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

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Title:

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

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Abstract

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

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

1. Introduction

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

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

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

2. Materials and Methods

2.1. Culture conditions and breeding design

Approximately 400 *Gammarus fossarum* adults were collected within an upstream location of the River Bourbre, Isère, France. Recent demographic and ecotoxicological follow-ups led us to consider the sampled population as not impacted by environmental contamination in recent years. After 1 month of acclimatisation to laboratory conditions (natural water; temperature: 13°C; conductivity: 300 µS cm^{-1}; photoperiod: 15/9 h light/dark), 24 pairs of breeders with females in the last stage of their reproductive cycle were isolated in individual 500-mL beakers with constant water flow. Individuals were fed with alder leaves and weekly supplies of *Tubifex* larvae. Attention was paid to female size homogeneity. The identification of mating pairs was easy for this amphipod because of the formation of a characteristic amplexus (precopulatory guarding behaviour) and the identification of the reproductive status of females was achieved by direct observation (hatched juveniles in brood pouch, visible gonads, guarding male). After a few days, 19 females released the juveniles present in their brood pouches. These 140 neonates – each with an identified mother and an unknown father (fertilisation during the laboratory acclimatisation) – constituted a first set of 19 broods produced in March (Supp. figure 1). Juveniles were isolated in individual 50-mL tubes (BD FalconT) beginning on the day of their emergence. It should be noted that the accuracy of reproductive stage determination successfully translated into good synchronisation of offspring release (79% of neonates emerged within 3 days; Supp. figure 1). Soon after neonate release females moulted and shed eggs which were fertilised by the guarding males. These eggs developed into juveniles released 26 days later (duration between median dates of emergence). They formed a second set of 14 broods from two 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 µg 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 µg 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 µg 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 ($CdCl_2.H_2O$, Sigma Aldrich, Saint Quentin Fallavier,
159 France) in ultrapure water acidified (0.2% HNO_3) 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

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

3. Results

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

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

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

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

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

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

4. Discussion

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

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

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

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

From a broader perspective, the hypothesis of a weakness of additive components in the 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

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

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434

Tables

observational variance component		*REML estimator*	
sires	σ_S	**$2.4\ 10^{-5}$**	***not significant***
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			

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

445

observational variance component		*REML estimator*	
dams	σ_S	**$3.9\ 10^{-5}$**	***not significant***
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

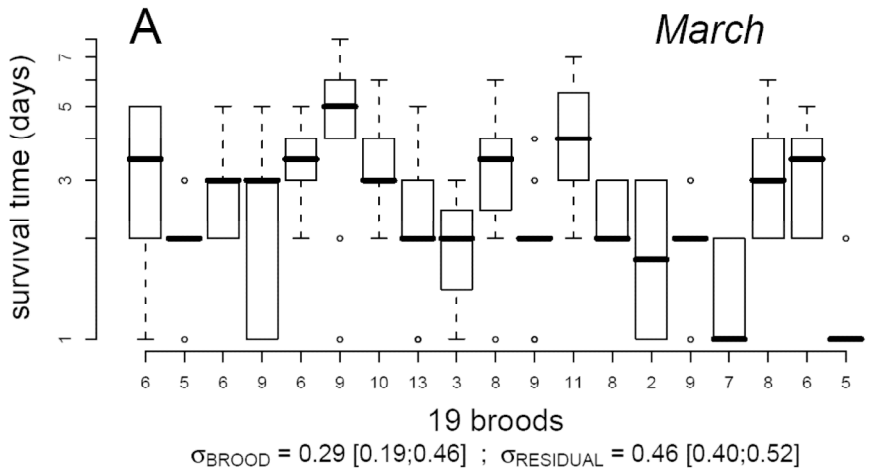
Figure captions

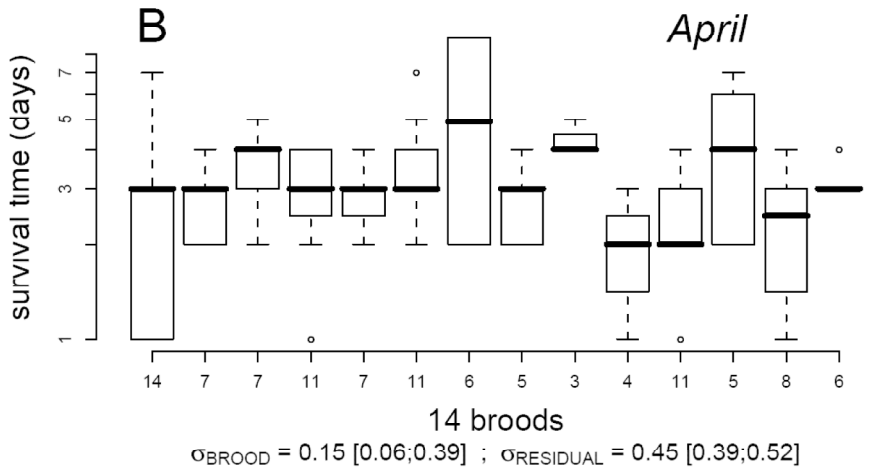
Figure 1. Between-brood heterogeneity in Cd-tolerance. Box-and-whisker plots of log-transformed lethal times of neonates during 20-µg Cd L^{-1} exposure. The three panels (A, B, C) correspond to the records of the three monthly sets of broods (March, April, May). Labels of horizontal axes report the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

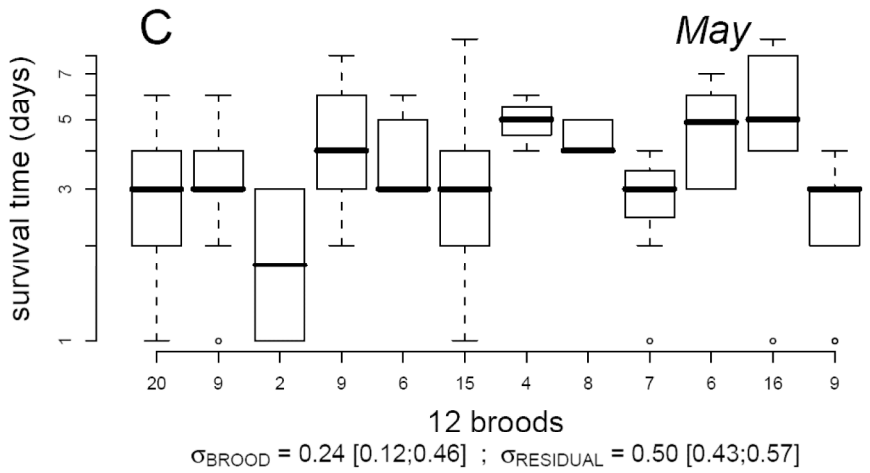
Figure 2. Paternal half-sib design. Box-and-whisker plots (same conventions as figure 1) of log-transformed lethal times (20-µg Cd L^{-1} exposure) of 157 neonates from the breeding of ten males called sires (one grey level per sire) with two successive different females called dams (two successive boxes).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

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

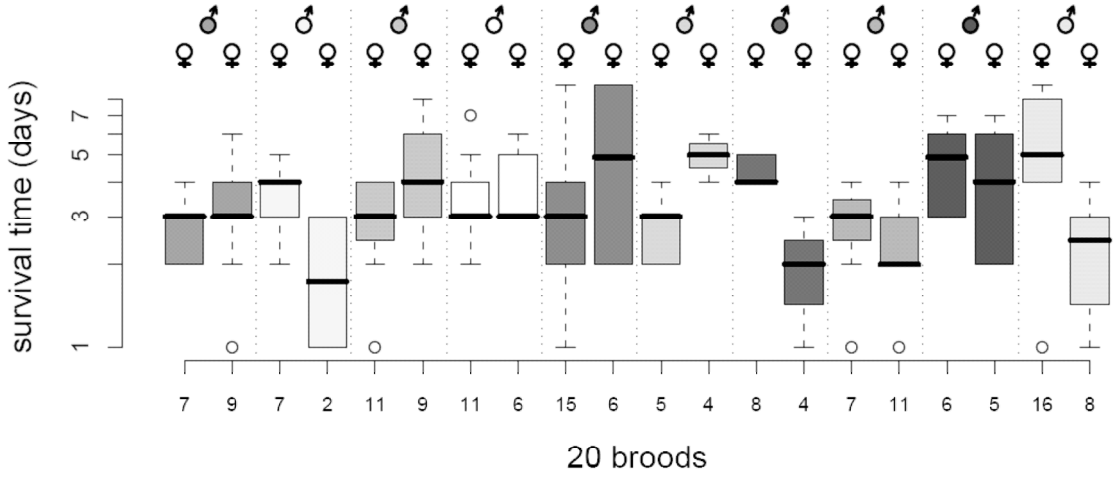
Supp. figure 1. Breeding design. Following one month of acclimatisation, mating pairs of *Gammarus fossarum* breeders were selected with females in the last stage of their reproductive cycle (hatched juveniles in brood pouch). After a few days, neonates were released away (pink arrows) and were collected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


A
March
survival time (days)
1
3
5
7
6 5 6 9 6 9 10 13 3 8 9 11 8 2 9 7 8 6 5
19 broods
σBROOD = 0.29 [0.19;0.46] ; σRESIDUAL = 0.46 [0.40;0.52]

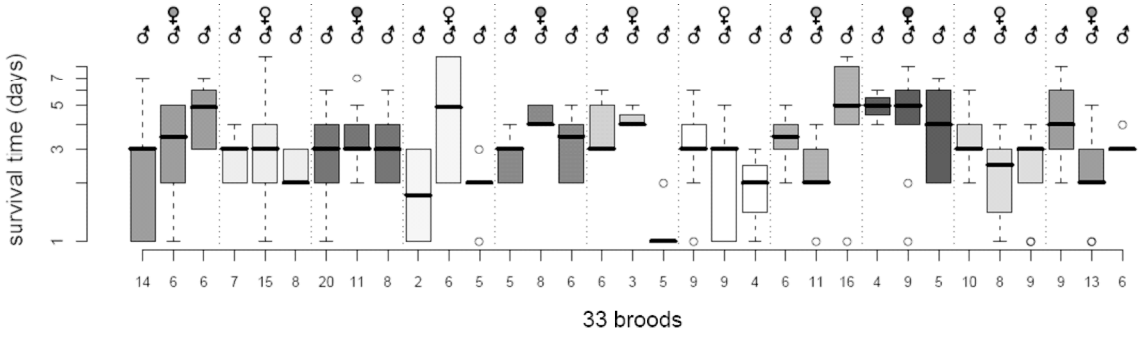

B
April
survival time (days)
1
3
5
7
14 7 7 11 7 11 6 5 3 4 11 5 8 6
14 broods
σBROOD = 0.15 [0.06;0.39] ; σRESIDUAL = 0.45 [0.39;0.52]


C
May
survival time (days)
1
3
5
7
20 9 2 9 6 15 4 8 7 6 16 9
12 broods
σBROOD = 0.24 [0.12;0.46] ; σRESIDUAL = 0.50 [0.43;0.57]

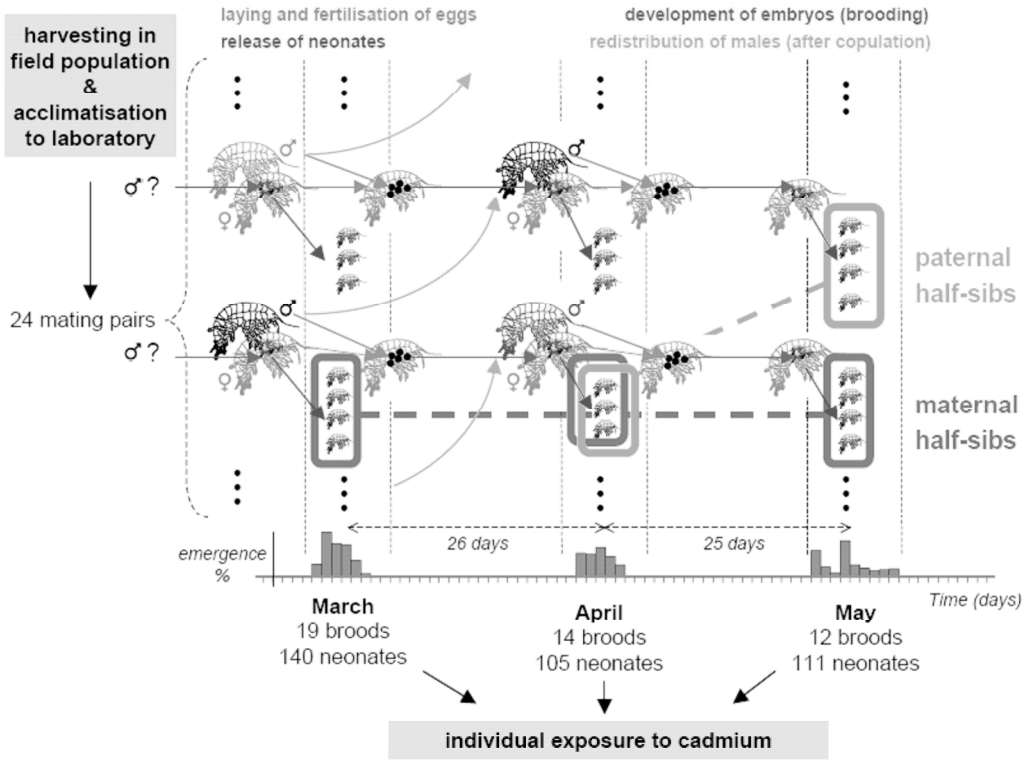
489


survival time (days)
1
3
5
7
7 9 7 2 11 9 11 6 15 6 5 4 8 4 7 11 6 5 16 8
20 broods

490


survival time (days)
1
3
5
7
14 6 6 7 15 8 20 11 8 2 6 5 5 8 6 6 3 5 9 9 4 6 11 16 4 9 5 10 8 9 9 13 6
33 broods

491


harvesting in field population & acclimatisation to laboratory
laying and fertilisation of eggs
release of neonates
development of embryos (brooding)
redistribution of males (after copulation)
♂ ?
24 mating pairs
♂ ?
paternal half-sibs
maternal half-sibs
26 days
25 days
emergence %
Time (days)
March
19 broods
140 neonates
April
14 broods
105 neonates
May
12 broods
111 neonates
individual exposure to cadmium

492