The initial tolerance to sub-lethal Cd exposure is the same among ten naïve pond populations of *Daphnia magna*, but their micro-evolutionary potential to develop resistance is very different

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Abstract

Genetic variation complicates predictions of both the initial tolerance and the long-term (micro-evolutionary) response of natural *Daphnia* populations to chemical stressors from results of standard single-clone laboratory ecotoxicity tests. In order to investigate possible solutions to this problem, we aimed to compare the initial sub-lethal tolerance to Cd of 10 naïve natural pond populations of *Daphnia magna* as well as their evolutionary potential to develop increased resistance. We did so by measuring reproductive performance of 120 clones, i.e. 12 clones hatched from the recent dormant egg bank of each of 10 populations, both in absence (Cd-free control) and presence of 4.4 µg Cd/L. We show that the *initial tolerance*, defined as the reproductive performance of individuals of the first generation exposed to Cd relative to that in a Cd-free control was not significantly different among the 10 studied pond populations and averaged 0.82 ± 0.04 over these populations. Moreover, these populations' initial tolerances were also not significantly different from the mean initial tolerance of 0.87 ± 0.08 at 4.0 µg Cd/L measured for a group of 7 often-used laboratory clones, collected from a range of European ecotoxicity testing laboratories. This indicates that the initial response of naïve natural pond populations to sub-lethal Cd can be relatively accurately predicted from ecotoxicity test data from only a handful of laboratory clones. We then used estimates of broad-sense heritability of Cd tolerance (H^2) - based on the same dataset - as a proxy of these populations' capacities to evolutionarily respond to Cd in terms of the development of increased *resistance*, which is here defined as the increase with time of the frequency of clones with a higher Cd tolerance in the population (accompanied with an increase of mean Cd-tolerance of the population above the initial tolerance). We show that the populations' estimated H^2 values of Cd-tolerance cover almost the entire theoretically possible range, ranging from not significantly different from zero (for five populations) to between 0.48 and 0.81 (for the five other populations). This indicates that, unlike the initial tolerance to Cd, the (long-term) micro-evolutionary response to Cd may be very different among natural pond populations. Therefore, we conclude that it may be very difficult to predict the long-term response of an unstudied population to chemical stress from tolerance data on a sample of other populations. It is therefore suggested that new methods for forecasting long-term responses should be explored, such as

the development of predictive models based on the combination of population-genomic and tolerance time-series data.

Key words

Daphnia; genetic adaptation; micro-evolution; lab-to-field extrapolation; heritability; chemical stress

Highlights

- Sub-lethal chronic Cd toxicity varies 14-fold between 7 laboratory clones of *Daphnia*
- Initial sub-lethal Cd tolerance is the same among 10 natural *Daphnia* populations
- Cd tolerance of natural populations can be predicted from data with laboratory clones
- Evolutionary potential under Cd stress varies strongly between populations

1. Introduction

Extrapolating results of laboratory ecotoxicity tests to predict effects of chemicals on natural populations in the field remains one of the big challenges in ecotoxicology and risk assessment (Forbes et al., 2001; Van den Brink et al., 2008). One reason for this is that standard ecotoxicity tests are often conducted with laboratory populations, which often harbor little genetic diversity (Medina et al., 2007; Barata et al., 1998). This is for instance the case with *Daphnia magna*, which is one of the most sensitive and most frequently used species in aquatic ecotoxicity testing (Von der Ohe and Lies, 2004). Indeed, most ecotoxicity tests with *D. magna* and other *Daphnia* spp. are performed with isoclonal populations of female individuals that are maintained in a laboratory culture through parthenogenetic reproduction. This situation contrasts profoundly with the fact that, in the field, *Daphnia* populations can be genetically highly diverse (De Meester et al., 2006). This contrast brings about two important problems for predicting the response of a naïve (i.e., previously unexposed) natural *D. magna* population that is challenged with a chemical stressor (Medina et al., 2007; Morgan et al., 2007; Klerks et al., 2011).

First, an ecotoxicity test with a single laboratory clone will usually not be predictive of the *initial* (i.e., *proximate*) response of a natural *Daphnia* population consisting of many genetically different clones (Barata et al., 1998; Barata et al., 2002a), with the *initial* response being defined throughout our paper as the effect of the chemical (relative to a control) on the individuals of the first exposed generation.

Second, under multi-generational exposure, genetically diverse natural *Daphnia* populations may exhibit natural selection of more tolerant clones, i.e. those clones that experience a smaller adverse effect of the chemical (relative to a control) (Ward and Robinson, 2005; Lopes et al., 2006). This may eventually result in selection-mediated increased *resistance* of the natural population, where increased *resistance* is defined throughout our paper as an increased frequency of occurrence in the population of clones with a higher tolerance to the chemical (i.e., *genetic adaptation*; Morgan et al. 2007). This is

91 a micro-evolutionary response that cannot be predicted from single-generation ecotoxicity tests with a
92 single laboratory clone.
93
94 A possible solution to these two problems would be to be able to make predictions of initial and
95 micro-evolutionary responses based on tolerances determined for a set of clones isolated from a set of
96 naïve natural populations. Indeed, some studies are suggestive that such an approach would enable at
97 least some broad predictions.
98
99 First, Hoffmann and Parsons (1991, 1997) postulated that differences in tolerance to toxicant stress
100 among populations should reflect local genetic adaptation to selective pressures experienced in their
101 local habitats. Populations from distinct habitats with no history of pollution with a toxicant (i.e. *naïve*
102 populations) are therefore expected to show similar levels of tolerance to that toxicant. This has been
103 confirmed by Barata et al. (2002a, 2002b), who reported similar reproductive effects of sub-lethal
104 exposure of natural *D. magna* populations to an insecticide (λ-cyhalotrin) and a metal (Cd) in a study
105 involving three and four populations, respectively. Thus, extrapolating initial responses to chemicals
106 from one natural population to another may provide reasonably accurate predictions
107
108 Second, given that, (i) standing genetic variation (e.g., when measured as heritability) of chemical
109 tolerance within a natural population is considered a valuable proxy of the micro-evolutionary
110 potential and may be used to calculate the rate of adaptation to chemical stress through natural
111 selection (Chaumot et al., 2009; Klerks et al., 2011; Messiaen et al., 2012) and (ii) that the heritability
112 of many traits is often similar among different populations of the same species (Visscher et al., 2008),
113 the micro-evolutionary response of naïve natural populations to chemical stress may indeed be
114 sufficiently similar to extrapolate observations with one population to other populations. Initial
115 support for this has been provided by Barata et al. (2002b), who reported similar broad-sense
116 heritability values (H^2) of sub-lethal (reproductive) Cd tolerance in two Cd-naïve *D. magna*
117 populations.
118

In the present study we aimed to build further on the earlier work of Barata et al. (2002a, 2002b), also using Cd as the model chemical, but using a much larger set of 10 naïve natural *D. magna* pond populations in order to address two main questions: (i) is the *initial* sub-lethal tolerance to a nominal concentration of 5 µg Cd/L (expressed as reproductive performance relative to a control) the same or different among 10 natural populations (i.e., is the *proximate* effect of a standardized exposure to a chemical similar or different among populations)?, and (ii) is the evolutionary potential (based on measurements of broad-sense heritability as its proxy) of sub-lethal Cd tolerance similar or different in these populations? The investigated populations were established from the dormant, ephippial egg bank from a broad variety of habitats in terms of their abiotic and biotic characteristics, and have been shown to differ substantially in genetic composition (Orsini et al., 2012). They were also confirmed to be naïve with respect to Cd exposure in the sense that all habitats are characterized by low to very low concentrations of Cd (Table 1).

In order to be able to discuss our observations in a broader regulatory context, we chose to perform our experiments at a regulatory relevant low sub-lethal effect level, by exposing the natural populations to a Cd-concentration close to the geometric mean of the 21-day 10% effective concentration based on reproductive performance (21d-EC10, see *Materials and Methods* for details) of 7 laboratory clones, collected from 7 different ecotoxicity testing laboratories across Europe. The geometric mean of multiple chronic (or sub-lethal) EC10s for the same species is often used in EU chemicals legislation as a basis for derivation of predicted no effect concentrations (PNEC) or environmental quality standards (EQS) of chemicals (ECHA, 2008; EC, 2011a). The results from these laboratory clones also provide information on the range of tolerances to a model toxicant among often-used laboratory clones.

2. Materials and Methods

2.1. Seven Daphnia magna laboratory clones

147 Several individuals from 7 different *D. magna* laboratory clones were shipped from different
148 ecotoxicity testing laboratories across Europe to the UGent laboratory (see *Supportive Information*,
149 Table S1, for details of the origin of the clones). One individual of each clone was randomly picked
150 out to establish our own in-house isoclonal cultures of these clones. These in-house cultures were first
151 established in April 2009, which allowed the clones ample time to acclimate to our in-house culture
152 conditions (see further) before actual experiments started in October 2009.
153
154 ***2.2. Clones from ten natural Daphnia magna populations***
155 The recent dormant egg bank (ephippial eggs) of 10 pond populations in Flanders (Belgium)
156 was sampled between January and March 2007 by collecting the upper 2 centimeters of the sediment
157 using a sediment corer. The genetic diversity of the dormant egg bank of *Daphnia* spp. at the sediment
158 surface layer is commonly considered to be representative of the genetic diversity of the actual (live)
159 population that is established by hatching of ephippial eggs at the start of a new growing season (e.g.,
160 Wolf and Carvalho, 1989; Antunes et al., 2003; Orsini et al., 2012).
161
162 Seven ponds were located in the province of Flemish-Brabant (near Leuven) and three ponds
163 were located in the province of Western Flanders (near Knokke). Details on location and
164 characteristics of each pond are provided in Table 1 and as *Supportive Information* (Table S2).
165
166 The investigated ponds represented a broad variety of habitats in terms of their biotic and abiotic
167 characteristics (Orsini et al., 2012). In addition to those characteristics, we also sampled water and
168 sediment from the ponds to determine metal concentrations, to ensure that the ponds were not
169 contaminated with Cd (or other metals) (see 2.5. for analytical methods). Comparison of Cd measured
170 in water and sediment with natural background Cd concentrations confirmed the absence of Cd
171 pollution in all ponds (Table 1), which was already expected based on the absence of important
172 sources of Cd in the vicinity of the ponds. Furthermore Cd concentrations in the water are in all ponds
173 below the hardness-corrected EQS values (derived following EC, 2005), meaning that no adverse
174 effect of Cd on ecological structure and function is expected in either of the ponds (Table 1).

175 Comparison of other metal concentrations (Zn, Ni, Cu, Pb) in water and sediment with background
176 concentrations and with dissolved organic carbon (DOC) normalized HC5 values (hazardous
177 concentration for 5% of the species) for Zn, Ni, Cu (Zwolsman and De Schamphelaere, 2007) or with
178 EQS for Pb (EC, 2011b) suggests very limited to no metal pollution and no metal-induced ecological
179 effects in any of the ponds (See *Supportive Information*, Table S2). In addition, total Cd
180 concentrations in water (0.012 to 0.099 µg/L, Table 1) are well below the geometric mean hardness-
181 corrected 21d-EC10 for laboratory clones (0.8 to 11.2 µg/L, Table 2), suggesting that the current (low)
182 Cd exposure in the ponds is expected not to affect *D. magna* reproductive performance in the ponds.
183
184 Cladoceran dormant eggs were isolated by means of the sugar flotation method (Onbe, 1978; Mareus,
185 1990). Briefly, sediment was transferred together with an oversaturated sugar solution (1000 g sugar in
186 1000 mL of distilled water) into 50 mL Falcon tubes. These tubes were centrifuged (10 minutes at
187 3000g) and decanted twice. Most ephippial eggs then floated in the decanted sugar solution and were
188 easily isolated. The remaining sediment in the tubes was inspected visually and any remaining
189 dormant eggs were picked out manually. All isolated *D. magna* eggs were put individually in ADaM
190 medium (Aachener Daphnien Medium; Klüttgen et al., 1994) in a climate room at 20°C and under a
191 16:8 light:dark photoperiod. Medium was refreshed every 8 to 9 days and hatchlings were isolated
192 daily. A single hatchling from each ephippium was selected to establish a clonal lineage (Ebert et al.,
193 1993). As dormant eggs of *D. magna* are produced by sexual reproduction, each clonal lineage
194 hatched from an ephippium can be considered genetically distinct (Barata et al., 2000). Clonal lineages
195 were first maintained at KULeuven in 300 mL of tap water and were fed two times a week with 10^8
196 cells of *Scenedesmus obliquus*. In December 2008, twelve randomly selected clones from each of the
197 ten natural pond populations (120 clones in total) were transported to UGent for establishing in-house
198 cultures of these clones, which were maintained until the actual experiments.
199
200 ***2.3. Maintenance cultures of D. magna clones***
201 The 7 laboratory clones and the 120 clones hatched from dormant egg banks were maintained at 20°C
202 and under a light:dark photperiod of 16h:8h. The culture medium was a modified M4 medium, which

203 is different from the original composition of M4 medium (Elendt and Bias, 1990) as follows:
204 Na_2EDTA and $FeSO_4$ were omitted and replaced with natural dissolved organic matter (DOM). The
205 DOM was collected from a small creek (Ruisseau de St. Martin, Bihain, Belgium) using a portable
206 reverse osmosis system (PROS/2) (Sun et al., 1995). This modified M4 medium has a hardness of 250
207 mg $CaCO_3$/L, a pH of 7.6, and a concentration of dissolved organic carbon (DOC) of 4 mg/L.
208 Individuals of each clone were kept in polyethylene vessels in 50 mL of M4 medium. Once every
209 week, 1 or 2 juveniles and 1 or 2 adults (daphnids carrying parthenogenetic eggs) of each clone were
210 transferred to fresh medium. Each clone was fed daily with a 3:1 mixture (based on cell numbers) of
211 the algae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*, at an amount of 500 µg
212 dry wt per day and per 50 mL. Maintenance of all clones continued in this way until October 2009,
213 when the actual experiment was initiated.
214
215 ***2.4. Experimental design***
216 The experiments with the laboratory clones and the clones hatched from dormant egg banks were
217 identical with only two exceptions. First, while each laboratory clone was exposed to 6 treatments (5
218 Cd concentrations and a control), only two treatments (1 Cd concentration and a control) were
219 imposed on the clones hatched from dormant egg banks. Second, while 6 individuals of each
220 laboratory clone were tested in each treatment, only 3 individuals were used in each treatment for each
221 clone hatched from natural dormant egg banks.
222 From each clonal lineage, a single, randomly selected third- or fourth-brood juvenile (<24h old) from
223 the maintenance culture was put individually in 50 mL of modified M4 medium without added Cd as
224 the F0 grandmother generation. Following maturation of this individual, six (for the laboratory clones)
225 or three (for the clones hatched from dormant eggs) of its third- or fourth-brood offspring (<24h old)
226 were put individually in 50 mL of modified M4 medium without added Cd and were allowed to
227 mature to F1-mothers. From each of these mother individuals, one third- or fourth-brood juvenile
228 (<24h old) (=F2 experimental generation) was randomly assigned to one of the six (laboratory clones)
229 or two treatments (natural population clones). As such, every replicate in each treatment was
230 represented by a single F2-individual, produced by a different F1-mother. In this way we minimized

interference from maternal effects that would otherwise potentially inflate our estimates of genetic variance of traits within populations (Lynch and Walsh, 1998; Messiaen et al., 2012) (see also 2.5.5).

Life-table experiments with all F2-individuals, clones and treatments were initiated simultaneously to avoid temporal effects. All exposures were conducted in modified M4 medium (one individual per test vessel) at 20°C and under a 16h:8 light:dark photoperiod. Laboratory clones were investigated in six treatments, a control (no Cd added) and nominal Cd concentrations of 1, 2.2, 4.6, 10 and 22 µg Cd/L. Clones hatched from dormant egg banks were investigated in two treatments, a control and a nominal Cd concentration of 4.6 µg Cd/L. The latter concentration was chosen with regard to the aim of the present study to be able to interpret our observations in a broader regulatory context (see *Introduction*), because preliminary experiments had indicated that this concentration was close to the concentration that caused an average 10% reduction of reproductive performance in the 7 laboratory clones. The modified M4 medium was always spiked with the desired Cd concentration (added as $CdCl_2 \cdot H_2O$) 24h to 48h prior to transfer of the daphnids into the medium.

Throughout the entire experiment (P, F1, and F2 generation), organisms were fed daily with a 3:1 mixture (based on cell numbers) of the algae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* equivalent to 250 µg dry wt/*Daphnia*, 500 µg dry wt/*Daphnia* and 750 µg dry wt/*Daphnia* in the first, second and third week of their life, respectively. The medium was renewed completely three times a week (Monday, Wednesday, Friday).

Each individual of the F2-generation was monitored during the life-table experiment for 21 days. Survival and the number of juvenile offspring were recorded daily. During the life-table experiment, samples of new (fresh) and old medium were taken at regular intervals for analysis of filtered (0.45µm) Cd (using graphite-furnace AAS) and DOC concentrations (using Shimadzu TOC analyzer).

2.5. Data analysis

2.5.1. Intrinsic rates of increase

Intrinsic rates of increase (r_m) were calculated per individual replicate from age-specific fecundities recorded during the life-table experiment, by fulfilling the condition (Caswell, 2001):

257 $\sum_{x=1}^{21} F_x e^{-r_m x} = 1$ (Eq. 1)

258 Where x = the number of days since the start of the life-table experiment, F_x = age-specific fecundity
259 (i.e. number of live offspring recorded on day x). Replicates holding a male F2 individual or in which
260 no reproduction occurred (mostly due to parent mortality) were excluded from analysis, as the r_m
261 equals -∞ in these cases. An r_m calculated in this way for *Daphnia* spp. was called 'reproductive
262 performance' by Jansen et al.(2011) and Van Doorslaer et al. (2009). We choose to work with r_m
263 because this is considered a relevant measure of fitness in parthenogenetically reproducing *Daphnia*
264 spp. populations, at least under non-limiting conditions (Lynch and Walsh, 1998; Hooper et al., 2008).
265
266 *2.5.2. Tolerance*
267 Since F2 individuals from each clone in each Cd treatment always had a 'sister' that was exposed to
268 the control (i.e. they shared the same F1 mother, see 2.4 *Experimental Design*), we were able to
269 determine replicate "observations" of tolerance for a given Cd treatment based on r_m values for such
270 'sister' pairs, of which one was exposed to Cd and the other to the control:
271
272 $\text{Tolerance(Cd)} = r_m(\text{Cd}) / r_m(\text{control})$ (Eq. 2)
273
274 As such, for each Cd treatment, a number of tolerance "observations" was available equal to the
275 number of replicates per treatment (and equal to the number of F1 mothers used per clone, see 2.4).
276
277 *2.5.3. Further data analysis with results of laboratory clones*
278 All analyses started from calculated tolerances (See Eq. 2). Second-order polynomial regression was
279 used to calculate 21d-EC10 values for each clone (Barata et al., 2002b), except for the K6 clone where
280 a linear regression was more appropriate. Averages of measured dissolved Cd concentrations were
281 used as the independent variable; observed tolerances as the dependent variable. The jack-knife
282 method was used to estimate approximate 95% confidence limits. The regression analyses was
283 performed with Statistica 7 software (Statsoft, Tulsa, OK, USA).

284

285 *2.5.4 Further data analysis with natural populations (population means)*

286 To test the hypothesis that naïve populations have similar mean (initial) *proximate* tolerances, we
287 compared Cd tolerances among populations. To this end, we first calculated the clone-mean r_m and Cd
288 tolerance for each clone (as the mean of three replicate observations). All further statistical analyses
289 and comparisons were performed using these clone means as the dependent variables. In order to test
290 if exposure to 4.6 µg Cd/L had a significant initial (proximate) effect on each of the 10 natural
291 populations (compared to the control), we performed a t-test for dependent samples ($p<0.05$). In case
292 the normality assumption was not met (Shapiro-Wilkinson W, $p<0.05$), the non-parametric alternative,
293 i.e. the Wilcoxon matched pairs test, was conducted. The Cd-tolerance was statistically compared
294 among the ten populations with the Kruskall-Wallis test ($p<0.05$).

295

296 *2.5.5. Further data analysis with natural populations (heritabilities and evolutionary potential)*

297 Heritability, a simple dimensionless measure of the importance of genetic factors in phenotypic
298 differences between individuals, enables predictions about the response to selection in populations and
299 can be compared among populations (Visscher et al., 2008). As *Daphnia* reproduce asexually
300 (parthenogenetically) most of the year, clonal selection is a strong factor in *Daphnia* microevolution
301 and broad-sense heritability (H^2) is an appropriate parameter to determine the potential short-term
302 response to (clonal) selection in natural populations (Ebert et al., 1998; Stirling and Roff, 2000). High
303 heritability of tolerance traits in *Daphnia* predicts the capacity for rapid evolution by clonal selection
304 (Messiaen et al., 2012). Because both fitness (reproduction, survival) under chemical stress itself (e.g.
305 Messiaen et al., 2012; Chaumot et al., 2009; Klerks and Moreau, 2001), as well as relative tolerance
306 (defined as relative fitness under chemical stress compared to a control, see Eq. 2, as in Barata et al.,
307 2002b) have been put forward as useful tolerance traits for predicting evolutionary potential, we
308 considered both in the present study. Relative tolerance as defined here is equivalent to a slope of a

reaction norm (Stirling and Roff, 2000) and heritability of relative tolerance as defined here is therefore equivalent to heritability of plasticity, reflected in a significant genotype by environment interaction (Stirling and Roff, 2000). Replicate observations of r_m of all clones were used to determine broad sense heritability (H^2) of r_m(control), r_m(Cd) and of Cd-tolerance following Messiaen et al. (2010). For each population, the genetic variance (V_G) and the environmental (or residual) variance (V_E) were estimated from the observed among-clone (MS_C) and within-clone mean squares (MS_E), using the method of the moments with appropriate accounting for unequal sample sizes among clones, as follows (Table 18.1 in Lynch and Walsch, 1998):

$$MS_C = \frac{\left[\sum_{i=1}^{N} n_i(\bar{z}_i - \bar{z})^2\right]}{(N-1)} \qquad \text{(Eq. 3)}$$

$$V_E = MS_E = \frac{\left[\sum_{i=1}^{N}\sum_{j=1}^{n_i}(z_{i,j} - \bar{z}_i)^2\right]}{(T-N)} \qquad \text{(Eq. 4)}$$

$$V_G = (MS_C - MS_E) / n_0 \qquad \text{(Eq. 5)}$$

$$n_0 = \frac{\left[T - \left(\sum_{i=1}^{N} \frac{n_i^2}{T}\right)\right]}{(N-1)} \qquad \text{(Eq. 6)}$$

where T = the total number of observations for the population, N = the number of clones studied for the population, n_i = the number of replicates for the i^{th} clone, $z_{i,j}$ is the observed value for the j^{th} replicate of the i^{th} clone, $\bar{z}_i$= the mean of all (n_i) observed values for the i^{th} clone, $\bar{z}$ = the mean of all (T) observed values for the population, and n_0 is a weighted number of replicates per clone to account for unequal sample size among clones in the calculation of V_G (Searle et al., 1992).

H^2 was calculated as V_G / (V_G+V_E). Construction of confidence intervals and hypothesis testing was performed using non-parametric bootstrap re-sampling (5000 samples) with replacement of clones (Lynch and Walsh, 1998; Messiaen et al., 2010). If in a run the V_G turned out to be negative, it was set to zero for further calculations (Lynch and Walsh, 1998). The median values (50^{th} percentile) and the 5^{th} and 95^{th} percentile of H^2 are reported for r_m(control), r_m(Cd) and for tolerance. Statistical tests were then constructed using the bootstrap output. First, if more than 95% of the bootstrap samples yielded

an $H^2>0$ (equivalent to a one-sided test at $p<0.05$ level), we considered that there was an H^2 significantly >0, reflecting significant evolutionary potential. Second, if more than 95% of the bootstrap samples yielded $H^2(Cd) > H^2(control)$ (equivalent to a one-sided test at $p<0.05$ level), we considered the H^2 in the Cd treatment to be statistically significantly higher than in the control. Third, when more than 97.5% of the bootstrap samples yielded a higher (or lower) H^2 for one population than for another, those two populations were considered to have a statistically different H^2 value for the trait considered (equivalent to a two-sided test at $p<0.05$ level). All calculations were performed in MATLAB 7.5.0.342 software (Mathworks Inc).

It has been argued that the interpretation of H^2 is complicated by the fact that it depends both on genetic variance (V_G) and environmental (residual) variance (V_E) in the observations, as $H^2=V_G/(V_G+V_E)$ (Klerks et al., 2011). Likewise, it has also been argued that contaminant-driven genetic erosion by directional selection is more likely in populations with a combination of high V_G and low V_E of tolerance to the contaminant (Ribeiro and Lopes, 2013). However, in our study, when considering the results of all populations together, H^2 is strongly correlated with V_G and not with V_E (Figure S12) indicating that any differences found in H^2 among populations mainly have a genetic cause and are not an artifact of uncontrolled differences in environment between replicates or residual experimental error. For this reason, we only report and discuss H^2 (and not V_G or V_E) in the present paper.

2.3. Metal concentrations in water and sediment of the study ponds

In addition to those pond characteristics already recorded and reported previously (Orsini et al., 2012), we sampled water and sediment from the ponds in April 2009 to determine metal concentrations. The upper layer (approximately 10 cm) of the sediment was sampled to determine Ni, Cu, Pb, Zn and Cd. Sediment was acid-digested with the aid of a microwave oven. Ni, Cd, Cu, Zn and Pb were analyzed using flame AAS (Spectra AA 100-Varian) or a graphite furnace AAS (Zeeman, Spectra AA300-Varian). To determine Cu, Ni, Pb, Cd, Na, Ca and Mg concentrations in the water, triplicate samples of 50 mL were collected into Falcon tubes, which had been acid washed and rinsed three times with

359 pond water at each location. Samples were centrifuged for 15 minutes at 2000g in the lab (Centra 8,
360 Thermolife Sciences, Belgolab) and the total concentration of Cu, Ni, Pb, Cd, Ca and Mg in the
361 supernatant were measured with ICP-MS (inductive coupled plasma mass spectrometry, Perkin-Elmer
362 Elan DRC-e, Wellesley, MA, USA).

363 **3. Results**

364 ***3.1. Cd tolerance of seven laboratory clones***

365 Details of the chemical analyses in the exposures of the seven laboratory clones are reported as
366 *Supportive Information* (Table S3). Across all Cd treatments (including the control), pH was on
367 average 7.7 (range 7.6-7.9) and DOC was on average 5.3 mg/L (range 4.6-6.0 mg/L). In fresh medium,
368 measured dissolved Cd was between 84% and 90% of the nominal concentration. Measured dissolved
369 Cd in old medium was 4% to 31% lower than in fresh medium. At the nominal Cd concentration of
370 4.6 µg/L, the mean dissolved Cd concentration was 4.0 µg/L.

371

372 Table 2 shows the differences in Cd tolerance among the seven laboratory clones. Detailed
373 concentration response data and fitted concentration response models are presented as *Supportive*
374 *Information* (Figure S1). The 21d-EC10s for the laboratory clones varied 14-fold between 0.8 (clone
375 K6) and 11.2 µg/L (clone SE), with a geometric mean of 3.7 µg/L. In the 4.0 µg Cd/L treatment, i.e.
376 within less than 10% of the geometric mean EC10 of 3.7 µg Cd/L, the observed Cd tolerance varied
377 between 0.74 (Clone K6) and 0.96 (clone SE), with a mean of 0.87 (S.D. 0.08, n=7).

378

379 ***3.2. Fitness and Cd tolerance of 10 field populations***

380 Details of the chemical analyses made during the exposures of the 10 field populations are reported as
381 *Supportive Information* (Table S4). The pH was on average 7.7 (range 7.6-7.8) and DOC was on
382 average 5.7 mg/L (range 4.0-7.6 mg/L). At the nominal Cd concentration of 4.6 µg/L, the mean
383 dissolved Cd concentration was 4.4 µg/L. Measured dissolved Cd in old medium was 9% lower than
384 in fresh medium.

385

386 Figure 1 depicts the mean r_m under the control (<0.1 µg Cd/L) and the Cd environment (4.4 µg Cd/L)
387 of each individual clone within each population (reaction norms) and also each population mean
388 (mean of clone means). Figure 2 depicts the clone means and population means of Cd tolerance. Table
389 S5 in *Supportive Information* provides the population mean values corresponding with these figures.
390 The r_m were significantly lower at 4.4 µg Cd/L than in the control for each of the 10 populations, using

391 a paired analysis and using clone identity to pair data ($p<0.05$ by t-test for dependent samples or
392 Wilcoxon Matched Pairs test, details in *Supportive Information,* Table S6). The population means of
393 Cd tolerance were all between 0.75 (KNO17 and TER2) and 0.87 (TER1) (average ± S.D.: 0.82 ±
394 0.04), which corresponds to a reduction of the r_m between 13% and 25% (Figure 2, Table S5).
395
396 Across all populations, observed tolerances per clone ranged between 0.17 (most sensitive clone in
397 LRV) and 1.13 (least sensitive clone in KN052) (Figure 2). Cd tolerances (clone means) were not
398 normally distributed in 5 of 10 populations (i.e., in LRV, OHZ, OM3, TER1 and ZW4; see *Supportive*
399 *Information*, Table S7) (Shapiro-Wilkinson W, $p<0.05$). As the null-hypothesis of homoscedasticity
400 (equal variances) was not rejected (Levene, $p>0.05$), but as none of the classic transformations
401 (logarithmic, inverse, square) were able to remediate the non-normality issue, an appropriate non-
402 parametric test under these conditions to test for differences in tolerance among populations is the
403 Kruskall-Wallis test. This test did not reveal any significant differences of the mean population
404 tolerance among the populations ($n=106$, $df=9$, $p=0.715$). Other statistical testing alternatives, albeit
405 less robust ones for our dataset, lead to an identical conclusion (See *Supportive Information*, SI8). This
406 leads to the conclusion that there are no statistically significant differences in mean sub-lethal Cd
407 tolerance among the 10 natural pond populations.
408
409 ***3.3. Comparison of tolerance of natural pond populations with 'population' of laboratory clones***
410 None of the 10 field populations showed a significantly different mean Cd tolerance at around 4 µg/L
411 compared to the group of laboratory clones (i.e., the mean of the collection of seven laboratory
412 clones). This conclusion is based on the result of t-tests for independent samples with p-values
413 between 0.159 and 0.963 or of Mann-Whitney U tests (in case of non-normality or unequal variance)
414 with p-values between 0.135 and 0.735 (see *Supportive Information*, Table S9 for details).
415
416 ***3.4. Heritability of r_m and Cd tolerance of 10 natural pond populations***
417 A broad range of median estimates of H^2 was observed across populations for r_m under control and Cd
418 exposure (Figure 3) and for Cd tolerance (Figure 4) (see *Supportive Information*, Table S10 for all

values). In the control $H^2(r_m)$ varied between -0.59 (OHZ) and 0.75 (KNO17) across all ten investigated populations, with a mean (± S.D.) of 0.36 ± 0.39. In the Cd treatment $H^2(r_m)$ varied between 0.23 (KNO15) and 0.85 (KNO17), with a mean of 0.60 ± 0.21. $H^2(r_m)$ in the Cd environment was significantly higher than 0 for seven populations, and for five of those populations $H^2(r_m)$ was also significantly higher than 0 in the control environment (with $H^2(r_m)$ between 0.44 and 0.75) (Figure 3, Table S10). For most populations (except OHZ and TER2) median estimates of H^2 were similar between the control and Cd environment (Figure 3). Considering all populations together, H^2 shows no significant trend of being higher in the Cd environment than in the control (Wilcoxon matched pairs test, p=0.074, n=10). When populations are considered separately, only in the TER2 population the H^2 in the Cd environment is significantly higher than in the control (non-parametric bootstrapping, p=0.011). Pair-wise comparisons of the H^2 values between populations but within the same environment, revealed significant differences for 8 of 55 possible population pairs in the control environment (OHZ differs from 8 other populations except TER2) and for 4 population pairs in the Cd environment (i.e., KNO15-KNO17, KNO15-TER2, KNO17-KNO52, and KNO17-OM2) (All comparisons performed with non-parametric bootstrapping, $p<0.05$, see *Supportive Information*, Table S11 for all pair-wise p-values).

Across all ten populations, H^2 of Cd tolerance varied between 0.11 (KNO52) and 0.81 (TER2), with an average of 0.49 ± 0.26. We found that H^2 of Cd tolerance was significantly higher than 0 in 5 of these 10 populations (with H^2(Cd-tolerance) between 0.48 and 0.80) (Figure 2). Each of these five populations also had $H^2(r_m)$ significantly higher than 0 in the Cd environment. Pair-wise population comparisons revealed significant differences of H^2(tolerance) between six population-pairs, i.e. KNO15-KNO17, KNO17-KNO52, KNO17-OM2, KNO52-OM3, KNO52-TER2, and OM2-TER2 (Non-parametric bootstrapping, $p<0.05$, see *Supportive Information*, Table S11 for all pair-wise p-values).

445 **4. Discussion**

446 The cyclical parthenogen *Daphnia magna* is one of the most frequently-used model organisms in
447 ecotoxicology and for risk assessment of chemicals. Yet, genetically determined variation of chemical
448 tolerance traits among parthenogenetically (asexually) reproducing clones complicates predictions of
449 ecologically realistic responses of natural *D. magna* populations from results of typical laboratory
450 ecotoxicity tests, which are usually conducted with a single laboratory clone (Barata et al., 2002a,
451 Messiaen et al., 2010). In this context, our study provides three pieces of information that are
452 important in the context of the implications of this issue for ecologically relevant risk assessment, each
453 of which will be discussed below.

454

455 First, we found a 14-fold difference in 21d-EC10 values of Cd among 7 *D. magna* laboratory clones
456 maintained in ecotoxicity testing laboratories across Europe, with 21d-EC10 values of these clones
457 ranging between 0.9 and 11 µg Cd/L (Table 2). This observed inter-clonal variation is in line with and
458 even slightly larger than the earlier-reported 4 to 10-fold inter-clonal variation of sub-lethal toxicity of
459 a variety of substances (Cd, Cu, NaBr, fluoranthene, dichloro-aniline, parathion, λ-cyhalotrin) among
460 laboratory clones of *D. magna* based on feeding or reproductive traits (Baird et al., 1990; Soares et al.,
461 1992; Barata et al., 2000). Our finding reinforces the statement of Barata et al. (2002a) that the
462 conclusions from a risk assessment (or the derivation of water quality criteria) for a given chemical,
463 based on chronic ecotoxicity test data with only one *D. magna* clone, may be strongly dependent on
464 the clone that was tested. Although risk assessment using data from a single clone is a crucial first
465 step, our and earlier observations provide strong arguments for the implementation of multiple clone
466 testing.

467 Second, we found no significant differences of the sub-lethal Cd tolerance at 4.6 µg Cd/L among 10
468 Cd-naïve field populations, with reductions of r_m in all populations ranging between 13% and 25%,
469 relative to the control (Figure 2, Table S5). This result supports and extends earlier findings of small
470 differences (<1.6 fold) in reproductive EC10-values for Cd and λ-cyhalotrin between 3 natural *D.*
471 *magna* populations (Barata et al., 2002a) and of small, non-significant differences in effects of Cd on

472 fitness among 4 natural *D. magna* populations exposed to a sub-lethal Cd concentrations between 0.5
473 and 2 µg/L (Barata et al., 2002b). In both these earlier studies and ours, all populations were collected
474 from habitats with no indication of current or historical Cd pollution (see Table 1, see 3.2). As
475 opposed to what has been reported for sub-lethal toxicity, more and a wider variety of results have
476 been reported for exposures of naïve *D. magna* populations to lethal chemical concentrations. On the
477 one hand, small (1.6-fold) and insignificant differences of acute copper and zinc toxicity (measured as
478 median effective concentrations) have been found among two and three natural *D. magna* populations,
479 respectively (Bossuyt et al., 2004; Muyssen et al., 2005). On the other hand, other studies did find
480 significant inter-population differences of lethal toxicity. Barata et al. (2002b) found significant inter-
481 population differences in longevity, ranging between about 3 and 10 days, among 4 naïve *D. magna*
482 populations when exposed to 10 µg Cd/L. Coors et al. (2009) found 2.1-fold differences in acute 48h-
483 EC50s of $K_2Cr_2O_7$ among 10 *D. magna* pond populations, and these were also significant. Finding the
484 explanation for this difference in among-population observation between lethal and sub-lethal toxicity
485 is an interesting avenue for further research.
486
487 In addition, we found that none of our ten study populations appeared to show a different mean
488 tolerance when compared with the mean tolerance of the group of 7 laboratory clones when exposed to
489 the same Cd concentration of 4.6 µg Cd/L (Table S9). Collectively, this means that the mean sub-
490 lethal tolerance of naïve natural populations to Cd (a measure of the *proximate* or *initial* relative
491 response to Cd, see *Introduction*) could be relatively accurately predicted by the mean tolerance of a
492 collection of only a hand-full of often-used ecotoxicology laboratory clones. If this finding would be
493 confirmed for a broader range of toxicants, it could clearly aid in the improvement of lab-to-field
494 extrapolation in risk assessment practice, at least for naïve populations. This would be an important
495 improvement of the current situation, where risk assessment continues to be dominated by largely
496 arbitrary assessment factors (e.g., EC, 2011; ECHA, 2008).
497
498 Third, while all populations exhibit a similar mean tolerance and are thus all predicted to experience a
499 similar *proximate* reduction of population mean r_m when exposed to Cd, the within-population genetic

variation of sub-lethal Cd-tolerance traits (i.e., r_m(Cd) and tolerance), measured as broad-sense heritability (H^2), does show significant differences among some of these populations (Figure 3, Figure 4, Table S11). Thus, several populations show genetic variation in Cd tolerance, and the degree they do so differs among populations. This finding with Cd as the stressor is in line with Ebert et al. (1998), who reported - for a set of four *D. magna* populations - significant within-population genetic variation of several traits that are indicative of tolerance to a bacterial parasite stressor. The finding of differences in H^2 values among populations predicts different capacities for micro-evolutionary responses among populations upon exposure to Cd. Based on whether or not H^2 values of r_m(Cd) or of relative Cd-tolerance were significantly greater than zero, we can broadly classify populations in three groups (Table S10). Below, we describe expected micro-evolutionary effects of Cd for each of these in terms of the potential for *resistance* development (as defined in the introduction) and changes in genetic (clonal) composition.

A first group of populations (KNO17, LRV, OM3, TER1, TER2) all exhibit significant genetic variation of Cd-tolerance (Figure 4, Table S10), with H^2 values between 0.48 and 0.81 (mean ± sd: 0.70 ± 0.13). This means that in group I, inter-individual variation of relative Cd-tolerance is significantly, and to a large extent (48%-81%) determined by genetic factors and that the relative Cd-tolerance trait (as defined in Eq. 2) can respond to selection (Klerks et al., 2011; Visscher et al., 2008). All these populations also exhibit a significant H^2 of r_m(Cd), with H^2 values between 0.52 and 0.85 (mean ± sd: 0.74 ± 0.13) (Figure 3, Table S10), and their clone means for Cd-tolerance are highly significantly and positively correlated with the clone means for r_m(Cd) (product-moment correlations $r=0.91$-0.98, $p<0.001$). As a result, multi-generational exposure of these populations to Cd is expected to lead to increasing frequencies of clones with higher Cd-tolerance and, thus to an increasing mean population Cd-tolerance (i.e., increased resistance *sensu* Morgan et al., 2007). Overall, this first group of populations also exhibits a higher H^2 of r_m(Cd) compared to H^2 of r_m(control) (Wilcoxon matched-pairs test $p=0.043$). This suggests, that these populations may show more rapid changes in genetic (clonal) composition in the presence than in the absence of Cd and that they may also show faster clonal erosion (reduction of clonal diversity) in the presence than in the absence of Cd (Van Overbeke

528 and De Meester, 2010; see also Prugnolle et al., 2005). Barata et al. (2002b) also reported significant
529 H^2 of sub-lethal Cd-tolerance and of fitness under 0.5 to 2.0 µg Cd/L exposure in two natural, naïve *D.*
530 *magna* populations and also concluded that there was a strong potential in these populations to select
531 for more Cd-tolerant genotypes (clones) under Cd exposure, and thus to evolve resistance to Cd stress.
532
533 In contrast to Barata et al. (2002b), however, in our larger survey of populations we have also
534 observed populations that do show different characteristics (Figure 3, Figure 4, Table S10). Indeed, a
535 second group of populations in our study (comprising ZW4 and OM2) exhibit no significant H^2 of Cd-
536 tolerance, but they do show significant H^2 of r_m(Cd) and of r_m(control) (Figure 3, Figure 4, Table S10).
537 This implies that these two populations have the capacity to show micro-evolutionary responses in r_m
538 both in the Cd and the control environment, but that development of *resistance* (increasing frequency
539 of more Cd-tolerant clones) under Cd exposure is not expected. In addition, changes in genetic
540 composition and reduction in clonal diversity in these two populations are expected to take place at a
541 similar rate, based on similar median estimates and non-significant differences of $H^2(r_m)$ between the
542 control or the Cd environment (Figure 3, Tables S10 and S11). A third group of populations (KNO15,
543 KNO52, and OHZ) show no significant heritabilities in any of the traits (Figure 3, Figure 4, Table
544 S10). Our results therefore indicate that these population have no or at most very low evolutionary
545 potential to genetically respond to Cd exposure.
546
547 Collectively, while all naïve populations studied showed similar mean *initial* (*proximate*) sub-lethal
548 tolerance to Cd in our experiments, our results also indicate that they may differ substantially in their
549 capacity to evolutionarily respond to long-term, multi-generational exposure to Cd, in terms of the rate
550 of change in clonal composition and/or in terms of the development of genetically determined
551 *resistance*. Overall, in half of the studied populations (i.e. those in the second and third group of
552 populations, as discussed in the paragraph above) the capacity to develop increased Cd tolerance under
553 long-term Cd stress is weak. Together with the observation that the mean *initial* Cd tolerance of all
554 field populations is similar to the mean tolerance of 7 laboratory clones, this means that the long-term
555 effect of Cd (relative to a control) in these five populations could be relatively accurately predicted

556 from the mean tolerance of only a handful of laboratory clones. However, this is clearly not the case
557 for the other half of the studied populations that do show a strong evolutionary capacity to develop
558 increased tolerance. In addition, it is noted that the observed H^2 values of tolerance cover a large
559 portion of the theoretically possible range of H^2 values, i.e. they range from non-significantly different
560 from zero (for five populations) to between 0.41 and 0.81 (for the other five populations). It will
561 therefore be difficult or even impossible to predict the long-term, micro-evolutionary response of
562 unstudied naïve populations from data on tolerance (and genetic variation thereof) obtained with a
563 sample of other populations. Thus, we suggest that other approaches will likely be needed to forecast
564 long-term effects of chemical on field populations, such as the development of predictive models
565 based on the combination of long-term time-series of population-genomic (DNA sequences) and
566 tolerance data, e.g. as they are archived in dormant egg banks (see Orsini et al., 2013 for details).

5. Conclusions

We observed that 21d-EC10s of 7 often-used laboratory clones of *D. magna* varied 14-fold (between 0.9 and 11 µg Cd/L, with a geometric mean of 3.7 µg/L). This indicates that risk assessment of chemicals and derivation of EQS should preferably be based on ecotoxicity test results from multiple clones rather than from single clones. Further, when exposed to a concentration of 4.4 µg Cd/L, which is close to the geometric mean EC10, ten naïve natural pond populations of *D. magna*, collected from a wide variety of habitats, did not exhibit any significant difference in their mean sub-lethal, reproductive tolerance (with observed mean tolerances ranging between 0.75 and 0.91). Furthermore, the tolerance of none of these ten populations differed from the mean tolerance of the 7 laboratory clones (which was 0.87). Together, this means that the mean initial (*proximate*) response of an unstudied naïve natural *D. magna* population to Cd exposure (relative to a control) can be predicted relatively accurately from the mean tolerance of another naïve natural population or from the mean tolerance of only a handful of investigated laboratory clones. This makes risk assessment using a multiple clone approach feasible and relevant, at least to predict initial effects of pollutants. For longer-term effects that also involve the possibility of micro-evolutionary adaptation, we find that predictions will be much more difficult or even impossible, because evolutionary potential was shown to differ substantially among populations. Thus, evolutionary potential should preferably be measured for each focal study population separately and different approaches for forecasting micro-evolutionary responses of naïve natural populations to chemical exposure should be explored, such as the development of predictive models based on the combination of long-term genomic and tolerance time-series data.

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753 **TABLES FOR MAIN MANUSCRIPT**

754 **Table 1 Overview of natural pond populations and some habitat characteristics**

Pond ID	Lat (N)	Long (E)	Hardness (mg $CaCO_3$/L)	EQS(dissolved Cd)[1] (µg/L)	Total Cd in H2O (µg/L)	Total Cd/ Dissolved EQS	Total Cd / Total bgc [2]	Cd in sediment mg kg^{-1} dry wt	Cd(sed)/bgsed[3]
KNO15	51°20'05.52"	03°20'53.63"	158	0.211	0.061	0.29	0.15	1.2	0.39
KNO17	51°21'01.97"	03°19'49.58"	286	0.328	0.063	0.19	0.15	1.8	0.60
KNO52	51°20'11.27"	03°20'55.31"	201	0.252	0.059	0.23	0.14	1.4	0.46
LRV	50°49'42.08"	04°38'20.60"	185	0.237	0.012	0.05	0.03	3.3	1.05
OHZ	50°50'22.09"	04°39'18.16"	142	0.195	0.042	0.22	0.10	1.2	0.40
OM2	50°51'47.82"	04°43'05.16"	132	0.185	0.058	0.32	0.14	2.3	0.74
OM3	50°51'47.32"	04°43'05.16"	184	0.236	0.018	0.08	0.04	1.1	0.37
TER1	50°49'22.98"	04°35'38.17"	74	0.120	0.099	0.83	0.24	1.5	0.48
TER2	50°49'18.24"	04°36'04.50"	242	0.290	0.086	0.30	0.21	1.6	0.52
ZW4	50°49'24.68"	04°39'53.46"	235	0.283	0.012	0.04	0.03	2.5	0.81

755 [1] Cd EQS = $0.09 \times (\text{Hardness}/50)^{0.7409}$ (European Commission, 2005)

756 [2] Total bgc = 90th percentile of Cd natural background concentration; values have been reported for unpolluted freshwater in Netherlands = 0.41 µg/L (total
757 Cd) (Crommentuijn et al., 1997) and for Northern European lowlands = 0.78 µg/L (total Cd) (Zuurdeeg,1992); the calculated ratio is based on the bgc of 0.41
758 µg Cd/L

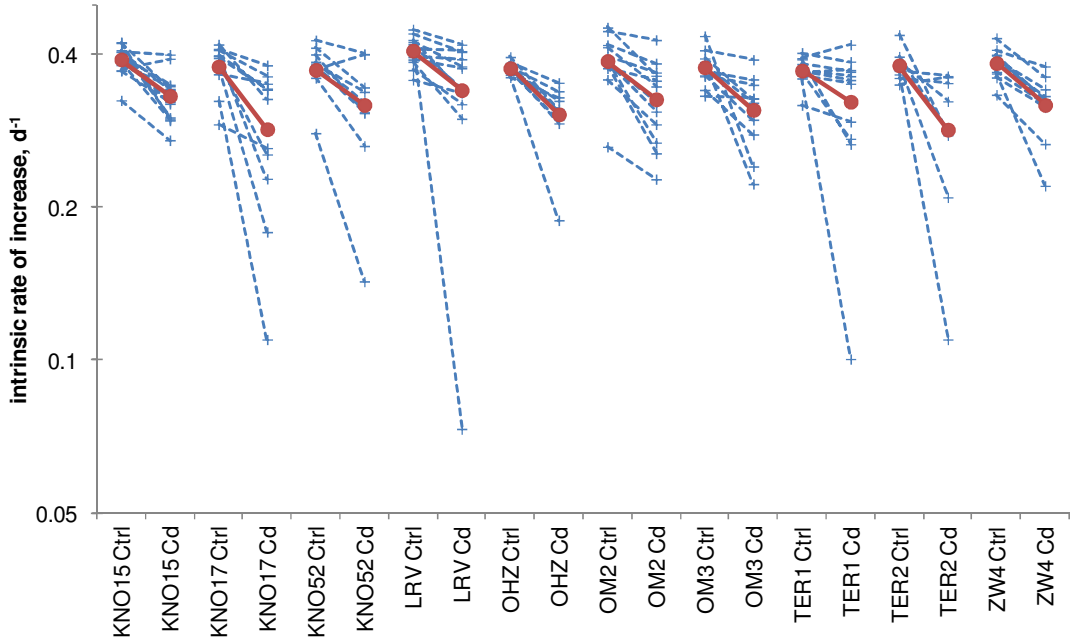
759 [3] bgsed is based on mean + 1 standard deviation of background sediment Cd concentration in Belgian freshwater sediments (mean = 1.6 mg/kg, stdev = 1.5
760 mg/kg) = 3.1 mg/kg (Swennen et al., 1998)

761 **Table 2 The 21-day 10% effect concentrations (EC10) of Cd, the intrinsic rate of increase (r_m)**
762 **under control (no Cd added) and 4.0 µg Cd/L, and Cd tolerance (r_m@Cd / r_m@control) for 7**
763 **isoclonal laboratory populations as shown by. See supportive information (Table S1) for origin**
764 **of laboratory clones.**

Laboratory clone	EC10 (µg/L) (95% C.I.)	r_m @ control ± S.D	r_m @ 4 µg Cd/L ± S.D	Tolerance @ 4.0 µg Cd/L ± S.D
CZ	7.8 (5.3 - 10.0)	0.39 ± 0.02	0.37 ± 0.03	0.94 ± 0.09
Clone K6	0.8 (0.6 - 1.1)	0.38 ± 0.02	0.28 ± 0.03	0.74 ± 0.07
SE	11.2 (9.2 - 13.0)	0.40 ±0.02	0.39 ± 0.02	0.96 ± 0.09
DK	3.9 (3.4 - 4.5)	0.38 ± 0.04	0.34 ± 0.01	0.92 ± 0.08
Clone F	3.2 (2.5 - 4.1)	0.37 ± 0.03	0.31 ± 0.04	0.86 ± 0.18
Clone A	2.0 (1.9 - 2.1)	0.36 ± 0.01	0.28 ± 0.03	0.78 ± 0.06
Clone IRCHA-5	4.9 (4.9 - 5.1)	0.36 ± 0.01	0.32 ± 0.01	0.90 ± 0.04
Geometric mean	3.7			
Mean ± S.D.				0.87 ± 0.08

765 EC10 = 10% effective concentration, 95% C.I. = 95% confidence interval, S.D. = standard deviation

766 **FIGURES FOR MAIN MANUSCRIPT**



767

768 **Figure 1 Reaction norms for the different clones (crosses, dashed lines) and population reaction**
769 **norms (thicker dots, full lines) for 10 natural *D. magna* pond populations exposed to a control**
770 **(Ctrl) and Cd (4.4 µg Cd/L). Blue crosses represent the mean phenotypes of individual clones**
771 **within a population (clone means). Red dots represent the population mean (mean of clone**
772 **means). Values for population means are available in *Supportive Information* (Table S5). Note**
773 **the (log_2-based) logarithmic vertical axis. A steeper slope corresponds with a lower tolerance as**
774 **defined in Eq. 2 and as shown in Figure 2.**

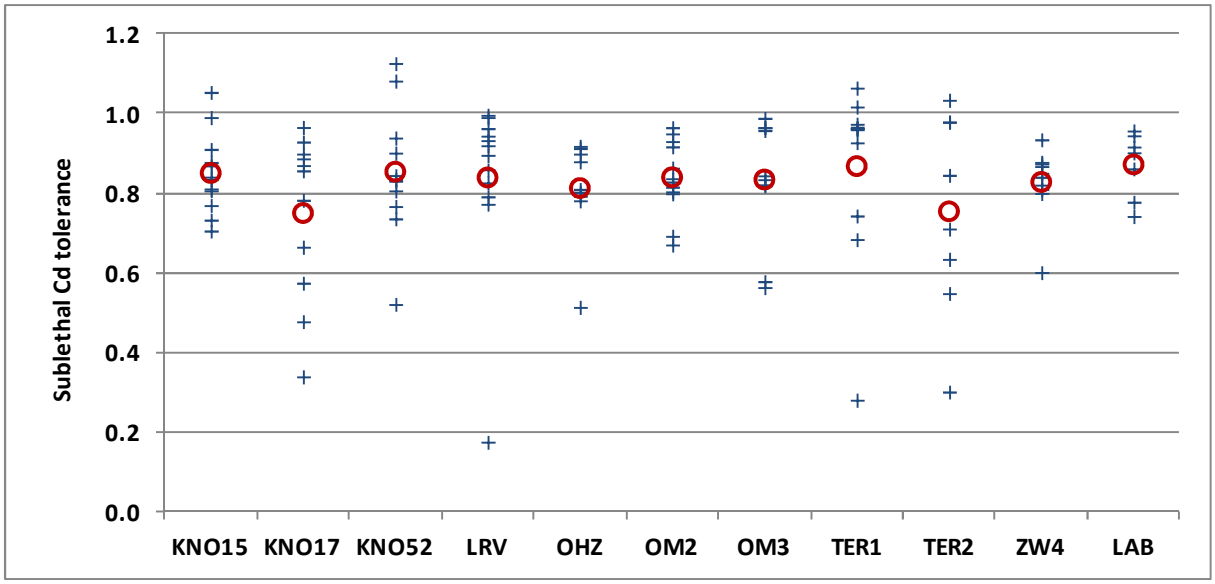


Figure 2 Sub-lethal Cd tolerance in 10 natural populations (KNO15 to ZW4) and in a collection of 7 isoclonal laboratory populations from ecotoxicology laboratories (LAB). Crosses represent means of each clone within a population. Circles are the population means (mean of clone means). Population means are available in *Supportive Information* (Table S5). Cd tolerance is calculated as r_m in the Cd treatment divided by r_m in the control treatment (Eq. 2).

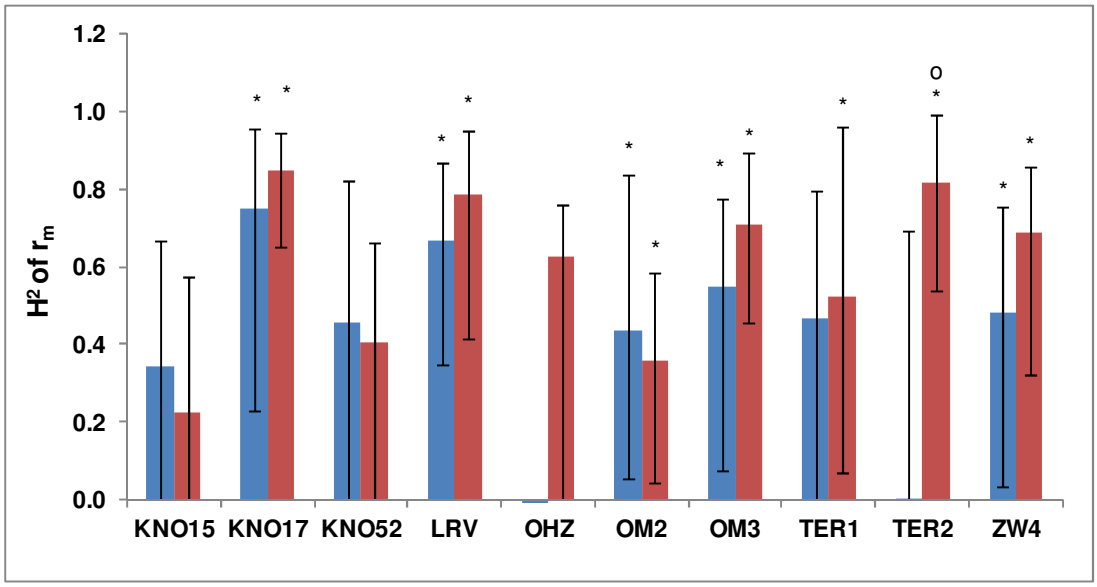


Figure 3 Median broad sense heritabilities (H^2) of intrinsic rates of increase (r_m) under control (blue) and Cd environments (red) in 10 natural *D. magna* populations. Error bars indicate 90% confidence interval. Values of H^2 and confidence limits are available in *Supportive Information* (Table S10). An asterisk (*) indicates $H^2 > 0$ in this population x environment combination. An open circle (o) indicates that H^2 is significantly different between the control and the Cd environment within a population (only the case for TER2).

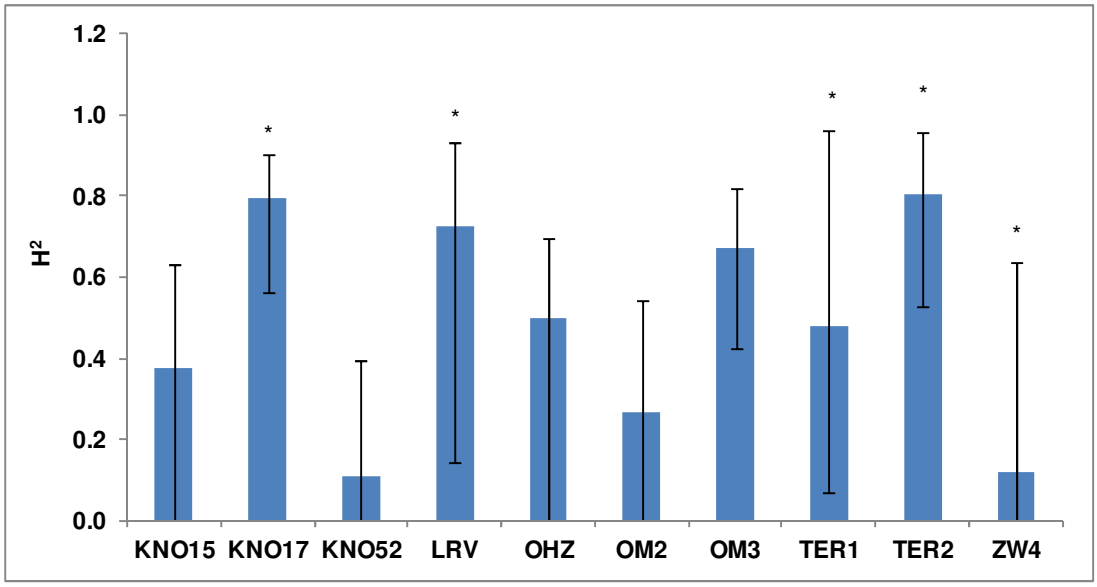


Figure 4 Median broad sense heritabilities H^2 of sub-lethal Cd tolerance in 10 natural *D. magna* populations. Values of H^2 and 90% confidence limits are available in *Supportive Information* (Table S10). An asterisk (*) indicates H^2 >0 and, thus, that there is a significant genetic component to Cd tolerance and a significant evolutionary potential of this sub-lethal tolerance trait in this population.