

1 **Title: Habitat stability, predation risk and ‘memory syndromes’**

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26 **Abstract**

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28 Habitat stability and predation pressure are thought to be major drivers in the evolutionary
29 maintenance of behavioural syndromes, with trait covariance only occurring within specific
30 habitats. However, animals also exhibit behavioural plasticity, often through memory
31 formation. Memory formation across traits may be linked, with covariance in memory traits
32 (memory syndromes) selected under particular environmental conditions. This study tests
33 whether the pond snail, *Lymnaea stagnalis*, demonstrates consistency among memory traits
34 ('memory syndrome') related to threat avoidance and foraging. We used eight populations
35 originating from three different habitat types: i) laboratory populations (stable habitat,
36 predator-free); ii) river populations (fairly stable habitat, fish predation); and iii) ditch
37 populations (unstable habitat, invertebrate predation). At a population level, there was a
38 negative relationship between memories related to threat avoidance and food selectivity, but
39 no consistency within habitat type. At an individual level, covariance between memory traits
40 was dependent on habitat. Laboratory populations showed no covariance among memory
41 traits, whereas river populations showed a positive correlation between food memories, and
42 ditch populations demonstrated a negative relationship between threat memory and food
43 memories. Therefore, selection pressures among habitats appear to act independently on
44 memory trait covariation at an individual level and the average response within a population.

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51 **Introduction**

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53 Predation pressure exerts a significant selective pressure on behaviour, both in terms of
54 evading predators, but also avoiding unnecessary antipredator responses that may reduce time
55 available for foraging and reproduction¹. In habitats where predation pressure is stable, local
56 adaptation to predation environments may occur where innate responses to cues from a
57 predator are enhanced in populations that overlap in distribution with that predator²⁻⁵.
58 Predation pressure also exerts selection on a range of other traits within individuals, with
59 populations from low-risk environments typically demonstrating increased boldness and
60 activity levels reflecting lack of risk in their environment⁶⁻⁸. Predators may also exhibit strong
61 indirect effects on prey behaviour⁹. One factor that has received considerable attention is how
62 predators influence the foraging behaviour of their prey in tri-trophic systems, with
63 ecological interactions among species occupying three trophic levels, predators, prey (a
64 herbivore) and plants^{10,11}. Foraging behaviour is often indirectly affected by predation risk
65 via trait mediated indirect interactions (TMIIIs), such that the foraging behaviour of a
66 herbivore, for example, alters due to the presence of a predator. Therefore the predator may
67 indirectly impact on plant growth in the habitat. Prey may choose to forage in less risky
68 habitats or during different time periods when faced with predation threat^{12,13}, and prey often
69 become less selective about food resources in the presence of predation threat¹⁴.

70

71 Selection on plasticity in foraging and antipredator traits may act in two different ways.
72 Firstly, it may act on the overall trait plasticity, i.e. how plastic an animal is in response to
73 food resources or the predation environment. Secondly, plasticity in behavioural traits may be
74 linked via covariation in memory formation across traits (i.e. memory syndromes), where the
75 degree of plasticity an animal exhibits in response to its environment co-varies across

76 different types of behaviour (e.g. Fig. 1a). Memory formation may also differ across
77 behavioural traits, for example only altering a single behavioural trait but still maintaining
78 covariance between behavioural traits in individuals (Fig. 1b). Alternatively, memory
79 formation that differs either in the degree to which it alters behavioural traits (Fig. 1c) or
80 among individuals within a population as well as across different traits (Fig. 1d) could either
81 break down or enhance covariance among behavioural traits altering behavioural syndromes.
82 For example, in wild *Gasterosteus aculeatus* (three-spine stickleback), high predation risk
83 selects for correlations among suites of behaviours related to exploratory and risk-related
84 behaviours; whereas low-risk populations demonstrate a lack of correlation among these
85 traits^{7,8,15,16}. However, recent exposure to a novel predation threat has been shown to both
86 enhance¹⁷ and break down¹⁸ correlations among behavioural traits in *G. aculeatus*. Therefore,
87 whilst some traits may be fixed, plasticity in traits may also form an important element of
88 behavioural syndromes.

89

90 Habitat stability is predicted to exert differing selection pressures on behavioural flexibility
91 among populations¹⁹. Plasticity in behavioural traits can occur through memory formation,
92 allowing animals to react to their environment. Whilst memory ability is frequently assessed
93 as a single trait in the context of behavioural syndromes, or the effect of experience is
94 measured on a suite of unrelated 'personality' traits, co-variation in memory forming ability
95 across different traits (a memory syndrome) has yet to be demonstrated in wild populations²⁰⁻
96 ²². Memory traits can be defined by the ability of an animal to demonstrate flexibility in a
97 behaviour following experience based on learned responses in different behavioural contexts
98 rather than as a result of other physiological or morphological changes that may take place.
99 Similarly to other traits an animal possesses, we might predict that memory will also differ in
100 a consistent manner among individuals. A strong linkage between different memory traits

101 would be predicted where a balanced response in both traits carries a greater fitness value
102 than responding to each independently, whereas memory forming ability across traits may
103 become disassociated if there is no fitness value to this linkage.

104

105 To test whether habitat type affects memory syndromes across different traits, we used the
106 great pond snail, *Lymnaea stagnalis*. This species has two distinct advantages. Firstly, there
107 are a number of well-defined memory traits that have been assessed using this species in the
108 context of neurobiology and ecology^{23,24}. Secondly, we have access to multiple populations
109 and laboratory strains that come from different backgrounds of habitat stability and predation
110 pressure. River populations experience a relatively stable habitat with predatory fish, whereas
111 ditch populations come from relatively unstable habitats experiencing predation threat from a
112 wide range of invertebrate predators. These factors may act independently in their selective
113 pressure on memory formation; however, it is also possible that they will interact to affect
114 memory. As these factors co-vary within the habitats from which wild populations were
115 sourced, the current study does not attempt to isolate individual effects. These wild
116 populations do exhibit innate differences in antipredator behaviour relative to the predator
117 regime they experience, indicating that predation pressure has a significant effect on
118 behavioural traits in this species⁴. Populations from each habitat type were bred through to
119 the F1 generation using wild-caught adults (minimum of 50 to establish laboratory
120 populations), and the F1 adults were used to assess memory traits. Laboratory strains have
121 also been established for studies in genetics and neurobiology, allowing access to populations
122 that have lived in very stable predation-free environments over many (≥ 14) generations.
123 Adults from each habitat type were tested for long-term memory formation in three traits, two
124 food-related (food aversion and food appetitive conditioning) and operant conditioning of
125 aerial respiration. It has been proposed that operant conditioning is a threat aversion

126 behaviour, related to antipredator behaviour²⁵. Whilst adults do not demonstrate overt
127 antipredator responses²⁶, juveniles of this species do, and have been shown to form
128 associative memory of predation threat²⁴. To confirm if there is population level co-variance
129 in juvenile antipredator behaviour and operant conditioning, we also assessed memory of
130 predation threat in F2 juveniles from the river populations.

131

132 Memory formation across traits in *L. stagnalis* was therefore evaluated by: i) testing whether
133 the average memory forming ability across the different traits in adults is consistent within
134 habitat types; ii) determining whether memory of operant conditioning in adults was linked
135 with memory of predation threat in juveniles at the population level; iii) assessing whether
136 memory formation across adult memory traits covaries at an individual level (i.e. a ‘memory
137 syndrome’); and iv) determining if the strength of covariation in memory formation is
138 affected by habitat of origin. We predicted that in snails originating from habitats where
139 relatively stable ecological problems (i.e. foraging and predation risk) co-occur, there would
140 be stronger selection for memory syndromes (covariation among memory traits). In a
141 relatively stable environment, retaining information about past experience is predicted to de-
142 value at a slow rate as memory of recent experiences maintain a benefit for longer. Therefore
143 river snails were predicted *a priori* to demonstrate better memory retention across all traits.
144 Consequently, we also expected to find the strongest memory syndromes in river populations,
145 i.e. where memory formation across traits shows strong positive covariation, and conversely
146 we predicted little covariation among memory traits in the unstable ditch populations. In
147 contrast, as potential to form memory carries costs in other species²⁷, we predicted that
148 laboratory populations that have been under relaxed selection for multiple generations would
149 demonstrate poorer memory forming capabilities relative to river populations. Additionally, if

150 selection in wild populations is maintaining co-variation among traits, this would also be lost
151 in laboratory populations.

152

153 **Results**

154

155 *Adult memory among populations*

156

157 Adult memory in F1 snails from 8 populations (2 laboratory; 2 ditch; and 4 river) was tested
158 using three traits, operant conditioning of aerial respiration (decrease in breathing behaviour
159 = memory), aversive food conditioning (decrease in bite rate = memory) and appetitive food
160 conditioning (increase in bite rate = memory). Each individual received all three training
161 regimes one week apart over three weeks. Controls, where snails received the same number
162 of stimuli but non-contingently, were used to determine memory formation. The estimated
163 difference (including 95% confidence intervals) in response between contingent vs. non-
164 contingent training and effect size for each population are given in Table 1.

165

166 Operant conditioning: the response to training differed among populations (Fig. 2; 2-way
167 interaction: training regime*population(origin): $F_{5,15,11} = 3.55$, $P = 0.026$; $\eta^2_p = 0.540$). Half
168 of the populations tested demonstrated a significant decrease in breathing attempts 24 h
169 following contingent training compared to those receiving non-contingent training (Fig. 2;
170 Table 1), indicating that these populations had formed long-term memory. The order in which
171 adult snails received training did not affect memory formation. There was also no significant
172 effect of habitat of origin on memory formation.

173

174 Aversive conditioning: there was a significant response to training regime during aversive
175 conditioning, with only contingently trained snails demonstrating a significant reduction in
176 bite rate (Fig. 2; main effect of training: $F_{1,5.05} = 8.01$, $P = 0.036$; $\eta^2_p = 0.613$; difference
177 between control vs. trained = -3.100, CI: -1.647,-4.553). There was also a non-significant
178 trend towards an effect of population on how snails responded to training (2-way interaction:
179 training regime*population(origin): $F_{5,15.11} = 2.75$, $P = 0.059$; $\eta^2_p = 0.476$), which is
180 substantiated by a significant difference between non-contingently and contingently trained
181 animals in half of the populations tested (Fig. 2; Table 1). The order in which adult snails
182 received training did not affect memory formation. There was also no significant effect of
183 habitat of origin on memory formation.

184

185 Appetitive conditioning: the response to training differed among populations (Fig. 2; 2-way
186 interaction: training regime*population(origin): $F_{5,15.05} = 4.75$, $P = 0.008$; $\eta^2_p = 0.612$). Half
187 of the populations tested demonstrated a significant increase in bite rate in response to amy
188 acetate exposure 24 h following contingent training compared to those receiving non-
189 contingent training, indicating that these populations had formed long-term memory (Fig.2;
190 Table 1). The order in which adult snails received training did not affect memory formation.
191 There was also no significant effect of origin on memory formation.

192

193 Overall, there was a pattern for population variability in long-term memory formation across
194 the three traits. Populations that demonstrated good long-term memory following operant
195 conditioning were poor at forming food related memories and vice versa (Fig. 2; Table 1).
196 Habitat type populations originated from did not appear to affect which memories the snails
197 are good at forming.

198

199 *Juvenile memory*

200 Juvenile memory of a predation event was assessed in F2 individuals from the four river
201 populations used to test adult memory. Juvenile snails were pre-exposed to predation or
202 control cues and then tested using either predator kairomones or control pond water to
203 determine if their antipredator behaviour (crawling out of the water) increased indicating
204 memory of recent predation threat. The data were analysed including the phenotype of the F1
205 generation derived from adult memory traits: phenotype A came from populations where
206 adults demonstrate good food memories, but poor operant conditioning memory, phenotype
207 B came from populations exhibiting poor food memories, but good operant conditioning
208 memory.

209

210 Crawl out behaviour differed between the two phenotypes dependant on both pre-exposure
211 conditions and exposure during the behavioural trial (Fig. 3; 3-way interaction:
212 phenotype*pre-exposure*behavioural trial exposure: $F_{1,2} = 66.63$, $P = 0.015$; $\eta^2_p = 0.972$).
213 Phenotype A snails (from populations that demonstrate poor operant conditioning memory)
214 demonstrated an elevated crawl out response to tench cue during behavioural trials relative to
215 snails that had received control conditions throughout (SNK: $P < 0.05$; difference 0.441, CI:
216 0.255,0.627; $\eta^2_p = 0.164$), though pre-exposure did not significantly increase the crawl out
217 response to tench cue alone (SNK: $P > 0.05$). For phenotype B snails (from populations that
218 demonstrate good operant conditioning memory) there was no significant difference among
219 groups pre-exposed to control conditions (irrespective of behavioural trial conditions) and
220 those pre-exposed to tench plus alarm but exposed to control cues during the behavioural trial
221 (SNK: $P > 0.05$). However, phenotype B snails pre-exposed to tench plus alarm cues then
222 exposed to tench cues during the behavioural trial demonstrated a significantly elevated crawl
223 out response to tench cues relative to phenotype B snails pre-exposed to control conditions

224 (SNK: $P < 0.05$; difference 0.643, CI: 0.362,0.923; $\eta^2_p = 0.273$). This indicates that the
225 phenotype B snails have retained information about predation threat from their experience 24
226 hours previously and this memory of a recent predation event has elevated their response to
227 the predator cues. There was no significant effect of population nested within phenotype on
228 crawl out behaviour.

229

230 Phenotype A snails failed to demonstrate associative conditioning of predation threat 24 h
231 following exposure, whereas phenotype B snails demonstrated a significantly elevated crawl
232 out behaviour to tench cues following cue association learning. This indicates that they
233 adjusted their antipredator behaviour based on recent experience as found in previous work²⁴.
234 Therefore, we concluded that operant conditioning in adults can be used as a proxy for
235 memory about predation threat at a population level, as postulated in our previous study²⁵.

236

237 *Memory syndromes*

238 The data from memory formation in adult snails was also assessed at an individual level to
239 determine if ability to form memory co-varied across the different adult memory traits, i.e. a
240 ‘memory syndrome’, using their responses to operant conditioning, aversive conditioning and
241 appetitive conditioning. All data were converted such that a positive value in the trait would
242 be an indicator of good memory formation; therefore a positive correlation means that
243 individuals that were good at memory formation in one trait were also good at memory
244 formation in the other.

245

246 Following non-contingent training snails did not show any consistency in how they altered
247 their behaviour between training and testing. However, following contingent training there
248 was a significant relationship between how well snails formed memory in each memory trait.

249 This relationship was negative between memory formation in operant conditioning and the
250 two food related traits (operant vs. aversive: $r = -0.23$ (CI $-0.379, -0.069$), $P = 0.007$; operant
251 vs. appetitive: $r = -0.21$ (CI: $-0.361, -0.048$), $P = 0.012$; $N = 143$), but there was a positive
252 relationship between the two food memory traits ($r = 0.22$ (CI $0.058, 0.37$), $P = 0.008$, $N =$
253 143).

254

255 When data from each habitat type (laboratory, ditch and river) were analysed separately,
256 there were differences in consistency in memory formation across traits compared to the
257 overall pattern. Again, non-contingently trained individuals did not demonstrate consistency
258 in how they altered behaviour between training and testing, indicating that without memory
259 formation there is no evidence of behavioural syndromes across traits. However, following
260 contingent training, habitat of origin affected the level of consistency among memory traits.
261 Laboratory reared snails demonstrated no strong link among traits (Fig. 4; $N = 38$). Ditch
262 origin snails showed a negative correlation between their ability to form food-related
263 memories and their ability to form memory of operant conditioning (operant vs. aversive: $r =$
264 -0.39 (CI: $-0.058, -0.648$), $P = 0.024$; operant vs. appetitive: $r = -0.37$ (CI: $-0.032, -0.633$), $P =$
265 0.034 ; $N = 33$), but no individual consistency in response across the two food-related
266 memory traits (Fig. 4). Whereas river populations demonstrated a positive correlation
267 between their ability to form memory in the two food-related memories ($r = 0.24$ (CI:
268 $0.01, 0.447$), $P = 0.041$), and a negative association between appetitive conditioning and
269 operant conditioning ($r = -0.24$ (CI: $-0.45, -0.014$), $P = 0.039$), but no consistency in response
270 between operant and aversive conditioning (Fig. 4; $N = 72$). Overall these data show that
271 consistency in how well individual snails perform across different memory traits is linked to
272 the habitat they originate from, demonstrating habitat specific memory syndromes
273 irrespective of the mean population response to training.

274

275 **Discussion**

276

277 This study demonstrated that memory formation across four fitness-related traits differs
278 significantly among *Lymnaea stagnalis* populations. Populations that exhibited strong
279 memory in threat avoidance traits (predator cue association and operant conditioning)
280 exhibited poor memory in foraging-related traits (food aversive and appetitive conditioning).
281 Conversely, those that exhibited good food memories were inflexible in their threat
282 avoidance behaviour. These population-level responses were not habitat specific, as might be
283 predicted based on work with other species differing in predator regime^{28,29}, but were
284 distributed equally across different habitat types for the eight populations tested. If memory
285 formation carries significant costs²⁷, removing the benefits of memory under the relaxed
286 selection conditions in the laboratory might be predicted to result in poorer memory
287 formation in these individuals. A lack of effect of habitat of origin indicates these laboratory
288 populations do not differ significantly in their ability to form memory relative to their wild
289 counterparts. This suggests that either there are low costs associated with memory potential
290 for these traits, or that the conditions in the laboratory, with food provided *ad libitum*, easy
291 mating opportunities, little need to move far and a lack of predators, negate the costs
292 associated with memory potential.

293

294 Why populations differ in their ability to form memory across the different traits is still to be
295 determined. It could be that differences in physiology, including metabolic rate, alters
296 whether animals are able to demonstrate plasticity. For example, metabolic rate may
297 determine the scope an animal has to alter its feeding behaviour or the time it is able to
298 allocate to threat avoidance. Differences in memory formation may also result from

299 attentional bias rather than underlying differences in physiology or neural capability to form
300 memory *per se*³⁰. How individuals respond to stress for example, is highly likely to alter their
301 memory retention^{31,32}, and may affect the way an individual behaves in the novel
302 environment used to train the snails. There is a strong correlation between the
303 neurophysiological changes that take place in *L. stagnalis* and the change in behavioural
304 phenotype following memory formation in both operant³³ and appetitive conditioning³⁴. This
305 indicates that differences in how individuals respond to training are not due to behavioural
306 masking of memory formation, but are instead due to underlying differences in the ability of
307 the animals to form memory across the different traits. There is also evidence that
308 neurophysiological differences among populations may determine how well the snail forms
309 memory in response to operant conditioning at least³⁵, indicating that underlying differences
310 among individuals in their physiology drives the population variability we see in memory
311 formation.

312

313 The ability of animals to perform consistently across a range of contexts, termed animal
314 personality when assessed by the same trait over time or behavioural syndrome when
315 assessed across different traits, has received significant interest in recent years^{22,36},
316 particularly the role that this co-variation may play in population ecology³⁷. How an animal
317 responds to its environment can also be plastic, and the ability to learn and remember
318 experiences can play an important role in this plasticity²¹. So far, evidence for individual
319 consistency in memory formation across different traits among natural populations has
320 proved elusive³⁸. However, in *L. stagnalis*, we found evidence that covariation among
321 memory traits - memory syndromes - do exist in wild populations. Individual consistency was
322 identified across populations in the negative relationship between memory of threat
323 avoidance and memory in food-related traits, which reflected the population level

324 relationships among traits. Similarly, a positive relationship between the two food-related
325 traits was also found. The effect sizes of these relationships were relatively low ($r = 0.21-$
326 0.23), though within the normal range of individual levels of consistency in behaviour across
327 many studies of behavioural syndromes³⁹. However, when individual responses were
328 assessed within habitat type, a different pattern becomes evident, demonstrating an effect of
329 habitat in the strength of trait covariance as we would have expected *a priori* (see
330 introduction). Nevertheless, the observed pattern did not conform to our habitat specific
331 predictions for wild populations, and was considerably more complex than expected.

332

333 Pace-of-life syndromes, where individuals within populations differ in behavioural tendencies
334 depending on metabolic and life-history requirements³⁶, may explain why the strength of
335 correlation among memory traits differs among habitat types in the opposite direction to our
336 initial prediction. In unstable habitats with fluctuating predation threat, where refuge use
337 becomes unreliable due to a diverse range of predator foraging activities, there is likely to be
338 strong selection on life-history traits that allow survival in the face of continuous and variable
339 threat. Unpredictable conditions may strongly favour individuals exhibiting alternative
340 memory phenotypes, benefitting either fast growth rate and high reproductive output or long-
341 lived threat aversive individuals. The relatively strong negative relationship (effect size $r = -$
342 0.37 to -0.39) between threat aversion and food memories supports this hypothesis. As an
343 individual, it is beneficial in ditch habitats to either demonstrate plasticity in response to
344 foraging related cues, allowing fast growth and earlier reproductive output, or respond to
345 predation threat, increasing longevity. Individuals that demonstrate a middle ground, between
346 these two life-history strategies, may be disadvantaged.

347

348 In stable habitats, individuals may exhibit some degree of innate recognition of resources or
349 predation threat. For example, there is strong evidence for innate predator recognition by *L.*
350 *stagnalis* in river habitats found here and elsewhere⁴. Whilst some populations are clearly
351 capable of altering their response following experience of predation cues²⁴, those that do not
352 are still afforded some degree of protection through this innate antipredator behaviour. Where
353 predators are easily avoided through refuge use, selection on plasticity of avoidance
354 mechanisms may be relatively weak if animals are able to demonstrate adaptation of innate
355 responses. Instead, selection may act primarily on foraging behaviour, where animals are able
356 to make use of food patches in safe places and can demonstrate a greater degree of selectivity
357 based on food quality in stable habitats. Selection on pace-of-life phenotype may therefore be
358 relaxed to some degree. In river populations, there is a positive relationship in food memory
359 formation across the two traits, and also a negative relationship between threat aversion and
360 food appetitive conditioning with similar effect sizes ($r = 0.24$) to the combined data, but the
361 strength of these relationships is lower than that found in the ditch populations.

362

363 In laboratory populations, despite population level consistency in how well snails formed
364 memory across the traits, there was little evidence of individual consistency in memory
365 formation. A non-significant trend ($P = 0.089$) with a relatively strong effect size ($r = 0.29$)
366 was found between the two food memory traits, indicating that laboratory rearing had not
367 completely eliminated this linkage. However, there was no relationship between threat
368 aversion and food traits. In the absence of predation threat (other than scientists) and a
369 constant food supply, there is no selective benefit derived from memory formation across
370 these traits. Whilst strain differences have been maintained over many generations in the
371 laboratory environment, individual consistency in the relationship among memory traits
372 appears to have been lost. This is unlikely to be a result of rearing conditions only, as all

373 populations tested were F1 laboratory reared, but more likely a result of relaxed selection for
374 this linkage between traits⁴⁰. Together these data suggest that selection pressures within each
375 habitat type are acting differently on links between memory traits, mirroring environmental
376 effects on behavioural syndromes among populations¹⁶.

377

378 Memory syndromes may link in with the overall behavioural syndrome, not just in terms of
379 how memory alters behavioural traits, but also how other behavioural traits may predict
380 memory formation across different contexts. For example, a timid individual may form better
381 threat aversion memories but poor food memories in a novel context where fear is elevated; a
382 bold individual may be equally capable of forming food and threat related memories in the
383 same novel context. However, in safer, familiar surroundings, both individuals may perform
384 equally well. Memory syndromes are therefore likely to play a key role in understanding the
385 evolutionary and ecological relevance of behavioural syndromes in wild populations²¹.
386 Together these data point towards the importance that ecological background can play in
387 determining the strength of covariation among traits underpinning behaviour⁴¹, whilst not
388 having any apparent effect on the mean population behavioural responses.

389

390 **Methods**

391

392 *Animal origin and maintenance*

393

394 Pond snails, *Lymnaea stagnalis*, were used from eight original different sources. Two strains
395 originated from laboratory populations that had been maintained under constant conditions in
396 the laboratory for a minimum of 14 generations (L1-L2). Four strains were F1 laboratory
397 reared adults originating from adults collected from river populations (R1-R4), and two

398 strains were F1 laboratory reared adults originating from adults collected from ditch
399 populations (D1-D2). Both river and ditch populations were collected on the Somerset
400 Levels, U.K using sweep netting in aquatic vegetation, with a minimum of 50 adults collected
401 per population and contributing to each generation. *Lymnaea stagnalis* is a preferentially out-
402 crossing hermaphrodite mating frequently in the laboratory⁴², ensuring the maintenance of
403 genetic variation in the laboratory populations. River populations are exposed to high levels
404 of fish predation, with *Tinca tinca* (tench), a specialist molluscivore present at all sites. Ditch
405 sites have no predatory fish present but experience invertebrate predation from bugs, leeches
406 and beetles. Juveniles from ditch and river sites have been found to differ in their innate
407 response to fish predation threat in previous work⁴. The ditch sites are also subject to frequent
408 infilling from rotting vegetation, followed by dredging by farmers, so fluctuate in terms of
409 vegetation available for food, water depth and oxygen availability (particularly during
410 shallow, in-filled periods) to a greater extent than river populations²⁵.

411

412 Adult snails (spire height 25 ± 1 mm) used for all experiments were reared under constant
413 conditions in the Aquatic Resource Centre at the University of Exeter. They were held at $20 \pm$
414 1°C on a 14:10 light:dark schedule in aerated artificial pond water (Ca^{2+} [80 mg/l]; Mg^{2+} [4.9
415 mg/l]; NaHCO_3 [3.75 mg/L]; KCL [1.0 mg/L]; Marine salts (Crystal Sea® *Marinemix*,
416 Baltimore, U.S.A) [20 mg/L]) and fed lettuce and trout pellets ad libitum. F2 juveniles (spire
417 height 6 ± 0.5 mm) were reared under identical conditions to the adults.

418

419 *Training – adult memory*

420

421 Adults were trained using three different methods: operant conditioning of aerial
422 respiration⁴³, food aversion conditioning⁴⁴, and food appetitive conditioning⁴⁵. Individuals

423 from each population were randomly allocated to the contingent (trained) or non-contingent
424 (control) group (see below for details). If changes in behaviour were due to memory
425 formation, it was predicted that only trained snails that had received contingent stimuli would
426 demonstrate a significant change in behaviour. Individual snails were exposed to all three
427 training methods, randomly assigned to one of four orders in which they received each
428 training method (contingently trained or non-contingent control). The four possible orders in
429 which they received training were: 1) operant > aversive > appetitive, 2) operant > appetitive
430 > aversive, 3) aversive > appetitive > operant and 4) appetitive > aversive > operant. The
431 order in which they receive the different training methods was included in the subsequent
432 analyses to assess whether forming memory under one regime altered memory formation
433 under other regimes.

434

435 *Operant Conditioning*

436 Snails are trained to associate a spontaneous behaviour (aerial respiration in hypoxic
437 conditions) with a negative tactile stimulus. Memory is demonstrated by a reduction in
438 breathing behaviour in hypoxia in trained animals but not in non-contingent controls.

439

440 Contingent (trained): 500 ml of artificial pond water was placed in a 1 l glass beaker. N₂ was
441 then vigorously bubbled through the water for 20 min to make the water hypoxic (< 5%
442 [O₂]). N₂ bubbling was reduced and continued at a low level to maintain hypoxic conditions
443 without disturbing the animals. Snails were then introduced into the beaker in small groups of
444 5 to 6 individuals and allowed to acclimate for 10 min before the start of training. Training
445 was carried out for 30 min (TR1), whereby the snail receives a tactile stimulus (a poke) on
446 the pneumostome each time it attempts to open it at the water's surface⁴³. This poke is
447 sufficient to cause the pneumostome to close, but does not cause the snail to withdraw into its

448 shell. To test for long-term memory (LTM) the snails received an identical procedure to the
449 training session 24 h later.

450

451 Non-contingent (control): Training was identical to the contingent training above except that
452 during training the control snail was poked in the vicinity of the pneumostome each time the
453 snail with which it was paired received a poke, i.e. the control snail received an identical
454 number of stimuli, but they were not contingent with pneumostome opening. During testing
455 the control animal received contingent stimuli.

456

457 *Food aversion conditioning*

458 Snails are trained to associate a recognised food resource that stimulates feeding behaviour
459 (carrot) with a negative stimulus (exposure to KCl). Memory is demonstrated by a reduction
460 in feeding behaviour in response to the carrot stimulus in trained animals.

461

462 Contingent (trained): Snails were food deprived for 48 h prior to training. They were placed
463 individually into a small Petri dish (60 mm diameter) in 18 ml of artificial pond water and
464 allowed to acclimate for 10 min. During the first session, 1 ml of artificial pond water was
465 then added, followed 1 min later by a further 1 ml of pond water. The snails were then
466 returned to their home aquaria. During the second session, 1 h following the first, snails were
467 again acclimated to the small Petri dish in 18 ml of artificial pond water for 10 min. 1 ml of
468 5% carrot (w/v) water was then added and the bite rate (number of rasps) counted for 1 min.
469 Following 1 min in carrot, 1 ml of 100 mM KCl was added and the snails were left in the
470 resulting solution (0.5% carrot; 10 mM KCl) for a further 1 min. They were then removed
471 and placed in their aquaria. To test for long-term memory 24 h later, snails were again placed
472 in 18 ml of artificial pond water a small Petri dish and allowed to acclimate for 10 min. 1 ml

473 of pond water was then added and the bite rate over 1 min counted, immediately followed by
474 adding 1 ml 5% carrot solution and the bite rate counted for a further minute.

475

476 Non-contingent (control): To control for exposure to both carrot and KCl stimuli control
477 training was carried out as above, except stimuli were presented non-contingently on the first
478 day. Individual snails were placed in a small Petri dish (60 mm diameter) in 18 ml of artificial
479 pond water and allowed to acclimate for 10 min. During the first session, 1 ml of artificial
480 pond water was then added, followed 1 min later by a further 1 ml of 100 mM KCl and
481 exposed for 1 min. The snails were then returned to their home aquaria. During the second
482 session, 1 h following the first, snails were again acclimated to the small Petri dish in 18 ml
483 of artificial pond water for 10 min. 1 ml of 5% carrot (w/v) water was then added and the bite
484 rate (number of rasps) counted for 1 min. This was immediately followed by addition of 1 ml
485 of artificial pond water; snails were left in the Petri dish for a further 1 min then returned to
486 their aquaria. The memory test was identical to trained (contingent) animals above.

487

488 *Food appetitive conditioning*

489 Snails are trained to associate a neutral stimulus that does not normally stimulate feeding
490 behaviour (the odour of amyl acetate) with a food resource (exposure to sucrose solution).
491 Memory is demonstrated by an increase in feeding behaviour in response to amyl acetate.

492

493 Contingent (trained): Snails were food deprived for 48 h prior to training. They were placed
494 into a large Petri dish (140 mm diameter) in 90 ml of artificial pond water and allowed to
495 acclimate for 10 min. During the first session, 5 ml of artificial pond water was then added,
496 followed 2 min later by a further 5 ml of pond water and given a 2 min exposure period. The
497 snails were then returned to their home aquaria. During the second session, 1 h following the

498 first, snails were again acclimated to the large Petri dish in 90 ml of artificial pond water for
499 10 min. 5 ml of 0.08% amyl acetate water was then added and the bite rate (number of rasps)
500 counted for 2 min. Following 2 min in amyl acetate solution alone, 5 ml of 13.4% sucrose
501 solution was added and the snails were left in the resulting solution (0.004% amyl acetate;
502 0.67% sucrose) for a further 2 min. They were then removed and placed in their aquaria. To
503 test for long-term memory 24 h later, snails were again placed in 90 ml of artificial pond
504 water a large Petri dish and allowed to acclimate for 10 min. 5 ml of pond water was then
505 added and the bite rate over 2 min counted, immediately followed by adding 5 ml 0.08%
506 amyl acetate and the bite rate counted for a further 2 min.

507

508 Non-contingent (control): To control for exposure to both amyl acetate and sucrose stimuli,
509 control training was carried out as above, except stimuli were presented non-contingently on
510 the first day. Snails were placed in a large Petri dish (140 mm diameter) in 90 ml of artificial
511 pond water and allowed to acclimate for 10 min. During the first session, 5 ml of artificial
512 pond water was then added, followed 2 min later by a further 5 ml of 13.4% sucrose solution
513 and the snails left for 2 min. The snails were then returned to their home aquaria. During the
514 second session, 1 h following the first, snails were again acclimated to the large Petri dish in
515 90 ml of artificial pond water for 10 min. 0.08% amyl acetate water was then added and the
516 bite rate counted for 2 min. This was immediately followed by addition of 5 ml of artificial
517 pond water; snails were left in the Petri dish for a further 2 min then returned to their aquaria.
518 The memory test was identical to trained (contingent) animals above.

519

520 *Data analysis – adult memory*

521

522 Data analyses were carried out using SPSS 21 (SPSS Inc., Chicago, IL, USA). Adult memory
523 performance at a population level was analysed using the change in behaviour between
524 training and testing for each memory trait as follows: operant conditioning (breaths during
525 memory test – breaths during training); aversive conditioning (bites during the memory test –
526 bites during training); appetitive conditioning (bites during the memory test – bites during
527 training). Data were analysed using ANOVA with training regime (contingent vs. non-
528 contingent), order they experienced training regimes (4 levels) and origin (laboratory vs.
529 ditch vs. river) as fixed factors, and population nested in origin as a random factor in the
530 model, using the Satterthwaite approximation to estimate the degrees of freedom⁴⁶. Student-
531 Newman-Keuls pair-wise comparisons were used to carry out posthoc analyses.

532

533 To test for memory syndromes, all data on changes in behaviour between training and testing
534 were converted so that they were on a positive scale, i.e. the greater the positive value the
535 stronger the memory, and are presented in this format. Data were analysed using Pearson's
536 correlation.

537

538 *Training – juvenile memory*

539

540 Operant conditioning was previously proposed to relate to threat avoidance behaviour in *L.*
541 *stagnalis*²⁵. To confirm whether memory following operant conditioning is indeed related to
542 threat memory at a population level, cue association memory of predation threat in juvenile
543 snails was tested using F2 juveniles from the same populations tested for adult memory traits.
544 Juveniles were obtained by randomly selecting offspring from 50 F1 randomly selected adult
545 snails (3-4 months old) per population that were not used to assess memory formation but
546 retained as laboratory stock. Only river populations were used to assess this, as habitat type

547 can significantly alter antipredator traits⁴. Juvenile *L. stagnalis* from river populations (R1-
548 R4) were tested for memory of predation threat using methods adapted from Dalesman et. al.
549 ²⁴. Juvenile F2 snails (spire height 6 ± 0.5 mm) were pre-exposed to either control conditions
550 or tench (*T. tinca*) plus alarm cue, their memory of predation threat was then tested 24 h
551 following pre-exposure by exposing them during behavioural trials to either tench cues alone
552 or control conditions. Tench cue was produced by holding three tench (10 ± 1 cm length) in 4
553 l of artificial pond water for 1 h; tench plus alarm cue was produced by crushing three
554 juvenile snails (spire height 6 ± 0.5 mm) in 4 l of tench cue ²⁴. Control water was artificial
555 pond water alone.

556

557 Pre-exposure was carried out by placing 15 juvenile snails selected at random from the
558 laboratory population into either 2 l of control water or tench plus alarm cue for 24 h. Water
559 was fully aerated throughout, and snails were fed lettuce ad libitum during exposure.
560 Following 24 h exposure to cue or control water, all snails were moved into new aquaria
561 containing 2 l of control water for a further 24 h.

562

563 On the day of the behavioural trial, snails were randomly assigned to individual behavioural
564 arenas 165mm diameter x 60mm depth (A.W.Gregory & Co. Ltd., U.K.) containing a central
565 shelter, a longitudinally sectioned white PVC pipe, 36mm long, 30mm diameter, attached
566 open side down to the centre using non-toxic sealant (Wickes Ultimate Sealant and
567 Adhesive[®], Wickes Building Supplies Ltd., U.K.) in 630 ml of control pond water and
568 allowed to acclimate for 2 h. Following acclimation, either 70 ml of tench water or 70 ml of
569 control water was added to each chamber in a randomised block design, such that an even
570 number of snails were exposed to each of the pre-exposure conditions received either control
571 or tench cue exposure during the behavioural trial. The position of each snail was recorded

572 initially, and then every 5 min for 1 hour. Crawling above the water line is the primary
573 antipredator response of juvenile *L. stagnalis*^{4,24,47}, and so the proportion of time spent
574 crawled out over the 1 h behavioural trial was used to assess antipredator behaviour.

575

576 Memory phenotype for each population was designated based on memory of adults snails in
577 the F1 generation (see results Fig. 1): phenotype A: R1 and R2 (no evidence of memory
578 following operant conditioning memory, but memory following food conditioning); and
579 phenotype B: R3 and R4 (memory formation following operant conditioning but no evidence
580 of food conditioning memory). Proportional data for time spent crawled out of the water were
581 arcsine square-root transformed prior to analysis. Data were analysed using ANOVA with
582 memory phenotype (A vs. B based on adult memory), pre-exposure conditions (control or
583 tench plus alarm cue) and behavioural exposure conditions (control or tench cue) as fixed
584 factors in the analysis, and population nested in phenotype as a random factor. Student-
585 Newman-Keuls tests were used for posthoc pair-wise comparisons where overall significant
586 effects were found.

587

588 **References**

589

590 ¹ Lima, S. L. and Dill, L. M., Behavioural decisions made under the risk of predation: a
591 review and prospectus. *Can. J. Zool.* **68**, 619 (1990).

592 ² Laurila, A., Behavioural responses to predator chemical cues and local variation in
593 antipredator performance in *Rana temporaria* tadpoles. *Oikos* **88**, 159 (2000).

594 ³ Åbjörnsson, K., Hansson, L. A., and Brönmark, C., Responses of prey from habitats
595 with different predator regimes: Local adaptation and heritability. *Ecology* **85**, 1859
596 (2004).

597 ⁴ Dalesman, S., Rundle, S. D., and Cotton, P. A., Predator regime influences innate
598 anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Fresh. Biol.*
599 **52**, 2134 (2007).

600 ⁵ Giles, N. and Huntingford, F. A., Predation risk and inter-population variation in
601 antipredator behavior in the 3-spined stickleback, *Gasterosteus aculeatus* L. *Anim.*
602 *Behav.* **32**, 264 (1984).

603 ⁶ Herczeg, G., Gonda, A., and Merila, J., Predation mediated population divergence in
604 complex behaviour of nine-spined stickleback (*Pungitius pungitius*). *J. Evol. Biol.* **22**,
605 544 (2009).

606 ⁷ Brydges, N. M., Colegrave, N., Heathcote, R. J. P., and Braithwaite, V. A., Habitat
607 stability and predation pressure affect temperament behaviours in populations of
608 three-spined sticklebacks. *J. Anim. Ecol.* **77**, 229 (2008).

609 ⁸ Bell, A. M., Behavioural differences between individuals and two populations of
610 stickleback (*Gasterosteus aculeatus*). *J. Evol. Biol.* **18**, 464 (2005).

611 ⁹ Paterson, R. A. et al., Predator cue studies reveal strong trait-mediated effects in
612 communities despite variation in experimental designs. *Anim. Behav.* **86**, 1301 (2013).

613 ¹⁰ Werner, E. E. and Peacor, S. D., A review of trait-mediated indirect interactions in
614 ecological communities. *Ecology* **84**, 1083 (2003).

615 ¹¹ Peckarsky, B. L. et al., Revisiting the classics: Considering nonconsumptive effects in
616 textbook examples of predator-prey interactions. *Ecology* **89**, 2416 (2008).

617 ¹² Orrock, J. L., Danielson, B. J., and Brinkerhoff, R. J., Rodent foraging is affected by
618 indirect, but not by direct, cues of predation risk. *Behav. Ecol.* **15**, 433 (2004).

619 ¹³ Sura, S. A. and Mahon, H. K., Effects of competition and predation on the feeding
620 rate of the freshwater snail, *Helisoma trivolvis*. *Am. Mid. Nat.* **166**, 358 (2011).

621 ¹⁴ Wada, Y., Iwasaki, K., and Yusa, Y., Changes in algal community structure via
622 density- and trait-mediated indirect interactions in a marine ecosystem. *Ecology* **94**,
623 2567 (2013).

624 ¹⁵ Bell, A. M. and Stamps, J. A., Development of behavioural differences between
625 individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* **68**,
626 1339 (2004).

627 ¹⁶ Dingemanse, N. J. et al., Behavioural syndromes differ predictably between 12
628 populations of three-spined stickleback. *J. Anim. Ecol.* **76**, 1128 (2007).

629 ¹⁷ Bell, A. M. and Sih, A., Exposure to predation generates personality in threespined
630 sticklebacks (*Gasterosteus aculeatus*). *Ecol. Lett.* **10**, 828 (2007).

631 ¹⁸ Smith, B. R. and Blumstein, D. T., Structural consistency of behavioural syndromes:
632 does predator training lead to multi-contextual behavioural change? *Behaviour* **149**,
633 187 (2012).

634 ¹⁹ Wolf, M. and Weissing, F. J., An explanatory framework for adaptive personality
635 differences. *Philos. Trans. R. Soc. B-Biol. Sci.* **365**, 3959 (2010).

636 ²⁰ Reale, D., Reader, S. M., Sol, D., McDougall, P. T., and Dingemanse, N. J.,
637 Integrating animal temperament within ecology and evolution. *Biol. Rev. Camb.*
638 *Philos. Soc.* **82**, 291 (2007).

639 ²¹ Sih, A. and Del Giudice, M., Linking behavioural syndromes and cognition: a
640 behavioural ecology perspective. *Philos. Trans. R. Soc. B-Biol. Sci.* **367**, 2762 (2012).

641 ²² Bell, A. M., Future directions in behavioural syndromes research. *Proc. R. Soc. B-*
642 *Biol. Sci.* **274**, 755 (2007).

643 ²³ Benjamin, P. R., A Systems Analysis of Neural Networks Underlying Gastropod
644 Learning and Memory in *Invertebrate Learning and Memory*, edited by R. Menzel
645 and PR Benjamin (Elsevier Academic Press Inc San Diego, 2013)

646 ²⁴ Dalesman, S., Rundle, S. D., Coleman, R. A., and Cotton, P. A., Cue association and
647 antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim. Behav.* **71**, 789
648 (2006).

649 ²⁵ Dalesman, S., Rundle, S. D., and Lukowiak, K., Microgeographic variability in long-
650 term memory formation in the pond snail, *Lymnaea stagnalis*. *Anim. Behav.* **82**, 311
651 (2011).

652 ²⁶ Rundle, S. D. and Brönmark, C., Inter- and intraspecific trait compensation of defence
653 mechanisms in freshwater snails. *Proc. R. Soc. B-Biol. Sci.* **268**, 1463 (2001).

654 ²⁷ Burns, J. G., Foucaud, J., and Mery, F., Costs of memory: lessons from 'mini' brains.
655 *Proc. R. Soc. B-Biol. Sci.* **278**, 923 (2011).

656 ²⁸ Brydges, N. M., Heathcote, R. J. P., and Braithwaite, V. A., Habitat stability and
657 predation pressure influence learning and memory in populations of three-spined
658 sticklebacks. *Anim. Behav.* **75**, 935 (2008).

659 ²⁹ Kelley, J. L. and Magurran, A. E., Learned predator recognition and antipredator
660 responses in fishes. *Fish. Fish.* **4**, 216 (2003).

661 ³⁰ Lacreuse, A., Schatz, K., Strazzullo, S., King, H. M., and Ready, R., Attentional
662 biases and memory for emotional stimuli in men and male rhesus monkeys. *Anim.*
663 *Cogn.* **16**, 861 (2013).

664 ³¹ Schwabe, L., Wolf, O. T., and Oitzl, M. S., Memory formation under stress: Quantity
665 and quality. *Neurosci. Biobehav. Rev.* **34**, 584 (2010).

666 ³² Shors, T. J., Learning during stressful times. *Learn. Mem.* **11**, 137 (2004).

667 ³³ Dalesman, S., Sunada, H., Teskey, M. L., and Lukowiak, K., Combining stressors that
668 individually impede long-term memory blocks all memory processes. *PLoS One* **8**,
669 e79561 (2013).

670 ³⁴ Marra, V., O'Shea, M., Benjamin, P. R., and Kemenes, I., Susceptibility of memory
671 consolidation during lapses in recall. *Nat. Commun.* **4**, 1578 (2013).

672 ³⁵ Braun, M. H., Lukowiak, K., Karnik, V., and Lukowiak, K., Differences in neuronal
673 activity explain differences in memory forming abilities of different populations of
674 *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* **97**, 173 (2012).

675 ³⁶ Reale, D. et al., Personality and the emergence of the pace-of-life syndrome concept
676 at the population level. *Philos. Trans. R. Soc. B-Biol. Sci.* **365**, 4051 (2010).

677 ³⁷ Dall, S. R. X., Bell, A. M., Bolnick, D. I., and Ratnieks, F. L. W., An evolutionary
678 ecology of individual differences. *Ecol. Lett.* **15**, 1189 (2012).

679 ³⁸ Thornton, A. and Lukas, D., Individual variation in cognitive performance:
680 developmental and evolutionary perspectives. *Philos. Trans. R. Soc. B-Biol. Sci.* **367**,
681 2773 (2012).

682 ³⁹ Garamszegi, L. Z., Marko, G., and Herczeg, G., A meta-analysis of correlated
683 behaviors with implications for behavioral syndromes: relationships between
684 particular behavioral traits. *Behav. Ecol.* **24**, 1068 (2013).

685 ⁴⁰ Lahti, D. C. et al., Relaxed selection in the wild. *Trends Ecol. Evol.* **24**, 487 (2009).

686 ⁴¹ Dall, S. R. X. and Griffith, S. C., An empiricist guide to animal personality variation
687 in ecology and evolution. *Front. Ecol. Evol.* **2**, 3 (2014).

688 ⁴² Koene, J. M., Tales of two snails: sexual selection and sexual conflict in *Lymnaea*
689 *stagnalis* and *Helix aspersa*. *Integr. Comp. Biol.* **46**, 419 (2006).

690 ⁴³ Lukowiak, K., Ringseis, E., Spencer, G., Wildering, W., and Syed, N., Operant
691 conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **199**,
692 683 (1996).

693 ⁴⁴ Sugai, R. et al., Taste discrimination in conditioned taste aversion of the pond snail
694 *Lymnaea stagnalis*. *J. Exp. Biol.* **209**, 826 (2006).

695 ⁴⁵ Alexander, J., Jr., Audesirk, T. E., and Audesirk, G. J., One-trial reward learning in
696 the snail *Lymnea stagnalis*. *J. Neurobiol.* **15**, 67 (1984).

697 ⁴⁶ Fai, A. H. T. and Cornelius, P. L., Approximate F-tests of multiple degree of freedom
698 hypotheses in generalized least squares analyses of unbalanced split-plot experiments.
699 *JSCS* **54**, 363 (1996).

700 ⁴⁷ Dalesman, S. and Rundle, S. D., Cohabitation enhances the avoidance response to
701 heterospecific alarm cues in a freshwater snail. *Anim. Behav.* **79**, 173 (2010).

702

703

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706

707 **Author contributions:**

708 S.D. and A.R. posed research questions and contributed to analytical results; SD analysed
709 numerical results and prepared the figures; S.D. and S.R.X.D. wrote the main text of the
710 manuscript. All authors reviewed the manuscript.

711

712 **Additional information**

713 The authors declare no competing financial interests.

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720 **Figure Legends**

721

722 Figure 1: Demonstrating the relationship between behavioural syndromes (covariance
723 between behavioural traits) and memory syndromes (covariance in plasticity across traits
724 following memory formation) using two behavioural traits, e.g. antipredator behaviour and
725 foraging behaviour (solid line and dotted line) in three individuals (red, blue and black).
726 Arbitrary trait value (behaviour) is shown before and after memory formation. Panels
727 demonstrate potential scenarios in which: a) memory formation is equal across behavioural
728 traits and individual covariance between traits is maintained, demonstrating both a memory
729 syndrome across traits and maintains the behavioural syndrome; b) memory only affects one
730 trait, i.e. no memory syndrome across traits, but whilst it alters the mean difference between
731 traits animals still demonstrate a behavioural syndrome following memory formation; c) all
732 individuals demonstrate memory formation and the degree to which an individual alters its
733 behaviour across traits is equal in within each trait (i.e. all individuals demonstrate a memory
734 syndrome), however the behavioural syndrome is broken down; and d) no covariance among
735 traits before memory formation and not all individuals demonstrate memory formation (i.e.
736 no memory syndrome), however, after memory formation there is now significant covariance
737 among behavioural traits (behavioural syndrome).

738

739 Figure 2: Population level memory response in adult snails across three traits. Populations
740 derived from laboratory reared stock (L1-L2), ditch habitats (D1-D2) or river habitats (R1-
741 R4). Mean change in behaviour (operant conditioning: breathing rate; aversive conditioning:
742 bite rate; appetitive conditioning: bite rate) following non-contingent (white columns) or
743 contingent (grey columns) training. * = significant effect of training (contingent vs. non-

744 contingent) on the response (Student-Newman-Keuls pair-wise comparisons: $P < 0.05$). (N =
745 15-23 per treatment group)

746

747 Figure 3: Antipredator behaviour of juvenile snails from four river populations (R1-R4)
748 following cue association. Mean proportion of time spent crawled above the waterline in
749 response to control pond water (white columns) and tench cue (grey columns) following pre-
750 exposure to pond water alone (control) or tench and alarm cues. (N = 15 per treatment group)

751

752 Figure 4: Correlation for individual memory formation among memory traits in snails derived
753 from three habitat types. Positive value on x- or y-axis shows strength of memory formation
754 (higher positive value = stronger memory in the trait). Trend line is included where Pearson's
755 correlations were significant ($P < 0.05$).

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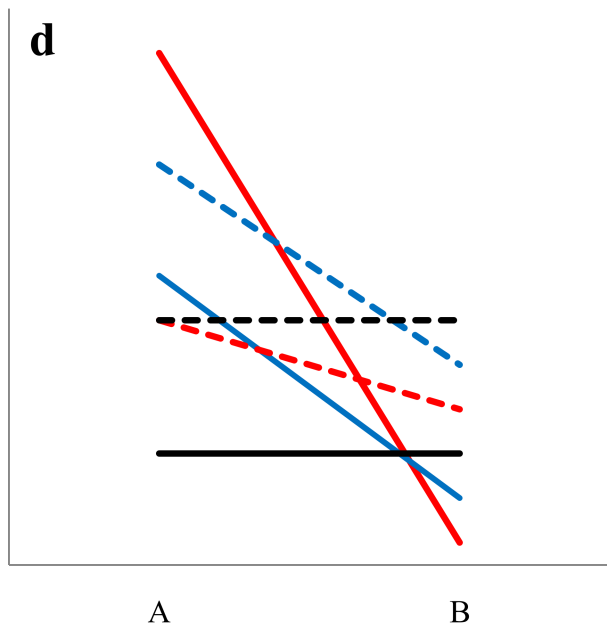
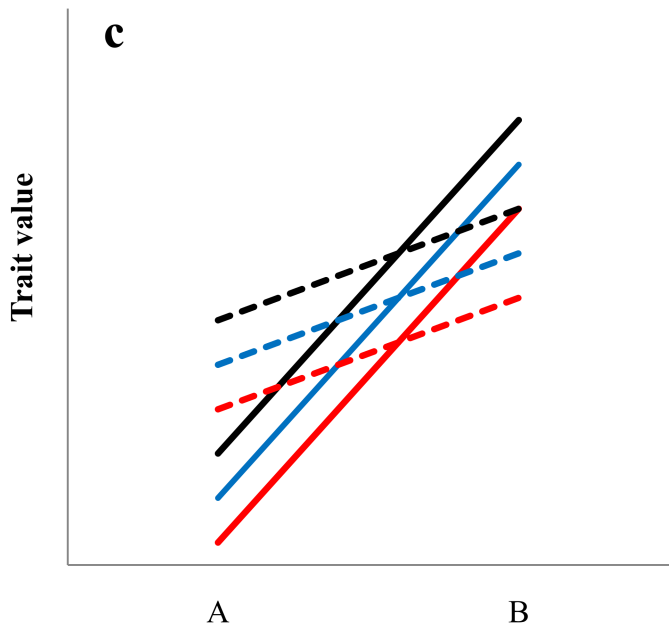
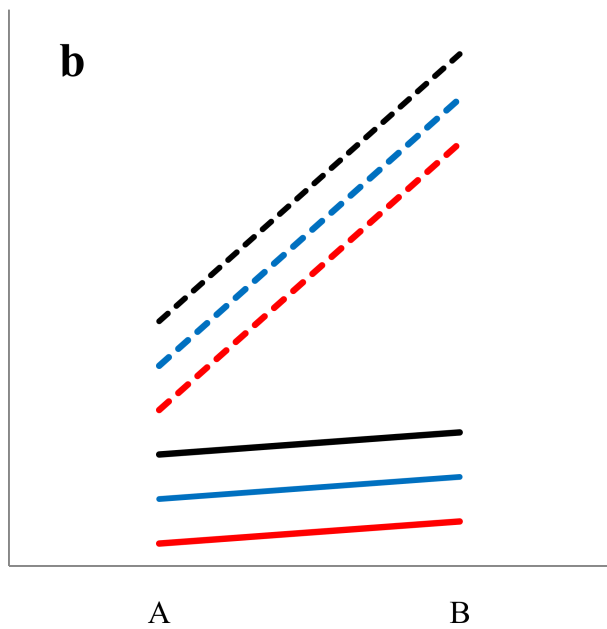
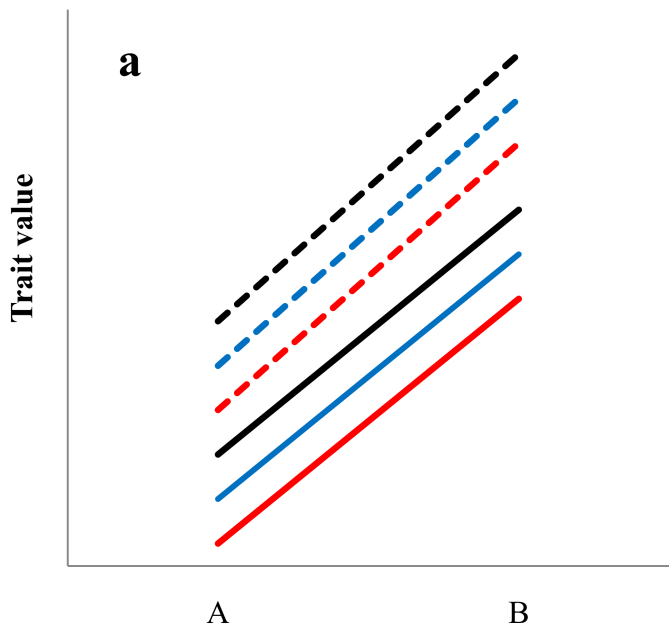
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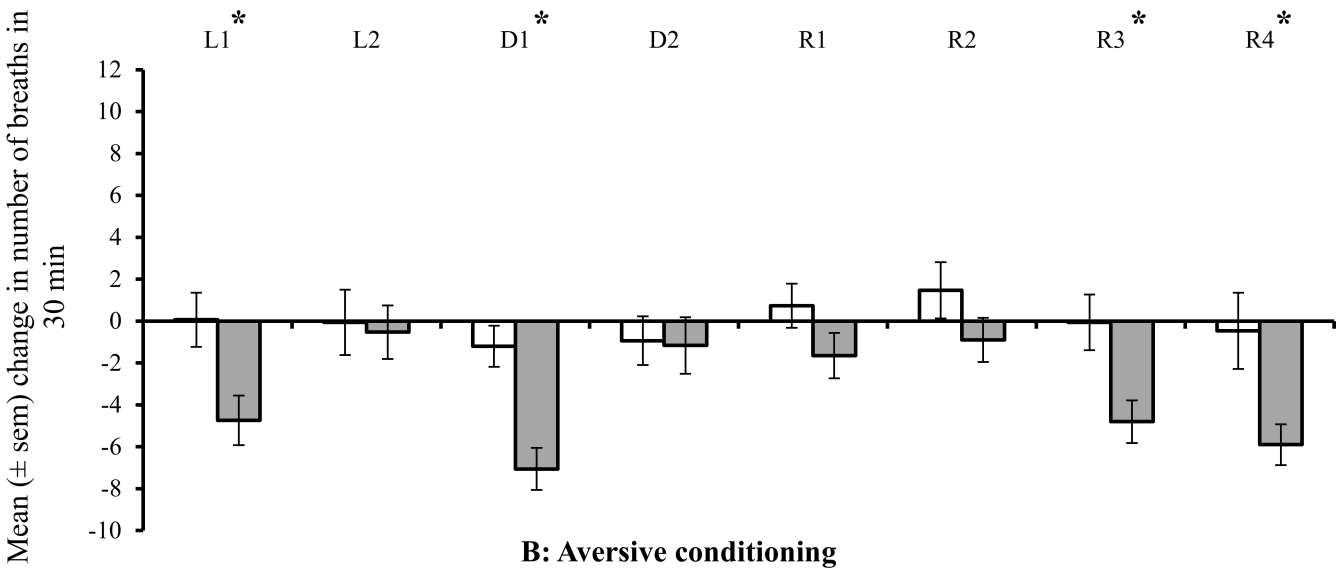
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769 Table 1: Comparison of contingent training (trained) versus non-contingent training (control)
 770 within each individual population for each adult memory trait, showing the mean difference
 771 (trained - control), 95% confidence interval (CI) for the difference and effect size. * =
 772 significant difference found in posthoc pair-wise tests (SNK: $P < 0.05$).
 773

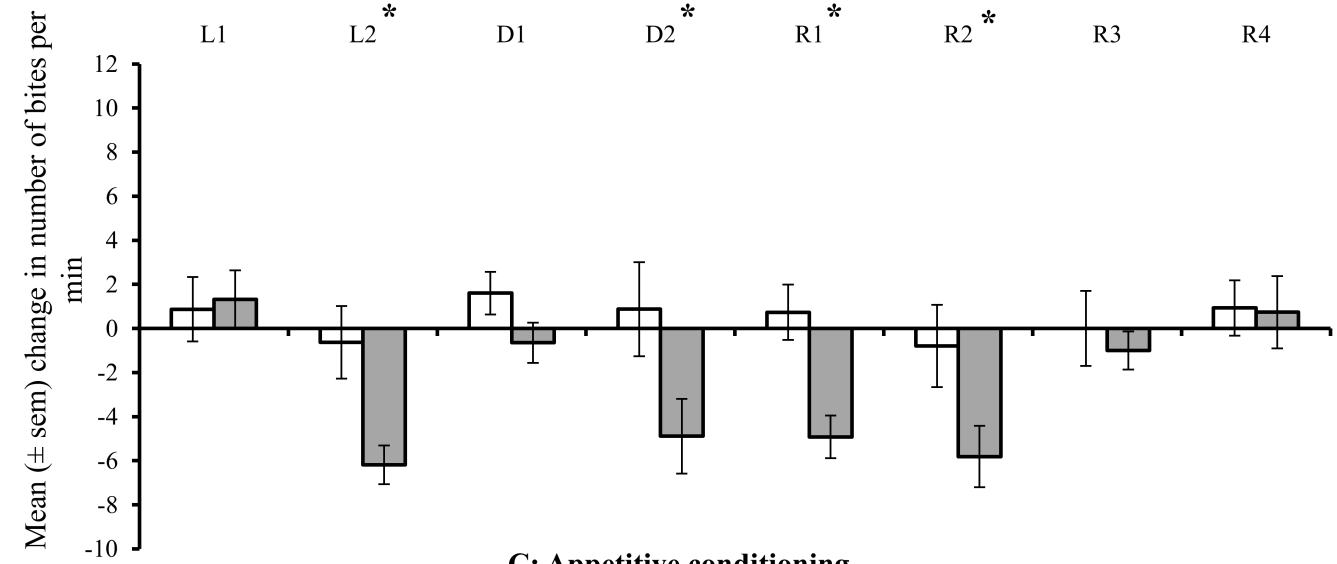
Source	Operant conditioning		Aversive Conditioning		Appetitive conditioning	
	Mean difference (95% CI)	η^2_p	Mean difference (95% CI)	η^2_p	Mean difference (95% CI)	η^2_p
Laboratory 1	-4.806* (-8.457,-1.155)	0.165	0.452 (-3.615,4.518)	0.001	3.557 (-1.275,8.388)	0.058
Laboratory 2	-0.464 (-4.521,3.593)	0.002	-5.563* (-9.370,-1.755)	0.229	6.035* (0.781,11.288)	0.146
Ditch 1	-5.859* (-8.739,-2.979)	0.365	-2.247 (-4.959,0.465)	0.087	0.298 (-2.940,3.563)	0.001
Ditch 2	-0.220 (-3.919,3.479)	<0.001	-5.764* (-11.266,-0.262)	0.125	6.765* (0.741,12.789)	0.145
River 1	-2.380 (-5.469,0.708)	0.076	-5.653* (-8.862,-2.445)	0.251	9.528* (4.612,14.444)	0.283
River 2	-2.361 (-5.794,1.071)	0.058	-5.012* (-9.739,-0.286)	0.140	8.000* (3.525,12.475)	0.300
River 3	-4.737* (-8.069,-1.406)	0.197	-1.000 (-4.656,2.656)	0.009	-0.388 (-3.245,2.470)	0.002
River 4	-5.433* (-9.356,-1.511)	0.194	-0.196 (-4.591,4.198)	<0.001	1.333 (-0.257,2.942)	0.081



A: Operant conditioning



B: Aversive conditioning



C: Appetitive conditioning

