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3

4 **Title:**

5 Additive vs non-additive genetic components in lethal cadmium tolerance of *Gammarus*
6 (Crustacea): novel light on the assessment of the potential for adaptation to contamination.

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19

19 **Abstract**

20 Questioning the likelihood that populations adapt to contamination is critical for ecotoxicological
21 risk assessment. The appraisal of genetic variance in chemical sensitivities within populations is
22 currently used to evaluate *a priori* this evolutionary potential. Nevertheless, conclusions from this
23 approach are questionable since non-additive genetic components in chemical tolerance could
24 limit the response of such complex phenotypic traits to selection. Coupling quantitative genetics
25 with ecotoxicology, this study illustrates how the comparison between cadmium sensitivities
26 among *Gammarus* siblings enabled discrimination between genetic variance components in
27 chemical tolerance. The results revealed that, whereas genetically determined differences in lethal
28 tolerance exist within the studied population, such differences were not significantly heritable
29 since genetic variance mainly relied on non-additive components. Therefore the potential for
30 genetic adaptation to acute Cd stress appeared to be weak. These outcomes are discussed in
31 regard to previous findings for asexual daphnids, which suggest a strong potency of genetic
32 adaptation to environmental contamination, but which contrast with compiled field observations
33 where adaptation is not the rule. Hereafter, we formulate the reconciling hypothesis of a
34 widespread weakness of additive components in tolerance to contaminants, which needs to be
35 further tested to gain insight into the question of the likelihood of adaptation to contamination.

36
37 **Keywords:** adaptation, evolutionary ecotoxicology, *Gammarus*, genetic components,
38 quantitative genetics.

39

39 **1. Introduction**

40 Questioning whether populations can adapt to contamination is critical for predictive
41 ecological risk assessment since it is based on the extrapolation of laboratory bioassays to natural
42 context (Medina *et al.* 2007; Millward and Klerks 2002). Although reported in numerous aquatic
43 species for different toxicants, some authors conclude from the examination of published
44 impacted field population surveys that genetic adaptation globally appears to be infrequent,
45 notably in aquatic animal populations (Klerks 2002; Millward and Klerks 2002; Woods and
46 Hoffmann 2000). Theoretical explanations are proposed: first, the local scale of water ecosystem
47 contamination compared to the large home ranges of aquatic animal populations could lead to the
48 impossibility of genetic isolation due to important gene flows between pristine and contaminated
49 locations (see Groenendijk *et al.* 2002 for a case study); second, possible fitness costs of
50 adaptation could counterbalance the selective advantage of increased resistance to contamination;
51 third, genetically determined differences in resistance to toxic compounds could be insufficient to
52 permit Darwinian selection. Concomitantly, these last two open questions – the existence of
53 fitness costs and the lack of genetic variability – are central hypotheses tested by predictive
54 approaches tackling the question of the evolution of genetic resistance to contaminants.

55 This predictive assessment of adaptive abilities could be performed via multi-generation
56 artificial selection experiments (*e.g.* Vogt *et al.* 2007; Ward and Robinson 2005; Xie and Klerks
57 2003). Nevertheless, these protocols imply substantial experimental efforts and they are feasible
58 only with short life-cycle species. Moreover, the interference with evolutionary processes
59 induced by laboratory rearing conditions could complicate the interpretation of outcomes (Athrey
60 *et al.* 2007; Barata *et al.* 2000; Medina *et al.* 2007; Nowak *et al.* 2008; Reznick and Ghalambor
61 2005; Ward and Robinson 2005). A second approach adopts a more predictive viewpoint; it
62 focuses on the identification of genetically determined differences in tolerance to a specific

63 compound. Indeed the existence of such differences is a prerequisite for Darwinian selection.
64 This genetic variation can first be assessed by comparing the sensitivities of genetically
65 homogenous strains (highly inbred lineages or clones); daphnids as one of the foundation taxons
66 for aquatic ecotoxicology are extensively employed in this context (Baird *et al.* 1991; Barata *et*
67 *al.* 1998, 2000, 2002a, 2002b; Lopes *et al.* 2004, 2005; Soares *et al.* 1992). It is noteworthy that
68 this was done originally for regulatory standardisation purposes rather than to address
69 evolutionary issues. An alternative methodology consists in comparing the sensitivities of
70 relatives (*e.g.* parents *vs* offspring or between siblings). Surprisingly this quantitative genetics
71 approach is very little exploited in aquatic ecotoxicology (see Klerks and Moreau 2001 for one
72 example). Yet it is achievable with long life-cycle species (sib analysis) and it does not require
73 lab-specific lineages. In this framework, the potential for adaptation is quantified through the
74 concept of heritability (Falconer and Mackay 1996), which embraces two components: first the
75 amount of genetic variability and second the potential to transmit the differences sustaining this
76 variability. Testing these two prerequisites of Darwinian selection (variation and heredity) is in
77 fact imperative since what is genetically determined is not necessarily heritable due to the
78 possibility of non-additive genetic interactions, *i.e.* dominance and combined epistatic effects
79 (Falconer and Mackay 1996). These mechanisms have been reported in the inheritance of
80 susceptibility to toxicants through polygenic epistatic systems (Woods and Hoffmann 2000) or
81 dominance effects (Labbé *et al.* 2007). Yet these two components (variation and heredity) are not
82 assessed in the former clone approach: in such specific cases the existence of genetically
83 determined variations is sufficient to guarantee the possibility of selection since transmissibility
84 from parents to offspring is obvious. Nevertheless the question of transmissibility has never been
85 elucidated in field populations for aquatic species with sexual reproduction.

86 We wished to test the feasibility of such quantitative genetics protocols for
87 ecotoxicological concerns with a case study. We chose the freshwater amphipod *Gammarus*
88 *fossarum* because we control the reproductive cycle of this long life-cycle species in the
89 laboratory: this offered the opportunity to produce individuals with known pedigrees for
90 quantitative genetics protocols. Moreover, *Gammarus* being a crustacean, we could refer to
91 studies conducted with daphnids (Baird *et al.* 1991; Barata, *et al.* 1998, 2000, 2002a, 2002b;
92 Lopes *et al.* 2004, 2005; Soares *et al.* 1992; Ward and Robinson 2005). Then we could compare
93 outcomes from procedures based on clonal and sexual modes of reproduction. Given that
94 important genetically determined differences in tolerance to Cd have been demonstrated in
95 *Daphnia* (Baird *et al.* 1991; Barata *et al.* 1998, 2000, 2002a, 2002b) and adaptation to lethal Cd
96 exposure has been studied for this crustacean genus (Ward and Robinson 2005), this metal was
97 our model contaminant. Therefore, adopting a quantitative genetics approach, our study
98 illustrates how the comparison of sensitivities among siblings can yield insight into the potential
99 of a *Gammarus* population to adapt genetically to Cd exposure. For this, neonates were produced
100 from successive breeding events of acclimatised mating pairs from a non-compromised field
101 population. Pairing was controlled to supply half siblings. Then we tested the two prerequisites of
102 Darwinian selection (and thus a potential to evolve resistance) by answering two questions
103 employing either full-sib or half-sib designs (Falconer and Mackay 1996): (i) Are there
104 genetically determined differences in lethal Cd-tolerance within a native population of *G.*
105 *fossarum*? If yes, (ii) are these genotypic differences heritable?

106

106 **2. Materials and Methods**

107

108 **2.1. Culture conditions and breeding design**

109 Approximately 400 *Gammarus fossarum* adults were collected within an upstream
110 location of the River Bourbre, Isère, France. Recent demographic and ecotoxicological follow-
111 ups led us to consider the sampled population as not impacted by environmental contamination in
112 recent years. After 1 month of acclimatisation to laboratory conditions (natural water;
113 temperature: 13°C; conductivity: 300 $\mu\text{S cm}^{-1}$; photoperiod: 15/9 h light/dark), 24 pairs of
114 breeders with females in the last stage of their reproductive cycle were isolated in individual 500-
115 mL beakers with constant water flow. Individuals were fed with alder leaves and weekly supplies
116 of *Tubifex* larvae. Attention was paid to female size homogeneity. The identification of mating
117 pairs was easy for this amphipod because of the formation of a characteristic amplexus
118 (precopulatory guarding behaviour) and the identification of the reproductive status of females
119 was achieved by direct observation (hatched juveniles in brood pouch, visible gonads, guarding
120 male). After a few days, 19 females released the juveniles present in their brood pouches. These
121 140 neonates – each with an identified mother and an unknown father (fertilisation during the
122 laboratory acclimatisation) – constituted a first set of 19 broods produced in March (Supp. figure
123 1). Juveniles were isolated in individual 50-mL tubes (BD FalconT) beginning on the day of their
124 emergence. It should be noted that the accuracy of reproductive stage determination successfully
125 translated into good synchronisation of offspring release (79% of neonates emerged within 3
126 days; Supp. figure 1). Soon after neonate release females moulted and shed eggs which were
127 fertilised by the guarding males. These eggs developed into juveniles released 26 days later
128 (duration between median dates of emergence). They formed a second set of 14 broods from two
129 identified parents (105 neonates) still with good synchronisation (70% of releases within 3 days).

130 Taking advantage of the separation of the amplexus after copulation, males were redistributed
131 between beakers during the reproductive cycle in order to produce a third brood from each female
132 with a different father (Supp. figure 1). This third set of 11 broods (111 neonates in May)
133 emerged 25 days after the second one and synchronisation was less but still manageable to
134 consider neonate exposure as a single experimental group (67% of emergences within 4 days).
135 Over the 3 months, 356 neonates were thus collected the day of their emergence; the 45 broods
136 presented a median brood size of eight neonates per female (min=2, $Q_{25\%}=6$, $Q_{75\%}=9$, max=20).
137 The controlled mating scheme involving successive reproductive cycles yielded both paternal and
138 maternal half siblings (Supp. figure 1).

139

140 **2.2. Offspring Cd-sensitivities**

141 The 356 neonates were exposed to a lethal concentration of $20 \mu\text{g Cd L}^{-1}$ the day after
142 their release from the maternal brood pouch. The lethal response was chosen as the endpoint in
143 order to maximise the expression of possible genotypic differences in the toxicological responses
144 since genetic variability for sublethal responses to Cd tend to be lower than acute responses for
145 crustacean daphnids (Barata *et al.* 2000). The concentration of $20 \mu\text{g Cd L}^{-1}$ was determined in a
146 preliminary test (five Cd concentration levels with three replicates of ten neonates) with the aim
147 to ensure both sensitivity and specificity (not a too high concentration in order to scatter
148 mortalities in time, and not a too long exposure in order to attribute mortalities to Cd). During the
149 preliminary test, $20 \mu\text{g Cd L}^{-1}$ gave rise to mortalities scattered from 12 h to seven days of
150 exposure, while only one death occur in the controls. Here, survival was monitored daily during
151 semi-static exposure in 50-mL tubes randomly ordered within a thermoregulated tank with one
152 renewal of the water solution every 48 h; mortality was identified via the pleopod ventilatory
153 activity. Complementary to the individualisation of exposure and to the random spatial

154 distribution of individuals within the experimental system, independence between exposure
155 conditions and pedigrees was ensured by following a blind protocol and using a common Cd
156 solution for each of the three monthly sets of broods. To accomplish this, the total volume of
157 exposure solutions was prepared each month by a unique dilution in culture water of a common
158 stock solution of 100 mg L⁻¹ of cadmium (CdCl₂.H₂O, Sigma Aldrich, Saint Quentin Fallavier,
159 France) in ultrapure water acidified (0.2% HNO₃) for storage. Exposure Cd solutions were
160 conserved during neonate exposure in high-density polyethylene bottles with bubbling to
161 guarantee oxygen saturation. In total, considering the 356 exposed neonates, a median (minimum,
162 maximum, respectively) lethal time of 3 days (1, 9 days, respectively) was recorded.

163

164 **2.3. Sib analyses**

165 All statistical procedures were carried out with the R software (R Development Core
166 Team 2007). In order to employ linear models, log transformations of survival times were
167 required to verify the homoscedasticity of brood sensitivities (checked by Levene tests: $P > 0.05$),
168 agreeing with previous findings on the log-normal distribution of Cd-sensitivities in daphnids
169 (Barata *et al.* 1998, 2002b).

170 First, in order to test the existence of genetically determined differences in Cd-tolerance,
171 broods were compared in terms of survival time by means of non-parametric tests (Kruskal-
172 Wallis rank sum tests). The brood effect, *i.e.* similarity among full sibs, was also quantified by
173 variance decomposition using linear mixed-effect models (Pinheiro and Bates 2000) as
174 implemented in the nlme package within the R environment. The significance of a brood effect
175 on Cd-tolerance was assessed by inspecting confidence intervals of the restricted maximum
176 likelihood (REML) estimators of variance components from a model including a random brood
177 effect (Falconer and Mackay 1996; Pinheiro and Bates 2000). The possibility of a maternal effect

178 (Falconer and Mackay 1996) was assessed through the examination of correlations (Spearman
179 correlation tests) between tolerance and maternally mediated parameters tracing a possible
180 heterogeneity in maternal investment in progenies (brood size, mean length of offspring, mother
181 length).

182 Second, the similarity in survival times among half-sibs was investigated in order to
183 assess whether possible genotypic differences in Cd-tolerance are partly heritable. This required
184 shaping a paternal half-sib design (Falconer and Mackay 1996) taking into account the survival
185 of 157 neonates from 20 broods corresponding to the progenies of ten males which reproduced in
186 April and May (Supp. figure 1): ten sires mated with two different dams in quantitative genetics
187 terms. The heterogeneity in brood size involved an unbalanced nested design for the analysis of
188 variance. Linear mixed-effect models are suitable in such cases (Pinheiro and Bates 2000). We
189 therefore built a model considering (i) a random sire effect, (ii) a random dam effect nested
190 within the sire effect and (iii) a residual environmental variance. The significance of the sire
191 effect (*i.e.* the similarity among half sibs) was tested by comparing the likelihood of this nested
192 model with the likelihood of a model considering only one random brood effect (likelihood ratio
193 test implemented in the nlme package in R). REML estimators were computed only for
194 significant variance components. Furthermore, we controlled the possibility of a confounding
195 factor induced by the heterogeneity of exposure conditions between April and May by testing the
196 introduction of a fixed month effect in these models (ANOVA test) (Pinheiro and Bates 2000).
197 Complementarily to this paternal half-sib analysis, the survival times of 269 neonates
198 corresponding to the progenies of 11 females during March, April and May were analysed in the
199 same manner but as a maternal half-sib design (11 dams mated with three different sires). The
200 interpretation of resemblance among maternal half-sibs (dam effect) is quite different from the

201 paternal case because maternal effects could be involved in addition to additive genetic
202 components (Falconer and Mackay 1996).

203

203 **3. Results**

204

205 **3.1. Genetic determination of Cd-sensitivity: full sib analyses**

206 The lethal Cd-sensitivities between broods were compared separately for the three
207 monthly sets of offspring. This excludes any confounding effect due to a possible heterogeneity
208 in environmental or exposure conditions between months. Differences in tolerance were observed
209 between broods (Figure 1): for instance, the most sensitive brood in March presented a median
210 lethal time of 1 day, while it reached 5 days for the most tolerant one (Figure 1A). Kruskal-Wallis
211 rank sum tests concluded that these differences could not be explained only by a sampling effect
212 (induced by small and unequal brood sizes) in March and May ($P < 10^{-3}$). In April, however, a
213 weaker between-brood heterogeneity ($P = 0.08$) was observed (Figure 1B). The number of broods
214 considered here was too small to guarantee the detection of between-brood differences by means
215 of poorly powerful non-parametric tests. This is supported by the significant brood effect for the
216 three monthly data sets in the analysis of variance using linear mixed-effect models (see REML
217 estimators of variance components on Figure 1). We note that the 95% confidence intervals of the
218 REML estimators of the standard deviation associated with this brood effect are large; the limited
219 number of examined broods also explains this lack of precision. Nevertheless, these combined
220 results attest that there are significant differences in Cd-tolerance between broods.

221 Before concluding on the genetic determination of these toxicological differences, we first
222 ruled out a possible confounding effect related to neonate size. Even if a strong between-brood
223 heterogeneity in neonate size was detected (Kruskal-Wallis rank sum test: $P < 10^{-8}$), individual
224 survival time did not correlate with individual size (Spearman correlation test: $P > 0.05$). This
225 agrees with findings for *Daphnia* neonates exposed to Cd (Barata *et al.* 1998). Therefore,
226 considering that any common environment effect was excluded as a result of the experimental

227 protocol (individual exposure, randomised design, a single Cd solution, 1-day-old neonates), the
228 similarity among the sibs of a given brood could only be explained by shared genotypic features
229 or by a maternal effect (during egg production and brooding). Nevertheless, even if the latter
230 effect cannot be excluded, we have no evidence in favour of it. Firstly, no correlation was
231 detected between the size of a female and the Cd-tolerance of her offspring (Spearman
232 correlation test: $P > 0.05$). This is notably because breeders were selected aiming to limit female
233 size variability (min=9.1 mm, $Q_{25\%}$ =10.0 mm, $Q_{75\%}$ =10.1 mm, max=11 mm). Secondly, median
234 brood survival times did not correlate with any of the examined maternally mediated parameters
235 employed to evaluate the maternal investment in brood (mean length of offspring, brood size)
236 (Spearman correlation tests: $P > 0.05$). Thirdly, previous reports for *Daphnia* have suggested a
237 clear genetic determination of acute Cd-sensitivity (Baird *et al.* 1991; Barata *et al.* 1998, 2000,
238 2002a; Ward and Robinson 2005) and suspect an absence of maternal effects (Barata *et al.* 1998).
239 Then all these findings lead to the inference that the between-brood variability observed within
240 the three sets of *Gammarus* neonates are likely explained by genetically determined differences
241 in Cd-tolerance.

242

243 **3.2. Heritability of Cd-sensitivity: half sib analyses**

244 Only the additive part of this genetic variance – heritability in the narrow sense (Falconer
245 and Mackay 1996) – can be regarded as a potential for genetic adaptation since dominance and
246 combined epistatic interactions are not transmissible between generations in the case of sexual
247 reproduction. Because the phenotypic similarity within half-sibs (contrary to full-sibs) translates
248 only a significant additive variance component (plus the probability of a maternal effect for
249 maternal half-sibs), we assessed the heritability of Cd-sensitivity within the sampled *Gammarus*
250 population by means of classical half-sib designs (Falconer and Mackay 1996). Ten males, called

251 sires, were mated with two successive females, called dams, in order to produce 157 neonates
252 individually exposed to Cd (April and May; Supp. figure 1). The results of this paternal half-sib
253 design demonstrate that the between-brood variability in neonate survival time is not significantly
254 explained by a between-male heterogeneity (Figure 2). A likelihood ratio test of the linear mixed-
255 effect models confirms the non-significant sire effect, in other words a weak similarity between
256 paternal half-sibs (Table 1). We conclude that this pattern does not result from a confounding
257 effect induced by heterogeneity in experimental conditions between April and May (fixed month
258 effect; ANOVA: $P > 0.05$). Our initial conclusion was that Cd-tolerance is not heritable because
259 the major part of the genetic variance in Cd-sensitivity (brood effect) appears to be determined by
260 non-additive components. Analysis of the maternal half-sib design involving 269 individual
261 records of offspring from 11 dams mated with three successive sires (Supp. figure 1) corroborates
262 this finding: there was no significant resemblance within maternal half-sibs – *i.e.* a dam effect –
263 (Figure 3; Table 2), and no confounding effect related to monthly heterogeneity in experimental
264 conditions could conceal this resemblance (fixed month effect; anova: $P > 0.05$). Furthermore,
265 the absence of a noticeable dam effect not only confirmed the weakness of the additive genetic
266 variance in offspring Cd-sensitivity but also revealed that no maternal effect (at least mother-
267 specific deviations) contributes to the between-brood differences in Cd-tolerance, reinforcing the
268 hypothesis of genetic determination.

269 We also examined the possibility that lab rearing conditions select resistant breeders,
270 hence reducing inheritable genetic variation. From Figure 1, it appears that tolerance to Cd
271 increased in the assayed broods in May, as detected by linear mixed-effect modelling (fixed
272 month effect, ANOVA: $P=0.018$). Nevertheless, this increase has to be interpreted as
273 heterogeneity in experimental conditions between months. The possibility that Cd-resistant
274 genotypes among breeders were favoured in the lab is indeed not consistent with the unchanged

275 between-brood variability from March to May (random month effect on between-brood
276 variability, likelihood ratio test: $P > 0.05$). Note that this month effect was no more detected within
277 subsets of broods used in the half-sib designs.
278

278 **4. Discussion**

279 Genetic determination of the variability in acute Cd-sensitivity was demonstrated here
280 within a *Gammarus fossarum* population. This finding agrees with reported between-clone
281 differences in the lethal response to Cd for crustacean daphnids (Baird *et al.* 1991; Barata *et al.*
282 1998, 2000, 2002a; Ward and Robinson 2005) and other metals (Baird *et al.* 1991; Barata *et al.*
283 1998, 2000; Lopes *et al.* 2004). Strikingly, the within-population genetic variability observed in
284 lethal time of Cd-exposed neonates for *Gammarus* was comparable in magnitude to observations
285 on natural populations of *Daphnia* (Barata *et al.* 2002a). However, the interpretation of this
286 genetically determined variability in terms of potential to evolve resistance to prolonged acute
287 stress diverges: the quantitative genetics methodology applied here to assess whether these
288 genetic differences in lethal tolerance are heritable between generations (half-sib analyses)
289 reveals that the observed genetic variance in Cd-sensitivity is mostly explained by large non-
290 additive variance components. As a consequence, the heritability in the narrow sense, which
291 quantifies responsiveness to selection, is negligible.

292 This failure to detect any significant additive genetic variance could result from the
293 restricted number of breeding pairs employed in the study, which could give rise to weak
294 statistical power. Nevertheless, this conclusion, even if based on a negative result, has to be
295 analysed considering the strong between-brood variability translating clearly detectable genetic
296 variability in our data set. We can therefore state that large non-additive genetic components are
297 present in the determination of lethal Cd-sensitivities within the sampled population, whereas
298 additive components in the observed genetic variance are weak. That leads to the preliminary
299 conclusion that such a population would not adapt genetically to prolonged acute Cd exposure
300 despite the existence of a significant genetic variability in Cd-sensitivity. Nevertheless, keeping
301 in mind that only a lethal response was considered in this pilot study, and that this parameter

302 provides a non-exclusive measure for chemical tolerance, similar questions on the genetic
303 determination of sublethal responses should be scrutinised before concluding.

304 This finding with crustacean gammarids seems to contrast with the outcomes from studies
305 with parthenogenetic crustacean daphnids, which describe genetically determined differences in
306 resistance to metals (Baird *et al.* 1991; Barata *et al.* 1998, 2000, 2002a, 2002b; Lopes *et al.* 2004,
307 2005; Soares *et al.* 1992) and suggest a possible increase in mean tolerance of populations during
308 multi-generation artificial selection experiments (Ward and Robinson 2005) or within
309 historically-contaminated field contexts (Lopes *et al.* 2004, 2005). Nevertheless, as pointed out
310 by Lopes *et al.* (2004, 2005), the apparent increase in mean metal tolerance resulted from the
311 disappearance of sensitive clones and not from the appearance of resistant ones within
312 populations. This pattern, where no higher resistant genotypes are revealed during selection
313 induced by exposure, is not falsified regarding the outcomes from the artificial selection test with
314 Cd (Ward and Robinson 2005), notably considering that only asexual reproduction was allowed
315 in this experiment. The fact that after decades of metal exposure no clones with higher resistance
316 emerge within field daphnid populations (Lopes *et al.* 2004, 2005) could be surprising
317 considering that genetically determined differences in tolerance exist. Nevertheless, in view of
318 our findings, these observations could be explained by a weakness of additive components in the
319 genetic variance of lethal metal tolerance. In that case, the apparent adaptation (increased
320 tolerance in contaminated populations) would be a transitory state reached each year in field
321 populations due to the loss of sensitive clonal lineages. Therefore, because of a lack of
322 heritability, this state of apparent adaptation would disappear at each sexual reproduction event
323 occurring when ephippial eggs are produced before each winter in field populations.

324 From a broader perspective, the hypothesis of a weakness of additive components in the
325 variability of sensitivity to contaminants is also consistent with the reported similarity between

326 the range of sensitivities observed among different laboratory populations and the variability
327 among or within field populations (Barata 2002b). This potentially explains why selective
328 processes related to laboratory rearing or to natural habitat conditions do not result through
329 pleiotropic effects in population divergence in terms of resistance to contaminants, whereas
330 genetic variability is attested. Strikingly, the importance of non-additive genetic components in
331 stress tolerance is also reported in other arthropods for the resistance against parasite infection
332 (Wegner *et al.* 2008). These results are also pertinent to the current debate on the relative part of
333 additive components in the determination of the variation of complex phenotypic traits (Hill *et al.*
334 2008; O'Hara 2008). Because the genetic architecture of complex traits implies polygeny and
335 epistasis, this question of additive versus non-additive components is of primary importance to
336 understanding the specific evolutionary behaviour of complex traits, as exemplified by disease
337 susceptibilities (Blekhman *et al.* 2008). Then, analogous questioning should investigate tolerance
338 to contaminants.

339
340 Overall, the identification of genetic variability in tolerance to contaminants within field
341 or laboratory populations does not necessarily indicate a strong potential to evolve genetic
342 resistance; indeed non-additive components seem to be able to contribute substantially to the
343 genetic variance of tolerance. The involvement of large non-additive effects may not be restricted
344 to the model of polygenic inheritance of contaminant susceptibility through multiple minor genes
345 implying epistasis. Even in case of inheritance through single major genes (Barata *et al.* 1998;
346 Woods and Hoffmann 2000), non-additive effects through dominance interactions could also
347 operate (*e.g.* Labbé *et al.* 2007). The hypothetical widespread weakness of additive components
348 in chemical tolerance could reconcile on one hand the observation that genetic adaptation in
349 contaminated contexts is infrequent (Klerks 2002; Millward and Klerks 2002; Woods and

350 Hoffmann 2000) and on the other hand the frequent reports of genetically determined differences
351 in tolerance to toxic compounds. Then, following this hypothesis and as previously claimed from
352 the apparent mismatch between laboratory and field outcomes on the inheritance of insecticide
353 resistance (Reznick and Ghalambor 2005), the exceptional cases of adaptation of field
354 populations to contamination would be permitted only by the fixation of rare alleles of major
355 genes (Woods and Hoffmann 2000). Thus, quantitative genetics protocols could provide insight
356 into the question of genetic adaptation for ecological risk assessment. Indeed it is crucial to test
357 the hypothesis that additive genetic variation for tolerance to contaminants is generally weak, and
358 to consider the possibility that evolution of tolerance relies more often on exceptional events in
359 which rare alleles become fixed in exposed populations.

360

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364

364 **References**

- 365 Athrey, N.R.G., Leberg, P.L., Klerks, P.L., 2007. Laboratory culturing and selection for
366 increased resistance to cadmium reduce genetic variation in the least killifish,
367 *Heterandria formosa*. Environ. Toxicol. Chem. 26, 1916-1921.
- 368 Baird, D.J., Barber, I., Bradley, M., Soares, A.M.V.M., Calow, P., 1991. A comparative study of
369 genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. Ecotox.
370 Environ. Safe. 21, 257-265.
- 371 Barata, C., Baird, D. J., Markich, S. J., 1998. Influence of genetic and environmental factors on
372 the tolerance of *Daphnia magna* Straus to essential and non-essential metals. Aquat.
373 Toxicol. 42, 115-137.
- 374 Barata, C., Baird, D. J., Minarro, A., Soares, A., 2000. Do genotype responses always converge
375 from lethal to nonlethal toxicant exposure levels? Hypothesis tested using clones of
376 *Daphnia magna* Straus. Environ. Toxicol. Chem. 19, 2314-2322.
- 377 Barata, C., Baird, D. J., Mitchell, S.E., Soares, A., 2002a. Among- and within-population
378 variability in tolerance to cadmium stress in natural populations of *Daphnia magna*:
379 Implications for ecological risk assessment. Environ. Toxicol. Chem. 21, 1058-1064.
- 380 Barata, C., Baird, D.J., Soares, A., 2002b. Determining genetic variability in the distribution of
381 sensitivities to toxic stress among and within field populations of *Daphnia magna*.
382 Environ. Sci. Technol. 36, 3045-3049.
- 383 Blekhman, R., Man, O., Herrmann, L., Boyko, A.R., Indap, A., Kosiol, C., Bustamante, C.D.,
384 Teshima, K.M., Przeworski, M., 2008. Natural selection on genes that underlie human
385 disease susceptibility. Curr. Biol. 18, 883-889.
- 386 Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics, Longman, London.

387 Groenendijk, D., Lucker, S.M.G., Plans, M., Kraak, M.H.S., Admiraal, W., 2002. Dynamics of
388 metal adaptation in riverine chironomids. *Environ. Pollut.* 117, 101-109.

389 Hill, W.G., Goddard, M.E., Visscher, P.M., 2008. Data and theory point to mainly additive
390 genetic variance for complex traits. *PLoS Genet.* 4, e1000008.

391 Klerks, P.L., 2002. Adaptation, ecological impacts, and risk assessment: Insights from research at
392 Foundry Cove, Bayou Trepagnier, and Pass Fourchon. *Hum. Ecol. Risk Assess.* 8, 971-
393 982.

394 Klerks, P.L., Moreau, C.J., 2001. Heritability of resistance to individual contaminants and to
395 contaminant mixtures in the sheepshead minnow (*Cyprinodon variegatus*). *Environ.*
396 *Toxicol. Chem.* 20, 1746-1751.

397 Labbé, P., Berticat, C., Berthomieu, A., Unal, S., Bernard, C., Weill, M., Lenormand, T., 2007.
398 Forty years of erratic insecticide resistance evolution in the mosquito *Culex pipiens*. *PLoS*
399 *Genet.* 3, e205.

400 Lopes, I., Baird, D.J., Ribeiro, R., 2004. Genetic determination of tolerance to lethal and
401 sublethal copper concentrations in field populations of *Daphnia longispina*. *Arch.*
402 *Environ. Contam. Toxicol.* 46, 43-51.

403 Lopes, I., Baird, D.J., Ribeiro, R., 2005. Resistance to metal contamination by historically-
404 stressed populations of *Ceriodaphnia pulchella*: Environmental influence versus genetic
405 determination. *Chemosphere* 61, 1189-1197.

406 Medina, M.H., Correa, J.A., Barata, C., 2007. Micro-evolution due to pollution: Possible
407 consequences for ecosystem responses to toxic stress. *Chemosphere* 67, 2105-2114.

408 Millward, R.N., Klerks, P.L., 2002. Contaminant-adaptation and community tolerance in
409 ecological risk assessment: Introduction. *Hum. Ecol. Risk Assess.* 8, 921-932.

410 Nowak, C., Czeikowitz, A., Vogt, C., Oetken, M., Streit, B., Schwenk, K., 2008. Variation in
411 sensitivity to cadmium among genetically characterized laboratory strains of the midge
412 *Chironomus riparius*. Chemosphere 71, 1950-1956.

413 O'Hara, B., 2008. Journal club. Nature 452, 785-785.

414 Pinheiro, J., Bates, D., 2000. Mixed-effects models in S and S-PLUS, Springer, New York.

415 R Development Core Team, 2007. R: a language and environment for statistical computing, R
416 Foundation for Statistical Computing, Vienna.

417 Reznick, D.N., Ghalambor, C.K., 2005. Selection in nature: Experimental manipulations of
418 natural populations. Integr. Comp. Biol. 45, 456-462.

419 Soares, A.M.V.M., Baird, D.J., Calow, P., 1992. Interclonal variation in the performance of
420 *Daphnia magna* Straus in chronic bioassays. Environ. Toxicol. Chem. 11, 1477-1483.

421 Vogt, C., Nowak, C., Diogo, J.B., Oetken, M., Schwenk, K., Oehlmann, J., 2007. Multi-
422 generation studies with *Chironomus riparius* - Effects of low tributyltin concentrations on
423 life history parameters and genetic diversity. Chemosphere 67, 2192-2200.

424 Ward, T.J., Robinson, W.E., 2005. Evolution of cadmium resistance in *Daphnia magna*. Environ.
425 Toxicol. Chem. 24, 2341-2349.

426 Wegner, K.M., Berenos, C., Schmid-Hempel, P., 2008. Non-additive genetics components in
427 resistance of the red flour beetle *Tribolium castaneum* against parasite infection. Evolution
428 62, 2381-2392.

429 Woods, R.E., Hoffmann, A.A., 2000. Evolution in toxic environments: Quantitative versus major
430 gene approaches, in: Kammenga, J., Laskowski, R. (Eds), Demography in ecotoxicology,
431 John Wiley & Sons Ltd, pp. 129-145.

432 Xie, L.T., Klerks, P.L., 2003. Responses to selection for cadmium resistance in the least killifish,
433 *Heterandria formosa*. Environ. Toxicol. Chem. 22, 313-320.

434 **Tables**

435

<i>observational variance component</i>		<i>REML estimator</i>	
sires	σ_S	2.4 10⁻⁵	<i>not significant</i>
dams within sires	σ_D	0.24	[0.14;0.39]
residual	$\sigma_{RESIDUAL}$	0.47	[0.42;0.53]

157 neonates; ten males mated with two females

436

437

438 **Table 1.** Paternal half-sib analysis. REML estimators of the variance components from a linear
 439 mixed-effect model considering log-transformed lethal time of 157 neonates from the paternal
 440 half-sib design (figure 2) as a response explained by (i) a random sire effect, (ii) a random dam
 441 effect nested within the sire effect and (iii) a residual environmental variance. The significance of
 442 the sire effect (*i.e.* the similarity among paternal half-sibs) was tested through a likelihood ratio
 443 test comparing this nested model and a model with only one random brood effect. 95%
 444 confidence intervals are provided for significant effects.

445

445

<i>observational variance component</i>		<i>REML estimator</i>	
dams	σ_s	3.9 10⁻⁵	<i>not significant</i>
sires within dams	σ_D	0.26	[0.18;0.38]
residual	$\sigma_{RESIDUAL}$	0.49	[0.44;0.53]

269 neonates; 11 females mated with three males

446

447

448 **Table 2.** Maternal half-sib analysis. REML estimators of the variance components from a linear
449 mixed-effect model considering log-transformed lethal time of 269 neonates from the maternal
450 half-sib design (figure 3) as a response explained by (i) a random dam effect, (ii) a random sire
451 effect nested within the dam effect and (iii) a residual environmental variance. The significance
452 of the dam effect (*i.e.* the similarity among maternal half sibs) was tested through a likelihood
453 ratio test comparing this nested model and a model with only one random brood effect. 95%
454 confidence intervals are provided for significant effects.

455

455 **Figure captions**

456

457 **Figure 1. Between-brood heterogeneity in Cd-tolerance.** Box-and-whisker plots of log-
458 transformed lethal times of neonates during 20- $\mu\text{g Cd L}^{-1}$ exposure. The three panels (A, B, C)
459 correspond to the records of the three monthly sets of broods (March, April, May). Labels of
460 horizontal axes report the brood sizes (*i.e.* the number of neonates tested per brood). The REML
461 estimators of variance components of a linear mixed-effect model considering a random effect of
462 brood on log-transformed lethal time are presented below each panel (standard deviation and
463 95% confidence interval). Boxes extend from the first to the third quartile of lethal times within
464 each brood with a bold segment for the median lethal time; the whiskers extend to the most
465 extreme data points which are no more than 1.5 times the interquartile range, and open circles
466 represent outliers.

467

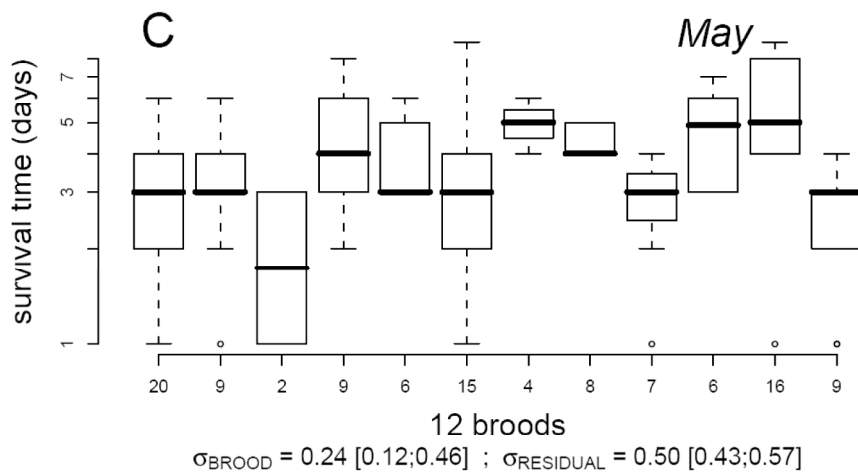
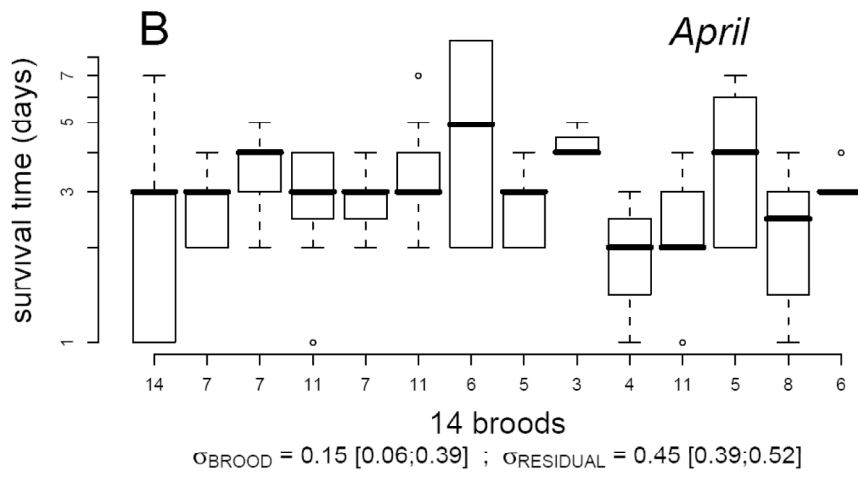
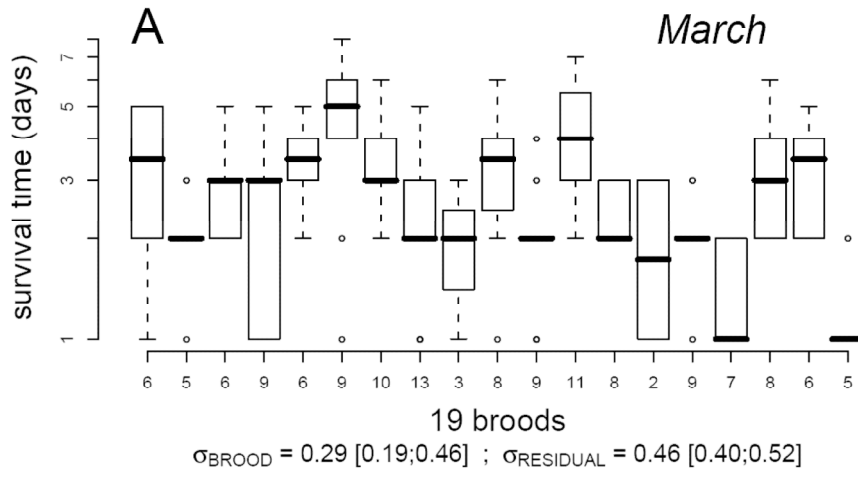
468 **Figure 2. Paternal half-sib design.** Box-and-whisker plots (same conventions as figure 1) of
469 log-transformed lethal times (20- $\mu\text{g Cd L}^{-1}$ exposure) of 157 neonates from the breeding of ten
470 males called sires (one grey level per sire) with two successive different females called dams
471 (two successive boxes). Labels of the horizontal axis report the number of neonates tested per
472 brood.

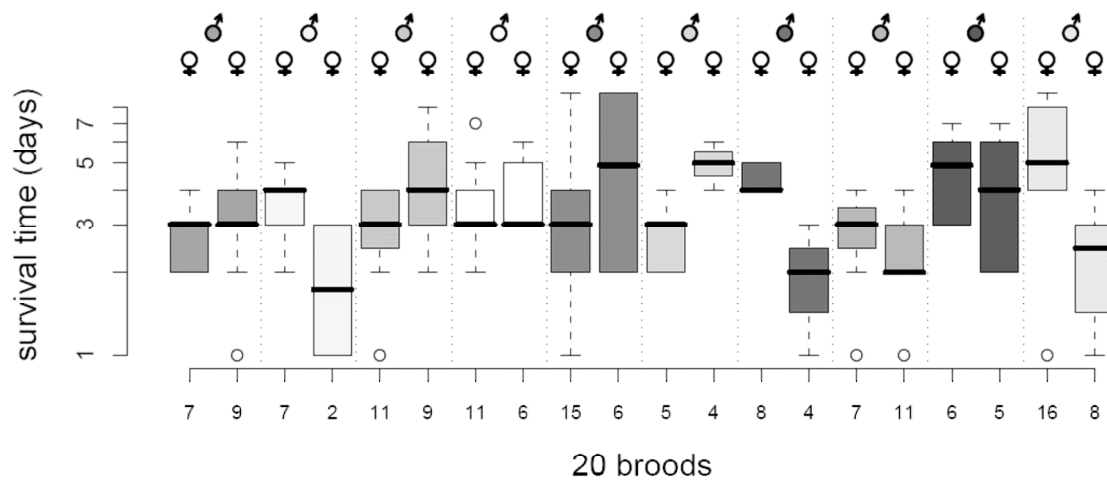
473

474 **Figure 3. Maternal half-sib design.** Box-and-whisker plots (same conventions as figure 1) of
475 log-transformed lethal times (20- $\mu\text{g Cd L}^{-1}$ exposure) of 269 neonates from the breeding of 11
476 females called dams (one grey level per dam) with three successive different males called sires
477 (three successive boxes). Labels of the horizontal axis report the number of neonates tested per
478 brood.

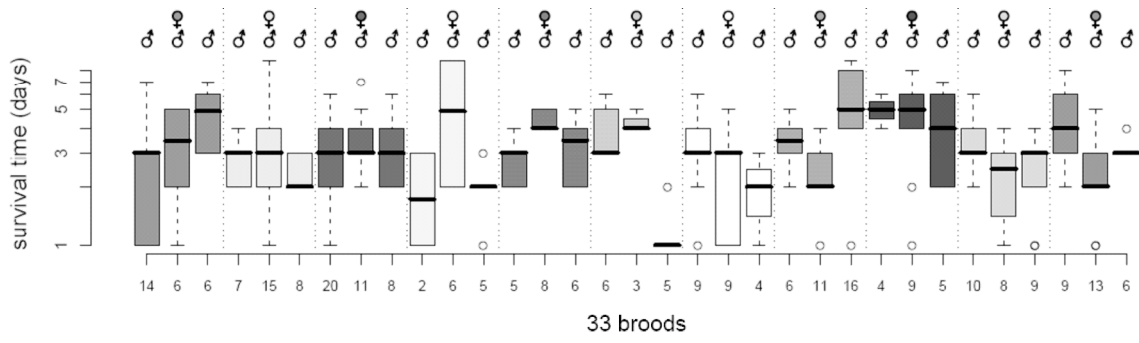
479 **Supp. figure 1. Breeding design.** Following one month of acclimatisation, mating pairs of
480 *Gammarus fossarum* breeders were selected with females in the last stage of their reproductive
481 cycle (hatched juveniles in brood pouch). After a few days, neonates were released away (pink
482 arrows) and were collected to form a first set of broods (in March) for Cd exposure. Afterward,
483 females moulted and shed eggs, which were fertilised by the guarding male (green arrows) and
484 yielded neonates (violet arrows) 3 weeks later (second set of broods in April). After copulation,
485 males were redistributed (orange arrows) in order to supply a third brood from each female
486 (emergence in May). In total, 356 neonates were tested individually. Coloured boxes
487 (respectively blue and pink) underline sib relatedness between broods from the different months
488 (respectively paternal and maternal half sibs).

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