Spatial navigation strategies in *Peromyscus*: a comparative study

Eldin Jašarević, Scott A. Williams, R. Michael Roberts, David C. Geary, Cheryl S. Rosenfeld

A male advantage in spatial abilities is predicted to evolve in species where males rely on expansion of home territory to locate dispersed mates during the breeding season. We sought to examine mechanistic underpinnings of this evolved trait by comparing spatial navigational abilities in two species of *Peromyscus* that use widely different reproductive strategies: deer mice, *Peromyscus maniculatus bairdii*, in which males engage in territorial expansion and mate search, and California mice, *Peromyscus californicus insignis*, in which males do not engage in territorial expansion and mate search. Males and females from outbred stocks of each species were administered tasks that assessed spatial learning and memory, and activity and exploratory behaviours. The maze used for these studies included four spatial cues that could be used to aid in locating one of 12 potential escape holes. As predicted, male deer mice outperformed conspecific females and male California mice in maze performance and memory, and this difference appeared to be due to extent to which animals used spatial cues to guide maze navigation. Consistent with territorial expansion as a component of competition for mates, male deer mice were more active and engaged in more exploratory and less anxiety-related behaviours than conspecific females and male California mice. The results have implications for understanding and studying the cognitive and behavioural mechanisms that have evolved through male–male competition that involves territorial expansion and mate search.

Sex differences in spatial ability are predicted to evolve in mating systems where males and females differ in home range size during the breeding season, but these sex differences will be minimal in mating systems in which sexually mature males and females share a home range (Gaulin 1992). Comparative studies with various species of *Microtus* support this hypothesis (Gaulin & FitzGerald 1986, 1989). During the breeding season, male meadow voles, *Microtus pennsylvanicus*, expand their home range four- to five-fold and compete by searching for multiple females that are distributed in the habitat (Stickel 1968; Baird & Birney 1982). Home range expansion is considered to be a feature of sexual selection and male–male competition for meadow voles because the sex difference in range size does not appear until puberty and disappears during the nonbreeding season (Madison & McShea 1987; Wolff 1989). Additionally, these behaviours may yield a reproductive benefit for males (Spritzer et al. 2004, 2005). Male prairie, *Microtus ochrogaster*, and woodland voles, *Microtus pinetorum* (previously referred to as the pine vole), in contrast, do not expand their home range during the breeding season but rather share overlapping home ranges of similar size with their mate. As predicted, field and laboratory studies demonstrate that male meadow voles have enhanced spatial ability relative to conspecific females and male prairie and woodland voles, with no sex differences evident in the two latter species (Gaulin & FitzGerald 1986, 1989).

Studies to date have largely confirmed that sex differences in home range size in natural settings correspond to sex differences for a variety of laboratory measures, including maze tasks designed to measure spatial learning (Williams et al. 1990; Galea et al. 1994, 1996). Maze learning performance is typically assessed by measuring latency to reach the escape hole, path length, errors (i.e. entering a blind hole) and search strategy. The three broad categories of search strategy may be considered as (1) random/mixed...
search, characterized by unorganized hole searches separated by multiple crossings of the maze centre before locating the escape hole, (2) serial/thigmotaxic search, characterized by moving along the periphery of the maze in a clockwise or anticlockwise direction and visiting at least two blind holes prior to locating the escape hole, and (3) direct search, which involves moving directly to the quadrant in which the escape hole is located and either immediately entering this hole or moving to an adjacent blind hole prior to entering the correct hole. Predictably, animals that use a direct search strategy demonstrate shorter latencies and path lengths and fewer errors than animals using random or serial strategies (Harrison et al. 2006; Mueller & Bale 2007; Rodriguez et al. 2010; Jasarević et al. 2011).

For many of these laboratory maze tasks, males show shorter latencies and path lengths, commit fewer errors and acquire direct search strategies more rapidly than females (e.g. Galea et al. 1994, 1996; Rodriguez et al. 2010; Jasarević et al. 2011), although these sex differences are absent in many inbred strains of mice (O’Leary et al. 2011). Direct assessment of the relation between these sex differences and the home range hypothesis (Gaulin 1992) has been confounded by the use of different tasks across studies and species. In the present study, we used a comparative approach and tested the hypothesis that the male advantage in spatial ability associated with home range expansion is due to males’ more efficient use of spatial cues to guide navigation. We also tested the hypothesis that home range expansion is associated with higher activity levels, increased exploratory behaviour and decreased anxiety-like behaviour.

The genus Peromyscus provides valuable animal models for testing these predictions because it includes closely related species that in response to environmental pressures have evolved the full range of mammalian mating systems (Dewsbury 1981). Similar to male M. pennsylvanicus, polygynous male deer mice, Peromyscus maniculatus bairdii, expand their home range during the breeding season to search for mates. In contrast, monogamous male California mice, Peromyscus californicus insignis, do not expand their home range but rather show overlapping territories and pair with a single conspecific female (Stickel 1968; Wolff 1989; Ripple & Salvioni 1990). Laboratory studies have confirmed a male advantage in spatial abilities in polygynous Peromyscus species (Galea et al. 1994, 1996; Jasarević et al. 2011), which only emerges during the breeding season (Pyter et al. 2005; Walton et al. 2011). In particular, male deer mice outperformed conspecific females in navigating to a submersed platform in the Morris water maze (Galea et al. 1996) and in spatial learning as assessed in a Barnes maze (Jasarević et al. 2011). One possibility is that the males’ advantage was related to use of distant cues located outside the water maze to find the platform (Williams et al. 1990), although this strategy was not directly assessed. On the other hand, Bredy et al. (2004) determined that performance of male and female California mice in the Barnes maze did not differ, consistent with the prediction that shared ranges and a lack of male–male competition that involves range expansion is associated with similar spatial abilities in males and females. Together, the findings suggest that the sex differences observed in deer mice are related to differences in reproductive strategy rather than in foraging strategy.

These combined results are consistent with the home range hypothesis (Gaulin 1992), but use of contrasting methods among studies potentially weakens their comparative power. Herein, we report the first direct cross-species comparison of sex differences in spatial learning, activity levels, exploratory and anxiety-like behaviours for deer mice and California mice. Moreover, the use of the Barnes maze and automated tracking of escape strategy and error patterns enabled inferences to be drawn about the relative use of spatial cues during spatial learning. If the home range hypothesis is correct, then we predicted the following. (1) Male deer mice should show decreased latency, fewer errors and shorter path lengths to the escape hole, and a more direct search strategy than conspecific females in the Barnes maze. Males should also show a greater frequency of exploratory and overall activity levels and less anxiety-like behaviours in the elevated plus maze (EPM). (2) No comparable sex differences should be detected for these measures in California mice. (3) Male deer mice should demonstrate an advantage on all of these measures relative to male California mice.

METHODS

Animals

We obtained 26 (15 males and 11 females) adult deer mice (60–100 days of age) and 21 (12 males and 9 females) adult California mice (age 120–150 days) from the Peromyscus Genetic Stock Center at the University of South Carolina (Columbia, SC, U.S.A.). These polygynous and monogamous species differ considerably in the timing of development and reproductive maturation; thus, the age of the animals at the time of testing corresponds to species differences in average age of sexual maturity (King 1968; Layne 1968). At these ages, California mice are substantially larger (males: 44.36 ± 2.10 g; females: 44.39 ± 1.56 g) than deer mice (males: 17.04 ± 0.24 g; females: 16.76 ± 0.33 g). From the time the animals were originally captured from the wild, each species has been carefully bred by the facility to maintain their outbred status, although duration of captivity has varied across these two species with the founder P. maniculatus bairdii and P. californicus captured in 1948 and 1987, respectively. All experiments were approved by University of Missouri Animal Care and Use Committee and performed in accordance with National Institutes of Health Animal Care and Use Guidelines.

Mice were housed in white polypropylene cages (27.8 × 7.5 × 13 cm) with a 16:8 h light:dark cycle (lights on at 0600 hours Central Standard Time, CST, lights off at 2200 hours CST) to simulate the breeding season, when sex differences are most apparent in P. maniculatus (Galea et al. 1996). The animals were maintained at a constant temperature and humidity (22 ± 3 °C and 50 ± 10%, respectively), and provided ad libitum access to food (AIN–93G rodent diet supplemented with 7% corn oil; Harlan Teklad, Indianapolis, IN, U.S.A.) and filtered water (Jasarević et al. 2011). Prior to behavioural testing, mice were maintained in same-species and same-sex housing conditions with no more than three siblings, and were singly housed for 1 week prior to behavioural testing to reduce any social housing effects (Holmes et al. 2000; Palanza et al. 2001). All animals were gonadally intact.

Spatial Learning and Memory

The Barnes maze was used to test spatial learning and memory (Barnes 1979), but modified for Peromyscus, as described previously for deer mice (Jasarević et al. 2011). This dry-land, circular maze measures a rodent’s ability to learn intramaze spatial cues to escape the platform into a home cage (Barnes 1979). The animal is motivated to solve the maze by aversive stimuli, including bright lights and the recording of a natural predator.

The maze consisted of a circular platform (95 cm diameter) constructed of black polypropylene, with 12 escape holes, one placed every 30° and surrounded by a 50 cm high black curtain barrier to prevent escape and viewing of extramaze cues (e.g. distal objects in the testing room that the animals could possibly use to locate the target exit hole; Harrison et al. 2006). Although previous reports have shown that mice show a preference for using distal
cues, even when proximal cues are present (Harrison et al. 2006), distal cues are less likely to remain constant during the course of training than proximal cues. The circular maze was elevated 100 cm above the floor on a polypropylene stand. A small polypropylene ramp was attached to the correct exit hole and led to the home cage of the animal. The correct exit hole was of the same colour and texture as the blind exit holes such that it was indistinguishable from the remaining 11 holes from the centre of the maze. Four spatial geometric cues (triangle, square, circle and star) were placed at the same height (~10 cm) every 90° inside the maze wall. Three 100 W lights (encased in aluminium shells) were suspended approximately 150 cm above the platform to motivate the mice to escape from the brightly lit open surface after release onto the platform. As pilot data indicated individual differences in habituation to bright lights, after 30 s, a recording of barn owl, Tyto alba, screech was included as an ecologically relevant aversive stimulus (see below).

Each mouse was assigned an escape hole number, with hole numbers for consecutively tested mice alternated by 90° (i.e. 3, 6, 9 and 12) to eliminate odour cues. The escape box location remained constant for any individual mouse over all test trials. At the beginning of each training day, the maze was rotated 90° and disinfected with 70% ethanol to eliminate odour cues for consecutively tested mice, but the exit hole number and the positions of the spatial cues relative to the escape hole remained fixed for any individual animal across all acquisition trials and the probe trial. At the beginning of each test day, animals were transferred from the vivarium to the testing room 30 min prior to behavioural testing to minimize any confounding stress. All testing occurred in the light phase (between ca. 1200 and 1500 hours CST), and animals were returned to the vivarium immediately after testing. Animals received two shaping trials followed by 7 days of two-trial evaluations per day for 5 min (300 s) each, with a 30 min intertrial interval. A trial consisted of carefully placing the mouse in the centre of the maze, but randomly relative to the location of the spatial cues, in an opaque starting box to allow the tracking system (see below) to detect the centre body point. The starting box was lifted and a trial was initiated once the mouse had begun to move in the maze. If the animal failed to enter the escape box within 5 min, the observer gently guided the animal to the escape hole. A stimulatory light measuring 1200 lx (versus ca. 400 lx for vivarium 5 min, the observer gently guided the animal to the escape hole. A trial consisted of carefully placing the mouse on the centre of the platform and allowed to explore the maze for 300 s. After each test, the apparatus was cleaned with 70% ethanol. Each trial was recorded with EthoVision XT software (Noldus Technologies, Leesburg, VA, U.S.A.), and latency to enter escape hole and path length were tracked by using accompanying automated tracking EthoVision XT software. Latency performance was averaged across trials on the same day for each individual. However, as noted, California mice are roughly three times the size of deer mice (Layne 1968), thereby, confounding the across-species latency measure. To control for species differences in body size, we focused on differences in path length, escape errors and search strategy which were quantified from the video recordings and tracking image composites produced by the EthoVision XT software, respectively. Figure 1a illustrates the spatial strategies used in these assessments (Harrison et al. 2006; Jašarević et al. 2011).

The random search strategy (coded 1) was operationally defined as localized searches of holes separated by maze centre crosses. Serial search strategy (coded 2) was defined as a systematic search of consecutive holes in a clockwise or anticlockwise direction. Finally, direct search strategy (coded 3) was defined as navigating directly to the target quadrant without crossing the centre of the maze more than once and with three or fewer errors. Caution must be used for this empirical classification of the direct strategy, however, because it is possible that animals that are dependent on a serial strategy will have some of their trials coded as direct, as, for example, would occur if the animal started the serial search by chance in the correct quadrant. In this situation, up to 25% of serial strategy trials could be incorrectly scored as a direct strategy. As a result, reliable use of the direct strategy was only attributed to groups (such as deer mice males) that consistently used this strategy on more than 25% of the trials.

The combination of trial-by-trial strategy and error information allowed us to infer whether and how the intramaze spatial cues were used by the mice to find the correct escape hole. Use of directional cues would entail finding the correct hole based on its location between two of the four intramaze cues. If the animal never entered an incorrect hole on any given trial more than once, a completely random search strategy would, on average, result in 5.5 errors (11 incorrect holes/2). Therefore, the average number of errors for this strategy would be more than 5.5 if the animal entered the same incorrect hole more than once in the trial. A serial strategy would result in shorter latencies but the same error rate (5.5), presuming the animals were unable to use spatial cues to narrow the range of potential exit holes. If the animal was able to use spatial cues to narrow the search to nine, six or three escape holes, the corresponding error rates (assuming incorrect holes are entered only once) would be 4.0, 2.5 or 1.5, respectively. Use of a single cue as a landmark would in effect serve as a positional cue and would result in an average of 0.5 errors for the eight exit holes adjacent to a cue and an average of 1.0 error for the four holes that were not adjacent to a cue. The overall error rate would then be 0.67. The position of the exit hole was necessarily constant for individual animals, as noted. However, these predictions should be useful when within-strategy errors rates are averaged over individuals within species by sex.

Exploratory and Anxiety-like Behaviour

One week after the animals were tested in the Barnes maze, their exploratory and anxiety-like behaviours were measured by using the elevated plus maze (EPM), as described previously (Fountain et al. 2008; Jašarević et al. 2011). The EPM was constructed of black polypropylene in a plus configuration with two opposite open arms (30 cm), a middle platform (5 × 5 cm) and two opposing closed arms (30 cm). The maze was supported 100 cm above the floor by a stand constructed of polypropylene. Each animal was placed on the centre of the platform and allowed to explore the maze for 300 s. After each test, the apparatus was cleaned with 70% ethanol. Each trial was recorded with EthoVision XT software (Noldus Technologies), which automatically scores total time spent in open and closed arms and number of closed and open arm entries and centre entries. Arm entry was defined as both front paws and shoulders placed into the area. On the occasion an animal jumped off the maze, it was gently placed back in the centre and the trial was continued.

Statistical Analysis

Barnes maze path length and errors were analysed with a 2 (sex) × 2 (species) × 7 (day) repeated measures ANCOVA, with trial
and the proportion of time in closed and open arms and time spent immobile in the EPM as covariates. Latency to reach the target exit hole was assessed separately for California mice and deer mice with a 2 (sex) × 7 (day) repeated measures ANCOVA, with trial and the EPM variables as covariates. The discrete Barnes search strategy that was coded 1 (random), 2 (serial) or 3 (direct) was analysed with a 2 (sex) × 2 (species) × 6 (day) logistic analysis. Day 1 was excluded from these analyses because there was only a single use of strategy 3 on this day, thus preventing accurate contrasts of the probability of using different strategies. For days 2–7, the significant three-way sex-by-species-by-day interaction was followed by 2 (sex) × 2 (species) logistic analyses for each day, with trial and the EPM variables as covariates. The outcome of interest was the probability of using strategy 3 (direct strategy), and thus strategy 1 and strategy 2 (random and serial strategies, respectively) were combined in the final logistic analyses and contrasted with strategy 3 by using a binomial distribution. Mean number of errors associated with each of the three strategies (i.e. random, serial and direct) was calculated and contrasted with predicted error rates by using one-sample t tests (see Table 2). Finally, the probe trial was analysed with a 2 (sex) × 2 (species) ANCOVA, with trial and the EPM variables as covariates.

The EPM variables were included as covariates to ensure that differences in activity level and anxiety-like and exploratory-related behaviours were not influencing performance on the Barnes maze. None of the EPM variables significantly correlated with strategy, path length or probe variable data (Ps > 0.08) and thus were not included in the final reported analyses. Several effects emerged for latency and error, and thus the EPM variables were retained in the final analyses. Trial effects were not significant for any of the Barnes variables (Ps > 0.1472), except for strategy (P = 0.0003), which correlated with significant trial effects for days 2, 3 and 5 (Ps < 0.0433); trial was not significant for the remaining training days: Ps > 0.2207). The associated means and SEs for sex and species were adjusted for effects of EPM variables and trial for analyses in which these variables were included. The proportion of total EPM time spent in open and closed arms and spent immobile, as well as total number of arm entries were submitted to a 2 (sex) × 2 (species) ANOVA.

RESULTS

Barnes Maze

Animals in all groups predominantly used a random search strategy on the first day of acquisition training (Fig. 1b). Following the first day, the significant day-by-sex-by-species effect (F5, 479 = 3.14, P = 0.0085) indicated the groups differed in their patterns of strategy use across training. From day 5 forward, male deer mice had a significantly higher probability of using the direct search strategy (probabilities = 0.56–0.83) than did female deer mice (probabilities = 0.09–0.15) and male California mice (probabilities = 0.06–0.21; Ps < 0.018; Table 1). Male and female California mice did not differ in strategy use on any day (Ps > 0.1274).

Consistent with the across-day shift in search strategy, latencies to reach the target exit hole decreased across days (Fig. 2a). Within-species comparisons confirmed that male deer mice located the correct exit hole faster (X = 33 ± 6.8 s) than female deer mice (81 ± 8.1 s) (F1, 46 = 17.47, P = 0.0001), and no overall sex difference was evident for California mice (females: 40 ± 6.5; males: 34 ± 6.1 s; F1, 32 = 0.41, P = 0.526).

During the probe test of spatial memory retention (Fig. 2b), male deer mice spent 56% of their time in the quadrant of the correct exit hole (P < 0.0001, compared to 25% chance), whereas animals in the other three groups spent between 26% and 28% of their time in the correct quadrant, which did not differ from chance performance (Ps > 0.50). The corresponding sex-by-species interaction was significant (F1, 83 = 25.99, P = 0.0001), with male deer mice spending significantly more time in the correct quadrant than female deer mice and male California mice (Ps < 0.001).

Figure 1. Barnes maze escape strategies of male and female deer mice and California mice. (a) Schematic diagram of navigational strategies (random: top; serial: middle; direct: bottom) used to locate the correct exit hole. (b) Distribution of spatial strategies (random: yellow; serial: green; direct: black) across sex, species and day of training.
In terms of error rates, the sex ($F_{1, 78} = 8.37, P = 0.0049$) and sex-by-species ($F_{1, 78} = 11.82, P = 0.0009$) effects were significant (Fig. 2c). Male deer mice committed fewer overall errors ($\bar{X}/SE = 3.0/0.45$) than female deer mice ($5.4/0.61, P = 0.0104$) and male California mice ($5.8/0.42, P = 0.0001$). Error rates of male and female ($5.0/0.50$) California mice did not differ ($P = 0.5945$).

Mean number of errors (i.e. incorrect holes) associated with each of the three strategies (random, serial and direct) were then contrasted with the predicted error rates for such strategies (Table 2). For example, a purely random search strategy would be expected to result in 5.5 errors, while a narrowing of the search to nine, six or three escape holes would provide errors of 4.0, 2.5 or 1.5, respectively. During random searches, the number of errors committed by male California mice did not differ from the predicted 5.5 error rate, but the error rates for all other groups exceeded this value ($P_s > 0.05$); that is, they made more errors than expected.

In serial searches, the mean number of errors committed by male and female California mice did not differ from 5.5, again indicating that the majority of these mice were unable to use spatial cues to narrow the range of potential exit holes. Nevertheless, the confidence interval for male California mice and female deer mice encompassed the 4.0 value, suggesting that some narrowing of the search by some of the mice might have occurred. For male deer mice, the error rate during serial searches fell between 2.5 and 4.0, indicating that the animals had narrowed their search to between six and three escape holes.

Error rates for the direct search have to be interpreted with caution for male and female California mice and female deer mice because of the small number of trials in which they used this strategy (Table 2). Moreover, as noted previously, a serial search could be miscoded as a direct search if the animal started the search in the quadrant with the escape hole. Accordingly, the potential for miscoding could occur in up to 25% of the trials, and only male deer mice exceeded this threshold (42% of trials were coded as direct searches, $P < 0.0001$, relative to 25% chance). During direct searches, the error rates of male deer mice did not differ from the predicted 1.5 value associated with use of spatial cues to narrow the search to one quadrant of the maze.

Across days of acquisition training, male deer mice had shorter path lengths ($\bar{X}/SE = 615/75$ cm) than female deer mice ($844/83$ cm; $F_{1, 46} = 5.82, P = 0.0199$) and significantly shorter...
path lengths than male California mice on day 5 (454 ± 106 versus 887 ± 134 cm; \( t_{41} = 2.54, P = 0.0150 \)) and day 6 (442 ± 98 versus 815 ± 124 cm; \( t_{41} = 2.36, P = 0.0233 \); Fig. 3). Across days, male California mice had shorter path lengths (607 ± 94 cm) than female California mice (1123 ± 112 cm; \( F_{1, 26} = 8.19, P = 0.0082 \)). The advantage of male California mice was statistically significant, however, on only 2 of the 7 days of acquisition training (day 1: \( t_{41} = 3.29, P = 0.0029 \); day 3: \( t_{41} = 2.17, P = 0.0397 \)).

**Elevated Plus Maze (EPM)**

The EPM results revealed sex-by-species effects for the proportion of time spent in open (\( F_{1, 43} = 6.89, P = 0.012 \)) and closed (\( F_{1, 43} = 5.89, P = 0.0195 \)) arms, as well as time spent immobile (\( F_{1, 43} = 17.85, P < 0.0001 \)). Male deer mice spent proportionately more time in the open arms (64 ± 6.4% of total time) than female deer mice (40 ± 9.5%; \( P = 0.0048 \)) and male California mice (37 ± 7.1%; \( P = 0.0135 \); Fig. 4), and proportionally less time in closed arms (30 ± 4.4% of total time) than female deer mice (56 ± 9.9%; \( P = 0.0141 \)) and male California mice (55 ± 8.6%; \( P = 0.013 \)). Male deer mice also spent less time immobile (118 ± 6.6 s) than female deer mice (158 ± 7.5 s; \( P = 0.0007 \)) and male California mice (230 ± 9.2 s; \( P < 0.0001 \)) and made more total arm entries (76 ± 12.5 entries) than female deer mice (37 ± 12.5 entries; \( P = 0.0089 \)) and male California mice (40 ± 4.8 entries; \( P = 0.0125 \)). Male and female California mice did not differ in terms of proportion of time spent in open and closed arms, or in total number of arm entries (\( P < 0.2226 \)), but females spent less time immobile (201 ± 10.0 s) than males (230 ± 9.2 s; \( P = 0.0409 \)).

**Covariates of Elevated Plus Maze for Barnes Maze Performance**

The proportion of time spent in open and closed arms and spent immobile in the EPM was used as a covariate for Barnes maze performance. The only statistically significant correlations that emerged between the EPM and Barnes maze results were for Barnes maze error and latency. The proportion of time spent in the open arms of the EPM was related to frequency of Barnes maze errors across days of acquisition training (\( F_{6, 468} = 2.73, P = 0.0129 \)). Lower proportional time spent in the open arms was associated with fewer errors on day 4 (\( r_{93} = -0.27, P = 0.0077 \)) and day 7 (\( r_{93} = -0.37, P = 0.0003 \)). Proportion of time spent in closed arms of the EPM was also related to frequency of errors across days of training (\( F_{6, 468} = 2.53, P = 0.0205 \)). Higher proportional time in closed arms was associated with more errors on day 4 (\( r_{93} = 0.36, P = 0.0003 \)) and day 7 (\( r_{93} = 0.28, P = 0.0057 \)).

For California mice, the proportion of time spent immobile in the EPM interacted with latency across days of acquisition training (\( F_{6, 192} = 2.83, P = 0.0115 \)). Higher proportional time spent immobile was associated with faster latencies on day 2 (\( r_{43} = -0.32, P = 0.0389 \)), day 5 (\( r_{43} = -0.39, P = 0.0109 \)) and day 6 (\( r_{43} = -0.33, P = 0.034 \)). No other statistically significant Barnes maze latency effects correlated with time spent in the open or closed arms or with time spent immobile for either species (\( P > 0.87 \)).

**DISCUSSION**

Our study provides the first side-by-side and sex-by-species comparisons for P. maniculatus bairdii and P. californicus insignis that enables a systematic testing of the home range hypothesis (Gaulin & FitzGerald 1986). It confirmed the overall predictions that sex differences in spatial ability and learning would be observed in deer mice, that no such sex differences would be observed in California mice, and that male deer mice would outperform male California mice. Male deer mice performed better on the Barnes maze, as observed by fewer errors, adoption of a direct search strategy during acquisition training and better spatial memory during probe testing than conspecific females and male California mice. Consistent with our predictions, male and female California mice did not differ from each other on these measures. The only exception was a potential male advantage for path length, but this was statistically significant for only 2 of the 7 acquisition training days. The result may merit a follow-up study to determine whether a consistent sex difference exists for path length in California mice. The critical point though is that path length is only one component of maze learning and performance, and, for all other components analysed (search strategy, errors, latencies and probe-trial memory), male and female California mice did not differ.

Moreover, our assessment of anxiety-like and exploratory-related behaviours and overall activity level on the EPM extend previous studies on spatial ability in these two species (Galea et al. 1994, 1996; Bredy et al. 2004). These measures were included to

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**Table 2**

Error rates across species, sex and search strategy contrasted with predicted values for use of spatial cues by California mice and deer mice to narrow their search for the escape hole\(^a\)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Number of trials</th>
<th>Mean number of errors (SE)</th>
<th>CI</th>
<th>Predicted value within CI^(b)</th>
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<td></td>
<td></td>
<td></td>
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<td><strong>Random strategy</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>California</td>
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<td>62</td>
<td>7.56 (0.56)</td>
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<td></td>
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<td>6.28 (0.49)</td>
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</tr>
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<td>Deer</td>
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<td><strong>Serial strategy</strong></td>
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<tr>
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<td>2.78 (0.66)</td>
<td>1.25–4.30</td>
<td>No</td>
</tr>
<tr>
<td>Deer</td>
<td>Female</td>
<td>14</td>
<td>1.71 (0.32)</td>
<td>1.02–2.41</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>95</td>
<td>1.55 (0.16)</td>
<td>1.23–1.87</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\) A random search strategy would, on average, result in 5.5 errors, if the animal never entered the same hole more than once. A serial strategy would result in shorter latencies but the same error rate (5.5), presuming the animals were unable to use spatial cues to narrow the range of potential exit holes. If the animal used spatial cues to narrow the search to nine, six or three escape holes, the corresponding error rates would be 4.0, 2.5 and 1.5. A ‘Yes’ in one of the last four columns indicates that the error rate did not differ significantly from the predicted value (\( P < 0.05 \); i.e. the predicted value was within the confidence interval, CI, for the group), and a ‘No’ indicates that the rate was significantly different (\( P < 0.05 \)).

\(^b\) The proportion of time spent in open and closed arms and spent immobile for either species (\( P > 0.87 \)).
test the hypothesis that territorial expansion should, in addition to enhancing spatial ability, be associated with less anxiety-like behaviours and more exploratory-related behaviours in male deer mice than in female conspecifics and male California mice. We also predicted no sex differences for these measures in California mice. We confirmed these hypotheses and determined that the spatial learning and spatial memory results were not influenced by anxiety-like or exploratory-related behaviours.

Despite evidence that sex differences in spatial ability have evolved as a result of differences in the size of the home range, it is not known whether differences in spatial ability are related to performance (i.e. latency, path length or error), or due to differences in spatial search strategies, or a combination of these parameters (Jonasson 2005; McCarthy & Konkle 2005). Our study indicates that sex differences in spatial learning are related to both performance and search strategy. During acquisition training, female deer mice spent more time and, as a group, demonstrated greater variability than males in locating the target exit hole. These females also committed more errors across training days and spent less time in the quadrant with the correct exit hole in the probe.
trial, suggesting poorer memory retention for solving the maze. Male and female California mice performed comparably to each other in terms of errors and time spent in the target quadrant, yet, male California mice were still able to locate the target exit hole about as efficiently as the male deer mice, a finding that we attribute to greater size of the California mice compared to deer mice, which confounded interpretation of latency measures.

Notably, sex-by-species differences in spatial strategy and search error rates emerged across training (Figs 1, 2), and these data permit inferences to be drawn about how the animals used intramaze spatial cues to locate the target exit hole. During random searches, the error rates for animals in all of the groups were either consistent with the predicted random search error rate of 5.5 (male California mice) or exceeded this value (female California mice and male and female deer mice), suggesting these were truly random searches (Harrison et al. 2006). The higher values for random searches were probably due to certain animals entering the same incorrect hole more than once during some trials. Animals that used serial searches showed shorter latencies than those using random searches, but the same error rate (5.5) would be predicted if the animals were not using intramaze cues to narrow the search for the correct exit hole. Error rates associated with serial strategies of male and female California mice were consistent with this prediction, although the error rate for California males was also within the range expected if intramaze cues were used to narrow the search to nine of the 12 exit holes. Future studies will be needed to determine whether male California mice use intramaze cues.

Error rates associated with serial strategies of female deer mice suggest that these mice may have been able to use intramaze cues to narrow their search to fewer than nine exit holes, but they were still not as effective as male deer mice, even when using the same search strategy. Error rates of male deer mice using the serial search strategy, in fact, suggested that these mice were beginning to use intramaze cues to narrow their search to between nine (expected error rate of 4.0) and six (expected error rate of 2.5) exit holes. This outcome is not surprising if acquisition training leads to steady improvements in use of intramaze cues rather than a sudden switch in categorical search strategy. The results for the direct search strategy were clear-cut and indicate a more rapid response, across days, and an overall improvement in maze learning for male deer mice compared to female deer mice and male California mice.

Critically, the frequency of use of the direct search strategy was higher than chance (i.e. 25% potential miscoding of serial searches) only for male deer mice, and the associated error rate (1.55) was strongly consistent with use of intramaze cues to narrow the search to one quadrant. This conclusion is further supported by the above-chance performance of male deer mice on the probe trial, and suggests that male deer mice use intramaze cues in much the same way that male rats, Rattus norvegicus, can use extramaze cues to guide maze escape or food search strategy (Williams et al. 1990; Cheng & Newcombe 2005; Cheng 2008; Rodriguez et al. 2010). The overall pattern supports the notion that male deer mice use intramaze cues to guide navigation more effectively than conspecific females and male California mice. The efficient use of intramaze cues by male deer mice contributed to their advantage over conspecific females and male California mice in the Barnes maze (e.g. in use of direct strategy).

The observed sex differences in spatial learning and memory corresponded with sex and species differences in activity levels and anxiety-like and exploratory-related behaviours (Fig. 4). Male deer mice showed higher overall activity levels, more exploratory behaviour and less anxiety-like behaviour in the EPM than conspecific females and male California mice, with minimal sex differences for these parameters in California mice. These results are consistent with earlier findings of breeding season increases in open field activity for male meadow voles (M. pennsylvanicus) (Turner et al. 1983). Such increased activity levels and exploratory behaviours are necessary components of male—male competition that requires territorial expansion, and the reduced anxiety-like behaviours may be required to face the increased risk of predation that these males would be subjected to while searching for prospective mates (Clarke 1983). Consequently, anxiety-like behaviours, as measured by time spent in the closed arms of the EPM, may be correlated with activities that reduce predation risk in the wild. In essence, given that female deer mice and male California mice do not gain reproductive benefits by territorial expansion, their lower activity levels and higher anxiety-like behaviour may reduce predation risks in natural settings.

Overall, our results demonstrate that male deer mice show enhanced memory retention and greater use of spatial cues to guide search for an escape hole in the Barnes maze than conspecific females. Male deer mice also showed increased activity and exploratory behaviours and less anxiety-like behaviours than conspecific females in the EPM. These sex differences probably evolved as components of the polygynous mating system of P. maniculatus bairdii and male—male competition that involves territorial expansion to search for multiple, widely dispersed prospective mates. Support for this hypothesis is strengthened by simultaneous assessment of the related P. californicus, a species in which males mate monogamously and do not expand their territory to search for additional mates (Gubernick & Teferi 2000). As hypothesized, the sex differences observed within deer mice were not evident in California mice, and male deer mice demonstrated the same advantages over male California mice. The striking sex differences in deer mice as well as species differences between the males in spatial learning and anxiety-like behaviours supports the hypothesis that these behaviours have been shaped by sexual selection.

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References


