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Artificial selection for reversal learning reveals limited repeatability and no heritability of cognitive flexibility in great tits (*Parus major*)

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1 **Artificial selection for reversal learning reveals limited repeatability and no heritability of**
2 **cognitive flexibility in great tits (*Parus major*)**

3
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11
12
13 **Abstract**

14 Cognitive flexibility controls how animals respond to changing environmental conditions. Individuals
15 within species vary considerably in cognitive flexibility but the micro-evolutionary potential in animal
16 populations remains enigmatic. One prerequisite for cognitive flexibility to be able to evolve is
17 consistent and heritable among-individual variation. Here we determine the repeatability and heritability
18 of cognitive flexibility among great tits (*Parus major*) by performing an artificial selection experiment
19 on reversal learning performance using a spatial learning paradigm over three generations. We found
20 low, yet significant, repeatability ($R = 0.15$) of reversal learning performance. Our artificial selection
21 experiment showed no evidence for narrow-sense heritability of associative or reversal learning, while
22 we confirmed the heritability of exploratory behaviour. We observed a phenotypic, but no genetic,
23 correlation between associative and reversal learning, showing the importance of prior information on
24 reversal learning. We found no correlation between cognitive and personality traits. Our findings
25 emphasize that cognitive flexibility is a multifaceted trait that is affected by memory and prior
26 experience, making it challenging to retrieve reliable values of temporal consistency and assess the
27 contribution of additive genetic variation. Future studies need to identify what cognitive components
28 underly variation in reversal learning and study their between-individual and additive genetic
29 components.

30
31 **Keywords:** cognition, artificial selection, animal personality, quantitative genetics, reversal learning

32 **Introduction**

33 The need for animals to adjust their behaviour to environmental changes has selected for the evolution
34 of cognitive abilities, that is, the abilities to acquire, process, store and act on information from the
35 environment [1]. Animals show consistency during foraging [2,3] and are capable of memorizing food
36 source features, such as their location or shape [1,4]. However, the ability to re-evaluate this behaviour
37 can be equally essential if, for example, a source no longer provides food. One particularly relevant trait,
38 therefore, is cognitive flexibility, the executive cognitive function that allows animals to update goal-
39 directed behaviour in response to changing environmental conditions [5]. Frequent fluctuations in
40 ecological resources, such as in spatially complex and heterogeneous environments, should select for
41 greater cognitive flexibility, allowing individuals to respond more rapidly to changes in their
42 environment [1,6]. However, whether cognitive flexibility can respond to selection is currently
43 unknown. To understand this evolutionary potential of cognitive flexibility, we need to quantify the
44 extent of stable individual differences in cognitive flexibility and whether that individual variation has
45 a genetic basis [7–9].

46 Determining stable individual differences in cognitive traits is crucial when investigating their
47 adaptive nature, nevertheless, this has received relatively little attention in non-human animals [10],
48 although the literature is expanding [11]. To test whether individual differences in performance are
49 consistent over time, studies so far have focused on the existence of consistent among-individual
50 variation, or *repeatability* of cognitive traits [12], finding that repeatability is usually below 0.28, with
51 a significant publication bias in favour of reporting higher repeatabilities [11]. Cognitive flexibility is
52 generally assessed with a reversal learning task [5]. In this assay, a previously rewarded option becomes
53 non-rewarding and vice versa, in order to assess how flexible an animal can adapt its learned response
54 [13]. There is mixed evidence that reversal learning performance is repeatable: studies have reported
55 significant repeatability in great tits (*Parus major*) [14,15] and Australian magpies (*Cracticus tibicen*
56 *dorsalis*) [16,17], but in blue tits (*Cyanistes caeruleus*) [14] and song sparrows (*Melospiza melodia*)
57 reversal learning was not significantly repeatable [18]. In the latter case, the authors suggest the lack of
58 repeatability is due to a change in the nature of a task when it is measured repeatedly. Because of

59 familiarity with the task, the performance might improve due to (rule-)learning or increased motivation
60 [10,13]. They also argue that potentially the same performance measure assesses a different cognitive
61 trait when the task is repeated [18]. Nonetheless, repeated measures of cognitive performance are needed
62 to estimate consistent among-individual variation, in order to support the idea that there are individual
63 differences in performance [10].

64 Repeatability estimates provide a fundamental upper limit for heritability, but consistency can
65 also arise from early environmental or maternal effects [19]; but see [12]. Therefore, demonstrating that
66 a phenotypic trait is heritable is a fundamental next step in understanding the trait's evolutionary
67 potential [20]. Since reversal learning performance is always tested following an associative learning
68 task, they are generally considered in concert. Assessing the extent of among-individual covariance
69 between the two learning phases will be crucial to understand what drives, possibly heritable, among-
70 individual differences in reversal learning performance. Following the hypothesized trade-off between
71 learning and cognitive flexibility, animals that are quick at forming initial associations learn subsequent
72 similar information less well [21–23]. Several studies have investigated both the phenotypic correlations
73 between and the heritability of associative and reversal learning performance [24–31]. Current evidence
74 suggests low to moderate heritability of reversal learning performance across species, but how it relates
75 to associative learning appears to vary between species. Identifying the existence of a trade-off between
76 learning and cognitive flexibility is important, because this has been hypothesised to maintain cognitive
77 variation [22].

78 In line with this, personality traits and cognitive performance have been hypothesized to be
79 correlated at the among-individual level [10]. Fast explorers would learn faster but inflexibly, meaning
80 that they have more difficulty learning a new cue-reward association [32,33]. However, even though
81 correlations have been found between personality traits and (reversal) learning performance, this
82 relationships direction is unclear [32,34], and generally raw phenotypic correlations with no partitioning
83 of among- and between-individual components are calculated. Knowing to what extent variation in
84 personality traits relates to reversal learning performance, will help us understand whether cognitive
85 flexibility and personality variation are controlled by the same underlying factors [35].

86 Experimental evidence for heritable variation in cognitive flexibility, or in any cognitive trait,
87 is lacking in non-human animals [36]. Artificial selection experiments provide a powerful method for
88 obtaining strong, direct evidence for naturally occurring, heritable individual variation in cognitive
89 performance [7]. Therefore, we here performed bi-directional artificial selection on natural variation in
90 spatial reversal learning ability under controlled laboratory conditions in hand-reared great tits (*Parus*
91 *major*) collected from a natural population, while including repeated measures to estimate consistent
92 among-individual differences. Previous artificial selection experiments in great tits have successfully
93 demonstrated heritability of exploratory behaviour, risk-taking behaviour and reproductive timing [37–
94 39]. Here we estimated (i) individual consistency and repeatability of performance on associative and
95 reversal learning tasks, (ii) the covariation between performance on the associative and reversal learning
96 task and two personality traits, and (iii) the narrow-sense heritabilities of these cognitive traits. We
97 predicted significant, but low to moderate, individual consistency and heritability, since within-
98 individual variation in cognitive performance is likely influenced by environmental effects, experience,
99 and intrinsic motivation. We expected to find associations between associative learning, reversal
100 learning, and personality traits; negative associations would support the above suggested trade-offs,
101 while positive correlations could indicate they are determined by similar underlying mechanisms.

102 **Methods**

103 *Study subjects*

104 We conducted this study from May 2018 to April 2021 spanning 3 years. Each year in May, we hand-
105 reared great tit (*Parus major*) nestlings until independence. As a starting population, we collected 322
106 10-day old nestlings from 42 broods originating from the nest box population Boslust (Groot
107 Warnsborn) near Arnhem, the Netherlands (5°850 E, 52°010 N), a 70 ha field site consisting of mixed
108 pine-deciduous forest. We brought them to the aviary facilities at the Netherlands Institute of Ecology
109 (NIOO-KNAW) and hand-reared the nestlings until independence according to the methods described
110 by [37]. Briefly, we transferred nestlings in sibling groups of three to four birds to a wooden box, each
111 box containing three compartments with in each a natural parasite-free nest. Upon fledging, around 17-
112 20 days after hatching, birds were transferred to small wire-mesh cages in groups of three. Around day
113 35 after hatching, they were completely independent and transferred to standard individual cages of 0.9
114 m × 0.4 m × 0.5 m with solid bottom, top, side, and rear walls, a wire-mesh front and three perches.
115 Birds were kept under natural light conditions in acoustic and visual contact with each other.

116 After two days of individual housing, in June/July, we performed a novel environment test and
117 a novel object test to test early exploratory behaviour ([37]; further details below). In July, we took a
118 blood sample for sex determination. In September, after their first moult, we transferred individuals to
119 single-sex groups (maximum 7 males or 8 females) in semi-open outdoor aviaries (2 m x 4 m x 2.5 m),
120 their standard housing outside the breeding season. Food consisted of a homemade mixture of ground
121 beef heart, egg, calcium and a multivitamin solution, supplemented with mealworms, apple, and
122 sunflower seeds and fat balls in winter, and was available ad libitum. In October, when birds were full-
123 grown, we measured tarsus with sliding callipers to the nearest 0.1 mm. From October-February, birds
124 were tested for associative and reversal learning performance to assess their cognitive flexibility. Based
125 on their performance in the reversal learning task, we selected individuals for the ‘reversal learning line’
126 breeding pairs in the same semi-open aviaries to produce the next generation in captivity (for details see
127 “*Selection procedure and breeding*”).

128 Eggs produced by this parental (P) generation and the subsequent, first (F1) generation were
129 placed in a natural nest at our field site to be incubated by wild foster females. Ten days after hatching,
130 nestlings were collected, brought to our indoor facilities and hand-reared as described above (224 F1
131 chicks from 29 P pairs and 181 F2 chicks from 27 F1 pairs). These chicks were tested for exploratory
132 behaviour for associative and reversal learning performance.

133

134 ***Reversal learning task***

135 In total, we successfully tested 105 animals from 33 pairs (P), 149 (F1) and 131 (F2) individuals for
136 reversal learning performance. From these birds, we tested 52 P, 55 F1 and 15 F2 individuals a second
137 and 52 P individuals a third time to obtain repeated measures, from here on we refer to these repeated
138 measures as ‘testing rounds’. Figure S1 and Table S1 provide an overview of the sample sizes and
139 timeline for the selection procedures and repeated measures.

140

141 ***Experimental apparatus***

142 We used automated feeders to assess learning performance, using a three-choice spatial learning
143 procedure. During each experiment, one feeder would provide a reward (freeze-dried mealworm) in a
144 triangular array with two other unrewarding feeders, requiring individuals to learn the rewarded feeders
145 location. A triangular array with three feeders is different from most setups of typically two choices.
146 Spatial reversal learning paradigms involving additional choice options [14,15,23,40,41], make it
147 possible to distinguish between error types and foraging strategies [13]. We used two feeder types for
148 these experiments, the second type being a refined version that allowed for automated reversal
149 switching.

150 *Feeder type one* (Figure S2a): For the first testing round of the P generation, we used radio-
151 frequency identification (RFID) controlled automated feeders [14]. Access to food (freeze-dried
152 mealworms) was controlled by a transparent plastic door at the feeder opening, held in place by a
153 solenoid. The solenoid would release for two seconds upon detecting a specific Passive Integrated
154 Transponder (PIT) tag, allowing an individual access to one food item by pushing open the door. RFID
155 readings and solenoid activation were controlled by a custom program loaded onto a printed circuit

156 board ('Darwin Board', Stickman Technologies Inc., UK). All visits and their timestamps were
157 monitored with the RFID antenna throughout the experiment.

158 *Feeder type two* (Figure S2b): In subsequent experiments (P generation testing rounds two and
159 three, F1 and F2 all testing rounds), we substituted the above-described feeding stations for automated
160 "smart" feeders, designed by NatureCounters (Maidstone, UK). These feeders functioned similarly to
161 the Type one devices, and were also equipped with an RFID antenna. Opening and closure of a
162 transparent plastic door was controlled with a micro-servomotor. Upon registration of a PIT-tag, the
163 door opened for a set duration of two seconds. The three feeders were connected through a network
164 cable, allowing for integrated responses between the feeders.

165

166 ***Training***

167 Eight days prior to testing, individuals were trained to use the feeders in semi-open outdoor aviaries.
168 Before training, birds were weighed and provided with a PIT-tag. During this training period, food and
169 water were available ad libitum, but mealworms were excluded from the diet. On training day one, we
170 placed one or two feeders in the aviary, with the doors permanently open so that birds could learn to eat
171 from the open door. On days two to seven, the feeder door was closed and set to a mode where it could
172 only be opened when triggered by a successful transponder reading of any of the birds. On day eight,
173 birds were selected for testing if during that week, they had >80 visits per day at least once, >30 visits
174 per day at least twice (mean of maximum visits per bird on the day it had the most visits = 268 visits),
175 see Figure S3a for distribution) or >30 visits per hour at least once (mean of maximum visits per bird in
176 the hour it had the most visits = 60 visits, Figure S3b). Out of 546 trained individuals 54 did not pass
177 this criterion. This selection criterion was chosen because the median number of visits that was needed
178 to complete both the associative and reversal task was 31 visits. Hence, most individuals would be apt
179 enough at using the devices to finalize the task within one day.

180

181 ***Learning experiment***

182 We tested all birds between 9:00h and 15:00h. We caught individuals from the group, weighed them,
183 and released them individually in a testing aviary. In this testing aviary, water was available ad libitum

184 and food was not available to maintain the motivation to take part in the learning test. Testing took place
185 with three feeders positioned in a triangular array, doors facing inwards.

186 In the associative learning phase, we *a priori* randomly chose the rewarded feeder. This assigned
187 feeder was reinforced until individuals reached a criterion level of six correct trials out of seven. This
188 success rate is significantly different from the expectation if birds selected feeders at random (binomial
189 test, $p < 0.01$). A trial was defined as a landing on the perch.

190 After completing the associative learning phase, the reward contingencies were reversed to one
191 of the other two feeders for the reversal learning task, which was randomly chosen *a priori*. All
192 experimental tests were observed live through a one-way screen and upon reaching criterion, the
193 observer initiated the reversal manually. Again, birds had to reach the learning criterion of six correct
194 visits over seven trials to the new feeder. Both phases were filmed using a GoPro Hero5 (GoPro inc.)
195 placed inside the aviary in case rewatching was necessary. After the test, birds were caught, weighed
196 and released back in their home aviary. Associative and reversal learning performance were scored as
197 the total number of trials required to reach the learning criterion, including those last seven visits.

198 After testing the parental generation with the first feeder type, we refined our testing feeder to
199 allow for automated high-throughput phenotyping. The following changes were implemented for feeder
200 type two: (i) For the associative learning phase, the first feeder that a bird visited was rewarded, instead
201 of a random feeder. This ensured that any variation in reversal learning performance would not be due
202 to variation in preference. (ii) The devices were programmed such that the rewarded feeder switched
203 automatically to initiate the reversal phase when the learning criterion was reached. Birds continued
204 serial reversals until caught from the aviary at 15:00. For all analysis in this paper we only included the
205 first reversal of each testing round. (iii) Due to a difference in design, the doors faced away from the
206 centre of the three devices, instead of inwards. (iv) Energetic state and satiation can affect evaluation of
207 the food reward and learning speed [42]. Therefore, we offered dry egg food ad libitum in the test aviary
208 during the experiment. Great tits strongly prefer the dried mealworm rewards in the feeders over dried
209 egg food. Despite these differences, the basic test principles remained the same. In all analyses, we
210 controlled for feeder type used to measure learning performance.

211

212 ***Exploratory behaviour and boldness***

213 To test birds for exploratory behaviour, we conducted a novel environment test in the observation room
214 after two days of individual housing (for more details, see [37,43]) We used the total number of flights
215 (movements between trees) and hops (movements between branches within trees) within the first two
216 minutes as ‘exploratory score’ [44]. We measured the response to novel objects on day one and day two
217 after the novel environment test ($R = 0.54$, $CI = [0.4,0.6]$, $p < 0.001$). We measured the latency to
218 approach the object and the shortest distance to it within 120 s, and the results for each test were
219 converted linearly to a 0–5 scale. Scores were given as follows: 0 when the bird did not land on the
220 object perch within 120 s, 1–4 depending on the time it took the bird to land on the perch and the minimal
221 distance to the novel object, 5 when the bird pecked the object. We summed scores from both novel
222 object tests as an index of boldness (0–10, ‘boldness score’).

223

224 ***Selection procedure and breeding***

225 From the parental generation, individuals were selected to breed based on their reversal learning
226 performance in the first testing round. Individuals were ranked on the number of trials they required to
227 reach learning criterion. For both the ‘fast’ and ‘slow’ learning lines we selected 18 males and 18 females
228 and created ‘fast’ and ‘slow’ pairs. For each sex, we included a maximum of three siblings per line.
229 Within each line, we ranked the selected birds for reversal learning performance for the two sexes
230 separately. We then paired males and females that had contrasting reversal learning performances within
231 each line (e.g. for the fast line a relatively fast performing fast male with a relatively slow performing
232 fast female), avoiding full-sib mating. In this way we avoided creating most variation between broods
233 within a line rather than between lines. Mid-parent values and performance ranges for the pairs that
234 produced offspring that was subsequently successfully tested are reported in Table S2.

235 From February onwards, breeding pairs were housed in semi-open outdoor aviaries
236 (approximately 2.0 m x 4.0 m x 2.5 m) under natural photoperiod and temperatures. Each aviary
237 contained four nest boxes. From March onwards, daily additional light was supplied from a single
238 full-spectrum daylight fluorescent lamp (58W, 5500K, Truelight, the Netherlands) per aviary. Lights
239 were on from 2.5 hr before sunrise (but never earlier than 02:00) until 24:00 to synchronize their

240 breeding with the wild population. Moss and dog hair was supplied from mid-March onwards as nesting
241 material.

242 Nest boxes in the aviaries were checked twice a week for nest building. When nests were
243 complete, nest boxes were checked daily for egg laying. Freshly laid eggs were collected, replaced with
244 dummy eggs, and stored for maximum 14 days in an egg-turner. After five days of incubation, the nests
245 and the dummy eggs were removed from the nest box and females were allowed to relay. All eggs
246 incubated by wild foster females (~14 days) at the nest box population in Boslust. For the F1 generation,
247 in order to increase hatching success in the wild, about one-third of broods was first incubated by the
248 genetic mother for five days in the aviary and then placed in a wild nest to be further incubated by foster
249 females (additional ~9 days). Around 50% of clutches was fostered as one unit, the other 50% was split
250 up across two or more foster nests.

251 To produce the F2 generation, we also formed 18 pairs for each line from the F1 generation as
252 described above for the P generation. Not all pairings bred successfully. We obtained 86 chicks from 16
253 ‘fast’ broods and 63 chicks from 12 ‘slow’ broods from the P generation, and 37 chicks from 10 ‘fast’
254 broods and 94 chicks from 16 ‘slow’ broods from the F1 generation (Figure S1).

255

256 *Statistical analysis*

257 For both associative and reversal learning performance, we used the number of trials to reach learning
258 criterion as performance measure. Across 56 out of 573 reversal learning tests, the criterion was not
259 reached before 3:00 PM, meaning trials to criterion was censored. To investigate if performance differed
260 between associative and reversal learning while including censored individuals, we compared survival
261 curves between the associative and reversal learning task. We used the functions *Surv* and *Survfit* from
262 the ‘survival’ package [45] to compute Kaplan-Meier survival curves. We employed the Kaplan-Meier
263 method to estimate number of trials to criterion for censored individuals. We used an individuals’
264 maximum number of trials for “follow up time”, and success (0 = criterion not reached, 1 = criterion
265 reached) for “event”. We used the function *survdifff* [45] to compare the two survival curves of the
266 associative and reversal learning task, by computing a log-rank test employing a chi-square test statistic.

267 As expected, individuals needed more trials to finish the reversal learning task compared to the
268 associative learning task (log-rank: $\chi^2_1=136$, $p < 0.001$, Figure S4).

269 To include censored data in the linear regression models to calculate heritability and
270 repeatability, we computed so-called pseudo-values of the restricted mean trials to criterion from the
271 Kaplan-Meier survival curves [46]. As a measure of trials to criterion, pseudo-values were computed
272 for all observed trials, both censored and uncensored, using the function *pseudo* [45]. Pseudo-values
273 were evaluated at the maximum trial number reached by the individual with the highest trials to criterion.
274 How the values for trials to criterion changed from raw to the pseudo values is illustrated in Figure S5.
275 To prevent confusion, the pseudo-values will hereafter be referred to as ‘trials to criterion (TTC)’.

276 To test whether variation in associative and reversal learning performance can be explained by
277 feeder type, test round, start time, body condition (residuals of the regression of body weight at start of
278 the experiment against tarsus) or sex, we constructed linear mixed models with the function *lmer* in the
279 ‘lme4’ package [47] with these explanatory variables and trials to criterion (ln-transformed) as the
280 dependent variable. To account for multiple observations, animal ID was included as random intercept.
281 We created separate models for associative and for reversal learning performance. Significance of fixed-
282 effects was tested using Type II ANOVAs Kenward-Roger degrees of freedom with the function *Anova*
283 in the ‘car’ package [48]. We evaluated terms using F-tests.

284

285 ***Heritability and repeatability of associative and reversal learning performance***

286 We used two approaches to assess narrow-sense heritability of associative and reversal learning.
287 Narrow-sense heritability (h^2) is the proportion of total phenotypic variance (V_P) that is attributed to the
288 additive effect of genes, i.e., additive genetic variance (V_A) [19]:

$$289 \quad h^2 = \frac{V_A}{V_P} \quad (1)$$

290 First, we estimated narrow-sense heritability of reversal learning performance using the response to
291 artificial selection on performance on the first testing round. Because we only tested the parental
292 generation with the first feeder type, we could not control for this using only data from the first testing
293 round. Therefore, we used reversal learning performance corrected for feeder type, by using the residuals

294 from the following model as response variable: a linear model of the complete dataset including all
295 testing rounds (in later testing rounds, the parental generation was tested with feeder type two), with
296 trials to criterion (ln-transformed) as the dependent variable, and feeder type as the fixed effect.
297 Estimates of heritability based on the response to selection are referred to as realized heritabilities [19],
298 and can be estimated from the breeder's equation,

$$299 \quad R = h^2 S \quad (2)$$

300 By calculating the ratio of observed response to selection (R), the difference between mean offspring
301 value and mean parent value, to observed selection differential (S), the difference between mean value
302 of selected parents (weighted for number of tested offspring) and mean parent value, we estimated the
303 realized heritability:

$$304 \quad \hat{h}_r^2 = \frac{R}{S} \quad (3)$$

305 To estimate realized heritability over several generations of selection, we summed R and S over
306 successive generations respectively, to acquire the cumulative selection response $R_C(t)$ and cumulative
307 selection differential $S_C(t)$. Then, by regressing $R_C(t)$ on $S_C(t)$, we estimated realized heritability as the
308 slope of this regression [19]. This approach allowed us to show the selection differentials in relation to
309 the response to selection in our experiment. However, it also returns a larger error around the estimates
310 and does not allow for further variance partitioning. Therefore, we additionally estimated heritability,
311 and repeatability of associative and reversal learning performance, by fitting univariate Bayesian mixed-
312 effect animal models with the 'MCMCglmm' package [49]. These models included the following
313 random effects: individual identity to account for repeated measures and estimate permanent
314 environment effect (V_{PE}), individual identity linked to the pedigree to estimate additive genetic variance
315 (V_A) and maternal identity to estimate maternal variance (V_M). In addition, feeder type was included as
316 fixed effect. For these univariate models, we used the complete data set including all repeated measures.
317 For both associative and reversal learning performance, we used trials to criterion as pseudo values
318 rounded to the nearest integer as response variable.

319 Estimates of genetic variance (V_A), were extracted from the full animal model and scaled by the
 320 total phenotypic variance (V_P , calculated as the sum of the estimated variance components and the
 321 residual variance, V_R). As such, heritability (h^2), was estimated as:

$$322 \quad h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_A + V_M + V_{PE} + V_R} \quad (4)$$

323 Repeatability was calculated as the proportion of phenotypic variance explained by the individual [19],
 324 with V_I representing among-individual variance ($V_A + V_{PE} + V_M$) and was estimated as:

$$325 \quad R = \frac{V_I}{V_P} = \frac{V_A + V_M + V_{PE}}{V_A + V_M + V_{PE} + V_R} \quad (5)$$

326 For personality, response variables were either ‘exploratory score’ or ‘boldness score’. We had no
 327 repeated measures, so estimated only additive genetic variance (V_A) and maternal variance (V_M). The
 328 estimate of genetic variance was extracted from the animal model and scaled by the phenotypic variance
 329 to yield estimated heritability (h^2).

330 We modelled all four latent traits using a Poisson error distribution, and therefore used a
 331 parameter expanded prior suitable for univariate poisson models (Fisher prior, $V = 1$, $\nu = 1$, $\alpha \cdot \mu = 0$, $\alpha \cdot V = 1000$) that is more informative toward the expected small variances, and puts less weight
 332 in small values close to zero [50]. Note that although the trials to criterion data shows overdispersion,
 333 the MCMCglmm Poisson model includes a residual term that takes up any overdispersion remaining
 334 after accounting for the variance explained by the fixed and random effects. We present the estimates as
 335 posterior means with the associated 95% CI on the latent scale and also converted these estimates to the
 336 observed scale (R_{obs} using the *QGicc* function, h^2_{obs} using the *QGparams* function, from the “QGglmm”
 337 R package [51]). Since variance components are constrained to be positive in MCMCglmm, we used
 338 closeness of the lower bound of the credible interval to zero to estimate confidence in a non-zero
 339 proportion of the variance components. For fixed effects, differences were considered significant when
 340 95% credible intervals (CrI) for effect sizes did not overlap with zero.

342

343 ***Pairwise correlations between associative learning performance, reversal learning performance,***
 344 ***exploratory behaviour and boldness***

345 To estimate genetic and non-genetic covariance between associative and reversal learning
 346 performance, we fitted two bivariate animal models using the complete data set including all repeated
 347 measures: one with feeder type as a fixed effect, and a second model where we partitioned the variance-
 348 covariance at the two feeder type levels. These models both included individual identity (V_{PE}) and
 349 individual identity linked to the pedigree (V_A) as random effects. We left out maternal identity (V_M)
 350 from the second model to avoid overfitting and because maternal effect was estimated to be near zero
 351 for both traits. To estimate covariance between the learning and personality traits, we fitted bivariate
 352 animal models. Because of the lack of repeated measures for the personality traits, we included only
 353 performance on the first test round for the learning traits. These models included a fixed effect of feeder
 354 type on learning traits and individual identity linked to the pedigree (V_A) as random effect.

355 We used a Poisson error distribution, and a parameter expanded prior suitable for bivariate
 356 poisson models that is weakly informative for the small variances expected for the Poisson distribution
 357 ($V = \text{diag}(2)$, $\text{nu} = 2$, $\text{alpha.mu} = c(0,0)$, $\text{alpha.V} = \text{diag}(2)$) [50]. We calculated the phenotypic, genetic,
 358 permanent environment and/or maternal correlations by dividing the covariance between each two traits
 359 by the product of the square root of their variances, e.g.:

$$360 \quad \text{Genetic correlation} = \frac{\text{additive genetic covariance}}{\sqrt{(V_{A, \text{Trait1}} * V_{A, \text{Trait2}})}} \quad (6)$$

361 We used the 95% CrI to assess statistical significance of the covariances, which were considered
 362 significant when not overlapping with zero. We ran each model for 100,000-200,000 iterations, with a
 363 burn-in of 10,000-20,000 and a 50-iteration thinning interval, to obtain sample sizes >1000 (with two
 364 exceptions being 266 and 488) and autocorrelation <0.10 (with some exceptions <0.20). Convergence
 365 of MCMC sampling and autocorrelation of chains were assessed visually following [50].

366 To visualize the relationship between associative and reversal learning performance, we
 367 employed a linear model using function *lm* from the ‘stats’ package [52], with trials to criterion of
 368 reversal learning performance (ln-transformed) as the dependent variable and trials to criterion of
 369 associative learning performance (ln-transformed), feeder type and their interaction as fixed effects, and
 370 used the function *interact_plot* from the ‘jtools’ package to plot the significant interaction. Additionally,
 371 for feeder type one, where we had randomly assigned the rewarded feeder a priori, we additionally

372 explored how the relationship between reversal learning performance and associative learning
373 performance depended on the number of trials it took a bird until the first correct visit was made, which
374 we called ‘correct visit lag number’. If the correct visit lag number equalled 1, the first feeder that an
375 individual chose to land was also the rewarded feeder. If the correct lag visit number equalled 2 or
376 higher, the individual first visited other unrewarded feeders. A higher correct visit lag number might
377 indicate slow learning or a strong tendency to visit the other unrewarded feeder(s). For this, we used a
378 linear model using function *lm* with trials to criterion (ln-transformed) of the reversal learning phase as
379 the dependent variable, and trials to criterion (ln-transformed) of the associative phase, ‘correct visit lag
380 number’ and their interaction as fixed effects. Significance of fixed- effects was tested using Type III
381 ANOVAs and Kenward-Roger degrees of freedom. We evaluated terms using F-tests. Post hoc
382 comparisons were performed with the function *emtrends* command in ‘emmeans’ package [53] to
383 compare estimates of slopes of the trials to criterion trend for each level of feeder type and mean \pm SD
384 of ‘correct visit lag number’, using the Tukey test to correct for multiple comparisons. We used the
385 function *interact_plot* from the ‘jtools’ package to visualize the interaction.
386
387 All analyses were conducted in R version 4.0.3 [52].

388 **Results**

389 *Heritability and repeatability of associative and reversal learning performance*

390 The significance and magnitude of fixed effects varied across task performances. These effects are not
391 directly relevant to hypotheses being tested but are reported in full in Table S3. Feeder type had a
392 significant effect and was therefore controlled for all in subsequent models. The repeatability (with 95%
393 CrI) of associative learning performance was 0.105 (0.015, 0.198) on the latent and 0.065 (0.011, 0.128)
394 on the observed data scale (Table 1). For reversal learning, the repeatability was 0.153 (0.038, 0.287)
395 on the latent and 0.116 (0.028, 0.220) on the observed data scale (Table 1). There was no support for
396 heritable variation in associative and reversal learning performance, with narrow-sense heritability
397 estimates of 0.020 (0.000, 0.074) on the latent and 0.012 (0.000, 0.044) on the observed data scale for
398 associative learning performance, and 0.030 (0.000, 0.099) on the latent and 0.022 (0.000, 0.072) on the
399 observed data scale for reversal learning performance and the 95% CrI for these h^2 values included zero.
400 On the contrary, narrow-sense heritability estimates for exploratory behaviour were 0.499 (0.239, 0.766)
401 and 0.265 (0.114, 0.399) on the latent and observed scale, and 0.463 (0.175, 0.714) and 0.252 (0.110,
402 0.410) on the latent and observed scale for boldness. In all cases, permanent-environment effects and
403 maternal effects explained less than 5% of the phenotypic variance and the lower limit of the 95% CrI
404 was zero (Table 1).

405

406 *Realized heritability of reversal learning performance*

407 Great tits from the 'fast' and 'slow' line did not differ in reversal learning performance after one (t-test:
408 $t_{151} = 0.46$, $p = 0.65$) and two (t-test: $t_{129} = -0.89$, $p = 0.37$) generations of selection. The realized
409 heritability (response to selection as proportion to selection differential) was 0.02 ± 0.03 and did not
410 differ significantly from zero (Linear regression: $F_{1,4} = 0.55$, $P = 0.50$, $R^2 = 0.12$, **Error! Reference**
411 **source not found.**).

412

413 *Covariances among associative learning performance, reversal learning performance and*
414 *personality traits*

415 We found a negative phenotypic correlation between associative and reversal learning performance,
416 indicating that individuals that were slow during associative learning showed fast reversal learning. This
417 phenotypic correlation is feeder type specific, with a significant negative correlation for feeder type 2,
418 and a non-significant positive correlation for feeder type 1 (Figure 2a, Table 2). This correlation could
419 not be explained at the genetic, permanent environment or maternal level, since these estimates were
420 low with relatively large 95% CrIs (but note that the permanent environment correlation is similar to the
421 phenotypic correlation; Table 2). We found no credible correlations between associative and reversal
422 learning performance and the personality traits, neither at the phenotypic nor the genetic level (Table 2).

423

424 Using data from feeder type 1, we explored whether the fact that an individual needed to learn
425 that a preferred or a random feeder was rewarded could explain the difference in direction of the
426 relationship between associative and reversal learning. Indeed, there was a trend that the effect of
427 associative learning on reversal learning performance depended on the latency to the first correct visit
428 that was made during associative learning (LM; TTC associative*Correct visit lag number: $F_{3,100} = 3.58$,
429 $p = 0.06$, Table 3). Post hoc comparisons show a significant positive relationship between associative
430 learning and reversal learning performance when the ‘correct visit lag number’ is high (not learning
431 initial choice, at mean +SD 0.5 ± 0.21 $t_{100} = 2.30$, $p = 0.02$), but that the slope is less steep when this
432 number decreases (learning initial choice, at mean -SD -0.11 ± 0.18 , $t_{100} = -0.63$, $p = 0.52$) (Figure 2b).

433

434

435 **Discussion**

436 To investigate the evolutionary potential of cognitive flexibility, we here estimated the among-
437 individual variation and additive genetic variation of reversal learning and related traits using a bi-
438 directional selection experiment of reversal learning performance in the great tit (*Parus major*). We
439 found significant, but low, repeatability of reversal learning performance, but no evidence for narrow-
440 sense heritability of reversal learning performance. We argue that this suggests a relatively low
441 evolutionary potential of cognitive flexibility, but also points to difficulties with obtaining measures of
442 cognitive flexibility.

443 The fact that reversal learning performance has a significant but relatively low repeatable
444 component ($R_{\text{lat}} = 0.153$; 95% CrI = 0.038,0.287), shows that performance on this task is to some extent
445 consistent over time. Because we used relatively long measurement intervals (mean = 345 days), our
446 repeatability estimates may be somewhat conservative. Over longer time periods, the environment of an
447 individual is more likely to change relative to the last measurement, increasing within-individual
448 variation in performance [17]. Our findings are in line with other studies finding low repeatability of
449 reversal learning performance in great tits [11,14,15]. An important reason for this low repeatability is
450 the challenging nature of measuring cognitive traits accurately in general. Because individuals need to
451 be trained in taking rewards before being tested, memory, motivation and prior experience are likely
452 confounding effects on the performance on subsequent cognitive tests [10,18]. This is especially true
453 for reversal learning tasks, because subsequent reversals induce a change in prior knowledge, essentially
454 changing the trait that is measured [13]. Our results show that slight differences in the test setup between
455 test rounds already affected the correlation between prior experience and reversal learning performance.
456 This prior experience may be driven by chance effects, such as the first feeder on which a bird lands. In
457 the future, a better understanding of the role of prior experience and memory needs to be identified. But
458 also, knowledge on the reasons why there is a link between performance in the associative phase and
459 the reversal phase is important in order to explain the large within-individual variance in reversal
460 learning over consecutive trials.

461 Reversal learning performance did not respond to artificial selection over generations, and
462 despite the low but significant repeatability, we found no evidence for heritable variation. The fact that,
463 in line with previous research, we found evidence for heritability of both personality traits we measured,
464 boldness towards a novel object and exploratory behaviour, shows that the power of our pedigree was
465 sufficient to pick up moderate heritabilities [37,54,55]. The low heritability estimates and lack of
466 response to selection as we found here, suggest that reversal learning performance has little to no
467 evolutionary potential. Potentially, genetic variation may have been selected against for cognitive
468 flexibility, since traits closely linked to fitness are expected to have little additive genetic variance,
469 possibly lost through strong directional selection [8]. However, considering the broad phenotypic
470 variation in cognitive flexibility in our study, that does not seem to be the explanation for the results in
471 this study. Instead, considering the high residual variance, there is still a great deal of individual variation
472 unaccounted for, and we speculate on some possible sources of variation in the discussion below.

473 Other studies did find moderate heritability in reversal learning performance, in red junglefowl
474 (*Gallus gallus*) and house mice (*Mus musculus*), [24–26], although no heritability was found in Aegean
475 wall lizards (*Podarcis erhardii*) [30]. What can explain the different findings among studies? In other
476 work on the great tit, problem solving was shown to be a moderately repeatable composite behaviour
477 [56] but not heritable [57]. Furthermore, repeatability in problem solving was shown to be explained
478 entirely by the underlying behaviours (motivation, experience, selective attention; [58]), possibly
479 explaining the lack of heritability in problem solving performance. Similarly, reversal learning
480 performance is a composite trait made up of different cognitive components [59]; its heritability is
481 therefore dependent on the additive genetic variation of each of these components, and on their
482 covariance (as detailed below). We summarized reversal learning performance as the number of trials
483 to reach a learning criterion. This is a commonly used performance measure, but it does not allow to
484 disentangle the different cognitive components that determine performance. If different task types,
485 experimental setups, or species, lean more towards usage of one of the components, our chosen
486 performance measure, trials to criterion, might be the cause of the differences between our and other
487 studies. Furthermore, since each cognitive component itself may also be influenced by environmental
488 variation, total additive genetic variation on a summary measure like trials to criterion may be masked.

489 Reversal learning performance tests a combination of cognitive components, that trials to
490 criterion may not adequately capture [60] such as: (1) the degree of proactive interference, where
491 previously learnt, but now irrelevant, information interferes with learning and remembering newly
492 relevant information [23,61]. Proactive interference results in perseverative choices and explained a
493 large part of the among-individual variation in reversal learning performance in great tits [15]; (2)
494 attentional allocation, the ability to notice that the change has occurred, resulting in a potential failure
495 to detect task transitions [13]. For example, high resistance against external sources of interference
496 (external attention) impairs reversal learning performance [62]; and (3) learning the new cue-reward
497 association through reinforcement learning [15]. Reinforcement learning is involved in both associative
498 and reversal learning performance since both phases require individuals to learn from positive
499 reinforcements, increasing the excitatory strength of a stimulus, and to learn from non-reinforcements,
500 decreasing approach towards non-rewarded locations [59]. Sensitivity to negative and positive feedback
501 are stable traits [63]. Each of these constituents may be separate components of reversal learning
502 performance that selection can act upon, but the extent to which these components make up reversal
503 learning and the heritabilities of these traits remain an important avenue of further study.

504 These different cognitive components are also important for understanding our findings on the
505 relationships between associative learning and reversal learning performance. The relative importance
506 of proactive interference suggests that reversal learning performance can be strongly dependent upon
507 the outcome of the associative learning task. We found that associative and reversal learning
508 performance are negatively correlated, in line with a hypothesized trade-off between learning and
509 cognitive flexibility, in which animals that are quick at forming initial associations learn subsequent
510 similar information less well [21–23]. However, we only observed this negative correlation when
511 individuals were rewarded for their first choice during the associative learning task, which may be their
512 preferred feeder. Individuals that were quick to learn their initial choice possibly had a stronger
513 preference for this feeder, developed a stronger memory, and experienced more proactive interference
514 during the reversal task. Strong preferences are difficult to reverse [64], and faster and stronger
515 associative learning might make it more difficult to inhibit the response towards that cue [65,66]. On
516 the other hand, when a randomly allocated feeder was rewarded first, with a positive trend for associative

517 learning to correlate with reversal learning performance, if individuals were forced to learn a non-
518 preferred feeder during the associative learning phase. Perhaps a stronger memory is formed if an
519 individual takes a long time to learn a feeder to which it does not have a strong initial tendency to visit,
520 causing more proactive interference of that memory, explaining the positive correlation between
521 associative and reversal learning performance [27].

522 Following the speed-accuracy trade-off hypothesis [33], we predicted that more exploratory and
523 bolder individuals, who are supposedly less sensitive to new information, would therefore be worse at
524 reversal learning performance while being better at associative learning tasks. However, contrary to
525 other studies [67–70] but see [71], our findings do not support a speed-accuracy trade-off as we find no
526 evidence for either phenotypic or genetic covariance between learning and personality traits. Perhaps
527 this relationship cannot be identified at the resolution of the performance criterion we used here. For
528 example, bolder sticklebacks performed better during associative learning and made more mistakes
529 during reversal learning, but only during the first few trials, whereas these differences were not visible
530 at trials to criterion [72]. This again suggests that dissecting the different mechanisms underlying
531 reversal learning performance and relating them to associative learning and personality traits will be a
532 crucial step towards better understanding trade-offs between learning, flexibility and personality.

533 In summary, our results demonstrate that reversal learning performance is somewhat repeatable
534 over longer time periods but seems to have no additive genetic variation. Although this suggests a
535 limited evolutionary potential, the intricate relationships with associative learning performance suggests
536 a more complex interplay of experiences that results in task performance, possibly explaining the lack
537 of additive genetic variation here. Future studies should disentangle the different components that likely
538 underlie individual variation in reversal learning performance; reinforcement learning, proactive
539 interference, and attentional allocation. This will allow for separately quantifying the repeatability and
540 heritability of these components and their covariances, and relationships with other behavioural traits
541 and ultimately fitness. This should preferably be done across a range of environmental conditions to
542 explore the possibility that selection acts on the level of reaction norms rather than on the reversal
543 learning performance itself.

544

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551

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556 **Tables and figure descriptions**

557 **Table 1** Estimates of fixed effects, variance components, repeatability and heritability of cognitive and personality
 558 traits, obtained from univariate animal models. Repeatability and heritability are given on the latent (R_{lat} , h^2_{lat})
 559 and observed scale (R_{obs} , h^2_{obs}). Estimates are given as posterior mean with 95% credible intervals. Significant
 560 estimates (95% CrI does not include zero) are in bold.

Variable	Associative learning	Reversal learning	Exploratory behaviour	Boldness
Fixed effects				
Intercept	2.989 [2.833,3.122]	3.369 [3.246,3.498]	0.909 [0.731,1.132]	1.034 [0.829,1.226]
Feeder Type (2)	-0.603 [-0.755,-0.441]	-0.288 [-0.416,-0.146]		
Variance components				
Additive genetic variance, V_A	0.010 [0.000,0.036]	0.011 [0.000,0.037]	0.304 [0.116,0.521]	0.263 [0.080,0.460]
Residual variance, V_R	0.420 [0.355,0.487]	0.302 [0.241,0.356]	0.269 [0.137,0.406]	0.278 [0.141,0.430]
Permanent environment effect variance, V_{PE}	0.018 [0.000,0.059]	0.031 [0.000,0.076]		
Maternal effect variance, V_{MA}	0.022 [0.000,0.053]	0.013 [0.000,0.036]	0.030 [0.000,0.096]	0.022 [0.000,0.074]
Variance ratio				
R_{lat}	0.105 [0.015,0.198]	0.153 [0.038,0.287]		
R_{obs}	0.065 [0.011,0.128]	0.116 [0.028,0.220]		
h^2_{lat}	0.020 [0.000,0.074]	0.030 [0.000,0.099]	0.499 [0.239,0.766]	0.463 [0.175,0.714]
h^2_{obs}	0.012 [0.000,0.044]	0.022 [0.000,0.072]	0.265 [0.114,0.399]	0.252 [0.110,0.410]

561

562 **Table 2** Results from bivariate linear mixed models. Reported are phenotypic, genetic, permanent environment
 563 and/or maternal correlations between each pair of traits. All correlations are given as posterior means with
 564 95% credible intervals. Significant correlations (95% CrI does not overlap zero) are in bold.

Traits	Phenotypic correlation	Genetic correlation	Permanent environment correlation	Maternal correlation
Associative learning /Reversal learning	-0.097 [-0.190,-0.007]	-0.003 [-0.485,0.507]	-0.022 [-0.865,0.828]	-0.074 [-0.914,0.802]
Associative learning /Reversal learning (Feeder Type 1)	0.115 [-0.076,0.317]	-0.005 [-0.822,0.736]	0.069 [-0.778,0.943]	
Associative learning /Reversal learning (Feeder Type 2)	-0.201 [-0.309,-0.095]	-0.020 [-0.589,0.553]	-0.233 [-0.964,0.702]	
Associative learning/Boldness	0.128 [-0.006,0.260]	-0.046 [-0.802,0.661]		
Associative learning/Exploration	0.027 [-0.109,0.165]	0.022 [-0.831,0.947]		
Reversal learning/Boldness	-0.029 [-0.165,0.098]	0.008 [-0.950,0.962]		
Reversal learning/Exploration	-0.106 [-0.228,0.029]	-0.074 [-0.755,0.673]		

565

566 **Table 3** Associative learning performance in the first testing round as predictor of reversal learning
 567 performance for feeder type 1 ($n = 104$), fitted with a linear mixed effect model with 'correct visit lag number'
 568 as fixed effect.

Variable	Estimate	SE	F-statistic	p-value
(Intercept)	3.79	0.59		
TTC associative	-0.14	0.19	$F_{1,100} = 0.56$	0.46
Correct visit lag number	-0.37	0.20	$F_{1,100} = 3.33$	0.07
TTC associative*Correct visit lag number	0.11	0.06	$F_{1,100} = 3.59$	0.06

569

570

571

572 **Figure 1 Response to selection per generation.** (a) Boxplot with horizontal lines representing median and
573 interquartile range of trials to criterion (**ln-transformed**), circle and triangles indicate mean trials to criterion,
574 corrected for feeder type. Eye plots represent density distributions for trials to criterion. Dots indicate number
575 of individuals within each bin. (b) Realized heritability (h^2) of reversal learning performance in an artificial
576 selection experiment. Cumulative response to selection (R) is plotted as function of the cumulative selection
577 differential (S) for each line selected on number of trials to reach learning criterion for two generations. The
578 realized heritability (h^2) was calculated as the slope of the linear regression between R and S (black solid line).
579 Error bars represent standard errors. Dashed line indicates expected function if slope (h^2) were 0.15. Orange,
580 downward pointing arrows: fast line; blue, upward pointing arrows: slow line.

581
582 **Figure 2 Relationship between the number of trials to criterion (ln-transformed) in the reversal learning**
583 **phase and the number of trials to criterion (ln-transformed) in the associative learning phase.** Higher values
584 = slower learning. This is shown for a) feeder type 1 (Light grey, continuous line and dots) and type 2 (dark
585 grey, dashed line and triangles) and for b) the interaction between associative learning and the 'correct visit
586 lag number' (grouping levels based on standard deviation; +1 SD is dark grey solid line and -1 SD is light grey
587 dashed line). Darker dots reflect a higher 'correct visit lag number'. For both a) and b), plotted lines are
588 marginal effects of the interactions term. Lines and shaded regions represent the model prediction (\pm 95 %
589 confidence interval). The points of the scatterplot represent the actual data points.
590

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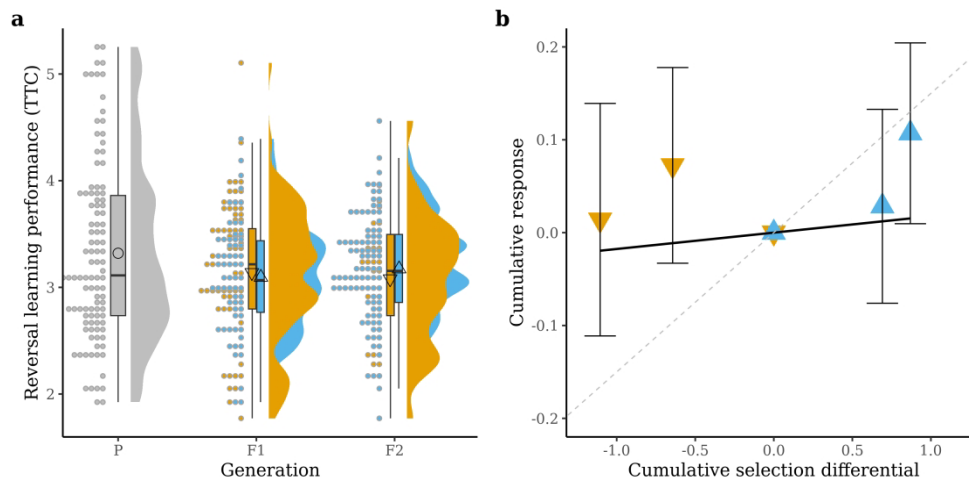


Figure 1 Response to selection per generation. (a) Boxplot with horizontal lines representing median and interquartile range of trials to criterion (ln-transformed), circle and triangles indicate mean trials to criterion, corrected for feeder type. Eye plots represent density distributions for trials to criterion. Dots indicate number of individuals within each bin. (b) Realized heritability (h^2) of reversal learning performance in an artificial selection experiment. Cumulative response to selection (R) is plotted as function of the cumulative selection differential (S) for each line selected on number of trials to reach learning criterion for two generations. The realized heritability (h^2) was calculated as the slope of the linear regression between R and S (black solid line). Error bars represent standard errors. Dashed line indicates expected function if slope (h^2) were 0.15. Orange, downward pointing arrows: fast line; blue, upward pointing arrows: slow line.

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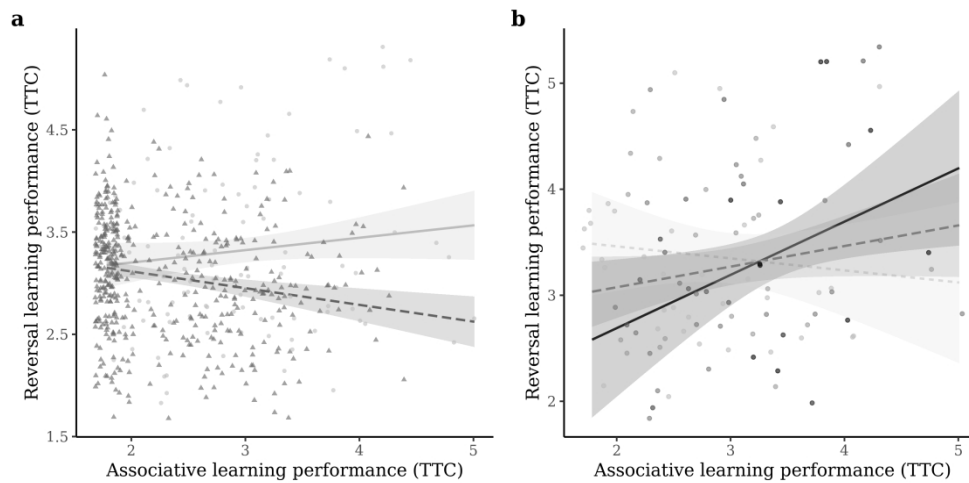


Figure 2 Relationship between the number of trials to criterion (ln-transformed) in the reversal learning phase and the number of trials to criterion (ln-transformed) in the associative learning phase. Higher values = slower learning. This is shown for a) feeder type 1 (Light grey, continuous line and dots) and type 2 (dark grey, dashed line and triangles) and for b) the interaction between associative learning and the 'correct visit lag number' (grouping levels based on standard deviation; +1 SD is dark grey solid line and -1 SD is light grey dashed line). Darker dots reflect a higher 'correct visit lag number'. For both a) and b), plotted lines are marginal effects of the interactions term. Lines and shaded regions represent the model prediction (\pm 95 % confidence interval). The points of the scatterplot represent the actual data points.

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