

1 **How squirrels protect their caches: Location, conspicuousness during caching, and**
2 **proximity to kin influence cache lifespan**

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10 **Abstract**

11 Scatter-hoarding animals cannot physically protect individual caches, and instead
12 utilize several behavioral strategies that are hypothesized to offer protection for caches.
13 We validated the use of physically altered, cacheable food items, and determined that
14 intraspecific pilfering among free-ranging fox squirrels ($N = 23$) could be assessed in the
15 field. In this study we were able to identify specific individual squirrels who pilfered or
16 moved caches that had been stored by a conspecific. We identified a high level of
17 pilfering (25%) among this population. In a subsequent study, we assessed the fate of
18 squirrel-made caches. Nineteen fox squirrels cached 294 hazelnuts with passive
19 integrated transponder tags implanted in them. Variables collected included assessment
20 and cache investment and protection behaviors; cache location, substrate, and
21 conspicuousness of each cache; how long each cache remained in its original location,
22 and the location where the cache was finally consumed. We also examined whether
23 assessment or cache protection behaviors were related to the outcomes of buried nuts.
24 Finally, we measured the population dynamics and heterogeneity of squirrels in this
25 study, testing the hypothesis that cache proximity and pilferage tolerance could serve as a
26 form of kin selection. Polymer chain reaction (PCR) was used to analyze hair samples
27 and determine relatedness among 15 squirrels, and the potential impact of relatedness on
28 caching behavior. Results suggested that cache protection behaviors and the lifespan of a
29 cache were dependent on the conspicuousness of a cache. Squirrels may mitigate some of
30 the costs of pilfering by caching closer to the caches of related squirrels than to those of
31 non-related squirrels.

32 **Keywords:** scatter-hoarding, food-storing, memory, pilfering, kin selection

33 **Introduction**

34 Scatter-hoarding animals cannot physically protect individual caches, and instead
35 utilize several behavioral strategies that are hypothesized to offer protection for caches.
36 These behaviors include assessing food items to appropriately allocate cache effort (e.g.,
37 Preston & Jacobs, 2009), caching out of sight of conspecifics (e.g., Dally, Emery, &
38 Clayton, 2004), caching food items at low density (e.g., Male & Smulders, 2007), or at a
39 great distance from the food source (Vander Wall, 1995a), or spending more time
40 carefully covering caches (e.g., Leaver, Hopewell, Caldwell, & Mallarky, 2007). How
41 these behaviors contribute to the survival and retrieval of these caches or might reduce
42 pilferage from conspecifics is still unknown. In fact, little is known about what factors do
43 contribute to whether a cache is stolen, forgotten, or retrieved by the animal who cached
44 it.

45 Many behavioral mechanisms that scatter-hoarding animals could use to protect
46 caches have yet to be examined in detail, such as the adaptive use of food assessment.
47 Several animal species display food assessment behaviors including squirrels, primates,
48 birds and fish (Jablonski, Fuszara, Fuszara, Jeong, & Lee, 2015; Kislalioglu & Gibson,
49 1976; Melin et al., 2009; Preston & Jacobs, 2009). These behaviors help animals select
50 higher quality food items, as demonstrated in scatter-hoarding Western scrub jays
51 (*Aphelocoma californica*) and Piñon jays (*Gymnorhinus cyanocephalus*), who use bill
52 clicking and item handling to choose heavier seeds (Langen & Gibson, 1998; Ligon &
53 Martin, 1974).

54 In the case of food-storing animals, assessment may provide information that allows
55 for the adjustment of cache investments to the value of individual food items. Fox

56 squirrels (*Sciurus niger*) use two overt behaviors to assess food items, head flicks and
57 paw manipulations. These behaviors may help squirrels assess the quality, weight, and
58 perishability of food items before caching or eating them (Delgado, Nicholas, Petrie, &
59 Jacobs, 2014; Preston & Jacobs, 2009). For example, fox squirrels are significantly more
60 likely to cache than eat items after they perform a head flick (Delgado et al., 2014;
61 Preston & Jacobs, 2009). Because many scatter-hoarding animals, including squirrels,
62 jays, mice, and chipmunks, adjust cache distance to the value of food (e.g., Delgado et
63 al., 2014; Jokinen & Suhonen, 1995; Moore, McEuen, Swihart, Contreras, & Steele,
64 2007; Tamura, Hashimoto, & Hayashi, 1999; Waite & Reeve, 1995), it follows that they
65 should have some means of assessing individual food items to determine their value.

66 Several scatter-hoarding animals, including squirrels, are sensitive to the presence of
67 other animals and adjust caching behaviors when competitors are present (Dally, Clayton,
68 & Emery, 2006; Dally, Emery, & Clayton, 2005; Emery, Dally, & Clayton, 2004). Birds
69 in the corvid and parid families eat food items and reduce the number they cache, or wait
70 to cache until after competitors have left (Goodwin, 1956; James & Verbeek, 1984; Lahti
71 & Rytönen, 1996; Leaver et al., 2007; Stone & Baker, 1989). Western scrub jays cache
72 out of view or move their caches several times when conspecifics are present, presumably
73 to reduce visual cues available to competitors (Dally et al., 2004; Dally et al., 2005).
74 Eurasian jays (*Garrulus glandarius*) may even reduce acoustic information available to
75 competitors by caching in quieter substrate (Shaw & Clayton, 2013), as other jays appear
76 to use auditory information to locate and steal caches made by other jays (Shaw &
77 Clayton, 2014). Scatter-hoarding tree squirrels also vary several behaviors in the presence
78 of competitors: the amount of time and effort spent traveling to a cache site (Delgado et

79 al., 2014; Hopewell, Leaver, & Lea, 2008; Leaver et al., 2007), the number of holes dug
80 before selecting a final cache location (Delgado et al., 2014; Steele et al., 2008), and time
81 spent covering a cache site with available substrate such as dirt or leaves (Delgado et al.,
82 2014; Hopewell & Leaver, 2008).

83 These behaviors suggest that there is a risk to the caching animal when burying food
84 in the presence of competitors. Pilfering is assumed to be common, but because an animal
85 who is pilfered from also likely pilfers from others, scatter-hoarding despite the risk of
86 theft is considered a viable and stable strategy (Vander Wall & Jenkins, 2003).

87 Attempts to quantify the amount of pilfering have mainly assessed the rate of
88 disappearance of human-made caches. In a three-week study of fox squirrels, results
89 suggested pilfering rates of up to 9.4% per day, although a second study used shallower
90 caches, and reported pilfering rates of up to 33% per day (Stapanian & Smith, 1984).
91 Studies of congeneric eastern gray squirrels (*Sciurus carolinensis*) suggested that
92 squirrel-made and human-made caches were removed from the ground at similar rates,
93 although it was not known if the cache owner was also the cache retriever for squirrel-
94 made caches (Thompson & Thompson, 1980). A more recent study of caches made by
95 gray squirrels suggested that all were depleted in less than six days (Steele et al., 2014).
96 However, another study demonstrated that by removing the caching animal from the area
97 immediately after they cached (and thus mimicking predation), caches survived up to 27
98 days (Steele et al., 2011). This provided evidence that a caching animal holds some
99 advantage in cache recovery but tells us little about what factors led to the pilferage of
100 nuts that were removed in the absence of the animal who original stored them.

101 Reducing cache density has not shown consistent results in preventing pilferage. In
102 some cases, the loss of human-made caches is reduced by decreasing density (Daly,
103 Jacobs, Wilson, & Behrends, 1992; Male & Smulders, 2008; Male & Smulders, 2007),
104 but in other studies it has had little effect (e.g., Galvez, Kranstauber, Kays, & Jansen,
105 2009). However, if cache density does increase pilfering, the impact of cache density or
106 of caching close to the caches of other squirrels may be mitigated when pilferers are close
107 relatives. Stapanian and Smith (1978) found that squirrels tended to cache in unique
108 areas, and cached slightly closer to their own previous caches than to those made by other
109 squirrels.

110 Food theft may be tolerated in animals with overlapping ranges because it is a form of
111 reciprocal exchange that avoids the behavioral costs of cache defense, vigilance, and
112 aggression (Stevens & Stephens, 2002). We currently know very little about the potential
113 effects of kin selection on the pilferage of scatter-hoarded food in free-ranging tree
114 squirrels. One study showed that related male-female and female-female pairs had closer
115 range centers than those of unrelated squirrels. However, the same study found that
116 within a restricted search area (a 50 x 50-m area around the food source), relatedness did
117 not influence the proximity of caches made by different squirrels (Spritzer & Brazeau,
118 2003). Another study reported a low degree of relatedness within groups of fox squirrels,
119 due to natal dispersal, which is influenced both by age and sex (Koprowski, 1996). Low
120 relatedness would make the question of kin selection less relevant. Population density
121 and dispersal patterns may be adapted to local conditions, however, and it is not clear
122 what group relatedness would be in urban squirrels who are provisioned with food

123 (Penner et al., 2013) or live in fragmented landscapes (Sheperd & Swihart, 1995), both
124 which can impact dispersal.

125 Reciprocal theft tolerance among related food-storers has been demonstrated in
126 larder-hoarding animals such as woodpeckers (*Melanerpes formicivorus*; Koenig, 1987)
127 and beavers (*Castor canadensis*; Novakowski, 1967). Among scatter-hoarders, there
128 could be fitness benefits in relaxing cache protection strategies in the presence of closely
129 related individuals.

130 This study had several objectives. The first was to determine if levels of pilfering
131 could be assessed in the field, including identifying specific individual squirrels who
132 pilfer or move caches. If it was possible to observe pilfer events, and determine who was
133 stealing from whom, further study into how behavioral and genetic factors could
134 influence the outcome of caches would be justified.

135 The second goal was to determine the fate of squirrel-made caches, including how
136 long caches remain where buried, and whether they are pilfered, re-cached, eaten or
137 forgotten. An additional question was whether assessment or cache protection behaviors
138 are related to the outcomes of buried nuts. Despite numerous studies of cache protection,
139 there is little direct evidence that these strategies labeled as cache protection help animals
140 recover their caches, or deter theft by others. We predicted that food assessment and
141 cache protection behaviors should be related to a longer cache life.

142 The final objective was to examine the population dynamics and heterogeneity of
143 squirrels in the study, including testing the hypothesis that cache proximity and pilferage
144 tolerance could serve as a form of kin selection. Where theft did occur, we predicted

145 there would be an increased likelihood of theft by offspring and other closely related
146 individuals and higher tolerance of pilferage by closely related conspecifics.

147

148 **Experiment 1: Testing squirrel responses to stimuli**

149 In order to observe cache movements in the field, we painted 350 caching stimuli
150 (intact hazelnuts) with two coats of yellow non-toxic acrylic paint (Sargent Art, Hazleton,
151 PA). We first tested the squirrels' ability to discriminate between painted and unpainted
152 hazelnuts to determine whether the paint might make it easier or more difficult for
153 squirrels to locate cached nuts.

154

155 **Methods**

156 *Study Site*

157 The study was conducted outside of Tolman Hall on the University of California,
158 Berkeley campus.

159

160 *Study Animals*

161 Eight free-ranging, marked fox squirrels participated in the study. The research was
162 approved under a protocol submitted to the Animal Care and Use Committee of the
163 University of California, Berkeley.

164

165 *Procedure*

166 Playground sand (Quikrete Cement and Concrete Products, Atlanta, GA) was placed
167 in a 50.8 x 50.8 x 14-cm plastic container at a depth of approximately 5-cm. The

168 container had a latch on one end that allowed the side to be lowered to allow easy access
169 into the box. The apparatus was divided into sixteen 12.7 x 12.7-cm quadrats, numbered
170 from one to sixteen.

171 Data were collected between October 14 and November 5, 2014. We lured one
172 marked squirrel at a time into the apparatus by calling to them and placing small pieces of
173 peanuts nearby and on top of the sand. Once the squirrel was habituated to entering the
174 apparatus, the peanut pieces were removed.

175 Four painted nuts, and four unpainted nuts were placed in quadrats chosen by a
176 random number generator (random.com), such that no quadrat had more than one nut in
177 it, and on any given trial, half of the quadrats contained a buried nut. Each hazelnut was
178 covered with enough sand that it could not be detected visually. The focal squirrel was
179 allowed to sniff around and dig in the sand, until it found a hazelnut. Some squirrels did
180 not locate a hazelnut and left.

181 When a squirrel first located a hazelnut, the following data was recorded: the name
182 of the squirrel, the quadrat the nut was removed from, and whether the nut was painted or
183 unpainted. All squirrels that found hazelnuts carried them away and cached them.
184 Between trials, all nuts were removed from the apparatus, the sand was stirred around to
185 reduce olfactory cues, and nuts were placed in new locations as predetermined by random
186 number generation.

187

188 **Results of Experiment 1**

189 Six squirrels completed at least 20 trials. A total of 118 trials were conducted. In 64
190 (55%) of the trials, the squirrel found a painted hazelnut first; in the remaining 54 trials,

191 the squirrels found an unpainted hazelnut first. Using a binomial probability, this
192 detection rate for painted nuts is not different from chance (binomial test, $p = .52$). From
193 this result, we conclude that the painting of the nuts did not give off odor cues that would
194 influence the difficulty or ease in locating cached nuts when compared to unpainted
195 hazelnuts.

196

197 **Experiment 2: Assessing pilferage in the field**

198 The purpose of the pilot study was to determine whether pilferage between
199 individual squirrels could be assessed in the field.

200

201 **Methods**

202 *Study Site*

203 The study was conducted on the University of California, Berkeley campus. This area
204 is relatively open and flat, with oak, pine and other trees, lawns, ivy ground cover and
205 campus buildings. The study area was approximately 0.09 km².

206

207 *Study Animals*

208 Twenty-three free-ranging fox squirrels who regularly frequented the study site
209 participated in the study. All squirrels were individually marked with fur dye (Nyanzol-
210 D, American Color and Chemical Corporation, Charlotte, NC). We chose one adult
211 female (Flame) as the focal subject, because she was frequently seen foraging in the
212 testing area. The research was approved under a protocol submitted to the Animal Care
213 and Use Committee of the University of California, Berkeley.

214

215 *Procedure*

216 The study was conducted between the hours of 10:00 and 16:00 on each weekday
217 from June 16th to July 25th, 2014. The caching stimuli were whole hazelnuts, in the shell,
218 which had been painted bright yellow with two coats of non-toxic acrylic paint as in
219 Experiment 1). The focal squirrel recognized the painted hazelnuts as food items, eating
220 or caching all nuts.

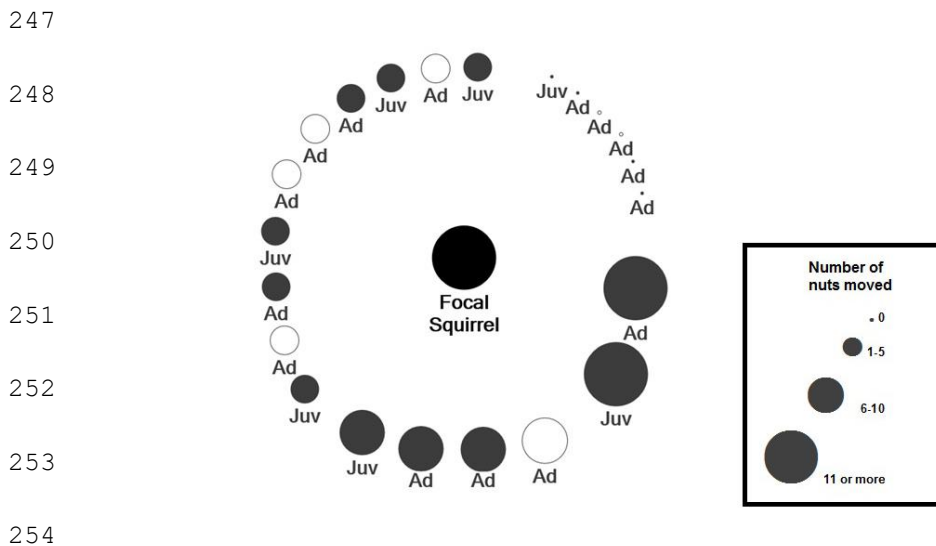
221 On each morning of testing we dispensed up to 15 nuts, one nut at a time, and
222 observed the focal squirrel while she either ate or cached the nut. The number of nuts
223 dispersed was dependent on the presence of the focal squirrel. On some days, she left the
224 study site before all 15 nuts were presented. If a nut was cached, we marked the number
225 of the nut and the location of the cache on a paper map. We also took a GPS waypoint for
226 each cache location. The focal squirrel cached 340 painted hazelnuts.

227 While nuts were dispersed, researchers noted which other squirrels could be observed
228 in the area. Each day, after dispersing all nuts to the focal squirrel, we used binoculars to
229 observe the squirrels in the study site for several hours each day. The yellow paint
230 allowed for increased visibility of the food items while carried by squirrels. Because the
231 nuts were painted, and all squirrels in the area were marked, when a squirrel was seen
232 moving or eating a yellow hazelnut, we were able to note the identity of the squirrel
233 carrying the painted nut. We also noted where nuts were re-cached.

234

235 **Results of Experiment 2**

236 During 125 hours of observation, 102 nuts were observed being moved by a squirrel. We
237 observed the focal squirrel moving and either eating or re-caching 16 of these nuts. The
238 remaining nuts were pilfered by other squirrels, suggesting an overall pilfering rate of at
239 least 25 percent. Our observations suggested that although several individuals were
240 pilfering small amounts from the focal squirrel, some squirrels were more likely to pilfer
241 nuts than others, with two squirrels pilfering 14 and 15 nuts respectively (Figure 1). For
242 22 caches (25% of stolen caches), nuts were pilfered within 20 minutes of being cached,
243 allowing us to note the specific identity of that cache. Of the two squirrels that frequently
244 stole nuts, one was a juvenile male often spotted in the same tree as the focal squirrel.
245 Behavioral observations suggested this juvenile may have been the offspring of the focal
246 squirrel.



255 **Figure 1. Pilfering of caches made by the focal squirrel.** Circles represent theft by
256 either male ● or female ○ adult (Ad) or juvenile (Juv) squirrels. The size of circles
257 represents number of nuts moved.

258 **Field Study**

259 The pilot data from Experiment 2 demonstrated that it was possible to quantify
260 pilfering in the field, and to identify which squirrels are pilfering specific nuts. The
261 purpose of the current study was to determine (1) what happens over the lifespan of a
262 cache – how many times, where and when is a nut moved before it is finally eaten; (2) the
263 influence of assessment behaviors on cache lifespan and outcomes; and (3) the effect of
264 relatedness of caching behaviors.

265

266 **Methods**

267 *Study Site*

268 The study was conducted on the University of California, Berkeley in the same
269 general area as the previous experiment. The study area was approximately 0.10 km².

270

271 *Study Animals*

272 Nineteen free-ranging fox squirrels who regularly frequented the study site
273 participated in the study. All squirrels were individually marked with Nyanzol-D
274 (American Color and Chemical Corporation, Charlotte, NC). The research was approved
275 under a protocol submitted to the Animal Care and Use Committee of the University of
276 California, Berkeley.

277

278 *Experimental Stimuli*

279 First, 350 hazelnuts were checked to determine that they had no cracks in their shell.
280 A small hole was drilled in each nut using a Dremel Multipro 395 hand-held tool fitted

281 with an 1/16" drill bit. A 12-mm 134.2 kHz pit tag (Biomark, Boise, ID) was placed in
282 each nut, and the hole was filled with Elmer's wood glue. The surface of the nut was
283 leveled when necessary by ensuring the hole was entirely filled with glue, and scraping
284 away any excess glue. After the glue was dry, the nuts were painted with two coats of
285 bright yellow paint (Sargent Art, Hazleton, PA). Due to experimental oversight, forty of
286 the nuts were painted light green with the same brand of acrylic paint. After the nuts were
287 dried, they were numbered 1 to 20 with a non-toxic marker, and placed in bags of 20 nuts
288 each that were labeled alphabetically, such that each nut had a unique alphanumeric code
289 (for example, A1, A2...B1, B2, etc.). All nuts were scanned with a BioMark HPR Plus
290 reader to verify that their pit tag was functional. We weighed each nut, and entered each
291 nut's alphanumeric code, pit tag code, and weight into a database.

292

293 *Procedure*

294 A total of 350 pit-tagged nuts were distributed to squirrels from February 11, 2016
295 until April 5, 2016, between 9:45 and 16:00 hours. On most days, 20 nuts were handed
296 out (10 in the morning and 10 in the afternoon), dependent on weather, lab staffing, and
297 squirrel participation.

298 A uniquely marked squirrel was solicited for each trial by an experimenter gesturing
299 or calling to the squirrel. One experimenter videotaped all sessions with a Canon FS300
300 handheld camcorder, noting the squirrel, and alphanumeric code of the nut for each trial.
301 The purpose of videotaping each cache was to record food assessment and cache
302 investment behaviors. If a squirrel could not be easily filmed, experimenters dictated any
303 change in behaviors when they could be observed.

304 A second experimenter gently tossed the nut on the ground toward the squirrel, and
305 kept records of the subject, time, and the other squirrels present for each trial. The third
306 experimenter scanned the nut at the start and end of the trial with the Biomark HPR Plus
307 reader, which also collected GPS information for the start location and the final cache
308 location.

309 The squirrel either cached or ate each nut. When the squirrel cached the nut, all
310 experimenters followed the squirrel from a distance until the nut was cached. At that
311 point, the third experimenter scanned the cache location to verify that the nut had been
312 cached and could be detected. The location of the cache was drawn on a map, and the
313 location of the cache was measured from at least two landmarks, noting both distance and
314 bearing (determined by a handheld compass or cell phone compass application) from the
315 landmarks.

316 Trials were repeated until 10 nuts were handed out for the session or until there were
317 no squirrels available to participate. We alternated between different individual squirrels
318 between trials if multiple subjects were available and willing to participate.

319 When not handing out nuts, experimenters observed the squirrels to note if there
320 were any cache movements. We used the BioMark HPR Plus to search for previously
321 cached nuts, initially scanning for all cached nuts that had a known location at least every
322 two to three days. Other testing areas were scanned regularly using either the handheld
323 HPR loop antenna, or with a portable antenna that had been mounted on a dolly with
324 wheels to facilitate the rapid search of large, open areas where squirrels often cached.
325 Constraints included weather, staffing, and the battery power of the pit tag reader.

326 We tracked nuts that had been stolen or re-cached, including their new locations, and
327 if observed, who moved the cache. Cache life was defined as the number of days a cache
328 stayed in its original location. We also recorded any nuts that were detected in a
329 previously unknown location, and then checked those nuts routinely until they
330 disappeared or were still present at the end of the experiment and assumed forgotten. Any
331 new microchips that were detected six months after the end of the experiment were dug
332 up to determine if they were still embedded in a nut or if the nut had been eaten.

333 All videos of the sessions were coded using The Observer XT (Noldus, Leesburg,
334 VA). There were three video coders, and inter-rater agreement on onset, timing and
335 presence of behaviors between the pairs of coders was high (agreement for coded videos
336 ($n = 9$) averaged Cohen's kappa, $\kappa = .91$, range: 0.75 to 1). The variables recorded for all
337 cached nuts included: the number of head flicks for each nut, the amount of time spent
338 paw manipulating, the amount of time spent digging, tamping, and covering the nut, and
339 the amount of time the squirrel spent handling the nut, from initial receipt of the nut until
340 the cache was completed.

341 One rater assessed the level of concealment of all cache events, whether open (the
342 entire squirrel could be observed caching), partially concealed (more than half of the
343 squirrel was covered by ivy or other plant matter), mostly concealed (less than half of the
344 squirrel's body could be seen), or completely concealed (none of the squirrel could be
345 observed while caching, such as if the squirrel was caching in a hedge). To determine
346 reliability, a second rater coded 60 of the cache events. Inter-rater agreement for the level
347 of concealment of the cache was $\kappa = .84$.

348 GIS data was used to determine the distance traveled for each cache, and the
349 proximity of an individual squirrel's caches to their own caches and those of other
350 squirrels.

351

352 *Statistical Analyses*

353 All data were analyzed using mixed models in JMP 12.0 (SAS Institute, Cary, NC).
354 All models included squirrel identity as a random effect. The alpha level for all analyses
355 was set at 0.05 and Tukey's HSD tests were conducted for any pairwise comparisons.

356 The first model determined if there were effects of nut weight and assessment on
357 distance traveled to cache. A second model examined the effects of assessment and
358 investment behaviors, and social competition on cache life. The independent variables
359 were number of headshakes, time spent paw manipulating, distance traveled, time spent
360 on cache, concealment of cache, time spent digging, tamping, and covering the cache,
361 and the number of other squirrels in the area. A third model was run to determine if
362 squirrels adjusted investment behaviors (digging, tamping, and covering their caches)
363 depending on the level of concealment of the cache location or the presence of other
364 squirrels.

365 Spatial data were analyzed using ArcGIS version 10.3 (ESRI, Redlands, CA), and
366 JMP Pro12.0 (SAS, Cary, NC. Waypoints were entered into ArcGIS with the WGS 1984
367 Geographic Coordinate System, and with the State Plane NAD83 California Zone III
368 projection. We calculated the distance traveled for each cache, the proximity of each
369 squirrel's cache to their own caches and all caches made by other squirrels.

370 **Experiment 3: DNA Collection and Analysis**

371 In order to assess the effects of relatedness on fox squirrel caching behaviors, hair
372 samples were collected during the same testing period as the rest of the experiment.

373

374 **Methods**

375 *Study Site*

376 The study was conducted on the University of California, Berkeley campus in the
377 same general area as the previous experiments. The study area was approximately .09
378 km².

379

380 *Study Animals*

381 Hair samples were collected from 14 of the free-ranging, marked fox squirrels who
382 participated in Experiment 3. Hair samples were collected from an additional eight
383 squirrels who were not in the field study. The research was approved under a protocol
384 submitted to the Animal Care and Use Committee of the University of California,
385 Berkeley.

386

387 *Procedure: Hair Collection*

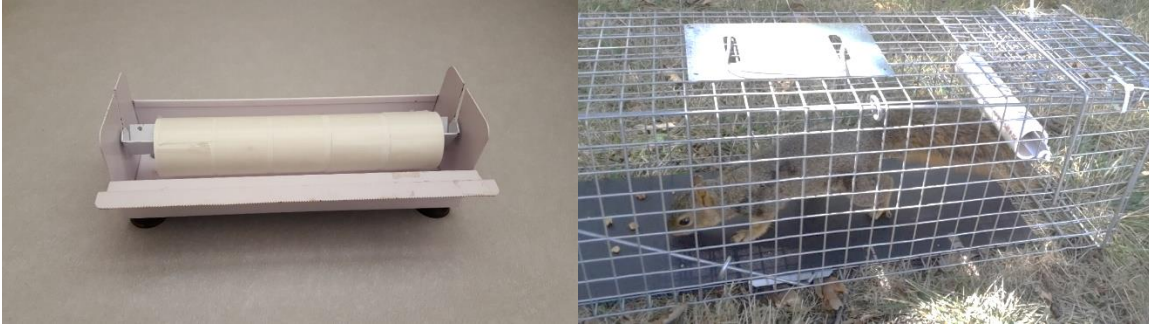
388 Hair collection was based on methods previous described in multiple studies of free-
389 ranging mammals (Finnegan, Hamilton, Perol, & Rochford, 2007; Reiners, Encarnaçãõ,
390 & Wolters, 2011). Squirrels were first desensitized to entering a Tomahawk Flush Mount
391 Squirrel trap for food. Both doors of the trap were secured open with zip ties, so the trap
392 would not be set off when an animal entered it. A 60.96 x 20.32-cm black strip of plastic

393 was placed at the bottom of the trap to allow for easy baiting with small pieces of walnuts
394 and peanuts. Once the squirrel entered the trap readily, the trap was set to collect hair.

395 Experimenters wore latex gloves during all handling of hair collection materials to
396 reduce the risk of contamination. All equipment was sanitized between uses in the field
397 or in the lab with rubbing alcohol. Five 3.58 x 13-cm strips of double-sided Ace brand
398 heavy-duty carpet tape were placed on a piece of PVC tubing (20.32-cm long, diameter
399 4.11-cm). The tubing was suspended in a storage box by placing it over the center core of
400 a multi-roll tape dispenser (Figure 2a). The storage box and a pair of sanitized tweezers
401 were taken out to the field site.

402 A marked squirrel was recruited for hair collection. Other squirrels were kept away
403 from the trap by tossing them peanuts. The release liner of the carpet tape was removed
404 with tweezers and PVC tubing was inserted at one end of the trap. The tube was
405 suspended by either a piece of wire affixed to both sides of the trap, or by the core of the
406 tape dispenser (Figure 2b). The tube was suspended low enough that if a squirrel passed
407 underneath it, their tail would touch the exposed tape. The squirrel was lured into the trap
408 several times with walnut pieces, until an adequate number of hairs with follicles were
409 collected from the tail. The tube was removed from the trap and returned to the tape
410 dispenser holder in the plastic storage container. The name and sex of the squirrel, and
411 the date of collection were marked on a label on the container. The container was sealed
412 and stored until hair samples could be processed.

413



414

415

416 **Figure 2. Hair collection procedures.** (a) PVC tubing prepared for hair collection. (b) A

417 marked fox squirrel in the trap baited for hair collection.

418

419 *Procedure: Preparation of Hair Samples*

420 Hair samples were later prepared for polymer chain reactions (PCR) in a clean
421 environment where no other biological materials were handled. Experimenters wore
422 gloves, a gown, a face mask and a hair net, which were all changed between samples. The
423 surface was sanitized with Sanizide Germicidal Solution (Safetec, Buffalo, NY) and then
424 a large piece of butcher paper was placed on the surface.

425 The tape dispenser with the hair sample was removed from the plastic storage
426 container. The experimenter removed individual hairs from the tape, inspected them
427 carefully for a follicle, and then cut the hair approximately 2 mm below the follicle. The
428 follicles were placed in an individual Fisherbrand glass threaded 15 x 45-mm, 3.7 mL
429 vial (Fisher Scientific, Chicago, IL) containing ethanol (200 proof Ethyl Alcohol,
430 Spectrum Chemical Mfg. Corp., Gardena, CA). Once an adequate number of hair
431 follicles were collected (generally between 30 and 40 follicles, but fewer if the sample
432 from the squirrel was scant), the tube was sealed and labelled with the squirrel's name

433 and sex, and the date. In between processing samples, all materials were sanitized with
434 rubbing alcohol, and any other materials (tape, butcher paper, hairs, gloves, gowns, etc.)
435 were disposed of in an individual trash bag that was sealed.

436

437 *DNA Amplification, PCR, and Sequencing*

438 Genetic relatedness and diversity of 15 fox squirrels was inferred from PCR
439 amplification and analysis of 12 microsatellite loci (Table 1). These markers were
440 previously identified as polymorphous in fox squirrels (Fike & Rhodes Jr, 2009). Primers
441 for the 12 loci were acquired from Sigma-Aldrich (The Woodlands, TX).

442 DNA from 5-10 hair follicles for each individual was extracted using standard
443 methods via a DNEasy Blood and Tissue kit (QIAGEN, Valencia, CA). We amplified the
444 DNA utilizing a polymerase chain reaction process in a BIO-RAD icycler thermal cycler
445 (BIO-RAD, Hercules, CA).

446 Each 10- μ L reaction mixture contained 3 μ L of DNA material, 0.3 μ L each of the
447 forward and reverse primer, 0.3 - 0.55 μ L MgCl₂ (adjusted for specific primer pairs, see
448 Table 1), 0.25 μ L of dNTP, 1.0 μ L reaction buffer (Tango, Carlsbad, CA) and 0.12 μ L of
449 Taq polymerase (Invitrogen, Carlsbad, CA). The forward primer for primer pairs was
450 fluorescently labeled with either 6-FAM or HEX dye. PCR reactions were run through
451 three steps: (1) denaturation at 95°C for 4 min; (2) 36 cycles of denaturation at 95°C for
452 45 s, annealing at 54-58°C (adjusted for specific primer pairs, see Table 1) for 30 s and
453 elongation at 72°C for 45 s; and (3) final elongation at 72°C for 10 min.

454 Successful reactions were prepared for sequencing with 2 μ L of PCR product, diluted
455 with 9.8 μ L of formamide and combined with 0.2 μ L of an internal size standard (LIZ

456 500, Applied Biosystems, Foster City, CA, U.S.A.). Fragments were determined via
457 sequencing using a Thermo Fisher 3730 DNA Analyzer (Thermo Fisher, Waltham, MA).
458 Base pair lengths were labeled using Geneious 10.1, with the Microsatellite Plugin 1.4
459 (Biomatters Limited, Newark, NJ).

460

461 *Statistical Analyses*

462 Pairwise relatedness between each pair of subjects in the study were estimated using
463 the program ML-Relate 5.0 (Kalinowski, Wagner, & Taper, 2006), which calculates
464 maximum likelihood estimates of relatedness and the most likely relationship between
465 pairs of individuals. Expected and observed heterozygosity (the probability that an
466 individual will be heterozygous at a given locus) were calculated using the “adegenet”
467 package in R 3.3.0 (Jombart, 2008).

468

469 **Results**

470 *Cache outcomes*

471 A total of 292 nuts were cached. No video was obtained for three caches and some
472 data was missing for these caches. Twenty nuts were eaten at the time they were
473 distributed to squirrels, and 36 nuts had an unknown outcome because the squirrel could
474 not be tracked until they ate or cached the nut.

475 The average lifespan of a cache was 38.38 days (Median = 4 days, range: 0 to 482
476 days). The number of nuts cached and cache life by individual are depicted in Table 2.
477 Four hundred and eighty-two days after the start of the experiment, 12 nuts remained in
478 their original cache locations and were assumed forgotten. This suggests an overall

Table 1. Motifs and oligo sequences for twelve polymorphic microsatellite loci used in the study to determine relatedness among fox squirrels. Annealing temperature, magnesium chloride (MgCl₂) concentrations used during PCR, expected (H_E) heterozygosity as reported by Fike and Rhodes (2009).

Locus	Motif	Oligo sequence (5' to 3')	PCR Product (base pairs)	Annealing Temperature (°C)	MgCl ₂ (μL)	H _E
FO 06 - F	AC(9)	AGAATGGAACTTAACCTAACTGTGG	218–222	57	.40	.632
FO 06 - R		CATTATTTTCGCAGGCTTTGG				
FO 11- F	AG(22)	CCATTTATGAGGGAGGTAGGG	258–296	57	.40	.843
FO 11- R		TTGAATCTGTAGATTGGGTAGTATGG				
FO 26- F	GT(14)	TTTAGAGTCTCGGCTGCTATCC	346–354	57	.50	.561
FO 26- R		GCTATGGAACCAACCTAAGTGC				
FO 33- F	AG(21)	ATTTCCCTGGGTTCAATTCC	149–171	54	.30	.850
FO 33- R		GTGGTTGCTTCCATAATGAGG				
FO 35- F	AC(12)	TTCTGATGCCTCTCAGGTAGC	246–252	55	.40	.535
FO 35- R		TCCATCCAAGGACATTACTGG				
FO 36- F	CT(9)	TGGATTGCTTTAGTCCACTTTG	106-122	55	.40	.601
FO 36- R		AAATTATGTTCTGCTGGGTTGTATAG				
FO 46- F	AC(16)	TGTTCCTTTTCCAAGACCAAA	214-224	57	.35	.238
FO 46- R		CACAAGTTGGTGCCAATTCA				
FO 58- F	CT(21)	CTCCTAATTAATTCAAGGACAAGC	158-182	58	.35	.795
FO 58- R		CCATGACCAAATTAATCAAAAGC				
FO 62- F	GT(19)	GAGCCAGGAACAAGGTAAAGC	198-208	57	.55	.689
FO 62- R		CCTTCCTGGCAGTTATCACC				
FO 63- F	CT(30)	CATAGTCACTTTCAAAGACTATTGATT	132-166	57	.30	.812
FO 63- R		TTGATTATGGGATACTCTGTAATTC				
FO 65- F	AC(14)	TTTGCTTTCTTTCTTTTCTTGC	233-243	57	.45	.642
FO 65- R		CCAAGTGAACATAACCCAGACC				

Locus	Motif	Oligo sequence (5' to 3')	PCR Product (base pairs)	Annealing Temperature (°C)	MgCl₂ (μL)	H_{E1}
FO 67- F	CT(19)	CTTGAGGTTTCAGATCTCATGG	183-199	57	.45	.746
FO 67- R		AAATGATCAGCATATTTCTCAGC				

480

481 forgetting rate of around four percent. An additional 18 nuts remained in new locations
482 that they had been moved to at some point during the experiment, a further loss of six
483 percent. Seven instances of pilfering and one recaching event (by the squirrel “Three”)
484 were observed. Pilfering events between squirrels are noted in Table 4.

485 The only variable that was related to the length of time a cache stayed in its original
486 location was the level of concealment ($F(3, 232) = 3.32, p = .021$) such that caches that
487 were placed in mostly concealed areas had longer cache lives ($n = 39, M = 93.38$ days,
488 95% CI [40.92, 145.83 days], Median = 8 days) than caches placed in open ($n = 175, M =$
489 32.80 days, 95% CI [19.81, 45.77 days], Median = 4 days) or partially concealed areas (n
490 $= 56, M = 21.30$ days, 95% CI [5.33, 37.27 days], Median 3.5 days). Caches placed in
491 totally concealed areas had a lifespan of 26 days ($n = 20, 95\%$ CI [-4.47, 56.47 days],
492 Median = 5.5 days) and were not statistically different from other cache concealment
493 categories.

494 Weight and the number of headshakes were weakly related to the distance from the
495 food source that a squirrel traveled to cache, such that heavier nuts and more headshakes
496 were associated with a longer distance traveled but the effect in both cases was not
497 statistically significant (weight: $F(1, 275) = 3.14, p = .08$; headshakes $F(1, 75.78) = 2.91,$
498 $p = .09$).

499 Finally, squirrels adjusted cache protection behaviors depending on the level of
500 conspicuousness of the cache. They spent more time caching nuts when in open locations
501 ($F(3, 269.4) = 3.76, p = .011$), or when other squirrels were present ($F(7, 265.2) = 2.72, p$
502 $= .010$; Figure 3). Squirrels spent the most time digging ($F(3, 254.1) = 4.43, p = .005$),
503 and covering their caches ($F(3, 256.5) = 13.68, p < .001$) when they cached in an open

504 location, and spent the least amount of time on all cache protection behaviors (digging,
505 tamping, and covering caches) when in a concealed location. See Figure 4.

506

507 *Microsatellite analysis*

508 The number of alleles per locus ranged from 3 to 16, and single locus
509 heterozygosities ranged from 0.20 to 0.92 (Table 3), suggesting an overall high level of
510 genetic diversity in the tested population. From 10000 randomized simulations performed
511 in ML-Relate, a possible heterozygote deficiency was found at one loci ($F_{62}, p = .059$;
512 Table 3). Observed heterozygosity was slightly higher than expected ($t_{11} = -2.09, p =$
513 $.06$).

514 Based on estimates of the most likely relationships between individuals (unrelated,
515 half siblings, full siblings or parent-offspring), there were likely six full siblings, five half
516 siblings, and three parent-offspring relationships between the fourteen individuals in the
517 study for whom we had DNA samples (see Table 4).

518

519 *Spatial distribution of caches*

520 Geospatial data was used to assess the proximity of a squirrel's caches to their own
521 caches, and to those of other squirrels, based on relatedness between individuals. When
522 treated as a continuous variable, there was an negative linear relationship between
523 probability of relatedness and cache distance ($F(1, 105) = 9.77, p = .002$, Figure 5a), but
524 this effect was largely driven by the inclusion of the distance each squirrel tended to
525 cache from their own other caches.

526

527 **Table 2.** Number of nuts cached by each squirrel, and average cache life (both mean and
 528 median) in days.

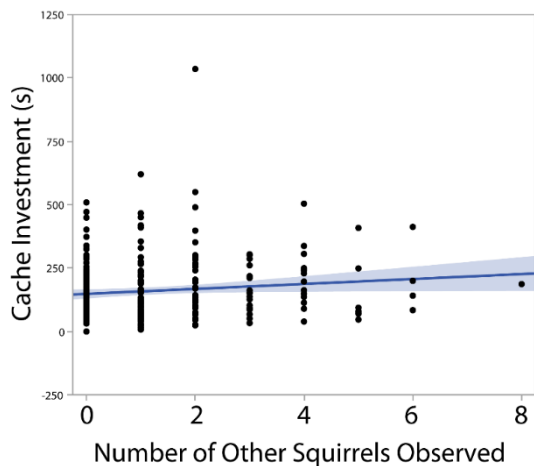
Squirrel	Number of nuts cached	Average cache life (days) (SD)	Median cache life
Biggie ^a	37	55.89 (115.75)	4
Billy Ray	3	18.00 (26.00)	4
Blake	18	28.89 (39.40)	11.5
Chubs ^b	23	91.65 (177.05)	5
Curly	1	24.00 (NA)	24
December	1	0.00(NA)	0
Fermata	7	23.57 (24.41)	22
Flame	16	26.31 (69.75)	4
Gwen	1	3 (NA)	3
Jewel	4	2.75 (3.50)	1
Joker	5	2.40 (1.14)	2
Mermaid	1	1.00 (NA)	1
Roger	21	21.29 (61.26)	5
Scarf	16	29.25 (63.69)	4
Squiggle ^c	43	41.40 (105.11)	4
Stool ^a	28	67.54 (136.01)	9.5
Stovetop	15	4.53 (5.83)	3
Teddy Bear	2	2.00 (1.41)	2
Three	47	24.57 (73.52)	3
Walter	3	2.33 (2.08)	3

529 ^aForgot three caches

530 ^bForgot four caches

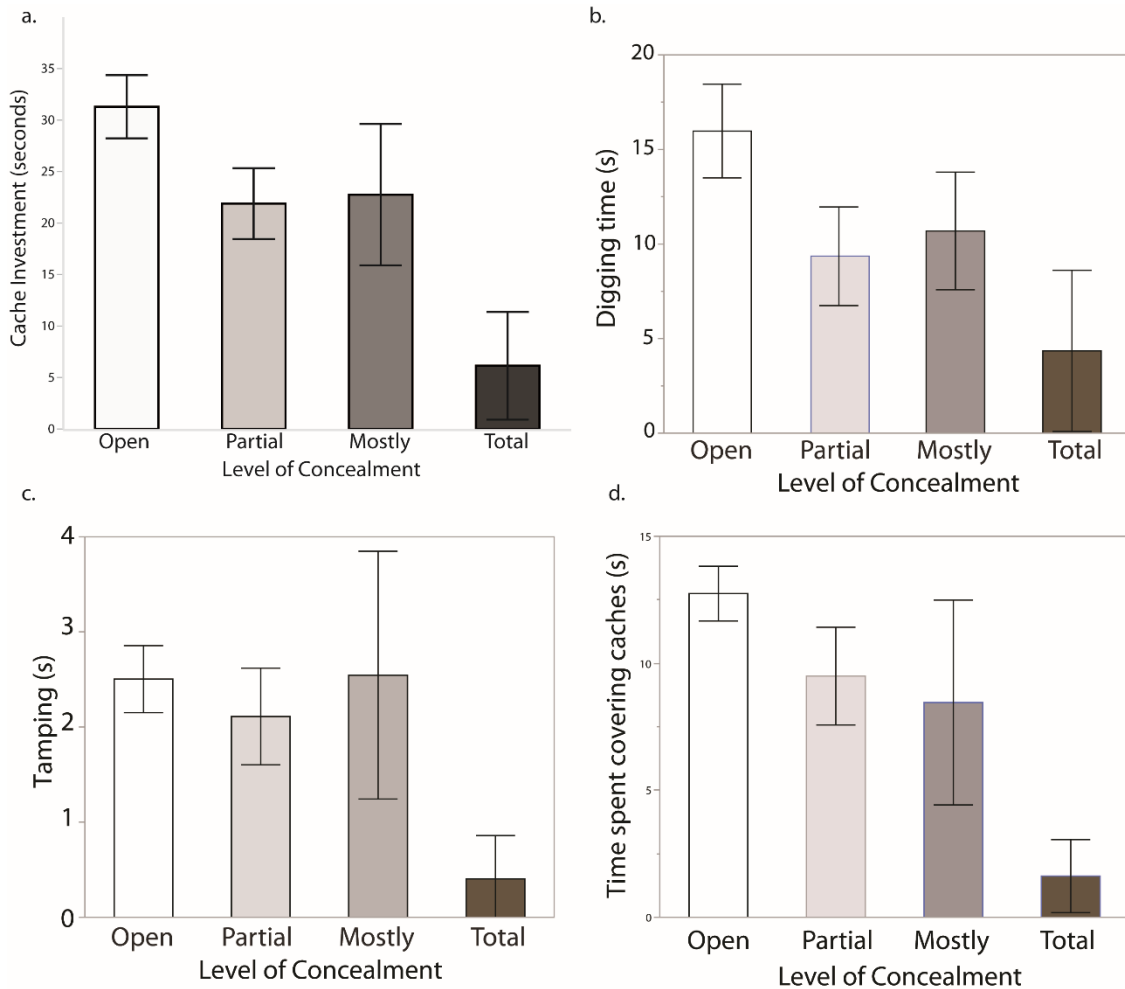
531 ^cForgot two caches

532



533

534 **Figure 3.** Total cache time (seconds) in the presence of other squirrels. Squirrels tend
 535 to spend more time caching as the number of competitors (other squirrels) increases.



536

537 **Figure 4. Cache investment and protection at different levels of cache**

538 **conspicuousness.** Squirrels spent more total time caching (a), more time digging (b), and
539 more time covering (d) caches made in open locations compared to completely concealed
540 locations. Squirrels spent the least amount of time tamping caches (c) made in completely
541 concealed locations.

542

543 When assessed as a categorical variable (self, related, unrelated), there were
544 differences between groups on average distance between caches ($F(2, 99.42) = 10.71, p <$
545 $.001$). Squirrels tended to cache closer to their own caches ($M = 59.14$ m, 95% CI [44.87,

546 73.41]) than to those of other squirrels, particularly when compared to those of unrelated
547 squirrels ($M = 91.28$ m, 95% CI [84.26, 98.3]). The average distance between related
548 squirrels was $M = 81.93$ m, 95% CI [67.37, 96.49]. See Figure 5b.

549 Squirrels also tended to disperse their caches more as the experiment continued. The
550 distance traveled from food source to cache increased during each consecutive week of
551 the experiment, ($F(1, 290) = 7.70$, $p = .006$, Figure 6). The density of nuts decreased as
552 the experiment continued (Table 5), although squirrels continued to cache in the central
553 area that they cached in during week 1 throughout the remainder of the experiment
554 (Figure 7).

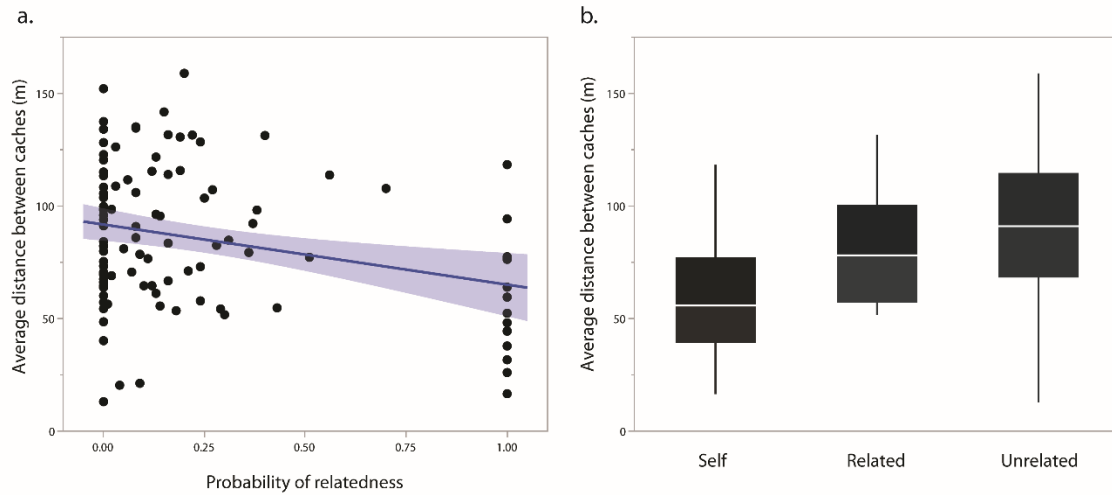
555

556 **Table 3.** Expected (H_E) and observed (H_O) heterozygosities at the twelve loci analyzed.

Locus	H_E	H_O
F06	0.64	1.00
F26	0.71	0.95
F11	0.92	1.00
F33	0.73	1.00
F35	0.71	0.59
F36	0.60	0.71
F46	0.71	0.95
F58	0.81	1.00
F62	0.34	0.20
F63	0.68	0.90
F65	0.64	0.86
F67	0.72	0.95

557

558

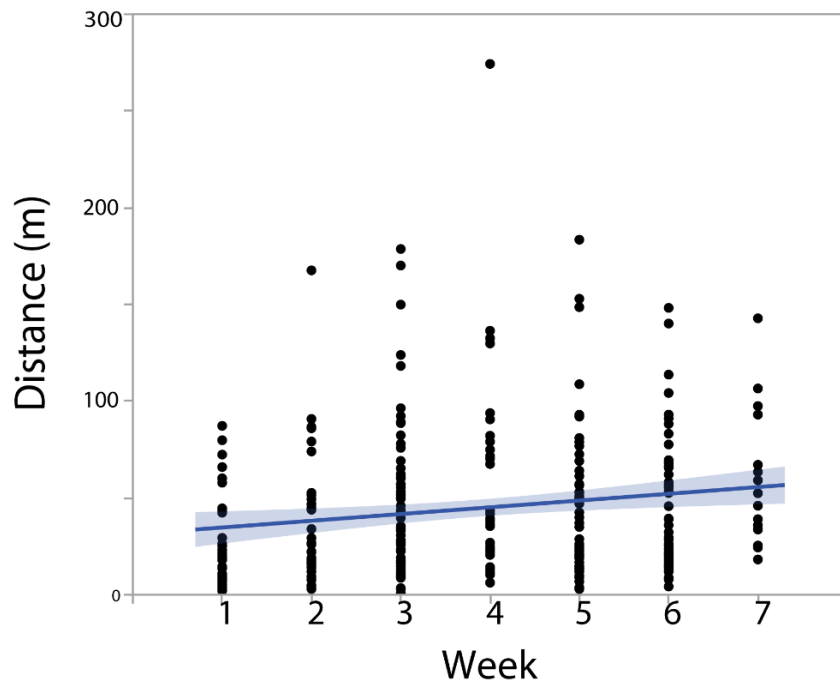


559

560 **Figure 5. The relationship between relatedness and distance between caches.**

561 Relatedness decreases distance between caches (a); squirrels tend to cache closer to their

562 own previously made caches than to those of other squirrels.



563

564 **Figure 6. Distance traveled for each cache buried by each week of the experiment.**

565 Squirrels increased distance traveled from the food source as the experiment continued.

566 Table 4. Probabilities of relatedness between individuals in the study, as calculated by ML-Relate.

	Biggie	Roger	Teddy	Fermata	Walter	Stool	Joker	Blake	Flame	Jewel	Three	Squiggle	Curly	Chubs
Biggie	1													
Roger	0.07	1												
Teddy	0.08	0.13 ^{c*}	1											
Fermata	0	0.06	0.36 ^b	1										
Walter	0.19	0.03	0.11	0.13	1									
Stool	0.19	0.08	0.14	0	0.25	1								
Joker	0.01	0	0	0.08	0.2	0.13	1							
Blake	0.27	0	0	0.16	0	0	0.15	1						
Flame	0.51 ^b	0	0	0	0.02	0.05	0	0.70 ^b	1					
Jewel	0	0	0.09	0.31	0	0	0	0.56 ^a	0.43 ^a	1				
Three	0.29 ^{b*}	0.30 ^c	0	0	0.16	0.38 ^a	0	0	0.12	0	1			
Squiggle	0.28	0	0.10 [*]	0.12	0.24	0.37 ^b	0	0.08	0.24	0.16 [*]	0	1		
Curly	0	0	0	0.09	0	0	0	0.08	0.18	0.04	0	0.21 ^c	1	
Chubs	0.02 [*]	0	0	0	0	0.22 ^b	0.24 ^c	0.4	0.13	0.03	0	0.14 ^c	0	1

567

568 ^aLikely parent-offspring relationship

569 ^bLikely full-sibling relationship

570 ^cLikely half-sibling relationship

571 ^{*}Pilfering event observed between these two individuals

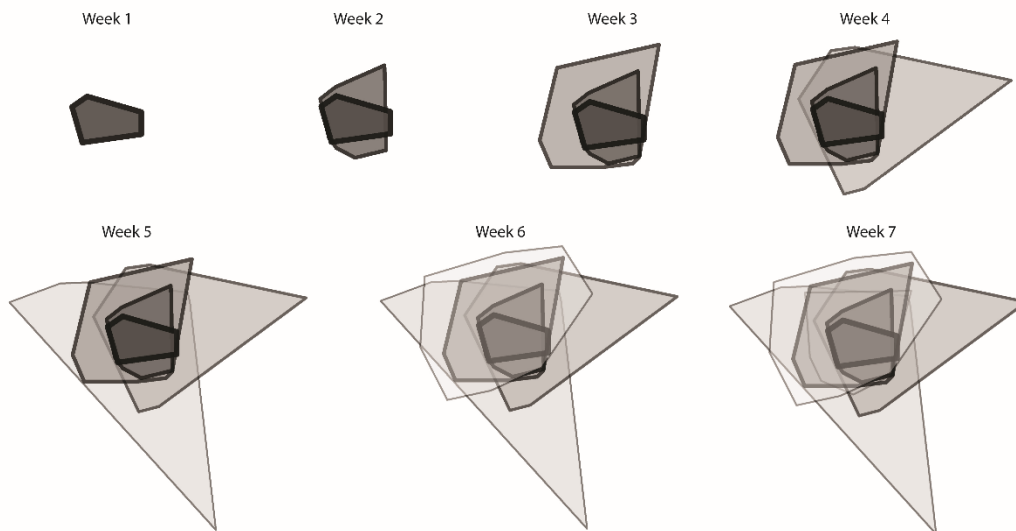
572

573 **Table 5.** Nearest Neighbor Distances throughout the experiment. NN ratios larger than one
 574 indicate nuts that were cached at a lower density than expected if randomly distributed. Observed
 575 distances between nuts tended to increase as the experiment continued.

Week	NN Ratio	Z-statistic	p-value	Observed distance (m)	Expected distance (m)
1	1.04	0.42	.680	8.43	8.13
2	.88	-1.34	.180	9.41	10.64
3	.88	-1.89	.060	10.48	11.87
4	.84	-1.71	.090	20.44	24.28
5	.80	-2.75	.006	19.37	24.20
6	2.03	1.15	.040	21.34	18.50
7	1.52	4.08	<.001	32.45	21.38

576

577



578

579 **Figure 7.** Polygons depicting the minimum bounding geometry for caches made by all
 580 squirrels for each week of the experiment. Squirrels utilized a larger area to cache in as the
 581 experiment continued, but also continued to cache in a core central area.

582

583 **Discussion**

584 The results of this study suggest that the most important factor contributing to the fate of
585 caches made by fox squirrels, strictly measured as how long a cache remained in its original
586 location, is the conspicuousness of the cache. Caches that were placed in open areas were moved
587 sooner than other caches. Squirrels also spent more time digging, tamping and covering caches in
588 open areas, compared to more concealed caches.

589 This study also supported previous findings that squirrels are sensitive to food item value and
590 the social context when caching. Squirrels showed a tendency to travel further for heavier
591 hazelnuts, even though the range of nut weights in this study was very small ($x = 3.94$ g, range:
592 2.3 to 5.5 g). Several studies that have shown that tree squirrels tend to travel further for heavier
593 nuts, nuts that provide more nutritional content, and nuts that are at lower risk of perishability
594 (Delgado et al., 2014; Moore et al., 2007; Preston & Jacobs, 2009; Stapanian & Smith, 1984;
595 Steele, Hadj-Chikh, Agosta, Smallwood, & Tomlinson, 1996), and this study demonstrates that
596 this may also happen on a very fine-grained scale, even when there are small differences in
597 quality between food items.

598 Squirrels traveled further away from the food source to cache when greater numbers of
599 competitors were present. They also showed some tendencies to cache closer to their own
600 previously made caches, and closer to the caches made by related squirrels than unrelated
601 squirrels. This supports that squirrels, although generally considered solitary (Steele &
602 Koprowski, 2001) are sensitive to the social context they are caching within.

603 It has been assumed that the time squirrels spend covering caches is somehow related to
604 preventing conspecific theft. Covering caches has been previously described as a method of
605 disguising caches or as cache protection (e.g., Delgado et al., 2014; Hopewell & Leaver, 2008;

606 Steele et al., 2008). The current study showed that more time covering caches was not a predictor
607 of cache life and in fact the inverse may be true. Squirrels spent more time covering caches that
608 were in open areas, and those caches also tended to stay in place the shortest amount of time. In
609 order to fully understand this effect, it would be necessary to assess the effect of substrate on
610 covering time; it is possible that caches in open areas were placed in a more compact, tighter
611 substrate that required more digging and covering than a looser soil.

612 If in fact caches are recovered by the squirrel who cached them, then cache covering may
613 serve as protection. But even if the food-storing animal retrieves their own caches, the function
614 of covering needs to be disentangled between different possible hypotheses. Covering caches
615 could provide protection by creating scent cues or consolidating the memory of the food-storer,
616 making retrieval easier for the caching animal. It could also provide protection by making it
617 more difficult for a competitor to find and pilfer a cache.

618 However, in Experiment 1, 25% of pilfered nuts were stolen shortly after they were cached.
619 This suggests that squirrels may be observing each other cache; in which case, spending more
620 time covering could provide a signal to competitors that a nut is being buried, and give pilferers
621 more time to observe the cache location. The function of cache covering behavior merits further
622 exploration, but most importantly how the outcome of caches is related to covering behavior
623 needs to be determined.

624 The results of this study demonstrated that pilfering between individual squirrels can be
625 quantified in the field. Unfortunately, we were unable to observe many instances of pilfering or
626 recaching in the final experiment. Given the results from the pilot study, this was surprising.
627 However, in the pilot study, we only provided one squirrel with nuts to cache. This limited the
628 area that needed to be observed, as the focal squirrel cached most of the nuts she was provided

629 with in a central area. Provisioning her with nuts each day may have artificially inflated the
630 pilfering rate by changing the caching behavior of only one individual in the study area.

631 Conversely, in the final study, because several squirrels were caching, the cache areas were
632 distributed across a larger area of the testing area (Figure 7), which made observation difficult as
633 the experiment continued. Furthermore, because we were providing squirrels with nuts in both
634 the morning and afternoon, this limited our total observation time. Because many nuts were
635 moved within a short period of time, the lack of pilfer and recache observations does not suggest
636 that squirrels were not pilfering and recaching nuts; they just did so in times and places that were
637 not being directly observed.

638 A previous study suggested that the experimental provision of food for squirrels could
639 increase pilferage (Penner et al., 2013). In that study, researchers first provided squirrels with ad
640 libitum food in one plot and did not offer food in a control plot. Later, pecans were buried at
641 identical densities in both plots, and pilfering was statistically higher in the previously
642 provisioned plot. We have not quantified how providing the squirrels with food in our study may
643 have inflated pilferage; however, the current study did not include any provision of food prior to
644 the experiment. During the study, squirrels were provided with nuts primarily where they were
645 observed, thus the provisioning location frequently changed. No specific area of the study site
646 should have been seen as more desirable for foraging or searching for previously made caches.

647 Squirrels buried the majority (almost 60%) of their caches in an open area, which suggests
648 there may be some benefits to caching in an open area, such as ease of retrieval for short term
649 storage. That said, five out of seven of the observed pilferage events were of nuts were originally
650 cached in open areas. In a previous study (Steele et al., 2014), human-made caches under canopy
651 were moved more than caches made in the open. Based on the limited data we acquired in this

652 study, fox squirrel caches in open areas may be pilfered more frequently. It is possible that since
653 gray squirrels spend more time under canopy in comparison to fox squirrels (Steele &
654 Koprowski, 2001), they were more likely to discover human-made caches under canopy than in
655 the open.

656 In the current study, half of all cached nuts were moved within four days of being buried.
657 That said, 25% of cached nuts had a life span longer than 20 days. A previous study of squirrel-
658 cached acorns found that of 57 cached nuts, all were moved between one and six days after
659 burial. No relationship was found between cache life and distance nuts were buried from cover.
660 Because it is unknown in both studies if short lifespans are due to pilfering or recaching, it is
661 difficult to say whether this life span is beneficial or detrimental to caching animals.

662 Approximately 10% of cached nuts remained in place a year after they were cached or re-
663 cached. Based on observations of nuts that were dug up six months after the end of the
664 experiment, they were likely no longer edible. Perhaps the squirrels could detect this and
665 abandoned caches, or these forgotten caches may represent what percent of nuts is typically
666 forgotten by food-storers. Cahalane (1942) found that fewer than two percent of nuts buried by
667 fox squirrels were forgotten over the winter, but as he marked caches with stakes, he may have
668 provided additional visual cues to the original food-storers or to pilferers that made these nuts
669 easier to locate.

670 A key function of seed dispersers is to propagate tree species (Price & Jenkins, 1986; Sun &
671 Zhang, 2013; Vander Wall, 1990), and squirrels have co-evolved with their food sources
672 (Stapanian & Smith, 1978; Steele, Wauters, Larsen, & Forget, 2004; Vander Wall, 2010). Thus,
673 some forgetting of cached nuts provides benefits to both the tree species, and the food-storer, in
674 terms of guaranteeing future food sources for kin. It is not possible to test the duration of

675 memory for caches with human-made caches, and so pit-tagging of nuts provides an excellent
676 methodology for further testing what percent of nuts may be forgotten by caching animals.

677 The microsatellite analysis of DNA collected for subjects in this study demonstrated that
678 despite a fragmented habitat, human-made structures, and likely artificial supplementation of
679 food, there is a similar level of genetic diversity among the study population as the populations
680 of fox squirrels sampled in their native habitat (Fike & Rhodes Jr, 2009). We were able to use a
681 non-invasive method to obtain hair samples from free-ranging squirrels that provided adequate
682 DNA for sequencing and analysis. This analysis found expected levels of heterozygosity at 11
683 out of 12 loci.

684 More importantly, microsatellite analysis allowed us to explore how relatedness impacts
685 caching behavior. Although we were not able to determine the relationship between probability
686 of relatedness and likelihood of pilfering between individuals, results suggested that squirrels
687 may cache nuts closer to caches made by relatives than unrelated squirrels. If squirrels are more
688 likely to pilfer within or close to their caching territory, then this would suggest some form of kin
689 selection could be at work. This could also prevent pilfering from non-related individuals. Given
690 the small sample size, and the fact that the effect was small, we should interpret these results
691 with some caution; further studies should examine this possibility in much more detail.

692 Ideally, this study would be replicated with fewer caching subjects and more time to observe
693 individual cache movements. Alternatively, the focal squirrel could be rotated, testing just one
694 individual at a time, to allow for a more fine-grained observations and analysis of the
695 relationship between caching behaviors, relatedness and cache fate. Ideally, hair samples would
696 be collected from all participating squirrels in the study, in addition to sampling squirrels in other

697 locations surrounding the test area, to better assess the level of dispersal among this population
698 of squirrels.

699 To summarize, this study established or validated several methods for testing the caching
700 behavior and population dynamics of a group of free-ranging, scatter-hoarding tree squirrels. The
701 results demonstrate the flexibility of squirrels when storing food and show that they adjust
702 behaviors according to several environmental and social factors. They also point to the need for a
703 greater understanding of how these behaviors are related to the outcomes of caches that are
704 stored for future use, a question that turned out to be much more challenging to answer than
705 anticipated.

706

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714 with data collection.

715

716 **Conflict of interest**

717 The authors declare that they have no conflict of interest.

718

719

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