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Female mound-building mice prefer males that invest more in building behavior, even when this behavior is not observed

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Abstract

Through behavioral correlations, mate choice could influence the evolution of traits that are not directly selected for, or even observed. We addressed whether mound building, a unique collective behavior observed in *Mus spicilegus*, could be favored by female mate choice, irrespective of whether females were able to observe the behavior. First, we introduced mixed sex groups of wild-born mice in large terraria with building materials, and assessed male variation in building investment. Second, we presented females with a choice between males that invested the most versus the least in building. Females were either able to observe the males during building or not. Third, because overwintering juveniles rely on mound protection, we hypothesized that building could be a form of paternal care, and assessed whether males that invested more in building also invested more in direct offspring care. We showed that females were more attracted to males that invested the most in building, even when these behaviors were not observed. In addition, direct offspring care was negatively correlated with males' investment in building, suggesting that two alternative paternal care strategies (mound building versus direct offspring care) may exist. Our study supports the hypothesis that building could be detectable by phenotypic cues that differ from building behavior *per se* and that mate choice may influence the evolution and maintenance of mound building that several authors describe as a common good.

Keywords: cues, parental investment, preference, personality, sexual selection, cooperation

Significance statement

In the mound-building mouse, individuals gather to build a common mound within which juveniles will spend the winter months. As some males invest more in building than others, we questioned whether females would prefer males that invest more in building behaviors, even though females could not observe males' building behaviors before mate choice. We assessed male investment in building and conducted choice tests. Females were more attracted to males that invested more in building, even when building was not observed. Hence, building investment seems detectable by phenotypic cues that differ from building behavior *per se.* Further, the males' investment in building was negatively correlated with their direct offspring care assessed during retrieval trials. Our findings indicate that two alternative paternal care strategies may exist in this species, and that mate choice might influence the evolution of their remarkable collective building.

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Introduction

Behavioral traits often form a suite of correlated traits, so-called behavioral syndromes (Sih et al. 2004a, b; Réale et al. 2010). For example, aggressiveness is associated positively with exploratory behavior and boldness in a number of species (Sih et al. 2004a, b; Groothuis and Carere 2005; Boon et al. 2008; Réale et al. 2009), and negatively associated with parental care (Mutzel et al. 2013). These behavioral correlations could result from natural and sexual selection that favor particular trait combinations (Sih et al. 2004a; Réale et al. 2010; Schuett et al. 2010, 2011; Pruitt et al. 2011; Kortet et al. 2012). Because of such associations, mate choice could indirectly select for a behavioral trait that is not a mate choice criterion, or even for signals or cues of behaviors that mates did not observe. For example, by preferring exploratory males, zebra finch females (*Taeniopygia guttata*) simultaneously select for more aggressive males due to an association between these traits (Schuett et al. 2011). Here we address whether individuals' investment in a common good could be indirectly selected for by an association of such behaviors with other sexually selected traits or cues. Such an association would indicate a potential role of mate preference, and possibly sexual selection, in the evolution of collective behavior and cooperation (Putland 2001; Tognetti et al. 2012; Van Vugt and Iredale 2013; Arnocky et al. 2017).

Our study model, *Mus spicilegus,* is a socially monogamous mouse, endemic to southeastern Europe, which possesses a mound-building behavior that is unique among mice (Tong and Hoekstra 2012). After a period of reproduction from spring to late summer, during which each adult breeding pair produces 4 to 5 litters in individual burrows of simple design (Sokolov et al. 1998), several individuals gather in late summer to collectively build a common structure in which juveniles born in late summer and early autumn will overwinter in a nest chamber located under the mound (Muntyanu 1990; Garza et al. 1997; Poteaux et al. 2008; Canady et al. 2009; Tong 2012). Several studies indicate that juveniles in a given mound may originate from several breeding pairs (up to 4) where the females are genetically related but the males are unrelated (Garza et al. 1997; Poteaux et al. 2008). In early spring, these mice leave their mounds, disperse (alone or in small kin groups) and form breeding pairs

(Muntyanu 1990; Sokolov et al. 1998; Simeonovska-Nikolova and Gerasimov 2000; Poteaux et al. 2008). In the northern part of the species' range, construction of mounds takes 14-21 days and occurs from mid-August to mid-November (Muntyanu 1990; Sokolov et al. 1998; Canady et al. 2009). The mounds are built by the accumulation of plant materials covered with earth and are up to four meters in diameter and typically 0.5 meters high when freshly built (Muntyanu 1990; Sokolov et al. 1998), which supports the view that they are built collectively (a single mouse cannot build such a large structure alone (Szenczi 2012)).

The precise age structure of mound builders has yet to be established in natural conditions (Hölzl et al. 2009; Tong 2012; Tong and Hoekstra 2012). Indeed, direct observations of mound building in nature are scarce. Furthermore, trapping success can be relatively low during mound building. For example, observations done during the mound-building period in semi-natural conditions suggest that, even when they were known to be present, the adults were particularly difficult to trap (Orsini 1982). Moreover, indirect evidence through trapping around the mounds revealed that adults could represent 40% to 60% of the population of mice captured from late August to late October (Simeonovska-Nikolova 2007; Canady et al. 2009; Simeonovska-Nikolova 2012; Tong 2012) indicating that, although often not detected, adults could be present during the mound construction and that juveniles could also contribute to building (Sokolov et al. 1998; Canady et al. 2009). From November, although some adults are still found around the mounds (Canady et al. 2009; Tong 2012), the population of juveniles increases substantially (Garza et al. 1997; Milishnikov et al. 1998; Canady et al. 2009; Tong 2012), which could reflect further gathering of mice and/or events of reproduction during the building season.

Altogether, based on the available literature, it seems that the age structure of mound builders may vary, and that one or several breeding pairs and their juveniles of 3-4 weeks old, or only the juveniles, may contribute to build the mound. Hence, selection may drive mound building behavior directly and/or indirectly by increasing survival of juveniles. Here we address whether males that invest more in mound building are preferred by females.

The propensity to build a mound is genetically heritable (Orsini 1982), and investment in mound building in captivity varies between individuals (Simeonovska-Nikolova and Mehmed 2009; Serra et al. 2012; Hurtado et al. 2013). Moreover, mound size is highly variable in natural conditions (Hölzl et al. 2009) and is positively correlated with insulation against water and temperature fluctuations (Hölzl et al. 2011; Szenczi et al. 2011, 2012). Since small energy savings influence animal survival even in temperate climate (Vogt and Lynch 1982; Geiser 2004), mound size is thus expected to influence the probability of offspring survival during the European continental winter (Hölzl et al. 2011; Szenczi et al. 2011, 2012). Hence, it is likely that females would benefit from choosing hardworking builders as such partners would invest more in mound building and/or transmit their building ability to their offspring and therefore improve the probability of offspring survival during winter.

However, female mate choice takes place before mound building (for adult builders) or after the wintering period (for juvenile builders) and, thus, before the potential display of male building behavior (Muntyanu 1990; Sokolov et al. 1998). Hence, the ability of females to detect male building behavior through other signals/cues should be positively selected for. Alternatively, females could indirectly select for male building behavior through their preferences for other traits associated with males' investment in building. Furthermore, investment in mound building might be seen as a form of parental care. Behavioral experiments in *Mus spicilegus* revealed that males invest in offspring attendance, and that highly attendant fathers increase not only their own reproductive success but also that of their mate (Patris and Baudoin 2000; Feron and Gouat 2007). Parental investment was shown to be advertised by phenotypic traits in several species (Hill 1991; Östlund and Ahnesjö 1998; Préault et al. 2005; Hill and McGraw 2006; Gleason and Marler 2010; Bartsch et al. 2015) and could thus allow females to select good fathers. Hence, a secondary aim of this study was to test whether males that invest highly in mound building also attend more to their offspring, or if these two forms of investment are uncoupled.

We designed our experiment to test whether females preferred males that varied in their contribution in mound building when this behavior was observed versus unobserved, and whether

133 male investment in mound building was positively associated with direct paternal care. We assessed
134 male spontaneous building behavior exhibited during mound construction in captivity by wild mice
135 captured in agricultural fields in Bulgaria while juveniles. Then, we used two-way choice tests to
136 assess females' attraction to males that invest more in mound building versus males that invest less.
137 Finally, we evaluated post-mating paternal care exhibited by males with different investment in
138 building behavior using pup retrieval experiments: we recorded the number of pups each male
139 retrieved back to the nest after they were removed from it, and the latency to engage in the retrieving
140 behavior. We predicted that females would be more attracted to males that invest more versus less in
141 mound building even when the former did not observe them build. We also predicted that males that
142 invest more in building would invest more in direct paternal care.

143

144 **METHODS**

145 *Capture of wild mice*

146 Mound-building mice were captured in northern Bulgaria in September 2011 and were then kept in the
147 animal facilities of the University of Montpellier in France. We caught the mice in an agricultural area,
148 at least 2 km away from the village of Rakita (GPS coordinates: 43°16'13.171"N 24°16'16.447"E),
149 using Sherman live-traps. At this time of year, the mice had already built their mounds. Live-traps
150 were set around each mound. We weighed and measured all the mice from the nose to the base of their
151 tail upon capture. A total of 30 males and 28 females, all subadults, captured from 14 mounds were
152 used in this study. Same-mound mice, irrespective of their sex, were kept in the same laboratory cages
153 from their capture until the start of the experiments two months later.

154

155 *Male investment in mound building*

156 We measured the contribution of each male to mound and nest building in large terraria two
157 months after capture. This experiment lasted 8 weeks. Earlier studies have shown that even laboratory-

158 born mice of this species would engage in mound building when building material is made available,
159 suggesting that mound building is a spontaneous behavior (Orsini 1982; Hurtado et al. 2013). Ten
160 groups of three males and two females were constituted. Sex composition was based on the sex-ratio
161 observed in the wild during the mound building period (Simeonovska-Nikolova and Gerasimov 2000;
162 Simeonovska-Nikolova and Mehmed 2009). We placed each group in a large terrarium (1 x 1 x 0.8 m)
163 containing approximately 0.2 m^3 of earth and stones. The room was maintained at 21/23°C under a
164 12:12 light/dark cycle (corresponding roughly to the photoperiod observed in the field in
165 September/October) with lights on at 9.00 pm. Water was available *ad libitum*, and food was provided
166 on a weekly basis, although daily visits were made to check the well-being of the mice. At the
167 beginning of each observation session, straw and seeds were provided as building materials in addition
168 to the earth and stones already present in the terrariums. Both males and females participated in
169 building. However, we only recorded male behaviors. For each male, five categories of behavior were
170 recorded: the number of times it dug, carried straw, carried seeds, carried stones, and entered the nest
171 (Fig. S1). The latter was recorded because the mice were observed entering the nest to build it and
172 shape it from inside. A single investigator (AT) observed each group during 5 sessions of 1 hour each,
173 approx. one week apart. Males were ear tagged (7.8 x 2.8 mm, Fine Science Tools GmbH) for
174 identification. For each terrarium, the number of metallic earrings (1 or 2) and the side of the tag (right
175 ear, left ear, or both) allowed individual mice to be recognized without having to handle them.

176 We could constitute only five groups of same-mound individuals (three males and two
177 females). The other five groups included three same-mound males to limit aggression (Sokolov et al.
178 1998) and two same-mound females from a mound different to that of the males. Unfortunately, for
179 four out of these five groups, we observed high female mortality (n=8) during the first night in the
180 enclosure, most likely due to males' aggressiveness. Following this observation, we immediately
181 removed the two remaining females from the fifth terrarium and placed them in a separate cage.

182 We averaged the data collected during the five observation sessions for each male and for each
183 of the five behavioral items recorded during building (transport of straw, seeds, and stone, digging and
184 entering the nest). As highlighted in Fig. S1, mice build a nest as well as a structure closely resembling

185 a mound that had all the characteristics described in the field: i.e. seed storage covered with earth and
186 plants materials. However, although seed transport and storage did take place (Fig. S1), because 22 out
187 of 30 mice were not observed transporting seeds, we removed this variable from the analysis. A
188 principal component analysis (PCA) was applied to the four remaining behavioral items with the aim
189 of extracting a single factor reflecting male building investment (function *prcomp* in R).

190 At the end of this experiment, all individuals were transferred to laboratory cages, and females
191 and males of a given group were kept in separate cages to prevent reproduction. They were maintained
192 in laboratory conditions under a 13.5:10.5 h light/dark cycle (light at 6.30 pm), corresponding to the
193 photoperiod during the breeding season (early spring). Food and water were available *ad libitum*, and
194 cotton was provided as nesting material. They were kept in these conditions until the beginning of the
195 next experiment.

196

197 *Female attraction*

198 We measured female attraction to males in a Y maze following the procedure described in Smadja and
199 Ganem (2002) six months after their capture. The stimuli were composed of 5 pairs of males. Males of
200 each pair shared the same terrarium during the mound-building experiment and belonged to one of the
201 five terraria in which females were present. In each of these terraria, we selected among the three
202 males the male that invested the most and the male that invested the least in order to present to the
203 females a choice between contrasting levels of building behavior. To that aim, we used the PCA factor
204 reflecting the most male investment in building (see Results).

205 Each female was tested once. We tested female attraction to males that invested the most
206 versus the least in building when building was observed (females that shared an enclosure with 3
207 males during mound building were presented with a choice between the male that invested in building
208 the most or the least in the triad) (n=10 females), and when building was unobserved (females that did
209 not share an enclosure with males were given the choice between a pair of males composed of the
210 males that invested the most and the least from a triad randomly chosen) (n=10 females).

211 A single investigator (AT) conducted all the tests. All mice were more than 6 months old and
212 sexually mature. To maximize the expression of sexual attraction, females were tested while sexually
213 receptive (i.e., estrus or proestrus/estrus, assessed with vaginal smears).

214 The Y maze was transparent (plexiglas and plastic ware) and composed of a main branch (5
215 cm diameter, 35 cm long) connected to two secondary branches (5 cm diameter, 25 cm long). Boxes
216 (35 x 23 x 13 cm) with transparent perforated doors were connected at the end of each branch (for an
217 illustration of the apparatus see: Smadja and Ganem 2002). One week before the experiment, each
218 female was allowed to explore the empty Y maze for 15 minutes in order to become habituated.

219 At the start of each test, a female was placed in a box connected to the main branch of the
220 apparatus. The two stimuli were randomly assigned to one or the other peripheral boxes (the identities
221 of the stimuli was not known by the observer). We then opened the door of the female's box and
222 started to record its behavior when the female crossed the box door. In all tests, the females entered
223 both secondary branches of the Y maze. During the 10-minute observation, we recorded the time spent
224 by females: i) in each secondary branch (including when females were in contact with the perforated
225 door but when the male was not behind the door), and ii) interacting with the male, or sniffing or
226 licking the transparent perforated door when the male was just behind the door. This is a classical
227 method to measure female preference (e.g. Smadja and Ganem 2005; Latour et al. 2013) that was
228 shown to constitute a good proxy of female propensity to engage in mating with the preferred male
229 (Patris and Baudoin 1998; Smadja and Ganem 2002). We implemented General Linear Mixed Models
230 to test whether male attraction was influenced by their investment in building. For this purpose, we
231 used GLMMs with a quasi-binomial error structure (*glmmPQL* function of the *MASS* package in R) in
232 which we included the identity of each pair of males as a random effect. In the first model, the
233 response variable was the proportion of time spent by the female in the left versus right side of the
234 maze. In a second model, it was the proportion of time the female was in close contact with the male
235 of the left versus right side of the maze. In both models, we included two explanatory variables: (i) a
236 binary variable concerning male investment in building (1 if the male on the left was the one that
237 invested the most in building behaviors, 0 if it was the male that invested the least) and (ii) a binary

238 variable reflecting the treatment (1 if the female observed male building behaviors, 0 if not). We also
239 tested the interaction between these two variables to test whether observing male building influenced
240 female preferences. When the interaction was not significant, we removed it from the model.

241 At the end of the experiment, all individuals were returned to their home cage and maintained
242 under laboratory conditions until the next experiment.

243

244 *Direct paternal care*

245 We used a pup retrieval procedure, a test used commonly to measure parental care in rodents (Dudley
246 1974; Cohen-Salmon et al. 1985), including *Mus spicilegus* (Patris and Baudoin 2000). We first
247 randomly paired females (n=20) and 'unfamiliar' males (i.e., captured in different mounds in the
248 field). During the first week, the two members of the pair were maintained in the same cage but
249 separated with a wire net so that they could first become familiar with each other. We maintained them
250 in laboratory conditions under a 13.5:10.5 light/dark cycle (light at 6.30 pm), corresponding to the
251 photoperiod of the breeding season in the field. Food and water were available *ad libitum*, and cotton
252 was provided as nesting material. Ten of the 20 pairs successfully bred (a low breeding rate is often
253 observed for wild-born mice kept under laboratory conditions, GG pers. obs.) and could hence
254 participate in this experiment that began 18 months after capture. Litter size varied from 5 to 12 pups.

255 On the day of birth, we placed the mice in a large terrarium (70 x 30 x 30 cm). The terrarium
256 contained clean sawdust, food and water. One corner also contained cotton and cardboard rolls as nest-
257 building material.

258 All males were tested twice: approximately four days (mean ± SD: 3.7 ± 0.8 days) and six
259 days (6.3 ± 0.9 days) after the birth of their first litter. During these two sessions, we first removed the
260 breeding pair from the terrarium. We then removed three of the pups from the nest and placed them at
261 the opposite end of the terrarium. We isolated these three pups from their littermates and the nest by
262 placing a transparent plastic separation in the middle of the terrarium. The male was then put back in

263 the side of the terrarium containing the nest. After 30 seconds, we removed the plastic separation and
264 we measured (i) male latency before the start of retrieval of the isolated pups, and (ii) the number of
265 pups retrieved to the nest during the next fifteen minutes. Both of these measures were averaged over
266 the two test sessions, and we used Spearman correlation tests (two-tailed tests, function *cor.test* in R)
267 to assess their potential association with the male building score measured during the mound-building
268 experiment (first experiment above). For males that did not retrieve any pups, the male latency to
269 retrieve was set at 15 minutes (i.e., the duration of the experiment). Because the Spearman correlation
270 test relies on ranks, this choice did not influence the results.

271 Because this species is nocturnal and particularly active during the first few dark hours
272 (Simeonovska-Nikolova and Mehmed 2009), all observations (mound building, female preferences,
273 and paternal investment) were conducted one to two hours after the beginning of the dark period under
274 dim red light. To minimize observer bias, we used blind methods when behavioral data were recorded
275 (preferences tests and retrieval experiments). We recorded all observations using the Observer
276 software Version 5 (Noldus Information Technology). All statistical analyses were performed using
277 the R software, version 3.4.2 (R Core Team 2017).

278

279 **RESULTS**

280 *Male investment in building*

281 In total, during the 50 hours of observation, each male transported 0 to 70 pieces of straw (mean ± SD:
282 18.1 ± 19.4), and 1 to 99 (20.9 ± 23.9) stones (approximately 1 cm^3 in size). The frequency of digging
283 varied between 0 and 35 times (10.1 ± 8.9), and they entered their nest 2 to 35 times (12.6 ± 9.9). We
284 used a PCA to extract a single factor reflecting male global investment in mound building and
285 avoiding giving too much weight to a specific behavior. The first two axes of the PCA, including the
286 four building items, captured 80% (45% and 34% respectively) of the total variation. The first axis
287 was positively correlated with the number of times a male entered the nest (r = 0.42), negatively
288 correlated with stones transport (r = -0.66) and digging frequency (r = -0.62), and not correlated with

289 straw transport (r = 0.06). We hence considered that this axis could represent a measure of
290 specialization in building tasks (Serra et al. 2012; Hurtado et al. 2013) rather than a measure of global
291 investment. The second axis was positively correlated with all four variables: frequency of digging (r
292 = 0.28), number of stones (r = 0.17) and straw transported (r = 0.76), and number of entrances into the
293 nest (r = 0.57), and we considered that it could reflect male global investment in building and hence a
294 pertinent measure allowing to identify males that invested the most and the least in building behaviors.

295 Investment in building was not related to male weight (10.26±0.87 grams) or size (7.14±0.34
296 cm) at capture (Spearman correlation test: weight: $\rho = 0.11$, $p = 0.58$, size: $\rho = 0.01$, $p = 0.95$) nor to
297 their weight (11.64±1.6 grams) at the end of the building experiment ($\rho = 0.13$, $p = 0.53$). Moreover,
298 we did not detect any difference in individual building when comparing males belonging to mixed
299 groups (containing females) to males belonging to all-male groups (Wilcoxon rank sum test: W = 86,
300 $p = 0.43$).

301

302 *Female attraction*

303 We found no effect of the interaction between male investment in building and treatment in any
304 model, suggesting that observing male building behaviors did not influence female attraction
305 (glmmPQL with time spent by females (n=20) in the branches as response variable: t=1.00, *P*=0.92;
306 glmmPQL with time spent by females (n=15) in close proximity to the males as response variable: t=-
307 1.03, *P*=0.34).

308 Male investment in building did not influence the proportion of time females (n=20) spent
309 exploring the two branches (52% of time was spent on the side of the male that invested the most
310 versus the least; glmmPQL: t=1.30, *P*=0.21; Table S1; Fig. 1). However, it did significantly influence
311 the proportion of time females (n=15) spent in proximity to the males (75% of time was spent close to
312 the male that invested the most versus the least; glmmPQL: t=3.69, *P*=0.006; Table S2; Fig. 1):
313 females spent more time in close proximity to the male that invested the most in building behaviors
314 compared to the time spent with the male that invested the least (median [1st quartile; 3rd quartile] of

315 the difference of time spent with the male that invested the most versus the least in building
316 behaviors=8.4 [4.6; 11.7] s).

317

318 *Investment in mound building & direct paternal care*

319 The males (n = 10) retrieved 0 to 3 pups at each session (median [1st quartile; 3rd quartile]: 1.5 [0; 3]
320 for the first session; 2.5 [0.25; 3] for the second session). For each male, the number of pups retrieved
321 during the first versus the second session was not significantly different (paired Wilcoxon rank test: 0
322 [-0.75; 0] pups, V = 5.5, p = 0.68). For sessions in which at least one pup was retrieved (n=12), the
323 latency to retrieve the first pup was between 23 and 810 seconds (171 [36; 325] s).

324 Direct paternal care was negatively associated with male investment in building: across the 20
325 sessions, the males that invested more in building retrieved fewer pups back to the nest (Spearman
326 correlation test: n=10 males, ρ = -0.67, p = 0.04; Fig. 2a) and presented a higher latency to retrieve the
327 first pup (n=10 males, ρ = 0.71, p = 0.02; Fig. 2b).

328

329 **Discussion:**

330 Mate choice and sexual selection could favor the evolution of traits that are not directly selected for
331 (Schuett et al. 2010, 2011). Here we addressed whether mound building, a spontaneous behavior
332 observed both in wild- and laboratory-born individuals of *Mus spicilegus*, could be favored during
333 female mate choice even though females were not able to observe the building activity of males.
334 Indeed, the presence of adults and juveniles around mounds during the building period suggests that
335 the two cohorts (adults and juveniles when 3-4 weeks old) could be involved in building
336 (Simeonovska-Nikolova 2007, 2012; Canady et al. 2009; Tong 2012), further indicating that mate
337 choice would take place before mound building (for the adults) or after (for the juveniles) and involve
338 unfamiliar partners (Garza et al. 1997). Aiming to get insight into the mechanisms favoring building
339 behavior, we asked whether female mate choice could favor males that invest more versus less in

340 mound building behaviors and whether males that invest more are also more involved in direct
341 parental care using pup-retrieval experiments.

342 Our findings indicate that females were more attracted to males that invested more in building
343 behaviors over males that invested less, even when building behaviors were not observed. This
344 suggests that female attraction to males could be influenced by cues that are different from building
345 behaviors *per se*, or by attraction to traits associated with male investment in building. We also show
346 that direct parental care is negatively correlated with male investment in mound building, suggesting a
347 trade-off between these two behaviors.

348 Our findings raise the question of which signals or cues females are responding to when they
349 show an attraction to males that invest more in mound building. In our experiment, females were more
350 attracted to males that invested more versus less in building, even without having observed them build.
351 Hence, phenotypic cues advertising male investment in building may exist in this species. Such cues
352 could be based on acoustic, olfactory, or visual traits, since all of them are involved in mice
353 communication (e.g.: Hurst and Beynon 2004; Musolf et al. 2010) and were available to females in
354 our test apparatus. Their detection by females could influence their sexual preferences, as some sexual
355 pheromones do in several species, including mice (Roberts et al. 2010; Li and Liberles 2015). An
356 alternative explanation could also be that female attraction to males that invest more in building was
357 not directed to building *per se* but to other traits with which investment in building is associated. We
358 did not observe any link between male size or weight and investment in building, suggesting that male
359 condition is unlikely to be one of these traits. Interestingly, experimental studies in the mound-
360 building mouse found that agonistic behaviors between unfamiliar males and females are positively
361 linked with sexual motivation (Busquet et al. 2009; Simeonovska-Nikolova and Lomlieva 2012).
362 Because of the expected high cost of mound building and its important role in the success of
363 overwintering, individuals that invest more in mound building are likely to more aggressively protect
364 resources and defend their mound from intruders. We can therefore speculate that male aggressiveness
365 or dominance might be sexually selected traits associated with building, leading to the indirect
366 selection of building behavior through female mate choice. During our preference tests, we did not

observe agonistic behaviors between males and females through the perforated doors; furthermore, males that invested more in mound building were not observed to try to attract more female attention than males that invested less. However, chemosensory cues of dominance and aggressiveness are present in the urine and preputial glands of male mice and therefore could have been detected by females during their interactions with the males (Harvey et al. 1989; Hurst and Beynon 2004; Soini et al. 2009; Mucignat-Caretta and Caretta 2014). Follow-up studies should thus investigate which traits are used by female *Mus spicilegus* during mate choice that are associated with building behavior. Such studies may particularly focus on dominance, aggressiveness and other personality traits.

In *Mus spicilegus*, the inclination of females to prefer males that invest more in mound building could be an adaptive strategy. Choosing a male that invests highly in building could increase females' fitness in several ways. First, since mound size is positively correlated with water-insulation and soil temperature inside the mound (Hölz et al. 2011; Szenczi et al. 2011, 2012), choosing a male that invests more energy and time in mound building could increase offspring survival during the winter. Second, mound-building behavior seems to be genetically heritable (Orsini 1982). If the level of investment in building was also heritable, by choosing a male that invests more in building, females could improve the ability of their offspring to build a mound, and hence increase the chance of survival of their own progeny. Third, given the attractiveness of the males that invest more in building behaviors, females could also increase their sons' reproductive success (sexy son hypothesis: Weatherhead and Robertson 1979).

Interestingly, we found a negative correlation between direct paternal care and building investment. Previous experimental studies involving the mound-building mouse found intense paternal investment, such as covering and warming the pups (Patris and Baudoin 2000), and males that exhibited the highest levels of offspring attendance increased their reproductive success by reducing their mate's inter-litter intervals (Feron and Gouat 2007). As both parental investment and building are likely to be energetically costly, the negative association observed between them may indicate the existence of a tradeoff. In addition, since parents are likely to build a mound for their offspring, mound building may be considered a form of parental care (as defined by (Royle et al. 2012)). In this case,

394 this negative association might suggest the existence of two alternative parental care strategies. Hence,
395 an interpretation of our results could be that different behavioral traits may correspond to different
396 types of fathers with regard to how they take care of offspring. Such alternative strategies in offspring
397 care were previously observed in some cooperative breeding species, such as cichlids,
398 *Neolamprologus pulcher*, or the noisy miner, *Manorina melanocephala*, in which different types of
399 helpers seem to exist (Arnold et al. 2005; Bergmüller and Taborsky 2007), but to our knowledge, they
400 were not extensively studied in relation to parental care.

401 Cooperative behavior is defined as a behavior that benefits others while reducing the relative
402 fitness of the performer of that behavior and hence could be selected against (West et al. 2007).
403 Mound building possesses *a priori* those characteristics and we propose that it may be considered as a
404 cooperative behavior, favored by female mate choice as observed in humans (Farrelly et al. 2007;
405 Tognetti et al. 2014, 2016; Arnocky et al. 2017) and some cooperatively breeding birds (Reyer 1984;
406 Jones 1998; Doutrelant and Covas 2007).

407 A limitation of our study concerns the small sample sizes, due to restrictions on field captures,
408 and unexpected mortality events. Nevertheless, male investment in building significantly influenced
409 female attraction and this result is particularly promising for more extensive research.

410 In conclusion, our results raise questions about the mechanisms involved in the evolution of
411 mound building and provide support to the hypothesis that such behavior could be detectable by
412 phenotypic cues different from building *per se.* While the mechanisms enabling females to prefer the
413 males that invest the most in building behaviors in this species are still unknown, our results suggest
414 that mate choice might influence the evolution and maintenance of mound building. Last but not least,
415 several authors pointed out the collaborative aspect of mound building, and some evidence indicates
416 that several breeding pairs and their offspring could be involved in building this common good. Future
417 studies should test whether building could be considered a cooperative behavior and whether sexual
418 selection is another pathway by which cooperative building in this species might evolve.

419

Compliance with Ethical Standards

Ethical Approval

Mouse sampling was performed with the authorization of the Bulgarian Ministry of the Environment and Water (permit N°33-00-140). The study complied with all the relevant laws of France and mice handling and behavioral tests were authorised by the French authorities (Direction Départementale de la Protection des Populations de l'Hérault – Ministère de l'Agriculture, permit N°C34-265). This study also followed the ABS/ASAB guidelines for the ethical treatment of animals. We were particularly committed to limiting the number of mice trapped and tested, and mice were provided an enriched environment and diversified food to reduce their stress as much as possible.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data availability

The datasets analysed during the current study are available in the figshare repository, https://doi.org/10.6084/m9.figshare.5562664

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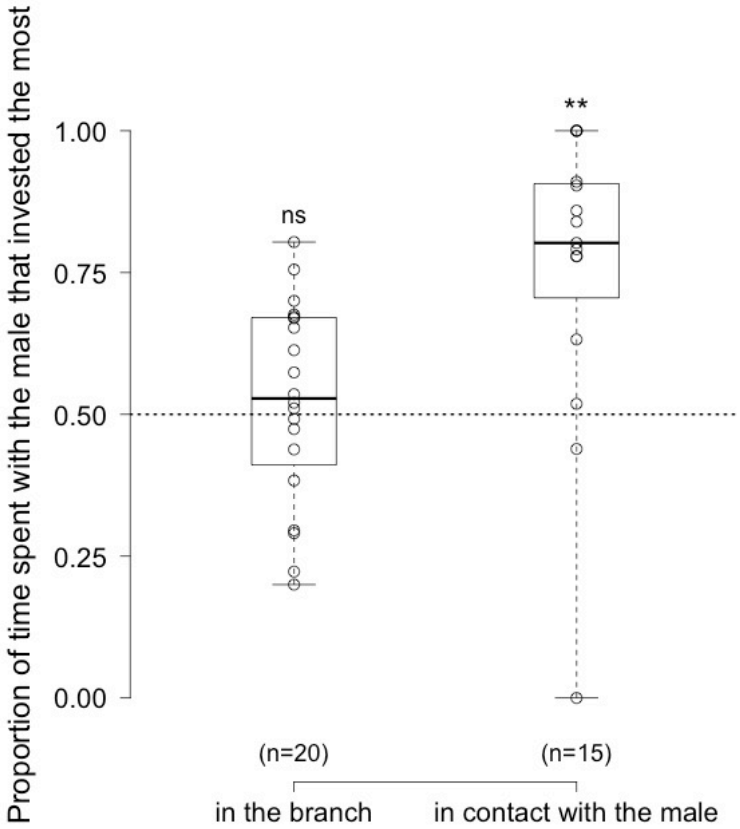

Fig. 1

Box plot representation of the proportion of time females spent exploring the branch of, or in proximity to the male most involved in building, relative to the total time spent exploring the two branches or in close proximity to the two males. The horizontal dotted line corresponds to a random choice. A value over 0.50 indicates preference for the male that invests the most in mound building. Medians (thick lines), first and third quartiles, whiskers representing the entire data range and individual data points are indicated. ns: non-significant, ** $p<0.01$

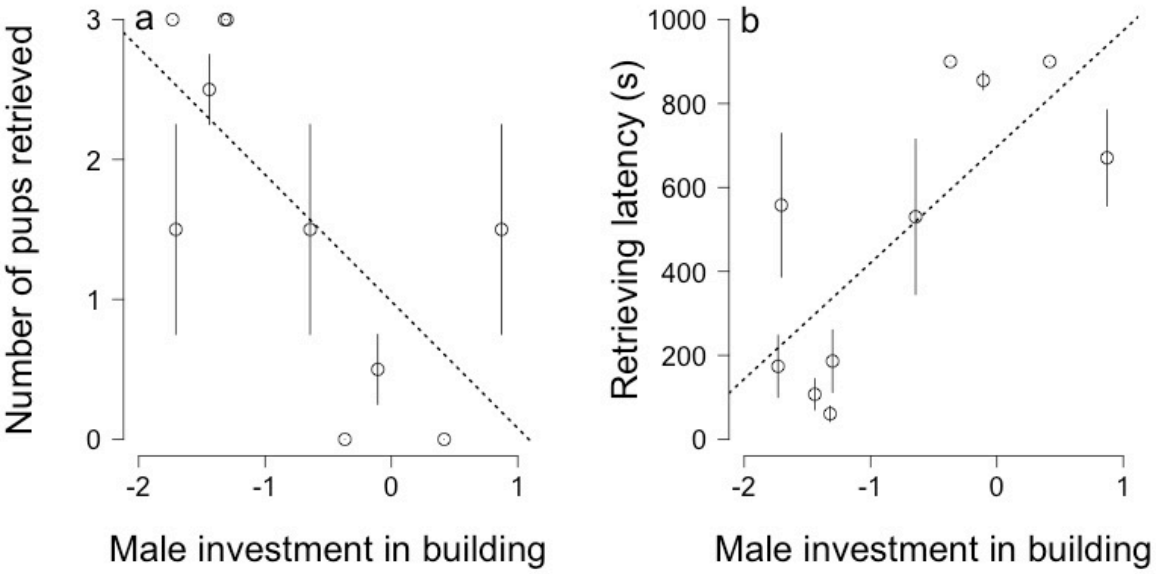

Fig. 2

Relationship between male investment in building and paternal care, measured as the average number of pups retrieved to the nest (a), and as the latency to retrieve the first pup to the nest (b). Error bars represent the standard error of the mean. Spearman correlation tests indicate that males investing more in mound building retrieved fewer pups back to the nest and presented a higher latency to retrieve the first pup.

608 Supplementary data: Tables S1 & S2.

609

610 **Table S1. Influence of male investment in building and treatment (observed building by the**
611 **female versus not) on the proportion of time females spent exploring the left versus right side of**
612 **the maze (n= 20).** We used a Generalized linear mixed model fitted by penalized quasi-likelihood
613 (glmmPQL, binomial error), with male pair's identity as a random effect. We included two
614 explanatory variables: a binary variable concerning male investment in building (1 if the male on the
615 left was the one that invested the most in building behaviors, 0 if it was the male that invested the
616 least) and a binary variable reflecting the treatment (1 if the female observed male building behaviors,
617 0 if not). The initial model also considered the interaction between male investment in building and
618 treatment, but it was excluded during model simplification due to its non-significance.

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620

Predictor variables	**Estimate**	**S.E.**	**df**	**t**	***P***
Intercept	-0.16	0.31	13	-0.52	0.61
Male investment in building	0.51	0.39	13	1.30	0.21
Treatment	–0.37	0.38	13	–0.97	0.35

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Table S2. Influence of male investment in building and treatment on the proportion of time the female spent in contact with the male of the left versus right side of the maze (n= 15). We used a Generalized linear mixed model fitted by penalized quasi-likelihood (glmmPQL, binomial error), with male pair's identity as a random effect. We included two explanatory variables: a binary variable concerning male investment in building (1 if the male on the left was the one that invested the most in building behaviors, 0 if it was the male that invested the least) and a binary variable reflecting the treatment (1 if the female observed male building behaviors, 0 if not). The initial model also considered the interaction between male investment in building and treatment, but it was excluded during model simplification due to its non-significance.

Predictor variables	**Estimate**	**S.E.**	**df**	**t**	***P***
Intercept	-1.16	0.59	8	-1.96	0.09
Male investment in building	2.51	0.68	8	3.69	0.006
Treatment	–0.16	0.70	8	-0.23	0.83

Figure S1: Photographs of mounds built in captivity

At the beginning of the experiment, the soil of each terrarium was uniformly covered with earth and stones. Additional material (straw and seeds) were added weekly at the center of each terrarium.

All mice built a nest made of straw in a corner of the terrarium (A), and constructed a 'mound like' stucture made of stone and straw (B). Under some parts of the 'mound like' structures we observed the presence of seeds indicating seeds transport, gathering and storage (C). Tunnels were also observed (D).

A

B

C

D