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# Quantitative genetics approaches to study evolutionary processes in ecotoxicology; a perspective from research on the evolution of resistance

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**Abstract** Quantitative genetic approaches are often used to study evolutionary processes in ecotoxicology. This paper focuses on the evolution of resistance to environmental contaminants—an important evolutionary process in ecotoxicology. Three approaches are commonly employed to study the evolution of resistance: (1) Assessing whether a contaminant-exposed population has an increased resistance relative to a control population, using either spatial or temporal comparisons. (2) Estimating a population's heritability of resistance. (3) Investigating responses in a laboratory selection experiment. All three approaches provide valuable information on the potential for contaminants to affect a population's evolutionary trajectory via natural selection. However, all three approaches have inherent limitations, including difficulty in separating the various genetic and environmental variance components, responses being dependent on specific population and testing conditions, and inability to fully capture natural conditions in the laboratory. In order to maximize insights into the long-term consequences of adaptation, it is important to not just look at resistance itself, but also at the fitness consequences and at correlated responses in characteristics other than resistance. The rapid development of molecular genetics has yielded alternatives to the "black box" approach of quantitative genetics, but the presence of different limitations and strengths in the two fields means that they should be viewed as complementary rather than exchangeable. Quantitative genetics is benefiting from the incorporation of molecular tools and remains an important field for studying evolutionary toxicology.

**Keywords** Quantitative genetics · Adaptation · Contaminant resistance · Evolution of resistance · Review

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## Ecotoxicology and evolutionary processes: contamination as a selective force

Environmental pollutants can affect a population's evolutionary trajectory via multiple processes (natural selection, mutation, gene flow, and genetic drift), though the most extensive evidence comes for cases where this is mediated by natural selection. When contamination affects survival and/or reproduction, natural selection will favor those individuals that are less sensitive to the contaminant. This selective force can result in the population as a whole evolving resistance if the resistant individuals differ genetically with respect to pollutant sensitivity. This paper focuses on population-level resistance, treating resistance as a quantitative trait. A quantitative trait is a characteristic that can be quantified on a scale; such traits are usually determined by many genes. A commonly used example is adult height in people. Genetically based changes in quantitative traits at the population level are addressed using quantitative genetics. Quantitative genetics has long found widespread application in agriculture, especially because of the insights provided for selective breeding (Falconer 1960). Quantitative genetics is also widely used

to study pesticide and antibiotic resistance in target species, in order to provide information on such practical issues as the rate at which resistance develops, and the chance for resistance spreading into susceptible populations (see e.g., Crow 1957; Firko and Hayes 1990; Georghiou 1990). Quantitative genetics has generally seen limited application in ecotoxicology, though a small number of studies on the development of resistance to environmental contaminants have been conducted throughout the history of this field. Early research includes work on insecticide resistance in fish (Boyd and Ferguson 1964; Vinson et al. 1963), on metal resistance in grasses on abandoned British metal mines (e.g., Antonovics et al. 1971), and on metal resistance in polychaetes from Restronguet Creek which drains these mining areas (e.g., Bryan and Hummerstone 1971). A review published in 1987 (Klerks and Weis 1987) appears to have contributed to a renewed interest among environmental toxicologists for studying adaptation to contaminants.

## Quantitative genetics approaches for addressing the evolution of resistance

Three quantitative genetics approaches are commonly used to obtain information on the evolution of resistance (Fig. 1). One approach to studying the evolution of resistance is to determine whether a population inhabiting a contaminated site has an increased resistance (relative to one or more control populations), which would provide evidence of past selection by the pollution. A second approach is to imitate the selection process in the laboratory, and determine whether (or how fast) a laboratory population responds to artificial selection for an increased resistance. A third approach investigates whether a population has genetic variation for resistance and thus harbors the genetic variation necessary for selection to operate. When investigating the evolution of resistance brought about by exposure to environmental contaminants, it is important to not just focus on resistance itself but also on correlated traits and fitness costs. This paper elaborates on these three approaches for looking at the evolution of resistance to contaminants, focusing not only on resistance itself but also addressing whether traits other than resistance are affected simultaneously and whether there are trade-offs between resistance, other population traits and fitness.

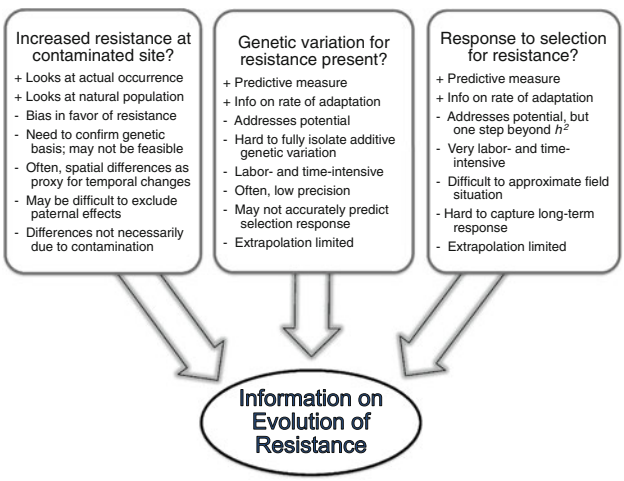


**Fig. 1** Three commonly-used quantitative genetic approaches for assessing the evolution of resistance to environmental contaminants, with their main strengths (+) and weaknesses (−)

## Evidence of adaptation in populations inhabiting contaminated sites

A population inhabiting a contaminated site may be subjected to selective pressure from the contamination, and this may result in an elevated resistance in this population, compared to resistance in a population of conspecifics living at a clean site. Comparing contaminant sensitivity of populations among sites may thus provide evidence of adaptation, if it can be shown that individuals from a contaminated site display elevated resistance and that among-population differences in resistance have a genetic basis. A common approach to examine the presence of genetic differences in resistance involves rearing individuals originating from different environments at a single location in order to eliminate most of the environmental variation as well as variation resulting from genotype by environment interactions. For example, seeds can be collected from plants at various sites and then sown at a common location (e.g., Eränen et al. 2009). This approach has also been used with populations of *Daphna magna* collected from different sites in Europe (Barata et al. 2002).

The occurrence of resistance differences among populations from sites differing in contamination status was investigated in a study of cadmium resistance in the tubificid oligochaete *Limnodrilus hoffmeisteri* from Foundry Cove, New York. This cove on the Hudson River (41°24′45.16″N, 73°57′6.85″W) was a Superfund site with a long history of contamination by metals from a facility producing nickel–cadmium batteries. Further background information on this site is provided by Knutson et al. (1987). Individuals were collected from three Foundry Cove locations differing in metal levels and from one location in the nearby control area, South Cove. After overnight depuration and water change, metal resistance of individuals was quantified as time-to-death (TTD) during an exposure to an aqueous solution with a mixture of three metals (Cd, Ni, and Co). Worms from the two sites with the highest sediment metal levels were less sensitive (had a higher mean TTD) than the worms from the two cleaner sites. No attempt was

made here to determine whether the resistance differences had a genetic basis or were the result of physiological acclimation. However, subsequent research with this worm from Foundry Cove and the control site confirmed a genetic basis—as resistance differences were still evident after populations were maintained in clean sediment for two generations (Klerks and Levinton 1989). Crosses between individuals of the two populations suggested that the resistance trait might be explained by one segregating genetic element (Martínez and Levinton 1996).

In the approach discussed above, geographic variation in resistance is used as a surrogate for temporal changes—it is assumed that the situation at the clean site represents the situation for the contaminated site prior to it becoming contaminated. There are some instances in which resistance can be directly compared on a temporal scale. Resting eggs of some aquatic invertebrates accumulate in the sediment and remain viable for decades or even longer (Weider et al. 1997). Derry et al. (2010) collected copepod eggs from sediment deposited during three time periods that differed in the degree of lake acidification. Comparisons of acid-tolerance among copepods hatched from the three groups of eggs provided some evidence of past selection for increased tolerance to low pH (Derry et al. 2010). However, such a "resurrection ecology" approach is feasible for just a small number of species.

## Subjecting laboratory populations to selection for increased resistance

Laboratory selection experiments investigating the development of resistance to environmental contaminants have been conducted for a variety of organisms and contaminants. Examples include selection for an increased resistance to cadmium in laboratory populations of *Daphnia magna* (LeBlanc 1982; Ward and Robinson 2005) and *Drosophila melanogaster* (Shirley and Sibly 1999), selection for resistance to a mixture of metals in laboratory populations of the same oligochaete used in the population comparisons described above (Klerks and Levinton 1989), and selection for zinc-resistance in laboratory populations of the flagfish *Jordanella floridae* (Rahel 1981). To provide more insight into procedures for such a study, a closer look is provided into the experiment where least killifish, *Heterandria formosa*, were selected for an increased resistance to cadmium (Xie and Klerks 2003). In each generation, about 240 immature fish from each of three laboratory selection lines and three control lines were exposed to a lethal concentration of cadmium till a mortality of 50% was obtained. This median time-to-death (MTTD) during this short-term exposure was used as a measure of their resistance. Survivors were transferred to clean water (delayed mortality resulted in a total Cd-induced mortality of about 75%) and allowed to reproduce. Each selection line was paired with a control line, control line fish were also exposed to cadmium to quantify their resistance, but only unexposed fish were used for establishing the next generation in these control lines. This was repeated for a total of six generations. Resistance to Cd showed a rapid response to selection; approximately a 3-fold increase in MTTD over the six generations of selection (Xie and Klerks 2003).

## Determining the presence of genetic variation for resistance

The amount of genetic variation for a trait that can be characterized on a quantitative scale is generally referred to as the trait's heritability. The heritability of resistance to a stressor indicates how well a population can respond to selection by the stressor, since such a response requires the presence of genetic variation. The term "heritability" usually denotes the "*narrow sense heritability*" (or $h^2$—though it is not a squared value), which is defined as the proportion of the total phenotypic variance in a population that is made up by the additive genetic variance. Thus $h^2 = V_A/V_P$ (Falconer and Mackay 1996). The additive genetic variance is the variance of breeding values; an individual's breeding value for a trait is the sum of the average effects of the genes it carries, summed over the pair of alleles at each locus and over all loci contributing to the trait (Falconer and Mackay 1996). More simply put, breeding value is the value of an individual as measured by the mean value of its progeny (Roff 1997). The narrow sense heritability is the best predictor of a population's response to selection. The "*broad sense heritability*" (or $H^2$) is the ratio of genetic variation to the total phenotypic variation—thus also referred to as the "*degree of genetic determination*" (Falconer and Mackay 1996). The broad sense heritability includes both additive and non-additive genetic variance, with the latter consisting of dominance variance resulting from alleles at a given locus not contributing to the trait in an additive manner, and interaction variance resulting from genes at different loci not contributing additively (or exhibiting epistasis). The broad sense heritability is not as useful a concept as the narrow sense heritability, but some methods or approaches do not allow the complete separation of additive and non-additive genetic components, such that the best that can be done in those cases is an estimate that falls somewhere between the broad and narrow sense heritability.

Heritabilities are usually determined using laboratory populations, with individuals separated by family. To estimate heritabilities from resemblances among relatives, various family relations can be used, such as offspring—

father, offspring—mother, mean offspring—mid-parent, full sibs, or half sibs. Some measures are biased by non-genetic effects. For instance, for the common situation in animals where mothers produce offspring and provide maternal care, offspring—mother resemblances are likely to be inflated because of maternal effects. Similarly, full-sib designs generally yield inflated estimates because resemblances include components other than additive genetic variation. Using half-sib families makes for a more complicated design (e.g., a number of males is each mated to multiple females) and more complex analysis, but enables the quantification of not only the additive genetic variation, but also the dominance variation and maternal effects (Falconer and Mackay 1996).

Examples of studies estimating heritabilities from resemblances in laboratory-reared families, are provided here for studies with the oligochaete *L. hoffmeisteri* (Klerks and Levinton 1993) and the sheepshead minnow *Cyprinodon variegatus* (Klerks and Moreau 2001). For the oligochaete, worms were raised in pairs, offspring removed regularly and raised separately, and resistance quantified in both parents and offspring once offspring were mature—with resistance measured as time-to-death when exposed individually to an aqueous solution with a mixture of cadmium, nickel and cobalt. The heritability was then determined from the regression of resistance in offspring (mean of offspring within a family) on that of average of the parents ("midparent") values. In this design, the heritability estimate is simply the regression coefficient. The heritability for survival time to cadmium exposure in *L. hoffmeisteri* was statistically significant and high ($0.92 \pm 0.12$; estimate $\pm$ S.E.), actually close to the theoretical maximum of 1.0 and consistent with the rapid response when the same laboratory population was selected for an increased resistance (Klerks and Levinton 1989). For the sheepshead minnow, heritabilities were determined for resistance to individual contaminants (phenanthrene or zinc) or mixtures (phenanthrene + zinc or a mixture of three metals and three polynuclear aromatic hydrocarbons). Sets of parents and offspring were again obtained in the laboratory. Fish were housed in pairs, eggs collected from each pair regularly, eggs allowed to hatch and offspring raised. Resistance in adult parents and in juvenile fish ($7 \pm 1$ day old) was again quantified as time-to-death in exposures to lethal levels of the contaminant(s). Heritability estimates were obtained from various parent-offspring regressions (offspring on father, offspring on mother, offspring on midparent). The estimates ranged from $-0.43 \pm 0.43$ to $0.55 \pm 0.50$, but none were statistically different from zero and estimates averaged only 0.09 (Klerks and Moreau 2001). Laboratory selection experiments with these fish and contaminants similarly failed to detect a consistent increase in resistance (unpublished data). Heritabilities have also been determined for metal resistance in plants (Antonovics et al. 1971), as well as for physiological characteristics and gene expression related to cadmium tolerance in the springtail *Orchesella cincta* (Posthuma et al. 1993; Roelofs et al. 2006). A recent paper by Chaumont et al. (2009) indicated a lack of additive genetic variance for cadmium tolerance in an amphipod. An example on heat tolerance, relevant in the context of climate change, is provided by the paper by Doyle et al. in this issue.

It is often advantageous to estimate heritabilities in natural populations rather than bringing the organisms into the laboratory (see discussion below). Traditionally, estimating heritabilities "in the wild" was not possible, except in the case where pedigrees were known—as in the long-term study of some great tit (*Parus major*) populations in Europe (see e.g., Van Noordwijk et al. 1980) where birds had been banded as adults and as young in nesting boxes for many generations. With today's widespread use of genetic markers, relatedness between individuals can now be determined with these markers and heritabilities estimated using various methods (Garant and Kruuk 2005). We are not aware of this approach having been used for contaminant resistance or related traits. An example in which the heritability of defense chemicals was estimated in a tree species, with the use of molecular markers for estimating relatedness, is provided by Andrew et al. (2005).

Heritabilities can also be determined from the response to selection, since the theoretical response to selection for a quantitative trait equals the product of the heritability of that trait in the population being selected and the selection differential ($R = h^2 \times S$). Thus *R*/*S* is the "*realized heritability*"; the heritability that was realized during the selection process (Falconer and Mackay 1996). The realized heritability is quantified from the regression of the cumulative response on the cumulative selection differential. The cumulative response is the total increase observed for the trait under selection. The cumulative selection differential is obtained by summing, over successive generations, the trait's difference between the population as a whole and the group of selected individuals. For example, in the previously discussed example where killifish (*H. formosa*) were selected for an increased resistance to cadmium and a rapid response to selection was obtained, realized heritability estimates averaged 0.50 (Xie and Klerks 2003). It is not uncommon for realized and regular heritabilities to differ, as a realized heritability may not provide a valid estimate of the heritability in the base population (Falconer and Mackay 1996). As an illustration of different outcomes, selection of the oligochaete *L. hoffmeisteri* yielded a realized heritability of 0.59 with S.E. of 0.14; still high but substantially less than the $0.92 \pm 0.12$ estimated from the parent-offspring regression (Klerks and Levinton 1989, 1993).

## Selection: correlated responses and fitness consequences

We saw earlier that resistance may change as a result of selection exerted by exposure to a contaminant. However, the selection may also affect other traits as a consequence of genetic correlations. Genetic correlations among traits can result from "linkage disequilibrium", potentially brought about by a variety of factors including selection and non-random mating among individuals in a population. However, this is generally not important in the long run, as linkage disequilibrium will eventually decay owing to recombination. A typically more-important cause of genetic correlations among traits is pleiotropy, when a gene affects more than one trait (Roff 1997). Thus changes in resistance to a specific contaminant will affect other traits that share one or more of the underlying genes. These traits can change in the same or in opposite directions, as it is possible for a specific gene to have a negative effect on the value of one trait but a positive effect on the value of another trait. Changes that occur in a trait that was not directly under selection, i.e., correlated responses, thus depend on the genetic correlation between the two traits and the heritability of the second trait. A common correlated response in cases of increased resistance is a simultaneously increased resistance to another contaminant (Brown 1978; Lopes et al. 2005; Macnair 1997; Xie and Klerks 2003). Various terms, including cross-resistance, co-resistance and co-tolerance, are used to refer to this situation. This may be a consequence of the two contaminants sharing a detoxification pathway, such as an increased induction of metallothioneins resulting in enhanced detoxification of both copper and cadmium. Putting this specific situation in quantitative genetic terms; copper- and cadmium resistance are genetically correlated and have metallothionein gene(s) in common.

A different emphasis in looking at changes accompanying an increase in resistance is one that focuses on changes in fitness (defined in a population genetic sense, reflecting contribution to the next generation). Adaptation to a contaminant is equivalent to an increase in fitness in the presence of the contaminant, but the adapted population's fitness may be decreased under normal conditions. Such a "fitness cost" or "fitness trade-off" may be a consequence of antagonistic pleiotropic effects (Shirley and Sibly 1999). With such a negative genetic correlation between resistance and a fitness-related trait, selection for an increased resistance will automatically decrease the fitness-related trait. Fitness costs may also be envisioned at the physiological level, arising from trade-offs in energy allocation—such as more energy going towards detoxification and less towards reproduction. The presence of fitness costs has been reported for many cases of pesticide resistance (see e.g., Scott et al. 1997), though in some cases they appear absent (Arnaud and Haubruge 2002). Similarly, in cases of resistance to environmental contaminants, fitness costs are reported in many cases (see e.g., Barata et al. 2002) but seem less obvious in others (Ward and Robinson 2005). A rapid loss of resistance once the population is no longer exposed to the contaminant is often considered to be an indication of fitness costs associated with the resistance (Levinton et al. 2003)—though in the latter case it became obvious later on that immigration of sensitive genotypes was responsible for the loss of resistance (Mackie et al. 2010). Resistance traits that are induced only in the presence of a contaminant might be explained by the presence of inducible duplicated genes, and therefore might not encumber a strong correlated cost in other traits. For example, the degree of duplication of metallothionein genes in *Drosophila melanogaster* reflects resistance to copper (Maroni et al. 1987), suggesting that the resistance response involved induction of multiple genes.

For the previously described experiment where least killifish were selected for an increased resistance to cadmium, a variety of fitness-related traits were measured for two pairs of control and selection lines (Xie and Klerks 2004). These traits were determined using offspring of the 6th generation. Fish were placed in pairs and monitored over their entire life-time, allowing quantification of the following traits: time to first reproduction, female length at first reproduction, male length at first reproduction, mean number of offspring per brood, mean brood interval (time between subsequent reproduction events), number of broods, lifetime fecundity, female longevity, and length of female's reproductive life (time between a female's first and last brood). Differences between control and selection lines were statistically significant for four of the traits. The trait that may best reflect fitness, lifetime fecundity, showed a reduction averaging 17% in the selection lines relative to the control lines (Xie and Klerks 2004). The same project also looked at cross-tolerance during several of the generations, and found that the cadmium-resistant lines were also more resistant to copper, but had a reduced resistance to heat (Xie and Klerks 2003).

## Discussion: strengths and weaknesses of quantitative genetics approaches

### Among-population differences in resistance as evidence of selection

Determining whether a population inhabiting a contaminated site has an increased resistance, is an approach with inherent strengths and weaknesses (Fig. 1). This approach benefits from the fact that it addresses resistance in a

population present at a contaminated site, thus potentially providing evidence that adaptation has occurred and has happened under "real-world" conditions (rather than a under specific laboratory conditions). However, since this approach starts with populations present at a contaminated site, the picture that emerges could easily be biased against species not able to adapt—and therefore not present at the contaminated site. When a population inhabiting a contaminated site has an increased resistance relative to a control population, further evidence is needed that this resistance difference has a genetic basis. While this is often addressed by studying offspring reared under clean conditions, this approach may not be feasible for a specific species of interests and options may be limited to maintaining individuals at a single location for a relatively short amount of time prior to measuring the quantitative trait. If resistance differences do not have a genetic basis, i.e., are a consequence of acclimation, they may disappear rapidly in individuals maintained under identical and clean conditions (see e.g., Klerks and Lentz 1998). Acclimation-based differences might be studied by examining descendants of individuals within the shared environment (Lonsdale and Levinton 1985). But if it is not possible to study offspring raised under clean conditions, it may be difficult to fully assess the relative importance of acclimation and adaptation in resistance differences.

Even in the case where it is possible to assess whether resistance differences are still present in offspring, finding such differences in offspring is no proof of a genetic basis. There is the potential for parental transfer of non-genetic variation from one generation to the next (Roff 1997); often in the form of maternal transfer. Maternal effects are common, and appeared present for a trait associated with cadmium resistance in the springtail *Orchesella cincta* (Posthuma et al. 1993). Because of such maternal effects it is preferable to compare second- or later-generation offspring. In the case of the oligochaete *L. hoffmeisteri* illustrated above, resistance differences between worms from Foundry Cove and South Cove were still present after two generations in clean sediment in the laboratory (Klerks and Levinton 1989). While it is often possible to experimentally quantify parental effects, this is not straightforward and results may be inconclusive (Roff 1997).

In the case where differences among populations for the quantitative trait have a genetic basis, the possibility remains that this is due to random genetic drift rather than selection. However, this can now be addressed using an elegant method that compares differentiation for the quantitative trait, measured as the $Q_{ST}$ value, to that for a neutral genetic marker, measured as $F_{ST}$ value (Leinonen et al. 2008). The logic being that, if the quantitative trait is under selection, then population differentiation for that trait will be more pronounced than it is for the neutral marker. In the absence of selection, $F_{ST}$ and $Q_{ST}$ values should be similar. This method has become popular and was recently applied to address differentiation in zinc tolerance among plant ecotypes, finding strong evidence that differentiation was due to selection (Jiménez-Ambriz et al. 2007).

## Heritability estimates to assess potential for responding to selection

Assessing whether populations have the genetic variation necessary for adaptation to occur, is again an approach with inherent strengths and weaknesses (Fig. 1). This approach can be used to provide information prior to a population becoming subjected to selection by the contaminant or other environmental stressor. Moreover, the quantification of the heritability of a resistance trait provides information about the rate at which adaptation may occur under specific conditions. However, using this approach to gain insight into adaptation to contaminants has several weaknesses.

As mentioned earlier, when using resemblances among relatives to quantify the proportion of phenotypic variation that is made up of additive genetic variation, it is often difficult to fully isolate the additive genetic component. Also, heritability estimates tend to be imprecise unless they are obtained using very large sample sizes (making it time- and labor-intensive). Consequently, many heritability estimates suffer inherently from low precision (i.e., have a large standard error). For example, the parent/offspring based heritability estimates in the previously mentioned study in the sheepshead minnow *C. variegatus* had standard errors averaging 0.37 (with the heritability estimates averaging 0.09) in spite of the use of 20–30 families for each estimate (Klerks and Moreau 2001). This situation can be improved by optimizing the experimental design and increasing the sample size, but can easily reach the point where it is not feasible from a logistical standpoint. For example, if parent-offspring regressions are used to estimate a heritability, one would need to quantify the trait in 400 parents and 400 offspring in order to achieve a standard error of 0.1 (Falconer and Mackay 1996). In addition to the imprecision, inaccuracy of heritability estimates is introduced when measurement of the trait is subject to measurement error. This error is expressed as the trait's *repeatability*—i.e., how consistent is the trait's value if measured multiple times on the same individual? A repeatability that is less than 100% will by itself lower a heritability estimate (Falconer and Mackay 1996).

Heritability estimates may have limited applicability. A specific heritability estimate is only valid for the trait, species and population for which it was determined. Estimates may also be dependent on environmental conditions—since these influence the environmental variance,

and therefore the total phenotypic variance of which it is a component, thereby altering the ratio of additive genetic to total phenotypic variance. Furthermore, stressful conditions may further influence a heritability estimate (Bubliy and Loeschcke 2002). Consequently, the issue arises whether or not the common procedure of estimating heritabilities on field-collected organisms brought into the laboratory for breeding, yields useful estimates. The general expectation is that the laboratory conditions reduce the environmental variance, causing the additive genetic variance to become a larger proportion of the phenotypic variance—thus resulting in an overestimate of the heritability relative to what would be expected for the natural population (Roff 1997). However, a literature review conducted by Weigensberg and Roff (1996) did not detect a consistent difference between field and laboratory estimates. It is possible that the situation is different for traits that are not closely related to fitness (Charmantier and Garant 2005). When heritability estimates for natural populations are determined using modeling techniques and models contain fixed effects, the outcomes can depend on the choice of included effects (Wilson 2008). Heritability estimates may also show a dependence on the method used for determining the trait (Chown et al. 2009).

Heritability estimates may not be the most relevant predictor of selection response. A heritability estimate provides a prediction of the *absolute* response to selection, via $R = h^2 \times S$ (see earlier). However, it may often be important to know how much of a *relative* change we can expect in a trait. Use of the additive genetic coefficient of variation ($CV_A$) instead of the heritability, would provide a prediction of the proportional response to selection (Houle 1992). The $CV_A$ adjusts for the mean of the trait, similar to the commonly used coefficient of variation, and is defined as the square root of the additive genetic variance divided by the mean value of the trait in the population studied (see also the paper by Coutellec et al. in this issue). Because of the potential usefulness of the $CV_A$, it is recommended to include trait means when reporting heritabilities (Houle 1992), allowing the $CV_A$ to be determined from the heritability. Use of $CV_A$ has the added benefit that the variable itself is not affected by the environmental variance.

The heritability of a single trait may not provide sufficient information to accurately predict the trait's responses to selection, and does not provide insight into other traits that may change simultaneously. As discussed earlier, the presence of genetic correlations means that a trait does not evolve independently from other traits (Houle 1991). These genetic correlations can bring about constraints to the former trait's evolution. For example, if the presence of a contaminant results in a selection pressure for increased resistance to that contaminant, and this trait has negative genetic correlations with a trait tied to fitness, then the response to selection will be slowed down by that negative genetic correlation. A recent meta analysis found no strong evidence for such constraints, as cases in which genetic correlations reduced adaptation were offset by cases in which the opposite pattern was observed (Agrawal and Stinchcombe 2009). Genetic correlations between traits may not only affect the response for the trait under selection, but will also cause traits other than the trait directly under selection to change. Another complication in predicting responses to selection is that a contaminant may in effect exert a selective pressure on multiple traits (e.g., selection on increased survival at the juvenile stage, selection on increased survival at the adult stage, selection on increased reproduction, etc.), and these traits are unlikely to be fully independent. All these situations mean that we would benefit from an analysis that takes genetic correlations into account. This can be accomplished in a multivariate approach in which the equivalence to the additive genetic index becomes the additive genetic variance–covariance matrix, also referred to as "G-matrix" or simply as "**G**" (Arnold 1994; Lande and Arnold 1983). It is complicated and logistically difficult to obtain estimates of **G**. Some studies on contaminant resistance have looked at genetic covariation among multiple traits (see e.g., Posthuma et al. 1993), but we are not aware of studies in which the G-matrix approach has been used for addressing evolutionary changes related to environmental contamination. The approach has been used in related contexts, including resistance to herbivory (Johnson et al. 2009) and effects from climate change (Garant et al. 2008).

## Response to selection in the laboratory to predict responses in the wild

Determining the response to selection in a laboratory population has some of the same strengths and weaknesses as the approach of estimating heritabilities of resistance traits (Fig. 1). Again, selection experiments can be used to predict a population's response prior to the actual exposure of a natural population, and the selection response provides a measure of the rate at which a population may be able to adapt. But it is again an approach that investigates the potential for adaptation, rather than providing evidence of past adaptation. However, since this approach measures the response to actual selection, it provides a better assessment of the potential for evolution of resistance than the information obtained from heritability estimates. Similarly to the situation for heritabilities, the potential for evolution of resistance is investigated for only a very specific set of conditions. Consequently, the applicability of laboratory selection experiments is again somewhat limited. The selection response is specific to the population and conditions being used in the laboratory selection experiment, and

these may not closely approximate natural conditions. It may be difficult to replicate field conditions in the laboratory, or actual exposure route in the field may be unknown. For example, in the Foundry Cove study it was very difficult to quantify the degree of exposure of worms to cadmium by feeding or exposure to dissolved cadmium in sediment pore waters. Moreover, exposure concentrations and resulting selection pressures may differ between laboratory and field. In order to obtain information from laboratory selection experiments in a reasonable time frame, these experiments often use a degree of exposure to a contaminant that is higher than what is likely at a contaminated site. For example, in the previously discussed least killifish selection study (Xie and Klerks 2003) only those fish that were in the top 25% of resistance potentially contributed to the subsequent generation (since 75% were "culled out" by the exposure). This can affect whether the selection will involve a small number of genes with large effect or many genes each with a small effect on resistance (Macnair 1991). Differences in exposure levels and other conditions also mean that the trait that is being selected for in the laboratory may differ from the trait that would be selected for at a contaminated site. For example, in the Xie and Klerks (2003) study, selection was based on survival during a short-term exposure to a relatively high cadmium level. In nature, selection is more likely to act over a much longer part of the organism's life span, may act at a different stage in the life cycle, or act on reproduction rather than (or in combination with) survival. Laboratory results often inherently assume that there is a correlation between the measured trait (e.g., survival time) and broader suites of life history traits in the field. Another complication with laboratory-to-field extrapolation from laboratory selection experiments, is that the long-term response to selection may differ from the shorter-term response observed in the laboratory. A strong selection intensity will mean that the eventual outcome with respect to selection response will be achieved faster, but an overly strong selection may bias the outcome with respect to the loss of genetic variation resulting from the selection (Fuller et al. 2005; Roff 1997). In the field, the response may slow down, or even reach a selection limit, due to a decrease in the amount of heritable variation over time as a consequence of fixation of alleles (Falconer and Mackay 1996). The gradual loss of genetic diversity in a field situation would not be captured very well by a relatively short-term laboratory experiment. Also, new genetic variation is likely to arise over the long-term, as a consequence of mutation or gene flow from nearby populations. Again, this would not be adequately captured by a laboratory selection experiment lasting a relatively small number of generations. Inbreeding in laboratory populations and resulting loss of genetic variation (see e.g., Athrey et al. 2007) may also cause a difference between outcomes in the laboratory from those in natural populations. In spite of the many shortcomings of laboratory selection experiments, they are nevertheless very useful tools for addressing various questions on evolutionary processes (Fuller et al. 2005).

## Quantitative genetics approach versus other approaches

As has become obvious from this treatment of quantitative genetics approaches in ecotoxicology with a focus on the evolution of resistance, these approaches provide numerous insights into the potential for contaminants to affect a population's evolutionary trajectory. A wealth of information can be obtained for a comprehensive trait like resistance, yielding predictions on issues such as the potential for resistance to evolve, the speed at which resistance may evolve, and the potential for other traits to change along with the resistance. That information is directly applicable for predicting a contaminant's impacts at the population level and for ecological risk assessment. Moreover, this can be done while treating the trait (e.g., survival time) as a "black box" with respect to underlying genetics. This is important, since detailed genetic information is lacking for the majority of species potentially at risk at contaminated sites. Modern quantitative genetic methods make it possible to use a multi-trait approach (see e.g., discussion about G-matrix), allowing research to account for the fact that traits generally do not evolve independently (McGuigan and Blows 2010).

Studying contaminants' effects on a trait like resistance, without knowing what specific genetic changes are responsible for this resistance, limits insights into underlying physiological or biochemical changes. Yet these mechanisms may be important for assessing risks to other organisms at a contaminated site, e.g., where the resistance mechanism affects food web transfer of the contaminant (Wallace et al. 1998). It is not surprising then that quantitative genetics studies of resistance are often accompanied by investigations of underlying mechanisms. Examples include studies on PAH-resistance in killifish *Fundulus heteroclitus* from the Elizabeth River (VA, USA), cadmium-resistance in the worm *L. hoffmeisteri* from Foundry Cove (NY, USA), cadmium-resistance in the springtail *Orchesella cincta* and the isopod *Porcellio scaber* from various contaminated sites in Europe, and pesticide-resistance in *Daphnia magna* from contaminated rice fields in Spain (Damásio et al. 2007; Donker and Bogert 1991; Klerks and Levinton 1989; Ownby et al. 2002; Posthuma 1990). These studies have yielded many insights into specific physiological and biochemical mechanisms contributing to, or associated with, the resistance (see e.g., Donker et al. 1990; Klerks and Bartholomew 1991; Meyer et al. 2003; Posthuma et al. 1992).

Evolutionary changes can now also be addressed using molecular approaches. The availability of a wide variety of neutral genetic markers has proven its usefulness in studying evolutionary processes (see e.g., Hoffmann and Willi 2008). For example, neutral markers can be very powerful for investigating effects of contamination on overall genetic diversity—though by definition their neutrality means that these markers by themselves are not affected by selection. However, the use of neutral markers requires a great deal of data collection; a meta-population model investigating various changes to genetic diversity resulting from anthropogenic activities found that it may take as many as 10–20 neutral genetic markers to provide the same information as a single quantitative genetic trait (Carvajal-Rodríguez et al. 2005).

Research on physiological and other mechanisms underlying quantitative genetic changes has already identified many genes that affect evolutionary responses to environmental contaminants. In some cases we have detailed information on genetic changes involved in the resistance (see e.g., Bhave et al. 1988; Powell et al. 2000; Wirgin et al. 2011) and a variety of approaches are now available for identifying other candidate gene sets (Hoffmann and Willi 2008). An important method combining quantitative genetics and molecular methods involves Quantitative Trait Loci (QTL) techniques, where trait differences can be assigned to specific sections on the chromosome delineated by genetic markers (Falconer and Mackay 1996). This approach is becoming more powerful with the increased availability of genetic markers and detailed linkage maps for more species—such as the recent first-generation one for *D. magna* (Routtu et al. 2010). Linking resistance to QTLs can ultimately result in the identification of resistance genes, as shown recently for cold tolerance in *Drosophila melanogaster* (Svetec et al. 2011). Once candidate genes are identified, a single-gene approach can now be used to study evolutionary responses to contamination (Hoffmann and Willi 2008). However, focusing on candidate genes means that one could miss some of the genetic changes responsible for an evolutionary response. In addition, detailed genetic information is still mostly limited to the typical model species such as *D. melanogaster*.

Another approach for understanding complex traits (such as resistance) is provided by proteomics, genomics and other "omics" techniques. For example, gene expression analysis is well suited for identifying the genetic differences between, for example, a control and resistant population. An example is provided by Meyer et al. (2005), using differential display and cDNA macroarrays to compare killifish (*Fundulus heteroclitus*) mRNA expression between a control and a PAH-resistant population. Such an approach can lead to the identification of resistance genes (Meyer et al. 2005), but has the drawback that we still lack the information to identify many of the genes that are differentially expressed. We refer to the publication by Van Straalen et al. in this special issue for an overview of molecular methods in ecotoxicology.

We conclude that quantitative genetics approaches provide powerful tools for studying evolutionary processes in environmental toxicology. However, the approaches are often complex and logistically demanding, and have their limitations in part from the fact that most work is done in a laboratory setting with conditions differing from those in the natural environment. The study of evolutionary processes in environmental toxicology is likely to benefit from the use of quantitative genetics as well as molecular approaches, utilizing each approach's strengths and benefitting from attempts to reconcile and complement the various insights obtained. Such an integration is ongoing in other fields (see e.g., Hoffmann et al. 2003) and will benefit the field of evolutionary ecotoxicology.

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## References

Agrawal AA, Stinchcombe JR (2009) How much do genetic covariances alter the rate of adaptation? Proc R Soc B 276:1183–1191

Andrew RL, Peakall R, Wallis IR, Wood JT, Knight EJ, Foley WJ (2005) Marker-based quantitative genetics in the wild? The heritability and genetic correlation of chemical defenses in *Eucalyptus*. Genetics 171:1989–1998

Antonovics J, Bradshaw AD, Turner RG (1971) Heavy metal tolerance in plants. Adv Ecol Res 7:1–85

Arnaud L, Haubruge E (2002) Insecticide resistance enhances male reproductive success in a beetle. Evolution 56:2435–2444

Arnold SJ (1994) Multivariate inheritance and evolution: a review of concepts. In: Boake CRB (ed) Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago, IL, pp 17–48

Athrey NRG, Leberg PL, Klerks PL (2007) Laboratory culturing and selection for increased resistance to cadmium reduce genetic variation in the least killifish, *Heterandria formosa*. Environ Toxicol Chem 26:1916–1921

Barata C, Baird DJ, Mitchell SE, Soares AMVM (2002) Among- and within-population variability in tolerance to cadmium stress in natural populations of *Daphnia magna*: Implications for ecological risk assessment. Environ Toxicol Chem 21:1058–1064

Bhave MR, Wilson MJ, Waalkes MP (1988) Methylation status and organization of the metallothionein-I gene in livers and testes of strains of mice resistant and susceptible to cadmium. Toxicology 50:231–245

Boyd CE, Ferguson DE (1964) Susceptibility and resistance of mosquito fish to several insecticides. J Econ Entomol 57:430–431

Brown BE (1978) Lead detoxification by a copper-tolerant isopod. Nature 276:388–390

Bryan GW, Hummerstone LG (1971) Adaptation of the polychaete *Nereis diversicolor* to sediments containing high concentrations

of heavy metals. I. General observations and adaptation to copper. J Mar Biol Assoc 51:845–863
Bubliy OA, Loeschcke V (2002) Effect of low stressful temperature on genetic variation of five quantitative traits in *Drosophila melonagaster*. Heredity 89:70–75
Carvajal-Rodríguez A, Rolán-Alvarez E, Caballero A (2005) Quantitative variation as a tool for detecting human-induced impacts on genetic diversity. Biol Conserv 124:1–13
Charmantier A, Garant D (2005) Environmental quality and evolutionary potential: lessons from wild populations. Proc R Soc B 272:1415–1425
Chaumot A, Gos P, Garric J, Geffard O (2009) 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. Aquat Toxicol 94:294–299
Chown SL, Jumbam KR, Sørensen JG, Terblanche JS (2009) Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct Ecol 23:133–140
Crow JF (1957) Genetics of insect resistance to chemicals. Ann Rev Entomol 2:227–246
Damásio J, Guilhermino L, Soares AMVM, Riva MC, Barata C (2007) Biochemical mechanisms of resistance in *Daphnia magna* exposed to the insecticide fenitrothion. Chemosphere 70:74–82
Derry AM, Arnott SE, Boag PT (2010) Evolutionary shifts in copepod acid tolerance in an acid-recovering lake indicated by resurrected resting eggs. Evolut Ecol 24:133–145
Donker MH, Bogert CG (1991) Adaptation to cadmium in three populations of the isopod *Porcellio scaber*. Comp Biochem Physiol 100C:143–146
Donker MH, Koevoets P, Verkleij JAC, Van Straalen NM (1990) Metal binding compounds in the hepatopancreas and haemolymph of *Porcellio scaber* (Isopoda) from contaminated and reference areas. Comp Biochem Physiol 97C:119–126
Eränen JK, Nilsen J, Zverev VE, Kozlov MV (2009) Mountain birch under multiple stressors—heavy metal-resistant populations co-resistant to biotic stress but maladapted to abiotic stress. J Evol Biol 22:840–851
Falconer DS (1960) Introduction to quantitative genetics, 1st edn. Oliver & Boyd, Edinburgh
Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Essex
Firko MJ, Hayes JL (1990) Quantitative genetic tools for insecticide resistance risk assessment: estimating the heritability of resistance. J Econ Entomol 83:647–654
Fuller RC, Baer CF, Travis J (2005) How and when selection experiments might actually be useful. Integr Comp Biol 45:391–404
Garant D, Kruuk LEB (2005) How to use molecular marker data to measure evolutionary parameters in wild populations. Mol Ecol 14:1843–1859
Garant D, Hadfield JD, Kruuk LEB, Sheldon BC (2008) Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population. Mol Ecol 17:179–188
Georghiou GP (1990) Overview of insecticide resistance. In: Green MB, LeBaron HM, Moberg WK (eds) Managing resistance to agrochemicals. American Chemical Society, Washington, DC, pp 18–41
Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. Nat Rev Genet 9:421–432
Hoffmann AA, Sørensen JG, Loeschcke V (2003) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J Thermal Biol 28:175–216
Houle D (1991) Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45:630–648
Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics 130:195–204
Jiménez-Ambriz G, Petit C, Bourrié I, Dubois S, Olivieri I, Ronce O (2007) Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France: role of gene flow, selection, and phenotypic plasticity. New Phytol 173:199–215
Johnson MTJ, Agrawal AA, Maron JL, Salminen J-P (2009) Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. J Evol Biol 22:1295–1307
Klerks PL, Bartholomew PR (1991) Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. Aquat Toxicol 19:97–112
Klerks PL, Lentz SA (1998) Resistance to lead and zinc in the western mosquitofish *Gambusia affinis* inhabiting contaminated Bayou Trepagnier. Ecotoxicology 7:11–17
Klerks PL, Levinton JS (1989) Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol Bull 176:135–141
Klerks PL, Levinton JS (1993) Evolution of resistance and changes in community composition in metal-polluted environments: a case study on Foundry Cove. In: Dallinger R, Rainbow PS (eds) Ecotoxicology of metals in invertebrates. CRC Press, Boca Raton, FL, pp 223–241
Klerks PL, Moreau CJ (2001) Heritability of resistance to individual contaminants and to contaminant mixtures in the sheepshead minnow (*Cyprinodon variegatus)*. Environ Toxicol Chem 20:1746–1751
Klerks PL, Weis JS (1987) Genetic adaptation to heavy metals in aquatic organisms: a review. Environ Pollut 45:173–205
Knutson AB, Klerks PL, Levinton JS (1987) The fate of metal contaminated sediments in Foundry Cove, New York. Environ Pollut 45:291–304
Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226
LeBlanc GA (1982) Laboratory investigation into the development of resistance of *Daphnia magna* (Straus) to environmental pollutants. Environ Pollut (Ser A) 27:309–322
Leinonen T, O'Hara RB, Cano JM, Merilä J (2008) Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. J Evol Biol 21:1–17
Levinton JS, Suatoni E, Wallace W, Junkins R, Kelaher B, Allen BJ (2003) Rapid loss of genetically based resistance to metals after the clean-up of a Superfund site. Proc Natl Acad Sci USA 100:9889–9891
Lonsdale DJ, Levinton JS (1985) Latitudinal differentiation in embryonic duration, egg size, and newborn survival in a harpacticoid copepod. Biol Bull 168:419–431
Lopes I, Baird DJ, Ribeiro R (2005) Genetically determined resistance to lethal levels of copper by *Daphnia longispina*: association with sublethal response and multiple/coresistance. Environ Toxicol Chem 24:1414–1419
Mackie JA, Levinton JS, Przeslawski R, DeLambert D, Wallace W (2010) Loss of evolutionary resistance by the oligochaete *Limnodrilus hoffmeisteri* to a toxic substance—cost or gene flow? Evolution 64:152–165
Macnair MR (1991) Why the evolution of resistance to anthropogenic toxins normally involves major gene changes: the limits to natural selection. Genetica 84:213–219
Macnair MR (1997) The evolution of plants in metal-contaminated environments. In: Bijlsma R, Loeschcke V (eds) Environmental

stress, adaptation and evolution. Birkhäuser Verlag, Basel, pp 3–24
Maroni G, Wise J, Young JE, Otto R (1987) Metallothionein gene duplication and metal tolerance in natural populations of *Drosophila melanogaster*. Genetics 117:739–744
Martínez DE, Levinton J (1996) Adaptation to heavy metals in the aquatic oligochaete *Limnodrilus hoffmeisteri*: evidence for control by one gene. Evolution 50:1339–1343
McGuigan K, Blows MW (2010) Evolvability of individual traits in a multivariate context: partitioning the additive genetic variance into common and specific components. Evolution 64:1899–1911
Meyer JN, Wassenberg DM, Karchner SI, Hahn ME, Di Giulio RT (2003) Expression and inducibility of aryl hydrocarbon receptor pathway genes in wild-caught killifish (*Fundulus heteroclitus*) with different contaminant-exposure histories. Environ Toxicol Chem 22:2337–2343
Meyer J, Volz DC, Freedman JH, Di Giulio RT (2005) Differential display of hepatic mRNA from killifish (*Fundulus heteroclitus*) inhabiting a Superfund estuary. Aquat Toxicol 73:327–341
Ownby DR, Newman MC, Mulvey M, Vogelbein WK, Unger MA, Arzayus LF (2002) Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. Environ Toxicol Chem 21:1897–1902
Posthuma L (1990) Genetic differentiation between populations of *Orchesella cincta* (Collembola) from heavy metal contaminated sites. J Appl Ecol 27:609–622
Posthuma L, Hogervorst RF, Van Straalen NM (1992) Adaptation to soil pollution by cadmium excretion in natural populations of *Orchesella cincta* (L.) (Collembola). Arch Environ Contam Toxicol 22:146–156
Posthuma L, Hogervorst RF, Joosse ENG, Van Straalen NM (1993) Genetic variation and covariation for characteristics associated with cadmium tolerance in natural populations of the springtail *Orchesella cincta* (L.). Evolution 47:619–631
Powell WH, Bright R, Bello SM, Hahn ME (2000) Developmental and tissue-specific expression of AHR1, AHR2, and ARNT2 in dioxin-sensitive and -resistant populations of the marine fish *Fundulus heteroclitus*. Toxicol Sci 57:229–239
Rahel F (1981) Selection for zinc tolerance in fish: results from laboratory and wild populations. Trans Am Fish Soc 110:19–28
Roelofs D, Overhein L, De Boer ME, Janssens TKS, Van Straalen NM (2006) Additive genetic variation of transcriptional regulation: metallothionein expression in the soil insect *Orchesella cincta*. Heredity 96:85–92
Roff DA (1997) Evolutionary quantitative genetics. Chapman & Hall, New York
Routtu J, Jansen B, Colson I, De Meester L, Ebert D (2010) The first-generation *Daphnia magna* linkage map. BMC Genomics 11:508
Scott JA, Plapp FW Jr, Bay DE (1997) Pyrethroid resistance associated with decreased biotic fitness in horn flies (Diptera: Muscidae). Southw Entomol 122:405–410
Shirley MDF, Sibly RM (1999) Genetic basis of a between-environment trade-off involving resistance to cadmium in *Drosophila melanogaster*. Evolution 53:826–836
Svetec N, Werzner A, Wilches R, Pavlidis P, Broman KW, Metzler D, Stephan W (2011) Identification of X-linked quantitative trait loci affecting cold tolerance in *Drosophila melanogaster* and fine mapping with selective sweep analysis. Mol Ecol 20:530–544
Van Noordwijk AJ, Van Balen JH, Scharloo W (1980) Heritability of ecologically important traits in the great tit. Ardea 68:193–203
Vinson SB, Boyd CE, Ferguson DE (1963) Resistance to DDT in the mosquito fish, *Gambusia affinis*. Science 139:217–218
Wallace WW, Lopez GR, Levinton JS (1998) Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar Ecol Prog Ser 172:225–237
Ward TJ, Robinson WE (2005) Evolution of cadmium resistance in *Daphnia magna*. Environ Toxicol Chem 24:2341–2349
Weider LJ, Lampert W, Wessels M, Colbourne JK, Limburg P (1997) Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. Proc R Soc B 264:1613–1618
Weigensberg I, Roff DA (1996) Natural heritabilities: can they be reliably estimated in the laboratory? Evolution 50:2149–2157
Wilson AJ (2008) Why $h^2$ does not always equal $V_A/V_P$. J Evol Biol 21:647–650
Wirgin I, Roy NK, Loftus M, Chambers RC, Franks DG, Hahn ME (2011) Mechanistic basis of resistance to PCBs in atlantic tomcod from the Hudson River. Sciencexpress. doi:10.1126/science.1197296
Xie L, Klerks PL (2003) Responses to selection for cadmium resistance in the least killifish, *Heterandria formosa*. Environ Toxicol Chem 22:313–320
Xie L, Klerks PL (2004) Fitness costs of resistance to cadmium in the least killifish (*Heterandria formosa*). Environ Toxicol Chem 23:1499–1503