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### Effects of captivity on house mice behaviour in a novel environment: implications for conservation practices

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# Effects of captivity on house mice behaviour in a novel environment: implications for conservation practices

## Abstract

Captive breeding programmes offer a method for preventing the extinction of threatened species, but often have difficulty establishing self-sustaining populations and generating individuals for release. This difficulty can arise because behaviour of captive-reared animals differs from wild animals. Whilst the effect of captivity on animal behaviour has been widely reported, few studies have explicitly compared differences between captive-reared and wild-caught animals. Even fewer have examined behavioural types (a composition of behavioural traits) displayed in novel environments, which is particularly relevant for determining reintroduction success. Further, the transgenerational effects on behavioural type, and potential differences between sexes in response to captivity, remain almost completely unknown. Using house mouse (*Mus musculus*) as a model for small mammals, we tested whether behavioural types displayed in a novel environment differed between captive-reared and wild-caught animals. In addition, it was tested whether behavioural types were subject to transgenerational effects in captivity, and whether there were sex-specific differences in behavioural types. We used an open field test to simulate a novel environment. Captive-reared mice were found to differ in their boldness and activity behavioural type compared to their wild-caught mice ( $p < 0.001$ ). There was marginal evidence for transgenerational effects on behavioural type in captivity, but three behavioural traits displayed a shift away from wild behaviours (% Time active:  $p < 0.001$ ; % Time mobile:  $p = 0.004$ ; Centre: maximum speed:  $p = 0.004$ ). Furthermore, behavioural types of individuals in captivity did not differ depending on sex (F0:  $p = 0.161$ ; F1:  $p = 0.665$ ), however behavioural type did differ between wild-caught females and males ( $p = 0.015$ ). These findings suggest that captivity can result in behavioural changes and loss of sex-specific behaviours. In addition, phenotypic plasticity may have a significant influence on behavioural type. This knowledge may be critical for developing methods to improve small mammal reintroduction programmes.

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1 Effects of captivity on house mice behaviour in a novel environment: Implications for conservation  
2 practices.

3

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14

15 **Short title:** Effects of captivity on behaviour

16 **Key words:** Captive breeding, behaviour, reintroduction, phenotypic plasticity, captivity,

17 transgenerational effects

18 **Highlights:**

- 19 - We investigated the effects of captivity on behaviour  
20 - Captive-reared mice differed in boldness and activity compared to wild-caught mice  
21 - There was limited evidence for transgenerational effects on behaviour in captivity  
22 - Behavioural responses in captivity did not differ depending on sex.

23

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29 *Effects of captivity on house mice behaviour in a novel environment: Implications for conservation*  
30 *practices.*

31

32 *Abstract*

33 Captive breeding programmes offer a method for preventing the extinction of threatened species, but  
34 often have difficulty establishing self-sustaining populations and generating individuals for release.  
35 This difficulty can arise because the behaviour of captive-reared animals differs from wild animals.  
36 Whilst the effect of captivity on animal behaviour has been widely reported, few studies have  
37 explicitly compared differences between captive-reared and wild-caught animals. Even fewer have  
38 examined behavioural types (a composition of behavioural traits) displayed in novel environments,  
39 which is particularly relevant for determining reintroduction success. Further, the transgenerational  
40 effects on behavioural type, and potential differences between sexes in response to captivity, remain  
41 almost completely unknown. Using house mouse (*Mus musculus*) as a model for small mammals, we  
42 tested whether behavioural types displayed in a novel environment differed between captive-reared  
43 and wild-caught animals. In addition, it was tested whether behavioural types were subject to  
44 transgenerational effects in captivity, and whether there were sex-specific differences in behavioural  
45 types. We used an open field test to simulate a novel environment. Captive-reared mice were found to  
46 differ in their boldness and activity behavioural type compared to their wild-caught mice ( $p < 0.001$ ).  
47 There was marginal evidence for transgenerational effects on behavioural type in captivity, but three  
48 behavioural traits displayed a shift away from wild behaviours (% Time active:  $p < 0.001$ ; % Time  
49 mobile:  $p = 0.004$ ; Centre: maximum speed:  $p = 0.004$ ). Furthermore, behavioural types of individuals  
50 in captivity did not differ depending on sex ( $F_0$ :  $p = 0.161$ ;  $F_1$ :  $p = 0.665$ ), however behavioural type  
51 did differ between wild-caught females and males ( $p = 0.015$ ). These findings suggest that captivity  
52 can result in behavioural changes and loss of sex-specific behaviours. In addition, phenotypic  
53 plasticity may have a significant influence on behavioural type. This knowledge may be critical for  
54 developing methods to improve small mammal reintroduction programmes.

55 **Key words:** Captive breeding, behaviour, reintroduction, phenotypic plasticity, captivity,  
56 transgenerational effects



58 *1. Introduction*

59 Captive breeding programmes (hereafter CBPs) are increasingly relied upon as an important  
60 conservation tool for threatened species management (Conde et al., 2011). Captive breeding  
61 programmes provide a controlled environment for the rearing, maintenance and preservation of many  
62 species challenged by key threatening processes in the wild (Thomas et al., 2004; Bryant et al., 1999).  
63 However, captive populations often produce behavioural phenotypes that differ from wild populations  
64 (Snyder et al., 1996). These behavioural changes may lead to captive individuals having reduced  
65 survivorship compared with their wild conspecifics, as well as reduced reproductive success following  
66 reintroduction (Johnson et al., 2014; Anthony and Blumstein, 2000; Philippart 1995). It is understood  
67 that the captive environment induces changes to the behavioural phenotype, but identifying specific  
68 mechanisms that cause such changes can be challenging, largely due to a multitude of abiotic and  
69 biotic differences between captive and natural environments. For instance, differences in behavioural  
70 phenotypes between captive-reared and wild individuals have been associated with environmental  
71 enrichment, habitat complexity and social learning environment (*see* Shier and Owings 2006;  
72 Bremner-Harrison et al., 2004; Geiser and Ferguson 2001; Carducci and Jakob 2000).

73

74 While the effects of the captive environment on behaviour have been widely reported (Snyder et al.,  
75 1996), few studies have quantified the particular composition of behavioural traits that an individual  
76 expresses (hereafter referred to as behavioural type; Bell 2007) in comparison to a control group of  
77 wild animals. Using an 'adaptive baseline' provides the ability to demonstrate and track the effects of  
78 captivity. That is, the scale of behavioural plasticity, the direction of change, and the specific  
79 behavioural traits that change (Jarvie et al., 2015; Mathews et al., 2005). For example, in a study  
80 comparing the behaviour of captive-bred versus wild-caught bank voles (*Clethrionomys glareolus*) it  
81 was found that captive-bred individuals displayed some wild-caught nest building and burrowing  
82 behaviours. However, captive-bred individuals were unable to utilise key food resources, and were  
83 less dominant in their interactions with conspecifics than wild-caught individuals. As a consequence,  
84 the captive-bred individuals were determined unsuitable for release (Mathews et al., 2005). Of note,

85 few studies have attempted to investigate behavioural types that may impact the fitness of individuals  
86 following reintroduction (Moseby et al., 2014; Smith and Blumstein 2008; McDougall et al., 2006).

87

88 Testing behaviour in a novel environment (e.g. open field test) is a commonly used tool for  
89 determining behavioural types, such as activity or boldness (Yuen et al., 2015; Rosemberg et al.,  
90 2011). Consequently, measuring behavioural types in a novel environment, and quantifying any  
91 changes resulting from maintenance in captivity may provide a valuable approach for increasing the  
92 success of captive-breeding and reintroduction programmes. Indeed, behavioural characterisation has  
93 been used as a criterion for selecting animals for reintroduction (Bremner-Harrison et al., 2004;  
94 Mathews et al., 2005). Specifically, boldness and activity relate to the tendency of an individual to  
95 take risks and explore novel environments (Coleman and Wilson 1998). In addition, boldness has been  
96 used to predict the probability that individuals survive and reproduce following reintroduction  
97 (Herborn et al., 2010; Wilson and Godin 2009). If changes in these behavioural types occur in  
98 captivity, the probability of an individual's survival and reproductive success might decline, and in  
99 turn, impact the likelihood that the reintroduction programme is successful. Based on optimality  
100 theory, an optimal level of boldness and activity would be expected for any given species in any given  
101 environment, with extremes on the axes of variation (shy-bold; inactive-active) being costly and  
102 selected against (Herborn et al., 2010). Boldness and activity can affect performance and fitness, and  
103 by determining these behavioural types, this information may be used to determine an individual's  
104 suitability for release (Mathews et al., 2005). Further, knowledge of behavioural changes occurring in  
105 captivity may be used to develop strategies to alleviate problems associated with domestication  
106 (Mason et al., 2013), or the effect of captivity on behaviours considered important for reintroduction  
107 success (McDougall et al., 2006).

108

109 How directional selection and phenotypic plasticity alter behavioural traits in the captive environment  
110 is only beginning to be investigated (Evans et al., 2014; Nelson et al., 2013). Developmental plasticity  
111 in behaviour allows individuals to alter their behavioural traits to suit their captive environment. In  
112 contrast, transgenerational effects in the captive environment influence the behavioural traits passed

113 from parents onto offspring (Evans et al., 2014). Due to changes in the strengths and targets of  
114 selection in captivity, and the heritable nature of behavioural traits, a shift in behaviour that increases  
115 fitness in the captive environment can be expected (McPhee 2004). Therefore, one might expect  
116 behaviour to shift away from the wild behavioural phenotype with each subsequent generation in  
117 captivity. Indeed, there is a growing body of evidence for transgenerational behavioural changes  
118 occurring in captivity. Previous research has shown that animals maintained in captivity for multiple  
119 generations usually display a consistent directional shift in behaviour away from the wild phenotype.  
120 Furthermore, these transgenerational behavioural changes have been shown to increase fitness within  
121 the captive environment (Johnson et al., 2014; Mason et al., 2013; Christie et al., 2012; MCPhee  
122 2004). Commonly reported transgenerational behavioural changes include loss of anti-predator  
123 responses and reduced exploratory behaviour (Håkansson and Jensen 2008). For example, refuge-  
124 seeking behaviour of oldfield mice (*Peromyscus polionotus subgriseus*) decreased in frequency with  
125 an increasing number of generations maintained in captivity (McPhee 2004).

126

127 The way behavioural traits change in captivity, and the direction of transgenerational effects, could  
128 depend on a multitude of factors, but one of the most important is likely to be sex. It is well  
129 established that behavioural types can differ between the sexes due to sexual selection favouring  
130 different trait values in each sex (Fresneau et al., 2014; Schuett et al., 2010). In general, it is expected  
131 that intra- and inter-sexual selection (male-male competition and female mate choice) will favour  
132 bolder and aggressive males and shy and discriminant females (Kokko 2005). However, such effects  
133 might be species- or taxon-specific. For example, a study investigating the effect of reproductive  
134 tactics on behavioural syndromes (i.e. personality) in African striped mice (*Rhabdomys pumilio*)  
135 found consistent sex-based differences in activity, boldness, exploration and aggression (Yuen et al.,  
136 2015). Given that sexual selection in behavioural types is evident across various taxa, captive-based  
137 research stands to benefit enormously from exploring the effects of captivity on the strengths and  
138 targets of sexual selection, and resultant behavioural differences between the sexes. A small number  
139 of behavioural studies on captive populations have examined the effects of captivity and sex on  
140 behaviour (*see* Benson-Amram et al., 2013; Herborn et al., 2010; Mathews et al., 2005; Bremner-



141 Harrison et al., 2004). Of these studies, only one examined the interaction between rearing  
142 environment and sex on behaviour, therefore more studies are required.

143

144 The overall aim of this study was to investigate whether behaviour in captive-reared and wild-caught  
145 animals differ using house mice (*Mus musculus*) as a model species. To address this overall aim, we  
146 had three specific aims i) to compare behavioural types displayed by captive-reared and wild-caught  
147 individuals in a novel environment; ii) to determine whether behavioural types are subject to  
148 transgenerational effects in the captive environment; and iii) to examine the behavioural types  
149 displayed by each sex. The respective predictions for these aims were i) the captive-reared animals  
150 would display differing trait values for boldness and activity behavioural types compared to wild-  
151 caught individuals; ii) the behavioural type would be subject to transgenerational effects in the captive  
152 rearing environment, with captive-reared individuals displaying behavioural types that do not  
153 significantly differ from their captive-reared parents, but do significantly differ from wild-caught  
154 individuals; and iii) the behavioural types would differ depending on sex. Further, the behavioural  
155 type displayed by each sex will be consistent across captive-reared and wild-caught individuals, with  
156 captive-reared animals displaying differing trait values for boldness and activity behavioural types  
157 regardless of sex.

158

## 159 2. *Methods*

### 160 2.1. *Ethical Note*

161 This study was conducted under University of Wollongong Animal Ethics Approval AE13/17.

162

### 163 2.2. *Study species*

164 The house mouse (*M. musculus*) is a small rodent species widespread throughout the world. The  
165 species has a short generation time, has an iteroparous reproductive strategy, displays clear sex roles,  
166 polygamous mating strategies and can be easily maintained in captivity. For these reasons, it is being  
167 increasingly used as a model to address questions related to small mammal captive breeding and  
168 reintroduction (Slade et al., 2014; Paproth 2011).

169

170 2.3. *Housing*

171 All individuals (wild-caught and captive-reared) were maintained separately in opaque plastic cages  
172 (32 x 18 x 12 cm; MB1 Mouse Box, Wiretainers Pty Ltd., Melbourne, Victoria, Australia) with a  
173 metal top. Wood shavings were used as cage substrate and all cages were provided with bedding  
174 material (shredded paper) and a 6 x 4 cm cardboard tube (toilet paper roll) for shelter. Water and food  
175 (Vella Stock Feeds brand Rat and Mouse Nut; The Vella Group, Glendenning, New South Wales,  
176 Australia) were available *ad libitum*. *Ad libitum* food quantities were determined as 20 grams of food  
177 per 100 g of body mass supplied daily (Hubrecht and Kirkwood 2010). Room temperature was  
178 maintained at  $22 \pm 2^\circ\text{C}$  on a reversed 12: 12 light: dark cycle, with full spectrum UV light provided.  
179 Housing conditions were based on conditions supplied to the original wild-caught founder generation  
180 and average temperatures in the field during the study period. Humidity was not controlled, but was  
181 monitored daily and recorded as  $75 \pm 10\%$ . Animals were monitored daily, with cages cleaned once a  
182 week by removing the occupant and placing them in a round escape-proof container (54 x 52 cm;  
183 Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia) then placing them in a new cage.

184

185 2.4. *Captive-reared parent generation (captive-reared  $F_0$ )*

186 Eleven sexually mature virgin male *M. musculus* and fifteen sexually mature virgin females were  
187 sourced from a captive population maintained at University of New South Wales, Sydney under  
188 Ethics Permit UNSW Reg. No. 12/88A. All individuals were third or fourth generation captive-  
189 maintained mice born between late-2012 and mid-2013. All animals had unrelated parents and  
190 grandparents from multiple litters that were descendants of an original wild-caught founder generation  
191 consisting of 42 females and 45 males captured between March and May 2011 at an agricultural site in  
192 the western Sydney area ( $34^\circ 4' 36.48''\text{S}$ ,  $150^\circ 34' 15.6''\text{E}$ ).  
193 Prior to this study, the captive-reared  $F_0$  mice were housed in a temperature ( $19 - 25^\circ\text{C}$ ) and light  
194 controlled room (12: 12 hr reverse light cycle, lights on at 9:00 AM AEST). Humidity was not  
195 controlled but was  $\sim 30\%$  (A. Gibson, personal communication, 17 January 2014). Males were housed  
196 separately at weaning to avoid aggression and physical injury but female siblings were housed

197 together in groups of up to three individuals. All animals were provided with food and water *ad*  
198 *libitum*. Mice were checked three times a week for changes in body condition, behaviour and injuries.

199

200 For this study, captive-reared F<sub>0</sub> individuals were collected from University of New South Wales on  
201 January, 17, 2014 and transported to the Ecological Research Centre at the University of Wollongong,  
202 Wollongong (34°24'24"S 150°52'46"E). Mice were weighed (grams) on digital scales (Mettler-Toledo  
203 PJ3600, Mettler-Toledo Ltd., Port Melbourne, Australia) and then housed individually (see *Housing*).  
204 Mice were acclimated in the individual housing for a maximum of 21 days (male: average 11 ± 2  
205 days; female: average 16 ± 5 days; due to the restrictions in processing mice through the behavioural  
206 characterisation). Once acclimated, the captive-reared F<sub>0</sub> mice were then entered into the behavioural  
207 characterisation assay (see *Behavioural Characterisation*) before breeding the captive-reared F<sub>1</sub>  
208 generation.

209

### 210 2.5. *Captive-reared offspring generation (captive-reared F<sub>1</sub>)*

211 Pedigree mapping was used to ensure unrelated individuals from the founder generation were paired  
212 so that captive-reared F<sub>1</sub> females and males had unrelated parents and grandparents. Each  
213 monogamous breeding pair was held together for one week in standard caging (see *Housing*). Water  
214 and food (Vella Stock Feeds brand Rat and Mouse Nut; The Vella Group, Glendenning, New South  
215 Wales, Australia) were available *ad libitum*, and temperature and light: dark cycles were uniform to  
216 those provided for the F<sub>0</sub> acclimation period.

217

218 Once mated, the captive-reared F<sub>0</sub> dams were monitored to check for young. Mice were checked once  
219 a day, commencing ten days following the male being removed, with the monitoring period lasting an  
220 average of 10 ± 2 days. Offspring were housed with their mother until they were weaned at 25 days of  
221 age; weaning age was kept uniform across all litters to reduce differences in maternal investment post-  
222 pregnancy. At 25 days of age, the captive-reared F<sub>0</sub> dam was removed from the breeding cage, and the  
223 litter was then housed for two days under *ad libitum* conditions to reduce stress on the litter following  
224 removal of the dam. Offspring were then housed individually in standard caging (see *Housing*). Upon

225 entry into the individual housing, individuals had their sex confirmed (13 males and 14 females for  
226 this study).

227

## 228 *2.6. Wild-caught population*

229 Eight sexually mature males and fifteen sexually mature females *M. musculus* were captured in  
230 October – November 2014, at the same agricultural site in the western Sydney area (34°4'36.48"S,  
231 150°34'15.6"E) as the source founder population for the captive-reared F<sub>0</sub> generation. Elliott traps (30  
232 x 10 x 8 cm; Sherman Traps Inc., Florida, USA) were set inside and outside sheds and surrounding  
233 vegetation. These were checked, emptied and reset daily in the early morning approximately 8:00 AM  
234 AEST. Elliott traps were baited with honey and peanut butter rolled oat balls. Once captured, animals  
235 were transported to the Ecological Research Centre at the University of Wollongong, Wollongong  
236 (34°24'24"S 150°52'46"E) and were housed in the same caging as the captive-reared generations (see  
237 *Housing*). Mice were weighed (grams) upon entry into the individual housing. To match the  
238 acclimation period of the captive-reared F<sub>0</sub> individuals, wild-caught mice were acclimated for a  
239 maximum of 21 days (male: average 11 ± 2 days; female: average 16 ± 5 days) prior to behavioural  
240 characterisation.

241

## 242 *2.7. Behavioural characterisation*

243 Behavioural characterisation occurred at sexual maturity for all wild-caught, captive-reared F<sub>0</sub> and  
244 captive-reared F<sub>1</sub> individuals (Captive-reared F<sub>0</sub> = 26; Captive-reared F<sub>1</sub> = 27; Wild-caught = 23). To  
245 ensure no effects of mating on behavioural characterisation, both captive-reared F<sub>0</sub> and captive-reared  
246 F<sub>1</sub> behavioural characterisations were conducted when individuals were virgins. As we were unable to  
247 determine whether wild-caught mice were virgins, all wild caught mice were acclimated for a  
248 maximum period of 21 days to reduce any effects of potential mating.

249

250 Behavioural characterisations for captive-reared F<sub>0</sub>, captive-reared F<sub>1</sub> individuals and wild-caught  
251 individuals were conducted in late Australian Spring/early Summer and in late Autumn/early Winter.

252 As behavioural analyses were unable to be run simultaneously for all populations, we assumed

253 acclimation period would account for any confounding effects associated with season. To determine  
254 how individuals displayed behavioural traits along the bold/shy and active/inactive axes of variation  
255 of the active and bold behavioural types, 14 behavioural traits were used (Table 1). These traits have  
256 previously been used to determine boldness or activity in the following empirical studies: Augustsson  
257 et al., (2005); Augustsson and Meyerson (2004); McPhee (2004). For full ethogram see Table 1.

258

### 259 2.8. Apparatus

260 We used an Open Field Test (henceforth OFT) to determine the behavioural types individuals would  
261 display in a novel environment. The OFT arena was constructed from an opaque rectangular LDPE  
262 plastic tank with an arena size of 90 x 60 cm with 60 cm high walls (Spacepac Industries Pty. Ltd.,  
263 Wollongong, NSW, Australia). Two PVC tunnels (6 x 4 cm) were placed in the central part of the  
264 arena at opposite ends (located 10 cm from the arena walls) to simulate shelter. Above each arena (n=  
265 4), a video camera (PRO-735 Camera, Swann Systems, Melbourne, Australia) was placed to record  
266 the entire OFT trial. Recorded videos were stored on a Digital Video Recorder (DVR8-4100, Swann  
267 Systems, Melbourne, Australia) and behaviour was analysed using ANY-maze® software (Stoelting  
268 Co., U.S.A). This analysis software is routinely used in vertebrate behavioural characterisation (*see*  
269 Rosemberg et al., 2011; Brenes et al., 2009; Walf and Frye 2007). The location and behaviours  
270 (duration) of the mice for the entire duration of the OFT were recorded. Trials were conducted at the  
271 same time of day and were conducted in the dark half of the light cycle. At the conclusion of the OFT  
272 observation period, a test subject was removed from the OFT arena and the OFT arena and shelters  
273 were thoroughly cleaned using 70% EtOH to remove any traces of animal scents.

274

### 275 2.9. OFT Procedure

276 Individual mice were transferred to the OFT arena and were placed in the estimated central point of  
277 the OFT arena. Following an acclimation period (2 minutes), behaviour was recorded for 20 minutes  
278 (1200 seconds). Fourteen behavioural traits were measured (Table 1).

279

### 280 2.10. Statistical Analysis

### 281 2.10.1. *Multivariate analysis*

282 To examine the effects of rearing environment on the behaviour of mice, we used multivariate  
283 analyses with Primer 7 (Clarke and Gorley 2015) and PERMANOVA+B version (Anderson and  
284 Gorley 2007). This non-parametric analysis accounts for any potential issues with small sample sizes.  
285 Of note, sample sizes used in this present study were comparable with other studies of this nature  
286 (Slade et al., 2014; Paproth 2011; Bremner-Harrison et al., 2004; McPhee 2004; Geiser and Ferguson,  
287 2001). To remove the effects of body mass on behaviour, we calculated the residuals of a least squares  
288 regression of each behavioural trait on body mass. We then normalised the behavioural trait data so  
289 that all behavioural traits would take values within the same limits (-2 to +2 to cover all entries). To  
290 test whether behavioural type varied between rearing environment and sex, a two factor  
291 PERMANOVA was used on the 14 behavioural traits, the factors were rearing environment (3 levels  
292 orthogonal and fixed; wild-caught; captive-reared F<sub>0</sub> and captive-reared F<sub>1</sub>) and sex (2 levels  
293 orthogonal and fixed; female and male) were used with acclimation period (number of days) as  
294 covariate. Interaction factors between acclimation period, rearing environment and sex were included  
295 to account for any interactive effects. Compositional differences in behavioural types between wild-  
296 caught; captive-reared F<sub>0</sub> and captive-reared F<sub>1</sub> were visualised using non-metric multidimensional  
297 scaling (nMDS) ordinations. All analyses used Euclidean similarity measures. Similarity percentage  
298 (SIMPER) analysis was used to identify the behavioural traits contributing most strongly to the  
299 compositional changes in behavioural type detected.

300

### 301 2.10.2. *Univariate analyses*

302 Behavioural traits that contributed >10% to compositional changes in behavioural types between wild-  
303 caught; captive-reared F<sub>0</sub> and captive-reared F<sub>1</sub> in SIMPER were then analysed using linear mixed  
304 effects model (LMMs; Table 4) to examine the effects of rearing environment and sex on the  
305 behavioural traits in mice. Rearing environment (wild-caught; captive-reared F<sub>0</sub> and captive-reared F<sub>1</sub>)  
306 and sex (female and male) were the fixed effects, acclimation period (number of days acclimated) was  
307 the covariate. An interaction factor between rearing environment and sex was also included. The  
308 residuals of a least squares regression of each behavioural trait on body mass were used. For all

309 behavioural data, Tukey's HSD pairwise comparison tests were used for post-hoc comparisons  
310 between treatments. All data were analysed in JMP 11.0.0 statistical package.

311

### 312 3. Results

#### 313 3.1. Effect of rearing environment and sex on behavioural type

314 There was a significant interaction between rearing environment and sex (PERMANOVA: Pseudo- $F_2$   
315 = 3.002,  $p = 0.008$ ; Table 2). The composition of behavioural types significantly differed between  
316 individuals from differing rearing environments (PERMANOVA: Pseudo- $F_2 = 5.102$ ,  $p < 0.001$ ; Table  
317 2) but did not significantly differ between male and female individuals (PERMANOVA: Pseudo- $F_2 =$   
318 0.415,  $p = 0.858$ ; Table 2). There were no significant interactions between acclimation period, rearing  
319 environment and/or sex and there was no significant effect of acclimation period on behavioural type  
320 (Table 2). SIMPER analysis revealed 8 behavioural traits contributed to the compositional differences  
321 in behavioural types between wild-caught; captive-reared  $F_0$  and captive-reared  $F_1$  and sex (only  
322 behavioural traits with  $>10\%$  contribution were considered; Supporting Information).

323

#### 324 3.2. Transgenerational effects in the captive environment on behavioural type

325 Transgenerational effects in the captive environment were defined as the behavioural type shifting  
326 away from the wild phenotype with each subsequent generation in captivity. Behavioural type  
327 significantly differed between captive-reared  $F_1$  females and captive-reared  $F_0$  females  
328 (PERMANOVA:  $t_{25} = 1.927$ ,  $p = 0.013$ , Table 3) and a marginally significant difference occurred  
329 between captive-reared  $F_1$  females and wild-caught females (PERMANOVA:  $t_{25} = 1.542$ ,  $p = 0.052$ ,  
330 Table 3). Behavioural type did not significantly differ between captive-reared  $F_0$  females and wild-  
331 caught females (Table 3). SIMPER analysis revealed that four behavioural traits (Perimeter: max  
332 speed, Perimeter: average speed, Mean speed, Distance) were driving the compositional differences in  
333 behavioural type between captive-reared  $F_1$  and captive-reared  $F_0$  females (only behavioural traits with  
334  $>10\%$  contribution were considered; Supporting Information).

335

336 There were no significant differences between captive-reared  $F_1$  males and wild-caught or captive-  
337 reared  $F_0$  males (PERMANOVA: captive-reared  $F_1$  and wild-caught:  $t_{17} = 1.429$ ,  $p = 0.096$ ; captive-  
338 reared  $F_1$  and captive-reared  $F_0$ :  $t_{20} = 1.389$ ,  $p = 0.107$ ; Table 3). Behavioural type significantly  
339 differed between captive-reared  $F_0$  males and wild-caught males (PERMANOVA:  $t_{15} = 2.810$ ,  $p$   
340  $< 0.001$ , Table 3). SIMPER analysis revealed four behavioural traits were driving the compositional  
341 differences in behavioural type between captive-reared  $F_0$  and wild-caught males (% Time active, %  
342 Time mobile, Centre: max speed, % Time freezing; Supporting Information).

343

### 344 *3.3. Sex-specific behavioural responses to rearing environment*

345 Pairwise comparisons between males and females in each rearing environment determined only  
346 behavioural type significantly differed between wild-caught individuals (PERMANOVA:  $t_{19} = 1.845$ ,  
347  $p = 0.015$ , Table 3). Between wild-caught males and females three behavioural traits were driving  
348 compositional differences in behavioural type (% Time active; % Time mobile; Centre: max speed;  
349 Supporting Information).

350

### 351 *3.4. Effect of rearing environment on behavioural traits*

352 Overall, seven of the eight behavioural traits contributing  $>10\%$  to compositional differences in  
353 behavioural type significantly differed between rearing environments and sex (Table 4, Supporting  
354 Information). There was a significant interaction between rearing environment and sex on % Time  
355 spent active and % Time spent mobile (LMM: % Time active:  $F_{2, 69} = 8.767$ ,  $p < 0.001$ ; % Time  
356 mobile:  $F_{2, 69} = 5.942$ ,  $p = 0.004$ ; Table 4). Compared with wild-caught male mice, captive-reared  $F_0$   
357 male mice spent more time active and mobile. There were no significant differences in time spent  
358 active or mobile in captive-reared  $F_0$  and wild-caught female mice. Post-hoc tests demonstrated the  
359 transgenerational effects in the captive environment were only evident in males, with % time spent  
360 active and mobile significantly differing between captive-reared  $F_1$  and wild-caught mice (Table 4, 5).

361

362 There was a significant difference between individuals from different rearing environments for five  
363 behavioural traits: Distance covered, % Time spent freezing, Mean speed, Centre: maximum speed



364 and Perimeter: mean speed (LMMs, Table 4). Compared with wild-caught mice, captive-reared  $F_0$   
365 mice covered more distance, spent less time freezing, displayed a faster mean speed and faster mean  
366 speed in the perimeter of the OFT arena. In addition, in the centre of the arena, captive-reared  $F_0$  mice  
367 displayed a slower maximum speed (Table 6). Post-hoc tests demonstrated that transgenerational  
368 effects in the captive environment were minimal, with only one behavioural trait (Centre: maximum  
369 speed) significantly differing between captive-reared  $F_1$  and wild-caught mice. Conversely, for four  
370 behavioural traits (Distance, % Time freezing, Mean speed, Perimeter: mean speed), captive-reared  $F_1$   
371 mice did not significantly differ from wild-caught mice, but did significantly differ from captive-  
372 reared  $F_0$  mice (LMMs, Table 4, 6). There were no significant effects of sex or acclimation period on  
373 any behavioural traits (LMMs, Table 4).

374

#### 375 4. Discussion

376 The aims of this study were threefold. Firstly, to investigate whether behavioural type in a novel  
377 environment differed between captive-reared and wild-caught individuals; secondly, to determine  
378 whether behavioural changes in captive-reared individuals were subject to transgenerational effects in  
379 the captive environment; and thirdly, to determine whether there were differences in behavioural types  
380 displayed between the sexes. Mice reared in captivity exhibited a different behavioural type compared  
381 with wild-caught conspecifics, providing support for the prediction that captive-reared animals would  
382 differ from wild-caught animals. There was evidence for transgenerational effects on behavioural type  
383 and as well as on some behavioural traits, providing some support, albeit limited, for the second  
384 prediction that the behavioural type would shift away from the wild phenotype with each subsequent  
385 generation in captivity. It was found that behavioural type did not significantly differ depending on  
386 sex. Furthermore, behavioural type of each sex did not differ in captive environments, but did differ  
387 between wild-caught females and males. This finding did not provide any support for the third  
388 prediction that each sex would display differing behavioural types.

389

##### 390 4.1. Effects of captivity on behavioural type displayed in a novel environment

391 Mice reared in captivity exhibited a different behavioural type compared with wild-caught  
392 conspecifics, providing support for the prediction that captive-reared animals would differ from wild-  
393 caught animals. Our findings provide support for the use of an ‘adaptive baseline’ by demonstrating  
394 the scale of behavioural plasticity occurring; the direction of change; and the behavioural traits that  
395 changed (Mathews et al., 2005). In this regard, we suggest that the magnitude and direction of change  
396 to behavioural types (such as boldness and activity used in this study) in an individual may reflect the  
397 way the animal behaves in a novel environment following reintroduction (Mason et al., 2013;  
398 McDougall et al., 2006).

399

400 The effect of captivity on animal behaviour has been reported across a variety of taxa (Wisely et al.,  
401 2008; Snyder et al., 1996). Differences in behaviour between captive-reared and wild populations may  
402 be expected due to the inherent differences in rearing environments, and associated differences in  
403 selection pressures (Mason et al., 2013). However, predicting which behaviours will be affected, and  
404 predicting the magnitude and direction of change in a given behaviour can be challenging. Indeed,  
405 past studies have shown that the captive behavioural phenotype can remain unchanged, or move  
406 toward or away from the wild behavioural phenotype (*see* Champagnon et al., 2012; Augustsson et al.,  
407 2005; McPhee 2004; Stoinski and Beck 2004; Geiser and Ferguson 2001; Carducci and Jakob 2000).  
408 In general, however, we might expect behavioural type to show adaptations to captivity (Mason et al.,  
409 2013). If behaviour in captivity shifts from an adaptive behavioural phenotype, it is valuable to  
410 determine the ongoing impact of these behavioural changes on individual fitness, particularly if these  
411 behavioural changes have consequences for the viability of captive populations, and/or impact the  
412 probability of reintroduction success. As such, future research might benefit from investigating  
413 whether behavioural changes occurring in captivity are maladaptive under natural conditions.

414

#### 415 *4.2. Transgenerational effects in the captive environment on behavioural type*

416 Between captive generations, there was limited evidence of transgenerational effects on behaviour,  
417 with captive-reared female behavioural types showing a marginal shift from the wild-caught  
418 behavioural type with each subsequent generation. There was evidence of transgenerational effects in

419 captivity for some but not all behavioural traits (in both females and males), with three behavioural  
420 traits in captive-reared F<sub>1</sub> mice significantly differing from wild-caught mice, however these did not  
421 significantly differ from captive-reared F<sub>0</sub> mice (Centre: maximum speed; % Time active and % Time  
422 mobile in males only). This result provided some support for the second of our predictions; that with  
423 each subsequent generation in captivity the behavioural type would shift away from the wild  
424 phenotype. Specifically, captive-reared F<sub>1</sub> mice significantly differed from wild-caught mice in only  
425 one behavioural trait (Centre: maximum speed). Captive-reared F<sub>1</sub> male mice significantly differed  
426 from wild-caught male mice in only two behavioural traits (% Time active, % Time mobile).  
427 Conversely, for four behavioural traits (Distance, % Time freezing, Mean speed, Perimeter: mean  
428 speed), captive-reared F<sub>1</sub> mice did not differ from wild-caught mice but significantly differed from  
429 captive-reared F<sub>0</sub> mice. Given the limited evidence for transgenerational effects on behavioural type  
430 and behavioural traits between captive-reared F<sub>0</sub> and F<sub>1</sub> mice, it is important to consider the  
431 experimental captive-reared population used in this study was derived from 3 – 4 previous captive-  
432 reared generations. Consequently, behavioural changes may have occurred relatively quickly in these  
433 previous generations, making it difficult to detect any additional changes in this study. However, we  
434 were able to demonstrate that captive-reared F<sub>1</sub> behavioural traits shifted from the wild-caught  
435 behavioural phenotype, indicating that transgenerational effects are likely to occur quickly.

436

437 Previous studies have reported transgenerational effects in the captive environment, with these studies  
438 focussing on particular behavioural traits rather than a composition of behavioural traits (behavioural  
439 type) that an individual would express (*see* Evans et al., 2014; Paproth, 2011; Håkansson and Jensen  
440 2008). For example, a past study investigating the temporal changes in behaviour of house mice in  
441 response to captivity reported a reduction in a single exploratory behaviour (time spent touching  
442 tunnels) after two generations (Paproth 2011). The lack of transgenerational effects on all behavioural  
443 traits that contributed to a behavioural type observed in the present study may have occurred because  
444 some, but not all, behavioural traits had an impact on individual performance (and potentially fitness)  
445 in the captive environment (McPhee 2004). Furthermore, transgenerational effects on behavioural type  
446 in the captive environment may have remained undetected simply because such effects require

447 multiple generations to manifest. This could occur if individual traits differ in how quickly they  
448 respond to change. Another possibility is that differences in social environment during early  
449 development may have masked transgenerational effects, resulting in a reduced ability to detect a shift  
450 towards ‘captive-like’ behavioural traits in subsequent captive generations. Consequently, although an  
451 identical captive-environment was used for all individuals, and an acclimation period was used to  
452 account for any effects of the prior environment for captive-reared and wild-caught mice, inadvertent  
453 differences in social rearing-environment may have occurred for the captive-reared F<sub>0</sub> and F<sub>1</sub> mice.  
454 Specifically, captive-reared F<sub>0</sub> females were group-housed prior to introduction to this study, whereas  
455 males and all captive-reared F<sub>1</sub> mice were separated at weaning age. Indeed, solitary housing has been  
456 shown to increase exploratory behaviour (a proxy for boldness) in house mice (Goldsmith et al.,  
457 1978). Likewise, early social experience has been shown to influence the expression of stereotypic  
458 behaviours in striped mice (*Rhabdomys sp.*), with early weaning (physical separation from the mother  
459 and siblings) increasing the incidence of stereotypic behaviours (Jones et al., 2010).

460  
461 Furthermore, captive-reared F<sub>1</sub> mice had behavioural traits that sat between captive-reared F<sub>0</sub> and  
462 wild-caught mice, this suggests that some behavioural traits did not shift away from the wild-caught  
463 phenotype. This may indicate a lack of transgenerational effects in the captive environment. Whilst  
464 age was not considered in this study (sampling behavioural types was unable to be conducted on  
465 same-age populations), age may have had a significant influence on the degree of behavioural change.  
466 That is, we may not have observed transgenerational effects in the captive-reared F<sub>1</sub> mice simply  
467 because behavioural traits were not fully developed. If we assume animals are held under consistent  
468 captive conditions during ontogeny and through to reproductive maturity, over their lifecycle the  
469 behaviour of individuals should adjust to the captive environment. Therefore, we suspect that the  
470 captive-reared F<sub>1</sub> behavioural types would change to reflect a behavioural type more similar to  
471 captive-reared F<sub>0</sub> mice, primarily due to similar captive environments and similar selective pressures.  
472 To substantiate whether behavioural types respond to captivity over an individual lifetime, and are  
473 subject to transgenerational effects, (i.e. behavioural type shifts away from a wild-caught phenotype  
474 over time and with each subsequent generation maintained in captivity) studies would need to measure

475 behavioural type throughout an individual's lifecycle, and across generations. Developmental  
476 plasticity in boldness has previously been documented in swift fox (*Vulpes velox*), with captive-bred  
477 adult foxes displaying a higher level of boldness compared with juveniles (Bremner-Harrison et al.,  
478 2004). To date, there have been limited efforts to determine how developmental plasticity influences  
479 transgenerational effects in the captive environment, but this may be a valuable inclusion in future  
480 research (Evans et al., 2014).

481

#### 482 4.3. Sex differences in behavioural type in captivity

483 Overall, it was found that the behavioural type did not differ significantly depending on sex,  
484 indicating each sex displayed similar behavioural types. This finding did not support our third  
485 prediction that each sex would display differing behavioural types. Further, the behavioural type of  
486 each sex did not differ in captive environments, but behavioural types were significantly different  
487 between wild-caught females and males. We suggest our findings indicate that there is a loss of sex-  
488 specific behaviours in captivity. Similarly, another study investigating the temporal changes in  
489 behaviour of house mice resulting from maintenance in captivity also reported no significant  
490 differences in exploratory or risk-taking behaviours between each sex, but unlike our study, there was  
491 no evidence for sex-specific behavioural differences in their wild-caught founder population (Paproth,  
492 2011).

493

494 Sex-specific differences in behavioural type occur because the strength and targets of sexual selection  
495 differ between sexes (Yuen et al., 2015; Fresneau et al., 2014; Biro and Stamps 2008; Stamps 2007;  
496 Sih et al., 2004). A lack of sex-specific differences in behavioural type in captivity may have occurred  
497 because the behavioural types examined in this study were subject to natural rather than sexual  
498 selective pressures (Dammhahn 2012; Coleman and Wilson 1998). Boldness and activity relate to a  
499 tendency for risk-taking particularly in novel environments (Coleman and Wilson 1998). Risk-taking  
500 may influence mate-selection, as well as other behaviours such as foraging, interactions with  
501 predators, conspecifics and the environment, all of which are experienced by both sexes (Coleman and  
502 Wilson 1998). As such, testing behaviour in a novel environment may not be appropriate for detecting

503 sex-specific differences of captive-reared animals, as sex-specific behavioural differences in a novel  
504 environment may not present an evolutionary advantage, unless there is an increased reproductive  
505 advantage in captivity. For example, wild grey mouse lemur (*Microcebus murinus*) males were  
506 consistently bolder than wild females, with boldness correlating with fecundity in males but not in  
507 females (Dammhahn 2012). Similarly, in wild African striped mice (*R. pumilio*) there were consistent  
508 differences in activity between females and males across reproductive tactics (group- to solitary-living  
509 in females, breeding to non-breeding males; Yuen et al., 2015). To the best of our knowledge, there  
510 remains a limited understanding of whether these sex-specific differences in behavioural type would  
511 be lost in captivity.

512

513 Despite emerging evidence that the sexes show behavioural differences prior to introduction to  
514 captivity, most previous studies investigating the effect of captivity on behaviour have ignored the  
515 effect of sex-specific differences, and associated differences in sexual selection pressure. Clearly,  
516 further investigation is required to determine whether captivity can result in losses of sex-specific  
517 behaviours. Such studies could focus on examining and comparing the behaviour of females and  
518 males in intra- and inter-sexual selection experiments (Chargé et al., 2014; Slade et al., 2014). If  
519 differences between the sexes can be consistently demonstrated, sex-specific management strategies  
520 may be required to improve CBPs. In recognition of this possibility, several recent studies have begun  
521 to explore whether sexual selection theory can be used to inform management strategies (Chargé et al.,  
522 2014; Slade et al., 2014).

523

#### 524 4.4. Implications for Captive Breeding Programmes

525 Our findings that captivity can result in the change of behavioural type and loss of sex-specific  
526 behaviours have significant implications for CBPs. Knowing how captivity changes behaviours across  
527 generations, and whether these changes differ between sexes, can help managers develop and refine  
528 approaches used in captive-breeding and reintroduction programmes.

529

530 The comparative approach (comparing captive-reared with wild-caught animals) used in this study  
531 allows predictions to be made about how behavioural types displayed in captivity may impact fitness  
532 of individuals following reintroduction (Mathews et al., 2005). Although we were unable to evaluate  
533 reintroduction success in the present study, past studies have reported links between behavioural  
534 change and post-reintroduction fitness (Bremner-Harrison et al., 2004). For example, evidence for  
535 maladaptive behavioural changes has been obtained for swift foxes (*V. velox*). A comparative study in  
536 this species revealed that a combination of habituation and directional selection resulted in individuals  
537 becoming bolder in captivity, and that the boldest individuals had a reduced probability of survival  
538 post release (Bremner-Harrison et al., 2004).

539

540 The evidence for transgenerational effects on behavioural type in the captive environment observed in  
541 our study highlights the potential for conservation biologists to manipulate the captive environment to  
542 induce phenotypic changes that may improve the fitness of animals following reintroduction. One  
543 approach may include providing natural conditions during early development, which may reduce the  
544 behavioural changes occurring in captivity (Evans et al., 2014). For example, in Atlantic salmon  
545 (*Salmo salar*) exposure of parents to natural conditions resulted in a two-fold increase in offspring  
546 survivorship in the wild, thereby mitigating the effects of captivity on descendants following  
547 reintroduction (Evans et al., 2014).

548

549 For most animal groups the effects of captivity on sex-specific differences in behaviour remain  
550 unknown. Our findings that captivity potentially may lead to the loss of sex-specific behavioural types  
551 provided important insights into the potential impacts of captivity on behavioural phenotypes.

552 Specifically, our results suggest that the sexes may need to be treated differently during the  
553 management of captive colonies, or when establishing reintroduction programmes. Gaining further  
554 information on sex-specific responses to captivity will assist with the development of effective sex-  
555 specific management strategies in captivity. Finally, incorporating knowledge of phenotypic traits  
556 such as behaviour into captive breeding and reintroduction programmes improves the likelihood of

557 minimising unfavourable phenotypic changes (Mathews et al., 2005; Smith and Blumstein 2008;  
558 Evans et al., 2014; Courtney Jones et al., 2015).

559

#### 560 4.5. Conclusions

561 This study aimed to determine whether behavioural types displayed in a novel environment differed  
562 between captive-reared and wild-caught house mouse (*Mus musculus*), to test whether these  
563 behavioural types were subject to transgenerational effects in captivity, and whether there were sex  
564 differences in behavioural types. Mice reared in a captive environment were found to differ in their  
565 boldness and activity behavioural type compared with their wild-caught conspecifics. After one  
566 generation there was evidence for transgenerational effects in captivity on behavioural traits but not  
567 behavioural type, and there was no evidence that changes in behavioural type were dependent on sex.  
568 Importantly, however behavioural type did differ between wild-caught females and males, suggesting  
569 that captivity resulted in the loss of sex specific behaviours. These findings contribute to a small but  
570 growing body of evidence that i) captivity can result in a change of behavioural type and the loss of  
571 sex-specific behaviours, and ii) phenotypic plasticity might have a significant influence on  
572 behavioural types across captive generations. This knowledge may prove to be important for  
573 developing methods to improve CBPs and reintroduction programmes.

574

#### 575 5. Supporting information

576 Additional supporting information may be found in the online version of this article at the publisher's  
577 website:

578

579 Table A1:

580 Behavioural traits that contributed most to similarity in behavioural type composition between rearing  
581 environment (captive-reared F<sub>0</sub>, captive-reared F<sub>1</sub> and wild-caught individuals) and sex (females and  
582 males; based on the SIMPER procedure using normalised data of residuals of least squares regression  
583 for each behavioural trait on body mass).

584



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590

591 7. *Author competing interests*

592 The authors declare no competing interests.

593

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733 **Table 1** Ethogram of behaviours measured in open field test.

Behavioural trait	Behavioural measure description	Functional category
Distance (m)	Total distance covered in OFT	Activity
Meandering ( $^{\circ}$ /m)	Absolute turn angle/Total distance travelled	Boldness
Mean speed (m/s)	Average speed during OFT	Activity
Maximum speed (m/s)	Maximum speed reached during OFT	Activity/Boldness
% Time mobile	% Total time spent mobile (Animal is in motion)	Activity
% Time active	% Total time spent active (Animal is mobile or performing some other behaviour)	Activity
% Time freezing	% Total time spent freezing (Animal is not moving, may be performing some other behaviour)	Boldness
Jumping: total number	Total count of jumps in OFT	Boldness
In tunnel: total time (s)	Total time spent in the tunnels (May include or exclude tail)	Boldness
% Centre: total time spent	% Time spent in the centre of the arena	Boldness
Centre: mean speed (m/s)	Average speed in centre zone of OFT	Activity
Centre: maximum speed (m/s)	Maximum speed in centre zone of OFT	Activity/Boldness
Perimeter: mean speed (m/s)	Average speed in perimeter zone of OFT	Activity
Perimeter: maximum speed (m/s)	Maximum speed in perimeter zone of OFT	Activity/Boldness

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735

736 **Table 2** PERMANOVA analyses comparing effects of rearing environment and sex on behavioural  
 737 type using multivariate behavioural trait data.

	d.f.	MS	Pseudo-F	P(perm)
Acclimation period x Rearing environment x Sex	2	23.701	1.989	0.058
Acclimation period x Rearing environment	2	11.494	0.964	0.447
Acclimation period x Sex	1	6.920	0.581	0.709
Rearing environment x Sex	2	35.769	3.002	0.008*
Rearing environment	2	60.794	5.102	<0.001*
Sex	1	4.947	0.415	0.858
Acclimation period	1	11.963	1.004	0.375
Residual	64	11.916		

738 **Table 3** PERMANOVA pairwise tests comparing behavioural type between rearing environments and  
 739 sex using multivariate behavioural trait data.  
 740

Pairwise Tests	t	Den. df	P (perm)
F <sub>1</sub> Female, F <sub>0</sub> Female	1.927	25	0.013*
F <sub>1</sub> Female, Wild Female	1.542	25	0.052
F <sub>0</sub> Female, Wild Female	1.269	26	0.161
F <sub>1</sub> Male, F <sub>0</sub> Male	1.389	20	0.107
F <sub>1</sub> Male, Wild Male	1.429	17	0.096
F <sub>0</sub> Male, Wild Male	2.810	15	<0.001*
F <sub>0</sub> Female, F <sub>0</sub> Male	1.312	22	0.161
F <sub>1</sub> Female, F <sub>1</sub> Male	0.811	23	0.665
Wild Female, Wild Male	1.845	19	0.015*

741



742 **Table 4** Effect of rearing environment and sex on behavioural traits in house mouse. Statistical output from linear mixed effects models (LMMs).

Behavioural trait	Rearing Environment x Sex											
	Sex			Rearing environment			Sex			Acclimation period		
	F	d.f.	p	F	d.f.	p	F	d.f.	p	F	d.f.	p
Distance	0.748	2, 69	0.477	5.409	2, 69	0.006*	0.267	1, 69	0.607	1.048	1, 69	0.309
% Time active	8.767	2, 69	<0.001*	13.009	2, 69	<0.001*	0.967	1, 69	0.328	2.883	1, 69	0.094
% Time mobile	5.942	2, 69	0.004*	11.546	2, 69	<0.001*	1.151	1, 69	0.287	0.758	1, 69	0.387
% Time freezing	2.447	2, 69	0.094	12.947	2, 69	<0.001*	1.586	1, 69	0.212	0.898	1, 69	0.346
Mean speed	0.754	2, 69	0.474	5.411	2, 69	0.006*	0.265	1, 69	0.608	1.044	1, 69	0.310
Centre: maximum speed	0.509	2, 69	0.603	6.031	2, 69	0.004*	1.140	1, 69	0.289	1.352	1, 69	0.249
Perimeter: mean speed	0.264	2, 69	0.768	6.067	2, 69	0.004*	0.145	1, 69	0.704	1.633	1, 69	0.205
Perimeter: maximum speed	1.089	2, 69	0.342	0.365	2, 69	0.695	0.831	1, 69	0.365	0.519	1, 69	0.473

743

744 **Table 5** Interactive effects of rearing environment and sex on behavioural traits in house mouse. Values are raw values mean  $\pm$  SE.

Behavioural trait	Wild Female (n = 15) Mean $\pm$ SE	Wild Male (n = 8) Mean $\pm$ SE	Captive F <sub>0</sub> Female (n = 15) Mean $\pm$ SE	Captive F <sub>0</sub> Male (n = 11) Mean $\pm$ SE	Captive F <sub>1</sub> Female (n = 14) Mean $\pm$ SE	Captive F <sub>1</sub> Male (n = 13) Mean $\pm$ SE
% Time active	89.753 $\pm$ 1.395 <sup>A</sup>	73.715 $\pm$ 4.322 <sup>B</sup>	91.858 $\pm$ 2.052 <sup>A</sup>	92.367 $\pm$ 1.467 <sup>A</sup>	90.237 $\pm$ 1.783 <sup>A</sup>	91.788 $\pm$ 2.320 <sup>A</sup>
% Time mobile	88.046 $\pm$ 2.414 <sup>A</sup>	72.289 $\pm$ 5.277 <sup>B</sup>	91.727 $\pm$ 2.088 <sup>A</sup>	92.367 $\pm$ 1.467 <sup>A</sup>	90.120 $\pm$ 1.810 <sup>A</sup>	91.238 $\pm$ 2.499 <sup>A</sup>

745 Post-hoc test (Tukey's HSD pairwise comparison tests) for differences among means; means labelled with differing letters are significantly different.

746

747 **Table 6** Effect of rearing environment on behavioural traits in house mouse. Values are raw values mean  $\pm$  SE.

Behavioural trait	Wild (n = 23)	Captive F <sub>0</sub> (n = 26)	Captive F <sub>1</sub> (n = 27)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Distance	137.106 $\pm$ 15.425 <sup>B</sup>	189.775 $\pm$ 24.627 <sup>A</sup>	139.658 $\pm$ 6.372 <sup>B</sup>
% Time freezing	49.141 $\pm$ 3.321 <sup>B</sup>	31.290 $\pm$ 2778 <sup>A</sup>	41.948 $\pm$ 2.250 <sup>B</sup>
Mean speed	0.114 $\pm$ 0.013 <sup>B</sup>	0.158 $\pm$ 0.021 <sup>A</sup>	0.116 $\pm$ 0.005 <sup>B</sup>
Centre: maximum speed	1.818 $\pm$ 0.112 <sup>B</sup>	1.390 $\pm$ 0.058 <sup>A</sup>	1.619 $\pm$ 0.071 <sup>A</sup>
Perimeter: mean speed	0.110 $\pm$ 0.012 <sup>B</sup>	0.166 $\pm$ 0.023 <sup>A</sup>	0.133 $\pm$ 0.008 <sup>B</sup>

748 Post-hoc test (Tukey's HSD pairwise comparison tests) for differences among means; means labelled with differing letters are significantly different.

## Supplementary data

Supplementary data associated with this article.

**Table A1.** Behavioural traits that contributed most to similarity in behavioural type composition between rearing environment (captive-reared F<sub>0</sub>, captive-reared F<sub>1</sub> and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

**Table A1** Behavioural traits that contributed most to similarity in behavioural type composition between rearing environment (captive-reared F<sub>0</sub>, captive-reared F<sub>1</sub> and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Wild Male - Average squared distance = 17.87						
Behavioural trait	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
Mean speed	-0.628	0.207	0.46	1.16	1.16	
Distance	-0.627	0.207	0.46	1.16	2.32	
Perimeter: mean speed	-0.63	0.255	0.45	1.42	3.74	
Centre: mean speed	-0.538	0.306	0.5	1.71	5.45	
Meandering	0.506	0.335	0.59	1.87	7.32	
In tunnel: time pressed	0.0649	0.637	0.39	3.56	10.89	
Perimeter: maximum speed	0.153	0.735	0.4	4.11	15	
% Time freezing	1.25	0.952	0.46	5.33	20.33	
Jump: number of presses	0.24	1.24	0.49	6.96	27.29	
% Centre: total time spent	-0.48	1.85	0.58	10.33	37.62	
% Time active	-1.59	1.93	0.53	10.82	48.43	
% Time mobile	-1.47	2.23	0.49	12.5	60.93	
Maximum speed	0.595	2.61	0.43	14.6	75.53	
Captive-reared F <sub>0</sub> Male - Average squared distance = 6.03						
	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
Perimeter: maximum speed	-0.117	0.0574	0.48	0.95	0.95	
In tunnel: time pressed	-0.276	0.0695	0.51	1.15	2.11	
% Time mobile	0.507	0.257	0.54	4.27	6.37	
% Time active	0.503	0.332	0.54	5.51	11.88	
Centre: maximum speed	-0.261	0.34	0.46	5.64	17.52	
Jump: number of presses	-0.191	0.35	0.42	5.81	23.33	
Maximum speed	-0.219	0.388	0.38	6.44	29.77	
% Time freezing	-0.575	0.446	0.57	7.4	37.17	
Perimeter: mean speed	0.359	0.465	0.42	7.71	44.88	
% Centre: total time spent	-0.0656	0.511	0.55	8.49	53.36	
Meandering	-0.48	0.572	0.47	9.49	62.86	
Mean speed	0.385	0.645	0.41	10.7	73.55	
Captive-reared F <sub>1</sub> Male - Average squared distance = 7.79						
	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
Perimeter: maximum speed	-0.273	0.0647	0.47	0.83	0.83	
Centre: mean speed	-0.325	0.181	0.45	2.33	3.16	
Mean speed	-0.255	0.199	0.51	2.56	5.71	
Distance	-0.256	0.2	0.51	2.56	8.28	
Perimeter: mean speed	-0.217	0.275	0.5	3.53	11.81	
Meandering	0.104	0.34	0.52	4.36	16.17	
Maximum speed	-0.35	0.353	0.36	4.52	20.7	

Centre: max speed	-0.116	0.489	0.5	6.28	26.98
% Centre: total time spent	0.134	0.658	0.46	8.44	35.41
Jump: number of presses	-0.158	0.669	0.52	8.59	44
% Time freezing	0.143	0.703	0.51	9.02	53.02
% Time mobile	0.191	0.793	0.39	10.17	63.2
% Time active	0.211	0.89	0.4	11.42	74.61

Wild-caught Female - Average squared distance = 12.12

	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
Perimeter: maximum speed	0.0261	0.126	0.43	1.04	1.04
Maximum speed	0.186	0.167	0.43	1.37	2.41
Centre: maximum speed	0.462	0.275	0.5	2.27	4.69
% Time active	0.0255	0.311	0.42	2.57	7.25
Perimeter: mean speed	-0.126	0.49	0.33	4.04	11.3
Mean speed	0.0506	0.678	0.36	5.6	16.89
Distance	0.0504	0.679	0.36	5.6	22.49
Meandering	-0.282	0.745	0.49	6.15	28.64
% Time mobile	-0.0831	0.761	0.35	6.28	34.92
% Time freezing	0.206	0.829	0.46	6.83	41.75
Centre: mean speed	0.269	1.07	0.42	8.81	50.57
% Centre: total time spent	-0.337	1.77	0.49	14.64	65.2
Jump: number of presses	0.618	1.82	0.45	14.99	80.2

Captive-reared F<sub>0</sub> Female - Average squared distance = 26.07

	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
In tunnel: time pressed	-0.186	0.225	0.46	0.86	0.86
% Centre: total time spent	-0.0694	0.516	0.49	1.98	2.84
Centre: maximum speed	-0.472	0.557	0.5	2.14	4.98
% Time mobile	0.322	0.621	0.39	2.38	7.36
% Time active	0.311	0.778	0.38	2.98	10.34
Jump: number of presses	-0.255	1.1	0.49	4.23	14.57
% Time freezing	-0.555	1.17	0.5	4.47	19.04
Maximum speed	0.421	2.29	0.35	8.8	27.84
Centre: mean speed	0.381	2.43	0.44	9.33	37.18
Meandering	0.0543	2.98	0.48	11.44	48.62
Distance	0.535	3.01	0.43	11.54	60.16
Mean speed	0.535	3.01	0.43	11.55	71.72

Captive-reared F<sub>1</sub> Female - Average squared distance = 5.07

	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
Mean speed	-0.335	0.117	0.42	2.3	2.3
Distance	-0.333	0.117	0.42	2.32	4.62
Centre: mean speed	-0.386	0.122	0.41	2.4	7.02
Perimeter: maximum speed	-0.268	0.171	0.32	3.37	10.38
Maximum speed	-0.494	0.202	0.48	3.98	14.36

Perimeter: mean speed	-0.197	0.249	0.51	4.91	19.27
Meandering	0.236	0.301	0.46	5.94	25.21
Jump: number of presses	-0.23	0.404	0.54	7.96	33.17
% Time mobile	0.0082	0.433	0.41	8.53	41.71
In tunnel: time pressed	0.0782	0.454	0.51	8.96	50.67
% Time freezing	-0.0181	0.457	0.52	9.01	59.68
% Time active	-0.0426	0.545	0.4	10.74	70.42

Wild-caught Male & Captive-reared F<sub>0</sub> Male - Average squared distance = 39.71

	Wild-caught Male	Captive-reared F <sub>0</sub> Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
% Time active	-1.59	0.503	6.38	1.04	16.08	16.08
% Time mobile	-1.47	0.507	6.1	0.83	15.37	31.45
Centre: maximum speed	0.695	-0.261	5.05	0.53	12.72	44.17
% Time freezing	1.25	-0.575	4.56	1.1	11.48	55.64
Maximum speed	0.595	-0.219	3.3	0.49	8.31	63.95
% Centre: total time spent	-0.48	-0.0656	2.25	1.07	5.67	69.62
Centre: mean speed	-0.538	0.379	1.97	0.59	4.96	74.58

Groups Wild Male & Captive-reared F<sub>1</sub> Male - Average squared distance = 33.09

	Wild-caught Male	Captive-reared F <sub>1</sub> Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cum.%
% Time active	-1.59	0.211	5.77	1	17.43	17.43
% Time mobile	-1.47	0.191	5.45	0.79	16.48	33.9
Centre: maximum speed	0.695	-0.116	4.93	0.54	14.91	48.81
Maximum speed	0.595	-0.35	3.5	0.49	10.58	59.39
% Time freezing	1.25	0.143	2.7	0.89	8.16	67.55
% Centre: total time spent	-0.48	0.134	2.6	0.97	7.85	75.41

Captive-reared F<sub>0</sub> Male & Captive-reared F<sub>1</sub> Male - Average squared distance = 15.90

	Captive-reared F <sub>0</sub> Male	Captive-reared F <sub>1</sub> Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
In tunnel: time pressed	-0.276	0.385	2.33	0.39	14.63	14.63
% Time freezing	-0.575	0.143	1.57	0.74	9.87	24.5
Centre: mean speed	0.379	-0.325	1.52	0.54	9.57	34.07
% Time active	0.503	0.211	1.21	0.52	7.6	41.67
Distance	0.385	-0.256	1.18	0.55	7.45	49.12
Mean speed	0.385	-0.255	1.18	0.55	7.42	56.54
Meandering	-0.48	0.104	1.17	0.7	7.39	63.93
% Centre: total time spent	-0.0656	0.134	1.11	0.75	6.99	70.92

Groups Wild-caught Male & Wild-caught Female - Average squared distance = 35.44

	Wild-caught Male	Wild-caught Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %

			Dist			
% Time active	-1.59	0.0255	4.6	0.95	12.98	12.98
% Time mobile	-1.47	-0.0831	4.59	0.75	12.96	25.94
Centre: maximum speed	0.695	0.462	4.14	0.59	11.67	37.61
% Centre: total time spent	-0.48	-0.337	3.29	0.82	9.29	46.9
Jump: number of presses	0.24	0.618	2.93	0.64	8.26	55.16
In tunnel: time pressed	0.0649	-0.0527	2.81	0.37	7.93	63.09
% Time freezing	1.25	0.206	2.69	0.79	7.59	70.68

Captive-reared F<sub>0</sub> Male & Captive-reared F<sub>0</sub> Female - Average squared distance = 31.07

	Captive-reared F <sub>0</sub> Male		Captive-reared F <sub>0</sub> Female				
Behavioural trait	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
Perimeter: maximum speed	-0.117	0.465	4.32	0.28	13.91	13.91	
Meandering	-0.48	0.0543	3.59	0.73	11.56	25.47	
Distance	0.385	0.535	3.42	0.57	11.01	36.48	
Mean speed	0.385	0.535	3.42	0.57	11.01	47.49	
Perimeter: mean speed	0.359	0.572	3.42	0.55	11.01	58.5	
Centre: mean speed	0.379	0.381	3.13	0.65	10.08	68.57	
Maximum speed	-0.219	0.421	2.9	0.42	9.35	77.92	

Wild-caught Female & Captive-reared F<sub>0</sub> Female - Average squared distance = 39.53

	Wild-caught Female		Captive-reared F <sub>0</sub> Female				
Behavioural trait	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
Perimeter: maximum speed	0.0261	0.465	4.24	0.29	10.73	10.73	
Perimeter: mean speed	-0.126	0.572	3.9	0.5	9.86	20.59	
Mean speed	0.0506	0.535	3.68	0.53	9.31	29.9	
Distance	0.0504	0.535	3.68	0.53	9.3	39.2	
Meandering	-0.282	0.0543	3.59	0.74	9.09	48.29	
Jump: number of presses	0.618	-0.255	3.49	0.65	8.82	57.11	
Centre: mean speed	0.269	0.381	3.28	0.63	8.3	65.41	
In tunnel: time pressed	-0.0527	-0.186	2.47	0.31	6.24	71.66	

Captive-reared F<sub>1</sub> Male & Captive-reared F<sub>1</sub> Female - Average squared distance = 12.43

	Captive-reared F <sub>1</sub> Male		Captive-reared F <sub>1</sub> Female				
Behavioural trait	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %	
In tunnel: time pressed	0.385	0.0782	2.34	0.44	18.84	18.84	
% Time active	0.211	-0.0426	1.39	0.63	11.19	30.03	
% Centre: total time spent	0.134	0.637	1.39	0.74	11.18	41.21	
Centre: maximum speed	-0.116	-0.0734	1.32	0.73	10.58	51.79	
% Time mobile	0.191	0.0082	1.17	0.61	9.39	61.18	
% Time freezing	0.143	-0.0181	1.1	0.76	8.84	70.02	

Wild-caught Female & Captive-reared F<sub>1</sub> Female - Average squared distance = 19.60

	Wild-caught Female		Captive-reared F <sub>1</sub> Female			
Behavioural trait	Av. Value	Av. Value	Av. Sq. Dist	% Contribution	Cumulative %	

			Sq. /SD	Contribution	%	
			Dist			
% Centre: total time spent	-0.337	0.637	3.14	0.81	16	16
Jump: number of presses	0.618	-0.23	2.79	0.57	14.23	30.23
In tunnel: time pressed	-0.0527	0.0782	2.68	0.36	13.67	43.9
Centre: mean speed	0.269	-0.386	1.54	0.53	7.85	51.75
Centre: maximum speed	0.462	-0.0734	1.41	0.96	7.17	58.92
% Time freezing	0.206	-0.0181	1.25	0.74	6.36	65.29
Meandering	-0.282	0.236	1.24	0.77	6.34	71.63

Captive-reared F<sub>0</sub> Female & Captive-reared F<sub>1</sub> Female - Average squared distance = 34.38

	Captive-reared F <sub>0</sub> Female	Captive-reared F <sub>1</sub> Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist /SD	% Contribution	Cumulative %
Perimeter: maximum speed	0.465	-0.268	4.63	0.29	13.46	13.46
Perimeter: mean speed	0.572	-0.197	3.77	0.48	10.98	24.43
Mean speed	0.535	-0.335	3.68	0.48	10.69	35.13
Distance	0.535	-0.333	3.67	0.48	10.68	45.8
Maximum speed	0.421	-0.494	3.17	0.42	9.21	55.01
Meandering	0.0543	0.236	3.1	0.77	9.01	64.02
Centre: mean speed	0.381	-0.386	2.97	0.5	8.65	72.67