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Research Article

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Effects of prior familiarity on female mate choice in the albino mouse (Mus musculus Linnaeus, 1758): Olfactory and live-encounter tests reveal context-dependent decoupling

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Abstract

Familiarity influences mammalian behaviour and mating decisions, including inbreeding avoidance. This study investigated whether prior familiarity affects female mate choice in albino mice (Mus musculus) using two experimental contexts: odour-based tests and live-encounter tests. Female-female tests were included as social controls. Female responses were measured as the number of visits and the total time spent investigating each stimulus. In the odour tests, females spent more time investigating bedding from unfamiliar males than from familiar males (P = 0.000), whereas the number of visits did not differ (P = 0.813). Thus, initial approach behaviour was similar, but sustained interest favoured unfamiliar male odours. In live encounters, females visited unfamiliar males more frequently than familiar males (P = 0.000), but the time spent with each male did not differ significantly (P = 0.074). Female-female control tests produced a different pattern: females spent more time investigating bedding from familiar females (P = 0.000), whereas live female-female encounters showed no significant differences in visit rate (P = 0.792) or time spent (P = 1.000). These findings indicate that familiarity affects female mate choice in a context-dependent manner. Unfamiliar male odours elicited longer investigation, which is consistent with inbreeding avoidance; however, this preference did not translate into longer interaction time during live encounters. Male behaviour may have influenced these outcomes. The study highlights the need for multiple testing approaches to understand mate choice and shows that familiarity effects are more complex than previously assumed.

Keywords: Familiarity; Mate Choice; Mus Musculus; Olfactory Cues; Inbreeding Avoidance; Dichotomous Choice Test.

INTRODUCTION

Familiarity shapes mating decisions in sexually reproducing mammals, and its effects appear to be highly context-dependent. It is well established that prior knowledge of an individual, whether as a neighbour or a relative, influences both social behaviour and mate choice (Tang-Martinez et al., 1993; Patris & Baudoin, 1998). One important function of familiarity is inbreeding avoidance: animals that recognize close kin tend to avoid mating with them, thereby reducing the genetic costs of inbreeding and helping to maintain genetic diversity (Fadao et al., 2000; Frynta et al., 2010). Thus, familiarity may act as a significant modulator of mate choice.

Familiarity is also important in the recognition of conspecifics. Animals that recognize their neighbours often behave less aggressively toward them than toward strangers, which may create mating opportunities for solitary individuals sharing adjacent territories (Tang-Martinez et al., 1993). However, responses observed in simplified laboratory settings, such as two-choice tests or small groups, may not fully represent behaviour in complex social environments. Female choice is interactive and context-dependent, and it may be influenced by factors such as the operational sex ratio and the intensity of male-male competition (Dunbar & Bever, 1998).

Most research on mate choice in vertebrates has focused on birds rather than mammals. In many avian species, males display elaborate ornaments, and females often select mates on the basis of physical traits that may signal genetic quality. By contrast, female mate choice in mammals has received less empirical attention. Existing mammalian studies have often emphasized mating decisions that improve female survival or reproductive success. More recently, however, evidence from rodents has shown consistent female preferences that may enhance offspring fitness. There is no strong reason to assume that female mammals have less developed mating preferences than female birds; rather, such preferences may be masked by male-male competition or by male behavioural strategies that limit female choice (Clutton-Brock & McAuliffe, 2009).

Although familiarity is theoretically important in shaping mating decisions, previous experimental studies have produced mixed results regarding its effects on female mate choice (Roberts & Gosling, 2004). This uncertainty indicates a gap in understanding how mammalian mate choice operates under different social and sensory contexts. The present study therefore examined the role of prior familiarity in female mate choice under controlled laboratory conditions, using the albino mouse (Mus musculus Linnaeus, 1758) as a model species.

It was hypothesized that familiarity with potential mates influences female mating preferences and that this effect would be detectable through two sensory contexts: olfactory cues and direct live encounters. Females were expected to respond differently to familiar and unfamiliar individuals, with differences reflected in two behavioural measures: the number of visits and the time spent investigating each stimulus.

The primary aim of this study was to determine whether prior familiarity influences female mate choice in the albino mouse. The specific objectives were to compare female behavioural responses, including visit rate and investigation time, to olfactory cues from familiar and unfamiliar males; to assess female preferences during direct live encounters with the same categories of males; and to repeat both olfactory and live tests using familiar and unfamiliar females as stimuli. The female-female trials were included as controls to distinguish general social responses from mate-choice-specific responses.

MATERIALS AND METHODS

Study Species

The albino mouse (Mus musculus Linnaeus, 1758) is polyestrous and breeds throughout the year, with only a modest reduction in fertility during winter. Females reach sexual maturity at approximately three to four weeks of age, and individuals older than eight weeks are considered reproductively active. Males mature slightly earlier than females, sometimes as early as five weeks, although sexual maturity more commonly occurs between six and eight weeks. The female oestrous cycle lasts four to six days. These traits make the albino mouse a useful model for studying how familiarity affects mating decisions because it shows clear mate-choice behaviour and adapts well to laboratory conditions.

Husbandry and Maintenance

All mice used in this study were outbred. A total of 20 males and 30 females were used across all experimental trials. Animals were kept in standard laboratory cages and divided into two experimental categories: familiar and unfamiliar. Males and females were housed separately in different cages on separate racks. Food was provided ad libitum as a seed mixture with insect additives (mainly millet and some sunflower seeds; Orlux Insect Patee, Versele-Laga, Deinze, Belgium). Water was supplied in dropper bottles. Wood shavings were used as bedding, and straw was provided for nest construction. Each female was tested only once across all trials, and no male was paired with the same female more than once to avoid pseudoreplication.

Experimental Procedure

To test whether familiarity influences female mate choice, a dichotomous choice design was used. The experimental arena was a clear glass terrarium measuring 28 × 35 × 58.5 cm. Three chambers were created using metal mesh dividers. The test female could move freely through the central area, whereas the stimulus males were confined to the side chambers and had no visual or physical contact with each other (Fig. 1). Before each trial, the side chambers were randomly assigned to stimulus individuals. After each trial, the entire arena was cleaned and disinfected.

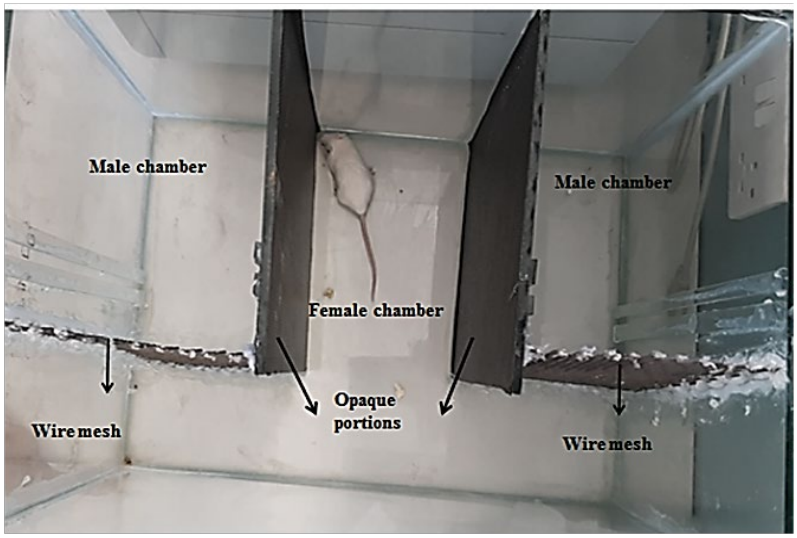

Figure (1). Experimental arena used for the dichotomous choice test.

Odour Preference Test

Soiled bedding was used as the olfactory stimulus from familiar and unfamiliar males. For six consecutive days, 100 g of bedding was collected from the cage of a male that had been housed on that bedding for one week and then placed in the female's cage, which was a clear glass terrarium. This procedure was intended to simulate a natural situation in which a female encounters the scent of nearby males without direct physical contact (Cheetham et al., 2008). After exposure, each female was assessed for oestrous status using a vaginal smear (McLean et al., 2012). The female was then placed in the central chamber of the arena and allowed to habituate for ten minutes. Soiled bedding from the test males (one familiar and one unfamiliar) was placed randomly in the two side chambers. Preference was measured by recording the number of visits to each side chamber and the total time spent in each chamber during a ten-minute observation period, measured to the nearest second.

The same odour procedure was repeated using soiled bedding from familiar and unfamiliar females as a control for non-sexual social interactions. Oestrous females were placed in the central chamber, allowed to habituate for ten minutes, and then exposed to bedding from familiar and unfamiliar females placed randomly in the side chambers. The number of visits and the time spent in each chamber were again recorded for ten minutes.

Live Preference Test

For the live preference test, females were first assessed for oestrous condition using a vaginal smear. Each female was placed in the central chamber of the arena and allowed to habituate for ten minutes. The same males used in the odour test, which had provided scent samples, were randomly assigned to the two side chambers; one was the familiar male and the other was the unfamiliar male. Female behaviour was recorded for ten minutes by measuring the number of visits to each side chamber and the total time spent in each chamber to the nearest second.

The live test was also repeated with familiar and unfamiliar females as controls. Oestrous females were placed in the central chamber, allowed to habituate for ten minutes, and then exposed to stimulus females (one familiar and one unfamiliar) placed in the side chambers. The same behavioural measures, visit frequency and duration of time spent, were recorded over a ten-minute period.

Statistical Analysis

All statistical analyses were performed using MINITAB (Version 17). Data normality was assessed with the Anderson-Darling test. Datasets that did not meet normality assumptions were transformed using square-root, rank, or cube transformations. The significance threshold was set at $\alpha = 0.05$. Independent-samples t-tests were used to compare the rate of female visits and the duration of time spent with familiar and unfamiliar stimulus individuals (males and females) in both the odour and live preference tests.

RESULTS

Odour Preference Tests

For male olfactory stimuli, no statistically significant difference was observed between the mean number of female visits to bedding from familiar males and that to bedding from unfamiliar males ($t_{37} = 0.24$, $P = 0.813$; Fig. 2). However, females spent significantly more time investigating bedding from unfamiliar males than bedding from familiar males ($t_{21} = -10.74$, $P = 0.000$; Fig. 3).

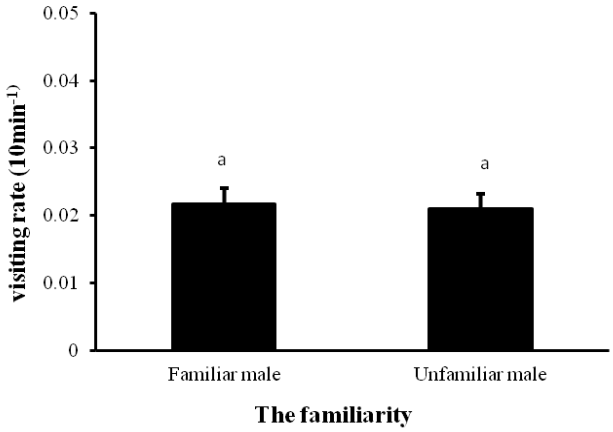

Figure (2). Visit rate of oestrous females (mean ± SE) to male bedding. Values with the same letter do not differ significantly.

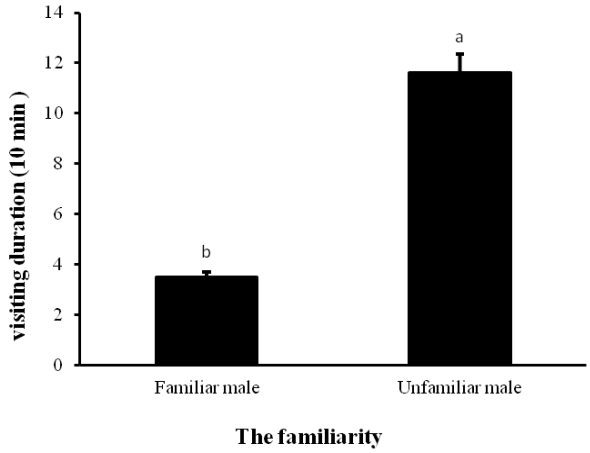

Figure (3). Visit duration of oestrous females (mean ± SE) to male bedding. Values with different letters differ significantly.

In the control odour test using female bedding, the rate of female visits did not differ significantly between familiar and unfamiliar female stimuli ($t_{35} = 0.84$, P = 0.407; Fig. 4). In contrast, the duration of investigation differed significantly: females spent more time investigating bedding from familiar females than bedding from unfamiliar females ($t_{19} = 5.94$, P = 0.000; Fig. 5).

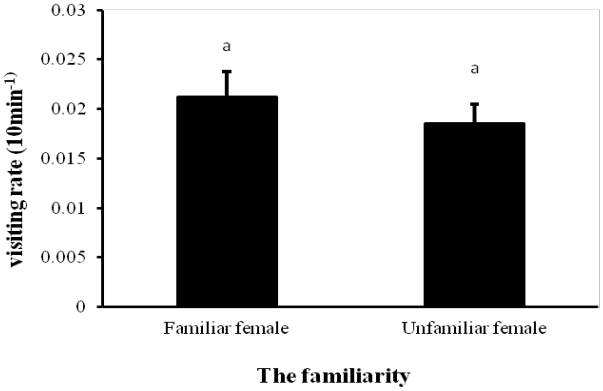

Figure (4). Visit rate of oestrous females (mean ± SE) to female bedding. Values with the same letter do not differ significantly.

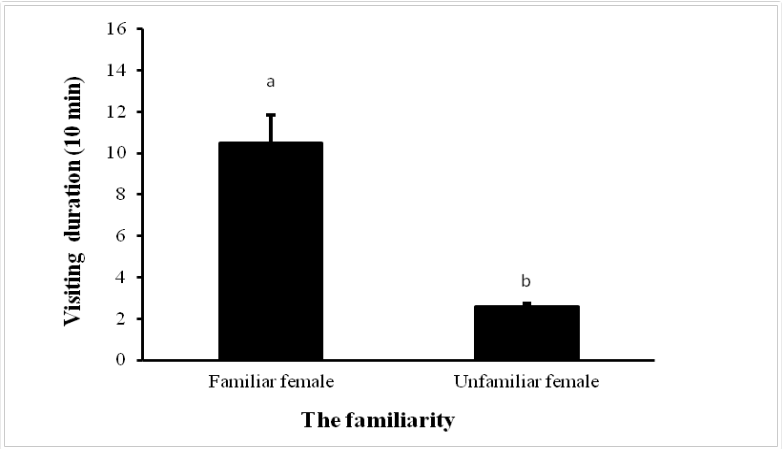

Figure (5). Visit duration of oestrous females (mean ± SE) to female bedding. Values with different letters differ significantly.

Live Preference Tests

In the live male preference tests, the frequency of female visits differed significantly between familiar and unfamiliar males. Females visited the chamber containing an unfamiliar male more often than the chamber containing a familiar male ($t_{20} = -7.90$, P = 0.000; Fig. 6). By contrast, no significant difference was detected in the duration of time females spent with familiar versus unfamiliar males ($t_{27} = 1.86$, P = 0.074; Fig. 7).

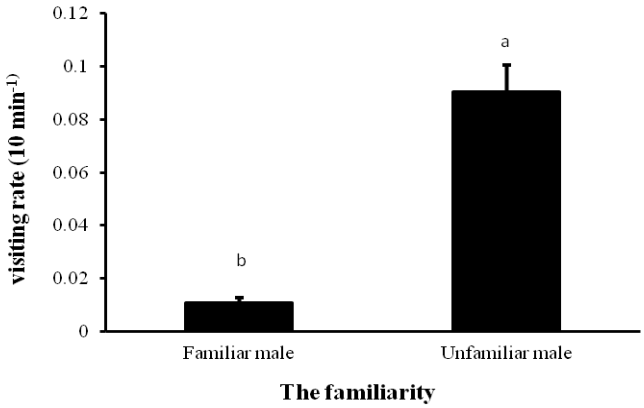

Figure (6). Visit rate of oestrous females (mean ± SE) to familiar and unfamiliar males. Values with different letters differ significantly.

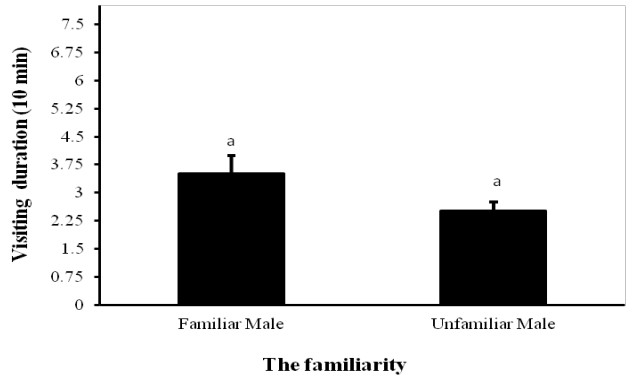

Figure (7). Visit duration of oestrous females (mean ± SE) with familiar and unfamiliar males. Values with the same letter do not differ significantly.

In the live female control tests, no significant difference was observed in the rate of female visits to familiar versus unfamiliar females ($t_{37} = -0.27$, P = 0.792; Fig. 8). Similarly, the duration of time spent did not differ between the two types of female stimuli ($t_{38} = 0.00$, P = 1.000; Fig. 9).

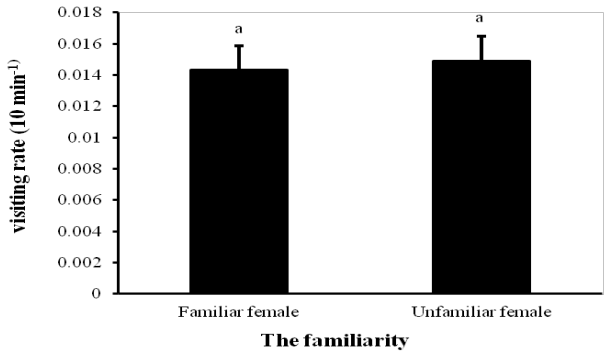

Figure (8). Visit rate of oestrous females (mean ± SE) to familiar and unfamiliar females. Values with the same letter do not differ significantly.

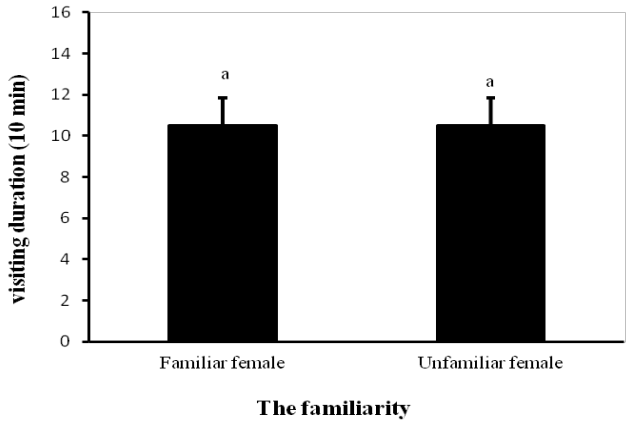

Figure (9). Visit duration of oestrous females (mean ± SE) with familiar and unfamiliar females. Values with the same letter do not differ significantly.

DISCUSSION

This study investigated the role of familiarity in female mate choice in albino mice (Mus musculus) using both olfactory cues and direct live encounters. The results revealed a context-dependent pattern: familiarity did not uniformly reduce or increase mating interest but interacted with the type of stimulus (odour versus live encounter) and the sex of the stimulus individual.

The odour preference tests produced the clearest evidence of mate-choice bias. Female mice spent significantly more time investigating bedding from unfamiliar males than bedding from familiar males, whereas visit rate did not differ. This indicates that initial exploratory motivation was similar, but the duration of investigation, a more sensitive measure of sustained interest, was biased toward novel male scents. This pattern supports the idea that olfactory cues from unfamiliar males may signal genetic novelty and possible outbreeding benefits (Milinski, 2016). Many mammalian species use scent to assess genetic compatibility or MHC dissimilarity without the risks of direct contact (Zala et al., 2015). Consistent with other rodent studies, the preference for unfamiliar male odours observed here may reflect an adaptive mechanism for avoiding inbreeding and increasing offspring heterozygosity (Lonsdorf et al., 2014; Schubert et al., 2021).

Control odour tests using female bedding produced a different pattern. Females spent significantly more time investigating bedding from familiar females than bedding from unfamiliar females. This suggests that social recognition may guide female-female interactions. Familiarity may promote greater olfactory inspection, possibly to reinforce social bonds or to assess the reproductive status of known group members (Hurst & Beynon, 2016). Unlike male-directed behaviour, which may be driven by sexual selection, female-directed olfactory investigation is likely to serve non-reproductive social monitoring. This interpretation is consistent with evidence that female rodents use scent marks to maintain social relationships and coordinate reproductive synchrony (Drickamer et al., 2018).

When the test context shifted from odour-only stimuli to live encounters, the pattern became less consistent. In the live male test, females visited unfamiliar males significantly more often than familiar males, but no significant difference was detected in the time spent with either male. This dissociation between visit frequency and duration is methodologically important. It suggests that unfamiliar males may initially attract more frequent sampling by females, whereas the actual time invested, perhaps reflecting more cautious or evaluative behaviour, did not differ once a live male was present. One possible explanation is that live encounters introduce additional variables, such as

male behaviour, aggression, and courtship attempts, which may override or obscure the olfactory-driven preferences observed in the simpler odour test (Clutton-Brock & McAuliffe, 2009). Male rodents often engage in competitive or coercive behaviours that can constrain female choice (Saleh et al., 2022). Under such conditions, the expression of female preference may be suppressed or altered (Stockley & Bro-Jørgensen, 2020).

No significant differences were observed in either visit rate or duration between familiar and unfamiliar females in the live female control tests. This result supports the interpretation that female-female interactions operate differently from female-male interactions. They are not primarily driven by novelty seeking or inbreeding avoidance; instead, they may be shaped by familiarity-based tolerance or hierarchy maintenance, which does not necessarily translate into differential spatial preference in a dichotomous choice arena (Goulet et al., 2017).

Taken together, these findings indicate that familiarity may play a sex-specific and context-dependent role in female mate choice. The clear preference for unfamiliar male odours supports the inbreeding-avoidance hypothesis, but this preference weakened during live encounters, suggesting that social dynamics should be incorporated into experimental designs (Wong & Candolin, 2015). As previous studies have noted, female preferences expressed in simplified laboratory settings may not always predict behaviour in more ecologically realistic environments, where male competition, female risk assessment, and prior social history interact (Ah-King & Gowaty, 2016).

A major strength of this study is the use of both odour and live tests, which allowed sensory evaluation to be distinguished from direct social interaction. The inclusion of female-female controls also strengthens the interpretation that the observed effects were specific to mate choice rather than a general response to novelty. Nevertheless, several limitations should be acknowledged. A dichotomous choice test cannot fully capture natural social relationships, and the use of outbred laboratory mice may limit generalisation to wild populations, where familiarity is often confounded with relatedness (Thünken et al., 2021).

Future studies should replicate these findings in semi-natural enclosures that allow more realistic mate encounter rates and male-male competition. The translation of preference for unfamiliar male odours into differential reproductive success should also be investigated by measuring outcomes such as litter size, offspring survival, or genetic diversity (Frommen, 2020). Female oestrous stage and previous mating experience may also influence the flexibility of female choice (Ramm, 2020).

CONCLUSION

Female albino mice preferred the scent of unfamiliar males, a pattern consistent with inbreeding avoidance. However, in live encounters, this preference did not translate into a longer duration of interaction with unfamiliar males. By contrast, female-female interactions showed no comparable novelty bias. These results contribute to the growing evidence that familiarity effects on mate choice are highly context-dependent and underscore the need for multifaceted experimental approaches in studies of mammalian sexual selection.

Declaration of competing interest: The authors declare that they have no competing interests associated with this manuscript.

Author contributions: M.A. and M.S. conceived and designed the experiments. H.F.A. conducted the experiments. M.A., M.S., and H.F.A. performed the analyses and interpreted the data. H.F.A.

prepared the original draft. M.A. and M.S. reviewed and edited the manuscript. M.A. supervised the project.

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