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Research Article **Open Access**

Social Density Modulates Male Courtship Behaviour Independently of Female Familiarity in Albino Mice Mus musculus

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Abstract

Understanding how social and ecological factors shape male reproductive behaviour in group-living mammals is a key question in behavioural ecology. This study examined whether prior familiarity with a female and social group density influence male courtship behaviour in albino mice *Mus musculus*, and whether these two factors interact. A controlled experiment used three density levels (small, medium, large) with a constant 1:1 sex ratio. Before trials, each female was exposed to olfactory cues only from the familiar male via his soiled bedding for six days; the unfamiliar male's scent was not presented. Courtship behaviour was recorded in the home terrarium for fifteen minutes per hour from early morning to late afternoon. The analysis yielded three principal findings. First, male courtship rate varied markedly across densities, being highest under medium density, lower under small density, and lowest under large density, thereby indicating a non-linear relationship. Second, familiarity status (familiar versus unfamiliar) did not produce a significant difference in courtship rate within any density group, nor did it interact with density. Third, a consistent diurnal pattern was observed, characterized by a courtship peak during early morning, followed by a steady decline through midday and afternoon across all densities. These results demonstrate that group density, but not prior familiarity with a female, significantly modulates male courtship effort in albino mice. The absence of a familiarity effect suggests that males do not adjust courtship intensity based on individual recognition under these conditions. The density-dependent pattern supports the concept of an optimal intermediate social density for reproductive behaviour.

Keywords: Familiarity; Courtship Behaviour; *Mus Musculus*; Group Density; Diurnal Rhythm.

INTRODUCTION

Understanding how animals select their mates has been a central theme in behavioural ecology for decades. Despite considerable progress, it is now recognised that both female choice and male reproductive effort are highly sensitive to situational and social variables. Among small mammals, particularly rodents, chemical signals and prior social experience are thought to play essential roles in regulating mating encounters (Zenuto et al., 2007; Carter et al., 2017). When two individuals share a history of cohabitation or repeated exposure, their subsequent interactions, which can range from aggressive to affiliative, can be substantially altered (Bielsky & Young, 2004; Weidt et al., 2008). On the female side, olfactory cues are known to be critical: female albino mice can discriminate between familiar and unfamiliar males, and such discrimination is linked to inbreeding avoidance and mate choice (Agbali et al., 2022; Agbali et al., 2023). However, the extent to which a male's courtship display is influenced by whether he is familiar or unfamiliar with a potential partner remains underexplored, especially when group size varies.

Male courtship in rodents typically comprises a sequence of behaviours such as anogenital sniffing, close following, and various body postures, sometimes accompanied by audible signals (Zenuto et al., 2007). Much of the existing literature has concentrated on female mating decisions as the principal factor driving male courtship intensity. Yet, it is equally plausible that male courtship reflects male-centred attributes, including his prior acquaintance with the female or his ability to perceive and respond to the surrounding social milieu (Zala & Potts, 2016; Ophir & Galef, 2004). In albino mice, female mate choice has been shown to be influenced by familiarity, with females displaying clear preferences in both odour and live tests (Agbali et al., 2022; Agbali et al., 2023). Moreover, male dominance status can affect female behaviour, but males themselves may not adjust their courtship based on the dominance of rivals (Saleh et al., 2022). At present, two interrelated questions lack clear answers: first, does the mere state of being familiar versus unfamiliar to a female alter the vigour of male courtship? Second, how does the density of the social group modify any such effect?

Population density is known to influence a wide range of behavioural traits, including stress physiology, competitive aggression, and the timing of reproductive activities (Weidt et al., 2008; Thünken et al., 2016). In crowded conditions, frequent male–male encounters might reduce the need for elaborate courtship rituals because mating opportunities become more numerous. In sparse populations, by contrast, a male might benefit from investing more time and energy in courting each available female. Intermediate densities could represent an optimum where social stimulation is sufficient to promote active courtship without triggering excessive conflict. These contrasting possibilities underline the need for empirical studies that systematically vary density while controlling for other factors such as sex ratio (Latham & Mason, 2004).

Despite the wealth of studies on female preference for familiar or unfamiliar males, very little is known about whether male courtship behaviour itself is contingent on familiarity, and how this might interact with group density. Most previous experiments have used pairwise or dichotomous choice designs that do not replicate the complex social dynamics of group living. Studies on female choice in albino mice have demonstrated that females use olfactory cues to evaluate male quality and that familiarity influences female preference (Agbali et al., 2022; Agbali et al., 2023). However, whether male courtship follows similar rules has not been systematically tested under varying densities**.** Consequently, whether density modulates male courtship independently of female choice remains an open question.

The albino mouse (*Mus musculus* Linnaeus, 1758) offers a practical and well-characterised model for such investigations. Its reproductive physiology, including a polyestrous cycle, short gestation length, and predictable postpartum oestrus, has been extensively documented (Guenet et al., 2015). Moreover, the species adapts readily to standardised laboratory housing, allowing researchers to manipulate familiarity and density under controlled conditions.

It was hypothesized that group density would exert a significant main effect on male courtship rate, with the highest courtship activity occurring at intermediate density due to optimal social stimulation. In contrast, we predicted that male familiarity (whether a male was familiar or unfamiliar to the female) would not independently affect courtship rate, nor would it interact significantly with density. This prediction was based on preliminary observations suggesting that male mice do not adjust courtship intensity according to familiarity when housed in mixed-sex groups.

Accordingly, the present study was designed to investigate the effects of male familiarity (familiar vs. unfamiliar) and group density (small, medium, large) on male courtship behavior in albino mice (*Mus musculus*) in a mate-choice context, and to determine whether there is an interaction between familiarity and density in shaping this behavior.

MATERIALS AND METHODS

Husbandry

All experimental procedures were conducted in the Zoology Department laboratories of the aforementioned university. This investigation utilized outbred albino mice (*Mus musculus*) obtained from the Faculty of Veterinary Medicine at Omar Al-Mukhtar University. Males and females were housed separately from the outset in standard laboratory cages placed on distinct racks to prevent any prior contact. Animals received an *ad libitum* diet consisting of a seed mixture (primarily millet and sunflower seeds) supplemented with insect-based components (Orlux Insect Patee, Versele-Laga, Belgium). Water was provided via dropper bottles. Wood shavings served as bedding material, and shredded straw was supplied for nest construction. To avoid pseudoreplication, no male was paired more than once with the same female.

Experimental Design: Female Response to Male Familiarity Across Group Densities

To investigate how familiarity with a specific male influences female choice under varying social densities, an experiment was designed to replicate naturalistic social conditions rather than using conventional dichotomous choice tests. A total of 60 individuals were used in this study. Prior to each trial, females were exposed indirectly to olfactory cues from the familiar male only, using his soiled bedding. Approximately 100 g of bedding previously occupied by the familiar male alone for one week was transferred daily for six consecutive days into the female's home terrarium (15 L capacity). This procedure simulated a natural scenario in which a female repeatedly detects the scent of a particular neighbouring male without direct physical contact. No such exposure was conducted for the unfamiliar male, ensuring that the female had no prior olfactory or physical experience with him.

Female preference for familiar versus unfamiliar males was evaluated across three distinct density treatments, with the operational sex ratio maintained at 1:1 (male:female). Three replicates were prepared for each density level: small (5 males + 5 females) (Fig.1), medium (10 males + 10 females) (Fig.2), and large (15 males + 15 females) (Fig.3). Each group was housed in a 15 L glass terrarium, but with different floor dimensions to achieve the desired density (small density 40×40 cm, medium density 56.5×56.5 cm, large density 69.2×69.2 cm).

Mate choice observations were conducted *in situ* within each group's home terrarium, with all individuals present, rather than in a separate choice apparatus. Each density level was assigned a dedicated experimental room, and three replicates per density were performed. Mice were randomly allocated to terraria. Prior to introduction, several males and females (including two males—one familiar and one unfamiliar to the focal female—and two females) were individually marked for behavioural identification. For visual identification, each mouse was briefly restrained, and a non-toxic, permanent marker was used to apply small coloured dots to a discrete area of the white fur. Unique colour-position codes (e.g., dorsal stripe, head spot) distinguished focal individuals. The ink dried within seconds, caused no distress, and remained visible throughout the study.

Figure (1). Experimental area for the small-density group

Figure (2). Experimental area for the medium-density group

Figure (3). Experimental area for the large-density group

Courtship Behaviour Recording

Individual behaviour was recorded for 15 minutes per hour from 08:00 to 16:00 (Libya local time). Courtship behaviour was operationally defined as a sequence of actions including approach, following, anogenital sniffing, and specific body postures. Olfactory cues were considered the primary mode of communication in this context. Positive female responses included neck exposure, tail lifting, and genital presentation during mutual chasing and sniffing, as described in previous ethological studies (Zenuto et al., 2007).

Statistical Analysis

All statistical analyses were performed using MINITAB (Version 14). Normality of the data was assessed using the Anderson–Darling test. Datasets that violated normality assumptions were transformed appropriately (square-root, rank, or cube transformations). The significance threshold was set at $\alpha = 0.05$. To compare courtship rates between familiar and unfamiliar males within each density level, independent-samples t-tests were employed. A two-way analysis of variance (ANOVA) was conducted to examine potential interaction effects between familiarity status and group density on male courtship behaviour. Finally, a one-way ANOVA was used to compare courtship intensity across the three density levels (low, medium, large) and to assess the temporal persistence of courtship behaviour throughout the observation period.

RESULTS

In the small-density condition, the courtship frequency of familiar males varied significantly across the hourly observation blocks (08:00–16:00) during the first 15-minute intervals (one-way ANOVA: $F_8 = 6.27$, $P = 0.001$), with the highest value recorded at 08:00 and the lowest at 12:00, 14:00, 15:00, and 16:00, as illustrated in Fig. 4. A similarly significant temporal fluctuation was observed for unfamiliar males in the same density ($F_8 = 2.89$, $P = 0.029$), peaking at 08:00 and reaching minima at 12:00 and 15:00 (Fig. 5).

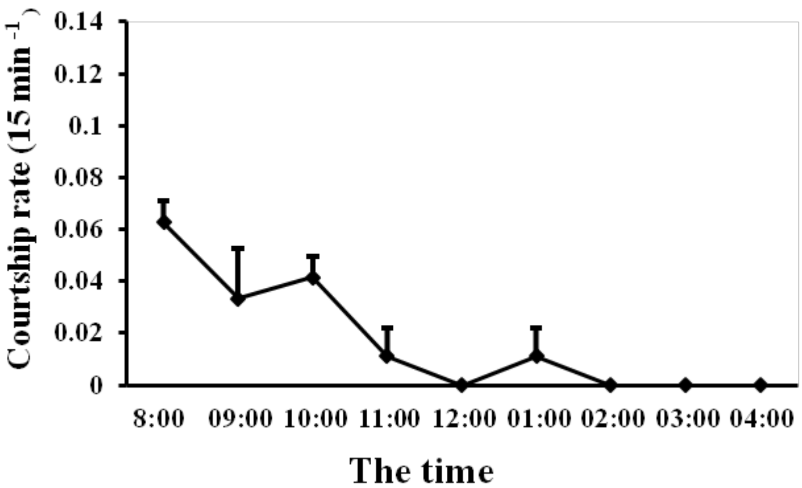

Figure (4). Courtship rate of familiar males, mean (±SE), in the small-density group.

Within the medium-density treatment, familiar males also exhibited a significant hourly variation ($F_8 = 2.75$, $P = 0.036$), with courtship peaking at 08:00 and showing the lowest rate at 19:00 (Fig. 6). Unfamiliar males in the same density displayed an even more pronounced temporal effect ($F_8 = 13.83$, $P < 0.001$), with maximum activity at 08:00 and minima at 15:00 and 16:00 (Fig. 7)

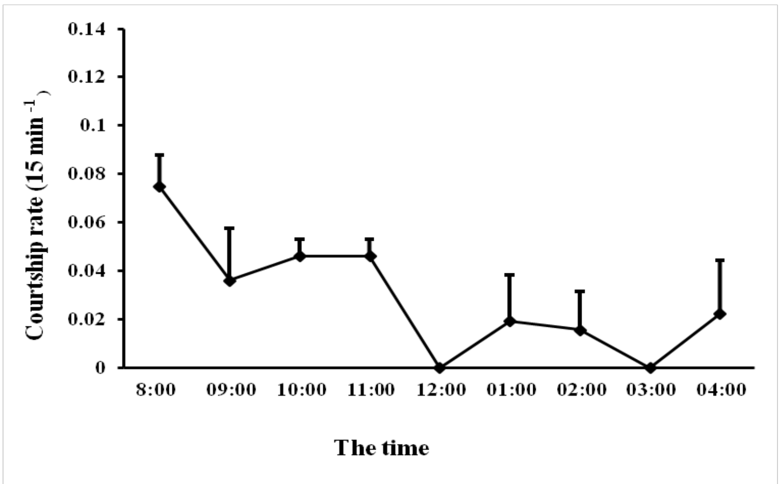

Figure (5). Courtship rate of unfamiliar males, mean (±SE), in the small-density group.

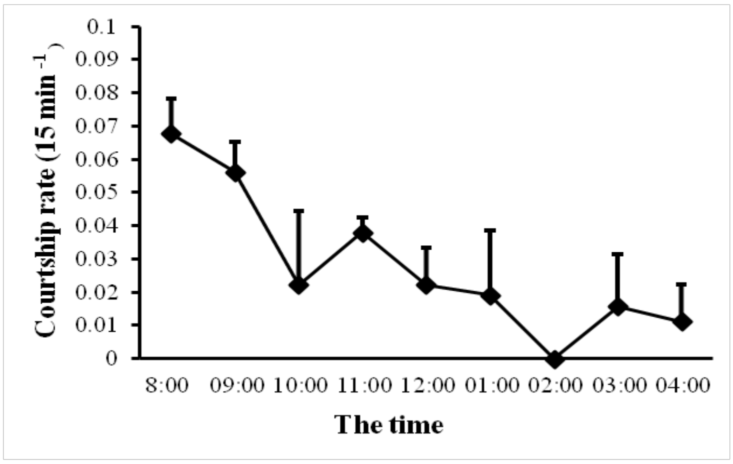

Figure (6). Courtship rate of familiar males, mean (±SE), in the medium-density group.

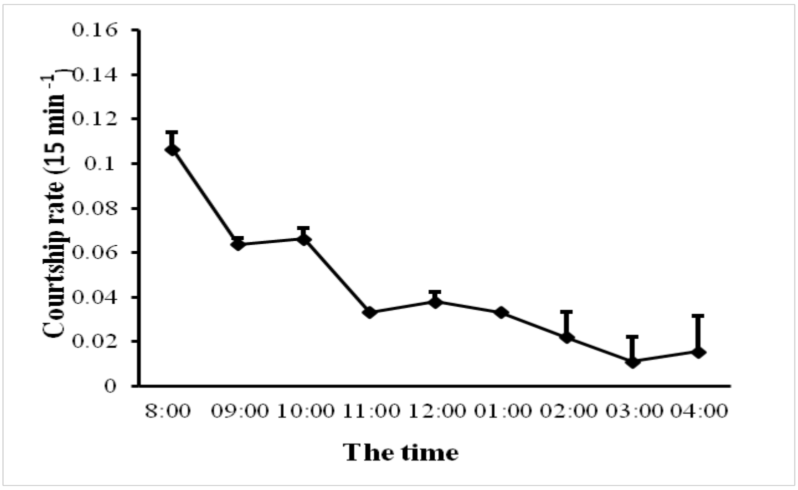

Figure (7). Courtship rate of unfamiliar males, mean (±SE), in the medium-density group.

For the large-density groups, familiar males demonstrated significant changes in courtship rate across hours (F_8 = 4.35, P = 0.005), where the peak occurred at 08:00 and the lowest frequencies were observed at 11:00, 13:00, 14:00, 15:00, and 16:00 (Fig. 8). Unfamiliar males in the large-density condition likewise showed significant hourly variation (F_8 = 3.12, P = 0.022), with the highest courtship at 08:00 and the lowest at 10:00, 12:00, 14:00, 15:00, and 16:00 (Fig. 9).

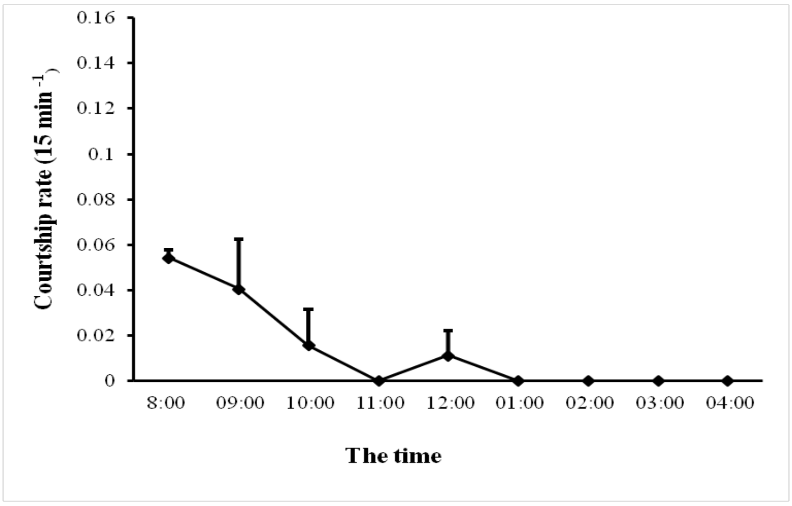

Figure (8). Courtship rate of familiar males, mean (±SE), in the large-density group.

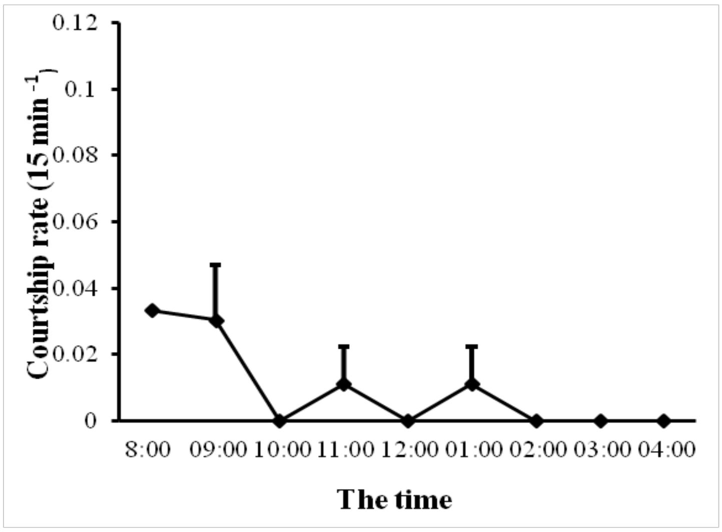

Figure (9). Courtship rate of unfamiliar males, mean (±SE), in the large-density group.

Within the small-density groups, no statistically significant difference emerged between the courtship rates of familiar and unfamiliar males (independent-samples t-test: t_{50} = –1.44, P = 0.157), as shown in (Fig.10)**.** In the medium-density groups, the difference also failed to reach significance (t_{51} = –1.87, P = 0.067) (Fig.11). Similarly, in the large-density groups, the courtship rates of familiar and unfamiliar males were comparable (t46 = 0.70, P = 0.488; Fig. 12). A one-way ANOVA revealed a significant main effect of group density on male courtship rate (F_2 = 10.75, P < 0.001). The mean courtship activity was highest under medium density (0.03571), followed by small density (0.02333), and lowest under large density (0.01153). These differences are presented in Fig. 13

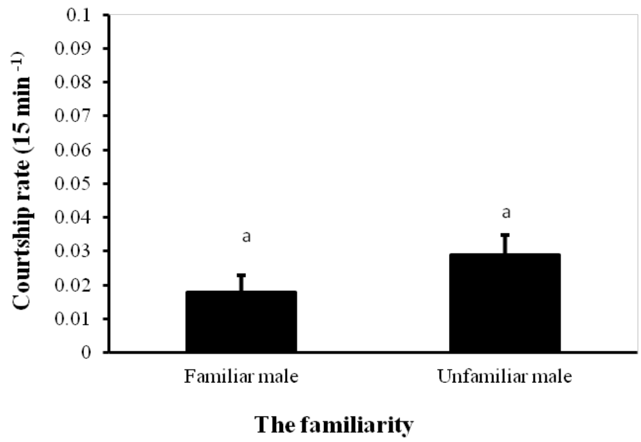

Figure (10). Courtship rate of familiar and unfamiliar males, mean (±SE), in the small-density group. Values with the same letter did not differ significantly.

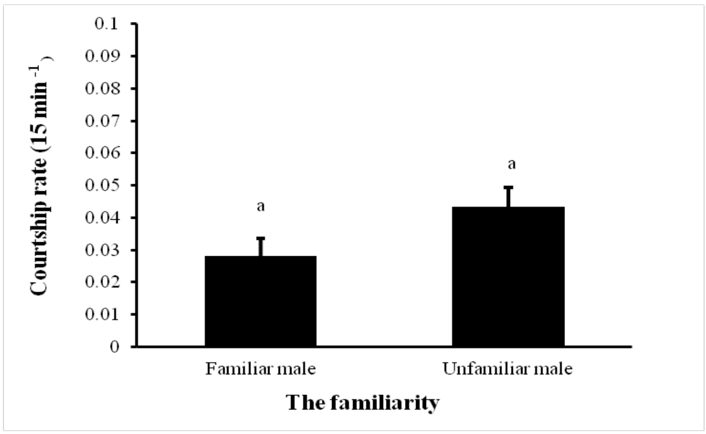

Figure (11). Courtship rate of familiar and unfamiliar males, mean (±SE), in the medium-density group. Values with the same letter did not differ significantly.

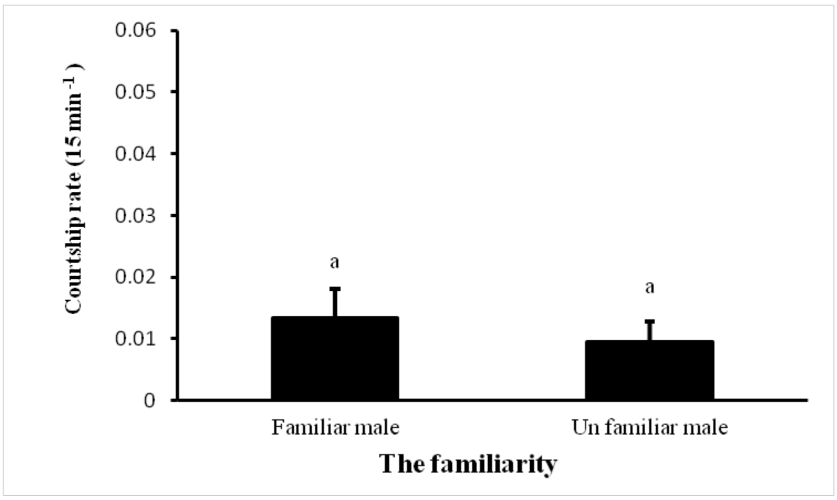

Figure (12). Courtship rate of familiar and unfamiliar males, mean (±SE), in the large-density group. Values with the same letter did not differ significantly.

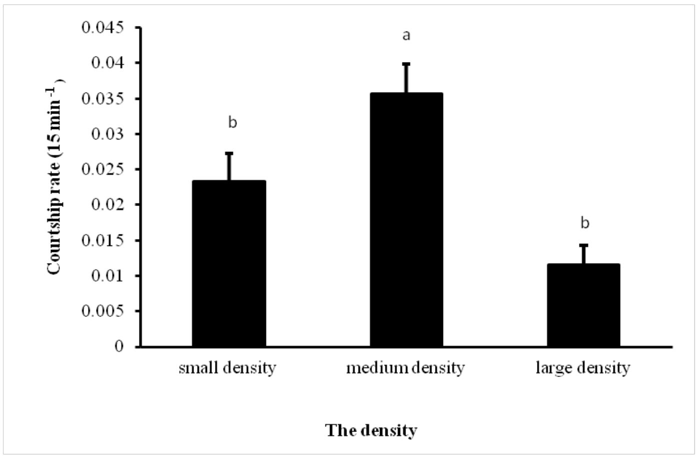

Figure (13). Courtship rate of males, mean (±SE), in different density groups. Values with the same letter did not differ significantly.

A two-way ANOVA was conducted to examine potential interaction effects. The results indicated no significant interaction between familiarity status and group density on male courtship rate ($F = 1.94$, $df = 2$, $P = 0.147$). In contrast, the main effect of group density remained highly significant ($F = 11.02$, $df = 2$, $P < 0.001$), while the main effect of familiarity alone did not reach statistical significance ($F = 3.18$, $df = 1$, $P = 0.077$). Detailed variance components are summarized in Table 1**.**

Table (1). Two-way ANOVA of mean courtship rates of male mice and interactions between these factors (familiarity and group size).

Source	df	SS	MS	F	P
Courtship rate					
Group size (G)	2	0.015792	0.0078959	11.02	0.000
Familiarity (f)	1	0.002275	0.0022754	3.18	0.077
Familiarity × group size (f × g)	2	0.002776	0.0013882	1.94	0.147
Error	156	0.111752	0.0007164		

DISCUSSION

This study yielded three main results. First, male courtship rate varied non-linearly with group density: it was highest under medium density and lower in both small and large groups. Second, familiarity with the female did not significantly affect courtship rate within any density, and no interaction between familiarity and density emerged. Third, courtship consistently peaked at 08:00 and declined through midday and afternoon across all densities.

The density-dependent pattern supports the hypothesis that social density non-linearly modulates male reproductive effort. Medium density likely provides optimal social stimulation without the negative effects of overcrowding, whereas low density limits encounter rates and high density elevates male-male competition and chronic stress (Latham & Mason, 2004). Comparable findings exist in other rodents: Hurst and Barnard (1992) reported more balanced social interactions at moderate densities in wild house mice, and Drickamer (2007) found higher scent-marking and approach rates in males from intermediate-sized groups. Thus, density acts as an ecological constraint shaping male courtship independently of female choice.

Contrary to predictions based on prior acquaintance, familiarity did not alter courtship rate. This contrasts with female-side results, where female albino mice show clear discrimination between familiar and unfamiliar males, especially in olfactory preference tests (Agbali et al., 2022; Agbali et al., 2023). Females also adjust their mate choice based on familiarity to avoid inbreeding and increase offspring fitness (Agbali et al., 2022). The lack of a familiarity effect in males aligns with the idea that male mice do not distinguish familiar from unfamiliar partners under group housing conditions. Constant mixed-sex interactions may make discrimination costly relative to any benefit, favouring a "generalist" courtship strategy that maximizes mating opportunities (Zala & Potts, 2016). Alternatively, the relatively short cohabitation period may have been insufficient for behavioural adjustment. However, female mice do use familiarity in mate choice (Weidt et al., 2008), indicating sex-specific strategies. Males may prioritise immediate mating over familiarity-based discrimination, especially when females are receptive. The absence of a familiarity-density interaction reinforces that overall social density drives courtship more than individual recognition.

A clear diurnal peak at 08:00 reflects the species' crepuscular/nocturnal activity pattern (Drickamer, 2007). This methodological insight emphasises the need for multi-time-point sampling; restricting observations to midday would underestimate courtship.

Consistent with our results, Ophir and Galef (2004) found that male house mice did not alter courtship intensity based on female familiarity. In contrast, male meadow voles spent more time investigating unfamiliar females (Parker et al., 2001), suggesting species-specific effects. Our work extends these findings to ecologically relevant densities.

The absence of a familiarity effect contrasts with female choice (Weidt et al., 2008). Sex-specific selection pressures likely explain this: females bear higher reproductive costs and benefit from discrimination, whereas males benefit from maximising mating attempts regardless of familiarity (Trivers, 1972). The strong density effect supports ecological factors over individual recognition. Future studies should examine more extreme densities, longer familiarisation periods, wild strains, and neuroendocrine mechanisms (e.g., oxytocin, vasopressin; Bielsky & Young, 2004). Simultaneous manipulation of familiarity, density, and female receptivity would further disentangle male and female drivers.

CONCLUSION

Group density non-linearly modulates male courtship behaviour in albino mice, with the highest courtship rate at medium density, whereas prior familiarity with a female does not affect courtship intensity and does not interact with density. A consistent diurnal peak at 08:00 was observed across all densities, underscoring the importance of temporal sampling. These findings indicate that ecological factors (social density) outweigh individual recognition in shaping male courtship, and that a generalist mating strategy may operate under group-living conditions, supporting the existence of an optimal intermediate density for reproductive effort. Future studies should examine more extreme densities, longer familiarisation periods, wild-derived strains, and neuroendocrine mechanisms to further disentangle the drivers of male courtship.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: M.S. and M.A. proposed and designed the experiments. H.F.A. conducted the experiments. M.A., M.S., and H.F.A. performed the analytic calculations. H.F.A. prepared the original draft. M.A. and M.S. reviewed and edited the manuscript. M.A. and M.S. reviewed and edited the final version of the manuscript. M.A. supervised the project.

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