Original paper

Partner preferences of three captive vole (*Microtus*) species derived from Eurasia and Japan

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Abstract: Mating systems, such as monogamous and promiscuous mating, can be inferred by assessing social behavior, particularly pair bonding. We used partner preference tests to infer pair bond formation in three captive vole species from Eurasia and Japan, i.e. *Microtus arvalis*, *M. montebelli*, and *M. levis*. In all three species, females exhibited a preference for their male partners, but no preference was observed by males for their female partners. These results suggest that the social system of these vole species is non-monogamous.

I. Introduction

Microtus species, a large genus of more than 60 species, are taxonomically closely related, but their social organization can vary markedly among them (Musser & Carleton 1993). For example, in the monogamous *M. ochrogaster* (prairie vole) and *M. pinetorum* (woodland vole) males and females form a perpetual mating pair and raise their pups cooperatively (Getz et al. 1981, FitzGerald & Madison 1983, Hofmann et al. 1984, Getz & Hofmann 1986, Getz & Carter 1996), whereas the non-monogamous *M. pennsylvanicus* (meadow vole) and *M. montanus* (montane vole) with reduced sociality lack pair bonding and nest sharing (Getz 1972, Jannett 1982, McGuire & Novak 1984, Gruder-Adams & Getz 1985). Studies on these voles have contributed to our understanding of social behaviors such as pair bonding, selective aggression, and paternal behavior, as well as their underlying neurobiological mechanisms (Carter & Getz 1993, Donaldson & Young 2008, Young et al. 2008, Phelps & Gustison 2020).

However, most such studies have been conducted on North American species. Clarification of sociality in more species of voles will provide valuable information to further understanding of the neural mechanisms underlying sociality. In this study, we investigated the partner preferences of three captive vole species from Eurasia and Japan.

II. Materials and Methods

Animals

The animals used in this study were from the Mar strain (*M. arvalis*, common vole), Mmon2 strain (*M. montebelli*, Japanese field vole), and MrosA strain (*M. levis*, East European vole), and were maintained under laboratory conditions at the Department of Zoology, Okayama University of Science. The origin of each vole strain is as follows. The Mar strain was derived from *M. arvalis* captured in Hungary in 1969-1970 (Kudo & Oki 1982). The Mmon2 strain was derived from *M. montebelli* captured in Okegawa, Saitama, Japan, in 1977 (Kudo & Oki 1982) and developed by introducing new wild individuals several times. The MrosA strain was derived from *M. levis* captured in St. Petersburg, Russia, in 1995 (Widayati et al. 2003). The housing conditions were set at room temperature ($23 \pm 2°C$) under a light/dark cycle of 14L10D. Animals were fed a combination of pellet feed for mice and rats (MR Breeder, Nosan Co., Yokohama), herbivores (ZF, Oriental Yeast Co., Ltd., Tokyo), and timothy hay (commercial product for pets). Food and water were given *ad libitum*.

All animal experiments were conducted in accordance with the Regulations for Animal Experiments of Okayama University of Science. Our experimental protocols, including those involving animals (Exp2020–004), were approved by the Animal Experiments Committee of our university.

Apparatus

The apparatus was constructed as in previous studies (Sadino et al. 2018, Tchabovsky et al. 2019) (Fig. 1). A roofless field of W75 cm × D25 cm × H25 cm in area was made with acrylic sheets. Acrylic partition boards further divided the field equally into three compartments (i.e.

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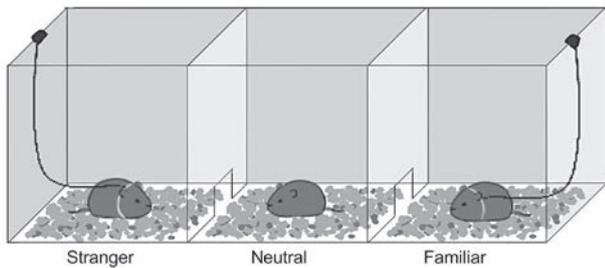*Correspondence: Kazuyuki MEKADA, Email: mekada@zool.ous.ac.jp

Fig. 1. Diagram of the acrylic-sheet testing apparatus (W75 cm × D25 cm × H25 cm) used to assess opposite-sex selective partner preferences in male and female voles. A test animal is allowed to freely move in a three-chambered apparatus with opposite-sex voles (the partner and a stranger, tethered on opposite ends).

W25 cm × D25 cm each). The two side compartments were used as stimulus animal holding areas and the middle compartment was used as the neutral area. Holes (5 cm × 5 cm each) were made in the center of the bottom of partitions to allow the test animals to move between areas. The outer sides of the apparatus were shaded so that the outside could not be seen. The bottom of the field was covered with wooden chips for use during the experiment.

Procedure

Adult females and males (3 to 9 months old) with no previous sexual experience were housed together for 1 week using a standard housing cage. Paired animals were designated as Familiar, and unrelated animals as Stranger. The combination of animals in each experiment was set up as follows: the test animal was a female, and the stimulus animals were a Familiar male and a Stranger male, or vice versa. When the test animals were females, 11 pairs of *M. arvalis*, 11 pairs of *M. montebelli*, and 10 pairs of *M. levis* were used. When the test animals were males, 10 pairs of *M. arvalis*, 10 pairs of *M. montebelli*, and 10 pairs of *M. levis* were used.

One week before the experiment, a collar was fitted around the neck of the stimulus animals under isoflurane inhalation anesthesia. The collar was made of soft plastic (commercially available 2.5 mm wide) and loosely fitted but not to allow the neck to get loose off. At the time of collar attachment and just before the experiment, the health conditions of the stimulus animals were observed by checking their coat, the presence or absence of external injuries, and body weight, and it was confirmed that there were no abnormalities.

First, the test animals were allowed to habituate to the field for 20 min. During this time, the animals were allowed to explore the field freely. Next, the test animals were removed from the field and the two stimulus animals were simultaneously habituated to the field for 20 min. During

habituation, a leash was connected to a collar attached to the animal, and the other end of the leash was fixed to the wall of each stimulus animal area. The length of the leash was set such that the stimulus animal connected to the leash could not enter the neutral area. After habituation of the stimulus animals, the test animals were placed in the neutral area (Fig. 1), and the behavior of the test animals was observed for 60 min. The behavior of the animals was recorded from the upper side of the field using a video camera (HC-V480MS, Panasonic Co., Kadoma, Osaka). The time spent in each area, duration of side-by-side contact, numbers of aggressive and mounting (for male) or lordotic response (for female) behaviors of the test animals were recorded based on the videos. Aggressive behavior was classified as the test animal engaging in charging, attacking, biting, or boxing with a stimulus animal (Ferkin 1988, Parker et al. 2001). After the experiment, the animals were returned to the housing cages, and those not participating in subsequent experiments had their collars removed. The apparatus was washed and dried after each trial.

The measurements obtained during the observation were statistically analyzed using Wilcoxon signed rank sum tests using the statistical Rcmdr package version 2.7–1 (Fox 2005, 2017) with R version 4.0.4 (R Core Team 2021).

III. Result

Box plots including scatter plots, generated for each sex of test animal using the experimental parameters outlined below, are shown in Figures 2-5.

Time spent in Familiar and Stranger animal areas

Among female test animals, *M. levis* spent a median of 42.6 min in the Familiar male area and a significantly shorter 4.9 min in the Stranger male area. *M. arvalis* and *M. montebelli* also tended to spend more time in the area of Familiar males, but some individuals stayed in the area of Stranger male. As a result, no significant differences were observed for these species (p = 0.067 and p = 0.147, respectively; Fig. 2). When test animals were males, there was no significant differences between time spent in the Familiar and Stranger female areas for any of the three species.

Side-by-side contact

For female test animals, the median side-by-side contact time with Familiar males was 1.0 min for *M. arvalis*, 18.8 min for *M. montebelli*, and 14.0 min for *M. levis*, while the median time with Stranger males was 0 min for all three species.
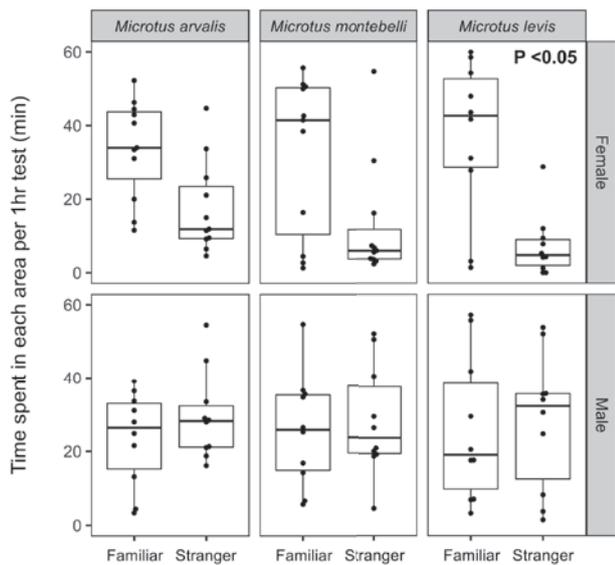
Fig. 2. Box plots including scatter plots of time spent in Familiar and Stranger animal areas during 1 h preference tests. In the upper row, the test animal is female and the stimulus animal is male; in the lower row, the test animal is male and the stimulus animal is female.
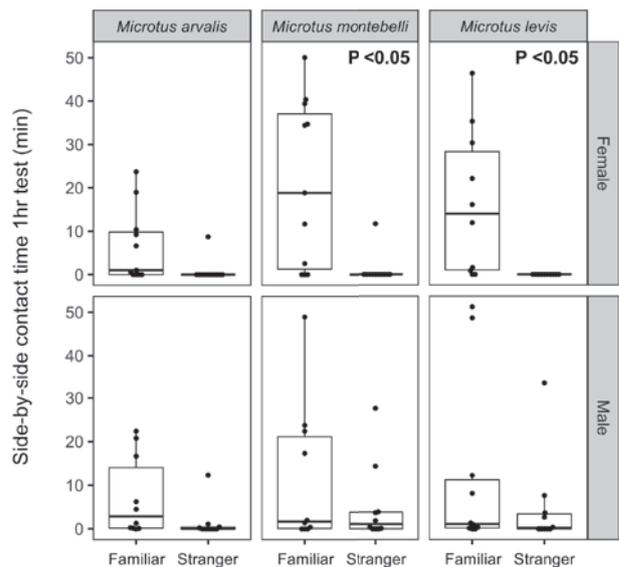

Fig. 3. Box plots including scatter plots of side-by-side contact with Familiar and Stranger animals during 1h preference tests. In the upper row, the test animal is female and the stimulus animal is male; in the lower row, the test animal is male and the stimulus animal is female.

There was a statistically significant difference (p < 0.05) in the side-by-side contact times between Familiar and Stranger males in *M. montebelli* and *M. levis* (Fig. 3). When the test animals were males, there were no significant differences in the side-by-side contact times between Familiar and Stranger females for any of the three species, although at the individual level, some males were found to have significantly longer side-by-side contact time with Familiar females. For example, in *M. montebelli*, a test male (ID: Mmon2–206A) spent 48.9 min with the Familiar female. Similarly, in *M. levis*, test males (ID: MrosA–435A and 439C) had side-by-side contact times of 48.6 and 51.2 min, respectively, for Familiar female. However, in *M. arvalis*, no male was observed to have side-by-side contact time with a Familiar female of more than half of the observation time.

Aggressive behavior
For female test animals, *M. arvalis* showed few or no attack behaviors against either Familiar or Stranger males (p = 0.181). *M. montebelli* and *M. levis* females showed more aggressive interactions against Stranger males than Familiar males (p < 0.05) (Fig. 4). For male test animals, there were no significant differences in the number of aggressive counts between Familiar and Stranger females for any of the three species.

Mounting or lordotic behaviors
For female test animals, lordotic responses were rarely observed in any of the three species, except in *M. levis*, where only two animals exhibited lordotic responses toward Stranger males.

For male test animals, no mounting behavior was observed toward Familiar females in *M. arvalis*, but mounting behavior was observed in 6 out of 10 males toward Stranger females (p < 0.05). *M. montebelli* exhibited mounting behavior to both Familiar and Stranger females. In *M. levis*, only one male exhibited mounting behavior toward a Familiar female (Fig. 5).

IV. Discussion

Comparing the time spent by *M. arvalis*, *M. montebelli*, and *M. levis* test animals in the stimulus animal areas, females spent more time in the area of Familiar than Stranger males, and this was more pronounced in *M. levis*. Furthermore, in the side-by-side contact time, the time spent with Familiar males was longer than that spent with Stranger males in *M. montebelli* and *M. levis*. This indicates that female *M. montebelli* and *M. levis* prefer Familiar males as their partners, as side-by-side contact is considered an amicable behavior (Williams et al. 1992). The selective aggression of females toward Stranger males observed in *M. montebelli* and *M. levis* may also support this preference. In monogamous *M. ochrogaster*, both females and males spend significantly more time in physical contact with their partners than with strangers (Aragona & Wang 2004). By contrast, no preference for partners is observed in non-monogamous *M. pennsylvanicus* males (Lim et al. 2004). Similarly, no selective preference for Familiar females was observed in the test males of the three vole species. Hence, we consider these three captive vole species are
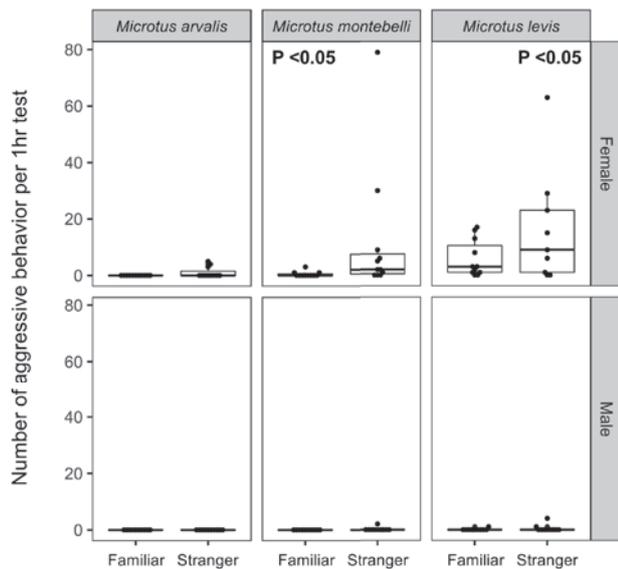
Fig. 4. Box plots including scatter plots of aggressive behavior against Familiar and Stranger animals during 1 h preference tests. In the upper row, the test animal is female and the stimulus animal is male; in the lower row, the test animal is male and the stimulus animal is female.
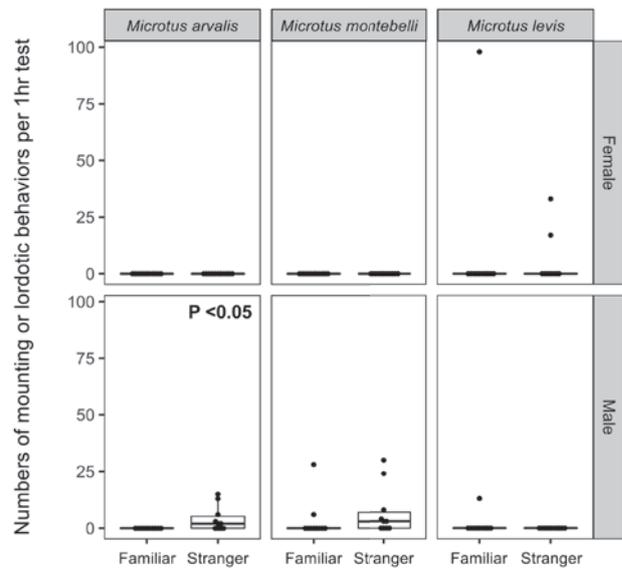

Fig. 5. Box plots including scatter plots of mounting (for male) or lordotic (for female) behaviors toward Familiar or Stranger animals during 1 h preference tests. In the upper row, the test animal is female and the stimulus animal is male; in the lower row, the test animal is male and the stimulus animal is female.

non-monogamous. In addition, test females of *M. arvalis* exhibited less marked preference for Familiar males than did females of the other two species. In *M. arvalis*, lordotic response behavior toward Stranger males was also observed, suggesting that *M. arvalis* may be more promiscuous than *M. montebelli* and *M. levis*.

M. ochrogaster is socially monogamous, but not sexually exclusive, and individuals vary in their degree of monogamous behavior (male sexual fidelity) (Ophir et al. 2008). Male fidelity is often thought to depend on spatial strategies which balance the demands of mate-guarding against the value of mating multiplication (Emlen & Oring 1977, Kokko & Rankin 2006). The variation in male fidelity in *M. ochrogaster* reflects differences in the expression, regulation and epigenetic status of a vasopressin receptor V1aR that functions in spatial memory (Okhovat et al 2015). Okhovat et al. (2015) found genetically related SNPs in the putative enhancer regions of the *Avpr1a* gene which encodes V1aR and correlated them with enhancer methylation. In this study, some animals showed significantly high partner preference. Genetic polymorphism within populations is expected to be high because the voles used in this study are maintained by random mating, not by sibling mating as in the genetically uniformed inbred strains of mice and rats. The animals which exhibited high sexual preference may represent a monogamous genotype, expressed as a social phenotype. Further analysis of these population at the genetic level is awaited in the future.

To understand the neurobiology of social be-havior, comparative studies on different species of voles are valuable. Although *M. arvalis*, *M. montebelli*, and *M. levis are* taxonomically close-ly related to the monogamous *M. ochrogaster*, they were demonstrated to have a non-monoga-mous strategy with no preference for female part-ners. The existence of a model animal repertoire of several closely related species with different social behavioral patterns is expected to provide further insights into the neuroanatomical, neuro-biological, and neurochemical underpinnings of social attachment.

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形山志歩・目加田和之：飼育下におけるユーラシア及び日本産ハタネズミ3種のパートナー選好性

要約

一夫一妻制や乱婚制などの交配システムは，社会行動，特にペアボンディングを評価することで推測することができる．本研究では，ユーラシア大陸と日本で飼育されているハタネズミ属3種（*Microtus arvalis*, *M. montebelli*, *M. levis*）のペアボンド形成を，パートナー選好試験を用いて推定した．その結果，3種とも，メスは相手のオスを好む傾向が見られたが，オスは相手のメスを好まない傾向が見られた．これらの結果は，これらの種の社会システムが非一夫一婦制であることを示唆している．