Concordance in mate choice in female mound-building mice

Émilie Beigneux*, Christophe Féron, Patrick Gouat

EA 4443, laboratoire d'éthologie expérimentale et comparée, université Paris-13, Sorbonne-Paris Cité, 99, avenue J.-B.-Clément, 93430 Villetaneuse, France

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ABSTRACT

Females must evaluate male quality to perform mate choice. Since females generally base their selection on different male features, individual females may differ in their choice. In this study, we show that concordance between females in mate choice decisions may arise without any experimental maximization of a particular attractive trait. Choice tests were performed in mound-building mice, Mus spicilegus, a monogamous species. Body odours of two male donors were presented to 12 female subjects individually. To determine female choice, the same pair of males was presented three times to a female. Four different pairs of male body odours were used. Male donors, not related to females, were selected at random in our polymorphic breeding stock. Using this two-way choice design, female mice displayed a clear choice and had a similar preference for particular males.

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RÉSUMÉ

Pour les femelles, le choix du partenaire passe par une évaluation de la qualité des mâles. Différents traits présents chez les mâles pouvant être impliqués dans l'appréciation de cette qualité, les femelles pourraient différer dans leurs critères de choix et ne pas toutes apprécier le même mâle. Dans cette étude, nous montrons qu'une préférence partagée entre femelles peut exister malgré des circonstances contraignantes où aucun facteur d'intérêt particulier n'a été expérimentalement magnifié. Douze femelles de souris glaneuse, une espèce monogame, ont été individuellement soumises à des tests de choix portant sur quatre paires différentes d'odeurs de mâles. Pour déterminer le choix des femelles, la même paire de mâles a été présentée trois fois à chaque femelle. Les mâles donneurs, non génétiquement liés aux femelles, ont été sélectionnés aléatoirement au sein de l'élevage. Malgré une procédure expérimentale conservatoire, les femelles ont effectué un choix clair et la majorité des femelles a préféré le même mâle.


1. Introduction

When females select their mate, they need to assess the quality of males. According to the optimal outbreeding theory, females usually do not have the same choice of mates [1–8]. Nevertheless, concordance between females should also occur when one male trait is of paramount importance for all the females. A shared preference for a given type of male has been shown in several species [9–12]. In these studies, emphasis was given to only one male trait by keeping the other traits as constant as possible. Moreover, to facilitate female choice the differences between males for the trait of interest were maximised, for example by surgically manipulating the sword length...
of males [9,10], by modifying the male diet [11], or even by infecting males with parasites [12]. Such experimental paradigm revealed to be fruitful and brought valuable information on male traits relevant to females. These experimental situations, nevertheless, only partially reflect the field situations because only extreme forms of the studied trait were used. In nature, male differences for a given criterion are usually more continuously distributed within the population [1]. The quality of males, moreover, results from several traits [13,14] promoting a greater diversity under natural than under laboratory conditions. One may then expect diversity in male traits to favour variation in female mate choice. The aim of our study was to test whether concordance between females in mate choice could arise under such conditions. In our study, potential mates were randomly selected from a polymorphic breeding stock of a wild species, the mound-building mouse *Mus spicilegus*.

Because of a short reproductive life not exceeding four months [15] and a monogamous mating system with exclusive pair bonds [16–18], mate choice is of paramount importance for female mound-building mice. These constraints should have promoted efficiency of mate choice processes in females. At the beginning of spring, mice disperse from over-wintering groups and reproduction begins [19]. According to field and genetic data, females are first to settle and are then joined by males originating from surrounding populations [19–22]. Females are very intolerant towards unfamiliar adult mice [23], whether male or female. Consequently, males must overcome the intolerance of females in order to gain access to reproduction. This period of pre-mating gives females the opportunity to evaluate the quality of visiting males.

When choosing their mate, female rodents generally use body odours to evaluate the quality of males [24]. Mound-building mice are capable of extracting information efficiently from individual body odours [25–28]. To test whether female mound-building mice displayed concordance in mate selection, male body odours were used as stimuli and presented to several females through individual two-way choice tests. To mimic field conditions, male donors and female subjects originated from two different populations and males were selected at random from our breeding stock.

## 2. Methods

### 2.1. Animals and general breeding conditions

The two strains used in this study come from two Hungarian populations that are at a distance of 100 km apart. Individuals of the ZHG strain originated from a stock of 80 wild mound-building mice captured in Gyöngyös (47.734705 N, 19.907913 E) in October 1999 and bred in captivity for nine generations. Individuals of the ZHK strain originated from a stock of twelve wild mound-building mice captured in Karcag (47.280161 N, 20.896683 E) in September 2004 and bred in captivity for two generations. Animals were bred under laboratory conditions (20 ± 1 °C) with a 14:10 light dark cycle. Mice were weaned at 28 days of age and housed in same-sex sibling groups from 35 days of age. Food (mice pellets type M20, Special Diet Services, Witham, Essex, UK) and water were provided ad libitum.

At least one week before the experiments, eight male donors – ZHK strain, seven months old (age: 192 ± 3 d), sexually naïve and originating from four different parental pairs – were placed by sibling pairs in standard polycarbonate cages (26 × 14 × 16 cm) with bedding material (sawdust and cotton). Twenty-four females – ZHG strain, 10 months old (age: 323 ± 9 d), sexually naïve and originating from twelve different parental pairs – were placed by sibling pairs under the same conditions. Mice were individually identified by hair clipping. Only twelve females were used as subjects, the other twelve females served as cage mates. Pair housing usually maintains females in constant anoestrus. In this species, a few days of cohabitation with a male is necessary to induce oestrus and fertile mating [29]. Because females may select their mate in an anoestrus state, testing was performed on anoestrus females only.

### 2.2. Experimental design

Soiled sawdust, with urine and droppings from donor mice, was used as odourant stimulus. This medium is known to convey different types of information in mound-building mice [26,30]. At the beginning of the sampling collection period, the males were placed into individual standard cages with 50 g of clean sawdust, cotton balls, food and water. After three days, the males were returned to their original cage with their cage mate. Soiled sawdust was collected and placed in freezer bags at −20°C until used. The sawdust was used in the first two weeks after collection so as to prevent any degradation [26]. As a consequence two sessions were necessary to collect the required soiled sawdust for each male donor.

Choice tests were carried out during the dark phase which corresponded to the period of activity of mice. The experimentation room was lit by dimmed red light during this phase in order to make observation possible. Females were placed in the test cage for a ten-minute aclimatisation period. The test cage (standard polycarbonate cage, 26 × 14 × 16 cm) contained 10 ± 1 g of sawdust. A plastic cover was placed on the cage to close it and to allow for observation. During aclimatisation period, only the necessary quantity of soiled sawdust was defrosted. Sawdust of each male was transferred respectively into two plastic dishes (diameter 3.5 cm) that were fixed onto a support (15 × 4.5 × 5 cm) so that the mice could not displace or overturn them [26]. After the aclimatisation period, the stimuli support was inserted into the test cage. The trial lasted five minutes and began with the first move of the subject. Investigation time of each of the two dishes was measured using two stopwatches. Investigation occurred when the nose of the mouse was 1 cm or less above the dish, or when the mouse was scratching in the dish. After each trial, the mouse was replaced with its cage mate and test apparatus was cleaned with a solution of Celsinald (Johnson Diversey).

To test concordance in mate selection in females, body odours of two male donors were presented to 12 female subjects individually. For each female, preference was
established by three successive trials using the same pair of male donors with an interval of two hours between each trial. The position of the two stimuli (right or left) was alternated between each trial. Preference for a given male was indicated when the female spent more time investigating olfactory stimulus of this male in each of the three trials. The trials were carried out on the same day in order to avoid an effect of this olfactory stimulation on the sexual receptivity of females [29]. Each female was tested with four different pairs of male donors. Males of each pair were not related (i.e. no grand-parent in common) and selected at random from our breeding stock. The presentation order of the four male pairs was alternated and balanced between females. A one-week period elapsed before a female was retested using a new pair of male donors.

2.3. Ethical note

All the experimental procedures were approved by the Ile-de-France Regional Ethics Committee in Animal Experiment number 3 (approval: p3/2007/02). Animals were kept in pairs, and were isolated only during the three-day period of sample collection (males) or during testing (females). As the mice were not used in other experiments and could not return to our breeding stock for sanitary safety, they were killed at the end of the experiment. They were put into a rising concentration of carbon dioxide.

2.4. Statistical analysis

Due to the small size of samples, non-parametric statistics with exact procedures were used [31]. Tests were performed on StatXact (Cytel Software Corporation, Cambridge, MA, USA). For each pair of males, the proportion of females that made a choice (i.e. preference for the same male donor during the three trials of a test) was compared to a distribution expected by chance with a probability of choice in each test of 0.25 (two chances out of eight to perform a choice) using binomial tests. The proportion of females making the same choice was compared to a distribution expected by chance with a probability of the same male choice in each test of 0.5 using the same procedure. To evaluate the responsiveness of the females to the experimental situation from the first to the fourth pair of males presented, the total investigation time spent by each female on both dishes of soiled sawdust during the three trials was compared between tests using permutation tests for paired samples. To test whether the repetition of tests affected female performance, the proportion of females that made a choice was compared between tests using a Chi square test. The same procedure was used to test if the proportion of females that made a choice differed between the four pairs of males.

3. Results

In a first analysis, we tested whether females’ behaviour changed throughout the repetition of tests. During the first test, females spent significantly more time investigating the two odorant stimuli than during the second test (means ± SE: test 1: 172.4 ± 16.7 s; test 2: 127.3 ± 18.5 s; P = 0.009); investigation time did not vary significantly during the following tests (test 3: 123.0 ± 23.0 s; test 4: 135.5 ± 22.3 s; P > 0.55 whatever the comparison). Nevertheless, the proportion of females making a choice (i.e. preference for the same male donor at each of the three trials of a test) remained high and did not vary significantly from the first to the fourth pair of males presented (respectively 10/12, 11/12, 7/12, 11/12; χ² = 5.88, d.f. = 3, P = 0.15).

According to the pair of males, 58% to 100% of females displayed a significant preference for one of the two males (Table 1). Half of the females made a choice no matter

Table 1
Choice of females for each of the four pairs of male donors.

<table>
<thead>
<tr>
<th>Males</th>
<th>Male pair A</th>
<th>Male pair B</th>
<th>Male pair C</th>
<th>Male pair D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental lineage</td>
<td>116</td>
<td>115</td>
<td>110</td>
<td>113</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A2</td>
<td>B2</td>
<td>C2</td>
<td>D2</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>B2</td>
<td>C2</td>
<td>D2</td>
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<td>C2</td>
<td>D2</td>
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<tr>
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<td>B2</td>
<td>C2</td>
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<td>A2</td>
<td>NC</td>
<td>C2</td>
<td>NC</td>
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<tr>
<td>Choice</td>
<td>12</td>
<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Binomial test</td>
<td>&lt; 0.001</td>
<td>0.029</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Concordance</td>
<td>12</td>
<td>7</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Binomial test</td>
<td>&lt; 0.001</td>
<td>0.016</td>
<td>0.012</td>
<td>0.004</td>
</tr>
</tbody>
</table>

For each pair of males (A to D), the identity and the parental lineage of each donor are given. The identity of the chosen male inside each pair of donors is given for each female (1 to 12) (NC: No Choice). The “chosen” male corresponds to the donor preferred at each of the three trials. Choice: number of females that made a choice and associated statistics in italics. Concordance: number of females that made the same choice and associated statistics in italics.
which pair of males was presented (females 1 to 6 in Table 1). In the other half, female choice could be assessed only for three (females 7 to 9 in Table 1) or two pairs of males (females 10 to 12 in Table 1). The proportion of females making a choice depended on the pair of males presented. The four pairs of males elicited different proportions of females that made a choice ($\chi^2 = 8.068$, d.f. = 3, $P < 0.05$) (Table 1), but the difference was significant only between the two more distant pairs (pairs A and B in Table 1) ($\chi^2 = 6.32$, d.f. = 1, $P = 0.037$). In each of the four pairs of males and when choice occurred, with only one exception (Table 1), females always chose the same male ($P < 0.016$, binomial test). Whereas choosing females investigated more the odours of preferred males, non-choosing females tended to spend a similar time investigating both odorous stimuli (Fig. 1). No statistical test was performed because the number of non-choosing females was always too small. The time spent investigating both stimuli did not vary greatly between choosing and non-choosing females (mean ± SE; male pair B, choosing females: 27.84 ± 5.74 s, n = 7; male pair B, non-choosing females: 22.16 ± 2.51 s, n = 5; male pair D, choosing females: 19.37 ± 3.20 s, n = 9; male pair D, non-choosing females: 22.41 ± 4.44 s, n = 3). The small number of non-choosing females precluded any statistical analysis.

4. Discussion

In our experiment, females had to choose between the body odours of two male donors. To determine female choice, the same pair of males was presented three times to a female. Choice was established by the female preference for a given male in each of the three trials of a test. Despite our conservative procedure, most Mus spicilegus females displayed a clear choice, whichever the pair of male donors used. Due to the novelty of the situation, females spent more time investigating the odorous stimuli during their first test. Nevertheless, females were efficient from the first test and experience did not affect their performance to choose. The short reproductive life of females in the wild may have exerted a strong pressure to select a mate in order to quickly initiate reproduction [32]. Our data revealed that the proportion of choosing females depended on the pair of males used. The contrast between male body odours may have been greater in some male pairs facilitating thereby female choice. Some females may have failed to choose when differences between males were too slight. These individual differences between females might have resulted from phenotypic differences in their sensory apparatus [33]. As a consequence, some females should be more efficient in perceiving the subtle differences between the body odours of two males and in performing a choice. The differences between females may also have occurred at a higher cognitive level: all females perceived efficiently the differences between body odours of donors but the information collected was not relevant enough for some females to induce a choice. Another hypothesis was that the difference in choosiness between females might well be due to a difference in motivation. In

![Fig. 1. Comparison of the time spent by Choosing (C) and Non-Choosing (NC) females investigating the olfactory stimuli. For each pair of male donors (A to D), investigation times (mean ± SEM) of the sawdust soiled by the preferred (black bars) and the non-preferred (open bars) donors are given for each category of females (Choosing and Non-Choosing females; number of females of each category between brackets). The “preferred” donor corresponds to the donor which soiled sawdust was the most investigated.](image-url)
this case, one may predict that non-choosing females should spend less time investigating odorous stimuli than choosing females. Although no statistical test could be performed, our data suggested, nevertheless, that it was not the case. The validity of hypothesis of a difference in motivation was then questionable.

Chemical signals provide cheat-proof displays that advertise honestly the quality of males and convey different types of information related to the donor [34]. Information transmitted is relative to the variable states of the donor (e.g. health and reproductive state, age, diet, social status) and the stable traits (e.g. species, individual identity, sex) of an individual. Our results clearly showed a strong concordance between Mus spicilegus females in mate selection whichever the pair of males used. This strong concordance between females suggested that they were able to detect relevant information and to extract it from male odours. In our experiment, males were bred under the same controlled laboratory conditions that minimized extrinsic characteristics such as diet or foraging efficiency. Consequently, males may have essentially differed by their intrinsic characteristics such as genetic, morphologic and physiological features. In nature, male diversity should be greater both by the range of variation of each trait and by the increased number of characteristics potentially taken into account by females. Concordance between females in mate choice may be limited therefore, unless one male trait is of paramount importance for all females or if females globally assess male traits using the same rules [35]. Additional studies are needed to investigate what information and criteria were used by Mus spicilegus females when they select their mate.

According to the optimal outbreeding theory, females search for complementary genetic features in males [1–7]. The overall condition of offspring may then be enhanced and the genetic diversity maintained in the population. In the field, male and female mound-building mice usually originate from different populations [21,22]. Females then do not have to avoid inbreeding when they select their mate. Consequently, the role of optimal outbreeding in this species is weakened. Instead of searching for complementary genetic features in males, Mus spicilegus females may choose to mate with the most heterozygous males as observed in other rodent species [36–39]. This alternative strategy might explain the concordance in mate choice observed between females in our study. Shared preferences might also result from mate choice copying which is an important strategy applied by females in several species [40–43]. Because female mound-building mice generally settle individually before mating [19,20] and are very aggressive towards other females [23], no social copying would occur in this species. On the other hand, contrary to previous studies that revealed a concordance in female choice [9–12], male traits were not manipulated in our experiment and male donors were selected at random from our polymorphic breeding stock. Our findings showed that a concordance between females in mate choice could be observed without necessitating any mate choice copying or manipulation of male traits. The experimental conditions of the present study tended to mimic a field situation and suggested that concordance between females may occur in nature.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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