



Sex and species differences in spatial memory in food-storing kangaroo rats

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Scatter-hoarding birds show superior performance in many spatial memory tasks and appear to relocate sites using different cues than nonscatter hoarders. However, there have not been similar studies of cue use and memory for locations by mammals that differ in their reliance on scatter hoards for survival. Here we report differences in spatial memory in two species of kangaroo rats that are predicted by foraging ecology. Merriam's kangaroo rat, *Dipodomys merriami*, a scatter hoarder that hoards intensively, showed accurate memory on a cache simulation task, which required the rat to find four predetermined locations after a 24-h delay. In contrast, the leaf-eating specialist Great Basin kangaroo rat, *D. microps*, which relies less on scatter hoarding, showed poorer accuracy under the same experimental conditions. The presence of local landmarks did not differentially affect recovery in the two species of kangaroo rats, unlike previous studies in birds. We did find a significant interaction between the presence of local landmarks and sex on target recovery. Female performance was significantly impaired by the absence of local landmarks, while male performance was unaltered. Spatial ecology predicted both species and sex differences in spatial memory in kangaroo rats.

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An important question about memory in food-storing animals is the exact nature of their spatial abilities. Avian scatter-hoarding species that hoard intensively have been found to encode the location of a feeder in relation to the feeder's absolute location in space rather than to its colour or position within the test array, while species less dependent on scatter hoarding do not show this same pattern (Brodbeck 1994; Clayton & Krebs 1994; Shettleworth & Westwood 2002). When the task is made more abstract, such as Olson's (1991) use of the delayed-non-match-to-sample operant task, intensive scatter-hoarding species are more accurate on spatial versions of this task compared with less intensive hoarders, but they are not more accurate on nonspatial versions of this task (Olson et al. 1995). In addition to differences in the cues used

to relocate sites, differences between intensive and less intensive scatter hoarders have also been detected in memory persistence. Using a touch-screen to simulate a feeder array, coal tits, *Periparus ater*, show greater memory persistence than do great tits, *P. major* (Biegler et al. 2001). Differences in spatial recall accuracy have been shown not only to exist between species, but also between populations of the same species: Alaskan black-capped chickadees, *Poecile atricapillus*, living in a harsher environment and hence more dependent on stored food, show superior spatial recall compared with individuals of the same species living in Colorado (Pravosudov & Clayton 2002).

It is still unclear, however, which cognitive process (or combination of processes) underlies these differences in spatial recall. Superior cache retrieval could result from differences in attention, encoding, memory capacity, memory organization or retrieval. One of the challenges a scatter hoarder faces is how to encode locations in such a way as to reduce the probability of changes in the landscape affecting its ability to relocate each site. Localized objects and texture gradients around food stores are

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subject to constant change. Additionally, many of the objects located near individual food stores are similar to other objects in the environment. In order to effectively search for a site, a scatter hoarder must be able to discriminate between similar locations. A preference for using an absolute location in space to code locations, for example, could affect the organization of memory (Jacobs 2003; Jacobs & Schenk 2003). This preference would allow an animal to integrate disparate locations in space into a single coordinate map, which in turn could affect both memory capacity and retrieval accuracy by reducing interference between similar sites. Using absolute location in space to code sites would also reduce the likelihood of changes in localized objects affecting recognition of the location.

Species Differences in Mammals

To further the comparative analysis of species differences in spatial memory (Kamil 1994), we can look for similar effects in scatter-hoarding mammals. Given our detailed knowledge of the neural and cognitive basis of spatial memory in the laboratory rat, *Rattus norvegicus*, and the laboratory mouse, *Mus domesticus* (Burgess et al. 1999), it is perhaps surprising that there are so few comparative studies of memory in mammals. To date, only two studies have compared spatial memory in mammalian species that vary in food-storing strategy. In a field study in Britain, introduced grey squirrels, *Sciurus carolinensis*, relocated artificial cache sites more accurately than did the native red squirrel, *S. vulgaris*, a species less dependent on scatter hoards for survival (Macdonald 1997). In the laboratory, Rebar compared spatial learning between the scatter-hoarding Merriam's kangaroo rat, *D. merriami*, and the sympatric rock pocket mouse, *Chaetodipus intermedius*, another species in the family Heteromyidae (Rebar 1995). In this study, kangaroo rats relocated seed trays more quickly after three successive foraging nights than did pocket mice, which, under natural conditions, forage over much more restricted areas. This pattern of spatial learning differences in mammals, showing a rough correlation between food-storing behaviour and habitat structure is therefore similar to that documented in birds, although clearly necessitating further investigation.

Sex Differences in Spatial Learning

In contrast to the few studies on species differences in spatial memory in mammals, there is a large literature on sex differences in spatial learning within species, in both laboratory and wild rodent species (Gaulin & FitzGerald 1986; Williams & Meck 1993; Jacobs & Schenk 2003). Gaulin first proposed that this sex difference arose through sexual selection for spatial navigation in polygynous males, as the reproductive success of males in these species depends on their ability to track the spatiotemporal pattern of receptive females. In voles (Rodentia: *Microtus*), the type of mating system predicted the presence or absence of a sex difference in maze performance: the sex

difference in maze learning was present in polygamous species but not in monogamous species (Gaulin & FitzGerald 1989). At the level of cognitive mechanism, sex differences in maze performance are a result of differential use of landmarks by females and males (Jacobs & Schenk 2003). For example, sex differences in errors on the radial arm maze in the laboratory rat can be manipulated by the presence or absence of different types of cues. Male (but not female) performance is impaired if the room shape is obscured with a curtain; female (but not male) performance is impaired if the location of objects near the maze is randomized (Williams & Meck 1991). A similar pattern has been shown in the desert kangaroo rat, *D. deserti*: here females used both the shape of the arena cues and the location of objects, while males relied only on geometric cues (Langley 1994). Females are slower than males to learn an active place avoidance task when intra-apparatus cues are useless for solving the task (Cimadevilla 2001). Finally, similar differences in cue use have been shown in humans. Males appear to use geometric, distal information for navigation, while females recall many more details of local landmarks and are severely disrupted in navigation without reliable local landmark information (Galea & Kimura 1993; Sandstrom et al. 1998; Gibbs & Wilson 1999; MacFadden et al. 2003).

Species and Sex Differences in Kangaroo Rats

The goal of the present study was to use a single task to compare spatial memory in closely related rodent species that differ in their food-storing behaviour and to compare sex differences in spatial memory in the same species. Kangaroo rats (Rodentia: Heteromyidae: *Dipodomys*) vary widely in their pattern of food storing, ranging from larder hoarding to a mixture of larder and scatter hoarding (Jenkins & Breck 1998). Within the genus, one species, the Great Basin kangaroo rat, *D. microps*, is specialized to eat the leaves of the saltbush, *Atriplex confertifolia*. By using its specialized grooved incisors, the Great Basin (GB) kangaroo rat is able to remove edible tissue from the otherwise inedible leaves. Eating this abundant food allows this species to rely less on scatter-hoarded seeds than the sympatric Merriam's (MR) kangaroo rat (Kenagy 1972; Kenagy 1973). In contrast, the MR kangaroo rat is an intensive scatter hoarder in the field (Daly et al. 1992a). In the laboratory, MR kangaroo rats use spatial memory to relocate their scatter hoards and they retrieve caches more efficiently than do pilfering conspecifics (Jacobs 1992). Their patterns of hoarding in the laboratory are highly sensitive to the presence of conspecific pilfering (Preston & Jacobs 2001) and pilfering by sympatric heterospecific competitors, such as the GB kangaroo rat (Preston & Jacobs 2005). This fine-tuning of cache decisions in response to competition suggests that scatter hoarding is an important, if not the primary, foraging strategy of this species. These patterns of behaviour would predict a significant species difference in spatial memory between the MR kangaroo rat, the *Dipodomys* species most dependent on scatter hoarding, and the GB kangaroo rat, the *Dipodomys* species least dependent on stored seeds.

In contrast to such species differences in space use, all kangaroo rats are polygynous, with males searching for females in space (Behrends et al. 1986a; Randall 1993). These sex differences in spatial orientation are correlated with sex differences in hippocampal size in the MR kangaroo rat and in the banner-tailed kangaroo rat, *Dipodomys spectabilis* (Jacobs & Spencer 1994). We therefore predicted sex differences in performance in both MR and GB kangaroo rats, particularly in response to trial-unique local landmarks, as females of polygynous mammal species appear to rely more heavily on such cues. In a previous study, the recovery of caches by the MR kangaroo rat after a 10-day delay was accurate only if a trial-unique configuration of cues was available during both the encoding and retrieval phases. In the absence of such landmarks, accuracy of spatial memory dropped sharply. There were no sex differences found in this study, however, sex differences might have been masked by variance in caching behaviour among individuals (Barkley & Jacobs 1998). In the present study, we devised a cache simulation method that equalized the number of sites to be remembered across all individuals. This allowed us to directly compare performance between individuals of the two *Dipodomys* species whose foraging behaviours had widely diverged (i.e. the scatter-hoarding MR kangaroo rat and the secondarily folivorous GB kangaroo rat). In the laboratory, both the proportion of seeds that are larder versus scatter hoarded and the size of the scatter hoards differ between these two species (Jenkins & Breck 1998). Additionally, using this task, we could directly compare the effect of landmarks on performance in female and male *Dipodomys*.

To simulate the memory demand of scatter hoarding, the spatial task required the kangaroo rat to learn four random locations in a rectangular field of 128 identical locations and to return to the learned locations 24 h later. In addition, there were two landmark conditions: either 16 trial-unique landmarks (16L) or zero trial-unique landmarks (no landmarks, NL). The visual environment varied among trials, but not within. We predicted that the scatter-hoarding MR kangaroo rat would show more accurate retrieval of targets than the GB kangaroo rat. We further predicted that as intensively hoarding birds show a stronger dependence on the absolute location of sites in space over local landmarks that the intensively hoarding MR kangaroo rat would be less sensitive to the presence of local landmarks than the GB kangaroo rat. Second, because female kangaroo rats should rely more heavily on landmark cues to relocate target sites, we predicted that the females in both species would be more affected by the presence or absence of such cues than would males.

METHODS

Animals

Twelve MR kangaroo rats (six females, six males) and nine GB kangaroo rats (five females and four males) were used in this experiment. All animals were wild-caught and trapped as adults. Of the MR kangaroo rats, one female was trapped in December 1995 near Palm Desert,

California, U.S.A., three females were trapped in October 1996 near Reno, Nevada, U.S.A., and the remaining two females and six males were trapped in November 1997 near Palm Desert, California. GB kangaroo rats were all trapped in October 1996 near Reno, Nevada. The animals were experimentally naïve at the start of this experiment and were in nonreproductive condition throughout testing. At the conclusion of this experiment, the animals were retained in the laboratory and tested in other experiments.

Animals were housed singly in plastic cages (46 × 24 cm) on sand with a small container (either a capped plastic pipe, semiopaque glass jar or metal can) as a nest chamber. Prior to the start of the experiment, all animals were placed on a reversed 12:12 h light:dark cycle, the lights being extinguished at 0800 hours. All heteromyid rodents, including kangaroo rats, are nocturnal, so we tested them during the dark phase of their light cycle. All animals were given ad libitum access to a diet of mixed birdseed and rodent chow. Lettuce was provided as a water source. Two days before the start of a trial, MR kangaroo rats were food restricted to 1.5 g of rolled oats/day and GB kangaroo rats were restricted to 1.5–2.2 g of rolled oats/day, depending on their reaction to the food deprivation. Lettuce was still provided ad libitum. Following each trial, animals were returned to free feeding for at least one day. Body weight was monitored throughout the experiment and on trial days; animals ranged in weight from 80% to 90% of their free-feeding baseline weight. The large range of weight was the result of individual reaction to the food deprivation regimen and variation in lettuce consumption.

Apparatus

Habituation arena

The habituation arena consisted of a black acrylic rectangular arena (80 × 100 × 50 cm high), with a plastic foam floor, fitted with four potential bait sites (i.e. plastic cup, 4-cm interior diameter, 3.25 cm deep, filled with sand). These sites were arranged in a rectangle, 25 cm from each corner of the box. Cups were either capped with a snug-fitting lid to make them unavailable to the animal for digging, or covered with a blue plastic poker chip that was easily removed by the animal. The light source for the arena was a single 60-W bulb located outside the arena, at one corner. The interior of the room (3.6 × 3.6 m) was visible from the arena. Visible cues inside the room consisted of a door on the north wall, a suspended cabinet on the east wall, and the experimenter, who sat on a stool on the west side of the arena.

Training and testing arena

Training and test trials were conducted in a much larger rectangular arena (118 × 179 × 47 cm high), previously described in Barkley & Jacobs (1998). This open box, also constructed from black acrylic plastic, was enclosed by white plastic curtains that were suspended from the ceiling and draped inside the arena. The floor consisted of eight galvanized steel plates (45 × 45 cm), divided into two parallel

rows of four plates each, and the rows were separated by a wood divider (29 × 179 cm). Each plate contained a four-by-four array of 4-cm drilled holes. Each hole was fitted with a cup (4-cm interior diameter; 3.25 cm deep), which fit snugly into the hole. These cups were filled with sand and represented potential food bait sites, producing a total of 128 potential sites. Each location could be covered with either a blue plastic poker chip (making the site available to the animal) or a snug-fitting cap that prevented animals from accessing the site.

Extra-apparatus cues were visible, such as curtain holes to allow videotaping (a small hole for the camera lens, a large hole for the suspended mirror, required to videotape the entire arena). Light from a single 60-W bulb, located behind the curtain, created a diffuse uneven light in the arena.

Landmarks were used in the arenas during habituation, training and each 16L test trial (four landmarks for habituation, eight for training and 16 for the 16L condition). The habituation arena was small, so we chose only four landmarks. For training, we used eight landmarks, which was intermediate between the two test conditions (zero and 16). In this way, the appearance of the arena during the training phase was equally dissimilar from the two test conditions. We chose objects with natural shapes and textures such as rocks, artificial cloth flowers, sticks and pinecones. In the habituation arena, landmarks were placed between the cup sites and around the edges of the arena. In the training and testing arena, landmarks were placed on the plates between cups, around the edges of the plates and on the centre divider. Landmarks were randomly chosen for each trial from a pool of approximately 100 objects.

Procedure

Habituation

There were three types of trials: habituation, training and test trials. During 3 days of habituation trials, each animal was placed in the small arena four times each day. They were transferred into the arena in a plastic pipe, which was then removed. For the first habituation trial, we placed a sunflower seed at the top of one of the cups, partially covered by a poker chip, and placed a second seed in the sand slightly lower than the top seed. All of the other cups were capped. A randomly selected cup was baited with two sunflower seeds, and the seeds were placed lower in the cup on each subsequent trial. By the end of day 2, the seeds were placed at the bottom of the cup. Four landmarks were randomly selected and placed in unique locations for each habituation trial.

Training and testing

General procedure. Each training and test trial was divided into two parts, learning (Part 1) and memory (Part 2). For each trial, we selected four locations as the baited target locations. We selected the sites by randomly selecting two plates in the arena, and then randomly selecting two sites on each of those two plates to be target locations using a random numbers table. We rejected plates that had been baited in the immediately preceding trial. Order of

presentation of configurations of target sites was counterbalanced across trial types. Eight landmarks were used for the training trials and zero or 16 landmarks for the test trials; landmarks were unchanged between Part 1 and 2 in any trial. Landmarks were placed in trial-unique configurations by randomly choosing the locations they would occupy using a random number table, with each landmark occupying a unique location ($N = 428$ potential locations).

In Part 1, the learning session, each target site was baited with a shelled sunflower seed on the top of the sand surface and one at the bottom of the cup, and covered with a poker chip. The remaining 124 cups were capped. Animals were allowed to search the arena until either all seeds had been removed or 15 min had elapsed. For training trials, if an animal failed to find the seeds in at least three of the four baited sites, it was returned to the arena 2 h later to find the seeds in the sites that they had missed on the previous attempt. In test trials, animals were returned to the arena if they had failed to retrieve seeds from all four of the baited sites. Animals were then removed from the experimental room for 1 day before Part 2 began.

In Part 2, the memory session, all 128 cups were sand-filled and covered with a loose poker chip, and thus, all looked identical. Both sunflower seeds were placed at the bottom of the cup. Part 2 sessions lasted until the animal had recovered seeds from all four baited sites or until 15 min had elapsed. In training trials, if the animal had failed to retrieve seeds from three-fourths of the baited sites, they were returned to the arena 2 h later to find the seeds in the missed locations. During Part 2 of testing, sessions were ended at 15 min and animals were not returned to the arena.

Between all sessions and trials, the plates and centre divider were thoroughly wiped with disposable, detergent-impregnated cleaning towels (i.e. 'baby wipes') to clean or mask odour trails left by the animals. Sand-filled cups were emptied and the number of seeds remaining in baited sites were counted. The cups were returned to the plates in new locations. All trials were separated by an intertrial interval of at least 3 days with no more than 5 days elapsing between trials.

Training. Training consisted of 10 trials, with eight unique landmarks per trial. Landmarks did not change within trials, only between trials. We determined this number of trials from a pilot study, where MR kangaroo rats on trials 8–10 found three out of four of the target sites in 20 or fewer searches. For the first three training trials, all sites except the baited locations were capped in both Part 1 (learning phase) and Part 2 (the memory phase) to facilitate learning of the task. In training trials 4–10, all cups were covered with poker chips in Part 2 and hence appeared identical.

Testing. Each animal was tested in two conditions (NL, 16L). Order of presentation of conditions to animals was counterbalanced. Note, as above, the type of landmarks and their arrangement did not change within a trial. Each test trial contained either no landmarks (NL) or 16 landmarks (16L) in both Part 1 (the learning phase) and

Part 2 (the memory phase). During Part 2 of each session, only two of the four target cups (50%) contained seeds. This allowed us to separately analyse sites that could be found using the odour of seeds from those that could not (i.e. that did not contain seeds). The location of the sites not rebaited was chosen using a random numbers table.

Analysis

Number of target sites recovered during training trials was analysed using a $2 \times 2 \times 2$ mixed design ANOVA with training trial as the repeated measure and sex and species as the between-subjects variables.

We devised three measures to analyse the test trial data for Part 2 of each test session. First, we calculated the total number of locations searched by each animal. Second, we calculated the number of target sites retrieved, which measured each animal's recovery of targets over the entire session. Third, we calculated the number of searches directed to target sites in the first four searches (Barkley & Jacobs 1998). There were four target sites in each session, so a score of 4 reflected perfect accuracy for relocating all targets. If a rat found two of the four target sites in the first four sites it searched, it was given an accuracy score of 2, and so on. We analysed the number of targets recovered over the whole session and retrieval accuracy separately for those sites that could be found using odour alone (rebaited) and those that could not be found using odour (unbaited). All of these measures were analysed using $2 \times 2 \times 2$ mixed design ANOVAs with landmark condition as the repeated measure. Significant interactions were further analysed using repeated measures ANOVAs with landmark condition as the repeated measure. In addition, because subjects showed considerable individual variability in the percentage of free-feeding body weight, we ran a Pearson correlation test between the percentage of free-feeding body weight and the proportion of targets recovered in Part 2 of each test session. Alpha level was set at 0.05.

RESULTS

The correlation between percentage of free-feeding body weight and proportion of target sites recovered during Part 2 of each test session was 0.189 ($\chi^2_{41} = 1.444, P = 0.23$), suggesting that percentage of free-feeding body weight did not affect the proportion of target sites recovered.

During training trials 4–10, in which all sites were available during retrieval, there was a steady increase in the number of target sites retrieved ($F_{6,102} = 2.71, P = 0.018$). A trend analysis revealed a linear ($F_{1,17} = 4.837, P = 0.042$) rather than a quadratic ($F_{1,17} = 0.79, P = 0.39$) pattern. This result suggests steady improvement over the course of trials. The effect of sex on number of target sites retrieved approached significance ($F_{1,17} = 3.94, P = 0.063$), with males recovering slightly more target sites through training than did females. There were no other significant effects (species: $F_{1,17} = 0.01, P = 0.90$; sex*species: $F_{1,17} = 0.72, P = 0.41$; trials*sex: $F_{6,102} = 0.84, P = 0.54$; trials*species: $F_{6,102} = 1.74, P = 0.12$; trials*species*sex: $F_{6,102} = 0.66, P = 0.69$).

MR kangaroo rats searched fewer sites during Part 2 of each test trial (on average, 15 sites searched per trial/128 sites available) than GB kangaroo rats (on average, 49 sites searched/128 available) ($F_{1,17} = 19.35, P = 0.0001$; Fig. 1). There was no effect of sex or landmark on the number of sites searched, nor were any interactions significant (sex: $F_{1,17} = 0.35, P = 0.56$; landmark: $F_{1,17} = 2.17, P = 0.16$; sex*species: $F_{1,17} = 0.00, P = 0.97$; landmark*sex: $F_{1,17} = 0.43, P = 0.52$; landmark*species: $F_{1,17} = 0.00, P = 1.00$; landmark*sex*species: $F_{1,17} = 0.01, P = 0.94$).

For number of target sites searched in Part 2 of each test trial, there was no effect of species or sex, nor was there a species*sex interaction (species: $F_{1,17} = 2.72, P = 0.12$; sex: $F_{1,17} = 0.13, P = 0.72$; species*sex: $F_{1,17} = 0.13, P = 0.72$; Fig. 2a). More target sites were searched in the 16L condition than in the NL condition ($F_{1,17} = 8.782, P = 0.009$). There was a significant landmark*sex interaction ($F_{1,17} = 7.547, P = 0.014$), but the interactions between landmark and species, and landmark, sex and species were not significant (landmark*species: $F_{1,17} = 0.25, P = 0.63$; landmark*sex*species: $F_{1,17} = 1.03, P = 0.32$). To determine the nature of the interaction between landmark and sex, we analysed the effect of landmark condition on recovery separately for males and females. For males, there was no effect of landmark condition ($F_{1,9} = 0.18, P = 0.68$). In contrast, females searched significantly more target sites when 16 landmarks were present than when no landmarks were present ($F_{1,10} = 14.694, P = 0.003$).

A separate analysis of unbaited targets searched (i.e. those that could not be located by odour), revealed a significant landmark*sex interaction ($F_{1,17} = 7.58, P = 0.014$; Fig. 2b), but no other significant effects (sex: $F_{1,17} = 0.00, P = 0.99$; species: $F_{1,17} = 3.14, P = 0.09$;

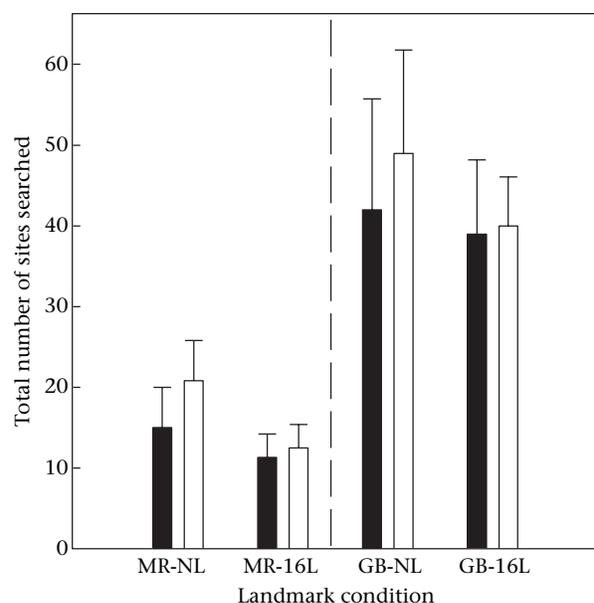


Figure 1. Mean \pm SE number of locations searched over the course of the entire memory session (Part 2) of each test trial, by landmark condition (NL = zero trial-unique landmarks, 16L = 16 trial-unique landmarks) and by species (MR = Merriam's, GB = Great Basin). ■: female; □: male.

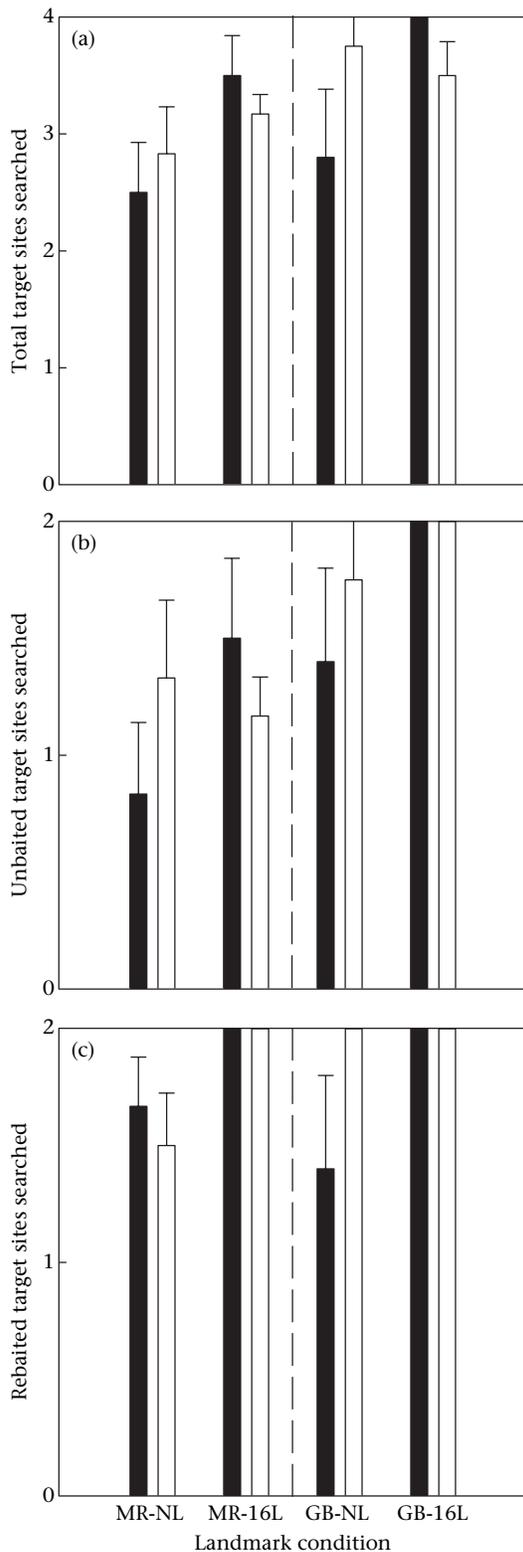


Figure 2. Mean \pm SE number of (a) total target sites searched, (b) unbaited target sites searched and (c) rebaited target sites searched in each memory session (Part 2) of a test trial, by landmark condition and by species. ■: female; □: male. Abbreviations as given in Fig. 1.

sex*species: $F_{1,17} = 0.10$, $P = 0.76$; landmark: $F_{1,17} = 1.93$, $P = 0.18$; landmark*species: $F_{1,17} = 0.06$, $P = 0.81$; landmark*species*sex: $F_{1,17} = 0.00$, $P = 0.98$). Separate analyses of males and females showed that females searched more unbaited targets in the 16L condition than in the NL condition ($F_{1,10} = 9.80$, $P = 0.011$), whereas males were unaffected by landmark condition ($F_{1,9} = 1.00$, $P = 0.34$).

Rats searched more baited targets (which could be found using odour alone) when landmarks were present than when no landmarks were present ($F_{1,17} = 7.39$, $P = 0.015$; Fig. 2c), but there were no significant effects of sex or species, nor were there any significant interactions (sex: $F_{1,17} = 0.68$, $P = 0.42$; species: $F_{1,17} = 0.20$, $P = 0.66$; sex*species: $F_{1,17} = 2.11$, $P = 0.16$; landmark*sex: $F_{1,17} = 0.68$, $P = 0.42$; landmark*species: $F_{1,17} = 0.20$, $P = 0.66$; landmark*sex*species: $F_{1,17} = 2.11$, $P = 0.16$).

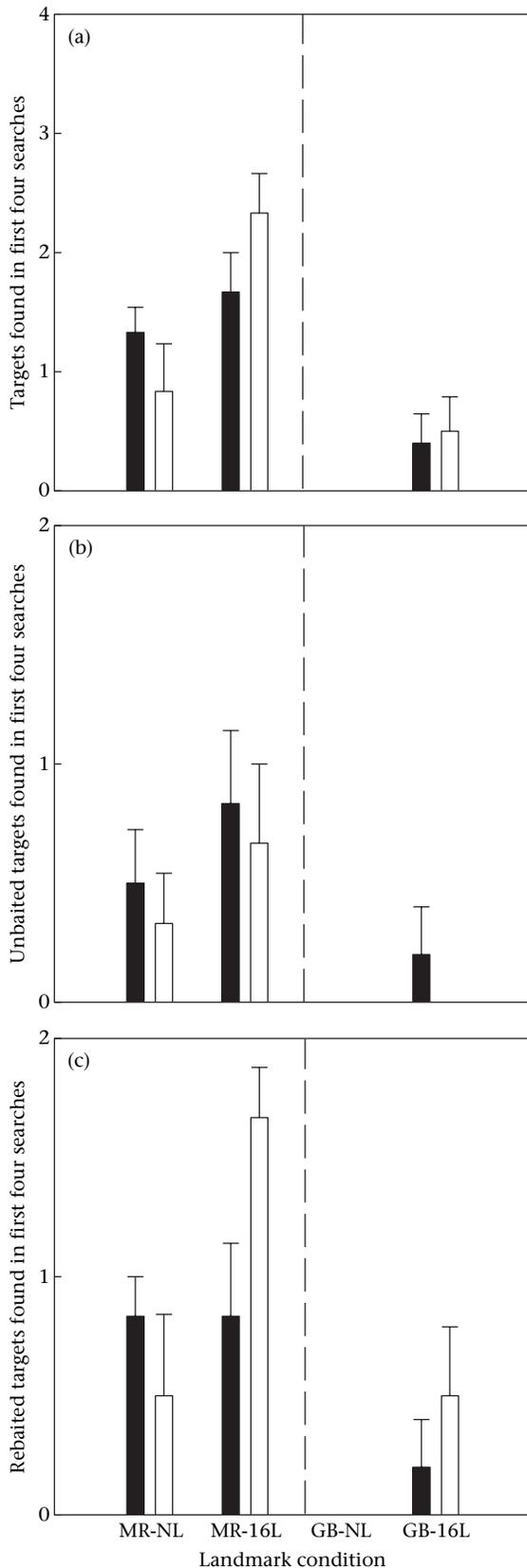
MR kangaroo rats found more targets in the first four searches than did GB kangaroo rats ($F_{1,17} = 28.6$, $P = 0.0001$; Fig. 3a). Across species, accuracy was better when 16 landmarks were present ($F_{1,17} = 18.0$, $P = 0.001$), but there was no effect of sex ($F_{1,17} = 0.073$, $P = 0.79$). Although it appeared that male MR kangaroo rats showed better accuracy in the 16L condition than females, the landmark*sex interaction only approached significance ($F_{1,17} = 3.86$, $P = 0.066$). No other interaction was significant (landmark*species: $F_{1,17} = 2.09$, $P = 0.17$; sex*species: $F_{1,17} = 0.01$, $P = 0.95$; landmark*sex*species: $F_{1,17} = 2.73$, $P = 0.12$).

MR rats searched more unbaited targets during the first four searches than did GB rats ($F_{1,17} = 6.99$, $P = 0.017$; Fig. 3b). No other significant effects were observed in the three-way ANOVA for unbaited targets found in the first four searches (sex: $F_{1,17} = 0.44$, $P = 0.52$; sex*species: $F_{1,17} = 0.027$, $P = 0.87$; landmark: $F_{1,17} = 2.98$, $P = 0.10$; landmark*sex: $F_{1,17} = 0.16$, $P = 0.70$; landmark*species: $F_{1,17} = 0.87$, $P = 0.37$; landmark*sex*species: $F_{1,17} = 0.16$, $P = 0.70$).

MR rats also searched more baited targets during the first four searches than did GB rats ($F_{1,17} = 17.81$, $P = 0.001$; Fig. 3c). More rebaited targets were found in the 16L condition than in the NL condition ($F_{1,17} = 8.76$, $P = 0.009$) and there was a significant landmark*sex interaction ($F_{1,17} = 5.41$, $P = 0.033$). No other effects were significant (sex: $F_{1,17} = 1.16$, $P = 0.30$; sex*species: $F_{1,17} = 0.073$, $P = 0.79$; landmark*species: $F_{1,17} = 0.55$, $P = 0.47$; landmark*sex*species: $F_{1,17} = 1.89$, $P = 0.19$). Separate analyses for males and females revealed that males found more rebaited targets in initial searches in the 16L condition than in the NL condition ($F_{1,9} = 10.57$, $P = 0.010$). Females showed no effect of landmark condition on retrieval of sites that could be found using odour in the first four searches ($F_{1,10} = 0.31$, $P = 0.59$).

DISCUSSION

Our first goal was to determine whether food-storing behaviour patterns predicted species differences in performance in a cache simulation task in mammals, as has been shown in passerine birds. We predicted that the



intensively scatter-hoarding MR kangaroo rat would show more accurate retrieval of targets than the species with alternate food sources (i.e. the secondarily folivorous GB kangaroo rat). The second goal was to determine whether females and males would differ in performance in this task. Based on work in other polygynous mammal species, we predicted that female performance in both species would be more accurate in the presence of rich landmark cues (i.e. 16L) than in the absence of these cues, whereas male performance would be unaffected by this cue manipulation.

As predicted, MR kangaroo rats showed significantly better performance in relocating sites after a 24-h delay than did GB kangaroo rats. MR kangaroo rats searched fewer overall locations in the arena (15/128 versus 49/128, on average) and located more targets in the first four sites they searched, although the total number of targets discovered did not differ between species. The GB kangaroo rats thus found the same number of seeds, albeit with significantly more search effort, as measured by the total number of sites searched to discover the four target locations. As we used a cache simulation task, we cannot determine how the differences observed here might relate to retrieval strategies for scatter caches. Although GB kangaroo rats typically hoard more of their seeds than do MR kangaroo rats, they have been observed making scatter hoards in the laboratory, and therefore, future research can explore whether memory differences exist in retrieval of scatter hoards.

There were no species differences in response to the presence or absence of local cues, however (16L versus NL). We cannot therefore conclude that the species differences arose from differential use of cues to find locations, as has been demonstrated in passerine birds (Brodbeck 1994; Clayton & Krebs 1994). Our task differs significantly from those tasks that have demonstrated species differences in reliance on localized information. Our task presented animals with an environment containing an array of localized objects or an environment bare of local objects. Tests that have found differential use of localized cues have typically presented birds with a feeder having a unique spatial location and a localized cue, which were then dissociated by moving the localized cues. Under these conditions, nonstoring birds show greater reliance on local cues than do storing birds (Clayton & Krebs 1994; Brodbeck 1994). The nonstorsers use the localized object as well as the spatial location, whereas the storers strongly prefer the spatial location. A further difference between this task and previous studies in birds is in the nature of the localized cues. In the bird studies, localized cues were contiguous with the goal, whereas our localized cues represented a spatial array of local cues that were not contiguous with the goal. Failure to find species differences in our landmark conditions might have

Figure 3. Mean \pm SE number of (a) target sites searched, (b) unbaited target sites searched and (c) rebaited target sites searched in the first four searches in each memory session (Part 2) of a test trial, by landmark condition and by species. ■: female; □: male. Abbreviations as given in Fig. 1.

resulted either from poor accuracy on the part of the GB kangaroo rats or from these differences in the nature of the experimental design.

Our analysis of sex differences in performance, in contrast, did reveal a significant effect of positional cues on retrieval accuracy, as predicted. Across species, females retrieved more target sites, and specifically unbaited targets, in the presence of 16 positional cues (trial-unique objects), while male retrieval of targets was unaffected by cue condition. This latter result would also be predicted from previous studies of sex differences in cue use in other species (Williams & Meck 1991; Langley 1994), given the strong directional cues present under both 16L and NL conditions (rectangular shape of arena, asymmetric light source). However, when we examined accuracy in terms of the number of targets found in the first four searches, male MR kangaroo rats showed a tendency to locate more target sites in the 16L condition than in the NL condition compared to female MR kangaroo rats. This pattern was most evident for rebaited sites, which could be found using odour alone. Future studies could examine this pattern in more detail; at present, we can only conclude that male MR kangaroo rats responded to visual cues differently than did females. We speculate, however, that the presence of landmark cues could have potentiated the use of odour cues (e.g. seed odour in baited targets) in males, as has been shown in laboratory rats (Lavenex & Schenk 1997), which could have increased their accuracy in the first four searches.

These results are also consistent with previous studies of the differences in female and male responses to the same visual environment. The arena contained clear and salient directional cues, the long rectangular shape and the asymmetric lighting. These features should have been used primarily by males to locate the simulated cache locations. Thus, the male strategy of orientating to directional cues would have been better adapted to the demand of this particular arena. Females, in contrast, lacked any local landmarks in the NL condition and therefore were forced to give up their preferred strategy, and to use a less preferred strategy to encode targets. Under these conditions females of both species showed a significant decrement in performance.

Conclusions

Our results suggest that differences in performance between MR and GB kangaroo rats resulted from differences in a spatial memory process, either in encoding, storage or retrieval of spatial location data, consistent with an ecological function. However, alternative explanations, in the framework introduced by Niko Tinbergen (Tinbergen 1963) and particularly in light of current debate (Macphail & Bolhuis 2001; Hampton et al. 2002), could be that our findings reflect phylogenetic differences within the genus *Dipodomys*, physiological differences between MR and GB kangaroo rats, and/or differences in developmental trajectories of the individuals in this study. Species differences in sensory physiology could account for differences in performance, apart from different capacities for learning and memory. Differences in

developmental history could also clearly influence performance of wild-caught individuals tested in the laboratory. Experience has a significant effect on spatial memory ability in captive-reared parids (Clayton 1995), and it is likely that the two species we studied, which have different patterns of food storage, also differed in their experience with relocating scatter hoards. Differences in experience could also account for the sex differences observed, because females and males in this genus differ in their space use patterns (Behrends et al. 1986b; Daly et al. 1992b). Sex differences in spatial encoding, however, are also frequently found in the laboratory rat and mouse, where spatial experience is restricted and equalized for females and males (Jacobs & Schenk 2003).

Studying species differences in cognition requires a multifaceted approach, using a variety of techniques, tasks and species (Kamil 1994; Shettleworth 2003). Ours is the first study to use a cache simulation task to compare memory in closely related food-storing mammalian species. Although our results are consistent with the predictions from an ecological function interpretation, we cannot rule out other interpretations; future studies are required to explore the roles of species differences in the development of spatial search strategies and/or spatial memory abilities. The many alternative explanations for species differences highlight the advantages of studying the interaction of sex and memory in food-storing animals. Sex differences in performance in both species suggest that females relocate simulated food caches, and presumably naturally constructed food caches, differently than do males. Cognitive sex differences therefore may be a valuable tool in the field of cognitive and brain evolution.

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