both preference and that the odors differed. The results show that the 2 races carry distinct odors and, irrespective of the race they belonged to, males preferred odors of females from the race occurring in habitats of lower quality (hereafter, race B), whereas females preferred odors of males from the race occurring in habitats of better quality (hereafter, race A). Further, preference in the hybrid zone was for race B odors, which differed significantly from that displayed by the 2 races (i.e., for race A odors). The relative influences of geography, ecology, and chromosomal compatibility are discussed, thus leading us to propose that habitat differences might play the most important role in shaping signal divergence and preference in this system. Key words: habitat variations, hybrid zone, Madeira Island, Mus musculus domesticus, odor preference, Rb chromosomes, urinary signals. [Behav Ecol 20:1252–1261 (2009)]
~1.5%). Because stable habitats provide steady resources, we expected them to have a higher value for mice, and, as a result, to trigger stronger competition and selection for highly competitive territorial males. Conversely, less stable habitats were expected to select for less competitive males. However, less competitive males may cope with frequent environmental changes (Van Zegeren 1980; Van Oortmerssen et al. 1985; Benus et al. 1991). Based on these considerations, we predicted that differences in habitat characteristics could result in a different selection regime within the 2 races. Because information on social status can be conveyed via odor cues present in the urine (Drickamer et al. 1992; Rich and Hurst 1999; Gosling et al. 2000; Roberts and Gosling 2003), we hypothesized that habitat quality, through its influence on the frequency of more competitive versus less competitive mice, might impact the odor characteristics of the males and lead to divergence between the 2 races.

Crosses between mice with distinct karyotypes are predicted to produce progeny with impaired fertility (e.g., Hauffe and Searle 1998; Piälek et al. 2001). Preliminary data indicated a decrease in fertility of hybrid progeny between the 2 chromosomal races involved in this study (Nunes-Oliveira 2007) and hence reduced compatibility between the 2 races.

Finally, the hybrid zone being located in a density trough (i.e., an area where habitat characteristics result in the species present in lower density, see Hewitt 1999), interactions between the 2 races are expected to be restricted to that area (Nunes et al. 2005); moreover, given that dispersal out of a density trough is expected to be rare, geographical isolation might occur between the 2 races.

Given the potential for divergence in our study system, we tested 1) preference for opposite sex odors in mice of the 2 races and the hybrid zone and 2) olfactory discrimination between mice of the 2 races. Our results indicate that divergence has occurred. We discuss 3 factors that could influence divergence: local adaptation, chromosomal incompatibility, and geographical isolation.

**MATERIALS AND METHODS**

**The chromosomal context**

Chromosomal radiation in the house mouse (M. musculus domesticus) is relatively widespread in Europe and considered to be a relatively recent event (Britton-Davidian et al. 1989; Nachman and Searle 1995), not older than 3500 years (Auffray 1993). The mouse is believed to have colonized Madeira not later than 1000 years ago (Britton-Davidian et al. 2005, 2007). Chromosomal races consist of populations of mice carrying different combinations of Robertsonian (Rb) centric fusions between acrocentric chromosomes (Piälek et al. 2005). The 2 races involved in this study carry a total of 8 Rb chromosomal mutations and differ by 1 Rb mutation (hereafter, the diagnostic fusion). Rb(6.7), that is, the fusion of chromosomes 6 and 7, characterizes populations of race A (the stable habitat race). Rb(7.15), that is, the fusion of chromosomes 7 and 15, characterizes race B (Figure 1). Race B presents a continuous distribution along the western coast of the island. Race A has a discontinuous distribution, with most populations inhabiting the southern part of Madeira and an isolated population occurring in the northern part of the island and separated from the northern populations of race B by a geographical barrier (Figure 1). The 2 races come into contact in the southwest, and race A is separated from another race (hereafter, race C) in the south by a 2-km long band of natural vegetation. The latter race occurs in similar stable habitats to race A, and their karyotypes differ by one Rb mutation: Rb(15.18) in race C and Rb(5.18) in race A (Britton-Davidian et al. 2005).

An important characteristic of our system is that races A and B are polymorphic for their racial diagnostic fusion, that is, individuals carrying the same diagnostic fusion in a homozygous or heterozygous state can coexist in a given population. This polymorphism led to 4 zones based on the relative frequency of mice carrying the racial diagnostic fusion Rb(6.7) or Rb(7.15). Nunes et al. (2005) found that race A comprised 2 zones: zone 1, where all the mice carry 2 copies of Rb(6.7), and zone 2, where at least some mice in a given population carry 1 or 2 copies of Rb(6.7) and none carry Rb(7.15). Zone 3 delimits the hybrid zone, where mice carrying both types of Rb can be found and where first generation hybrids were recorded (2 mice). Populations of race B that were used in this study belonged to a single zone, zone 4, where all populations are characterized by the presence of at least some mice carrying a single or 2 copies of Rb(7.15) and the absence of mice carrying Rb(6.7), which is the diagnostic fusion of race A (Table 2; Figure 1).

**Animals and the geographical context**

Mice involved in this study originated from the western part of the island of Madeira (32°37′–32°52′N, 16°39′–17°15′W). The
mice were trapped between June 2001 and September 2002 along a south/northwestern transect following the main road, which crossed the range of the 2 chromosomal races and their hybrid zone (Figure 1). They were then brought to Lisbon where they were kept in the facilities of the Centre for Environmental Biology of the Faculty of Science. Mice trapped as juveniles (i.e., $\leq 10$ g with bright fur) were not used in the behavioral tests. To prevent aggression, the males were isolated or housed in small groups composed of one male and several females. Mice were kept under a 12-h light:dark regime (light on between 07:00 and 19:00) with water and food provided ad libitum. A minimum of 2 weeks between their arrival in Lisbon and the use of the mice in the behavioral tests was established. All together, mice involved in the behavioral study were trapped in 27 sites along the road (Figure 1). Mice from very close trapping sites were considered to form a single population (based on potential for active dispersal between sites); hence, some sites were pooled and our sample was composed of 17 distinct populations, distributed over the 4 different chromosomal zones described above.

Odor stimuli

We aimed to obtain odor stimuli that would represent a race odor. Urine donors (a total of 84 mice) were selected from populations captured in 3 trapping sites within the range of each of the 2 races (represented by stars in Figure 1). The distance between the sites where urine donors were trapped varied roughly between 0.5 and 1.6 km, and female and male donors of a given race originated from the same trapping site. Hence, urine donors originated from distinct demes, although from a limited number of geographic localities.

Urine was collected after a minimum of 2 weeks of acclimatization to laboratory conditions. We obtained urine by gently pressing the belly of the mouse or by collecting drops left by the mouse on a cleaned surface (a minimum of $30\mu l$ per mouse). Urine samples were pooled across several individuals of a given sex and race. At the time of pooling, the donors’ karyotypes were not known. Pooling was aimed to minimize differences between odors of different individuals and demes of a given race (Penn and Potts 1998b; Smadja and Ganem 2008). Therefore, the only distinctive information present in the odor mixture is supposed to be one that is shared across individuals, that is, a population or a race signal. The pooling procedure significantly reduced the number of mice involved in the behavioral test as donors. Urine samples were kept at $2\pm 18^\circ C$ and were thawed just before testing during which they were kept on ice to reduce evaporative loss of volatiles. We used several pooled urine samples for each race. The pools varied by the number, identity, and karyotypes of donors (average number of donors in urine pools: $8.5\pm 0.5$). Because the 2 races were chromosomally polymorphic, having this variation in our urine pools was important, and we checked, at the end of the experiment, that it was the case. The study involved
As mentioned earlier, race A is also parapatric with race C. Although no contact zone or hybrid mice were known to occur between the 2 races, populations of the 2 races could have interacted in the past. If the latter were true, then karyotype incompatibilities could have favored assortative preference among mice of race A independently of interactions with mice of race B. To avoid a bias in our interpretation of divergence of odor preference between races A and B, we investigated preference of mice (15 males and 14 females) from zone 1 of race A (the closest to the third race) for a pair of urine stimuli composed of race A and race C (3 sampling sites shown as squares in Figure 1) versus a pair composed of odors of races A and B. A 6-month interval separated the 2 tests.

Behavioral apparatus and the choice test procedure

The behavioral apparatus was made of transparent Plexiglas and plastic ware. A Y maze (5 cm ø; main branch: 43 cm long; secondary branches: 25 cm long) was connected to a start box on one side and to 2 peripheral test boxes (35 × 23 × 13 cm) on the other side. At the beginning of each trial, the chooser was allowed to explore the whole system for a few seconds, after which it was isolated in the central box. Then, a sample of 20 μl of pooled urine was dropped with a micropipette onto square marks made by the observer on the test boxes’ floor at a distant corner diagonal to the entrance. During all tests, the observer was not aware of the origin of the 2 stimuli that were labeled anonymously. A sliding door separated the start box from the Y maze and impeded the access of the chooser to this box during the test. Recording started when the whole body of the mouse entered the main branch of the Y maze. Each test lasted 10 min during which the time spent on each side of the apparatus (arm + test box) and the time spent sniffing, licking or standing in close proximity to a stimulus (roughly ≤1 cm) was recorded with a Psion Organizer (recording accuracy = 0.1 s) and the Observer software (Observer Mobile, version 3.0; Noldus Information Technology, Wageningen, The Netherlands).

Most animals were tested once (except for mice involved in the second control test that were tested twice with a 6-month interval in between). Data from tests were not considered when the mouse remained immobile for more than 30% of the duration of the test. In this case, the mouse was tested again after a minimum of 1 week. For each trial, the potential effect of laterality was controlled by alternating the position of a given stimulus (left and right). Mice were euthanized and karyotyped (see Nunes et al. 2005) after the behavioral tests.

Data analysis

We evaluated odor preference by the relative time spent investigating one versus the other stimulus:

\[ R = \ln\left(\frac{\text{Time investigating race B stimulus}}{\text{Time investigating race A stimulus}}\right) \]

A ratio value of 0 corresponded to an absence of directional preference (equal time spent investigating the 2 stimuli), a negative ratio indicated that more time was spent investigating the race A stimulus, whereas a positive ratio indicated that more time was spent investigating the race B stimulus. R is given as the mean ± standard error (SE).

We tested the contribution of zone and individual chromosomal characteristics to variation of R. The 2 factors could not be tested in the same model. The first model included as fixed factors: zone, sex, zone × sex, and 2 random factors: population nested in zone and urine pool nested in sex. The second model included as fixed factors: karyotype, sex,
Table 3
Results of the mixed model ANOVA involving sex and zone as fixed factors and the ratio of attraction (R) as the dependent variable

<table>
<thead>
<tr>
<th>Factors in the ANOVA</th>
<th>n.d.f</th>
<th>d.d.f</th>
<th>F ratio or z* value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>12.5</td>
<td>7.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Zone</td>
<td>3</td>
<td>13.7</td>
<td>3.83</td>
<td>0.035</td>
</tr>
<tr>
<td>Sex × zone</td>
<td>3</td>
<td>82</td>
<td>0.73</td>
<td>0.54</td>
</tr>
<tr>
<td>Population (zone)</td>
<td>3</td>
<td>0.25*</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Pool (sex)</td>
<td>3</td>
<td>0.65*</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

Random factors are indicated in italics. n.d.f. is the numerator degree of freedom; d.d.f. is the denominator degree of freedom; z* test of covariance parameter estimate of random factors.

RESULTS

Patterns of odor preference across chromosomal zones

Odor preference (i.e., R) differed significantly between the sexes and the zones (Table 3). The females displayed a negative R indicating preference for odors of race A males (n = 126, R = −0.21 ± 0.09, test R ≠ 0: t125 = −2.33, P < 0.01), whereas the males displayed preference for odors of race B females (n = 140, R = 0.15 ± 0.06, test R ≠ 0: t139 = 2.41, P < 0.05). As far as the zones were concerned, mice in zone 3 (the hybrid zone) had a significantly different R from those in zone 1 (i.e., race A: P = 0.0005) and zone 4 (race B: P = 0.002). In particular, mice in zones 4 and 1 displayed preference for race A odor, whereas the opposite pattern was displayed by mice from the hybrid zone (Table 4).

Individual karyotype and odor preference

The ANOVA revealed a significant influence of sex and sex × karyotype (Table 5). However, the only significant post hoc tests pointed out significant differences between females Rb(7,15), which showed a significant preference for race A type odors, and females Rb(6.7) and Rb(0.0) that did not show a directional preference (Table 6, and, respectively, for the 2 comparisons: P = 0.005 and P = 0.025). Differences between the sexes mainly concerned mice carrying Rb(6.7) and Rb(7.5) (respectively; P = 0.01 and P < 0.0001), preference being more marked, and in favor of race A type odors among the females as opposed to the males, the preference of which was less marked and nonsignificantly directed toward race B odor (Table 6).
Control test 1: comparing patterns of attraction for race B stimuli of allopatric versus parapatric populations of race A

Mice from the northern populations of race A were tested with the same pair of stimuli as above (i.e., races A and B), and the R value was compared with that of the southern populations of race A either distant (zone 1) or close (zone 2) to the hybrid zone. R differed between the sexes ($F_{1,110} = 4.53$, $P = 0.03$) but only marginally between the 3 populations ($F_{2,110} = 2.6$, $P = 0.08$). Actually, unlike the mice in zone 1 which showed a preference for race A odor (zone 1: $n = 30$, $R = -0.41 \pm 0.17$, the 2 other populations did not show a directional attraction to any of the odors (zone 2: $n = 63$, $R = -0.006 \pm 0.10$; the northern population: $n = 26$, $R = 0.04 \pm 0.17$).

Control test 2: patterns of attraction of mice of race A for same-race urine when the heteroracial ureine was either of race B or of race C

The tested mice originated from zone 1 of race A (Figure 1). Total time spent investigating the pair of odors was significantly higher when the alternative to race A was a race C odor as compared with a race B one ($F_{1,55} = 7.38$, $P = 0.009$). The latter pattern was more marked among males than among females ($F_{2,55} = 19.8$, $P < 0.0001$) and was not influenced by the interaction between sex and odor origin ($F_{2,55} = 0.058$, $P = 0.81$). Mice of race A spent more time investigating race C odor as compared with race B odor (Wilcoxon, $Z = 2.87$, $P = 0.004$), whereas the time spent investigating race A odor was not significantly different in the 2 tests (Wilcoxon, $Z = 1.365$, $P = 0.17$). Consequently, odor attraction of race A mice was significantly assortative when the alternative was race B (Table 3), whereas it was not directional when the alternative was race C ($n = 29$, $R = -0.03 \pm 0.15$, $t_{29} = 0.2$, $P > 0.20$).

DISCUSSION

Different groups (or demes) of mice occurring contiguously on the same farm have distinct odor characteristics and can discriminate between each other using odor (Cox 1984, 1989). In our study, the odor donors originated from several demes, and each stimulus was a pool of odors from different individuals and demes. Such a procedure was expected to enhance heterogeneity within each stimulus and would tend to mask small differences between odors of the 2 races. Nevertheless, our results suggested that the 2 races have distinct odor bouquets. Further, patterns of preference were consistent within the sexes irrespective of the race they belonged to but differed between the sexes.

Males preferred odors of females of race B and females preferred odors of males of race A

Two factors might trigger enhanced investigation of an odor by a mouse: novelty and preference (i.e., attraction for a specific stimulus whether familiar or not?) but see Hoffmann et al. (2009) for the effect of freezing odor signals on the ability of a mouse to distinguish between novel and familiar odors. In this study, if investigation was triggered by novelty, we would have expected the 2 races to present opposite patterns of odor attraction and preference not to be consistent within the sexes. Yet, our results indicated that the females, irrespective of their race, prefer a race A odor, whereas the males prefer a race B odor.

Earlier studies have pointed out that preference can be displayed by both male and female house mice and that reproduction with a preferred mate would increase the pair’s reproductive success (Drickamer et al. 2000, 2003; Gowaty et al. 2003). Our results suggest that opposite patterns of preference between the sexes might occur. However, given the geographical range of the 2 races, a large proportion of the mice would never encounter their preferred odor in their natural environment. Perhaps, our study has revealed potential or “hidden preferences” resulting from a process of sensory drive (Endler and Basolo 1998; Fuller et al. 2005), rather than traits that have evolved during interactions between male and female signals and preferences.

Obviously, our results suggest that race-specific recognition signals, if they exist, do not drive preference here. They also suggest that information present in race A male odor attracts the females, whereas information present in race B female odor attracts the males. We see 2 possible interpretations for these results. The first one is that drift or selective mechanisms, not related to mate recognition, might have resulted in males of race A and females of race B evolving signals that exploit specific properties of the sensory system of the receiver and trigger stronger preference than the other signals (Endler and Basolo 1998). Alternatively, the patterns displayed in the 2 sexes across the races might reflect an ancestral preference for specific cues evolved in the ancestral population of the 2 races. Such mechanisms were invoked to explain heterospecific preferences in the swordtail Xiphophorus pygmaeus (Ryan and Wagner 1987). The latter assumption implied that the preferred cues carry information that are valued and recognized by the sensory system as valuable. We discussed in the

Table 6

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Sex</th>
<th>R ± SE</th>
<th>Student t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh(6.7)²</td>
<td>Males</td>
<td>0.11 ± 0.13 ($n = 43$)</td>
<td>$t_{42} = 0.85$, ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>−0.38 ± 0.13 ($n = 37$)</td>
<td>$t_{36} = −2.92$, $0.05 &lt; P &lt; 0.01$</td>
</tr>
<tr>
<td>Rh(6.7)</td>
<td>Males</td>
<td>0.31 ± 0.14 ($n = 27$)</td>
<td>$t_{26} = 2.21$, $0.02 &lt; P &lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.20 ± 0.24 ($n = 21$)</td>
<td>$t_{20} = 0.83$, ns</td>
</tr>
<tr>
<td>Rh(7.15)²</td>
<td>Males</td>
<td>−0.10 ± 0.13 ($n = 15$)</td>
<td>$t_{12} = 0.77$, ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>−0.145 ± 0.24 ($n = 12$)</td>
<td>$t_{11} = 0.60$, ns</td>
</tr>
<tr>
<td>Rh(7.15)</td>
<td>Males</td>
<td>0.25 ± 0.14 ($n = 19$)</td>
<td>$t_{18} = 1.78$, ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>−0.87 ± 0.24 ($n = 18$)</td>
<td>$t_{17} = −3.62$, $0.002 &lt; P &lt; 0.005$</td>
</tr>
<tr>
<td>Rh(0.0)</td>
<td>Males</td>
<td>0.125 ± 0.13 ($n = 38$)</td>
<td>$t_{37} = 0.96$, ns</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.025 ± 0.17 ($n = 38$)</td>
<td>$t_{37} = 0.15$, ns</td>
</tr>
</tbody>
</table>

Results of tests of deviation of $R$ from 0 are also indicated ($n =$ sample size). A negative $R$ indicates preference for race A type stimulus, whereas a nil $R$ indicates nondirectional preference. *Indicates significance after applying the sequential Bonferroni correction, ns, not significant.
introduction that, like in many other species (Qvarnström and Forsgren 1998), female mice tend to prefer dominant males (Oakeshott 1974; Wolf 1985; Moosman and Drickamer 1996; Rich and Hurst 1998; Roberts and Gosling 2003), even when they carry semilethal alleles (Coopersmith and Lenington 1992). Further, habitats of good quality (i.e., stability of resources and limited disturbance) seemed to favor males that can gain and keep the best territories (Van Zegeren 1980; Franks and Lenington 1986). So-called dominant versus subdominant males (within a given age class) correspond to different behavioral strategies and possibly genotypes the frequency of which in natural populations might vary with habitat conditions (Van Oortmerssen et al. 1985; Van Oortmerssen and Busser 1989). The 2 behavioral phenotypes (or personalities) might be maintained in a given population, one strategy being advantageous when habitat conditions are stable and the other when habitat conditions fluctuate (Benus et al. 1991, 1992). Under our model system, we expected the frequency of dominant type individuals to be higher among race A than among race B. Further, because the 2 strategies were associated with different levels of testosterone and male pheromones (Harvey et al. 1989; Novotny et al. 1990), we expected their odors to signal their differences. Hence, we propose that female preference for race A type odors could reflect a preference for males of the dominant type.

A recent study (Mills et al. 2007) involving the bank vole pointed out that the genetic benefits of females mating with dominant males varied with habitat stability: It was lower when the habitat became less favorable (i.e., less stable). Surprisingly, the females continued to show a preference for dominant males even when the habitat deteriorated, leading the authors to predict that the pattern of preference would change if the habitat remained unfavorable. In our study, females occurring in less favorable habitats (race B) might still prefer dominant males because they are rare in their social environment and very few, if any, experience reproduction with the "dominant type males." The only area where males of race A were found in habitats of lower quality and could be selected against was the hybrid zone. Hence, under our assumption, females in the hybrid zone (zone 3) should evolve a preference for odors of race B because they were the only ones to show a positive R (values of R of females in zone 1 = −0.62 ± 0.26; zone 2 = −0.14 ± 0.18; zone 3 = 0.145 ± 0.16; and zone 4 = −0.45 ± 0.15).

Preference of males for odors of females of race B suggested, like above, that they might carry traits that either exploit intrinsic properties of the male mouse sensorial system or present some advantages to the females. The latter implied that females living in suboptimal habitats might be more valued than those occurring in stable habitats, which might sound counterintuitive. Very little is known about the criteria used by male mice in their mate choice. Data available for other species demonstrated the importance of size and fecundity of females (Reading and Backwell 2007; Doutrelant et al. 2008b). Laboratory investigations on reproductive abilities of the house mouse under food and temperature constraints suggested that female house mice might be more resource dependent than males, particularly at low temperatures (Bronson 1984; Perrigo and Bronson 1985). Both food availability and average annual temperature were found to be slightly lower in areas where race B was sampled as compared with areas occupied by race A (Table 1). Survival and reproduction in suboptimal habitats might result in selection for females of higher fecundity in race B as compared with females of race A which thrive under environmental conditions where such selection might be relaxed (but see Clutton-Brock 2007). This hypothesis could be tested through a comparison of fitness characteristics of females in the 2 habitat types.

Contrasted preference in the hybrid zone and in the 2 races

Preference displayed in the hybrid zone was significantly different from that displayed in populations of races B and A. In the hybrid zone, mice were attracted to race B type odor, whereas mice of races B and A were more attracted to race A type odors. Contrasting preference strongly suggests that a different selective regime might be occurring in the hybrid zone as compared with the 2 other areas.

Populations of races B (zone 4) and A (zone 1 but not zone 2) preferred the odor of race A, suggesting that it might be more attractive than that of race B. We have discussed earlier how ecological constraints might explain patterns of divergence between the sexes; natural selection might have favored here, that is, higher quality of male donors of race A. Moreover, mice of race A (zone 1) did not show a preference in control tests where the alternative to race A type odor was a race C instead of a race B odor. Given that race C occurs in habitats similar to those of race A (i.e., good quality), our results suggest that the mice were assessing differences (or similarities) in habitat quality, rather than racial differences. This assumption could be tested by comparing behavioral traits (e.g., dominance) of mice from different races and habitats. Convergence of preference between the 2 races might also be explained by a mechanism of sensory bias for ancestral signals (Ryan and Rand 1995; Phelps and Ryan 2000). Indeed, given the geographical scheme of colonization of the island by the house mouse and the chromosomal specificities of Madeira system (Britton-Davidian et al. 2005), it is reasonable to consider that race B has evolved as a subpopulation of race A. Mice of race B might have retained an ancestral preference for the same odors. Although, as far as the signaling component of the recognition system is concerned, different ecological constraints and underlying natural selection might have favored, incidentally, rapid signal divergence between the 2 races.

The patterns of preference were not homogeneous within race A: Preference was stronger in zone 1 than in zone 2. This finding was contrary to our expectations, given that mice of zone 2 are supposed to be the most exposed to selection against hybridization and hence expected to have evolved the strongest assortative preference. Besides, we compared zone 2 and the northern population of race A as, unlike mice in zone 1, the 2 are polymorphic for the race A diagnostic fusion (Nunes et al. 2005). Both the northern populations and zone 2 did not show directional preference. Different patterns evidenced between mice of zones 1 and 2 might relate to their chromosomal differences. Alternatively, convergence of patterns of preference between the northern and zone 2 populations could be only an artifact, and the pattern shown in zone 2 might reflect a trace of past hybridization with race B. The latter explanation is consistent with the low genetic divergence of house mice from zone 2 from the hybrid zone and race B mice (Britton-Davidian et al. 2007). Investigations involving microsatellite markers might help to detect traces of past hybridization.

As mentioned earlier, preference in the hybrid zone contrasted with that in the 2 races. However, the hybrid zone being the only geographical area where mice of the 2 races meet, it was also the only suboptimal habitat where mice of race A are found and could be selected against. Indeed, although a "dominant type" behavioral strategy might be more beneficial for males in stable habitats, it might not be so in disturbed habitats where resources fluctuate (Benus et al. 1991, 1992; Mills et al. 2007). Along these lines, both natural and sexual selection might discriminate against males of race A in the hybrid zone and could have favored evolution of preference for race B type stimuli in the hybrid zone. The hybrid zone was also the only area where selection against hybridization could take
place. Nevertheless, if the latter mechanism were involved in shaping preference, given the larger proportion of race A as compared with race B type mice present in the hybrid zone (Table 2), we would have expected either not to detect a directional preference or to detect a preference for a race A odor, which is not the case.

Finally, specific chromosomal features might be involved in shaping preference between the races. Although Rb fusions do not involve important changes in genetic information, several authors have claimed that specific Rb fusions might produce behavioral modifications (references in Sans-Fuentes et al. 2005). However, to date, specific phenotypes could not be ascribed convincingly to chromosomal variation in the house mouse (Capanna et al. 1984; Mainardi et al. 1986; Corti et al. 1989; Ganem and Searle 1996; Carpineti and Castiglia 2004; Sans-Fuentes et al. 2005; Mathias et al. 2006). Regarding our study model, we could not test the influence of karyotype divergence per se on behavior (too many intermingled factors). Nevertheless, given the convergence of preference within 2 races characterized by different Rb mutations, the latter might not play a major role. Still, we cannot totally exclude a role of Rb mutations, which will have to be tested in controlled conditions.

CONCLUSION

The results of this study are unusual in showing nonconsist split preference between the sexes and the absence of own race preference. However, it raises several hypotheses that could be tested through exciting experimental perspectives. The role of environmental conditions in shaping signal and preferences has been reported in many species and for different sensorial recognition systems (Ryan 1980; Endler and Houde 1995; Britton-Davidian et al. 2004; Britton-Davidian and Britton-Davidian, 2007). Our results on the house mouse in Madeira suggest that environmental characteristics might constitute an important force shaping recognition systems based on olfaction.

FUNDING

Fundação para a Ciência e a Tecnologia (Project POCI/TSE/BSE/47019/02 partially financed by European FEDER funds; PhD Grant [FCT/SFRH/BD/3114/2000]); the French Government (Ministère de l’Éducation et de la Recherche: Convention de Cotutelle de Thèse; Ministère des Affaires Étrangères: coopération France-Portugal).

The authors are indebted to E. Calvet-Fichet, M. Perriat-Sanguinet, and L. Gaço for valuable field assistance, R. Capela for field technical support, and R. Oliveira for his help in the maintenance of mice in captivity. We wish to thank G. Ramalhinho, J. Catalan, and J. Britton-Davidian for mouse karyotype analyses and J. Britton-Davidian, J. B. Searle, and C. Smadja for comments on the manuscript. All the experiments were conducted in accordance with legal procedures in Portugal (Decree law Number 129/92, 197/96 and 1005/92, all based on the Directive Number 86/609/CEE). This is a contribution of UMR 5554, n° ISEM 2009-063.

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