# Prior experience of captivity affects behavioural responses to 'novel' environments

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15    **Abstract**

16    Information ecology theory predicts that prior experience influences current behaviour, even if

17    the information is acquired under a different context. However, when individuals are tested to

18    quantify personality, cognition, or stress, we usually assume that the novelty of the test is

19    consistent among individuals. Surprisingly, this 'gambit of prior experience' has rarely been

20    explored. Therefore, here we make use of a wild population of great tits (*Parus major*) to test if

21    prior experience of handling and captivity influences common measures of exploration (open

22    field tests in two novel contexts: room and cage arenas), social response (simulated using a

23    mirror), and behavioural stress (breathing rate). We found that birds with prior experience of

24    captivity (caught previously for unrelated learning and foraging experiments) were more

25    exploratory, but this depended on age: exploration and captivity experience (in terms of both

26    absolute binary experience and the length of time spent in captivity) were associated more

27    strongly in juvenile (first-winter) birds than adults were. However, there was no association of

28    prior experience of captivity with social response and breathing rate, and nor did the measures of

29    exploration correlate. Together our results suggest that re-testing of individuals requires careful

30    consideration, particularly for younger birds, and previous experiences can carry over and affect

31    behaviours differently.

33    **Introduction**

34    Consistent individual variation in behavioural expression is recognised to be common across the

35    animal kingdom with far-reaching ecological and evolutionary consequences for populations,

36    species, and communities (reviewed in Wolf & Weissing, 2012). Intrinsic differences among

37    individuals gives rise to individual-level variation in traits such as exploration behaviour,

38    aggressiveness, or risk-taking (Réale et al., 2007).  These differences can then influence

39    individual fitness components like survival and longevity (Smith & Blumstein, 2008), population

40    dynamics and dispersal (Dall et al., 2005; Cote & Clobert, 2007; Duckworth, 2008), parent-

41    offspring interactions (McGhee & Bell, 2014), as well as biological invasions and range

42    expansions (Carvalho et al., 2013). However, the validity of measurements of animal personality

43    depends on repeatedly testing individuals, to determine whether the differences in focal

44    behavioural responses between individuals are consistent over time and context. Repeatability of

45   so-called personality traits is often low (37% on average, Bell, Hankison & Laskowski, 2009),

46   suggesting that much of the variation in individual behaviour depends on context or other

47   environmental conditions. What contributes to this 'environmental noise' remains an open

48   question.

49

50   Although there are calls to partition variation from a wide range of behaviours to investigate

51   context-independent personalities and correlations among traits (Dingemanse & Dochtemann,

52   2013, Sánchez-Tójar, Moiron & Niemelä, 2022), many studies use a two-step approach

53   (reviewed in Niemelä & Dingemanse, 2014). Here, single behavioural responses are measured

54   under a single context before variation is related to ecological or fitness components of interest

55   (Niemelä & Dingemanse, 2014), and many of the assays derived to measure putative personality

56   traits involve capture of free-living individuals. This introduces important potential artefacts:

57   firstly, the types of individuals captured may not necessarily be a random sample of a study

58   population (unintended biased sampling; e.g. Biro & Dingemanse, 2008; Carter et al., 2012a;

59   Stuber et al., 2013), which becomes especially problematic when tests require re-capture of

60   individuals (Wilson et al., 1993; Garamszegi, Eens & Török, 2009). Secondly, tests in captivity

61   may generate variation in behavioural responses that might not manifest in the wild (Niemelä &

62   Dingemanse, 2014) or thirdly, tests in captivity may generate variation in behavioural responses

63   due to differences in how individuals respond to captivity itself (Thorpe, 1956; Butler et al.,

64   2006). For instance, in a foraging behaviour experiment on wild caught chaffinches (*Fringilla*

65   *coelebs*), Butler *et al.* (2006) found that birds were less successful at completing behavioural

66   trials when the time spent in captivity prior to the tests was longer than for individuals given a

67   shorter acclimation time. Furthermore, assays of animal personality often assume that the novelty

68   of the test is equal for all individuals (e.g. novel environment, novel object; Carter, 2013). This

69   may be because many of the tests were adapted from animal psychology studies (Réale et al.

70   2007) where subjects were captive throughout their lives and thus histories were known, but this

71   is not the case when working with wild animals.

72

73   Each of these artefacts and assumptions invokes an implicit 'gambit of prior experience' where

74   we assume that our tests on wild individuals will be sufficiently robust to detect individual

75   differences, despite the likelihood that no individual shares a similar past. Information ecology

76   theory predicts that information gathered from a range of sources prior to an experience will

77   influence current behaviour (Dall et al., 2005), and this may not necessarily vary consistently

78   among individuals according to the personality trait of interest (Rodriguez-Prieto, Martin &

79   Fernandez-Juricic, 2011; Dingemanse et al., 2012; Niemelä & Dingemanse, 2014; Smit & van

80   Oers, 2019). For instance, bold trout that lose experimental fights become more shy (increased

81   latency to approach a novel object) and shy trout that win become more bold (Frost et al. 2007);

82   therefore, estimates of boldness for wild-caught trout blind to prior experience would include

83   variation that could not be accounted for and affect conclusions. However, quantifying the prior

84   experience of wild animals, and how it may influence their response to behavioural tests, is

85   usually effectually impossible and an issue inherent across behavioural ecology. Nevertheless,

86   prior experience is likely to affect subsequent behavioural testing or repeated testing, and affect

87   the estimation of repeatability of personality traits.

88

89   Prior experience may manifest itself in the state of an individual, or be related to differences in

90   age. However, it is well known that individuals also vary in how they respond to captivity or

91   habituate to stimuli (e.g. Zimmermann et al., 2001; Carere & van Oers, 2004; Ellenberg, Mattern

92   & Seddon, 2009; Biro, 2012), and this may or may not reflect intrinsic variation in tolerance

93   (Martin & Réale, 2008; Allan, Bailey & Hill, 2020). It is therefore conceivable that experiences

94   during previous periods of captivity might affect the outcome of subsequent behavioural tests.

95   For instance, when exploring a novel environment an individual collects information about its

96   environment and properties (Renner, 1990; Greenberg & Mettke-Hofmann, 2001). However, the

97   perception of the testing environment may be different for animals that have experienced

98   captivity before. Studies on the two most commonly used inbred strains of lab-mice found that

99   prior experience and training affected behavioural aspects differently (McIlwain et al., 2001;

100  Võikar, Vasar & Rauvala, 2004). More specifically, mice that underwent previous handling and

101  testing showed reduced exploration activity, emotionality and contextual fear compared to naïve

102  mice. A previous experience of captivity may also have a carry-over effect by influencing the

103  time taken to acclimatize to the novel environment (e.g. Butler et al., 2006; Martin & Réale,

104  2008) and shape responses to novel stimuli. Although some attention has been given to the

105    effects of acclimation time on a focal behaviour (Butler et al., 2006) and reduced response

106    (sensitization: Groves & Thompson, 1970) due to habituation to behavioural tests (Martin &

107    Réale, 2008; Dingemanse et al. 2012), these artefacts of behavioural testing methods are only

108    beginning to be explored (Forss et al., 2021).

109

110    Here, we test how prior captivity influences three commonly measured behavioural traits:

111    exploration, social response and breathing rate. We took advantage of a population of great tits, a

112    model species for studies on animal personality, where many individuals were used previously in

113    unrelated foraging experiments (see *Table S1*). Exploration behaviour is one of the most

114    commonly tested traits in animal personality studies (Carter et al., 2012a) and in great tits, it

115    correlates with multiple important ecological variables (e.g. dominance: Dingemanse & de

116    Goede, 2004; space use: van Overveld & Matthysen, 2010; van Overveld, Adriaensen &

117    Matthysen, 2011). Exploration behaviour is assayed most often using a novel arena in a room

118    (e.g. Dingemanse et al., 2002) or cage (e.g. Stuber et al., 2013), and so here we used both types

119    of novel environments to estimate repeatability (Carter et al., 2012a) while reducing the potential

120    for habituation and learning effects (e.g. Réale et al., 2007; Uher, 2011). To assay social

121    response behaviour we used a mirror test that provides a perfectly matched competitor and

122    responses can vary from avoidance to attraction. Mirror tests originate from research on primates

123    but is increasingly used to quantify behaviour in birds (e.g. Henry et al., 2008; Carvalho et al.,

124    2013). Finally, we measured breathing rate to quantify response to handling stress (e.g. Carere &

125    van Oers, 2004). Breathing rate has been shown to correlate with exploration behaviour and

126    boldness in great tits (Carere & van Oers, 2004; Fucikova et al., 2009) and cage activity in blue

127    tits (*Cyanistes caeruleus*, Kluen, Siitari & Brommer, 2014). All behavioural assays were novel,

128    but nearly half of the birds had some prior experience of captivity and handling. We predicted

129    that birds with prior experience of captivity would be less stressed by handling and more

130    acclimatised to the testing environment (i.e. have a slower breathing rate), and thus more

131    exploratory and responsive to a conspecific. If prior experience had an absolute effect on

132    behaviour, we expected an association with a binary measure of captivity experience (i.e.

133    yes/no), or if the effect of prior experience increased linearly then we expected an association

134    with the number of days spent in captivity.

135

**Materials & Methods**

*Study site and catching methods*

138    During March 2018, we caught 50 great tits around the University of Jyväskylä Konnevesi

139    Research Station (62.616°N, 26.346°E) using permanent feeder-traps baited with peanuts and

140    familiar to the birds as they are open throughout the winter (see Ham et al., 2006 for details). An

141    additional three great tits were caught over two days in the city of Jyväskylä (62.253°N,

142    25.750°E), using the same type of peanut trap. Birds were transported to the laboratory within 10

143    minutes after trapping, or within two hours when caught in the city of Jyväskylä, and all birds

144    were caught between 9am (sunrise at 06:40am) and 12pm. We also used a commercially

145    available 'tent spring trap' (TW45 Moudry-trap (www.moudry.cz)) baited with seeds at

146    additional locations around the research station for a different study on blue tits and caught a

147    further three great tits as by-catch and used them in the current study (total sample size N = 56).

148    While trapping location was recorded, the trap style used for each bird was not. However, we

149    checked if differences in trapping location affected our results by repeating analyses with the

150    three birds from Jyväskylä omitted (*Table S2*). Results were qualitatively the same so we

151    retained them in our dataset.

152

*Housing*

154    Birds were weighed (mass to the nearest 0.25 g using a 30 g Pesola (www.pesola.com) spring

155    balance) upon arrival and released into individual plywood housing boxes (*Fig. 1A,* 65 cm length

156    x 60 cm width x 80 cm height) where they were in auditory, but not visual, contact (as used

157    during previous experiments, e.g. Hämäläinen et al. 2020). The housing boxes had a perch, were

158    illuminated by a light bulb (for no more than 12 hours per day), and the front consisted of a

159    sliding darkened Plexiglas wall that facilitated observation by observers but limited visual

160    contact for the bird as the boxes were in a dark room. All birds had access to drinking water and

161    food *ad libitum* (mix of peanuts, sunflower seeds and commercially available fat granules) and

162    were left undisturbed for at least one hour before the first behavioural assays commenced. All

163    behavioural assays for each bird were performed during one day (11am – 6pm) and took place

164    during natural daylight hours. Once the assays were completed, individuals were measured (wing

165    length to the nearest mm and mass as above), ringed with a numbered metal ring issued by the

166    Finnish Museum of Natural History (if un-ringed) and sex and age (young (first winter)/old)

167    were recorded based on plumage characteristics (Svensson, 1992). Birds were released during

168    daylight hours at the site of trapping before 4pm; when birds could not be released before 4pm,

169    birds were housed overnight and released the following morning. All animal care was provided

170    or supervised by a highly-trained animal care technician employed by the research station, and

171    no birds were harmed or euthanised during the experiment.

172

173    *Capture history*

174    Almost half (26 out of 56: 46%) of the trapped birds had been held previously in the research

175    facilities (from here on referred to as 'captivity experience'). They experienced similar housing

176    and handling procedures as described above and participated in a range of learning experiments

177    where they were offered food items of varying palatability in plywood boxes similar to their

178    housing box (*Table S1*). Birds were caught semi-randomly with respect to captivity experience,

179    although experienced birds tended to be caught more often earlier in the study (*Fig. S*1) as these

180    were needed for another experiment. Date was therefore included as a covariate in all analyses

181    (see data analysis methods).

182

183    *Exploration assays*

184    The behavioural assays performed in this study were novel to all birds. Exploration behaviour

185    was measured twice for each bird within one day, once in a room and once in a cage. The order

186    of these two trials was alternated and the birds were left undisturbed for at least one hour in their

187    housing box with food and water *ad lib.* between trials. The exploration trials and social response

188    assay were recorded and videos analysed using open-source software 'BORIS' (Friard and

189    Gamba 2016).

190

191   The room was intended to match as closely as possible to the layout described in detail by

192   Dingemanse *et al*. (2002), given logistical constraints and existing architecture (*Fig. 1B*). The

193   room measured 390 cm length x 340 cm width x 230 cm height, and contained five artificial

194   trees that were 150 cm tall, each with four 20 cm long horizontal 'branches'. At least one hour

195   prior to the start of the exploration assay, each bird was brought from its housing box in its

196   original resting place using a cotton bird bag and housed individually in one of the five boxes

197   (similar in layout and size to their original housing box) placed on the floor along one side of the

198   room. The fronts of the five housing boxes were covered in dark plastic to prevent visual contact

199   with the room.  Each trial was filmed using a remote controlled GoPro camera (model: Hero4

200   Session (GoPro, San Mateo, USA)) fixed to an upper corner of the room for later analysis, see

201   *Video S1* for an example video clip of room exploration ,while an observer followed the bird

202   through a one-way window in the door. Immediately prior to the start of the trial, an observer

203   entered the room in complete darkness, switched off the home box light and opened the sliding

204   front wall of the focal individual's house box. The observer then left the room and switched on

205   the room light remotely, marking the start of the trial. Each bird was individually tested, where

206   the focal bird was given 10 minutes to enter and explore the room while the observer scored its

207   movements through a one-way window in the room's door, see below for how the exploration

208   behaviour was scored. If a bird started to explore only after eight minutes of the trial, we

209   continued observations until two minutes of exploration had been completed. Once the trial

210   ended, the bird was coaxed to return to its home box by the observer switching off the lights in

211   the room and switching on the light in the box.

212   Following Dingemanse *et al.* (2002), we recorded the number of flights and hops a bird made

213   during the first two minutes of exploration in the room (i.e. when a bird emerged from its

214   housing box), through the one-way window. All flights and hops within a 'tree' (but not within a

215   'branch'), and between 'trees', floor, ceiling or walls were counted. One bird hopped mainly on

216   the floor of the room, this individual was scored according to approximate 'between tree

217   distance' covered, and 10 individuals did not explore during the assay and thus were assigned a

218   zero score for room exploration in the analysis.

219

220    Our second assay of exploration behaviour used a cage arena (*Fig. 1C*) adapted from previous

221    studies with great tits (Stuber et al., 2013) and other passerines (Herborn et al., 2010; Kluen et

222    al., 2012; Zhao & Sun, 2016). The cage (100 cm length x 35 cm width x 52 cm height) was

223    placed on a table in a dark room (a battery-powered led light at both ends of the cage provided

224    illumination), and all sides were opaque except for the front which consisted of metal mesh to

225    allow filming. A sliding wall (double layer, see mirror assay) divided the cage into two

226    compartments: an acclimation side (40 cm length x 35 cm width x 52 cm height) containing two

227    perches and an exploration side (60 cm length x 35 cm width x 52 cm height) containing five

228    perches. Each layer of the dividing wall could be moved using a plastic rod attached to the wall,

229    meaning the observer remained out of view of the bird. Each bird was tested individually. The

230    assay was filmed using a Canon PowerShot A2400 (Canon, Tokyo, Japan) camera mounted on a

231    tripod 150 cm from the cage, see *Video S2* for an example video clip of cage exploration.

232    Immediately prior to the trial, the bird was caught from its housing box (within one minute of

233    switching off the home box light) and released into the acclimation side of the cage for five

234    minutes. Filming of the trial started immediately after the bird was released. After five minutes,

235    both layers of the sliding wall were opened simultaneously by 10 cm, giving the bird 10 minutes

236    the opportunity to move into the exploration side of the cage.

237

238    From the video we recorded the number of hops and flights for two minutes once the bird

239    emerged from its acclimation side into the exploration side (either by choice or when coaxed

240    through after 10 minutes, see above). All flights and hops between (but not within) the perches,

241    floor or mesh were counted. Ten birds (32%) returned to the acclimation side during the two

242    minutes. For these birds, we decided to include all movements made, regardless of which side of

243    the cage the bird was in, rather than negatively bias their measures of exploration. This was

244    because the majority (9 out of 10) spent more time on the exploration side (one bird: 2%, seven

245    birds: 21 – 30%, one bird: 41%) and visits to the acclimation side were in short bouts of a few

246    seconds (except for one bird who returned to the acclimation side for 74% of the two minutes in

247    one 88s bout). All birds showed continuous movement on the acclimation side, exhibiting similar

248    behaviour to that on the exploration side. Three birds entered the exploration side of the cage just

249    before the end of the 10 minute assay and were erroneously assumed (during the assay) to have

250    been present for two minutes before the social response assay began. Therefore, the exploration

251    time for these birds was very short (22 s, 28 s and 46 s) and they had a shorter time than other

252    birds to familiarize with the cage before exposure of the mirror. We omitted these three birds

253    from all analyses. Twenty-two birds did not enter the exploration side of the cage within 10

254    minutes. These birds were assigned a zero score for cage exploration. The birds that did not

255    explore the cage were coaxed into the exploration area and were given five minutes to explore

256    this area, with the sliding doors closed to prevent return to the acclimation side, before the social

257    interaction assay was performed, to allow familiarization with the exploration area of the cage. In

258    general, these coaxed birds were all very inactive, where movements in two minutes ranged from

259    0 – 3 moves.

260

261    *Social response behaviour assay*

262    Immediately after the cage exploration trial, we conducted a mirror test to assay social response

263    behaviour following the methods of Carvalho *et al*. (2013). The exploration-side layer of the

264    sliding wall was removed to expose a rectangular mirror (17.5 cm x 25.0 cm) attached to the

265    (closed) acclimation side wall layer and positioned at the same height as three of the five perches

266    (*Fig. 1B*). Birds could not see their reflection from the other two perches as they were above the

267    mirror (i.e. due to the 'law of reflection'). Behaviour towards the mirror was filmed for five

268    minutes before the lights were switched off and the bird was caught from the cage by hand. See

269    *Video S3* for an example video clip of response towards the mirror.

270    From the videos we recorded: the number of pecks against the mirror and the time a bird spent

271    looking at the mirror (time spent looking at the mirror was counted when the bird's beak was at

272    maximum of 90 degrees angle towards the mirror, following Carvalho et al., 2013), the amount

273    of time (in seconds) birds spent on each perch and the number of movements (in the same

274    manner as for the exploration assay). We then calculated the proportion of time spent on each

275    perch as a percentage of the total time spent with the mirror. These values were used to calculate

276    the difference in time spent on each of the five perches relative to the time spent on these perches

277    before the social challenge (based on Kluen et al. 2012) using the following formula:

278

279          log [(X + *C* )/ (Y + *C*)]

280

281    where X = proportion of time spent on a perch during mirror exposure trial, Y = proportion of

282    time spent on the same perch during exploration trial, *C* = 1 was included to account for log-

283    transformation of zero values. The proportion of time spent on each perch during the exploration

284    trial was relative to the total amount of time a bird spent on the exploration side of the cage (i.e.

285    not including any time spent back on the acclimation side). For birds that were coaxed to the

286    exploration arena of the cage, we used the five minutes after coaxing to calculate this measure.

287    The same formula above was used to calculate difference in number of movements but instead of

288    proportion of time on each perch, we used movements per second. A score of zero for both

289    relative time spent on a perch and movements indicated no change in behaviour and positive

290    values indicated that the bird spent more time on the perch, or was more active, during the mirror

291    exposure than during exploration.

292

293    *Breathing rate*

294    Immediately after removing the bird from the cage, we recorded its breathing rate in the hand

295    following Kluen, Siitari & Brommer (2014). The bird was held with its back on the palm of the

296    observer's hand and its neck between the observer's index and middle fingers (i.e. ringers grip,

297    *Fig. 1D*), with one leg held between thumb and index fingers and the other by the middle and

298    ring fingers. We then measured the time it took a bird to take 30 breaths (fall and rise of the

299    chest) twice, using a stopwatch. Breathing rate was calculated as the average of these two

300    measurements and expressed as the number of breaths per second.

301

302    *Data and Statistics*

303    *Principal Component Analysis (PCA) on social response assay*

304    We used a Principal Component Analysis (PCA) to reduce the number of variables extracted

305    from the social response behaviour videos. To improve the principal component interpretation

306    we used data on two of the five perches in the PCA using the following reasoning; The birds'

307   own mirror reflection could not be seen from two out of the five perches, due to the angle

308   (perches high in the cage). Time spent on these two perches was negatively correlated (r = -0.59)

309   and we opted to choose only one of these perches in the PCA i.e. the perch furthest away from

310   the mirror. Furthermore, from the three perches at mirror level, we opted to leave out the two

311   perches furthest from the mirror as these contained many zero's (i.e. they were not often used

312   during the assay). The other variables in the PCA were: 'proportion of time spent looking at the

313   mirror', 'change in number of movements (compared to exploration)' and 'number of pecks at

314   the mirror'. The first Principal Component (PC1; 41%) represented interaction with the mirror:

315   number of pecks on the mirror, relative time spent on the perch closest to the mirror, and

316   increased movement were positively loaded while relative time spent on the perch furthest from

317   the mirror was negatively loaded (see *Table S3* for PC1 loadings). Subsequent PC's (PC2: 24%

318   & PC3: 23%, *Table* S3) were dominated by time spent looking at the mirror (PC2) and increased

319   movement (PC3), however these were not used in further analyses as PC1 already included these

320   behaviours (time spent looking at the mirror was loaded positively but weakly; *Table S3*).  We

321   used the scores of each bird on PC1 as the behavioural variable for the social response assay in

322   further analyses.

323

324   *Phenotypic correlations among behaviours and associations with prior experience of captivity*

325   We first explored phenotypic correlations between the different behaviours using a Pearson's

326   correlation tests. The two assays of exploration behaviour were included separately (i.e.

327   exploration in the room and exploration in the cage). Next, we tested for associations of prior

328   experience of captivity and behaviours using multivariate mixed models (MMMs). MMMs are

329   generalised linear models where multiple response variables are modelled using both fixed and

330   random effect terms. We did this in a Bayesian framework, using R-package MCMCglmm

331   (Hadfield, 2010). We ran 3 050 000 iterations, used a burn-in of 50 000 and sampled the chain

332   every 3000 iterations, ending up with 1000 samples of the chain. We used an inverse gamma

333   uninformative prior and, as we only had repeated measures for exploration, we set within

334   individual variance and within individual correlations for all other behaviours to zero following

335   Houslay & Wilson (2017). Convergence of the chains was checked using plots of the MCMC

336   samples. Exploration behaviour consisted of count data (number of movements in two minutes);

337    therefore, we used a Poisson error distribution in its model and a Gaussian distribution for all

338    other behavioural variables (i.e. PC1 and breathing rate). We ran our analyses twice to

339    investigate whether the length of captivity (i.e. continuous predictor, days in captivity, scaled to

340    the mean and unit standard deviation) or experience of captivity (i.e. binary predictor, yes/no)

341    influenced each behaviour. To assess whether any relationships were significant, we used the

342    estimated coefficients with their 95% Bayesian Credible Intervals (BCI). We considered a

343    significant effect for those covariates which credible intervals did not overlap zero. As a random

344    effect in both models, we used 'bird identity'. The fixed effects structure for both models was the

345    same, with the exception of the captivity variables, and included the following: 'body mass'

346    (scaled to their mean and unit standard deviation), 'sex' (male/female), 'age' (first winter/ older)

347    and 'catching date' (mean centered and scaled by the standard deviation). We also included an

348    interaction between age and each variable describing prior experience of captivity to account for

349    variation in life experience and the possible impacts of developmental processes underlying the

350    expressed behaviour (e.g. Stamps & Groothuis, 2010). When estimating effects on exploration

351    behaviour we included two additional variables: 'test-type' (cage or room, to account for

352    differences in the results due to the arena) and 'test order' (whether conducted first or second). In

353    addition, we checked whether 'test-type' influenced the effects of variables describing prior

354    experience of captivity (assessed using an interaction term in a univariate model; *Table S4*) but

355    as it was not significant, we did not include it in the MMMs. This indicates that any association

356    of captivity experience with exploration behaviour is not dependent on the arena in which

357    exploration was tested.

358

359    Finally we estimated the repeatability of our tests for exploration behaviour, conditional on the

360    fixed effects (adjusted repeatability), using the random effects (co)variances from the MMM

361    (following Houslay & Wilson, 2017) where prior experience of captivity was included as a

362    continuous measure (i.e. days in captivity). We estimated repeatability by dividing the between-

363    individual variance estimate by the sum of the between-individual and residual variance and used

364    the credible interval to assess significance. All statistical analyses were performed in R version

365    3.4.3 (R Core Team, 2017).

366

367 *Ethics*

368 All experiments complied with Finnish law on animal experimentations. Permits for the capture

369 and use of great tits in experiments were granted by the Central Finland Centre for Economic

370 Development Transport and Environment (ELY; VARELY/294/2015) and licensed from the

371 National Animal Experiment Board (ESAVI/9114/04.10.07/2014). Bird trapping was done only

372 under dry (no precipitation) weather conditions. Bird handling was done by skilled persons and

373 with highest possible care and all birds were trapped and released (at the trapping location)

374 during natural daylight hours.

375

376 **Results**

377 After excluding 3 birds with erroneous exploration measurements (see *Methods*), our dataset

378 included assays conducted with 53 great tits (*Table S5*), of which 26 had experienced captivity

379 before, ranging from 2 – 29 days spent in captivity spread out over 1 – 4 capture events (see *Fig.*

380 *S2*). Our dataset included 24 females and 29 males; for the age of the birds 32 birds were in their

381 first winter and 21 were adults.

382

383 *Phenotypic correlations between the behavioural variables*

384 Surprisingly, the two measures of exploration were not correlated, nor were any of the

385 behaviours significantly correlated with one another (*Table 1*). However, both exploration

386 measures tended to correlate positively with PC1 (i.e. more exploratory birds spent more time

387 close to the mirror and pecked against it) although these correlations were not significant (*Table*

388 *1*). We acknowledge that, to detect low significant correlations we should have a large sample

389 size and that ours may not be sufficient in doing so.

390

391 *Does prior experience of captivity influence exploration behaviour?*

392 We found that both the number of days spent in captivity and binary experience of captivity were

393 positively associated with exploration behaviour (*Table 2*, *Figs. 2A & 2B*). This relationship was

394 independent of the type of test used following from the interaction of days in captivity and test-

395    type and experience of captivity and test-type (*Table S4*). However, the effect of prior experience

396    of captivity on exploration behaviour did depend on age where older birds with experience of

397    captivity were less exploratory than younger birds that also had previous experience of captivity

398    following the interaction of age and the binary variable of captivity (*Table 2* and *Fig. 2B*).

399    Furthermore, for birds without previous experience of captivity, older individuals were more

400    exploratory than younger ones (*Fig. 2B*). This interaction with age was also significant for the

401    continuous variable of captivity (*Table 2 and Fig. 2A*), and was not driven by the 10 older birds

402    with a longer captivity experience than the younger birds (results not shown). Test-type was

403    significantly associated with exploratory behaviour, where birds explored less during the first

404    two minutes in the cage than in the room (*Table 2*). Days in captivity and experience with

405    captivity were not associated with social response behaviour (*Table 2*, *Figs. 2C & 2D*) or

406    breathing rate (*Table 2*, *Figs. 2E & 2F*). However, sex was significantly associated with

407    breathing rate, where males breathed slower than females (*Table 2*). In addition, there was a

408    tendency that heavier birds breathed faster following from the nearly significant association of

409    body mass and breathing rate (*Table 2*).

410

411    *Is exploration behaviour repeatable?*

412    The estimated adjusted repeatability of exploration behaviour was 0.12 (BCI 95%: [<0.01 -

413    0.34], *Table 2*) and the lower credible interval value was very close to zero, indicating that the

414    repeatability value was not significant.

415

416    **Discussion**

417    Here we took advantage of knowledge regarding wild individual great tits' capture histories to

418    examine whether the 'gambit of prior experience' may affect point measures of behaviour. We

419    predicted that birds with prior experience of captivity would be more likely to explore and

420    interact with a competitor (mirror image) because they were more habituated to the general

421    context of our testing environment. Our results supported these predictions to an extent: prior

422    experience of captivity influenced exploration behaviour, but this effect varied according to age.

423    Younger birds exhibited a stronger response to previous experience of captivity, exploring more

424    when they had spent more time in captivity before or had ever been caught. Adult birds, on the

425    other hand, showed limited variation in their exploration behaviour in response to prior captivity.

426    We found no difference in social response behaviour or breathing rate, suggesting either that

427    captivity has little effect on social responses or stress, or that intrinsic responses remain

428    consistent across capture events. The latter is plausible for breathing rate, as this measure is often

429    found to be repeatable (Fucikova et al., 2009; Kluen, Siitari & Brommer, 2014). Together our

430    results suggest that re-testing of individuals requires careful consideration, particularly for

431    younger birds, as a single event of captivity may be sufficient to affect the outcome of a

432    behavioural measurement, and previous experiences might carry over and affect behaviours

433    differently. This could be especially important when the behaviour of interest is related to

434    novelty.

435

436    Previous work has shown that exploration of a 'novel' environment scales with repeated

437    experience of the same test (e.g. Minderman et al., 2009; Dingemanse et al., 2012), yet here we

438    show that experience with captivity in general also has an effect on exploration. This relationship

439    may occur through two not mutually exclusive pathways. First, repeated capture may lead to

440    habituation, the lessening of a response after repeated exposure to the same stimulus (Thorpe

441    1956). In our case, habituation to prior experience of captivity could have enabled these

442    individuals to explore more actively because they were less stressed (e.g. Baugh et al., 2013) and

443    therefore assessed the risk associated with the captive situation differently (Augustsson &

444    Meyerson, 2004). However, we did not detect any correlation between breathing rate (i.e. stress,

445    Carere & van Oers, 2004) and exploration, or between breathing rate and prior experience of

446    captivity, but stress measured in the hand may capture a different aspect of stress than that

447    experienced during the acclimatization and exploration periods of our tests. Capture bias, a

448    widely recognised issue in animal behavioural testing (Biro & Dingemanse, 2008; Carter et al.,

449    2012a; Biro, 2012), provides an alternative explanation. Here, bolder, dominant or more

450    aggressive individuals may be more likely to be re-captured at feeding sites, whereas shyer and

451    less aggressive individuals may avoid these after having experienced capture. In this way, naïve

452    birds may be more random with respect to behavioural types. Hence, unintended biased sampling

453    may have occurred and capture history may be confounded. However, capture bias alone is

454  unlikely to explain our results as we also found an effect of length of captivity. Furthermore,

455  multiple individuals in our dataset were caught only once but held in captivity for longer than

456  birds caught multiple times (*Fig. S2*) and when we checked our key result, we found no

457  relationship between the number of times captured and exploration behaviour (*Table S7*).

458

459  The effect of prior experience of captivity on exploration behaviour may also reflect variation in

460  how birds value acquiring new information. It is notable that here we found young birds explored

461  more actively compared to adults with similar experience of captivity, including both in terms of

462  binary experience of captivity and the length of their experience. In some studies, young birds

463  continue to sample information about their surroundings, whereas adults rely more on previous

464  experience (e.g. Aplin, Sheldon & Morand-Ferron, 2013; Franks & Thorogood 2018; Franks et

465  al., 2020; Hämäläinen et al., 2021), and young birds may therefore be more flexible and continue

466  to explore despite prior experience of captivity (but see Penndorf & Aplin, 2020 for a meta-

467  analysis showing a lack of age effects in foraging behaviours). In addition, developmental

468  processes play a large role in the expression of behaviour across context and time (Stamps &

469  Groothuis, 2010). For instance, a study on ravens (*Corvus corax*) and crows (*Corvus corone,*

470  *Corvus cornix*) found that exploration diminishes over ontogeny (Miller et al., 2015) and

471  changes in exploration over age (juvenile/adult) in great tits were due to changes in the

472  underlying genetic architecture of exploratory behaviour during development (Class, Brommer

473  & van Oers, 2019). Similar age context-dependent effects were detected in great tits in terms of

474  dominance and exploration (Dingemanse & de Goede, 2004), providing further evidence that age

475  in combination with prior experience may affect behavioural responses differently.

476

477  While we cannot yet explain why prior experience with captivity influenced exploration, our

478  results do provide support that using single measures of behavioural traits may be unreliable.

479  Like many studies, we measured exploration twice across two measurement contexts, however,

480  like some other studies (Burns, 2008; Fox et al., 2009; Stuber et al., 2013; Kluen & Brommer,

481  2013; Fisher et al., 2015; Arvidsson et al., 2017), we found only mixed evidence that the

482  measures were repeatable. Accounting for capture experience only further reduced repeatability

483  (*Fig. S3*). This is because capture experience is associated with exploration behaviour and

484    therefore any variation in individual experience with captivity will add to differences between

485    individuals in exploration behaviour. Admittedly, our sample size was smaller than

486    recommended to be able to detect a low repeatability (Dingemanse & Dochtermann, 2013), but

487    this is a common problem (Dingemanse & Wright, 2020). Single behavioural observations

488    mainly reflect residual variation (reviewed in Niemelä & Dingemanse, 2018; Dingemanse &

489    Wright, 2020) coming from various contextual or environmental sources, and as our results of

490    prior experience with captivity indicate, this may also be context-dependent according to age.

491

492    Alternatively, methodological differences between the two test setups may underlie the lack of

493    repeatability; our two exploration arenas may have been testing different aspects of behaviour.

494    This too is a common problem in studies of animal personality (Carter et al., 2012a). While some

495    researchers use the same setup multiple times in the same contexts (e.g. Dingemanse et al. 2012),

496    others urge that measures should be done in different setups to ensure the validity of a trait

497    (Carter et al., 2012b). In our experiment, we followed the latter approach using a room and a

498    cage for exploration, however using the cage introduced differences in the way we handled the

499    birds. Birds tested in the room were housed in their home boxes and released from these boxes

500    into the exploration room, whereas the birds tested in the cage were moved by hand from their

501    home boxes and given only a five minute acclimation period before the exploration trial began. It

502    is possible that a component of the exploration behaviour that we measured in the cage was

503    actually a response to handling. Nevertheless, both of our measures of exploration tended to

504    correlate with social response behaviour (mirror test) in the same direction and strength (albeit

505    marginally non-significantly). This finding is comparable to a previous study (Moiron et al.,

506    2019) where exploration behaviour of great tits measured in a cage was weakly correlated with

507    territorial aggressive responses to a taxidermic mount of a male conspecific. Altogether, this

508    stresses the importance of taking into account contextual differences between behavioural

509    measurements, but also differences in prior experience of the tested individuals.

510

511    **Conclusion**

512  In conclusion, our results highlight an issue in studies of behavioural traits that has thus far been

513  largely overlooked, the gambit of prior experience. Using individual histories of captivity, we

514  found that the effects of prior experience on exploration behaviour varied with both the age of

515  individuals tested and the behaviours measured. Indeed, our results suggest that tests commonly

516  associated with novelty are likely to be most affected. Although we do not yet know the

517  mechanisms underpinning the effects of prior experience on exploration behaviour, our study

518  highlights that habituation to captivity may affect acclimatisation and information use, and

519  furthermore, these effects may be context-dependent on both age and testing methodologies. This

520  could be especially important when designing behavioural experiments, as from an ethical (the

521  three R's; Replacement, Reduction and Refinement (Russel & Burch, 1959)) and economical

522  point of view there is a need to reduce the number of animals used in experiments, and studies of

523  animal personality often emphasise the need to recapture and retest individuals to derive

524  appropriate measurements. Our results show that when making decisions on whether to use naïve

525  individuals or reuse individuals, it is important to establish whether previous, and perhaps

526  seemingly unrelated, experience may affect behavioural measurements in subsequent trials. In

527  wild animals, it is usually impossible to ascertain prior experience of tested individuals.

528  However, we suggest taking prior experience into account wherever possible.

529

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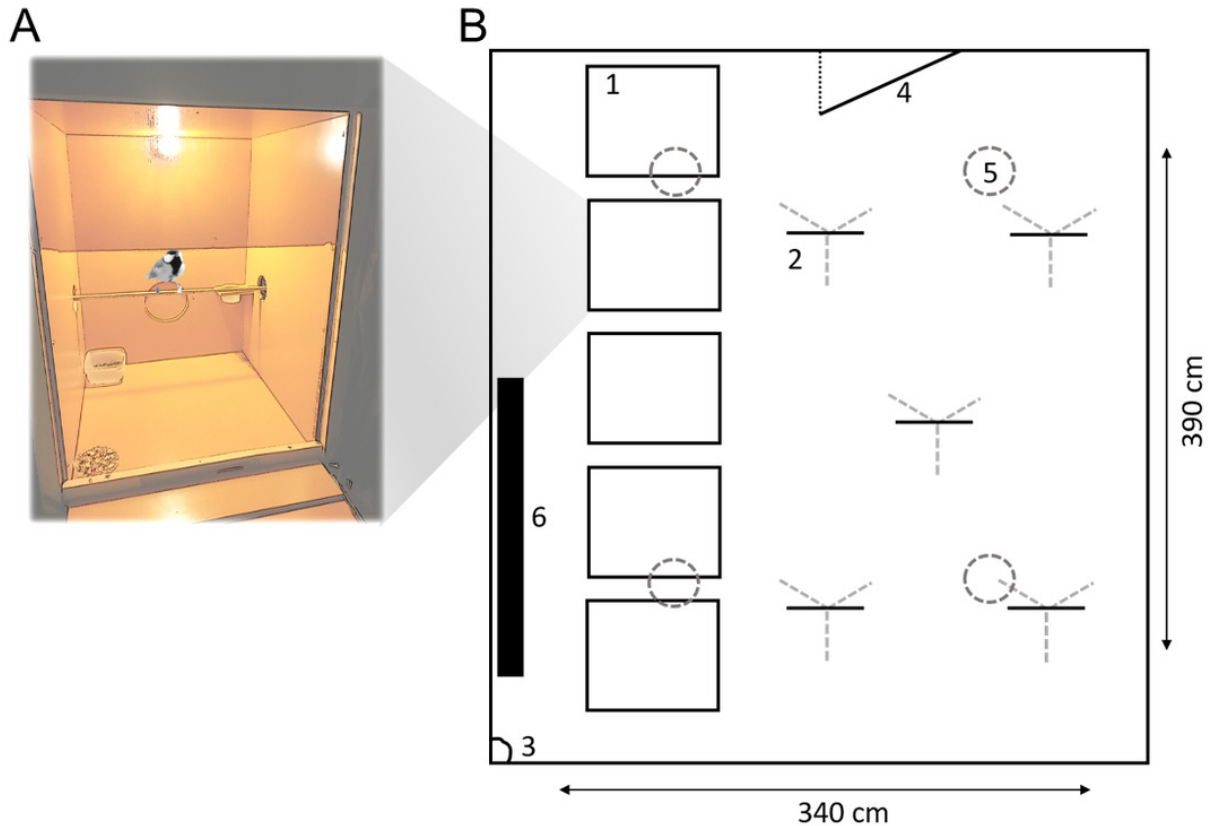
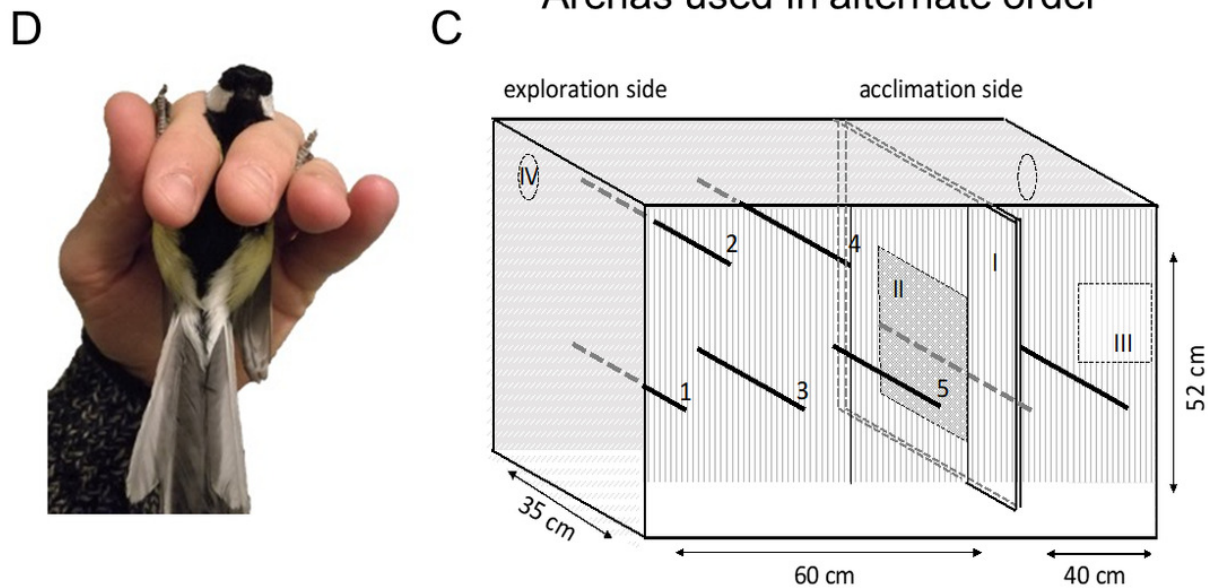
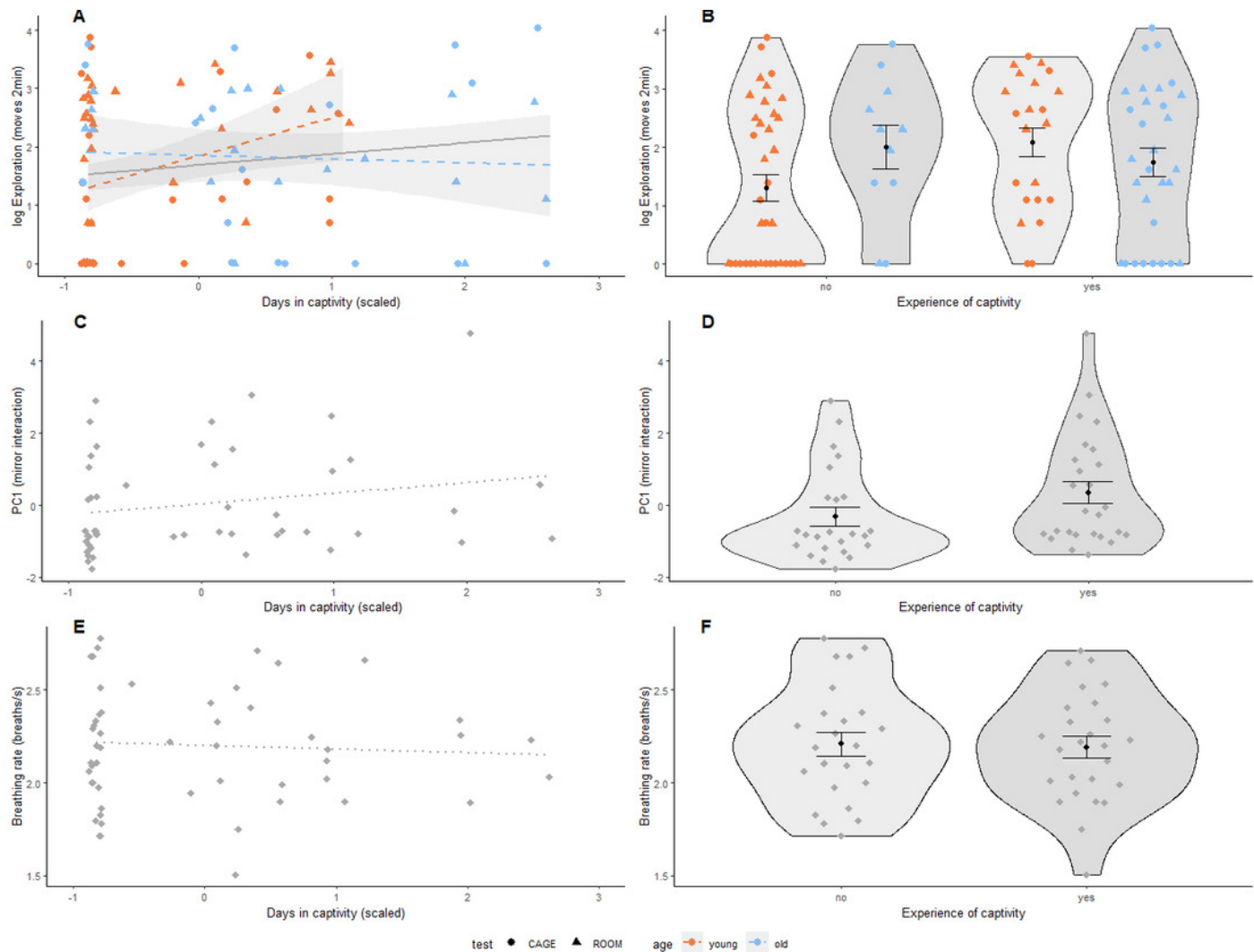
# Figure 1

Schematic of behavioural assays.

Great tits were housed in (A) boxes before and between behavioural assays. Exploration behaviour was assayed twice using: (B) a room arena (plan view) and (C) a cage (frontal view). In (B), birds were released individually from (1) housing boxes (A) to explore (2) five artificial 'trees' (metal tripod with wooden perches). Exploration was recorded by (3) camera and observation through a one-way window in the (4) door; birds could land on (5) ceiling lights but not (6) wall radiator (not in use). In (C), birds were placed in the 'acclimation side' before (I) sliding door was opened 10 cm to allow movement in the 'exploration side' (five perches, numbered 1-5) illuminated by (iv) two LED lights (not used as perches). A (II) mirror was exposed to assay social response behaviour before the bird was recaptured via a (III) door and (D) breathing rate recorded in the 'ringers grip'.

Arenas used in alternate order

# Figure 2

Prior experience of captivity and behaviour in great tits.

(A, B) Exploration behaviour (shown on log-scale as Poisson distributed) was measured in two arenas (room: triangle, cage: circle); (C, D) Social response behaviour was measured using a mirror, where PC1 represents interaction (number of pecks on the mirror, and time spent on the perch closest to the mirror and movements relative to behaviour during cage exploration assay); and (E, F) Breathing rate (breaths per second), N = 53 birds for all panels. Prior experience of captivity is shown as a continuous variable (mean centered and scaled by standard deviation) in (A, C & E), and as a binary variable in (B, D & F). The association of prior experience with captivity and exploration behaviour differed by age (i.e. significant interaction, Table 2) so data points and regression lines in (A & B) are indicated accordingly (first-winter: orange, adult: blue). Non-significant regressions are shown as dotted lines. In (B), (D), & (F), black dots indicate group means with standard error bars. Note that the interaction in (A) remains if adult birds with more than 12 days in captivity (scaled days in captivity greater than 1.5) are removed.

Phenotypic correlations between the three behavioural variables.

Pearsons correlations and their 95% confidence intervals between each of the behavioural variables measured on great tits (N=53). Where high PC1 values represented interaction with the mirror: number of pecks on the mirror, relative time spent on the perch closest to the mirror, and increased movement (compared to movements during exploration) were positively loaded while relative time spent on the perch furthest from the mirror was negatively loaded.

1    Table 1 Phenotypic Pearsons correlations and their 95% confidence intervals between each of the
2    behavioural variables measured on 53 great tits.  Where high PC1 values represented interaction with
3    the mirror: number of pecks on the mirror, relative time spent on the perch closest to the mirror, and
4    increased movement (compared to movements during exploration) were positively loaded while relative
5    time spent on the perch furthest from the mirror was negatively loaded.

|  | Exploration - cage | Exploration - room | Breathing rate |
|---|---|---|---|
| Exploration – cage | -- | | |
| Exploration – room | 0.04 (-0.23 – 0.31) | -- | |
| Breathing rate | -0.17 (-0.42 – 0.10) | 0.00 (-0.27 – 0.27) | -- |
| PC1 (interaction with mirror) | 0.19 (-0.08 – 0.44) | 0.24 ( -0.03 – 0.48 ) | -0.14 (-0.40 – 0.13) |

Multivariate generalized linear mixed effects models (MMMs) of prior experience of captivity on behaviour.

The fixed effect coefficients (β) and random effect parameters ($\sigma^2$) with their 95% credible intervals (CI) based on a multivariate mixed effect model on three behaviours in 53 great tits and the adjusted repeatability of exploratory behaviour based on model 1. (i) Model 1 included prior experience of captivity as a continuous variable (Days in captivity); Coefficients from a similar multivariate mixed effect model(ii) Model 2 where experience of captivity was included as a binary variable (0/1). Significant effects (credible intervals not crossing 0) are given in bold. The fixed and random effect coefficients of model 2 are presented in *Table S6*.

1    Table 2 The fixed effect coefficients (β) and random effect parameters (σ²) with their 95% credible
2    intervals (CI) based on a multivariate mixed effect model on three behaviours in 53 great tits and the
3    adjusted repeatability of exploratory behaviour based on model 1. (i) Model 1 included prior experience
4    of captivity as a continuous variable (Days in captivity); Coefficients from a similar multivariate mixed
5    effect model(ii) Model 2 where experience of captivity was included as a binary variable (0/1).
6    Significant effects (credible intervals not crossing 0) are given in bold. The fixed and random effect
7    coefficients of model 2 are presented in *Table S6*.

| | Exploratory behaviour moves in 2min N= 53 | Breathing rate breaths s⁻¹ N= 53 | Social interaction – PC1 (Close to and pecking at mirror, movements (+)) N= 53 |
|---|---|---|---|
| **Fixed effects** | β (95% CI) | β (95% CI) | β (95% CI) |
| Intercept | **1.75 (0.87 – 2.79)** | **2.37 (2.22 – 2.52)** | -0.52 (-1.34 – 0.21) |
| Bodymass | 0.05 (-0.46 – 0.47) | 0.09 (0.00 – 0.18) | -0.30 (-0.68 – 0.12) |
| Sex (male) | 0.45 (-0.36 – 1.35) | **-0.28 (-0.45 – -0.12)** | 0.50 (-0.28 – 1.35) |
| Age (old) | 0.09 (-0.97 – 1.04) | -0.05 (-0.23 – 0.13) | 0.72 (-0.26 – 1.56) |
| Catching date | 0.41 (-0.14 – 0.99) | -0.05 (-0.15 – 0.05) | -0.21 (-0.69 – 0.32) |
| Test type (cage) | **-0.91 (-1.69 – -0.14)** | -- | -- |
| Test order | -0.43 (-1.14 – 0.30) | -- | -- |
| *(i)  Model 1: continuous measure of captivity* Days in captivity | **1.50 (0.54 – 2.60)** | -0.08 (-0.27 – 0.09) | 0.27 (-0.71 – 1.06) |
| Days in captivity * Age (old) | **-1.46 (-2.60 – -0.35)** | 0.03 (-0.15 – 0.22) | -0.24 (-1.12 – 0.77) |
| *(ii) Model 2: binary measure of captivity* Experience of captivity | **2.15 (0.78 – 3.79)** | -0.04 (-0.31 – 0.24) | 0.44 (-0.81 – 1.95) |
| Experience of captivity * Age (old) | **-2.07 (-3.88 – -0.15)** | -0.13 (-0.48 – 0.21) | -0.30 (-2.07 – 1.38) |
| **Random effects** | σ2 (95% CI) | | |
| Bird_ID | 0.50 (<0.01 – 1.41) | | |
| Residual | 3.30 ( 1.94 – 5.03) | | |
| | **Link-scale approximation R (95% CI)** | | |
| **Repeatability (adjusted)** | 0.12 (< 0.01 – 0. 34)^& | | |

8    & because the variance components are constrained to be positive in MCMCglmm models, a lower bound of the
9    credible interval close to zero indicates low confidence in a non-zero proportion of the phenotypic variance in
10    exploratory behaviour being explained by differences between individuals.