RESISTANCE TO CADMIUM AND PARASITE INFECTION ARE INVERSELY RELATED IN TWO STRAINS OF A FRESHWATER GASTROPOD

CHRISTOPHER J. SALICE and GURITNO ROESIJADI*
University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, P.O. Box 38, Solomons, Maryland 20688, USA

(Received 7 May 2001; Accepted 19 November 2001)

Abstract—Phenotypes that are either resistant or susceptible to infection by the trematode parasite Schistosoma mansoni exist in the tropical freshwater snail Biomphalaria glabrata. We tested the hypothesis that a cost of parasite resistance in B. glabrata is greater sensitivity to cadmium toxicity, using parasite-resistant and parasite-susceptible strains exposed to cadmium in the laboratory. Survival analysis showed that time to death for cadmium was significantly shorter in eggs, juveniles, and adults of the parasite-resistant BS90 strain in comparison with the parasite-susceptible NMRI strain. Cadmium exposure increased time to hatch in both strains, but the effect was greater in BS90. Percentage hatch decreased with increased cadmium; BS90 was again more sensitive than NMRI. Comparison of the median effective concentration (EC50) for hatching and median lethal concentrations (LC50s) for survival of juveniles and adults showed that the order for cadmium resistance was adults > juveniles > eggs in NMRI and adults > eggs > juveniles in BS90. Cadmium resistance of F1 and F2 progeny of BS90/NMRI crosses was intermediate to that of parental strains. Numerical estimates indicated that a single genetic factor was responsible for the difference in cadmium resistance in the two strains. These findings were consistent with the hypothesis that greater sensitivity to cadmium is a cost of resisting parasitic infection.

Keywords—Gastropod Cadmium Metal resistance Parasite resistance Genetic difference

INTRODUCTION

Genetic diversity in susceptibility to pathogens results in the coexistence of susceptible and nonsusceptible individuals [1]. This polymorphism in disease susceptibility is part of the genetic background on which toxicant stress is superimposed. While disease-resistant individuals are capable of avoiding the adverse effects of infection per se, they may be predisposed to greater sensitivity to other stressors. The general phenomenon of physiological or ecological costs associated with increased resistance to pathogens appears widespread among organisms [2]. Costs of resistance to pathogens can manifest as reductions in fitness associated with altered reproduction [1,2] or competitive disadvantage in the presence of susceptible individuals [3] and are reported for the freshwater snail Biomphalaria glabrata [1,3]. Whether such costs can include heightened sensitivity to chemically imposed stress has yet to be fully investigated.

Biomphalaria glabrata is a freshwater planorbid snail that is prevalent in tropical environments. It is an intermediate host for the trematode parasite Schistosoma mansoni, the agent for schistosomiasis in humans [4]. Natural populations of B. glabrata are composed of parasite-susceptible and -resistant individuals. The isolation of strains of B. glabrata differing in susceptibility to infection by S. mansoni [4] provides an opportunity to study ecotoxicological consequences of differences in parasite resistance. Focus was placed on response to cadmium, a worldwide contaminant of concern in tropical environments [5,6]. Toxicity of cadmium to B. glabrata has been reported previously [7,8,9]. We hypothesized that resistance to chemical toxicity is a cost of increased resistance to disease and that relative resistance to each would be inversely related. This hypothesis was tested by determining cadmium resistance in various life history stages of parasite-resistant BS90 and parasite-susceptible NMRI strains of B. glabrata. Estimates were also made for the number of cadmium resistance genes that differentiate the two strains.

MATERIAL AND METHODS

Test animals

Biomphalaria glabrata is a self-fertilizing hermaphrodite but favors cross-fertilization when other individuals are present [10]. Eggs are laid in individual capsules that are cemented together to form an egg mass or clutch composed of about 15 to 30 eggs. Egg masses are attached to external substrates. Embryogenesis and larval development occur within the egg, and individuals hatch as juvenile snails in about one week. Snails become reproductively mature adults in approximately two months. The NMRI strain is a lab-derived line selected for high susceptibility to schistosome infection [11], and the BS90 strain is a parasite-resistant line descended from a Brazilian field isolate [12]. NMRI is albino, while BS90 carries a dominant gene for dark brown/black pigmentation. These characteristics facilitated identification of hybrid snails in crossbreeding experiments.

The founder individuals used to establish NMRI and BS90 colonies were gifts from Fred Lewis, Biomedical Research Institute (Rockville, MD, USA). The expected susceptibility of NMRI and BS90 to infection was confirmed by challenging 42 individuals of each strain to miracidia of S. mansoni: No BS90 were infected, while 41 NMRI (97.6%) were infected.

Stock snail cultures were maintained in acid-cleaned (0.1 N HNO3) plastic containers with 3 L artificial freshwater (1 mM NaCl, 1 mM MgSO4, 0.10 mM K2SO4, 0.1 mM FeCl3·6H2O, and 0.5 mM NaHCO3 in deionized water) at 23 to 25°C and a 12:12-h light/dark cycle. Snail density was approximately 20/L. The diet was romaine lettuce heated in a microwave oven.
oven, occasionally supplemented with an artificial snail food containing alginic acid, cerophyll or freeze-dried lettuce, wheat germ, fish food, and dried milk.

**Experimental design**

Snails were acclimated to 25°C and a 16:8 h light:dark cycle in a temperature- and light-controlled laboratory for one week before exposure. Eggs, juveniles, and adults were exposed to cadmium in static exposures. Exposure medium was prepared by adding cadmium (as cadmium chloride) from a stock solution of 1 mM cadmium in deionized water. Exposures were based on nominal cadmium concentrations in all cases. Snails were fed microwave-cooked lettuce during exposures. Assignment of egg masses and snails of the different experimental groups to various exposure concentrations and placement of exposure containers on the laboratory bench was done randomly.

**Egg exposures.** Egg masses were collected by floating Styrofoam cards, approximately 5 cm², in tanks containing adult snails, which preferentially use the cards as substrates for attachment of egg masses. Egg masses were removed from the cards within 24 h of being deposited and placed in acid-rinsed (0.1 N HNO₃) 24-well culture plates (Primaria 1837) containing 2.5 ml of test medium, one egg mass per well. Egg masses were exposed to control (no added cadmium) and 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 µM cadmium for 14 d at 26°C (n = six egg masses × 15–30 eggs each per concentration). Test solutions were replaced every second day. Observations for number normal, number abnormal, time to death, and time to hatch were made daily.

**Juvenile exposures.** Six-week-old juvenile NMRI and BS90 were exposed for 14 d to control (no added cadmium) and 0.125, 0.25, 0.5, and 1 µM cadmium in plastic beakers containing 300 ml test medium that was changed every second day (n = 30 snails per concentration). Observations for number alive and time to death were made daily. Snail length was measured at time of death. Qualitative assessments on amount of lettuce eaten and fecal material produced were also noted.

**Adult exposures.** Adult snails from both strains were placed in 3-L plastic aquaria and exposed for 14 d to control (no added cadmium) and 0.25, 0.5, 1, and 2 µM cadmium in 2 L test medium (n = 30 snails per concentration). Observations and data were recorded as described previously for juveniles.

**Number of genes responsible for the difference in cadmium resistance between BS90 and NMRI.** We used a quantitative method to estimate the minimum number of genetic factors contributing to differences in cadmium tolerance between BS90 and NMRI [13,14]. The minimum number of genetic factors, nₐ, contributing to phenotypic differences between parental stocks is estimated from the expression:

\[ n_a = \frac{(\mu_{p_2} - \mu_{p_1})^2}{8\sigma_2^2} < n \]

where \( n_a \) is the number of genetic factors that contribute to the differences in cadmium resistance between the parental populations of BS90 and NMRI raised under similar conditions. An \( n_a \) value of less than 1 can indicate either that multiple genes responsible for a given trait are not acting in the same direction [13] or that a single factor is contributing to differences in metal tolerance [14]. Cadmium resistance was measured as the mean time to death of BS90 and NMRI, represented by \( \mu_{p_1} \) and \( \mu_{p_2} \), respectively. The extra genetic variance segregating in the F2 cross beyond that in the F1 cross is \( \sigma_2^2 \) and can be calculated using the following equations:

\[ \sigma_2^2 = \sigma_{P_2}^2 - \sigma_{P_1}^2 \]

\[ \sigma_2^2 = \sigma_{P_2}^2 - [0.5\sigma_{P_1}^2 + 0.25\sigma_{P_1}^2 + 0.25\sigma_{P_1}^2] \]

where \( \sigma_{P_1}^2 \) and \( \sigma_{P_2}^2 \) are the variance in time to death associated with parental BS90 and NMRI, respectively, and \( \sigma_{P_1}^2 \) and \( \sigma_{P_2}^2 \) represent variance in mean time to death of the F1 and F2 progeny. Also, BS90/F1 and NMRI/F1 backcrosses were used to generate another estimate of \( n_a \). The numerical expressions for \( \sigma_2^2 \) using the backcrosses were as follows:

\[ \sigma_2^2 = 2\sigma_{B_2}^2 - \sigma_{B_1}^2 - \sigma_{B_2}^2 \]

\[ \sigma_2^2 = \sigma_{B_1}^2 + \sigma_{B_2}^2 - [\sigma_{B_1}^2 + 0.5\sigma_{B_1}^2 + 0.5\sigma_{B_2}^2] \]

where \( \sigma_{B_1}^2 \) is the variance in mean time to death of the BS90/F1 backcross and \( \sigma_{B_2}^2 \) is the variance in mean time to death of the NMRI/F1 backcross.

This method for estimating the minimum number of genes responsible for differences in a quantitative character was first introduced for inbred populations [15], expanded to include outbreeding or wild populations [13], and recently applied in studies in evolutionary toxicology [14]. The method assumes that one population is fixed with alleles increasing and the other fixed with alleles decreasing. Loci contributing to the trait are assumed to be unlinked, in random combination, and to contribute equally. If the assumptions are violated, the estimate for the number of loci contributing to the trait can be underestimated [16]. To minimize such a possibility, we estimated a mean \( n_a \) from individual \( n_a \) values obtained using all the equations for calculating variance described previously.

**Cadmium resistance and parasite susceptibility in gastropods.** Pigmentation in BS90 is dominant over albinism in NMRI, and all pigmented offspring produced from albino NMRI snails that have been crossed with BS90 can be identified as successful hybrids and not the result of self-fertilization. The F1 hybrids identified in this manner were used for propagation of F2 progeny.

Because the propagation of F1 and F2 progeny of NMRI/BS90 crosses is sequential, testing the F1 and F2 of the same age would entail exposing them to cadmium at different times. Testing them simultaneously to avoid such temporal variability would entail use of snails of different age. It is preferable to test snails of the same age, and, ideally, F1 and F2 progeny are tested at the same age and at the same time so that both temporal and age-dependent variance can be controlled. This would avoid variability introduced by testing different groups at different times and variability due to testing snails of different age, both of which can be substantial. Conducting tests in which the F1 and F2 snails were of the same age and tested simultaneously was simulated by propagating surrogate F1 hybrid progeny from the parental stock at the same time that the F2 were collected from the F1. Hence, to reduce variation due to temporal differences in response to cadmium, the F2 and surrogate F1 snails of similar age were exposed to cadmium simultaneously.

For propagation of snails for these experiments, NMRI/BS90 pairs were isolated for mating on two separate occasions: first to obtain the F1 progeny (designated F1₁) for subsequent propagation of the F2 (designated F2₁) and, later, to obtain the surrogate F1 (designated F1₂). F1₁ snails were obtained from five NMRI/BS90 pairs isolated from the main laboratory stocks. F2₁ snails were isolated as newly laid eggs from approximately 200 randomly mating F1₁. At the same time that the F2₁ eggs were produced from the F1₁, the surrogate F1₂ eggs were isolated from matings of 15 to 20 BS90/NMRI pairs...
Table 1. Mean time to hatch and percentage hatch of eggs from parasite-resistant BS90 and parasite-susceptible NMRI strains of the gastropod Biomphalaria glabrata exposed to cadmium

<table>
<thead>
<tr>
<th>Strain</th>
<th>[Cd] (µM)</th>
<th>Time to hatch (mean ± SD)</th>
<th>% Hatch (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS90</td>
<td>Control</td>
<td>5.98 ± 0.74</td>
<td>92.4 ± 0.088</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>5.89 ± 1.09</td>
<td>95.9 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>6.86 ± 1.51</td>
<td>85.4 ± 0.049</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>7.75 ± 1.93</td>
<td>63.2 ± 0.218*</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>10.00 ± 1.93</td>
<td>11.4 ± 0.151*</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>NMRI</td>
<td>Control</td>
<td>5.33 ± 0.50</td>
<td>95.7 ± 0.061</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>5.17 ± 0.52</td>
<td>96.5 ± 0.050</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>5.71 ± 0.70</td>
<td>96.5 ± 0.050</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>6.27 ± 0.86</td>
<td>93.6 ± 0.101</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>7.01 ± 1.35</td>
<td>86.5 ± 0.130</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>7.65 ± 1.69</td>
<td>50.3 ± 0.379*</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>7.70 ± 1.81</td>
<td>35.4 ± 0.297*</td>
</tr>
</tbody>
</table>

* Cadmium concentrations were nominal.
* Survival analysis of time to hatch of individual eggs showed a statistically significant effect of cadmium, strain, and cadmium × strain interaction.
* Analysis of variance showed significant effects of cadmium, strain, and cadmium × strain interaction.
* SD = standard deviation.
* Significantly different than control based on Fisher’s protected least significant difference (PLSD) posthoc test (α = 0.05).

and from each of the main laboratory stocks of BS90 and NMRI. F1₂, F2₁, and parental stock BS90 and NMRI snails were raised to the juvenile stage for exposure to cadmium and for estimating n₀ with Equations 1 and 2.

We also tested F2₂ progeny that were directly descended from the F1₁ snails in a second set of exposures to account for variation in parental gene pool introduced by comparing the F2 above to the surrogate F1. Approximately 200 additional F1₁ snails were used to produce F2₂ progeny, and approximately 15 isolated BS90/F1₁ pairs and NMRI/F1₁ pairs were also used to obtain backcrosses. For the second cadmium exposure, we obtained same-age juvenile F2₂, NMRI, BS90, and the BS90/F1₁ and NMRI/F1₁ backcrosses. Variance in time to death of the F1₁ snails was used where the variance for the F1 was required to estimate n₀ with backcrosses.

Sixty to 65 juvenile snails approximately six weeks old were used in each cadmium exposure to estimate numbers of genes. Exposure concentrations were control, 0.25, 0.5, and 0.75 µM cadmium. Because we found that the toxicity at 0.5 and 0.75 µM exceeded a threshold for expression of concentration-dependent differences in acute cadmium toxicity, results for only the 0.25-µM cadmium exposure were used for final time-to-death analysis. Time-to-death observations were made every 8 h for the first 5 d and every 24 h thereafter until all snails exposed to cadmium had died. Death was determined as lack of heartbeat or response to prodding.

We used the Kaplan–Meier survivor function (PROC LIFETEST; SAS Institute, Cary, NC, USA) to estimate mean and variance in time to death of snails since some snails were censored at the end of the exposure [17]. Time-to-death data are frequently nonnormally distributed; hence, standard methods for calculating mean and variance may not be appropriate.

Statistical analyses

Time-to-death data from egg, juvenile, and adult exposures were used to generate accelerated failure time models using the PROC LIFEREG survival time analysis procedure. The advantages of using survival analyses compared to more traditional methods for assessing toxicity includes greater use of available data and simplicity of experimental design. The model describes the relationship between time to death and specified covariates and has been applied in ecotoxicology [10]. Parameters provided by this procedure include β, chi-square, and p. The β is an estimated parameter that describes the changes in ln(time to death) as a covariate increases by one unit holding other covariates constant. A positive β indicates that time to death increases as the covariate increases; a negative β indicates that time to death decreases as the covariate increases [17,18]. The chi-square statistic tests the hypothesis that the slope of the relationship between a factor and time to death is zero. The larger the chi-square value, the more significant the factor as indicated by the p value. These estimated parameters are also used in determining other statistics, such as the median time to death.

Individuals that had not reached the toxicological endpoint during the course of the experiment were taken into account in the model through censoring. Individual eggs were censored on hatching or when the developing organism, although insufficiently developed for viable hatch, was still alive at the end of the experiment. Juvenile and adult snails still alive at the end of 14-d experiments were censored.

The PROC LIFEREG does not accommodate interactions. To test for interactions, we used the concatenation operator of SAS (II) to create a dummy variable that links two covariates for inclusion in the regression equation (G.A. Fox, personal communication). A log-logistic distribution was the most appropriate for all survival analyses as determined by comparing maximum likelihood estimates of various distributions and by comparing the linearity of the data fit to the various distributions [19]. Percentage hatch was analyzed using analysis of variance (ANOVA), following arcsine transformation. Length measurements of juvenile and adult snails were analyzed using ANOVA.

RESULTS

For the early developmental stages, cadmium, strain, and the cadmium × strain interaction (Table 1) significantly af-
affected time to hatch and percentage hatch. Time to hatch and percentage hatch in both strains exhibited concentration-dependent sensitivity to cadmium. Additionally, eggs of BS90 were more sensitive to cadmium compared to eggs of NMRI. Time to hatch was delayed to a greater extent in BS90, increasing from 6 to 10 \text{d} in treatments ranging from control to 0.2 \text{mM} cadmium; in NMRI, time to hatch increased from 5.3 to 7.0 \text{d} in treatments ranging from control to 0.3 \text{μM} cadmium. Similarly, for the same treatments, percentage hatch was reduced to a greater extent in BS90, decreasing from 96 to 87\% in NMRI and from about 92 to 11\% in BS90. In NMRI at the two highest cadmium concentrations of 0.25 and 0.3 \text{μM}, percentage hatch was 50 and 35, respectively. These concentrations were lethal to all eggs of BS90.

Survival analysis of the entire toxicity data set for the three life history stages, the survival curves, cadmium resistance of the F1 and F2 progeny of BS90/NMRI crosses at 0.25 \text{μM cadmium (nominal)}: eggs; juveniles; adults.

Time to hatch was delayed to a greater extent in BS90, increasing from 6 to 10 \text{d} in treatments ranging from control to 0.2 \text{mM} cadmium; in NMRI, time to hatch increased from 5.3 to 7.0 \text{d} in treatments ranging from control to 0.3 \text{μM} cadmium. Similarly, for the same treatments, percentage hatch was reduced to a greater extent in BS90, decreasing from 96 to 87\% in NMRI and from about 92 to 11\% in BS90. In NMRI at the two highest cadmium concentrations of 0.25 and 0.3 \text{μM}, percentage hatch was 50 and 35, respectively. These concentrations were lethal to all eggs of BS90.

Survival analysis of the entire toxicity data set for the three life history stages, the survival curves, cadmium resistance of the F1 and F2 progeny of BS90/NMRI crosses at 0.25 \text{μM cadmium (nominal)}: eggs; juveniles; adults.

While useful in comparing the relative resistance of NMRI and BS90 at different life history stages, the survival curves alone were not sufficient for comparing the relative toxicity of cadmium to the different stages. Comparing the response of different stages is complex because stage duration can limit exposure time, and biological characteristics associated with different stages (e.g., enclosure of embryonic and larval stages within the egg and rapid differentiation and/or growth in the egg and juveniles) can modify both exposure and response. In *B. glabrata*, the egg stage is approximately one week; hence, direct comparisons with juveniles and adults need to be based on responses that occur within a comparable period. Furthermore, we noted that successful hatch, which is directly related to viability of the early developmental stages, is a more sensitive measure of toxicity than survival during the course of egg exposure per se. Thus, to compare the relative cadmium sensitivity of the egg, juvenile, and adult stages, we first determined EC50s for percentage hatch of 0.15 and 0.26 \text{μM cadmium for BS90 and NMRI}, respectively, from data in Table 1. We then calculated the mean time to hatch at the EC50 concentrations as 8.3 and 7.56 \text{d} for BS90 and NMRI, respectively. For comparison, juvenile and adult LC50s were estimated for exposure times corresponding to the mean time to hatch for each strain. For BS90, the juvenile and adult LC50s were 0.127 and 0.306 μM, respectively, at 8.3 \text{d}. For NMRI, the juvenile and adult LC50s were 0.430 and 0.536 μM, respectively, at 7.6 \text{d}. BS90 was again shown to be more sensitive to cadmium, and the order of stage-specific resistance to cadmium differed in the two strains. For the different stages of NMRI, the rank order for cadmium resistance was adults > juveniles > eggs, while that for BS90 was adults > eggs > juveniles.

Other indications of cadmium toxicity included a reduction in food consumption in both juveniles and adults, noted as more uneaten lettuce and less fecal output, and reduced fecundity, noted as reduced egg capsule production. The onset of mortality was usually preceded by a reduction in both lettuce consumption and egg production.

Survival of BS90, NMRI, and the F1 and F2 progeny of BS90/NMRI crosses at 0.25 \text{μM cadmium was used to estimate the number of genes responsible for differences in cadmium toxicity of the two strains. Tests were made of juveniles. From the survival curves, cadmium resistance of the F1 and F2 progeny was intermediate to that of the parental strains (Fig. 2). The average value for $n_k$ of 0.75, the estimate for number of genes responsible for the difference in cadmium resistance between BS90 and NMRI, was not significantly different from 1 (Table
laboratory [42], cadmium concentrations higher than 0.3 inhibited hatching in BS90. Based on earlier findings in our lab, cadmium resistance is dependent on cadmium exposure concentration. For example, high concentrations of 1 and 2 μM cadmium caused rapid mortality in BS90 and NMRI adults, with no detectable differences in time to death. However, at 0.5 μM cadmium and below, the greater resistance of adult NMRI over adult BS90 was clearly evident. Above a threshold of exposure, the survival curves converge and show commonality in response because the expression of any differential response is obscured by the extreme level of toxicity. Exposures at low but toxic levels are likely to be more effective in eliciting strain-specific differences.

Strain-specific differences have been shown to occur in resistance to other metals [23]. For example, the M-line and PR-79 strains, both susceptible to S. mansoni infection [20], had similar LC50s when exposed to copper [23]. However, after protracted exposure, the M-line strain developed enhanced copper resistance after five generations of selection, while the PR-79 strain did not. This was suggestive of strain-specific differences in adaptation to copper. Hence, for B. glabrata, strain-specific responses to metals appear important in determining sensitivity to metal toxicity. Indeed, bioassay results are determined not only by the toxicity of the compound alone but also by the relative sensitivity of the clone or strain being tested [24].

A single gene appears to confer resistance to schistosome infection in adult B. glabrata, and genetic markers that segregate with this resistance have been identified [22]. Although the mechanistic basis for metal resistance has yet to be identified in B. glabrata, we showed that cadmium resistance is genetically based and, using a quantitative approach, that the number of genes responsible for differences in cadmium resistance between BS90 and NMRI is likely to be one or a few. The involvement of protective mechanisms is commonly associated with resistance to metals, and several studies have implicated metallothionein in the evolution of metal resistance in other taxa [14,25,26]. Differences in resistance to cadmium may also result from differential ability to eliminate metals or reduce metal uptake. Differences in pigmentation in B. glabrata, by itself, would not be expected to result in any differences in these processes, however [27]. It would be of great interest to determine the nature of the relationship between genes associated with parasite and metal resistance in this species.

Our findings are consistent with studies in plants showing that enhanced metal resistance is often attributable to a single gene [28,29,30]. It has been hypothesized that adaptation to metal exposure requires only a few genes to enable a rapid shift in the mean response to metal toxicity [31]. This hypothesis is consistent with the often, rapid spread of metal tolerance in populations experiencing selection for metal resistance [26,32,33]. In addition, the phenomenon is not restricted solely to metal resistance since for some organic toxicants a single genetic factor has been shown to account for enhanced tolerance [34]. Further studies on the genetics of differential toxicant tolerance may provide valuable insights into ecological and evolutionary responses to chemical stress. For example, the relationship between the genes responsible for enhanced toxicant resistance and the mechanism of resistance may provide a tenable link between molecular responses to toxicants and responses at higher levels of biological organization.

In this study, we showed that the parasite-resistant BS90 was significantly more sensitive to cadmium toxicity than the parasite-susceptible NMRI. The negative correlation between parasite resistance and metal resistance suggests that a trade-off may exist between the two traits. Costs of parasite resistance in B. glabrata have been shown to manifest as delayed maturity [1] and reduced fertility [35]. Costs of parasite resistance have

### Table 3. The number of genes contributing to the difference in cadmium resistance between parasite resistant BS90 and parasite-susceptible NMRI strains of the gastropod Biomphalaria glabrata exposed to 0.25 μM cadmium (nominal)

<table>
<thead>
<tr>
<th>Cross/exposure</th>
<th>( n_g )</th>
<th>Equation no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.03</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>0.99</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>0.44</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>0.49</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>1.23</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>0.30</td>
<td>4</td>
</tr>
</tbody>
</table>

\( SE = \) standard error.

a Equation number refers to one of four equations in the Methods section used to calculate the variance for number of genes estimate.

b A = estimates of \( \sigma^2 \) that were obtained from BS90, NMRI F1, and F2 snails simultaneously exposed to 0.25 μM cadmium (nominal), where the F2 were not direct descendants of the F1 tested. B = estimates of \( \sigma^2 \) that were obtained from exposures at two separate times where the F2 were direct descendants of the F1 tested. C = estimates of \( \sigma^2 \) obtained from BS90/F1 and NMRI/F1 backcrosses.

c \( SE \) = standard error.

d NS = not significant.

3), indicating that a single genetic factor was likely the basis for the difference in cadmium resistance in the two strains.

### DISCUSSION

Biomphalaria glabrata is sensitive to metal toxicity and has been proposed as an indicator of metal pollution in tropical environments [7,9]. Despite the existence of numerous strains of B. glabrata [4], little is known about strain-related differences in resistance to metals. Our findings showed that a parasite-resistant strain of B. glabrata, BS90, is highly sensitive to cadmium toxicity when compared with NMRI, a strain selected for parasite susceptibility. The relative cadmium sensitivities described here for BS90 and NMRI can be directly inferred from parasite and metal resistance in this species.

Our findings are consistent with studies in plants showing that enhanced metal resistance is often attributable to a single gene [28,29,30]. It has been hypothesized that adaptation to metal exposure requires only a few genes to enable a rapid shift in the mean response to metal toxicity [31]. This hypothesis is consistent with the often, rapid spread of metal tolerance in populations experiencing selection for metal resistance [26,32,33]. In addition, the phenomenon is not restricted solely to metal resistance since for some organic toxicants a single genetic factor has been shown to account for enhanced tolerance [34]. Further studies on the genetics of differential toxicant tolerance may provide valuable insights into ecological and evolutionary responses to chemical stress. For example, the relationship between the genes responsible for enhanced toxicant resistance and the mechanism of resistance may provide a tenable link between molecular responses to toxicants and responses at higher levels of biological organization.

In this study, we showed that the parasite-resistant BS90 was significantly more sensitive to cadmium toxicity than the parasite-susceptible NMRI. The negative correlation between parasite resistance and metal resistance suggests that a trade-off may exist between the two traits. Costs of parasite resistance in B. glabrata have been shown to manifest as delayed maturity [1] and reduced fertility [35]. Costs of parasite resistance have
manifested in other species as altered reproduction [36] and a decrease in larval competitive ability [37]. The interaction between disease and pollutant stress has not been well studied, although it has been shown that parasitic infection can potentiate metal toxicity [9,38]. The interaction of the traits for parasite and metal resistance has, to date, received little attention, and the mechanisms that underlie the relationship and the broader consequences are not clear. In *B. glabrata*, which is an intermediate host for pathogens responsible for human disease, an inverse relationship between parasite resistance and metal resistance suggests that contamination of natural environments with toxic chemicals may favor survival of individuals most likely to propagate disease and have societal consequences. Decreased metal resistance as a trade-off for increased parasite resistance, if generally applicable, can be an important consideration in assessing the effects of toxicants on the ability of natural populations of organisms to withstand disease or serve as reservoirs for disease transmission.

Acknowledgement—Support was provided by National Institute of Health training grant T32 ES-7263 to the Program in Toxicology, University of Maryland. Fred Lewis, Biomedical Research Institute, provided snails and invaluable advice on the biology and husbandry of *Biomphalaria glabrata*.

REFERENCES


3. Minchella DJ, Loverde PT. 1983. Laboratory comparison of the relative success of *Biomphalaria glabrata* stocks which are susceptible and unsusceptible to infection with *Schistosoma mansoni*. *Parasitology* 86:335–344.


