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Genetic differences for behaviour in juveniles from two strains of brown trout suggest an effect of domestication history

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Abstract:

Because captivity constitutes a drastic environmental change, domestication is expected to induce a rapid genetic selection for behavioural traits. In this study, we searched for genetic differences in behaviour among brown trout juveniles from two strains differing for their domestication history, i.e. an almost pure native wild Mediterranean population (W) and an Atlantic domesticated strain (D). In order to assess pure genetic differences among strains, males from the two origins were mated with Mediterranean females to produce two experimental crosses (WW and WD). The swimming activity characteristics of individual WW and WD juveniles were compared before and after the application of a stress (light switched off suddenly, followed by a 5-min period of darkness). For each of the fish observed, mating type origin (WW or WD) was unambiguously reassigned by genotyping. Behavioural responses differed between WD and WW fish. Angular velocity and the time spent immobile were greater for WW fish both before and after the short period of darkness, indicating higher reactivity. Once the light had been turned on again, mean velocity and total distance travelled were higher in WD than in WW fish. WD fish tended to recover levels of swimming activity higher than those before the dark period. This study therefore demonstrates an impact of genetic origin and domestication on swimming activity repertoire (higher reactivity in WW fish), a behavioural trait of particular importance for individual ecological performance. Owing to the contrasted domestication history of the two strains used in the comparison, we assume that the domestication level largely contributes to the behavioural changes observed.

Keywords: Domestication ; Genotyping ; Microsatellite ; Swimming activity ; Restocking

42 **1. Introduction**

43 Domestication is the process by which a population of individuals becomes adapted to
44 humans and to the captive environment, through the recurrence of environmentally induced
45 developmental events in each generation and genetic changes occurring over generations
46 (Price, 1999). These changes involve both deliberate and inadvertent selection, together with
47 random genetic changes known as genetic drift, in which genetic variation is lost due to
48 stochastic changes in allelic frequencies over several generations (Crow and Kimura, 1970).
49 According to Denis (2004), domestication can best be described as a continuum of genetic
50 transformations over generations constituting a general progression from the wild state to a
51 state of genetic adaptation to captivity. For most farmed animals, essentially large terrestrial
52 herbivorous and omnivorous mammals, domestication began 10 500 years ago (Diamond,
53 2002). By contrast, the domestication of 97 % of cultured fish species did not begin until the
54 start of the 20th century (Duarte et al., 2007). Farmed fish are therefore unlikely to differ
55 markedly from the corresponding wild ancestral forms and only a few would be expected to
56 be on the threshold of becoming domesticated (Balon, 2004). Nevertheless, domestication
57 effects can already be observed in some fish, in some cases within as little as one or two
58 generations of their removal from the natural environment (Dunham, 1996a). These
59 modifications concern first morphological and behavioural characters (Bilio, 2007).

60

61 In fish, the coexistence of wild and domesticated stocks of the same species provides an
62 opportunity to investigate the process and dynamics of the domestication process. A number
63 of studies have been conducted to investigate differences between wild and domesticated fish.

64

65 Investigations of behavioural traits in wild and domesticated fish constitute an effective
66 approach to studies of the domestication process, because these traits are likely to be among

67 the first affected (Swain and Riddell, 1990; Ruzzante and Doyle, 1991; Ruzzante and Doyle,
68 1993; Price, 1999), in some cases within a generation of initial domestication (Vandeputte
69 and Prunet, 2002; Bégout Anras and Lagardère, 2004; Huntingford, 2004). Antipredator
70 behaviour, one of the most studied of these traits, has been shown to be very sensitive to
71 artificial rearing (Johnsson and Abrahams, 1991; Berejikian, 1995; Dellefors and Johnsson,
72 1995; Johnson et al., 1996; Einum and Fleming, 1997; Fernö and Järvi, 1998; Johnsson et al.,
73 2001), and swimming performances have been shown to be poorer in domestic stocks
74 (Beamish, 1978). These differences between wild and cultured fish may be accounted for
75 partly by differences in experience in the life histories of individual fish (Huntingford 2004).
76 Farmed fish are faced with conditions that appear to be less challenging than natural habitats,
77 with structurally simpler environments, a ready supply of food and an absence of predators.
78 However, they also have to deal with high animal densities, space restrictions, artificial and
79 uniform food and frequent handling (Fernö et al., 2007). Another way of studying the impact
80 of domestication on fish is to investigate their behavioural responses to novel environments
81 and/or challenging situations. Indeed, the most important effect of domestication on
82 behaviour is a decrease in emotional reactivity or responsiveness to fear-evoking stimuli (*i.e.*
83 environmental change, Price, 2002). Measurements of behavioural reactivity are thus
84 sensitive indicators of the complex of biochemical and physiological changes occurring in
85 response to stress (Schreck et al., 1997). For example, environments such as the light/dark
86 plus maze, based on the tendency of fish to seek dark backgrounds (or to avoid light
87 backgrounds) in unfamiliar environments, can be used to study such behaviour (Serra et al.,
88 1999; Champagne et al., 2010; Gould, 2011; Steenbergen et al., 2011a). Another method
89 involves the sudden exposure of fish to darkness, which has been used as an acute
90 ecologically relevant challenge producing robust changes in locomotor activity in larval
91 zebrafish (Ali et al., 2011).

92 Brown trout, *Salmo trutta* L., is the most common salmonid in Europe and is of considerable
93 socio-economic importance and heritage value because of its intraspecies diversity (Caudron
94 et al., 2009). Two evolutionary lineages have been identified in France on the basis of
95 allozymes, mtDNA and nuclear DNA markers: the Atlantic lineage (AL), which occurs in
96 the rivers of the Atlantic catchment area, and the Mediterranean lineage, which occurs in the
97 rivers of the Mediterranean basin (Guyomard, 1989; Bernatchez et al., 1992; Launey et al.,
98 2003; Cortey et al., 2004).
99
100 An important issue in studies of the domestication of brown trout (and of many other fish) is
101 the replacement of many natural populations by domesticated stocks through intensive
102 stocking or escapees. For example, most of the native Mediterranean lineage in France has
103 been replaced by domesticated stocks belonging to the Atlantic lineage (Krieg and
104 Guyomard, 1985; Barbat-Leterrier et al., 1989; Guyomard, 1989; Beaudou et al., 1994;
105 Largiadèr et al., 1996; Poteaux et al., 1998; Berrebi et al., 2000; Launey et al., 2003) and this
106 is also probably true for most of the native Atlantic populations. Thus, in most comparisons
107 of domestic and wild stocks, it is not possible to exclude the possibility that the two
108 genotypes investigated originate from domesticated stocks and differ only in one of them
109 having being released into the wild for a short period of time. This may bias the experiments
110 carried out. Fortunately, remnant unstocked or almost unstocked native Mediterranean
111 populations of brown trout have been identified (Caudron et al., 2011) and provide a suitable
112 genetic source for studying the domestication process in this species. Furthermore, many
113 studies of phenotypic differences between wild and domesticated stocks of fish species do not
114 include the necessary fertilisation designs for separation of the effects of genetic and
115 environmental factors. It is also important to take into account maternal effects, which
116 contribute to phenotypic complexity for many traits, and may complicate attempts at

117 phenotypic analysis (Bernardo, 1996). Maternal effects in fish are due to the energy reserves
118 within the yolk (reviewed by Love 1980), which depend on the reserves the female is able to
119 commit to oogenesis and oocyte maturation (Kerrigan, 1997). Many studies comparing wild
120 fish with those from hatcheries fail to recognise the influence of maternal effects on egg
121 quality, with repercussions for the survival and behaviour of the offspring (Huntingford
122 2004). Gene-environment interactions can also be limited by placing all the fish in the same
123 environment (Dupont-Nivet et al., 2008) or by using replicates to assess tank effects.
124
125 There have been few studies of the domestication process based on differences in behaviour
126 between wild and domesticated fish and an experimental design allowing the identification of
127 lineages and the control of environmental sources of variation. However, a recent study on
128 zebrafish, *Danio rerio*, confirmed the potential of such studies for the QTL mapping of
129 behavioural traits and for dissecting the consequences of selection during domestication
130 (Wright et al., 2006).
131
132 In this study, we assessed the genetic variation underlying differences in behavioural traits
133 between genetically differentiated populations of juvenile brown trout. This study is
134 innovative in its use of genotypes corresponding to well characterized stages of
135 domestication. In particular, we compared juveniles produced by mating females from a near-
136 pure Mediterranean population with males from the same population (WW) or with males
137 from an Atlantic domesticated strain (WD). The swimming activity characteristics of
138 individual WW and WD fish were compared before and after a short period of darkness, and
139 each of the fish studied was genotyped to check its origin.
140
141 **2. Materials and methods**

142 *2.1. Experimental animals and housing conditions*

143 In December 2006, an experimental captive population was founded with 11 females and 16
144 males (effective population size = 22.8) caught in the Fier, a tributary of the Rhône inhabited
145 by an almost pure Mediterranean population (Guyomard and Caudron, unpublished data). We
146 checked that the parents had a Mediterranean genetic profile by genotyping with Str541 and
147 Str591, two microsatellites that have proved useful for differentiating between Atlantic stock
148 strains and Mediterranean populations (see Estoup et al., 2000 and Caudron et al., 2006, for
149 details on the method). Fish were fertilised, hatched and reared to sexual maturity at La Puya
150 fish farm (Annecy, Haute-Savoie, France).

151 In 2009, an experiment with a semi-factorial design was carried out with mature males and
152 females of the Fier captive population and males from the INRA strain (INRA experimental
153 fish-farm, Le Drennec, Finistère, France). We pooled the eggs from all females and divided
154 them up into the same number of groups as individual males used. Each group of eggs was
155 fertilized by an individual male. Fifteen minutes after fertilisation, all the eggs fertilised by
156 males of the same origin were pooled and each pool was divided into two replicates (R1 and
157 R2, Table 1). Eggs were incubated at temperatures of 6 to 10 °C, in small stainless steel
158 incubators, until the first feed, after which they were transferred to small concrete tanks until
159 the end of the experiment. The fry were initially fed with zooplankton, which was gradually
160 replaced with dry pellets. The fish were reared according to standard trout farming practice.
161 The behavioural experiment was started 280 days after fertilisation of the eggs.

162

163 *2.2. Experimental set-up*

164 Observations were made in a specially modified dark room dedicated to this experiment at La
165 Puya farm. The apparatus consisted of 12 transparent rectangular arenas (24.5 x 15 x 13.5 cm,
166 Aquabox® 3, AQUA SCHWARZ GmbH, Göttingen, Germany), each filled with 1.5 L of

167 water (the water used had characteristics identical to those of the water in the original tanks)
168 giving a water depth of 7 cm. The temperature and oxygen level were checked before and
169 after the observations. Temperature was 11.6 ± 0.6°C before and 12.8 ± 0.3°C after the
170 observations, whereas oxygen concentration was 7.4 ± 0.2 mg L^{-1} before and 7.2 ± 0.2 mg L^{-1}
171 after the observations. Arenas were numbered from 1 to 12 and placed on a waterproof
172 infrared casing (1 x 1 m, Noldus, The Netherlands) to assist the recording of videos in total
173 darkness. A frame made of opaque white cardboard was placed against each arena, preventing
174 visual interaction between fish. A camera (Imaging Source DMK 21AUO4) with a frame rate
175 of 30 Hz and a resolution of 640 x 480 pixels was positioned 87 cm above the infrared casing.
176 Three 120 W spot lights placed around the infrared casing were used to light the arenas
177 indirectly. The light intensity measured at the surface of the water in each arena was 150 Lux.
178 The spot lights were connected to a programmable timer, making it possible to turn the light
179 on or off automatically at specific times.
180
181 *2.3. Experimental protocol*
182 Six WW (3 R1 and 3 R2) and six WD (3 R1 and 3 R2) fish were gently collected from the
183 tanks with a dip net and individually transferred into arenas in random order. Care was taken
184 to select juveniles of the two origins that were visibly similar in size. The fish were allowed to
185 acclimatise to the arenas for 5 minutes before the start of video recording. The arenas were
186 filmed for 65 min: 30 min in the light, 5 min of darkness and then another 30 minutes in the
187 light. The light was turned off abruptly, resulting in sudden darkness, and then switched back
188 on, with the programmable timer. At the conclusion of each recording period, individuals
189 were placed in 12 numbered 1-litre beakers. They were anaesthetised with 2-phenoxyethanol
190 (0.3 ml L^{-1}) for measurement (total body length, BL, to the nearest mm) and weighing (BW,

191 to the nearest mg). Thereafter, the anaesthetised fish were sacrificed by severance of the
192 spinal cord.
193
194 Fin clips were taken from each of the juveniles observed and stored in 95% ethanol for
195 genotyping. DNA extraction and genotyping with Str541 and Str591 microsatellite markers
196 was performed as described by Caudron et al. (2006). We also took fin clips from each of the
197 males and females used to generate the juveniles studied.
198 This procedure was carried out eight times in all (*i.e.* 48 WW and 48 WD fish were observed
199 over two days).
200 The water in each arena was replaced, in its entirety, after each observation session.
201
202 *2.4. Video analyses and behavioural variables*
203 The video recordings were analyzed with EthoVision XT software (Noldus, The
204 Netherlands), which was used to track the swimming fish in each arena.
205 Each video recording was analysed in three sequences:
206 - L1: 30 minutes in the light.
207 - D: 5 minutes in the dark.
208 - L2: 30 minutes in the light.
209 For each sequence, the following variables of interest were used to characterise the swimming
210 behaviour of the fish:
211 • Distance moved: the distance travelled by the centre point of the subject between two
212 consecutive X–Y coordinates acquired (Dtot in mm),
213

214 • Mean velocity: the distance moved by the centre point of the individual fish per unit time

215 between two consecutive X–Y coordinates acquired expressed in body lengths per second

216 (Vel in BL s^{-1}),

217 • Time immobile: the total duration the fish displayed no movement (NotMov in second) with

218 a threshold of 1.7 cm s^{-1},

219 • The fish absolute angular velocity expressed in degrees per second (Vang in $\circ$ s^{-1}) was

220 calculated by the software as followed: $Vang_n = RTA_n/t_n - t_{n-1}$, where RTA_n is the relative turn

221 angle for sample n, and $t_n - t_{n-1}$ is the time difference between the current and previous

222 sample. Here, the rate of change in direction is unsigned. The turn angle is calculated as the

223 difference between two subsequent values for heading direction. This variable was an

224 indicator of the amount of turning per unit time and quantified the swimming path

225 complexity.

226

227 *2.5. Statistical analysis*

228 All variables were compared by parametric analysis of variance (ANOVA), after checking

229 that the normality and homoscedasticity requirements were met (Dagnélie, 1975). All

230 statistical analyses were conducted with Statistica 8 (Statsoft, USA) and, for all tests, $p < 0.05$

231 was considered significant.

232 BL and BW were compared in a one-way analysis of variance, with Replicate (R1 and R2) as

233 the fixed factor, and then in a one-way analysis of variance with Origin (WW and WD) as the

234 fixed factor.

235 The regression between each swimming variable and either BL or BW was analysed, to check

236 for allometric relationships in fish of the two origins during each period (L1, D and L2).

237 The effects of fish size were resolved, by comparing all variables relating to swimming

238 activity in a repeated measures analysis of covariance after checking for parallelism

239 (Dagnélie, 1975). Fish origin (WW and WD) was taken as a between-subject factor, Sequence
240 (L1, D and L2) as a within-subject factor and body size or body weight as a covariate.
241 Significant ANCOVA results were followed by a post-hoc multiple comparison test
242 (Newman–Keuls).
243
244 **3. Results**
245 *3.1. Biometry*
246
247 No significant differences in weight or size were found between replicates of the same origin
248 and data were therefore pooled for further analyses. However, WD fish were significantly
249 heavier and larger than WW fish (1.54 ± 0.52 and 1.19 ± 0.55 g, $F_{(1,94)}$ = 9.8, P = 0.002; 5.49
250 ± 0.63 and 5.08 ± 0.70 cm, $F_{(1,94)}$ = 8.8, P = 0.004, respectively).
251
252 *3.2. Genotyping*
253
254 The INRA males had only Atlantic alleles (Str541*132, Str591*150 and *152), whereas the
255 Fier males and females had only Mediterranean alleles (Str541*136, Str591*164, *166 and
256 *170), with the exception of a single female with one Atlantic allele (Str541*132). The
257 juveniles were therefore unambiguously reassigned to the WW or WD group by genotyping
258 for these two markers. All the genotyping results were consistent with the expected genotypes
259 of the fish analyzed.
260
261 *3.3. Swimming activity*
262
263 In most cases, correlations between swimming variables and BL or BW were weak and not
264 significant. However, two significant correlations were identified in WW fish during L1: a
265 correlation between Dtot and BL or BW (r^2 = 0.22, P = 0.001; r^2 = 0.18, P = 0.003
266 respectively) and a correlation between NotMov and BL or BW (r^2 = 0.22, P = 0.001 in both
267 cases). Dtot tended to decrease with increasing BL or BW and NotMov tended to increase

268 with increasing BL. There was also one significant correlation in WD fish, during L2,
269 between Vang and BL ($r^2 = 0.1$, $P = 0.03$). Vang tended to increase with increasing BL.
270
271 The hypothesis of parallelism was verified for each of the variables studied. This involved
272 that the slope of the regression line for swimming traits against fish size or fish weight did not
273 differ significantly between fish of different origins. Similar results were obtained whether
274 BL or BW was used as a covariate, so we present only the results obtained with BL below.
275
276 The difference in Vang between WW and WD was significant ($F_{(3,85)} = 10.9$, $P < 0.001$).
277 Further, Newman-Keuls tests showed that Vang was higher in WW fish than in WD fish
278 during L1 (but not significant: $P = 0.06$, Figure 1) and L2 ($P < 0.001$) but not during D ($P =$
279 0.55), when Vang decreased in fish of both origins. The significant difference observed
280 during L2 results from the combination of an increase in Vang during this period in WW fish
281 and a tendency for Vang to decrease in WD over the same period (Figure 1). If we consider
282 measurements taken every minute (Figure 2), Vang was always higher in WW fish than in
283 WD fish during L1 and L2. During the dark phase (D), the two groups of fish reacted
284 similarly, with an immediate decrease in Vang, which lasted from the beginning to the end of
285 D; Vang continued to decrease during the first minute of L2 and then increased thereafter.
286
287 Dtot differed significantly between WW and WD fish ($F_{(3,85)} = 11.4$, $P < 0.001$). Further
288 Newman-Keuls tests showed that the difference was significant only during L2 ($P < 0.001$).
289 In WD fish, Dtot increased more strongly in L2 than in L1, whereas similar values were
290 obtained for these two periods for WW fish (Figure 1).
291 Similarly, Vel differed significantly between WW and WD fish ($F_{(3,85)} = 11.9$, $P < 0.001$,
292 Figure 1). As for Dtot, the difference was significant only during L2, when Vel increased in

293 WD fish (P < 0.001, Figure 1). If we consider measurements taken every minute (Figure 2),
294 the two groups of fish reacted similarly, with an immediate decrease in Vel at the beginning
295 of D, followed by an increase until the end of D; Vel then decreased again at the beginning of
296 L2. However, during L2, Vel fell to values lower than those for L1 in WW fish, whereas it
297 remained higher than during L1 in WD fish.

298

299 Finally, NotMov differed significantly between WW and WD fish ($F_{(3,85)} = 13.9$, $P < 0.001$).
300 This difference was significant during L1 and L2 (P = 0.04 and P < 0.001, respectively,
301 Figure 1), but not during D (P = 0.48). WD fish were immobile for 50.4 ± 3.8% of the time
302 during L1 and for 43.1 ± 3.1 of the time during L2, whereas WW fish were immobile for 60.5
303 ± 3.3% of the time during L1 and 61.6 ± 2.7 of the time during L2.

304

305 **4. Discussion**

306 In this study, we compared the swimming behaviour (in a challenging situation) of two
307 groups of brown trout juveniles, the origin of which was clearly identified by genotyping.
308 These two groups differed in terms of the domestication history of the male parents. The
309 Atlantic INRA strain has a long history of domestication that largely predates the foundation
310 of the strain (more than 10 generations), which itself originates from a mixture of
311 domesticated stocks, whereas the Mediterranean La Puya strain has been in captivity for only
312 one generation. Since the two groups differed only in terms of the genetic background of the
313 male parents, the observed significant differences should provide an estimate of strictly
314 paternal genetic effects (both additive and dominance effects) for the behavioural traits
315 investigated. These genetic differences result from two sources of variation: 1) the two
316 populations used initially originated from two different well differentiated lineages and 2)
317 they also differ in terms of their domestication history. With the experimental design used, it

318 was not possible to dissect the relative contributions of the two factors, but the nature of the
319 differences between the two genotypes strongly support the hypothesis, discussed below, that
320 these differences partly reflect an impact of the domestication process on behavioural traits.
321
322 Growth performance is known to increase rapidly with the stage of domestication in fish
323 (Gjedrem, 1979). The results obtained for the WW and WD groups were consistent with this
324 observation. A substantial difference (~ 20 %) was already evident 180 days after fertilisation
325 (see table 1) and was maintained until sampling for the behavioural assay. Differences in
326 growth and survival rate were repeatedly observed between French wild and domesticated
327 brown trout populations at the advantage of the latter group (Guyomard, 1989, 1997). These
328 studies also showed that reciprocal hybrids were intermediate between the two parental
329 strains. The visual selection of juveniles was not sufficient to ensure that the WW and WD
330 groups were homogeneous in terms of size and weight. However, we found that swimming
331 activity was only weakly correlated with fish size or weight.
332
333 Significant differences were recorded between WD and WW fish for several behavioural
334 traits. Angular velocity and time spent immobile were greater in WW than in WD fish, both
335 before and after the light was turned off. Mean velocity and total distance travelled were
336 higher in WD fish, particularly during the 30-minute period after the light was switched back
337 on. These results reflect a lower swimming complexity in WD fish and a higher vigilance
338 threshold (Bégout and Lagardère 2004) or stronger fear response (Arai et al., 2007) in WW
339 fish, regardless of the challenge period (initial introduction into the test tank, or after the
340 period of darkness). These findings are consistent with previous studies comparing wild-
341 caught and domesticated sea bass juveniles under similar conditions (Benhaïm et al., 2012b)
342 and probably reflect an antipredator response that has already been shown to be eroded in
343 several farmed species, including Atlantic salmon, *Salmo salar* (Einum and Fleming 1997),

344 rainbow trout, *Oncorhynchus mykiss* (Johnsson and Abrahams 1991), brown trout, (Fernö and
345 Järvi 1998), and Atlantic cod, *Gadus morhua* (Nordeide and Svasand, 1990).
346 The observed differences are mostly accounted for by the inheritance of differentially selected
347 behavioural characters over several generations (Huntingford 2004). Indeed, during the
348 domestication process, the frequency distributions of various behavioural traits change, partly
349 because the fish selected from the source populations generally thrive in the predator-free and
350 food-rich hatcheries (Salvanes and Braithwaite, 2006).
351
352 The behavioural differences between WW and WD fish, which are likely to reflect only half
353 the genetic differences between the two strains, were quantitative rather than qualitative in
354 nature (see Price 2002), because both groups of fish reacted similarly to the two stimuli (light
355 off, light on). Indeed, the sudden onset of darkness triggered a decrease in mean velocity
356 rapidly followed by an increase, indicating avoidance of a potentially dangerous area and risk
357 assessment (Millot et al., 2009). A similar decrease in locomotor activity following a sudden
358 onset of darkness has been described in zebrafish, in which sudden changes in illumination
359 can temporarily override the activity levels set by the circadian clock (Steenbergen et al.
360 2011b). However, recovery differed between the two groups of fish: 30 minutes after the
361 second stimulation, WD fish had recovered higher levels of swimming activity, whereas the
362 level of swimming activity remained low in WW fish. Thus, WW fish remained fearful, as
363 reported for sea bass by Millot et al. (2009). Similarly, sudden exposure to darkness triggered
364 a decrease in angular velocity in both groups of fish. Thirty minutes after exposure to
365 darkness, only the WW fish displayed an increase in angular velocity, although values
366 remained lower than those before the first stimulation in both groups of fish.
367

368 Clearly, these differences have implications for the management of aquaculture systems and
369 for the success of restocking programmes (Huntingford 2004). Indeed, it is increasingly
370 recognized that restocking programmes often fail because of behavioural deficits in the
371 domesticated fish released (Olla et al., 1994; Brown and Laland, 2001; Brown, 2002;
372 Huntingford, 2004), resulting in lower fitness in natural environments (Einum and Fleming,
373 1997; Fleming and Einum, 1997; Garant et al., 2003; McGinnity et al., 2003; Metcalfe et al.,
374 2003). This is of particular importance in salmonids, given the long history of restocking and
375 farming of this taxonomic group (Salvanes and Braithwaite 2006) and one should indeed
376 favour fear related behavioural traits when doing restocking

377

378 This study constitutes one of the first attempts to demonstrate the existence of genetic
379 difference for behavioural traits between populations with different domestication profiles.
380 The behavioural traits identified here as relevant indicators of the domestication process in
381 brown trout are probably also applicable to other fish species. The experimental set-up
382 described here should be improved in several ways in future studies. One major improvement
383 would involve the use of full 2x2 factorial designs comparing F2-F3 captive Mediterranean
384 broodstocks with their wild source populations. This will make it possible to avoid bias due to
385 geographic variation and to assess domestication effects only. Moreover, we would estimate
386 the total differences between the two strains rather than the paternal effect only
387 (corresponding to half the expected additive genetic variability between strains).
388 The experiment carried out here focused on the behavioural response to a physical stressor,
389 but could be extended to other relevant traits such as early feeding behaviour, predator
390 avoidance, physiological response to handling or crowding. It would be of particular interest
391 in the perspective of understanding the genetic changes occurring in the course of the
392 domestication process. Finally, we suggest that particular attention should be paid to

393 determining how gene x environment interactions affect behavioural and physiological
394 adaptation in fish (Johnsson et al. 2001).
395
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589 **Tables**
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592 **Table 1.** Half-factorial design features: numbers of male and female brown trout used.

	13 Captive Fier ♀ x 9 captive Fier ♂		13 captive Fier ♀ x 10 INRA ♂	
	$[WxW]_1$	$[WxW]_2$	$[WxD]_1$	$[WxD]_2$
Fertilisation	500	500	350	350
Day 180	358 (0.062)	336 (0.155)	302 (0.199)	204 (0.091)
Day 220	150	150	150	150

593 Replicate sizes at fertilisation; from day 180 to day 220, replicate sizes were adjusted to 150.
594 In brackets: mean individual weight (in grammes) on day 180. W: wild captive Fier
595 (Mediterranean lineage); D: the domesticated INRA synthetic strain.
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600 **Figure captions**
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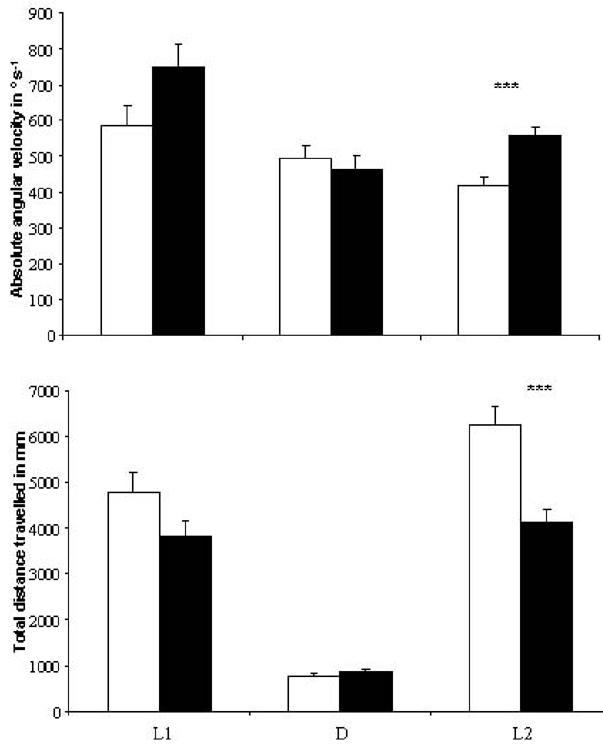
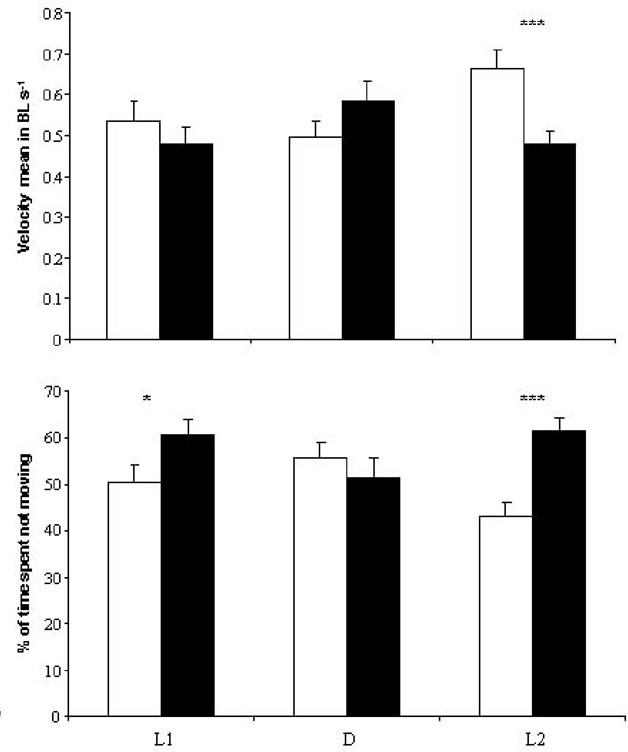
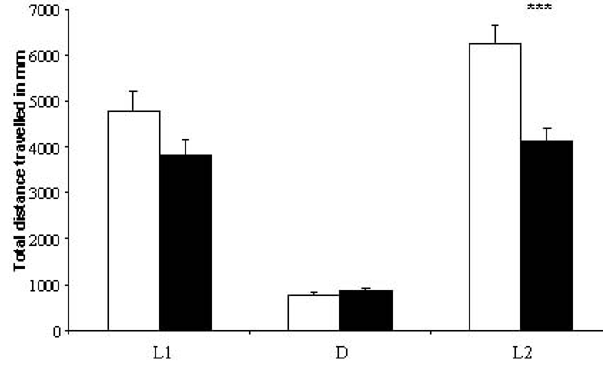
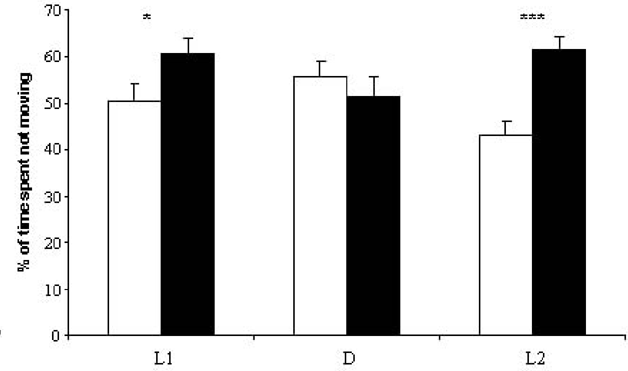
603 **Figure 1.** Mean ± SEM. Swimming variables in WD and WW fish during three sequences:
604 L1: 30 min in normal light conditions; D: 5 min in dark conditions; L2: 30 min in normal
605 light conditions. *P*<0.05, ***: *P*<0.001.
606
607 **Figure 2.** Mean ± SEM. Swimming variables in WD and WW fish during three sequences:
608 L1: 30 min in normal light conditions; D: 5 min in dark conditions; L2: 30 min in normal
609 light conditions. Data are given for each minute.









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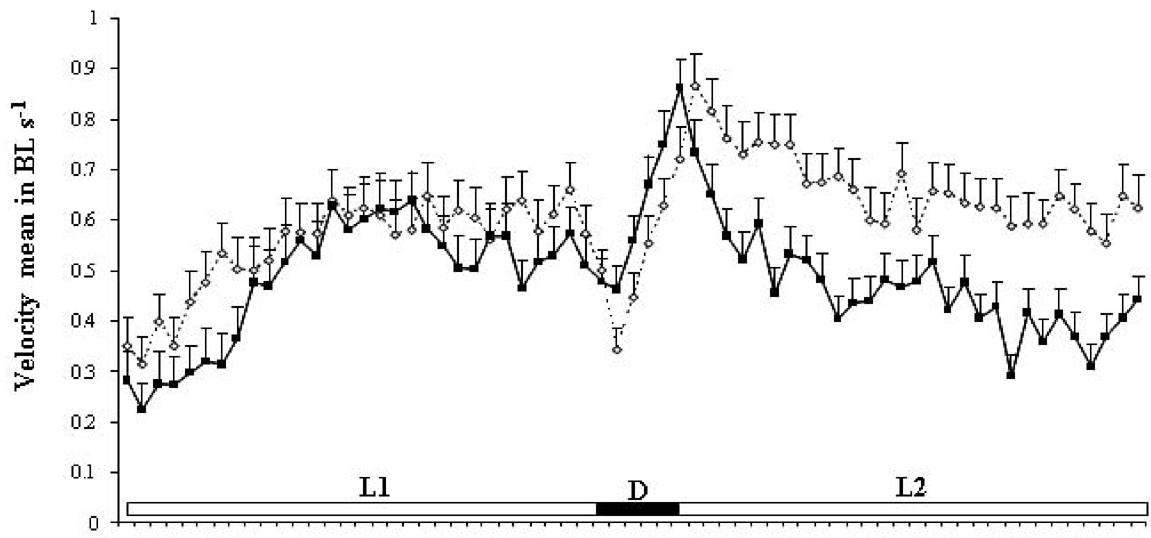

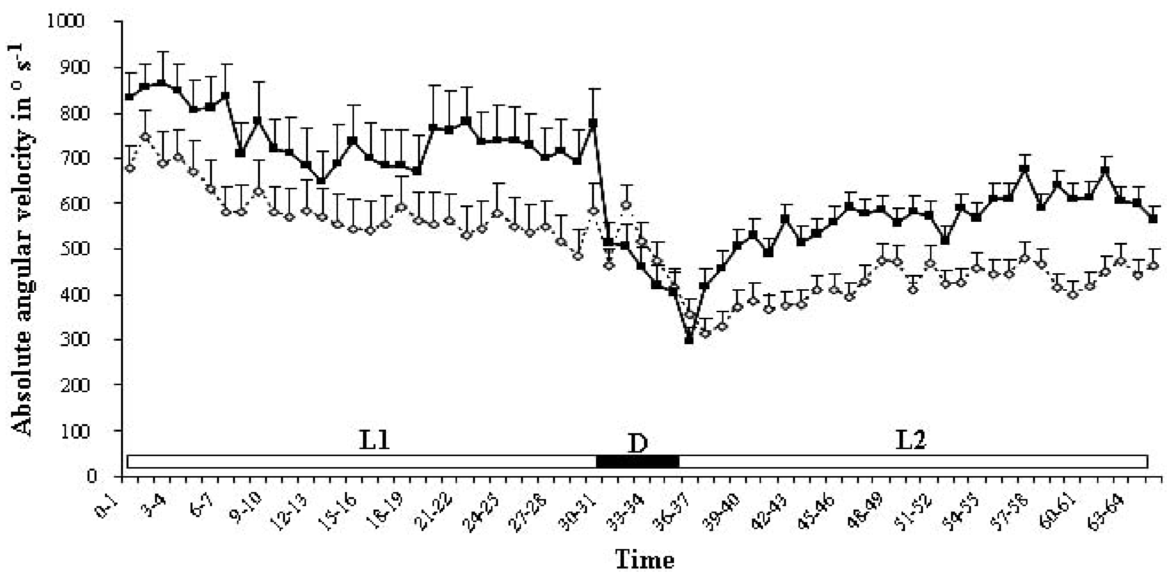

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