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Retrospective estimation of population-level effect of pollutants based on local adaptation and fitness cost of tolerance

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Abstract We present a novel framework for estimating site-specific effects of pollutants on natural populations. Our method is based on fitness optimization and uses observed differences in tolerance (sensitivity) to a particular pollutant between populations at contaminated and uncontaminated sites (i.e., target and reference populations). In addition, the method uses laboratory estimates of the fitness cost of tolerance, that is, the reduction of population growth rate (fitness) of a target population compared to that of a reference population when both are maintained in uncontaminated conditions. As a case study, we applied this framework to analyze observed genetic differentiation in tolerance to the pyrethroid insecticide fenvalerate between *Daphnia galeata* populations in Lake Kasumigaura and an adjacent agricultural pond. The estimated exposure level at the contaminated site was about 0.015 μg/L, and the population-level risk corresponded to about a 24 % reduction of the intrinsic rate of natural increase.

Keywords Tolerance cost · Adaptation · Ecological risk assessment · Genetic variance · Trade-off · Fitness optimization

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Introduction

Many organisms in natural environments have been able to adapt to the presence of pollutants by evolutionary acquisition of tolerance to the pollutants. The evolutionary acquisition of tolerance requires the tolerance trait of organisms to have genetic basis and maintain heritable variation within populations (Klerks et al. 2011). Many experimental studies, especially those concerned with tolerance to heavy metals and pesticides, have supported the assumption that these conditions are met (e.g., Klerks and Weis 1987; Mulvey and Diamond 1991; Ward and Robinson 2005; Lopes et al. 2006; Pease et al. 2010; Jansen et al. 2011).

Evolutionary acquisition of tolerance is likely to be the most common explanation for the frequently observed between-site differences in the tolerances of organisms to particular pollutants (Barata et al. 2002). From the standpoint of developing ecological risk assessments based on tolerance evolution, the higher tolerance acquired by a particular population living in a contaminated site compared to the tolerance of a population living in an uncontaminated site is strong evidence for the former population having suffered adverse effects from the toxicant (Grant 2002; Klerks et al. 2011). However, such an evolutionary approach in ecotoxicology has not been used for quantitative estimation of ecological risk from exposure to pollutants.

Many studies have shown that tolerance or a genotype that confers tolerance to a chemical pollutant is often accompanied by reduction of reproductive ability or other important traits that are associated with the fitness of individuals (i.e., there is a cost of tolerance in terms of fitness) (Carriere et al. 1994; Xie and Klerks 2004; Mackie et al. 2007; Agra et al. 2010; reviewed by Mouneyrac et al.

2011). We attempted to estimate the effect of pollutants on natural populations based on the trade-off between the benefit of tolerance and the reduction of fitness due to the acquisition of tolerance. For this purpose we made the basic assumption that evolution optimizes the mean tolerance of a population. This assumption is met if the fitness cost of tolerance causes a decline in the tolerance of a population, and the population becomes more sensitive to pollutants when the population does not suffer from exposure to pollutants for a long time (Hoffmann et al. 2001; Levinton et al. 2003).

This proposed novel approach was applied to a case study of observed differentiation of tolerance of *Daphnia galeata* to the pyrethroid insecticide fenvalerate. A previous study indicated that this species exhibited spatial or local genetic differentiation in terms of both molecular genetic markers and sensitivity to fenvalerate. The present study provided answers to two additional questions needed to estimate the ecological risk of the chemical to the population. The first question concerned how tolerance to fenvalerate affected fitness if fitness was measured in the absence of exposure. The second question concerned how a relevant dataset could be used to estimate the exposure level and ecological risk and thereby infer the evolutionary acquisition of tolerance and the fitness cost of the tolerance. Our main goal was to present a general framework for this analysis. We expect that there will be more elaborate extensions of the analytical methods based on more extensive case studies of particular chemicals, organisms, and study sites.

Materials and methods

Study site and test organisms

The study sites were Lake Kasumigaura, which is a large, shallow eutrophic lake located northeast of Tokyo (center of the lake: 36°02′35″N, 140°24′42″E), and Ohzen Pond (5.5 km east of the center of Lake Kasumigaura), which is an old reservoir pond (area: about 3.7 ha) surrounded by a protected forest in a prefectural natural park.

Three natural populations of *D. galeata*, two in Lake Kasumigaura and the other in Ohzen Pond, were sampled and are referred to as the Koise, Center, and Ohzen populations, respectively. The Koise population was sampled from the estuary of the Koise River, which flows into the lake, and the Center population was sampled around the center of the lake. The Koise River runs through agricultural areas and is heavily exploited for irrigation and drainage of paddy fields. Several agrochemicals, including fenvalerate, have been detected in the water of the Koise River (Hatakeyama 1998; Hatakeyama et al. 1999). In contrast, Ohzen Pond is not polluted with agrochemicals.

Daphnia galeata (water fleas) was chosen as the study organism because this species is widely distributed and is one of the most important zooplankton grazers in Japanese lakes and ponds. Moreover, among major cladoceran species *D. galeata* is known to be moderately sensitive to pyrethroid and organophosphorus insecticides (Mano et al. 2010).

Adults of *D. galeata* were collected from the study sites, and isofemale lines were established by selecting one ancestral female for each line. The *D. galeata* were maintained as isofemale clones in dechlorinated (Lake Kasumigaura water system) tap water (pH 6.9–7.2, dissolved oxygen 6.8–7.0 mg/L) at 20 °C and under a 16:8 (h:h) light–dark cycle. The daphnia of each isofemale line were maintained in two 200 mL glass bottles and fed *Chlorella vulgaris* throughout the study, including the life table experiments. The culture medium was replaced by fresh medium with sufficient food three times per week. Because at least two generations had passed (the offspring of the field-collected individuals were not used for the acute toxicity test and the life table experiment) before the start of experiments, no nongenetic, transgenerational effects due to parasites, diseases, and maternal effects confounded the analyses of between-line differences of performance.

We have previously detected consistent differences in molecular genetic markers (microsatellite DNA at six loci) and individual-level sensitivity to fenvalerate between populations of *D. galeata* (Tatsuta and Tanaka unpublished). The acute EC50 (48 h), measured as neonate immobility, for the Koise and Center populations was 5–10 times that of the Ohzen population (1.74 μg/L for Koise; 3.13 μg/L for Center; and 0.29 μg/L for Ohzen). We regarded the Ohzen population as the reference population for this insecticide, that is, the population from an uncontaminated site.

To estimate the fitness cost of tolerance, we conducted additional acute toxicity tests and life table experiments for each isofemale line. We sampled individuals of *D. galeata* with lake water from three to five sampling sites for each population and maintained them in the laboratory. Adult or semi-adult females were kept individually in rearing containers filled with dechlorinated tap water and *C. vulgaris* as food at 20 °C and on a 16:8 light:dark cycle.

Acute toxicity tests

To estimate quantitative relationships between tolerance and fitness, we conducted both acute toxicity tests and life table experiments with the same isofemale lines (some lines provided only acute toxicity data). For the acute immobility test, 30 neonates (individuals less than 24 h old) were arbitrarily chosen from each subgroup and kept

in three glass beakers (10 individuals each) with 200 mL of carbon-filtered tap water. Two beakers contained a predetermined nominal concentration (0.5 or 1.0 μg/L) of fenvalerate before the introduction of the test organisms (Tatsuta and Tanaka unpublished). The nominal concentrations were determined to be 0.5 and 1.0 μg/L because these concentrations induced an intermediate rate of immobility of *D. galeata* in preliminary tests, and we expected that genetic variability of tolerance was likely to be revealed at these concentrations. The test conditions were 20 °C on a 16:8 light–dark cycle. Immobile individuals, which were defined as animals that did not maintain an upright position when moving their secondary antennae, were counted after 48 h.

Each isofemale line was replicated before experiments (11, 5, and 5 isofemale lines for the Koise, Center, and Ohzen populations, respectively) and 10 or 11 sets of immobility tests were conducted for each replicate (two per line) of isofemale lines. The total number of sets of immobility tests was 433. Each immobility test consisted of two exposure treatments and a control treatment, with each treatment involving 10 neonate individuals. Immobility rates were checked 48 h after the start of exposure to fenvalerate by introducing the test organisms into test beakers that already contained medium with a specific concentration of fenvalerate.

The tolerance of each individual was defined as the common logarithm of the concentration of fenvalerate above which the individual became immobile (Cox 1987; Ashauer 2010; Tanaka et al. 2012). The tolerance for each isofemale line (genotypic value), which was equated to the mean threshold across all individuals within the isofemale line, was estimated with the maximum likelihood method described by Tanaka et al. (2012). The maximum likelihood estimates were derived with Mathcad's built-in function *Maximize* (Mathcad 14; Parametric Technology Corporation, Needham, MA), which returns local maxima of nonlinear functions with the conjugate-gradient method.

Life table experiments

We conducted two sets of life table experiments. The first experiment was narrowly focused on the two isofemale lines with the highest and lowest tolerances, the objective being to ascertain if there was any statistically significant difference in fitness between the two lines with the greatest difference of tolerance. The second experiment was conducted on all isofemale lines for which tolerance values had been estimated by the acute immobility test.

For the first experiment, neonates less than 24 h after birth were arbitrarily chosen from the most sensitive isofemale line (the Ohzen population) and the most tolerant isofemale line (the Koise population). The neonates were individually reared throughout their lifespan at 20 °C on a 16:8 light:dark cycle in glass vials containing 50 mL of water and an adequate supply of food (*C. vulgaris*). The culture medium containing the food was refreshed three times each week. The newborn neonates produced by each individual were counted every day. We obtained reproduction data for 92 and 77 parental females from the most sensitive and most tolerant lines, respectively. Individual fitness (the intrinsic rate of natural increase, r) was determined by numerically solving $\sum_{x=\alpha}^{\omega} m_x e^{-rx} = 1$ (α: the age of first reproduction, ω: the life span, and m_x: the fecundity at age x) for r.

In the second experiment, the schedule of reproduction and age-specific mortality rates were examined for as many isofemale lines as were relevant from all populations. Ten neonates less than 24 h after birth from the same isofemale culture were reared as a cohort group in a glass bottle containing 200 mL of water. The rearing conditions were the same as for the first experiment, except that neonates of the parent females were individually reared in the first experiment. The number of newborn neonates was counted and the survivorship of parents was checked when the medium water was exchanged. We obtained life table data for 29 cohort groups belonging to 12 isofemale lines. Replicates of the life table data for the same genotype (isofemale line) were treated as statistically independent data because the estimated tolerances of isofemale lines were regarded as fixed effects.

Risk estimation

We used fitness optimization (Fig. 1) to estimate the levels of stationary exposure in the environments inhabited by the examined populations and the effects in terms of reduction of the intrinsic rate of natural increase (reduction of the mean fitness) of the population.

Total fitness reflects a combination of the adverse effects of a pollutant and the fitness cost of tolerance. We therefore expressed total fitness by combining the response function simulating the effect of pollutant and the cost function of tolerance. To quantify the direct adverse effect of a pollutant, the intrinsic growth rate of a population with a tolerance z following exposure to a pollutant concentration x is described by the following equation:

$$r_{x,z} = r_{0,z}\{1 - R(x,z)\}, \quad (1)$$

where $r_{0,z}$ is the intrinsic growth rate under null exposure and $R(x,z)$ is the response function when a population having a tolerance z is exposed to a pollutant at concentration x in the common logarithmic scale. The response function quantifies the proportional reduction of the intrinsic rate of natural increase due to exposure to a specific pollutant concentration.

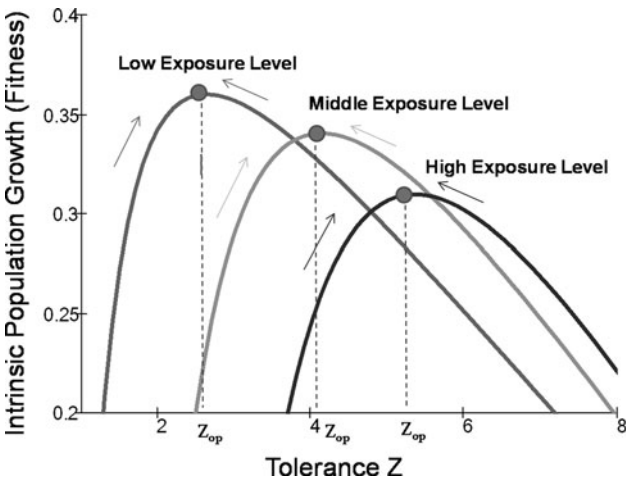

Fig. 1 A schematic drawing of hypothetical fitness profiles for the tolerance to pollutants under different exposure levels. For a particular exposure level, fitness has a specific peak balanced by the fitness gain by tolerance and the fitness cost by tolerance. Optimum tolerance values increase with exposure levels. Z_{op}: optimum tolerance

The shape of the response function is much more important than the value of the acute toxicity (EC50) for evaluating ecological risk at very low exposure levels (Tanaka and Nakanishi 2001). However, data were not available to estimate the shape of response of *D. galeata* to fenvalerate in terms of the intrinsic rate of natural increase. A previous study indicated that responses of the population growth rates of phytoplankton and zooplankton to various toxicants are well approximated by the power function $R(x,z) = (x/z)^{\beta}$, and the generic value of the power index β is about 2.24 (recalculated from Tanaka and Nakanishi (2001) after converting exposure concentrations to a logarithmic scale). We used this function and the generic estimate of β for the response of *D. galeata* to fenvalerate.

To quantify the cost of tolerance, we assumed fitness in the absence of exposure to decrease linearly with tolerance:

$$r_{0,z} = r_{\max} - c(z - z_{\text{ref}}), \tag{2}$$

where $r_{\max}$ is the maximum intrinsic growth rate (the growth rate achieved when there is no exposure to a toxicant and no fitness cost of tolerance), c quantifies the magnitude of the fitness cost and is the slope of the regression line that relates fitness to tolerance (cost coefficient), and z_{ref} is the tolerance of a reference population. This equation denotes the negative association (the trade-off) between tolerance and fitness when there is no exposure across different genotypes (isofemale lines).

We assumed that there was no interaction between the adverse effect of the pollutant and the tolerance cost of fitness. The total fitness of the exposed population is therefore the product of the two components of fitness (a Malthusian fitness is generally expressed as the product of fitness components if selection from each component acts additively; Lande and Arnold 1983):

$$r_{x,z} = \{r_{\max} - c(z - z_{\text{ref}})\}\left\{1 - \left(\frac{x}{z}\right)^{\beta}\right\}. \tag{3}$$

The optimal tolerance $\hat{z}$ that maximizes total fitness can be derived as a function of the exposure concentration. The local maximum of $r_{z,x}$ solves the equation $\frac{\partial r_{x,z}}{\partial z} = 0$, and leads to the value of $\hat{z}$, which solves the following implicit equation:

$$\left[c + \{r_{\max} - c(\hat{z} - z_{\text{ref}})\}\frac{\beta}{\hat{z}}\right]\left(\frac{x}{\hat{z}}\right)^{\beta} = c. \tag{4}$$

If the observed tolerance of the examined population remains at this optimal value of $\hat{z}$, the long-term and stationary level of exposure, $\tilde{x}$, which is assumed to make the tolerance of the examined population optimal, is estimated from the equation $\tilde{x} = \hat{z}\left[1 + \left\{\frac{r_{\max}}{c} - (\hat{z} - z_{\text{ref}})\right\}\frac{\beta}{\hat{z}}\right]^{-1/\beta}$. The fitness reduction due to exposure was evaluated by inserting the estimate of $\tilde{x}$ into Eq. (3) and comparing the estimated r with the maximum value $r_{\max}$, which we assumed to be unaffected by any fitness costs of tolerance and any adverse effects of pollutants.

The uncertainty of the estimates of exposure and risk level due to errors in the estimation of the tolerance cost based on life table experiments were evaluated with a bootstrap simulation, the jackknife procedure of Efron (1982). The data from the second life table generated 29 resampling units. Because the first life table experiment differed from the second life table experiment with respect to design and numbers of replicates, we could not define the same resampling units for the entire experiment (the first life table data were not relevant to estimating uncertainty).

Results

Distribution of tolerance across populations

There was overlap across populations in the distributions of the tolerances of individual isofemale clones (Fig. 2). The mean tolerances in each population were estimated to be 2.102 (log[ng/L]) for Ohzen, 2.685 (log[ng/L]) for Koise, and 2.665 (log[ng/L]) for Center. The tolerances were significantly lower on average among isofemale lines from the Ohzen population than from the other two populations. The broad-sense heritabilities, H^2, defined by the equation $H^2 = V_G/(V_G + V_E)$ (V_G: genetic variance estimated from between-isofemale line variance; V_E: environmental variance

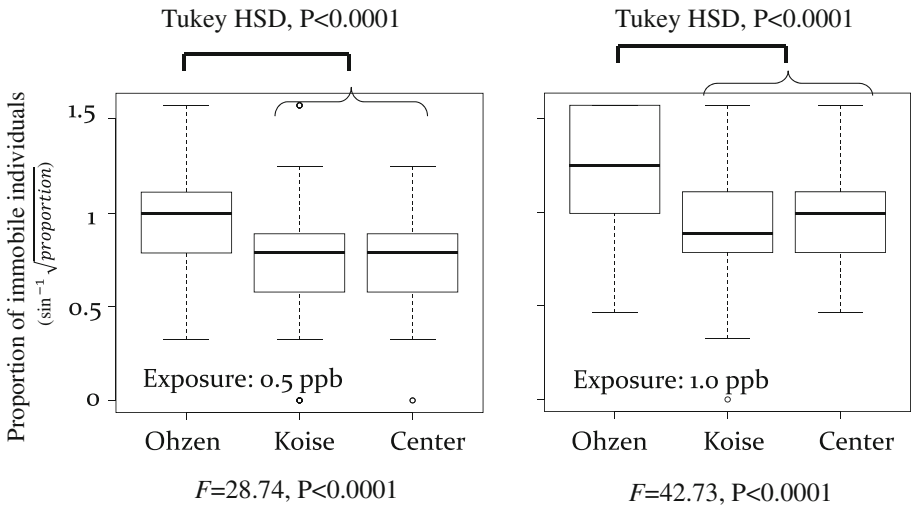

Fig. 2 Between-population differences in sensitivity to a pyrethroid insecticide (fenvalerate) by field samples of a cladoceran species *Daphnia galeata*, under two exposure concentrations (Box-and whisker diagram). The vertical axis denotes arcsin-transformed values of the immobility rates. The Ohzen population is significantly more sensitive than the other two populations (Tukey's Honesty Significant Difference test)

estimated from within-isofemale line variance; Tanaka et al. 2012), were estimated to be 0.070, 0.034, and 0.055 for the Ohzen, Koise, and Center populations, respectively.

Fitness cost of tolerance

The results of the first life table experiment indicated that the r of the most sensitive isofemale line was 0.297 ± 0.037 (mean ± standard deviation), whereas the r of the most tolerant line was 0.242 ± 0.046 (Fig. 3). These values were significantly different from each other if (genetic) effects of these lines were treated as fixed effects ($p < 0.001$; t test, Wilcoxon signed-rank test). The tolerances of these extreme lines were 1.738 and 2.900 (log[ng/L]), respectively, and the slope of the r versus tolerance relationship was therefore (0.297–0.242)/(1.738–2.900) = −0.047.

From the second life table experiment, 29 life table data were collected for 12 isofemale lines (some lines gave multiple life table data), which were a subset of all the isofemale lines for which we estimated tolerances. The regression slope of r versus tolerance was −0.042 (Fig. 2). This slope was not significantly different from 0, but nonetheless very close to the estimate from the first life table experiment. We used the mean value of the regression slopes from the two experiments, −0.045, for the estimate of the cost coefficient of tolerance (c in Eqs. 2–4).

The reconstructed population-level effect

Inserting the parameter values estimated to be $\hat{z} = 2.685$ for Koise and 2.665 for Center populations, $z_{\mathrm{ref}} = 2.102$, $c = 0.045$ (bias-corrected estimate ± standard error: 0.121 ± 0.042), $r_{\max} = 0.3$, and $\beta = 2.24$ into Eq. (4) and numerically solving the equation, we derived estimates for the environmental exposure concentrations, on the logarithmic scale, as 1.20 (log[ng/L]) for Koise and 1.19 (log[ng/L]) for Center populations. These values correspond to concentrations (±standard errors) of 0.015 (0.041 ± 0.015) μg/L and 0.014 (0.040 ± 0.015) μg/L, respectively. Based on Eq. (3), these estimates of exposure levels in the environment for the target populations predict decrements of r (Δr) due to exposure to fenvalerate to be 0.071 (0.186 ± 0.063) for Koise and 0.072 (0.183 ± 0.063) for Center populations in comparison to the maximal intrinsic rate of natural increase, which was assumed to be unaffected by toxicant effects and the fitness cost of tolerance. The proportional reductions of r, $\Delta r/r_{\max}$, were 0.238 (0.621 ± 0.209) for Koise and 0.234 (0.611 ± 0.206) for Center populations.

Discussion

One of the fundamental tenets in evolutionary theory is that individual or genetic fitness tends to increase by natural selection until it reaches a maximum (Fisher 1930). This rule is referred to as a maximum principle for natural selection (Crow and Kimura 1970) and has been used in evolutionary research to predict evolutionary changes or shifts of adaptive traits of organisms as a result of environmental changes or local differences in environmental factors (Bulmer 1994; Roff 2002). The more formalistic version of the hypothesis in the sense of theoretical population genetics is Fisher's Fundamental Theorem of Natural Selection (Fisher 1930).

The maximum principle for natural selection may be used to interpret local differences in the tolerance of wildlife to pollutants and to estimate ecological risk at the level of populations, with the caveat that this principle relies on some necessary conditions that are difficult to confirm. Our goal is to indicate the potential usefulness of an evolutionary approach in quantitative estimation of ecological risk, provided that the fitness cost of tolerance is reliably estimated.

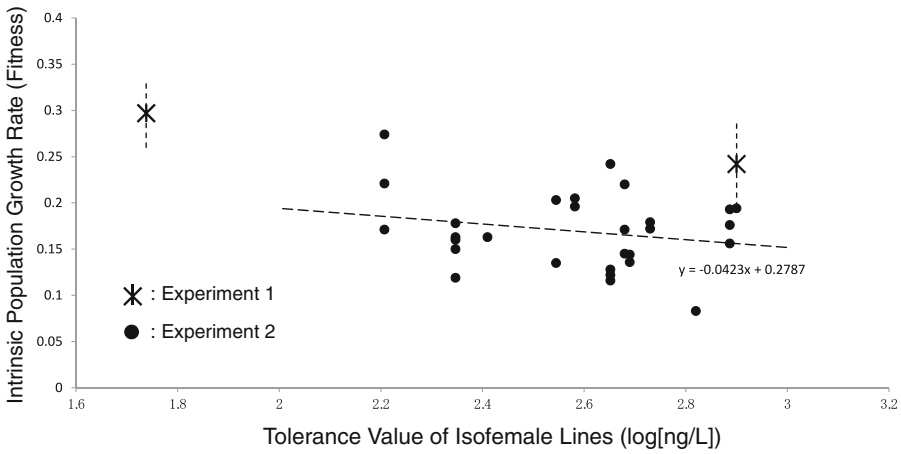

Fig. 3 Scatter plots of fitness as measured by intrinsic rate of natural increase from life table data against the mean tolerance values to fenvalerate among isofemale lines of *Daphnia galeata*. The whiskers on the plots for Experiment 1 range standard deviations of the individual-based estimates of fitness. The mean tolerance values are based on the definition given by the toxicant threshold model (Tanaka et al. 2012)

The major assumptions of fitness optimization in the special context of tolerance evolution are (1) additive genetic variances of tolerance are maintained, so that natural processes are effective in selecting tolerant genotypes over more sensitive genotypes within populations; (2) the gene flow between adjacent populations that have different exposure histories is sufficiently limited so that local adaptation of tolerance is possible; (3) the population size (the minimum number of mating individuals in a population) is large enough that random genetic drift does not largely confound the adaptive changes of tolerance; (4) the timeframe of changes in the exposure level is not much shorter than the timeframe of evolutionary change of tolerance in response to changes in exposure levels; and, most importantly, (5) the fitness cost of tolerance is quantitatively estimated.

Assumptions (1) and (2) can be confirmed if there are nonzero heritabilities of tolerance values within populations and if genetic differentiation on the basis of neutral genetic markers implies that gene flow is limited. If heritability estimates do not decline in contaminated versus uncontaminated sites, then there is reason to suggest that genetic variability of tolerance has not been depleted by persistent selection pressure on tolerance. Assumption (3) is associated with and is a necessary condition for assumption (1) to be true. Estimation of genetic heterozygosity within populations by use of neutral genetic markers can verify if the focal population has ever suffered strong genetic drift due to demographic bottlenecks (drastic declines of population size). We have previously examined genetic differentiation between populations and genetic heterozygosity within populations by using microsatellite DNA on the same field system. We concluded that gene flow was sufficiently limited between the investigated populations that local adaptation could cause tolerance to diverge between populations (Tatsuta and Tanaka unpublished). In addition, the mean heterozygosity within populations was sufficiently large to rule out the possibility that strong genetic drifts explained the observed differences of tolerance (see also Materials and methods, Study site and test organisms). We will address the last two assumptions later in this Discussion section.

Provided that these assumptions are met, a significant amount of variation in tolerance to chemicals between local populations is direct evidence that the populations have suffered a significant risk by the pollutants (Klerks and Levinton 1989; Barata et al. 2002; Ward and Robinson 2005; Lopes et al. 2006; Jansen et al. 2011). The present framework indicates that a retrospective estimation of the level of exposure and ecological risk from exposure can be very different between two chemicals, even though the acute or chronic toxicity of the pollutants (tolerance to these chemicals) is the same. The level of exposure to one chemical (or a category of chemicals that share a mode of action) to which the cost of tolerance is smaller than the cost of tolerance to a second chemical (or another category of chemicals) must be lower than the level of exposure to the second chemical to result in the same observed sensitivity, the result being a lower estimate of risk and level of exposure in the former.

Another advantage of the present approach is that it can measure population-level effects in terms of the intrinsic rate of natural increase (or the population growth rate). The intrinsic rate of natural increase is directly associated with population vulnerability and other related ecologically relevant measures of ecological risk (e.g., mean extinction time or probability of extinction; Tanaka and Nakanishi 2000; Nakamaru et al. 2002; Tanaka 2003; Raimondo and McKenney 2005; Meng et al. 2006) and can summarize any observed endpoints with proper weighting (Forbes and Calow 1999).

It is noteworthy that the decrements of the intrinsic rate of natural increase are decomposable into two parts, one due to the fitness cost of tolerance, and the other due to the response to adverse effects of pollutants. By definition, the former cannot be larger than the latter if fitness has been

optimized. The present case study indicated that the cost of tolerance explained as much as approximately one-third of the entire decrement of fitness. This indirect contribution from the cost of tolerance to population-level effects of pollutants must persist longer than the direct effects of exposure after environmental exposure is eliminated and may account for more than a small part of the impact of pollutants on populations.

If we apply this implicit evolutionary perspective in the context of regulating chemicals, we may reach different conclusions about the relative risks of chemicals versus rankings based on standard PEC/PNEC ratios. Even if two chemicals are equally toxic to a standard test organism (e.g., EC50s of acute immobility are the same for the chemicals) and if the environmental concentration also does not differ between the two chemicals, a natural population of the organisms may suffer a larger long-term risk from exposure to the chemical that induces a greater fitness cost than the other chemical, because it is difficult for a natural population to acquire tolerance to a chemical that requires a large tolerance cost. The PEC/PNEC ranking may even be reversed, depending on differences in the fitness cost of tolerance between chemicals.

Lastly, we should note that there are some technical and conceptual problems in the evolutionary approach presented here as regards limited precision of fitness cost, cotolerance, retrospectiveness, and differential time scales.

The indirect estimation of risk requires the fitness cost of tolerance to be precisely measured. However, a precise measurement is difficult because the measures of fitness include large uncertainties, and the association between fitness cost and tolerance is generally poor.

Chemical compounds that share a mode of action must have more-or-less cotolerance to each other. This issue may be shared with pollution-induced community tolerance (Grant 2002; Schmitt et al. 2006). The present approach is unable to discriminate effects of different chemicals that induce cotolerance by target organisms.

The present evolutionary approach may not be able to evaluate the ecological risk that a population is suffering at the present time if the rate of evolution is severely limited by a very low heritability, because our method assumes an evolutionary equilibrium. In fact, the target population may be out of equilibrium and be in the process of adapting to the present level of environmental exposure to the pollutants. Indicated risk levels may, in extreme cases, reflect the history of exposure from the remote past rather than risks due to current exposure.

If these difficulties are resolved in future studies, inclusion of the fitness cost of tolerance into an evolutionary analysis of tolerance to pollutants will provide a risk assessment framework that is an alternative to extrapolation methods based on laboratory test organisms.

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