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Short Communication

GENETIC VARIANCE OF TOLERANCE AND THE TOXICANT THRESHOLD MODEL

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Abstract—A statistical genetics method is presented for estimating the genetic variance (heritability) of tolerance to pollutants on the basis of a standard acute toxicity test conducted on several isofemale lines of cladoceran species. To analyze the genetic variance of tolerance in the case when the response is measured as a few discrete states (quantal endpoints), the authors attempted to apply the threshold character model in quantitative genetics to the threshold model separately developed in ecotoxicology. The integrated threshold model (toxicant threshold model) assumes that the response of a particular individual occurs at a threshold toxicant concentration and that the individual tolerance characterized by the individual's threshold value is determined by genetic and environmental factors. As a case study, the heritability of tolerance to *p*-nonylphenol in the cladoceran species *Daphnia galeata* was estimated by using the maximum likelihood method and nested analysis of variance (ANOVA). Broad-sense heritability was estimated to be 0.199 ± 0.112 by the maximum likelihood method and 0.184 ± 0.089 by ANOVA; both results implied that the species examined had the potential to acquire tolerance to this substance by evolutionary change. Environ. Toxicol. Chem. 2012;31:813–818. © 2012 SETAC

Keywords—Genetic variance Heritability Sensitivity Threshold model Tolerance

INTRODUCTION

The toxicity of a pollutant chemical to a particular population of a species changes genetically if the population acquires tolerance to the chemical by adaptive evolution under long-term exposure [1–5]. If evolutionary changes increase the tolerance of the population to the chemical, two problems may arise in the context of ecological risk assessment. First, the toxicity of the chemical may differ considerably among populations according to the past exposure histories of the populations, even within a species [1,4–7]. Second, assessing toxicity at the time of testing may result in overestimating the future ecological hazard posed by future exposure levels if a large genetic variance is maintained in the target population and rapid adaptation prevents local extinction [8]. These points imply that there are limitations to the extrapolation of toxicological data based on a particular population, to other populations, or even to the same population in the future. Evolutionary changes in toxicity should be taken into account in estimating the long-term ecological hazards of pollutants [9].

Adaptive change in organisms requires additive genetic variances to be maintained among individuals within populations [10,11], and it follows that an extremely low (close to zero) heritability in the tolerance to a substance indicates a lack of adaptability from the examined population to the target substance. If the heritability of tolerance to a toxicant mixture is considerably lower than the heritabilities of tolerance to the individual toxicants, it can be inferred, from genetic studies of the compound effects of pollutants, that exposure to the toxicant mixture will cause persistent adverse effects for a long time, because the population cannot acquire tolerance to the compound effects of the toxicants [12,13].

In an attempt to apply quantitative genetics—a standard method of estimating heritability based on the assumption that traits are normally distributed—to toxicological data, some previous studies have quantified the genetic variability of tolerance by measuring the responses of individuals to pollutants in terms of continuous measures, such as the time to death or the fitness [8,14,15], rather than relying on quantal endpoints. Nonetheless, appropriate continuous measures are not always relevant for replacing quantal responses. Here, we propose an analytical method that can be used to measure the genetic variance of tolerance for a wide range of toxicological data. This method is based on linking the concept of individual tolerance of effect dose in toxicology [16–20] and the threshold trait model in quantitative genetics [10,21,22]. We illustrate the proposed method with a case study of the acute toxicity of *p*-nonylphenol in a cladoceran zooplankton (*Daphnia galeata*).

MATERIALS AND METHODS

Quantitative genetics procedure

We briefly review quantitative genetics as a relevant analytical tool for estimating genetic variability of tolerance within populations. Applying quantitative genetics successfully requires the following assumptions to be met: that trait values are subject to the effects of genes at multiple loci, and that stochastic environmental factors, in conjunction with genetic factors, influence trait values such that a trait is continuously and normally distributed among individuals within populations. If these assumptions are met, it follows that with a proper experimental breeding design, the observed trait variance (the phenotypic variance), V_P, is decomposed into a genetic variance, V_G, and an environmental variance, V_E [10]. The genetic variance is further decomposed into the additive genetic variance, V_A, which is the fraction explained by the additive action of genes, and the nonadditive genetic variance, V_N, which is the fraction explained by the dominance effects of alleles and

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epistatic interaction between loci. The scaled measure of the genetic variance is referred to as *heritability*, which has two categories, the broad-sense heritability, H^2, equivalent to V_G/V_P, and the narrow-sense heritability, h^2, equivalent to V_A/V_P. Both heritabilities can range between 0 and 1. Broad-sense heritability denotes the upper limit of narrow-sense heritability and represents the fraction of the phenotypic variance that is under genetic determination. The present method, on the basis of clonal variances, estimates the broad-sense heritability. The estimation of narrow-sense heritability requires experiments with a regular breeding design, such as parent–offspring regression and a full-sib or half-sib design [10]. If dominance between alleles and epistasis between loci contributing to tolerance are not important, H^2 is a good approximation of h^2.

Quantitative genetics predicts an evolutionary rate per generation on the basis of the measured genetic variance and the selection intensity acting on the trait [10,11,23]. The additive genetic variance of tolerance is related to the potential evolutionary rate of the trait value per generation by the formula, $R = h^2S$, where R is the per-generation change in mean trait and S is the strength of selection, termed the *selection differential* [10].

Toxicant threshold model

Responses of test organisms to toxicant exposure are usually quantal data that are measured as binary (e.g., survival vs death, mobile vs immobile) or categorical (discrete symptoms of adverse responses to toxicants), whereas quantitative genetics assumes metric traits that vary continuously within populations. Some genetic studies in ecotoxicology have attempted to use time–concentration–response relationships [14,15] or other indiscrete measures of toxicity to measure individual sensitivity on a continuous scale (e.g., individual fitness [8]). Another more practical method is to treat the discrete responses of individuals as discrete expressions of a tolerance trait that is continuously distributed within populations to a toxicant in a threshold manner; the critical limit of the exposure concentration at which a particular individual exhibits a response is determined by the individual tolerance, which is continuously distributed in a population (Fig. 1a) [17–19,24]. The key concept of the threshold expression of individual tolerance in ecotoxicology is compatible with the threshold trait model separately developed in quantitative genetics (Fig. 1b) [10,21].

The model we present here is applicable to any response, including death, if the response is measured in a few discrete states and if the individual tolerance is continuously distributed among individuals within populations. We illustrate our method with the lethal effect of a pollutant, because such effects are among the most common and primary responses used as ecotoxicological data. The tolerance of each individual is defined as the threshold toxicant concentration beyond which the individual dies (lethal threshold) or (if the response is defined otherwise) the threshold beyond which the individual exhibits a predefined adverse response [17–19]. Because there is variation in tolerance values among individuals in a population owing to genetic or environmental factors, the tolerance values are assumed to be continuously distributed in the entire population. The normality assumption is not needed for the analysis described below under *Maximum likelihood method*. Such a scheme of trait expression has been studied as an environmental threshold model in evolutionary studies, which focus on the genetic variance of discrete traits,

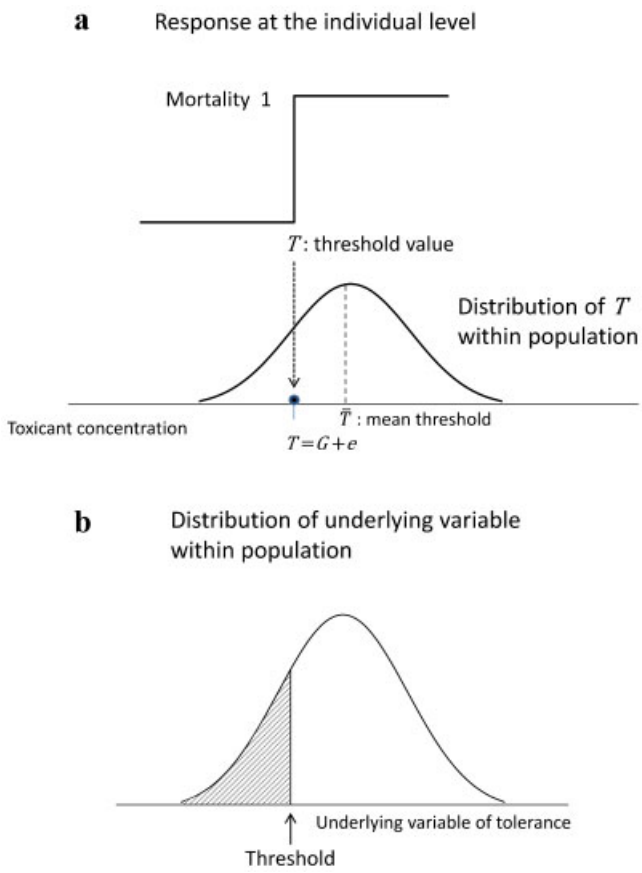

Fig. 1. Schematic drawings of the toxicant threshold model (TTM; **a**) and the threshold character model of quantitative genetics (**b**). In the TTM, the threshold concentration at which a quantal response is triggered is postulated for each individual, and the threshold value is continuously distributed among individuals in a population. The threshold value is a biotic trait (individual tolerance) that has genetic and environmental components. In the threshold character model, an underlying character, which is hypothetical and unmeasurable, is continuously distributed, and a single threshold point is assigned to a population. A quantal response is triggered if the individual trait value exceeds the threshold.

such as morphological dimorphism and sex determination, that depend on environmental factors [22,25,26] (see also review by Roff [11]); this can be referred to as the *toxicant threshold model* (TTM) in the context of ecotoxicology. The standard threshold character model in quantitative genetics assumes an underlying character that is continuously distributed but is not associated with any environmental variables such as the exposure concentration (Fig. 1b) [10]. The difference between the TTM and the threshold models in ecotoxicology [23] is that individual tolerance in the TTM has a decomposable structure and includes a stochastic component, in addition to the genetic component, among individuals; the former is due to environmental effects specific to individuals, but it does not include any temporal stochasticity within a particular individual.

Two calculation procedures, based on TTM and the standard threshold character model, are presented here to estimate the heritabilities of tolerance in the case when standard acute bioassays are conducted in several isofemale lines of a cladoceran species. These procedures are the maximum likelihood method and the analysis of variance (ANOVA) of binary scores assigned to quantal responses (the binary scoring method).

Maximum likelihood method

The maximum likelihood method based on a particular response function evaluates the mean tolerance value of each isofemale line and therefore determines the genetic variance of the tolerance. The tolerance value, T, on the logarithmic scale of an individual is assumed to consist of two components, the genetic value of the tolerance, G, which is equivalent to the expected mean tolerance of the isofemale line, and the random environmental deviation, e, such that $T = G + e$. We assumed that the random deviation e was subject to the normal distribution with mean 0, whereas no particular distribution was assumed for the genetic value of the tolerance G within a population. Because an individual of a particular isofemale line survives if the tolerance of the individual exceeds the exposure concentration, the probability of survival among individuals of this line at the exposure concentration x is

$$\int_x^{\infty} N(T|G,\, \sigma_e)\, dT \tag{1}$$

where $N(z|M, \sigma)$ denotes the normal density function of z with mean M and standard deviation σ, and σ_e is the standard deviation of e.

The genetic values, equivalent to the mean tolerance values of all lines, are estimated by finding the $\mathbf{G}$ value (the vector of G) that maximizes the following log-likelihood function

$$\ln L(\mathbf{G},\, \sigma_e|x) = \sum_k \sum_c \left[S_{k,c} \ln \left\{ \int_x^{\infty} N(T|G_k,\, \sigma_e)\, dT \right\} + D_{k,c} \ln \left\{ 1 - \int_x^{\infty} N(T|G_k,\, \sigma_e)\, dT \right\} \right] \tag{2}$$

in which k and c denote the isofemale line and the experimental lot within each isofemale line, respectively, and $S_{k,c}$ and $D_{k,c}$ denote the number of surviving and dead individuals, respectively. The exposure concentration, x, was set as log(75 μg/L) in the present case study (see *Acute toxicity assays*), because there was only a single exposure concentration. However, the number of exposure concentrations is arbitrary (x is allowed to vary) in this framework. We performed a parameter estimation with two steps. The first step assumed that the tolerance values of all lines were identical, to obtain a guess value for the environmental standard deviation σ_e and the mean tolerance value, $\overline{G}$, in the entire population. The second step maximized the above-mentioned model by using the guess values for $\mathbf{G}$ and σ_e as estimated in the first step. The maximum likelihood estimates were derived with Mathcad's built-in function Maximize (Mathcad 14; Parametric Technology), which returns local maxima of nonlinear functions with the conjugate-gradient method [27].

Binary scoring method

An alternative method is to assign hypothetical binary values (0 or 1) to individuals according to their responses and to convert the heritability of the 0/1 score to the heritability of the underlying variable, which reflects the individual tolerance [11,28]. Survival and death are assigned 0 and 1, respectively, and the estimated heritability of the 0/1 scores is transformed into the heritability of the underlying individual tolerance by the equation

$$H^2 = H_{tr}^2 \frac{p\,(1-p)}{z^2} \tag{3}$$

where H^2 and H_{tr}^2 are the heritabilities of the individual tolerance and the original 0/1 scores, respectively; p is the proportion of dead individuals; and z is the standard normal deviation corresponding to p [11,21]. The standard error of heritability is given by a similar formula.

$$SE(H^2) = SE(H_{tr}^2)\, \frac{p\,(1-p)}{z^2} \tag{4}$$

The heritability of the 0/1 score was determined from a component of the variance between isofemale lines by using ANOVA, in accordance with standard quantitative genetic methods [10]. A three-way nested ANOVA, based on the random-effect model, was used to estimate three variance components: the between-line component σ_b^2, the within-line between-replicate component σ_{rb}^2, and the residual within-replicate component σ_w^2. We estimated the between-replicate component to prevent common environmental effects that were specific to each isofemale line (if, in fact, these effects were present) from confounding the genetic component of variance between isofemale lines. To estimate variance components, we made a calculation program for three-way nested ANOVA following Snedecor and Cochran [29] in Mathcad 14. Statistical tests for variance components were performed in Statistica 2000 (StatSoft). The observed variance components are associated with genetic or environmental causal components as $\sigma_b^2 = V_G$, $\sigma_{rb}^2 = V_{Ec}$, and $\sigma_w^2 = V_{Ew}$, where V_{Ec} is the common environmental variance and V_{Ew} is the residual environmental variance [10]. The broad-sense heritability, H_{tr}^2, was derived from Equation 5.

$$H_{tr}^2 = \sigma_b^2 / \left(\sigma_b^2 + \sigma_w^2\right) \tag{5}$$

Test organisms

Adult test specimens of *D. galeata* were collected from Lake Sagamiko in Kanagawa Prefecture, Japan. Seven isofemale lines were made from the sampled individuals and were kept at 20°C under a 16-h:8-h light:dark regime in carbon-filtered tap water (pH 6.9–7.2, dissolved oxygen 6.8–7.0 mg/L, $CaCO_3$ 30 mg/L, Cd 0.009 μg/L, Cu 0.90 μg/L, Pb 0.053 μg/L, Zn 5.6 μg/L, As 0.27 μg/L, Fe 49 μg/L, Ni 2.3 μg/L, and Co 0.029 μg/L). Live *Chlorella vulgaris* was supplied as food.

Acute toxicity assays

For the assays, we arbitrarily chose one mature individual from each of the seven isofemale lines. After the females had produced a sufficient number of offspring, each clone group was divided into two subgroups, which were kept in different glass bottles, to evaluate common environmental effects within isofemale lines. The test populations were allowed to reproduce for a few generations before the start of the bioassay. During the bioassay, adult density per subgroup was kept at less than 10 individuals/L to prevent any density effects that could be transmitted to the offspring being tested.

For the acute immobility test, 20 neonates (individuals less than 24 h old) were arbitrarily chosen from each subgroup and were kept in a glass bottle with 200 ml carbon-filtered tap water or 75 μg/L *p*-nonylphenol solution dissolved in dimethyl sulfoxide (0.05 ml/L). This solvent does not induce any adverse responses in the same test organism at an exposure concentration of 0.05 ml/L [30]. We set the nominal concentration of *p*-nonylphenol at 75 μg/L, because this resulted in varied

responses (with an immobility rate in the entire population of ~50%) in a preliminary test (data not shown), and its administration was therefore likely to be useful in examining the genetic variability of tolerance. The test conditions were 20°C under a 16-h:8-h light:dark regime. Immobile individuals, which were defined as those that did not maintain an upright position by moving their secondary antennae, were counted after 48 h.

Statistical procedures

We used the jackknife method [31] to obtain standard estimation errors and bias-corrected estimates of H^2. The resampling unit was set as the isofemale line (groups $n = 7$). Because very few estimation biases were detected with the jackknife method using either estimation procedure, we disregarded the bias correction of heritabilities.

RESULTS

Variation of immobility rates among lines

The variation in immobility among lines was highly significant ($p < 0.001$, Kruskal–Wallis test; see Fig. 2), indicating that genetic or common environmental effects specific to isofemale lines influenced clone-specific susceptibility to the adverse effects of the chemical.

Heritabilities

The maximum likelihood estimates of the genetic values of log-transformed tolerance in the lines were 1.738, 1.814, 2.145, 2.02, 1.766, 1.856, and 1.987, and the standard deviation σ_e of the environmental effect was estimated as 0.301. By using $V_G = \mathrm{Var}(G)$ and $H^2 = V_G/(V_G + \sigma_e^2)$, the genetic variance and the broad-sense heritability were determined as $V_G = 0.0225 \pm 0.0101$ and $H^2 = 0.199 \pm 0.112$, respectively.

Three-way nested ANOVA based on the binary scoring indicated that there were significant variance components between isofemale lines ($p < 0.05$) and parental subgroups ($p < 0.001$). The variance components were determined as $\sigma_b^2 = 0.030$, $\sigma_{rb}^2 = 0.034$, and $\sigma_w^2 = 0.190$. The broad-sense heritability of the binary score was determined as $H_{tr}^2 = 0.117 \pm 0.057$. The converted estimate of heritability of the underlying tolerance trait was $H^2 = 0.184 \pm 0.089$.

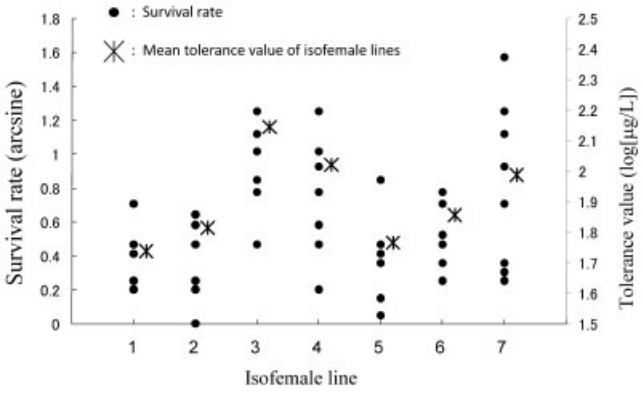

Fig. 2. Arcsine-transformed survival rates (1 − immobility rates) in each treatment cell and maximum likelihood estimates of the mean tolerance of each isofemale line. The tolerance value is defined as the threshold concentration in the logarithmic scale of the toxicant (log[μg/L]).

DISCUSSION

The merit of dealing with responses by organisms to chemical pollutants as threshold traits is based on the fact that many adverse responses are recorded as quantal or semi-quantitative data and not every observed quantal response can be replaced successfully by continuous measures such as the time to death. This implies that use of the analytical methods based on the threshold model may expand the range of data that can be analyzed with quantitative genetics. Conversion of quantal scores into continuous measures may be possible in some cases. However, the genetic variability of a continuous measure (e.g., time to death) may not reflect that of the short-term quantal responses (e.g., acute mortality).

Within the framework of the threshold manner of responses to toxicant, two methods were reviewed and presented here: the binary scoring method, based on the standard threshold character model; and the maximum likelihood method, based on the TTM. Substantial differences exist between the binary scoring method and the TTM. These differences result from the difference in the definition of the threshold between the two models: the standard threshold character model postulates that a continuously varying underlying variable (an unmeasurable trait) is possessed by each individual and assumes a fixed threshold point of the variable under a particular environmental condition, whereas in the TTM, the threshold point is assumed for each individual as a continuous character (Fig. 1).

For the purpose of analyzing the genetic variance of tolerance to pollutants, the TTM is superior to the standard threshold character model and the binary scoring method in some respects. The TTM directly estimates the tolerance of each genotype; this tolerance is defined as the genotype-specific threshold value of the ability to endure toxicant exposure and is continuously distributed in a population. However, the two methods are similar in that they assume threshold expression of responses to toxicants. The above-mentioned different properties of the TTM allow the analysis of the genetic variance of tolerance, even if the genetic value of tolerance does not follow a normal distribution. This is in contrast to the binary scoring method, which assumes that the underlying variable is normally distributed. The lack of a need for normality is especially useful for analyzing laboratory isofemale cultures of parthenogenetic species such as cladoceran test species, because the cultured genotypes are unlikely to be normally distributed. Another merit of the TTM is that precision is improved because multiple exposure concentrations are used, whereas the standard threshold character model is based on a single exposure concentration, because the threshold point, which is defined on the basis of a hypothetical underlying character, is fixed at a particular value for the entire population under a specific exposure concentration.

The last and most important merit of the TTM is its compatibility with the threshold model developed in ecotoxicology [17,20]. Ecological risk assessments based on the threshold dose–response model may be able to include the effects of genetic variability and evolutionary changes in tolerance by applying the threshold concentration to individuals and introducing genetic variance among the individual-specific threshold values. In contrast, the binary scoring method cannot deal with the effect of genetic variance and evolution at various exposure concentrations, which is essential for ecological risk assessment.

The heritability of tolerance to chemical pollutants has three implications in the context of ecotoxicology. The potentially rapid acquisition of tolerance, which is indicated by high heritability, may mitigate the long-term risks posed by pollutants to populations [2,3,5,6]. Therefore, a site-specific ecological risk assessment might have to take account of the mitigating effect of genetic variation of toxicity within populations [1,8,13].

The second implication of heritability estimation relates to biomonitoring based on variations in sensitivity to chemical pollutants as a parallel method to the pollution-induced community tolerance [23,32,33]. Higher tolerance (lower sensitivity) to a chemical in a particular population compared with other populations indicates that the population has a past exposure history if the following conditions are met: the genetic variance of tolerance has been maintained, despite the stronger selection pressure at the contaminated site than at the uncontaminated site; dispersal of tolerant individuals to other sites is limited; and the fitness cost associated with the tolerance keeps the individuals at the uncontaminated site susceptible to the pollutant [33]. The heritability estimates are relevant for examining the first condition and are associated with the last condition, because the fitness cost of tolerance must be examined as a genetic correlation (i.e., a correlation measure in quantitative genetics) between tolerance and fitness in the context of evolutionary change [23].

The third implication of heritability is related to the underlying biological mechanisms responsible for the dose–response function to toxicant exposure, the individual tolerance, and the stochastic response [18,20]. High (broad-sense) heritability strongly suggests that individual tolerance is the major source of the sigmoid cumulative response curve, because genetic variance contributes to the variation in tolerance among individuals. We cannot infer the stochastic response to be the major source, however, on the basis of low heritability, because the converse does not necessarily hold true. Environmental variance in quantitative genetics includes both the (stochastic) environmental effects among individuals and the stochasticity of responses in a particular individual. The former factor, stochastic in many contexts, may be included in individual tolerance in ecotoxicology research programs [18,34].

There are a couple of limitations to estimating heritabilities on the basis of the TTM using isofemale lines of zooplankton. Broad-sense heritability should be regarded, in fact, as the upper limit of narrow-sense heritability [10,23]. To estimate additive genetic variance and narrow-sense heritability, toxicity testing must include a specific breeding design, that is, a half-sib design or parent–offspring regression with sexual reproduction, both of which are difficult to practice in bioassays using parthenogenetic species such as cladocerans. The binary scoring method is superior to the TTM in this respect, because it can use all of the standard quantitative genetic methods, including those mentioned above. Further studies are needed to extend the TTM to species with sexual reproduction.

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