

Flexibility of cue use in the fox squirrel (*Sciurus niger*)

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Abstract Recent work on captive flying squirrels has demonstrated a novel degree of flexibility in the use of different orientation cues. In the present study, we examine to what extent this flexibility is present in a free-ranging population of another tree squirrel species, the fox squirrel. We trained squirrels to a rewarded location within a square array of four feeders and then tested them on transformations of the array that either pitted two cue types against one cue type, the majority tests, or all cue types against each other, the forced-hierarchy test. In Experiment 1, squirrels reoriented to the two-cue-type location in all majority tests and to the location indicated by the visual features of the feeders in the forced-hierarchy test. This preference for visual features runs contrary to previous studies that report the use of spatial cues over visual features in food-storing species. In Experiments 2–5 we tested squirrels with different trial orders (Experiments 2 and 3), a different apparatus (Experiment 4) and at different times of the year (Experiment 5) to determine why these squirrels had chosen to orient using visual features in the first experiment. Like captive flying squirrels, free-ranging fox squirrels showed a large degree of flexibility in their use of cues. Furthermore, their cue use appeared to be sensitive both to changes in the test apparatus and the season in which we tested. Altogether our results suggest that the study of free-ranging animals over a variety of conditions is necessary for understanding spatial cognition.

Keywords Spatial memory · Scatter-hoarding · Sciuridae · Orientation · Cognition · Majority strategy

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Introduction

Most animals orient in space using redundant sources of information (Schöne 1984). In some cases, such as when an animal adjusts its magnetic map using information from the setting sun, these redundant sources of information can be used to calibrate each other (Able 1993; Phillips and Moore 1992). In other cases, such as on cloudy days when honeybees switch from relying on a sun compass to relying on visual landmarks, it is clear that the redundancy acts as a critical backup for orientation in an unpredictable sensory environment (Dyer and Seeley 1994). Yet what is not clear—despite many studies in diverse species—is how animals use these different sources of information when they are in conflict with one another.

Several studies suggest that animals may use the information in a linear hierarchical manner. For example, in Brodbeck's study of black-capped chickadees (*Poecile atricapillus*) and dark-eyed juncos (*Junco hyemalis*), birds learned food was available at one of an array of four uniquely decorated feeders (Brodbeck 1994; see also Clayton and Krebs 1994; Herz et al. 1994; Sherry and Duff 1996). When feeders were shifted, chickadees oriented first to the feeder defined by landmarks outside of the array (i.e., the edges of the walls in the room). Their second choice was the feeder that was in the correct position in the array and their third choice was the feeder with the previously rewarded unique color pattern. Notably, chickadees did not visit the fourth location, which was not predicted by any cues during training. In contrast, juncos, a nonstoring passerine, did not show an ordered preference, although they also only visited three of four feeders, ignoring the feeder not predicted by any landmarks. This suggests that both species retained a memory of all available cues but only the chickadees used the cues in a linear hierarchal manner, first

searching in the location predicted by one cue type (distal room cues), followed by the location predicted by a second cue type (relative array position), and finally by the location predicted by a third cue type (array features).

More recently, Gibbs et al. (2007) found that southern flying squirrels (*Glaucomys volans*) chose the location indicated by the greatest number of cue types, rather than having a particular preference for one type of cue. Their behavior was not consistent with a linear hierarchical model of cue use. For example, when the line of feeders was rotated 180°, the feeder in the previously rewarded location relative to distal room cues now displayed a new set of visual features and was in a new array position. The feeder with the correct visual features was now in the previously rewarded array position, but was in a new location relative to the distal room cues. If the squirrels had followed a linear hierarchy, as did the chickadees, they would have first oriented to the location indicated by the distal room cues. Instead, they chose the feeder that was in the correct array position and had the correct visual features.

These results are consistent with a majority cue use strategy by which animals choose the location predicted by the greatest number of cue types rather than relying on an ordered hierarchy of cue types (Gibbs et al. 2007). This type of strategy would be also be consistent with a Bayesian model in which the two cue types that are least preferred in the linear hierarchy are weighted such that their sum would be greater than the weight given to the preferred cue type. Cheng et al. (2007) have recently proposed using a Bayesian framework to model how animals make orientation decisions. In such a model, sources of spatial information are weighted differentially according to their variance and the prior experience of the animal. Whether the majority strategy is optimal for combining multiple sources of spatial information, as viewed through a Bayesian lens, is clearly an important question and is in fact the focus of current research. The aim here, however, was first to determine the generality of the majority strategy.

The objective of the present study was therefore to determine if free-ranging fox squirrels (*Sciurus niger*) might also adopt the majority strategy seen in captive flying squirrels. If so, this would suggest that the strategy is not particular to the southern flying squirrel, the design of the Gibbs et al. experiment, or the conditions of captivity. Squirrels (Family Sciuridae) are suitable subjects for field studies of spatial memory because they are diurnal, habituate easily to human observers and novel experimental objects and have consequently been chosen as subjects in many experimental studies (Cahalane 1942; Lavenex et al. 1998; Devenport et al. 2000; Jacobs and Liman 1991; Jacobs and Shiflett 1999; Macdonald 1997; Vlasak 2006a, b). More important, fox squirrels, like southern flying squirrels, are obligate scatter hoarders (Stapanian and Smith 1978). Scatter hoard-

ing is a foraging strategy associated with specific cue use strategies, as in the comparison of chickadees and juncos discussed earlier. In general, scatter hoarding bird species rely preferentially on distant visual cues to reorient to a location (Brodbeck 1994; Clayton and Krebs 1994; Herz et al. 1994; Sherry and Duff 1996). In contrast, nonstoring bird species show no preference (see Hodgson and Healy 2005 for an exception).

Prior work has demonstrated that free-ranging fox squirrels, like chickadees and other food-storing birds, relied on distant visual cues to orient to a location on an outdoor vertical maze, while ignoring the color of maze pathways (Jacobs and Shiflett 1999). In another study of free-ranging fox squirrels, Lavenex et al. (1998) also found that squirrels relied on distant cues, and not the unique features of proximate cues, to orient to the spatial distribution of rewards in a large horizontal array of feeders. In field studies of free-ranging Columbian ground squirrels (*Spermophilus columbianus*), Vlasak again found that squirrels relied on distant visual cues to re-orient to a remembered location (Vlasak 2006a, b). Distant visual cues are large cues in the environment, such as trees and mountains, which are the most stable over time and across different viewing angles. Since these cues would have the least variance in a natural setting, relying on a linear hierarchical strategy that prefers these cues may be optimal when all cue types indicate different locations.

However, none of these studies confronted the study animals with a choice between a location indicated by two cue types and a location indicated by a single cue type. Under these conditions, the majority strategy would seem the most optimal since it is less likely for two types of cues to converge on the same incorrect location, while it is possible that the spatial relationship between a single cue type and the rewarded location might have changed. Relying on a hierarchical preference of cue types would lead the animal to choose the location indicated by the single cue type when it was the preferred one. We predicted that fox squirrels, as in previous studies, would use a linear hierarchical strategy if given no other option, but would use a majority strategy if available. If they did not, then the previous results with flying squirrels could be the result of species differences or the effect of orientation under captive conditions. If, however, free-ranging fox squirrels also use a majority strategy, then this points to a previously unsuspected degree of flexibility in cue use in scatter-hoarding mammals.

In our experiments, we trained squirrels to a rewarded location within a square array of four feeders and tested them on transformations of the array. Since it is well documented that squirrels can use a hierarchical strategy, the objective of Experiment 1 was to determine whether squirrels would show the same degree of flexibility as flying squirrels had in previous experiments, by using a majority

strategy when available. Experiment 2 examined whether the repetition of training trials might have caused the squirrels to switch from using a less neurologically efficient strategy based on spatial cues to a feature-based strategy in Experiment 1. In Experiment 3, we considered whether the order of testing trials might have caused this switch since we had been unable to counterbalance order of testing due to small sample sizes. Experiment 4 replicated Experiment 1 in the Spring to see whether squirrels were not using a majority strategy in Experiments 2 and 3 due to changes in trial order or seasonal changes. Finally, Experiment 5 replicated Experiment 1 at the same time of year, but using the apparatus from Experiments 2, 3, and 4, to determine if changes in the saliency of objects attached to the feeders or the method of opening the feeders between Experiments 1 and 2 might have caused squirrels to pay less attention to featural cues in Experiments 2, 3, and 4.

General methods

Study sites

All experiments were conducted on four wooded sites on the University of California, Berkeley campus. One site was located within a grove of mature bluegum eucalyptus (*Eucalyptus globules*), redwood (*Sequoia sempervirens*), and coast live oak (*Quercus agrifolia*) trees. The other three sites were located on groomed lawns with redwood and coast live oak (see Jacobs and Shiflett 1999, for details of study sites). We conducted Experiments 1 and 5 in late July and early August of 2005 and 2006, and Experiments 2, 3 and 4 in April and May of 2006. Data were collected from late morning to early afternoon. Experiments are presented in the order in which they were conducted.

Study animals

Adult male fox squirrels ($N = 42$) were individually marked with permanent black fur dye (Nyanzol D). Due to an intervening molt between Experiments 1 and 2, squirrels lost their markings and it is possible that the eight squirrels in Experiment 1 may have also participated in Experiments 2–5. After Experiment 2 we were able to continuously track all participants. However, squirrels that had previously participated were immediately obvious to the experimenter since they required no pretraining to learn how to manipulate the apparatus. These squirrels were excluded from participating. Furthermore, due to an increase in the mortality rate possibly as a result of the introduction of West Nile virus to California (Padgett et al. 2007) it is highly unlikely that the squirrels tested in Experiments 2–5 were the same squirrels that participated in Experiment 1.

Because of the prevalence of male squirrels on these sites, we excluded females from our study to reduce variance in the results. We selected squirrels that were available at the time of testing and that we could test without interference from or interactions with other squirrels. We excluded trials during which participants were interrupted due to social interactions with other squirrels or other disturbances (e.g., dog chases). All squirrels were highly habituated to human observers even at distances of one meter or less.

Experimental setup

As seen in Fig. 1, the array of feeders was presented to study animals on a small table (82 cm \times 82 cm \times 72 cm high), covered with vinyl sheeting. Feeders were small lockable containers topped with an object of a unique color and pattern, positioned in a square array (28 cm \times 28 cm). To preclude the use of orientation to odor cues, all feeders contained chopped nuts (chopped pecans, walnuts, and hazelnuts). In addition, the table surface was cleaned with disinfectant wipes between training and testing trials. The table was placed adjacent to a tree trunk, which served as

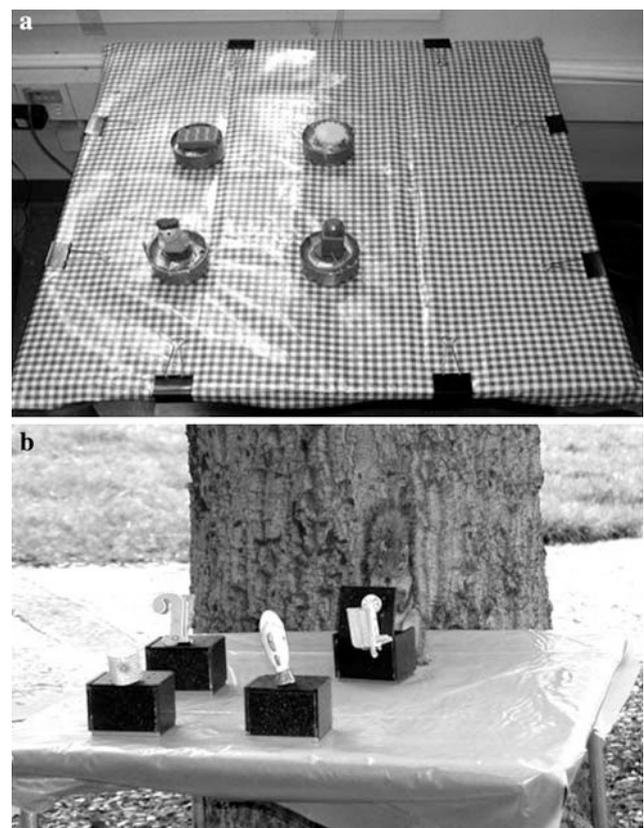


Fig. 1 Photographs of the experimental apparatuses in the training configuration. A plastic template was used at the beginning of each session to place the boxes in the correct positions. **a** The apparatus used in Experiment 1. **b** The apparatus used in Experiments 2–5. See [General methods](#) for more details

the only access point. This ensured that squirrels always approached the array from the same side.

In preliminary studies, we found that squirrels did not take the same route to the rewarded location, often examining the entire platform before making a choice. We could therefore assume that they were using allocentric information, such as landmarks in the external environment and visual features of the feeders, instead of self-motion cues or path integration. Since the global-local distinction is relative to the size of the experimental setup and is difficult to quantify in the field (see Hurly and Healy 1996; Thiele and Winter 2005 for more discussion on classification of cues in field studies), we classified the available spatial information based on its relationship to the experimental setup which we could control. We decided on three cue types: extra-array cues (EX), intra-array position cues (IN), and unique feature cues (UF). EX included any landmark outside of the testing platform, the testing platform itself, and the tree that provided access to the platform; these cues remained in the same locations throughout training and testing. IN referred to the position of the rewarded feeder as defined by its geometric relationship to the other three feeders in the square array without encoding the specific features of the feeders. UF referred to the features, such as shape and color, of the object located on the feeder and any residual odors on the feeder. The objects included small plastic toys and ceramic salt shakers (Fig. 1).

Pretraining

We trained squirrels to approach and eat nuts from an open feeder on the table. The squirrel was then shaped to open a feeder by gradually closing the feeder on subsequent pretraining trials. On average, squirrels were able to open and eat from a closed feeder after 5 min of pretraining.

Training

Pretrained squirrels approached the feeders and were given as much time as needed to find and open the correct feeder through trial-and-error learning. The location of the correct feeder was counterbalanced across subjects. The experimenter then gently urged the squirrel off the table and closed the now open feeder. To avoid experimenter cueing, the experimenter mimed closing the other three feeders in a pseudorandom order. To ensure that all squirrels were trained to the same criterion, training trials were repeated until the squirrel chose the correct feeder first in three consecutive trials. After reaching this criterion, squirrels were given only one testing trial and then retrained to criterion. Across all experiments, squirrels quickly reached criterion in initial training trials [mean \pm standard deviation (SD), 3.6 ± 1.0 ; range 3–7; N , 42]. After nonrewarded testing,

squirrels just as quickly returned to criterion in inter-testing training trials (mean \pm SD, 3.2 ± 0.8 ; range 3–7; N , 42).

Testing

There were two types of testing trials: the forced-hierarchy test and the majority test. In the forced-hierarchy test, the entire array of feeders was moved horizontally to the other side of the table and the previously rewarded feeder was switched with the diagonally opposite one (Fig. 2). Each cue type, EX, IN, or UF, now indicated a different feeder in the array. A fourth feeder served as a distracter. In the majority test, two of the three cue types (e.g., IN and UF) indicated one feeder—the majority location, while the third type (e.g., EX) indicated a second feeder—the minority location. The other two feeders served as distracters. The three possible majority tests are illustrated in Fig. 2.

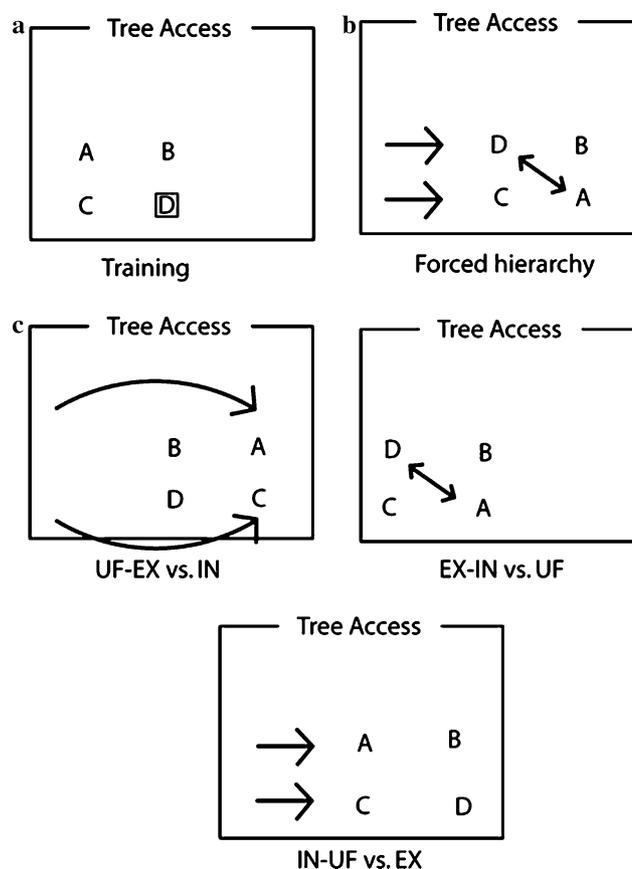


Fig. 2 **a** A schematic of the training trial in which the D position is rewarded. **b** A schematic of the forced-hierarchy trial based on this training setup. The entire array is moved horizontally to the other side of the table and the previously rewarded box (D) is switched with its diagonal opposite (A). D is now the location indicated by the *UF* cues, A is the location indicated by *IN* cues, and C is the location indicated by *EX* cues. **c** A schematic of the three majority tests based on this training setup

Training and testing trials for each subject were conducted in one session on the same day and in the same location. The same procedures were used for all experiments. The order of testing trials was not counterbalanced across subjects to reduce the variance within the small sample sizes. There was no evidence of a decrease in performance between testing trials.

Each squirrel took approximately 1 h to complete all trials, including time during which it would sometimes retreat and rest in the tree. The first feeder that the squirrel attempted to open was recorded for every trial. If the squirrel pushed the feeder over or tried to pry it open with its teeth, the behavior was recorded as a choice. All other interactions were noted but were not considered choices.

In each experiment, data were tested against chance using a two-tailed binomial test; the probability of choosing correctly by chance was 0.25. Additional analyses were run using two-tailed Fisher’s exact tests.

Experiment 1

Rationale

The purpose of this experiment was to determine what cue types free-ranging fox squirrels would use and whether they would use a majority strategy or a hierarchical strategy in the majority test. We first presented squirrels with all three versions of the majority test. Then we tested them in the forced-hierarchy test to determine what type of cue they would prefer. On the basis of the results from Gibbs et al. (2007) and Jacobs and Shiflett (1999), we expected fox squirrels to rely preferentially on EX cues in the forced-

hierarchy test and to use a majority strategy in the majority tests.

Methods

We recruited eight adult male fox squirrels at four testing sites. All squirrels were naïve to experimental conditions. We tested squirrels in this order of the three majority tests: EX-UF versus IN; IN-EX versus UF; UF-IN versus EX and then in the forced-hierarchy test (Fig. 3). Data were collected from July 23, 2005–July 26, 2005. All other procedures are as described in [General methods](#).

Apparatus

As seen in Fig. 1a, feeders were shallow circular aluminum containers (tuna cans; 9 cm diameter, 4 cm depth) filled with modeling clay with an inset plastic cup (4 cm diameter, 1.5 cm depth) in the center. A unique plastic object (large Lego™ block) was attached to the side of the aluminum container with steel wire and placed on top of the plastic cup. All four cups were filled with 5 g of chopped nuts. During training, three of the plastic cups were closed with tight-fitting lids, while one was left open; during testing all four cups were closed.

Results and discussion

In the three majority tests, squirrels significantly re-oriented to the majority and not the minority location (Table 1). In the EX-UF versus IN and IN-EX versus UF tests, five of seven squirrels and six of seven squirrels visited the majority

Fig. 3 **a** Schematic of the trial order in Experiment 1, beginning with the initial training trial and including all the inter-training trials. **b** Schematic of the trial order in Experiment 3, beginning with the initial training trial. The inter-training trials between testing trials are not included. Temporal sequence proceeds from *left to right*

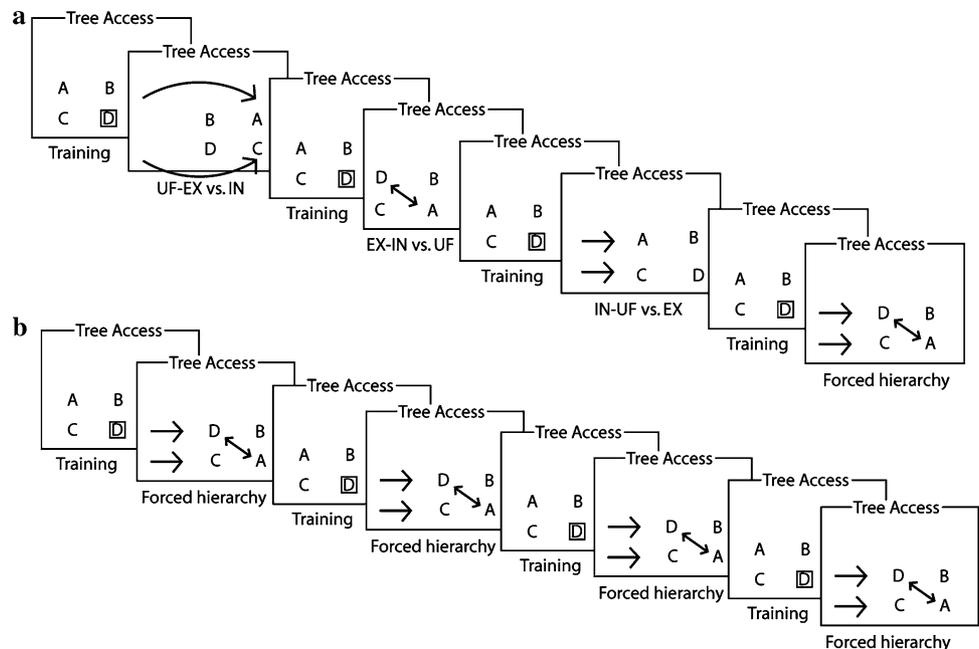


Table 1 Results of Experiment 1

| Trial type | Majority location | | Minority location | | Other locations % (Number) |
|-----------------------|-------------------|------------|-------------------|------------|-------------------------------|
| | Cue types | % (Number) | Cue type | % (Number) | |
| Majority tests | | | | | |
| EX-UF vs. IN | EX-UF | 71.4 (5) | IN | 14.3 (1) | 14.3 (1) |
| IN-EX vs. UF | IN-EX | 85.7 (6) | UF | 14.3 (1) | 0 (0) |
| UF-IN vs. EX | UF-IN | 80 (4) | EX | 0 (0) | 20 (1) |
| Cue type | EX | | IN | UF | Others |
| Forced-hierarchy test | | | | | |
| % (Number) | 0 (0) | | 0 (0) | 100 (5) | 0 (0) |

Trial and cue types defined in text. The n decreased because of the loss of squirrels during testing. Percentages and observed numbers of squirrels choosing are reported

location, respectively ($n = 7$, $P = 0.013$ and $P = 0.0013$). In the third majority test, UF-IN versus EX, four of five squirrels visited the majority location ($n = 5$, $P = 0.016$). In the forced-hierarchy test, five of five squirrels reoriented to the UF location ($n = 5$, $P < 0.001$).

Data from the majority tests suggest that squirrels are capable of using a majority strategy when orienting to locations. If the squirrels had used a rigid hierarchical strategy they would have chosen the minority location indicated by their preferred cue type in the corresponding majority test. For example, if UF is the preferred cue type in the forced-hierarchy test, they would choose the UF location instead of the IN-EX location in the IN-EX versus UF majority test. However, they did not. This confirms our prediction that fox squirrels do use a majority strategy.

On the other hand, data from the forced-hierarchy test suggest that the preferred cue type for this population of squirrels is UF. This conflicts with previous studies (Jacobs and Shiflett 1999; Vlasak 2006a, b) in which squirrels oriented using an allocentric frame of reference based on distal landmarks, i.e., EX in the current experiment. While the small sample size ($n = 5$) limits our ability to generalize from these data, the results of these previous studies would have predicted that the location indicated by UF cues would have been chosen below chance. In both the experiments cited, squirrels seemingly completely disregarded all featural information provided. Therefore, the results of Experiment 1 were more surprising than they would have been if the choice of UF had been at chance or at least equal to the other cue types. Instead, we had an overwhelming majority of the squirrels immediately indicate their preference for the UF location over both the EX and IN locations.

It is possible that the squirrels switched from a place navigation strategy by which they oriented using spatial cues, EX and IN, to orienting using an associative, feature-based search strategy by which they oriented using UF cues. Similar behavior has been previously seen in laboratory rats. It has been argued that this switch could arise because the neural mechanisms underlying a feature-based

cue encoding are faster than those of place navigation, making the feature-based search strategy more efficient (*Rattus norvegicus*; Chang and Gold 2003; Packard and McGaugh 1996). Thus, over time, if both strategies are equally accurate at solving the training task, the feature-based search may out-compete the slower place navigation (Chavarriga et al. 2005). Given that the forced-hierarchy test followed multiple testing trials, by this trial, the squirrels might have switched to using the feature-based strategy. We tested this hypothesis in Experiment 2.

Experiment 2

Rationale

To test the hypothesis that over-training through task repetition caused the squirrels to adopt a cue-directed search strategy, we tested each squirrel in the forced-hierarchy test four times in a row. If squirrels preferentially rely on UF cues they should choose the UF location throughout the whole experiment. If, however, they preferentially use spatial cues, but switch to a feature-based search after multiple repetitions of the same task, they should choose either the EX or IN locations in the initial testing trials and the UF location in the final testing trials.

Methods

We recruited eight adult male fox squirrels. All procedures were identical to those used in Experiment 1, except that only the forced-hierarchy test was used. Data were collected from April 8, 2006 to April 30, 2006. All other procedures are as described in [General methods](#).

Apparatus

To avoid problems with possible remnant odors in the original feeders, we used a new feeder design in this and subsequent experiments (Experiments 2–5). Each feeder was a

dark green acrylic box (10.5 cm × 7.5 cm × 7.5 cm high) with a hinged lid and a locking device located on the side (Fig. 1b). Boxes appeared identical whether they were locked or unlocked. We fastened ceramic figurines, different in both shape and color, to the top of each box using Velcro™. Each box contained 30 g of nuts.

Results and discussion

In the first presentation of the forced-hierarchy test, there was no one strategy used by the squirrels. An equal number of squirrels chose the IN as the EX location ($n = 3$ for both), the other squirrels chose the distracter and UF locations ($n = 1$ for both; Table 2). In the second, third, and fourth trials, squirrels chose the EX location ($n = 8$ for all, $P = 0.0042$, $P < 0.001$ and $P = 0.027$ respectively). Since the squirrels never chose the UF location, the data do not support the hypothesis that task repetition causes squirrels to switch to a cue-directed search, nor do they support the hypothesis that squirrels prefer UF cues.

The results of Experiment 2 suggest that EX is the preferred cue type and that task repetition does not cause squirrels to switch to a feature-based search strategy (i.e., preferring UF). It is also possible that specifically having the majority test trials precede the forced-hierarchy test in Experiment 1 caused squirrels to choose the UF location. Unlike in this experiment, in Experiment 1, the spatial locations were changing in every testing trial. Therefore the UF cue type might have appeared to be the most stable. We tested this hypothesis in Experiment 3.

Experiment 3

Rationale

The objective of this experiment was to test for the effect of the order of testing trials. We replicated the procedure of Experiment 1, but we introduced a forced-hierarchy test after each majority test. These new forced-hierarchy tests allowed us to assess the preferred cue type throughout the

experiment. If the preference for UF in Experiment 1 was due to order effects, then squirrels should initially choose the EX location in the forced-hierarchy trials and switch to the UF location in later trials. Otherwise, squirrels should choose the same location in all the forced-hierarchy trials.

Methods

We recruited nine adult male fox squirrels that had not participated in Experiment 2. The order of the majority tests was the same as in Experiment 1, except that after each majority test there was a forced-hierarchy trial (Fig. 3). Data were collected from April 30, 2006 to May 21, 2006. All other procedures are as described in [General methods](#).

Results and discussion

In all forced-hierarchy trials, squirrels chose the EX location ($n = 9$, in the last trial $n = 8$, $P = 0.0013$, $P = 0.0013$, $P = 0.027$, for the first, second, and third trials respectively; Table 3). Furthermore, while squirrels chose the majority location in the first two majority tests, EX-UF versus IN and IN-EX versus UF ($n = 9$, $P = 0.049$ and $P < 0.0001$, respectively), they chose the minority location (EX) in the third majority test, UF-IN versus EX ($n = 9$, $P = 0.049$).

These data suggest that trial order does not cause squirrels to switch from preferring EX to UF. Squirrels not only visited the EX location in all forced-hierarchy trials, but now there was also no evidence that they were using a majority strategy. In both of the tests in which they chose the majority location, that location was indicated by EX cues. This pattern of data made it impossible for us to determine whether they had used a majority strategy initially and then switched to a hierarchical strategy, or if they had only used a hierarchical strategy with EX as their preferred cue type the whole time. We chose to assume the simpler explanation, that they had used a hierarchical strategy the whole time. Therefore, we concluded from the data that the order of trials in Experiment 1 did not cause squirrels to use a cue-directed strategy; in contrast, if anything, the order induced them to use only spatial cues (i.e., EX and IN).

Finally, squirrels might have preferred UF cues in Experiment 1 and EX in Experiments 2 and 3 because the feeder construction in Experiment 1 had more salient UF cues than the feeder construction used in subsequent experiments. There were at least three possible sources of increased saliency: differences in the objects placed on the feeders, the manner of opening the feeders, and the feeders themselves. The objects in Experiment 1 were commercial plastic blocks that were simply colored objects with simple shapes, while in Experiments 2 and 3 the objects used were more visually complex. In Experiment 1, the squirrels had to remove the object from the feeder to access their reward,

Table 2 Results of Experiment 2

| Trial number | Cue type: % (number) | | | |
|-----------------------|----------------------|----------|----------|----------|
| | EX | IN | UF | None |
| Forced-hierarchy test | | | | |
| 1 | 37.5 (3) | 37.5 (3) | 12.5 (1) | 12.5 (1) |
| 2 | 75 (6) | 12.5 (1) | 12.5 (1) | 0 (0) |
| 3 | 87.5 (7) | 12.5 (1) | 0 (0) | 0 (0) |
| 4 | 62.5 (5) | 25 (2) | 12.5 (1) | 0 (0) |

Trial and cue types defined in text. Percentages and observed numbers of squirrels choosing are reported

Table 3 Results of Experiment 3

| Trial type | Majority location | | Minority location | | Other locations |
|-----------------------|-------------------|------------|-------------------|------------|-----------------|
| | Cue types | % (Number) | Cue type | % (Number) | % (Number) |
| Majority tests | | | | | |
| EX-UF vs. IN | EX-UF | 56 (5) | IN | 33 (3) | 11 (1) |
| IN-EX vs. UF | IN-EX | 100 (9) | UF | 0 (0) | 0 (0) |
| UF-IN vs. EX | UF-IN | 33 (3) | EX | 56 (5) | 11 (1) |
| Cue type: % (number) | | | | | |
| Trial number | EX | | IN | UF | None |
| Forced-hierarchy test | | | | | |
| 1 | 78 (7) | | 22 (2) | 0 (0) | 0 (0) |
| 2 | 78 (7) | | 22 (2) | 0 (0) | 0 (0) |
| 3 | 62.5 (5) | | 25 (2) | 12.5 (1) | 0 (0) |

Trial and cue types defined in text. Percentages and observed numbers of squirrels choosing are reported

while in the later experiments the object did not have to be removed (although many squirrels manipulated the objects prior to opening the feeder). In addition, the feeding containers in the first experiment were made from washed food cans, which might have contained remnant odors that would have increased their saliency to the squirrels. We tested the hypothesis that feeder construction had a significant effect on cue use in Experiment 4.

Experiment 4

Rationale

This experiment replicated Experiment 1 using the new acrylic box feeders from Experiments 2 and 3. If the feeder construction has a significant effect, squirrels should not use UF cues even though we were replicating the procedure of Experiment 1, in which they used UF cues.

Methods

We recruited eight male fox squirrels that had not participated in Experiments 2 or 3. Data were collected from May

20, 2006–May 21, 2006. All procedures were the same as in Experiment 1 (Fig. 3) and as described in [General methods](#).

Results and discussion

As in Experiment 3, squirrels consistently chose the EX location in the forced-hierarchy trial and showed no evidence of using a majority strategy. In the first majority test, EX-UF versus IN, squirrels showed no preference for either the minority or majority location (Table 4). In the second majority test, IN-EX versus UF, squirrels consistently chose the majority location ($n = 8$, $P < 0.0001$). In the last test, UF-IN versus EX, six of eight squirrels chose the minority location ($n = 8$, $P = 0.0042$). In the forced-hierarchy trial, five of eight squirrels chose the EX location ($n = 8$, $P = 0.027$) and only one visited the UF location.

The results from Experiments 2, 3, and 4, thus suggest that squirrels rely on EX cues and ignore UF cues when using the acrylic box feeders. However, one factor that is prominent in studying cognition in wild, free-ranging animals is that they are undergoing seasonal changes in behavior and physiology associated with reproduction. Such

Table 4 Results of Experiment 4

| Trial type | Majority location | | Minority location | | Other locations |
|-----------------------|-------------------|------------|-------------------|------------|-----------------|
| | Cue types | % (Number) | Cue type | % (Number) | % (Number) |
| Majority tests | | | | | |
| EX-UF vs. IN | EX-UF | 50 (4) | IN | 37.5 (3) | 12.5 (1) |
| IN-EX vs. UF | IN-EX | 100 (8) | UF | 0 (0) | 0 (0) |
| UF-IN vs. EX | UF-IN | 25 (2) | EX | 75 (6) | 0 (0) |
| Cue type | EX | | IN | UF | Other |
| Forced-hierarchy test | | | | | |
| % (Number) | 62.5 (5) | | 25 (2) | 12.5 (1) | 0 (0) |

Trial and cue types defined in text. Percentages and observed numbers of squirrels choosing are reported

seasonal changes are correlated with differences in performance on spatial orientation and memory tasks in males and females (deer mice: *Peromyscus maniculatus*, Galea et al. 1994; meadow voles: *Microtus pennsylvanicus*, Gaulin et al. 1990; Jacobs 1996; white-footed mice: *P. leucopus*, Pyter et al. 2006). In California, fox squirrels have two breeding seasons, the first from June to September and a second one from December to March (Byrne 1979; King 2004). Changes in cognitive strategies between the summer breeding season (Experiment 1) and late spring, which is at the end of the breeding season (Experiments 2–4) may have caused the changes in cue preference that we found. We examined this possibility in Experiment 5.

Experiment 5

Rationale

To determine whether it was seasonal changes or feeder construction that caused squirrels to prefer UF in Experiment 1 and EX in Experiments 2, 3, and 4, we decided to replicate Experiment 1 with the acrylic box feeders at the same time of year that Experiment 1 had been conducted, late summer.

Methods

We recruited 11 male fox squirrels that had not participated in Experiments 2, 3, or 4. Data were collected from July 28, 2006 to August 5, 2006. All procedures are as described in Experiment 1 (Fig. 3) and [General methods](#).

Results and discussion

In the three majority tests, squirrels chose the majority location ($n = 11$, $P = 0.007$, $P < 0.00001$, $P = 0.034$, for the EX-UF versus IN, IN-EX versus UF, UF-IN versus EX respectively; Table 5). Five of eight squirrels chose the EX location in the forced-hierarchy trial ($n = 8$, $P = 0.027$). Squirrels used a majority strategy in the majority tests as

they had done in Experiment 1, but chose the EX location rather than the UF location in the forced-hierarchy trial.

These results would be consistent with an effect of seasonal changes since the squirrels now attended to UF cues when using the acrylic box feeders as seen in their use of a majority strategy. However, using Fisher's exact test we found no significant differences between the pattern of choices in any of the majority tests in Experiment 4 and Experiment 5 (Fisher's exact test, two-tailed, $n = 19$, $df = 1$, for all, EX-UF vs. IN, $P = 0.38$; IN-EX vs. UF, $P = 1$; UF-IN vs. EX, $P = 0.35$). Therefore, we cannot conclude that there were seasonal differences across experiments.

On the other hand, when we compared the patterns of choices from the forced-hierarchy tests in Experiments 1 and 5, we found a significant difference (Fisher exact test, two-tailed, $n = 14$, $df = 3$, $P < 0.001$). There was no difference between experiments in any of the majority tests between Experiments 1 and 5 (Fisher exact test, two-tailed, $n = 55$, $df = 1$, $P = 1.0$). This suggests that the changes made to the feeders after Experiment 1 had significant effect on the weighting given to different cue types, but that the weightings still allowed for use of a majority strategy. Future experiments are needed to ascertain what elements of the feeder construction might have contributed to this effect.

General discussion

The objective of this study was to determine which cue types fox squirrels rely on to remember a location and whether they would use a majority strategy if available. In Experiment 1, as predicted from previous work on flying squirrels, squirrels reoriented to the majority rather than the minority location in all majority tests. However, in the forced-hierarchy test, they chose the UF location indicated by nonspatial features, rather than the IN or EX locations indicated by spatial information. This choice runs contrary both to studies of fox squirrels (Jacobs and Shifflett 1999; Lavenex et al. 1998) and to those of other bird and mammal species that predictably chose the EX location in forced-

Table 5 Results of Experiment 5

| Trial type | Majority location | | Minority location | | Other locations % (Number) |
|-------------------------------|-------------------|------------|-------------------|------------|-------------------------------|
| | Cue types | % (Number) | Cue type | % (Number) | |
| Majority tests | | | | | |
| EX-UF vs. IN | EX-UF | 73 (8) | IN | 18 (2) | 9 (1) |
| IN-EX vs. UF | IN-EX | 100 (11) | UF | 0 (0) | 0 (0) |
| UF-IN vs. EX | UF-IN | 54.5 (6) | EX | 45.5 (5) | 0 (0) |
| Cue type | EX | | IN | UF | Other |
| Forced-hierarchy tests | | | | | |
| % (Number) | 62.5 (5) | | 37.5 (3) | 0 (0) | 0 (0) |

Trial and cue types defined in text. Percentages and observed numbers of squirrels choosing are reported

hierarchy tests (flower bat: *Glossophaga commissarisi*, Thiele and Winter 2005; Columbian ground squirrel, Vlasak 2006a, b; rufous hummingbird: *Selasphorus rufus*, Healy and Hurly 1998; black-capped chickadee, Brodbeck 1994; western scrub jay: *Aphelocoma californica*, Watanabe 2005; lab pigeon: *Columbia livia*, Spetch and Edwards 1988). In Experiments 2–5, we tested different hypotheses as to why these squirrels had chosen UF cues as their preferred cue type rather than the predicted EX cues.

These experiments led to the following conclusions: that feeder construction could influence the attention to UF cues, that the time of year was related to trends in the data but its effect was not as strong as that of feeder construction and that finally, squirrels used a majority strategy when available and were most likely to choose the EX location in a forced hierarchy test. Our results are consistent with prior studies showing the use of EX cues in forced hierarchy tests in scatter-hoarding birds and mammals (e.g., Brodbeck 1994; Jacobs and Shiflett 1999). At the same time, our results confirm the flexible use of the majority strategy when this is an option, as previously demonstrated in southern flying squirrels (Gibbs et al. 2007).

The choice of the EX location as the preferred location is supported by data from all the experiments, except Experiment 1. In Experiments 2–5, the EX cue type appeared to exert the greatest influence on the squirrels' decision strategy. We found no differences in the results from the forced hierarchy test between Experiments 3, 4, and 5 (Fisher's exact test, two-tailed, $n = 58$, $df = 9$, $P = 0.97$). Across all the forced-hierarchy test trials, a majority of squirrels chose the EX location over the other three possible locations. In contrast, when we included the results of Experiment 1 in our comparison, we found a significant difference between the patterns of choices (Fisher's exact test, two-tailed, $n = 64$, $df = 16$, $P = 0.03$). Sources of increased saliency in Experiment 1 might have caused participating squirrels to rely on the more readily available UF cues rather than the spatial cue types (IN and EX).

The effect of the feeder construction on their spatial encoding is further supported by a comparison of the results of Experiment 1, using the aluminum can feeders, with those of Experiment 5, using the acrylic box feeders. Both experiments were conducted in late summer using the same procedure. In the former, squirrels chose the UF location, indicating UF as their preferred cue type in the forced-hierarchy test, while in the latter, squirrels chose the EX location (Fisher's exact test, two-tailed, $n = 14$, $df = 3$, $P = 0.003$). It is possible that remnant odor cues, differences in the squirrels' ability to discriminate between UF objects, and differences in the amount of direct contact with the UF objects contributed to this effect. Future work will have to determine the relative contribution of such factors to the squirrels' use of UF cues.

While EX appeared to be the preferred cue type in most of the forced-hierarchy tests, we found no evidence for the preference of one combination of cue types over the others in the majority tests. Overall, the combination of IN and EX cue types seemed to exert the most influence on the squirrels' orientation. In all the experiments, a majority of squirrels chose the IN-EX location over the UF location in the IN-EX versus UF majority test. However, data analyses including only those experiments in which the squirrels were using a majority strategy and therefore clearly attending to UF cues, Experiments 1 and 5, only confirmed a significant difference between the IN-EX versus UF and the UF-IN versus EX tests in Experiment 5 (Fisher exact test, $n = 22$, $df = 1$, $P = 0.035$). While this pattern of results implies that EX cues may be more influential than UF cues, we found no evidence that IN cues are more influential than UF cues. There was no difference between the IN-EX versus UF and the EX-UF versus IN tests in either Experiment 1 or Experiment 5 (Fisher's exact test Experiment 1: $n = 14$, $df = 1$, $P = 1$, and Experiment 5: $n = 22$, $df = 1$, $P = 0.21$). Thus, although IN-EX seemed to exert the greatest influence across experiments, we were unable to find support for a hierarchy of the different combinations of cue types.

Our pattern of results also suggests that encoding strategy varied by season of study. Although this pattern must be tested in the future with larger sample sizes, there was a remarkable change in the proportion of squirrels using a majority strategy in the UF-IN versus EX test. In the late spring experiments (Experiments 3 and 4), most squirrels chose the minority location (6 of 9 and 6 of 8, respectively). In contrast, in the late summer (Experiments 1 and 5), most squirrels chose the majority location (5 of 6 and 6 of 11, respectively). This suggests a weak seasonal effect that could become statistically significant with a larger sample size and hence the use of a higher power statistical test.

This pattern of results is also consistent with known seasonal changes in the brain of a congener species, the eastern gray squirrel (*S. carolinensis*). Adult male squirrels showed a significant increase in brain size in October, the height of the caching season, compared to adult squirrels captured in January or June (Lavenex et al. 2000). There was also an absolute increase in the hippocampal subfield (CA1), a structure selectively active during the encoding of small unique objects in the lab rat (Kemp and Manahan-Vaughan 2007, 2008). Thus, the male fox squirrel's greater attendance to UF cues in the late summer may be related to seasonal changes in how its hippocampus encodes a location. In addition, such a dissociation of hippocampal functions by cue class is consistent with the parallel map model of spatial encoding (Jacobs and Schenk 2003).

In conclusion, the series of experiments reported here on spatial encoding in a free-ranging, scatter-hoarding mammal,

the fox squirrel, both complement and challenge earlier studies of this phenomenon. While our results confirm squirrels' preference for distal spatial cues when all cue types are in conflict, they also suggest that there is a large degree of plasticity in the type of strategy used. This degree of plasticity was also recently reported in another scatter-hoarding squirrel, the southern flying squirrel. It appears that a single experiment, at a single time of year or using a single type of apparatus, may not be sufficient to characterize the spatial encoding strategy of a species. The great ecological diversity of spatial ecology among sciurids makes them an ideal group with which to further explore this topic. Future studies can make use of this diversity to systematically study spatial strategy among species with different foraging behaviors (scatter-hoarding vs. nonstoring species) and different breeding seasons, to determine what role ecological niche has played in the use and expression of spatial encoding strategy.

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