

1 **Species differences in egocentric navigation: The effect of burrowing ecology on a spatial-**
2 **cognitive trait in mice (In Press, Animal Behaviour May 2017)**

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24

25 **ABSTRACT**

26 Efficient navigation is a critical component of fitness for most animals. While most species use a
27 combination of allocentric (external) and egocentric (internal) cues to navigate through their
28 environment, subterranean environments present a unique challenge in that visually mediated
29 allocentric cues are unavailable. The relationship between egocentric spatial cognition and
30 species differences in ecology is surprisingly understudied. We used a maze-learning task to test
31 for differences in egocentric navigation between two closely related species of mice, the
32 eastern house mouse, *Mus musculus musculus*, and the mound-building mouse, *M. spicilegus*.
33 The two species are sympatric in Eastern Europe and overlap in summer habitat use but differ
34 dramatically in winter space use: whereas house mice occupy anthropogenic structures,
35 mound-building mice survive the winter underground in intricate burrow systems. Given
36 species differences in burrowing ecology, we predicted that *M. spicilegus* would learn the maze
37 significantly faster than *M. m. musculus* when tested in complete darkness, a condition that
38 eliminated allocentric spatial information and served as a proxy for the subterranean
39 environment. We found strong support for this prediction. In contrast, the two species
40 performed equally well when different mice were tested in the same maze with lights on. This
41 context-specific species difference in spatial cognition suggests that enhanced egocentric
42 navigation in *M. spicilegus* is an adaptation to the burrow systems on which the over-winter
43 survival of young mound-building mice depends. The results of this study highlight the
44 importance of ecological adaptations to the evolution of cognitive traits.

45 *Keywords:* Learning, memory, mound-building, *Mus spicilegus*, route-based navigation, spatial

46 ecology

47 **INTRODUCTION**

48 From the long distance migrations of pelagic seabirds, to a newborn wallaby's journey from
49 mother's birth canal to teat (Croxall, Silk, Phillips, Afanasyev, & Briggs, 2005; Egevang et al.,
50 2010; Schneider, Fletcher, Shaw, & Renfree, 2009; Tyndale-Biscoe & Renfree, 1987), the ability
51 to navigate from one location to another is a critical component of fitness for most non-sessile
52 organisms. To accomplish these non-random movements, animals use allocentric (external)
53 cues, such as the sun, stationary terrestrial objects, or odor trails, and egocentric (internal)
54 signals from the proprioceptive, vestibular or somatosensory systems (Shettleworth, 2010).
55 Whereas allocentric navigation can incorporate multimodal sensory information from both
56 local and distant cues, egocentric navigation relies on input generated by an organism's own
57 movement. Experimental studies subdivide egocentric navigation into path integration
58 (colloquially, 'dead reckoning'), and route-based navigation. While both rely on the ability to
59 update spatial position based on input from the proprioceptive and/or vestibular systems, path
60 integration is tested by displacing test subjects from a starting point and measuring homing
61 ability, whereas route-based navigation tests subjects' ability to learn and remember a series of
62 turns in a point to point system such as a maze (Benhamou, 1997; Shettleworth, 2010).

63 Few organisms use just one type of cue and most combine allocentric and egocentric
64 information to form a spatial representation, or cognitive map, of their surroundings (Etienne
65 et al., 1998, 1996; Shettleworth, 2010). Yet most work on the evolution and mechanistic basis
66 of vertebrate spatial abilities has focused on allocentric cue use. In this context, comparative
67 studies in a wide range of taxa suggest that species, population and sex differences in spatial
68 learning ability, and reliance on different types of external cues for navigation, are shaped by

69 differences in ecology as it relates to space use (e.g., social structure, Gaulin et al., 1990;
70 migratory behavior, Pravosudov et al., 2006; foraging ecology, Clayton & Krebs, 1994;
71 Pravosudov & Clayton, 2002; environmental complexity, Bruck & Mateo, 2010; du Toit et al.,
72 2012; predation pressure, Brown & Braithwaite, 2005). For example, seed-caching birds learn
73 the locations of hidden seeds with greater precision than non-caching species (Jones et al.,
74 2002), benthic three-spined stickleback learn to locate a hidden reward twice as fast as limnetic
75 ecomorphs that occupy less complex microenvironments (Odling-Smee et al., 2008), and
76 eusocial Damaraland mole-rats, a species with complex burrow architecture, learn a spatial task
77 faster and exhibit higher retention than Cape mole-rats, a solitary species with relatively simple
78 burrows (Costanzo et al., 2009).

79 Although path integration has been demonstrated in several mammalian orders,
80 including rodents (Alyan, 1996; Bardunias & Jander, 2000; Etienne, Maurer, Saucy, & Teroni,
81 1986; Kimchi & Terkel, 2004; Mittelstaedt & Mittelstaedt, 1980) and primates (Israël, Grasso,
82 Georges-François, Tsuzuku, & Berthoz, 1997), most work on egocentric navigation has been
83 conducted in invertebrates (e.g., Müller & Wehner, 1988; Wehner & Srinivasan, 1981; 2003;
84 reviewed in Srinivasan, 2015; c.f. Kimchi & Terkel, 2002; Presotto & Izar, 2010). Importantly,
85 ecologically-motivated tests for species differences in egocentric spatial ability are surprisingly
86 lacking.

87 We used a spatial learning task to test for differences in egocentric navigation between
88 two closely related, but ecologically distinct, species of Old World mice: the eastern house
89 mouse, *Mus musculus musculus*, and the mound-building mouse, *Mus spicilegus*. The two
90 species are sympatric throughout the range of *M. spicilegus* (Eastern Europe, from Hungary to

91 the Ukraine) and locally syntopic in crop fields during the spring and summer (Gouat et al.,
92 2003; Muntyanu, 1990; Poteaux et al., 2008) but exhibit major differences in burrowing
93 ecology. While house mice will dig and construct burrows under experimental conditions
94 (Bouchard & Lynch, 1989; Schmid-Holmes, Drickamer, Robinson, & Gillie, 2001), their
95 commensal relationship with humans typically precludes this behavior. In sympatry with *M.*
96 *spicilegus*, *M. m. musculus* overwinters in haystacks, farm buildings, and other anthropogenic
97 structures (Muntyanu, 1990). In contrast, *M. spicilegus* survives the winter in complex burrow
98 systems topped by mounds of soil and vegetation that serve a thermoregulatory function
99 (Szenczi et al., 2011; Szenczi, Kopcso, Bánszegi, & Altbäcker, 2012). The burrow systems
100 typically reach a depth of 1-2 m with exit holes up to 1.5 m away from the central mound
101 (Muntyanu, 1990; Szenczi et al., 2011). Construction takes several days to weeks and involves
102 multiple related individuals, primarily young of the year that delay reproduction till the
103 following spring (Garza et al., 1997; Muntyanu, 1990; Poteaux et al., 2008). In midwinter,
104 mounds can contain as many as 21 mice (Canaday et al., 2009). Mounds and burrows are
105 constructed during the autumn (September – November) and are occupied until spring (March
106 – April; Muntyanu, 1990; Szenczi et al., 2011). Thus, mound-building mice spend at least half of
107 the year living underground in a spatially complex and completely dark environment in which
108 allocentric cues are largely unavailable.

109 We tested for species differences in a maze-learning task performed in complete
110 darkness without access to allocentric cues. Given the specialized burrowing ecology of *M.*
111 *spicilegus* we predicted that this species would learn the task faster than *M. m. musculus*. To
112 control for more general species differences in spatial ability we repeated the experiment using

113 different individuals with lights on; i.e., with access to allocentric cues both inside and external
114 to the maze. Given that both species forage above ground and occupy the same habitat for part
115 of the year we did not expect to find species differences in maze learning with allocentric cues
116 available.

117

118 **METHODS**

119 *Animals*

120 A total of 27 *M. m. musculus* from 10 litters and 29 *M. spicilegus* from 16 litters were
121 used in this study. Both species were represented by wild-derived inbred strains, obtained from
122 Jackson Laboratory (*M. m. musculus*: PWK/PhJ) and the Montpellier Wild Mice Genetic
123 Repository (*M. spicilegus*: ZRU), and maintained at Oklahoma State University since 2013.
124 Subjects were sexually naïve young adults (*M. m. musculus*, 55 – 166 days; *M. spicilegus* 57 –
125 167 days) that had not been used in prior behavioral experiments. To minimize potential litter
126 effects (e.g., Lazic & Essioux 2013), we avoided using same sex litter mates in the same light
127 condition whenever possible.

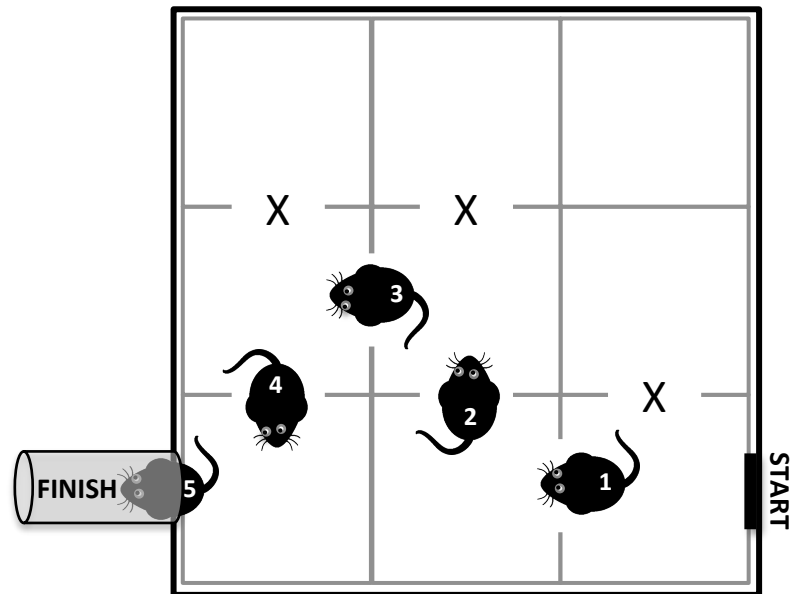
128 Mice were housed in polycarbonate cages bedded with Sani-chips® (Harlan Teklad,
129 Madison, WI, U.S.A.) and were provided with nesting material (cotton nestlets and alfalfa hay)
130 and *ad lib* water and chow (Rodent Diet 5001, Harlan Teklad). To enhance motivation for the
131 food reward (see below), seeds that were provided 2-3 times/week as enrichment to other
132 mice in the colony were not given to test subjects; animals were not otherwise food restricted.
133 The colony was maintained on a 12:12 h light:dark cycle (lights on at 0900) and maze trials were
134 run during the light phase (between 0900 and 1300). This schedule was chosen because *Mus*

135 species spend most daylight hours inside a nest or burrow, the environment we were
136 attempting to approximate with the maze.

137

138 *Apparatus and Procedure*

139 To test the subjects'
140 egocentric navigation abilities
141 we used performance learning
142 on a two-dimensional maze task.
143 The maze (Ware Manufacturing,
144 www.waremfginc.com)
145 consisted of a 3x3 grid of nine



146 13.5x13.5x11 cm boxes
147 with 6.5 cm diameter
148 holes for the animals to
149 move through (Figure 1)

Figure 1. Maze used to test for species differences in spatial learning and memory in *Mus spicilegus* and *M. m. musculus*. Numbers indicate the points at which mice could either take the correct route (indicated by the orientation of the mice) or make one or more errors. An error was scored each time a mouse backtracked in the maze or entered a dead end box (indicated with X's).

150 and a reward zone (a Habitrail® 5cm diameter plastic tube and endcap) with wild bird seeds and
151 bedding from each subject's home cage (see Mateo, 2008 for comparable methodology).

152 During pilot testing, the large number of errors that occurred with animals in the last box
153 before the reward tube led us to conclude that a navigation-useful odor gradient was not

154 perceptible by subjects. Furthermore, given the non-direct route of the maze (Figure 1) and its

155 open-air configuration, the use of an odor gradient would not be particularly informative for

156 subjects. Mice were tested in the maze under total darkness (0 lux, dark condition; measured

157 with Pyle Lux Meter PLMT21), or with lights on (150 lux, light condition). No subject was used in
158 both conditions.

159 Animals were brought from the colony room to the adjacent testing room in their home
160 cages immediately before each trial and were placed in the apparatus by an experimenter who
161 then left the room. For the dark condition, we used small strips of glow in the dark tape on the
162 outside of the apparatus to mark where the animals needed to be placed to start the maze. A
163 second experimenter timed and scored each trial from a different room using a remote live
164 video feed (Panasonic HC-W850 with night vision capability to a 32 inch Phillips 720p HDTV
165 model 32PF9631D or Samsung UN22F5000 LEDTV). Both experimenters were blind to sex and
166 species identity, although species differences in size were evident to experienced observers.

167 Mice were given a maximum of 10 minutes to complete the maze, defined as head
168 inside the reward tube. To minimize uneven experience with the maze and reduce handling
169 stress, animals were returned to their home cages in the reward tube within approximately one
170 minute of completion. To successfully run the maze, a mouse must have completed the task in
171 30 s or less with one or fewer errors. An error was defined as backtracking through the maze or
172 entering a dead end box (Figure 1). Entering a box was defined as the animal placing its head
173 through the hole between the sections. Each animal was run once daily until it either
174 completed the task successfully on two consecutive days, or until 21 days had elapsed. For mice
175 that met our criteria for successful maze completion, the number of days until the first
176 completion was taken as a dependent measurement (see Bruck & Mateo, 2010, for analogous
177 test criterion). Mice that did not meet our criterion received a nominal score of 21. Mazes were
178 washed with warm soapy water between each individual trial and maze orientation was rotated

179 180° daily to prevent the animals from using magnetic sensory input to navigate (e.g., Kimchi et
180 al. 2004; Muheim, Edgar, Sloan, & Phillips, 2006).

181 *Analysis*

182 The effects of species and sex on the number of trials required to successfully complete
183 the maze (trials to criterion) under each condition (dark or light) were explored with mixed
184 models in which litter ID was included as a random effect. Survival models were used for final
185 analyses because this approach accounts for incomplete or right-censored data; in the case of
186 this study, mice that did not meet criterion before the end of the three-week period. We fit a
187 parametric survival model with a Fréchet (inverse Weibull) distribution and tested for effects of
188 species, sex, and their interaction on trials to criterion under each condition. The same model
189 was used to test for an effect of condition within species. Mice that did not meet criterion by 21
190 days were coded as censored. Significance was evaluated with likelihood ratio tests (LRT).
191 Analysis of variance (ANOVA) was used for *post hoc* tests for sex differences within species. $P \leq$
192 0.05 was considered significant. All analyses were carried out in JMP 12 (SAS Institute Inc.).

193

194 *Ethical Note*

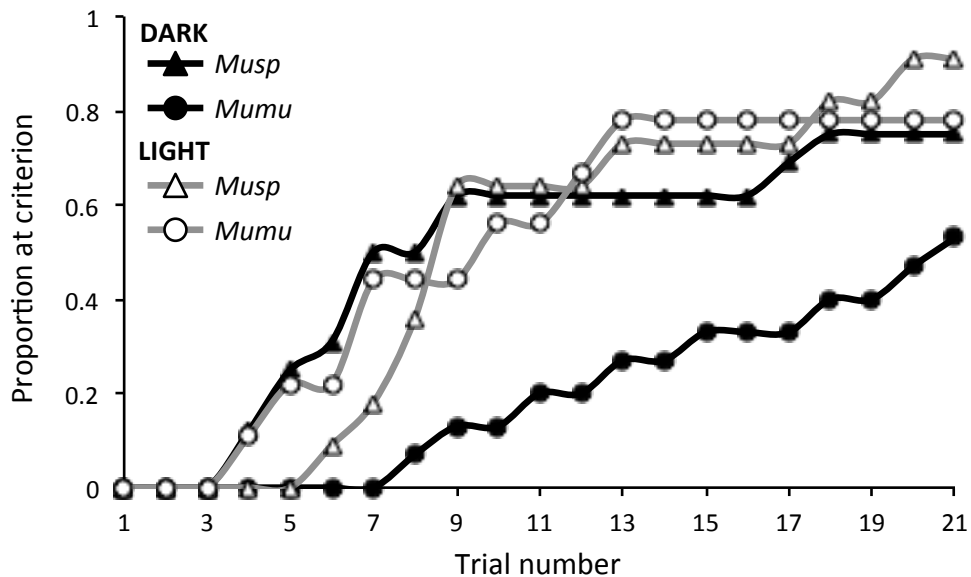
195 Animal care and experimental procedures were approved by the Oklahoma State
196 University Institutional Animal Care and Use Committee under protocol numbers AS1310 and
197 AS141. Mice were tested in the maze daily for a maximum of 22 days. During this time they
198 were housed singly. After serving in the experiment, mice were returned to the main colony
199 and used as breeders.

200

201 **RESULTS**

202 Of the 56 mice that started the maze trials, five were disqualified due to incorrect maze
 203 set up. Final sample sizes for the dark condition were 16 *M. spicilegus* (8/sex) from 10 litters
 204 and 15 *M. m. musculus* (8 males, 7 females) from five litters. Final sample sizes for the light
 205 condition were 11 *M. spicilegus* (5 males, 6 females) from seven litters and nine *M. m. musculus*
 206 (4 males, 5 females) from six litters. In the dark trials, 75% (12/16) of *M. spicilegus* reached
 207 criterion (maze completion in ≤ 30 s with ≤ 1 error on two consecutive days) before the end of
 208 the trial period whereas only 53.3% (8/15) of *M. m. musculus* reached criterion. In the light
 209 trials, 90.9% (10/11) of *M. spicilegus* and 77.7% (7/9) of *M. m. musculus* reached criterion (see
 210 supplemental materials for error and latency summaries).

211 The cumulative proportions of *M. spicilegus* and *M. m. musculus* that reached criterion under



212 each condition are shown in Figure 2. Summary statistics and sample sizes for each species split

Figure 2. The cumulative proportions of *Mus spicilegus* (triangles, *Musp*) and *M. m. musculus* (circles, *Mumu*) that met criterion for successful maze completion under dark (black lines, filled shapes) or light (grey lines, open shapes) conditions.

213 by sex and condition are in Table 1. Analysis with mixed models found a significant effect of
 214 species in the dark condition ($F_{1,31} = 5.71, P = 0.037$): *M. spicilegus* learned the maze faster than
 215 *M. m. musculus*. Neither sex nor the interaction between species and sex were significant in the
 216 dark (sex, $F = 0.30, P = 0.59$; species*sex, $F = 2.42, P = 0.13$), and none of these terms were
 217 significant in the light condition (species, $F_{1,20} = 0.002, P = 0.95$; sex, $F = 0.33, P = 0.58$;
 218 species*sex, $F = 0.21, P = 0.65$).

219 **Table 1.** Mean (SD) number of trials to reach criterion for *M. spicilegus* (*Musp*) and *M. m. musculus*
 220 (*Mumu*) males (M) and females (F) that successfully completed the maze task under dark or light
 221 conditions.

	Dark				Light			
	<i>Musp</i> F	<i>Musp</i> M	<i>Mumu</i> F	<i>Mumu</i> M	<i>Musp</i> F	<i>Musp</i> M	<i>Mumu</i> F	<i>Mumu</i> M
Trials to criterion	6.6 (4.54)	8.0 (5.24)	16.0 (3.61)	12.0 (5.7)	9.5 (5.24)	10.0 (4.69)	8.3 (3.86)	6.0 (3.0)
$n_{\text{criterion}}^*$	7	5	3	5	6	4	4	3
n_{total}	8	8	7	8	6	5	5	4
n_{litters}	7	8	4	4	3	5	4	4

222 * Number that reached criterion

223 Using survival analysis to account for mice that failed to reach criterion by the end of the
 224 trial increased the effect of species on trials to criterion in the dark (LRT: $\chi^2_{(1, n=31)} = 11.48, P =$
 225 0.0007). The effect of sex remained non-significant ($\chi^2 = 0.03, P = 0.9$) but the interaction
 226 between species and sex was marginally significant ($\chi^2 = 3.99, P = 0.046$). This interaction was
 227 explained by a tendency for *M. m. musculus* males that met criterion to do so earlier than
 228 females, whereas this pattern was reversed in *M. spicilegus* (Table 1). However, there was no
 229 significant difference between the sexes in either species (ANOVA: *M. m. musculus*, $F_{(1,8)} = 1.15,$
 230 $P = 0.3$; *M. spicilegus*, $F_{(1,12)} = 0.26, P = 0.6$). In the light condition there was no effect of species,
 231 sex, or their interaction (species, $\chi^2_{(1, n=20)} = 1.79, P = 0.2$; sex, $\chi^2 = 0.004, P = 0.95$; species*sex,
 232 $\chi^2 = 1.34, P = 0.3$). Within species, there was no effect of condition on trials to criterion for *M.*

233 *spicilegus* ($\chi^2_{(1, n = 27)} = 1.72, P = 0.2$), whereas *M. m. musculus* performed significantly better in
234 the light ($\chi^2_{(1, n = 24)} = 7.62, P = 0.006$).

235 While observing the dark trials we noticed that house mice seemed more hesitant than
236 mound-building mice in moving through the maze when placed in it for the first time. To
237 separate any species differences in initial response to a novel environment from differences in
238 ability to negotiate a dark environment, we tested for an effect of species on the times it took
239 for mice to leave the start box, and to complete the maze, on their first trial. The species did
240 not differ in the amount of time to leave the start box under either condition (ANOVA: dark,
241 $F_{(1,35)} = 1.04, P = 0.3$; light, $F_{(1,21)} = 0.03, P = 0.9$). However, *M. spicilegus* completed the maze
242 significantly faster than *M. m. musculus* on the first day they encountered it under dark
243 conditions, but not under light conditions (ANOVA, dark: $F_{(1,35)} = 16.36, P = 0.0003$; light: $F_{(1,21)} =$
244 $2.25, P = 0.2$).

245

246 **DISCUSSION**

247 We used a maze-learning task to test for differences in spatial ability between a pair of
248 sympatric but ecologically distinct species of mice, the eastern house mouse, *Mus musculus*
249 *musculus*, and the mound-building mouse, *M. spicilegus*. Given the specialized burrowing
250 ecology of *M. spicilegus* we predicted that this species would perform significantly better than
251 the commensal house mouse when tested in complete darkness without access to allocentric
252 cues. We found strong support for this prediction: in the dark condition, mound-building mice
253 moved through the maze faster when they first encountered it and learned the correct route
254 faster than did house mice. Moreover, there was no species difference when naïve individuals

255 were tested in the same maze with access to visual cues. Together, these results suggest that
256 enhanced ability to navigate using egocentric cues only is an adaptation to life in the burrow
257 systems that mound-building mice construct and occupy for up to half of the year in nature. We
258 discuss these findings in light of the evolutionary ecology and mechanistic basis of egocentric
259 navigation, and the opportunity for future studies of the genetic basis and neural architecture
260 of egocentric navigation and burrow construction in *M. spicilegus*.

261

262 *Ecological correlates of species differences in egocentric navigation*

263 There is robust evidence that cognitive ability, like any other complex trait, can evolve in
264 response to selection pressures in different environments or social contexts (reviewed in
265 Cauchoix & Chaine, 2016; Morand-Ferron et al., 2016). Species, population, and sex differences
266 in spatial learning and memory abilities have been particularly well studied in this ecological
267 framework (e.g., Bruck & Mateo, 2010; Gaulin et al., 1990; Kimchi & Terkel, 2004; Pravosudov
268 and Clayton, 2002). Yet few studies have asked whether differences in spatial cognition are
269 context- or task-specific (reviewed in Gibson & Kamil, 2009), and the relationship between
270 ecology and egocentric navigation ability has received little attention.

271 Mound and burrow construction in *M. spicilegus* is presumed to be an adaptation to
272 harsh seasonal environments: soil temperatures under mounds are elevated and stable relative
273 to the surrounding environment, and larger mounds have a higher proportion of mice that
274 survive the winter (Szenczi et al., 2011). Construction and occupation of mound/burrow
275 systems also shape life history and social structure in *M. spicilegus*. Most construction is carried
276 out by young of the year – animals that delay reproduction till they emerge from the mounds

277 the following spring – and there is evidence for individual task-specialization in the process of
278 mound construction (Serra et al., 2012; Hurtado et al., 2013). The results of this study add
279 enhanced egocentric navigation to the suite of behavioral traits that promote overwinter
280 survival in young mound-building mice.

281 We also found that species differences in spatial learning and memory depend on the
282 types of cues that are available. While house mice performed significantly better with access to
283 allocentric information, mound-building mice performed equally well with or without
284 allocentric cues. To the extent that the maze task approximated spatial problems that each
285 species encounters in nature, these results are consistent with the fact that the two species
286 overlap in aboveground space use where allocentric cues are available, whereas construction
287 and occupation of complex burrows is unique to *M. spicilegus*.

288 While sex differences in spatial ability are widely reported in lab mice and rats, which
289 sex performs better varies with task, genotype, and age (Ennaceur et al., 2008; reviewed in
290 Jonasson, 2005). Considering that male and female *M. spicilegus* overwinter in the same
291 burrow systems, do not differ in behaviors associated with mound construction (Hurtado et al.
292 2013), and mate after dispersal from mounds, we did not expect the sexes to differ in
293 egocentric navigation ability. Although we did not detect sex differences in either species under
294 either condition, there was a marginally significant interaction between sex and species in the
295 dark condition: female *M. spicilegus* tended to perform better than male *M. spicilegus*, whereas
296 the opposite was true for *M. m. musculus* females and males. Sex specific sample sizes in this
297 study were small; it is possible that increased sampling might reveal species-specific effects of

298 sex on egocentric navigation. Future studies should explore the intriguing possibility of opposite
299 patterns of sexual dimorphism in egocentric navigation ability in these closely related species.

300 Space use and burrowing behavior during the reproductive season are not well
301 characterized in natural populations of *M. spicilegus*. However, there is no evidence for
302 continued use of winter burrow systems; indeed, one study found a negative association
303 between the presence of mounds and capture rate for adult females (Gouat et al., 2003). Thus,
304 the proposed selective advantage of enhanced egocentric navigation as applied to learning a
305 fixed route may be specific to the life stage bounded by initial dispersal from the nest and first
306 reproduction. Given that parturition and lactation can enhance spatial cognition in female
307 rodents (e.g., Kinsley et al., 1999) it would be of particular interest to test for effects of
308 motherhood on performance of different types of spatial tasks. For example, path integration –
309 the ability to update spatial position relative to a starting point – relies on the same movement-
310 generated input and neural substrates (see below) as the route-based task used here, but also
311 requires flexibility in the formation of a cognitive map. Since updating her location relative to
312 the location of her nest is exactly what a foraging female must accomplish, we might expect this
313 aspect of egocentric navigation to be specifically enhanced in lactating mound-building mice
314 relative to pre-reproductive conspecifics of both sexes.

315

316 *Mechanisms of species differences in egocentric navigation*

317 *M. spicilegus* is slightly smaller than *M. m. musculus* and differs in tail length and several
318 cranial characters but does not exhibit any of the external phenotypes associated with sensory
319 adaptations to dark environments (e.g., specialized external pinnae, elaboration of vibrissae or

320 nasal soft tissue, modified foot pads or guard hairs) (Sokolov et al., 1998). Thus, species
321 differences in navigational ability in complete darkness are not readily explained by enhanced
322 auditory or tactile sensitivity in *M. spicilegus*. Indeed, maze dimensions were considerably
323 larger than the body width of the animals, such that extensive somatosensory stimuli were not
324 available as mice moved through the maze (e.g., Kimchi & Terkel, 2004). Likewise, by
325 thoroughly cleaning mazes after each trial and rotating maze position across days, we
326 eliminated cues that could provide allocentric information in the dark condition (e.g., odor
327 trails, extra-maze auditory cues, natural or artificial magnetic fields). These observations
328 suggest that enhanced egocentric navigation in *M. spicilegus* reflects more precise processing,
329 and consolidation into memory, of movement-generated input at the level of the central
330 nervous system.

331 While the capacity to generate and retain an internal representation of external spatial
332 relations is traditionally attributed to the hippocampus, work in lab mice and rats demonstrates
333 that interactions between the hippocampus and another forebrain region – the striatum – are
334 of particular importance to egocentric navigation (Mizumori et al., 2009; Chersi & Burgess,
335 2015). The dorsal and ventral regions of the striatum are critical to planned motor output and
336 reward-based learning, respectively. It has been suggested that these two striatal functions are
337 integrated in response learning, the association of body turns with reward (Chersi & Burgess,
338 2015). Within this circuitry, striatal dopamine is critical to egocentric, but not to allocentric,
339 navigation (Braun et al., 2015). In our study, mice learned to follow a route defined by a series
340 of points at which decisions involving body turns were required. The two species performed
341 equally well when allocentric visual cues were available, but *M. spicilegus* out-performed *M. m.*

342 *musculus* when these cues were eliminated and mice were forced to navigate using egocentric
343 input exclusively.

344 Given the close evolutionary relationship between house mice and mound-building
345 mice, and the fact that *M. spicilegus* is not a subterranean specialist, we would not expect to
346 find species differences in the size or structure of brain regions implicated in egocentric
347 navigation. Instead, the results of this study hint at greater hippocampal-striatal connectivity or
348 sensitivity in *M. spicilegus*, potentially mediated by dopaminergic activity. Co-localization of
349 immediate early gene and dopamine receptor activation by an egocentric navigation task would
350 provide a preliminary test of this hypothesis.

351 Finally, because the strains of mice used here to represent each species have been
352 maintained in the lab for many generations and individuals used in the experiment were reared
353 under identical standard conditions, our results indicate that species differences in spatial
354 cognition have a genetic basis. While *M. spicilegus* and *M. m. musculus* do not hybridize in
355 nature, crosses are still possible in the lab (Zechner et al., 1996). Therefore, traits unique to *M.*
356 *spicilegus* are amenable to genetic mapping. Work on the genetics of burrowing behavior in
357 *Peromyscus* mice (Dawson et al., 1988; Weber et al., 2013), nest construction in house mice
358 (Sauce et al., 2012), spatial navigation in rats (Herrera et al., 2013), and olfactory learning and
359 memory in *Nasonia* wasps (Hoedjes et al., 2014) and *Drosophila* (Nepoux et al., 2015),
360 demonstrates the feasibility of this approach for ecologically relevant cognitive traits.

361

362 *Conclusions*

363 Despite the extensive literature on spatial learning and memory in laboratory rodents,
364 and on patterns of space use in natural populations of many species, few studies have asked
365 whether there is a match between species-specific spatial ecology and species differences in
366 egocentric navigation ability. Here, we show that differences in spatial ability between
367 sympatric mouse species are exclusive to egocentric cue use, and that the direction of this
368 difference is consistent with species differences in burrowing ecology. These results highlight
369 the role of ecological selection in the evolution of cognitive traits, and pave the way for future
370 work on the genetic and neural substrates of behaviors that differ between mound-building
371 mice and their commensal relatives (Tong and Hoekstra, 2012).

372

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378 **REFERENCES**

- 379 Alyan, S. H. (1996). Evidence for resetting the directional component of path integration in the
380 house mouse (*Mus musculus*). *Ethology*, *102*, 629-638.
- 381 Bardunias, P. M., & Jander, R. (2000). Three dimensional path integration in the house mouse
382 (*Mus domestica*). *Naturwissenschaften*, *87*(12), 532-534.
- 383 Benhamou, S. (1997). On systems of reference involved in spatial memory. *Behavioural*
384 *Processes*, *40*, 149-163.
- 385 Bouchard, P. R., & Lynch, C. B. (1989). Burrowing behavior in wild house mice: Variation within
386 and between populations. *Behavior Genetics*, *19*, 447-456.
- 387 Braun, A. A., Amos-Kroohs, R. M., Gutierrez, A., Lundgren, K. H., Seroogy, K. B., Skelton, M. R.,
388 Vorhees, C. V., & Williams, M. T. (2015). Dopamine depletion in either the dorsomedial
389 or dorsolateral striatum impairs egocentric Cincinnati water maze performance while
390 sparing allocentric Morris water maze learning. *Neurobiology of Learning and Memory*,
391 *118*, 55-63.
- 392 Brown, C., & Braithwaite, V. A. (2005). Effects of predation pressure on the cognitive ability of
393 the poeciliid *Brachyraphis episcopi*. *Behavioral Ecology*, *16*, 482-487.
- 394 Bruck, J. N., & Mateo, J. M. (2010). How habitat features shape ground squirrel (*Urocitellus*
395 *beldingi*) navigation. *Journal of Comparative Psychology*, *124*, 176-186.
- 396 Canaday, A., Mosansky, L., & Stamlo, M. (2009). First knowledge of winter ecology of the
397 mound-building mouse (*Mus spicilegus* Petenyi, 1882) from Slovakia. *Acta Zoologica*
398 *Bulgarica*, *61*, 79-86.
- 399 Cauchoix, M., & Chaine, A. S. (2016). How can we study the evolution of animal minds?
400 *Frontiers in Psychology*, *7*, 358.
- 401 Chersi, F., & Burgess, N. (2015). The cognitive architecture of spatial navigation: Hippocampal
402 and striatal contributions. *Neuron*, *88*, 64-77.
- 403 Clayton, N. S., & Krebs, J. R. (1994). Memory for spatial and object-specific cues in food-storing
404 and non-storing birds. *Journal of Comparative Physiology A*, *174*, 371-379.
- 405 Costanzo, M. S., Bennett, N. C., & Lutermann, H. (2009). Spatial learning and memory in African
406 mole-rats: The role of sociality and sex. *Physiology and Behavior*, *96*, 128-134.
- 407 Croxall, J. P., Silk, J. R. D., Phillips, R. A., Afanasyev, V., & Briggs, D. R. (2005). Global
408 circumnavigations: Tracking year-round ranges of non-breeding albatrosses. *Science*,
409 *307*, 249-250.
- 410 Dawson, W. D., Lake, C. E., & Schumpert, S. S. (1988). Inheritance of burrow building in
411 *Peromyscus*. *Behavior Genetics*, *18*, 371-382.
- 412 du Toit, L., Bennett, N. C., Nickless, A., & Whiting, M. J. (2012). Influence of spatial environment
413 on maze learning in an African mole-rat. *Animal Cognition*, *15*, 797-806.

414 Egevang, C., Stnhouse, I. J., Phillips, R. A., Petersen, A., Fox, J. W., & Silk, J. R. D. (2010). Tracking
415 of Arctic terns *Sterna paradisaea* reveals longest animal migration. *Proceedings of the*
416 *National Academy of Sciences of the United States of America*, *107*, 2078-2081.

417 Ennaceur, A., Michalikova, S., van Rensburg, R., & Chazot, P. L. (2008). Detailed analysis of the
418 behavior and memory performance of middle-aged male and female CD-1 mice in a 3D
419 maze. *Behavioural Brain Research*, *187*, 312-326.

420 Etienne, A. S., Maurer, R., & Seguinot, V. (1996). Path integration in mammals and its
421 interaction with visual landmarks. *Journal of Experimental Biology*, *199*, 201-209.

422 Etienne, A. S., Maurer, R., Saucy, F., & Teroni, E. (1986). Short distance homing in the golden
423 hamster after a passive outward journey. *Animal Behaviour*, *34*, 696-715.

424 Etienne, A. S., Maurer, R., Berlie, J., Derivaz, V., Georgakopoulos, J., Griffin, A., & Rowe, T.
425 (1998). Cooperation Between Dead Reckoning (Path Integration) and External Position
426 Cues. *The Journal of Navigation*, *51*(01), 23-34.

427 Garza, J. C., Dalla, J., Duryadi, D., Gerasimov, S., Crosets, H., & Boursot, P. (1997). Social
428 structure of the mound-building mouse *Mus spicilegus* revealed by genetic analysis with
429 microsatellites. *Molecular Ecology*, *6*, 1009-1017.

430 Gaulin, S. J., FitzGerald, R. W., & Wartell, M. S. (1990). Sex differences in spatial ability and
431 activity in two vole species (*Microtus ochrogaster* and *Microtus pennsylvanicus*). *Journal*
432 *of Comparative Psychology*, *104*, 88-93.

433 Gibson, B., & Kamil, A. (2009). The synthetic approach to the study of spatial memory: Have we
434 properly addressed Tinbergen's "four questions"? *Behavioural Processes*, *80*, 278-287.

435 Gouat, P., Katona, K., & Poteaux, C. (2003). Is the socio-spatial distribution of mound-building
436 mice, *Mus spicilegus*, compatible with a monogamous mating system? *Mammalia*, *67*,
437 15-24.

438 Herrera, V. L., Pasion, K. A., Tan, G. A., & Ruiz-Opazo, N. (2013). Dahl (S x R) rat congenic strain
439 analysis confirms and defines a chromosome 17 spatial navigation quantitative trait
440 locus to <10 Mbp. *PLoS One*, *8*, e58280.

441 Hoedjes, K. M., Smid, H. M., Vet, L. E. M., & Werren, J. H. (2014). Introgression study reveals two
442 quantitative trait loci involved in interspecific variation in memory retention among
443 *Nasonia* wasp species. *Heredity*, *113*, 542-550.

444 Hurtado, M. J., Fénéron, R., & Gouat, P. (2013). Specialization in building tasks in the mound-
445 building mouse, *Mus spicilegus*. *Animal Behaviour*, *85*, 1153-1160.

446 Israël, I., Grasso, R., Georges-François, P., Tsuzuku, T., & Berthoz, A. (1997). Spatial memory and
447 path integration studied by self-driven passive linear displacement. I. Basic properties.
448 *Journal of Neurophysiology*, *77*, 3180-3192.

449 Jonasson, Z. (2005). Meta-analysis of sex differences in rodent models of learning and memory:
450 a reiew of behavioral and biological data. *Neuroscience and Biobehavioral Reviews*, *28*,
451 811-825.

452 Jones, C. M., Braithwaite, V. A., & Healy, S. D. (2003). The evolution of sex differences in spatial
453 ability. *Behavioral Neuroscience*, *117*, 403-411.

454 Kimchi, T., & Terkel, J. (2002). Seeing and not seeing. *Current Opinion in Neurobiology*, *12*, 728-
455 734.

456 Kimchi, T., & Terkel, J. (2004). Comparison of the role of somatosensory stimuli in maze learning
457 in a blind subterranean rodent and a sighted surface-dwelling rodent. *Behavioural Brain*
458 *Research*, *153*, 389-395.

459 Kimchi, T., Etienne, A. S., & Terkel, J. (2004). A subterranean mammal uses the magnetic
460 compass for path integration. *Proceedings of the National Academy of Sciences of the*
461 *U.S.A.*, *101*, 1105-1109.

462 Kinsley, C. H., Madonia, L., Gifford, G. W., Tureski, K., Griffin, G. R., Lowry, C., Williams, J.,
463 Collins, J., McLearn, H., & Lambert, K. G. (1999). Motherhood improves learning and
464 memory - Neural activity in rats is enhanced by pregnancy and the demands of rearing
465 offspring. *Nature*, *402*, 137-138.

466 Lazic, S. E., & Essioux, L. (2013). Improving basic and translational science by accounting for
467 litter-to-litter variation in animal models. *BMC Neuroscience*, *14*, 37.

468 Mateo, J. M. (2008). Inverted-U shape relationship between cortisol and learning in ground
469 squirrels. *Neurobiology of Learning and Memory*, *89*, 582-590.

470 Mittelstaedt, H., & Mittelstaedt, M.-L. (1980). Homing by path integration in a mammal.
471 *Naturwissenschaften*, *67*, 566-567.

472 Mizumori, S. J. Y., Puryear, C. B., & Martig, A. K. (2009). Basal ganglia contributions to adaptive
473 navigation. *Behavioural Brain Research*, *199*, 32-42.

474 Morand-Ferron, J., Cole, E. F., & Quinn, J. L. (2016). Studying the evolutionary ecology of
475 cognition in the wild: a review of practical and conceptual challenges. *Biological*
476 *Reviews*, *91*, 367-389.

477 Muheim, R., Edgar, N. M., Sloan, K. A., & Phillips, J. B. (2006). Magnetic compass orientation in
478 C57BL/6J mice. *Learning and Behavior*, *34*, 366-373.

479 Müller, M., & Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proceedings*
480 *of the National Academy of Sciences*, *85*(14), 5287-5290.

481 Muntyanu, A. I. (1990). Ecological features of an overwintering population of the hillock mouse
482 (*Mus hortulanus* Nordm.) in the south-west of the U.S.S.R. *Biological Journal of the*
483 *Linnean Society*, *41*, 73-82.

484 Nepoux, V., Babin, A., Haag, C., Kawecki, T. J., & Le Rouzic, A. (2015). Quantitative genetics of
485 learning ability and resistance to stress in *Drosophila melanogaster*. *Ecology and*
486 *Evolution*, *5*, 543-556.

487 Odling-Smee, L. C., Boughman, J. W., & Braithwaite, V. A. (2008). Sympatric species of
488 threespine stickleback differ in their performance in a spatial learning task. *Behavioral*
489 *Ecology and Sociobiology*, *62*, 1935-1945.

490 Poteaux, C., Busquet, N., Gouat, P., Katona, K., & Baudoin, C. (2008). Socio-genetic structure of
491 mound-building mice, *Mus spicilegus*, in autumn and early spring. *Biological Journal of*
492 *the Linnean Society*, *93*, 689-699.

493 Pravosudov, V. V., & Clayton, N. S. (2002). A test of the adaptive specialization hypothesis:
494 population differences in caching, memory, and the hippocampus in black-capped
495 chickadees (*Poecile atricapilla*). *Behavioral Neuroscience*, *116*, 515-522.

496 Pravosudov, V. V., Kitaysky, A. S., & Omanska, A. (2006). The relationship between migratory
497 behaviour, memory and the hippocampus: an intraspecific comparison. *Proceedings of*
498 *the Royal Society B*, *273*, 2641-2649.

499 Presotto, A., & Izar, P. (2010). Spatial reference of black capuchin monkeys in Brazilian Atlantic
500 Forest: egocentric or allocentric? *Animal Behaviour*, *80*, 125-132.

501 Sauce, B., de Brito, R. A., & Peripato, A. C. (2012). Genetic architecture of nest building in mice
502 LG/J x SM/J. *Frontiers in Genetics*, *3*, 90.

503 Schmid-Holmes, S., Drickamer, L. C., Robinson, A. S., & Gillie, L. L. (2001). Burrows and burrow-
504 cleaning behavior of house mice (*Mus musculus domesticus*). *American Midland*
505 *Naturalist*, *146*, 53-62.

506 Schneider, N. Y., Fletcher, T. P., Shaw, G., & Renfree, M. B. (2009). The olfactory system of the
507 tammar wallaby is developed at birth and directs the neonate to its mother's pouch
508 odours. *Reproduction*, *138*, 849-857.

509 Serra, J., Hurtado, M. J., Le Négrate, A., Féron, C., Nowak, R., & Gouat, P. (2012). Behavioural
510 differentiation during collective building in wild mice *Mus spicilegus*. *Behavioural*
511 *Processes*, *89*, 292-298.

512 Shettleworth, S. (2010). *Cognition, Evolution and Behavior* (2nd ed.). Oxford, U.K.: Oxford
513 University Press.

514 Sokolov, V. E., Kotenkova, E. V., & Michailenko, A. G. (1998). *Mus spicilegus*. *Mammalian*
515 *Species*, *592*, 1-6.

516 Srinivasan, M. V. (2015). Where paths meet and cross: navigation by path integration in the
517 desert ant and the honeybee. *Journal of Comparative Physiology A*, *201*, 533-546.

518 Srinivasan, M. V., Zhang, S. W., & Bidwell, N. J. (1997). Visually mediated odometry in
519 honeybees. *Journal of Experimental Biology*, *200*, 2513-2522.

520 Srinivasan, M. V., Zhang, S. W., Lehrer, M., & Collett, T. S. J. (1996). Honeybee navigation en
521 route to the goal: Visual flight control and odometry. *Journal of Experimental Biology*,
522 *199*, 155-162.

523 Szenczi, P., Bánszegi, O., Dúcs, A., Gedeon, C. I., Markó, G., Németh, I., & Altbäcker, V. (2011).
524 Morphology and function of communal mounds of overwintering mound-building mice
525 (*Mus spicilegus*). *Journal of Mammalogy*, *92*, 852-860.

- 526 Szenczi, P., Kocso, D., Bánszegi, O., & Altbäcker, V. (2012). The contribution of the vegetable
527 material layer to the insulation capacities and water proofing of artificial *Mus spicilegus*
528 mounds. *Mammalian Biology*, *77*, 327-331.
- 529 Tong, W., & Hoekstra, H. (2012). *Mus spicilegus*. *Current Biology*, *22*, R858-R859.
- 530 Tyndale-Biscoe, C. H., & Renfree, M. B. (1987). *Reproductive Physiology of Marsupials*.
531 Cambridge, U. K.: Cambridge University Press.
- 532 Weber, J. N., Peterson, B. K., & Hoekstra, H. E. (2013). Discrete genetic modules are responsible
533 for complex burrow evolution in *Peromyscus mice*. *Nature*, *493*, 402-405.
- 534 Wehner, R., & Srinivasan, M. V. (1981). Searching behavior of desert ants, genus *Cataglyphis*
535 (Formicidae, Hymenoptera). *Journal of Comparative Physiology A*, *142*, 315-338.
- 536 Wehner, R., & Srinivasan, M. V. (2003). Path integration in insects. In K. J. Jeffery (Ed.), *The*
537 *Neurobiology of Spatial Behaviour* (pp. 9–30). Oxford, U.K.: Oxford University Press.
- 538 Zechner, U., Reule, M., Orth, A., Bonhomme, F., Strack, B., Guenet, J. L., Hameister, H., &
539 Fundele, R. (1996). An X-chromosome linked locus contributes to abnormal placental
540 development in mouse interspecies hybrids. *Nature Genetics*, *12*, 398-403.

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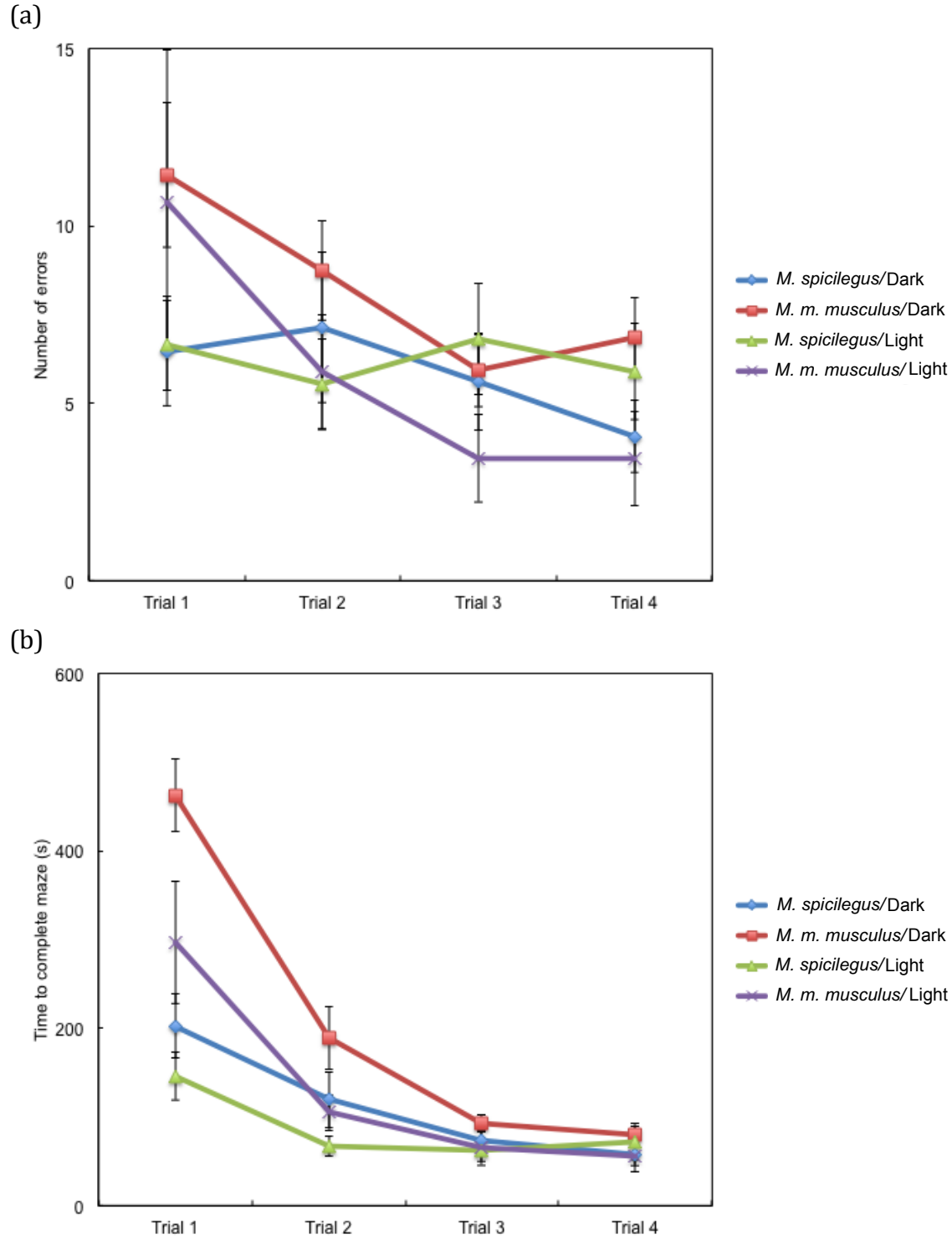


Figure S1. Mean number of errors (a) and time to complete maze (b) for *Mus spicilegus* and *M. m. musculus* under dark and light conditions during the first four trials. Because mice were removed from the study once they reached criterion for successful maze completion, errors and latencies to complete the maze for trials 5–21 are increasingly biased towards mice that performed less well, and are therefore not informative. Error bars are \pm SE.