Adaptation to Heavy Metals in the Aquatic Oligochaete Limnodrilus hoffmeisteri: Evidence for Control by One Gene

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ADAPTATION TO HEAVY METALS IN THE AQUATIC OLIGOCHAETE *LIMNODRILUS HOFFMEISTERI*: EVIDENCE FOR CONTROL BY ONE GENE

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This study is designed to understand the genetic architecture of resistance to metal in a metal-tolerant aquatic oligochaete. Our results suggest the control by one gene, and further support the genetic simplicity and potential evolutionary responsiveness of metal tolerance in animals. Toxic substances are potent media of natural selection and many field populations of resistant plants and animals have been discovered and described (Antonovics et al. 1971; Macnair 1987; Klerks and Weis 1987; Devonshire and Field 1991). Metals such as cadmium and lead are commonly released into terrestrial and aquatic environments, and metal-resistant populations have been found in both terrestrial plants (Antonovics et al. 1971; McNeilly 1968; Macnair 1983), terres-

trial invertebrates (Posthuma 1990), freshwater invertebrates (Klerks and Levinton 1989a,b), marine seaweeds (Russell and Morris 1970) and marine invertebrates (Bryan and Hummerstone 1971, 1973; Weis et al. 1981).

Although extensive evidence exists for a genetic basis of metal adaptation in plants (Antonovics et al. 1971), genetic studies of animals have been relatively uncommon, especially in field populations (Klerks and Weis 1987; Klerks and Levinton 1992). Allozyme frequencies have been found to correlate with metal exposure (e.g., Lavie and Nevo 1982; Nevo et al. 1984), although the direct biochemical significance of the selective effects is not clear. Posthuma et al. (1993) found that springtails exposed to metals had a heritable component

of resistance to cadmium. Klerks and Levinton (1989a, b) previously documented a strong heritable component of resistance to cadmium in the aquatic oligochaete *Limnodrilus hoffmeisteri*. Indeed, nearly all of the variation in resistance could be accounted for by genetic variation (Klerks and Levinton 1989b). High mortality of nontolerant conspecifics occurred when they were exposed to sediments rich in cadmium, whereas local adapted populations suffered low mortality. Selection experiments on laboratory control populations showed rapid responses in terms of cadmium resistance, and calculations (Klerks and Levinton 1989b) suggested that resistance in the field population had evolved in only a few generations. The strong selection intensity is accompanied by a very steep spatial-selection gradient; resistant populations are separated from intolerant populations over a spatial scale of a few hundred meters, with no apparent barrier of exchange between the populations (Klerks and Levinton 1989b, 1992). This situation resembles spatial gradients in terrestrial plants and suggests a selective disadvantage of metal-adapted genotypes, should they disperse to adjacent populations in metal-free microhabitats (McNeilly 1968; McNeilly and Bradshaw 1968).

The purpose of this study was to estimate the number of segregating genetic factors that might control the resistance of the oligochaete *L. hoffmeisteri*. Previous work suggested that individuals of resistant populations could synthesize high concentrations of a metal-binding ligand, which was most likely metallothionein (Klerks and Levinton 1989a; Klerks and Bartholomew 1991). It was therefore our purpose to learn more about the genetic architecture of metal resistance in *L. hoffmeisteri*. Specifically, we wished to see if the heritable resistance could be explained by one or just a few segregating genetic factors.

MATERIALS AND METHODS

Field Site.—Resistant individuals of the aquatic oligochaete *L. hoffmeisteri* were collected by scooping surface bottom mud from metal-rich sediments in Foundry Cove (near Cold Spring, New York; 87 km upriver from the Battery in Manhattan, New York). Foundry Cove received wastewater rich in cadmium, nickel, and cobalt during 1953–1971 (Klerks and Levinton 1989a; Knutson et al. 1987). Worms were sieved on a 1.0 mm screen. Non-resistant worms were collected in the same way from the nearby unpolluted South Cove.

Crosses and Estimates of Resistance.—*Limnodrilus hoffmeisteri* is a simultaneous hermaphrodite, reproducing sexually by crossfertilization, although uniparental reproduction has been reported (Gavrilov 1931; Kennedy 1966). We have not found single individuals to reproduce in the laboratory. The crosses and resistance assays were employed to estimate the minimum number of genes contributing to the difference in resistance to heavy metals between the *L. hoffmeisteri* populations at South Cove (P_1) and Foundry Cove (P_2). We used the method originally developed by Wright (Castle 1921; Wright 1952, 1968) and later generalized by Lande (1981). The estimate of the minimum number of genetic factors, n_E, is:

$$n_E = (\mu_{P_2} - \mu_{P_1})^2 / (8\sigma_s^2) \leq n$$

where n is the number of factors contributing to the phe-

notypic difference between samples from the parental populations 1 and 2 raised in a common environment; μ_{P_1} and μ_{P_2} are the mean phenotypes of the parental populations 1 and 2; σ_s^2 is the extra genetic variance segregating in the F_2 beyond that in the F_1 hybrids. Four estimates of σ_s^2 can be calculated using the parental phenotypic variances ($\sigma_{P_1}^2$ and $\sigma_{P_2}^2$) and the phenotypic variances of F_1 and F_2 or the backcrosses $B_1 = P_1 \times F_1$ and $B_2 = P_2 \times F_1$. We employed F_1 and F_2 data to estimate σ_s^2 as follows (Lande, 1981):

Estimate 1: $\sigma_s^2 = \sigma_{F_2}^2 - \sigma_{F_1}^2$

Estimate 2: $\sigma_s^2 = \sigma_{F_2}^2 - \left[\dfrac{1}{2}\sigma_{F_1}^2 + \dfrac{1}{4}\sigma_{P_1}^2 + \dfrac{1}{4}\sigma_{P_2}^2\right]$

These estimates were used to calculate the minimum number of segregating genetic factors n_E.

In the summer of 1990, parental worms were collected from populations at South Cove (Cold Spring, NY) and Foundry Cove (Garrison NY), sites approximately 2 km apart, on the east side of the Hudson River. The coves are tidal freshwater bays, bordered by marshes, and connected to the Hudson River through narrow openings, with vigorous tidal exchange. Foundry cove worms were collected from a site with high concentrations of cadmium, nickel, and cobalt in sediments (Cd: 5700 µg; Ni: 4300 µg; Co: 205 µg per gram of dry sediment; Klerks and Levinton 1989a). F_1 hybrids were produced from 120 mating pairs picked randomly from laboratory mass cultures among worms that showed no gonadal development. Pairs were maintained in plastic 4 oz containers with a layer of approximately 1 cm of South Cove sediment, which was low in metals. The sediment was collected from the field site, sieved through a 1 mm mesh and kept frozen at −80°C. Containers were covered with perforated lids and placed in plastic containers (30 × 16 × 8.5 cm, kept at 20°C) with Hudson River water collected approximately 1 km upstream from Foundry Cove.

Juvenile F_1 hybrids were sorted from the parental dishes and transferred to a common mass culture approximately four months after parental mating pairs were established. Some of the F_1 hybrids were reserved for the resistance assay, whereas others were used to produce the F_2 generation. The F_2 generation was produced from 49 F_1 mating pairs maintained under the same experimental conditions as the parental pairs. No backcrosses were performed here; thus, we used the parental and the F_1 and F_2 phenotypic variances to estimate the number of factors that would explain a difference in resistance between the two parental populations.

Resistance assays were performed using a solution of 9 µM cadmium, 10 µM nickel, and 0.5 µM cobalt in soft reconstituted fresh water (48 mg/l $NaHCO_3$; 30 mg/l $CaSO_4.2H_2O$; 30 mg/l $MgSO_4$; 2 mg/l KC_l). Each worm was exposed to the solution in a plastic tissue culture dish (60 × 15 mm). Control worms were exposed to reconstituted soft water without heavy metals. None of the control worms was affected. For each worm, we measured the time elapsed from exposure to death ("survival time"), which was considered to be an estimate of a worm's resistance to the heavy-metal solution (Klerks and Levinton 1989a,b). Parental worms (Foundry Cove, $n = 57$; South Cove $n = 64$) and F_1 hybrids
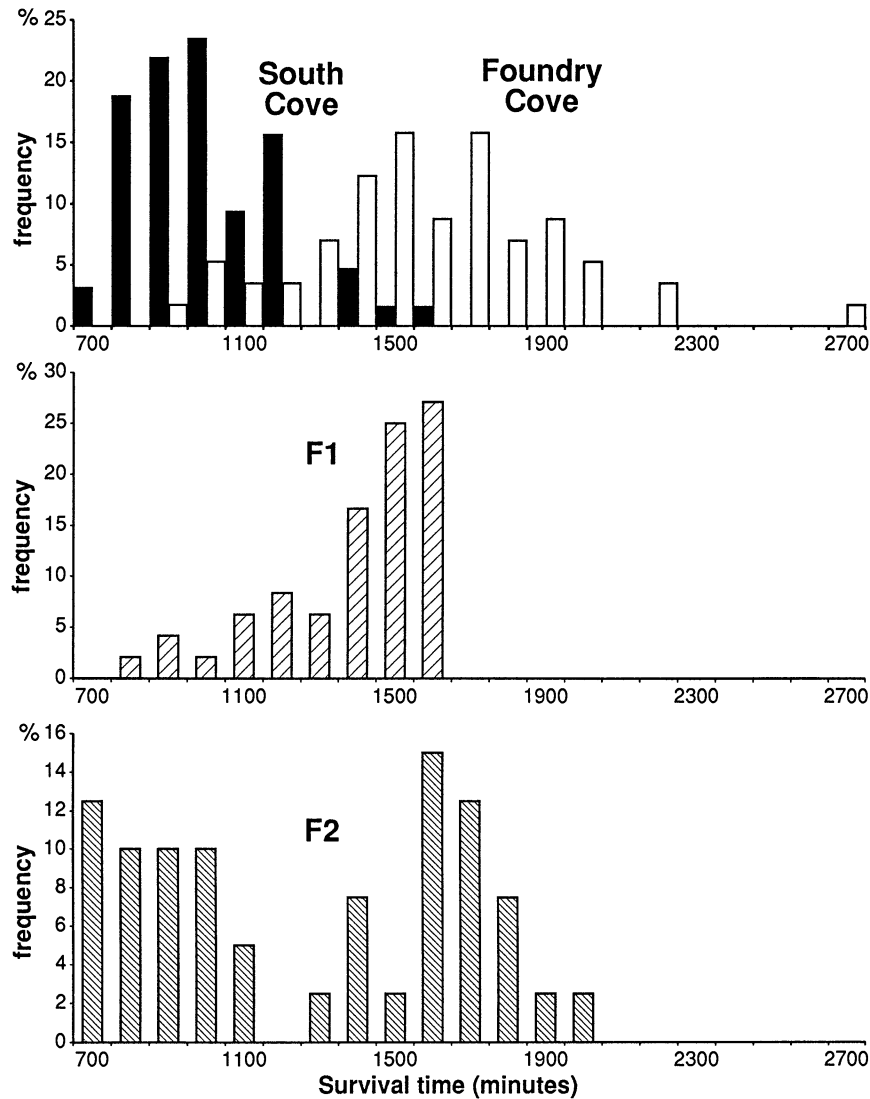
FIG. 1. The frequency of survival times for parental (South Cove and Foundry Cove) F_1 and F_2 worms. Survival times indicate the time elapsed from exposure of worms to a heavy metal solution until death.

(n = 48) were exposed at the same time. F_2 worms (n = 40) were assayed later under identical conditions.

RESULTS

As expected from previous studies (Klerks and Levinton 1989a,b) Foundry Cove worms survived longer in the metal-rich solution than did South Cove worms. F_1 worms had survival times that might seem intermediate between those of the parental populations (Fig. 1; Table 1). However, the F_1 distribution is skewed to the left, and its mode is well within the range of the Foundry Cove (resistant) population. That is, most F_1 worms seemed to be resistant to heavy metals, even though their survival times were never as great as some of those of the Foundry Cove parental population. Survival times for some of the F_1 worms strongly overlapped with the South Cove parental population. The shape of the F_1 curve may indicate the presence of dominance, which would be further supported by the bimodality observed in

the F_2 worms. The modes of the F_2 worms corresponded to those of the parental generation. The two estimates of the minimum number of genetic factors that contribute to the difference in resistance between the South Cove and Foundry Cove populations of *L. hoffmeisteri* were similar: 0.344 and 0.360. These values point to a major segregating factor that is likely to be responsible for the observed differences.

TABLE 1. Resistance to metal-rich solution estimated as survival time (minutes).

	n	Mean	SD
South Cove	64	945.16	187.04
F_1	48	1320.00	244.43
F_2	40	1223.00	426.39
Foundry Cove	57	1524.00	325.55

DISCUSSION

Our results suggest that a single segregating genetic factor underlies the resistance to heavy metals found in the Foundry Cove population of the oligochaete *L. hoffmeisteri*. Previous studies produced heritability estimates of approximately 0.9 (half-sib design) and ca. 0.6 (realized heritability calculated from a selection experiment—Klerks and Levinton 1989b). Both studies demonstrate the potency and general simplicity of natural selection in generating resistance to heavy metals in this species. Posthuma et al (1993) estimated a heritability of 0.33 in resistance to cadmium by a springtail, but we do not know of any other estimates of the number of segregating genetic factors in animal populations. Watkins and Macnair (1991) found that very few or perhaps one segregating factor formed the basis of arsenic resistance in a terrestrial grass species. Macnair (1983) found evidence that a single major gene determined copper tolerance in the yellow monkey flower, *Mimulus guttatus*.

The possibility of a single gene underlying the resistance of *L. hoffmeisteri* suggests a relatively simple mechanism of adaptation. Resistant individuals of *L. hoffmeisteri* in Foundry Cove have been found to have high concentrations of a metal-binding ligand, whose molecular weight is in the range of the metal-binding protein metallothionein (Klerks and Bartholomew 1991, Klerks and Levinton 1989a, 1992). The genetic basis of resistance may therefore lie in some aspect of evolutionary change in metallothionein genes. Maroni found that *Drosophila melanogaster* with mt gene duplications were more resistant to copper than those individuals with only single copies of the gene. Fly populations found at mines rich in metals did not, however, consist of individuals with more duplicates than populations found at unpolluted sites (Lange et al. 1990). We are now attempting to sequence the metallothionein gene in *L. hoffmeisteri* to further understand the molecular mechanism of adaptation.

This study and Watkins and Macnair's (1991) study on plants both suggest that metal adaptation, when it occurs, is under the control of very few genes. The work on *Drosophila* suggests that mechanisms of adaptation may be very simple, even involving the effects of gene duplications (Maroni et al. 1986, 1987). The function of metallothionein is strongly conserved, and environmental exposure to metals can result in increased transcription rates (e.g., Thiele et al. 1986; Shartzer et al. 1993). In *D. melanogaster,* alleles can differ fivefold to sixfold in rates of production of metallothionein tRNA (Theodore et al. 1991). These results suggest that metal adaptation, which has been so useful an evolutionary model (Antonovics et al. 1971), holds great promise for the understanding of the relationships of selection intensity, spatial gradients of gene frequencies, and molecular mechanisms of adaptation.

ACKNOWLEDGMENTS

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DEVELOPMENTAL BUFFERING AND SELECTION

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In higher organisms, buffering or developmental canalization is a ubiquitous phenomenon. As long as perturbations are weak, most morphological traits show little response. However, this tolerance breaks down suddenly under more extreme conditions or for traits already perturbed away from their optimum values (Waddington 1940; Rendel 1967; Scharloo 1988).

One manifestation of this buffering capacity is the masking of deleterious mutant alleles by dominant wild-type alleles. In organisms, for example, where the appropriate experimental tools are available, it has been shown that heterozygosity that reduces the amount of a gene product by half might fail to cause morphological effects. The complete absence of a gene product, however, has drastic effects on morphology and might result in lethal phenotypes. Addition of more copies of genes with similar phenotypic effect might save these lethals. In *Drosophila*, for example, double heterozygosity at the neurogenic loci *Delta* and *Notch* causes embryonic lethality, which is also observed in loss-of-function homozygotes at either of these loci. This lethality can be rescued by increasing dosage at a third neurogenic locus (Vässin et al. 1985; de la Concha et al. 1988).

Because direct modeling of this type of nonlinear, epigenetic interaction between genes leads to rather messy equations, I propose a simple two level model similar to Wagner's (1989). On a biochemical level, interactions between genes are simple and additive, and environmental variation is normally distributed and also additive. A nonlinear mapping function relates this biochemical level to the morphological level. Selection is assumed to act only on the morphological level. Typically, mapping functions show a gentle slope in the region of the optimum, while flanks are relatively steep. This results in buffering of the optimum phenotype (Fig. 1; Rendel 1967; Scharloo 1988). Here, I introduce the model. An in depth analysis and numerical simulations will follow later.

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THE MODEL

Consider the biochemical level first, and assume that a large number of loci, each with a small effect, contribute to the distribution of the biochemical trait X in the population. Assume further that environmental effects contribute additively to this biochemical trait. Let $f(x)$ be the density of X. For mathematical convenience, $f(x)$ must be a continuous and differentiable function.

To relate this biochemical trait X to a particular morphological trait Y, assume a mapping function S;

$$Y = S(X). \tag{1}$$

The mapping function S is assumed to be monotonic with steep flanks and a gentle slope in the middle region, and, therefore, the inverse function R of S exists over the entire character space of X such that

$$X = R(Y). \tag{2}$$

The fitness of individuals with phenotype y is given by the fitness function $w(y)$, and, hence, the mean fitness reads

$$\bar{w} = \int_{-\infty}^{\infty} w(y) f(R(y)) \frac{d}{dy} R(y)\, dy \tag{3}$$

or, alternatively,

$$\bar{w} = \int_{-\infty}^{\infty} w(S(x)) f(x)\, dx. \tag{4}$$

Consider a particular locus and let g_{ij} be the average deviation of individuals with alleles $A_i A_j$ from the population mean of the biochemical trait. If $l_{ij}(x)$ is the density of individuals with genotype $A_i A_j$ on the biochemical level, then the fitness of individuals with genotype $A_i A_j$ is (Nagylaki 1992, Ch. 10, and references therein):

$$w_{ij} = \int_{-\infty}^{\infty} l_{ij}(x) w(S(x))\, dx. \tag{5}$$

Assuming that single locus effects are small, we expand $l_{ij}(x)$