THE GENETICS OF COPPER TOLERANCE IN THE YELLOW MONKEY FLOWER, *MIMULUS GUTTATUS*. I. CROSSES TO NONTOLERANTS

MARK R. MACNAIR[1]

Department of Genetics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

Manuscript received May 24, 1978
Revised copy received September 12, 1978

ABSTRACT

The biometrical genetics of copper tolerance has been investigated in two Californian populations of *Mimulus guttatus* by crosses to a nontolerant British population. A simple biometrical model involving only additive and dominance effects is not sufficient. When the first order interactions are included, the model is shown to fit the data. Interactions between the dominance effects of different loci, and between dominance and additive effects, are the most important. These interactions can be explained either by a threshold model, or by postulating dominance modification.

ALTHOUGH the phenomenon of heavy metal tolerance in plants has been extensively studied (see ANTONOVICS, BRADSHAW and TURNER 1971 for a review), the genetics of this character is still little understood. Crosses between tolerant and nontolerant plants showed that tolerance was inherited in *Festuca ovina* (WILKINS 1960; URQUHART 1971), *Silene inflata* (BRÖKER 1963) and *Anthoxanthum odoratum* (ANTONOVICS 1966). These crosses also indicated that dominance was variable, and ANTONOVICS (1968) argued that this might arise because selection would favor an increase in the dominance of tolerance on copper mine soil. Diallel analyses of GARTSIDE and MCNEILLY (1974a,b) and URQUHART (1971) demonstrated that there was a highly significant additive component to tolerance in *Agrostis tenuis*, *Anthoxanthum odoratum* and *F. ovina*, and that dominance towards tolerance was also present.

ALLEN and SHEPPARD (1971) discovered that the yellow monkey flower, *Mimulus guttatus*, has developed races tolerant to copper in various localities in California. *M. guttatus*, in contrast to many of the species previously investigated, is an extremely good genetic organism, since it is readily emasculated and crossed, can produce more than 400 seeds at a time, is completely self-compatible, and can complete a single generation in less than 100 days.

In this paper I describe an experiment in which crosses were made between typical plants of two Californian populations, Copperopolis 4C and Penn, and a British nontolerant population from Cerig-y-drudion, and a biometrical analysis performed on the means of the F_1 and subsequent generations.

[1] Present address: Department of Biological Sciences, University of Exeter, Exeter, Devon.

THE MODEL

One of the most efficient ways of analyzing the genetics of a metrical character where chromosomal manipulation is not possible is from the generations available from a cross between two inbred lines. Following the notation and procedures of MATHER and JINKS (1971), we define the midpoint between the two lines as m, and the additive and dominance effects of the ith gene as d_i and h_i, respectively. The sums of the d and h effects of all loci at which the two lines differ are given the symbols $[d]$ and $[h]$. The six families, P_1, P_2, F_1, B_1, B_2 and F_2, provide sufficient statistics to estimate m, $[d]$ and $[h]$ and to test the adequacy of the simple genetic model of only additive and dominance effects.

However, where only plants from natural populations are available, this simple scheme is not sufficient, since it would be unwise to assume that any plant was homozygous for all revelant loci. But a nontolerant population can be assumed to be homozygous for the decreasing allele at all tolerance loci: this assumption is validated in Mimulus by the fact that neither ALLEN and SHEPPARD (1971) nor MACNAIR (1976) was able to detect any survivors in a total of 70,000 seeds of the Cerig-y-drudion population sown on mine waste.

A single tolerant plant can be said to have k loci that are homozygous for the tolerant allele, and k' loci that are heterozygous. If we define

$$[d] = \sum_{i=1}^{k} d_i \qquad [d]' = \sum_{j=1}^{k'} d_i$$

$$[h] = \sum_{i=1}^{k} h_i \qquad [h]' = \sum_{j=1}^{k'} h_i$$

the phenotype of the tolerant plant is then given by

$$m + [d] + [h]'$$

and of the nontolerant population by

$$m - [d] - [d]' \ .$$

The F_1 between the two will behave as a normal F_1 at the k homozygous loci, but as a backcross at the k' loci. Its mean is therefore given by

$$m + [h] - \tfrac{1}{2}[d]' + \tfrac{1}{2}[h]' \ .$$

If a sufficiently large F_1 is raised and k' is fairly small, it is likely that both extremes will be present in the F_1. Thus, if the most and least tolerant plants are selected from the F_1, it is likely that they will represent the two extremes, namely: high plant: heterozygous at k loci, heterozygous at k' loci; and low plant: heterozygous at k loci, homozygous for the decreasing allele at k' loci.

In the experiment reported here, 40 F_1 plants were raised and tested for copper tolerance. As long as $k' \leq 5$, there is a better than 0.5 chance of both extremes being present. (This is true because the chance of the family not containing one extreme is $(\tfrac{1}{2}^{k'})^n$, where n is the number in the family: the chance of it containing neither is given by the square of this.)

TABLE 1

Expectations of the means of the nine families in this experiment

Family	Expectation of the mean
P_1	$m + [d] + [h]'$
P_2	$m - [d] - [d]'$
F_1	$m + [h] - \frac{1}{2}[d]' + \frac{1}{2}[h]'$
LF_2	$m + \frac{1}{2}[h] - [d]'$
LB_1	$m + \frac{1}{2}[d] + \frac{1}{2}[h] - \frac{1}{2}[d]' + \frac{1}{2}[h]'$
LB_2	$m - \frac{1}{2}[d] + \frac{1}{2}[h] - [d]'$
HF_2	$m + \frac{1}{2}[h] + \frac{1}{2}[h]'$
HB_1	$m + \frac{1}{2}[d] + \frac{1}{2}[h] + \frac{1}{2}[h]'$
HB_2	$m - \frac{1}{2}[d] + \frac{1}{2}[h] - \frac{1}{2}[d]' + \frac{1}{2}[h]'$

P_1 : Tolerant parent, P_2 : nontolerant parent. $B_1 : F_1 \times P_1$, $B_2 : F_1 \times P_2$.
For definition of the symbols, see text.

The genetic expectations, in terms of the parameters defined above, of the families produced by selfing the two plants (to produce HF_2 and LF_2), and backcrossing them to the tolerant plant (HB_1 and LB_1) and to the nontolerant parent (HB_2 and LB_2) can then be calculated, and are given in Table 1. There are nine families produced in this experiment, and only five genetic parameters, so that these can be estimated and the model tested by the joint scaling test of CAVALLI (see MATHER and JINKS 1971).

MATERIALS AND METHODS

Details of the three populations involved, Cerig-y-drudion (nontolerant), Copperopolis 4C and Penn, are given in ALLEN and SHEPPARD (1971). The Copperopolis and Penn mines are about 14 miles apart, and the populations are apparently distinct. The Copperopolis population is much more tolerant to copper than is the Penn, but less tolerant to zinc. There are also several morphological and other characters distinguishing them (see MACNAIR 1976). One plant from each population, C10 and P9, typical of the samples of each population grown up and tested, was reciprocally crossed with a plant from the Cerig (= Cerig-y-drudion) population, and 20 plants of each of these four families were grown up and tested for tolerance. Maternal inheritance having been shsown to be insignificant (MACNAIR 1976), the two reciprocal F_1 families were pooled, and the most and least tolerant plants selected. These plants were selfed, and backcrossed to a Cerig plant and to the original tolerant parent, C10 or P9. C10 and P9 were cloned. Fifteen ramets of C10 and P9, 15 Cerig plants, and 12 individuals of each of the F_1, two F_2 and four backcross progenies were then grown in each of two randomized blocks. Because of the accidental destruction of one block before all plants had been tested, a third block with 20 of each family was raised later. The results from the three blocks have been pooled.

Tolerance testing was performed by the method of JOWETT (1958), in which root growth in a test solution is compared to growth in a control solution. Each test consisted of a cutting in $Ca(NO_3)_2$ solution (0.5 g/l) as control and a cutting in one or more test solutions of $Ca(NO_3)_2$ with either 0.125, 0.5 or 1.0 ppm copper added as $CuSO_4$. Tolerance indices are then calculated as

$$TI = \frac{\text{root growth in test solution}}{\text{root growth in control solution.}}$$

Cuttings were in the solutions for a week, the solutions being changed after the third and fifth days to maintain solution concentration and oxygenation. On the third day the rooting of the cuttings was checked, and any in the control or 0.125 ppm solution that had not rooted were retested. This procedure reduced the within-plant variance due to cuttings that failed to root at all. It could not be used for cuttings in solutions with more than 0.125 ppm, because plants are not necessarily expected to root at these levels. The *TI* for each plant at any level is the mean of two successful tests at that level.

Cuttings were rooted in expanded polystyrene cups with plastic lids, six cuttings to a cup. Each cup held 180 ml of solution. The position of each cutting, and the position of each cup in the growth chamber, was completely at random. Testing was performed at 23° ± 1° under constant illumination.

Ratios are not normally distributed, and thus for the purpose of statistical analysis the log transformation of the *TI* is to be preferred, since the difference of two normally distributed variables is still normal. MACNAIR (1976) showed that this procedure improved the normality of the *TI*s and, more importantly, removed the regression of residual on mean. In order to eliminate zeros, 1 mm was added to all root lengths. Cerig plants were not tested at levels greater than 0.125 ppm because previous tests had shown that they did not root at all at high copper concentrations: they were given a root length of 1 mm for all higher levels.

RESULTS

The means and weights (calculated as the reciprocal of the variance of the mean) of the nine families produced from both P9 and C10 are given in Table 2. Also given are the number of plants on which the means and variances were

TABLE 2

Means, weights, and numbers of plants tested of each family

	0.125 ppm			0.5 ppm			1.0 ppm		
	Mean	Weight	*n*	Mean	Weight	*n*	Mean	Weight	*n*
C10									
P_1	0.098	339.53	19	−0.060	301.64	19	−0.199	297.01	23
P_2	−1.883	111.64	33	−3.391	303.72	34	−3.391	303.72	34
F_1	0.066	475.03	23	−0.278	211.73	20	−1.222	42.08	26
HF_2	−0.195	166.34	25	−1.257	43.47	26	−1.781	34.28	25
HB_1	0.086	458.72	29	−0.477	149.22	26	−1.053	64.03	27
HB_2	−1.049	81.12	28	−2.578	43.96	25	−3.002	59.35	24
LF_2	−0.726	40.15	21	−1.409	16.82	19	−2.100	27.89	20
LB_1	0.042	480.58	33	−0.447	300.77	31	−1.041	85.53	33
LB_2	−1.345	61.71	38	−2.678	59.77	33	−3.179	121.65	33
P9									
P_1	−0.039	304.70	20	−0.919	151.53	16			
P_2	−1.883	111.64	33	−3.391	303.72	34			
F_1	−0.020	387.68	25	−1.129	61.33	27			
HF_2	−0.189	63.42	18	−2.169	8.03	16			
HB_1	−0.208	100.86	21	−1.284	36.87	18			
HB_2	−1.060	35.62	20	−2.283	23.38	19			
LF_2	−0.544	66.97	13	−1.574	42.23	9			
LB_1	−0.283	152.75	17	−1.993	27.10	17			
LB_2	−1.047	39.47	22	−2.322	28.21	22			

The weights for the Cavalli scaling test are calculated as the reciprocal of the variance of the mean.

based. It is apparent that the data for the C10 series of crosses is much better than that for P9, in that the weights and the family sizes are larger. The reason for this is that many of the plants from the P9 series, particularly from the F_2s, produced many thin straggly side shoots, and the plants were not testable for tolerance. One consequence of this is that the LF_2 has a higher mean than the HF_2 at 0.5 ppm, though this occurs only on log transformation. A certain degree of caution is therefore required when interpreting the data from this series.

The estimates of m, $[d]$, $[h]$, $[d]'$, and $[h]'$, and the $\chi^2_{(4)}$ for the goodness-of-fit of the model, are given in Table 3. The model fails to fit at all levels, but appears to get worse as the level of copper rises. The additive effects of the homozygous loci, $[d]$, are always large, while the dominance effects are also important at 0.125 and 0.5 ppm. The value of $[h]$ becomes nonsignificant at 1.0 ppm for C10: this agrees with the findings of ALLEN and SHEPPARD (1971) that the direction of dominance appears to shift as the concentration of copper increases. The effects of the heterozygous loci appear to be of lesser importance than those of the homozygous loci.

However, because the model does not fit, these conclusions must be viewed with suspicion. Particularly the nonadditive component can be seriously affected by factors that disturb the model. There are many possible causes of disturbance, particularly nonallelic interactions and genotype-environment interactions. It is possible to incorporate certain first-order interactions into the basic model.

MATHER and JINKS (1971) recognize three sorts of first-order interactions between a pair of loci: those between the additive properties of genes ($d \times d$), which they denote by the symbol i; interactions between additive and dominance components ($d \times h$), given the symbol j; and interactions involving only the dominance effects ($h \times h$), denoted by l.

Interactions could occur among those loci that were homozygous in the original parent, among loci originally heterozygous, or between pairs of loci, one of which was originally homozygous and the other heterozygous. Thus, we are in principle concerned with ten possible first-order interactions in this instance. However it

TABLE 3

Result of Cavalli test fitting the basic genetic model

	C10			P9	
	0.125	0.5	1.0	0.125	0.5
m	−0.8608±0.106	−2.2653±0.166	−1.9116±0.244	−0.9972±0.174	−2.6581±0.326
$[d]$	0.8537±0.079	1.3802±0.102	1.3769±0.117	0.6745±0.103	1.1101±0.141
$[h]$	0.9932±0.070	1.3377±0.078	(−0.0428±0.123)	0.9262±0.074	0.9462±0.127
$[d]'$	0.2789±0.099	(−0.1439±0.163)	(0.2150±0.226)	(0.1868±0.171)	(−0.3839±0.328)
$[h]'$	(0.1282±0.160)	0.8178±0.231	(0.3563±0.307)	(0.2524±0.229)	(0.5734±0.387)
$\chi^2_{(4)}$	13.22*	53.52***	50.33***	11.41*	23.29***

Terms which are less than twice their standard errors are in parentheses.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

is apparent from Table 3 that in both C10 and P9 the effects due to fixed loci are of greater magnitude than those due to heterozygous loci: it is thus reasonable to fit the interactions involving only these loci in the first instance.

Defining the sum of the i interaction effects over all the possible pairs of loci homozygous in the original tolerant parent as $[i]$ (see MATHER and JINKS 1971), and similarly for j and l effects as $[j]$ and $[l]$, the expectations of the means of the nine families can be specified. The expectations of both the high and low series are identical for these parameters, which are given in Table 4.

TABLE 4

Coefficients of the interaction parameters between fixed loci for the P_0, F_1, F_2, B_1 and B_2 generations

Family	Expectation of the mean
P_1	$+[i]$
P_2	$+[i]$
F_1	$+[l]$
F_2	$+\frac{1}{4}[l]$
B_1	$+\frac{1}{4}[i]+\frac{1}{4}[j]+\frac{1}{4}[l]$
B_2	$+\frac{1}{4}[i]-\frac{1}{4}[j]+\frac{1}{4}[l]$

There are now eight genetic parameters and nine equations, so that the Cavalli scaling test can still be used to produce estimates of the eight parameters and to test for these goodness-of-fit of this extended model. The results of this analysis are given in Table 5. In contrast to the simple model, this gives an excellent fit for C10, and a reasonable one for P9.

TABLE 5

Result of Cavalli scaling test fitting model including interactions

	C10			P9	
	0.125	0.5	1.0	0.125	0.5
m	(−0.2874±0.361)	(−0.8615±0.586)	−1.2567±0.572	(0.2703±0.452)	(−0.4252±0.871)
$[d]$	0.6097±0.137	1.5410±0.171	1.3858±0.146	0.7793±0.168	1.5594±0.244
$[h]$	(−1.0772±0.825)	−2.6446±1.286	−2.8648±1.254	(−1.9140±1.105)	(−2.1736±1.797)
$[i]$	−0.8673±0.359	−1.0369±0.598	(−0.7002±0.596)	−1.1283±0.505	(−0.5800±0.852)
$[j]$	0.8779±0.269	1.1470±0.285	1.0745±0.293	(−0.2060±0.355)	−1.8028±0.547
$[l]$	1.1688±0.493	3.0556±0.729	2.7381±0.755	1.7260±0.650	2.6194±1.051
$[d]'$	(0.1182±0.126)	(−0.0483±0.193)	(0.0481±0.290)	(0.2453±0.235)	(0.8265±0.447)
$[h]'$	0.6430±0.307	(0.2977±0.414)	(0.3719±0.458)	(0.0399±0.475)	(−1.4728±0.752)
$\chi^2_{(1)}$	0.56	0.05	0.20	0.84	6.20*

It is clear that many of the terms are nonsignificant. A minimum model for each level of copper was therefore fitted by progressively dropping terms from the analysis until all the remaining terms were significant (see Table 6). At 0.125 ppm, a better fit with C10 is obtained if $[h]'$ is included in the model: but

TABLE 6

Minimum models for each level of copper in C10 and P9

	C10			P9	
	0.125	0.5	1.0	0.125	0.5
m	—	−1.7255±0.041	−1.7953±0.041	—	−2.1466±0.045
$[d]$	0.9920±0.054	1.6657±0.041	1.5959±0.041	0.9009±0.051	1.2390±0.049
$[h]$	−1.3547±0.162	−0.5806±0.245	−1.6242±0.282	−1.6667±0.198	—
$[i]$	−0.8962±0.054	—	—	−0.9549±0.054	—
$[j]$	0.4701±0.194	0.9316±0.226	0.9671±0.235	—	−1.0633±0.386
$[l]$	1.4211±0.181	2.0285±0.256	2.1978±0.378	1.6463±0.217	1.0358±0.135
χ^2	12.88*	3.75	4.16	5.63	13.07*
d.f.	4	4	4	5	5

Both $[d]'$ and $[h]'$ are redundant.
* $0.05 > P > 0.01$.

since there is no other evidence from this experiment that this plant has any heterozygous copper tolerance genes, the simpler model is preferred. A reasonably consistent pattern is established. The term $[d]$ is always large and positive, while $[h]$ is consistently negative. The only interaction term to be significant at all levels of copper and in both plants is $[l]$. The value of $[j]$ is always significant in C10, but $[i]$ appears more important in P9 at 0.125 ppm.

A slightly different pattern at 0.125 ppm as compared to the higher levels of copper is not unexpected. At this level, all plants are able to root, and possibly genes that simply affect rooting performance could have more affect here than at higher levels where the rooting of many plants is inhibited (see below).

Number of loci: The number of loci is not normally an important feature of an analysis such as this. The methods available are too inaccurate and dogged by important and unlikely assumptions that they are rarely of more than academic interest. However, some of the data obtained from this experiment suggest that there may be only two loci involved in producing most of the tolerance observed in C10 (MACNAIR 1977), and since the interpretation of the biometrical analysis is made easier if we think in terms of two loci, this evidence will be briefly presented here.

The measurement of copper tolerance is highly inaccurate, principally because it is based on root growth, which is difficult to standardize. Thus, it is impossible to separate plants that have formed roots in a copper-containing solution into the definite classes needed for a Mendelian analysis; therefore, a biometrical analysis is essential for the comparison of plants with non-zero tolerance indices. However, it was found that at levels of copper higher than about 0.25 ppm, plants from the Cerig (nontolerant) population failed to root under the test conditions. Thus, any plant that forms roots at higher copper levels can be qualitatively distinguished from the Cerig plants, and said to be tolerant. However, it was not only the Cerig plants that failed to form roots at 0.5 ppm. Forty-four of the combined BC2 families from the C10 series also failed to form proper roots. These plants can thus be distinguished from the 14 plants of the backcrosses that did

root. This ratio (44:14) is not significantly different from 3:1, but is from 1:1 ($\chi^2 = 15.52$). A 3:1 ratio in a backcross can be obtained with two loci, but not with one or more than two.

This observation, therefore, makes a *prima facie* case for there being only two genes, though it is recognized that further crosses are needed to test this conclusion. These crosses are being performed.

DISCUSSION

These results shed some light on one of the most obscure aspects of the genetics of heavy metal tolerance: the question of dominance. Several authors have shown that dominance appeared to be variable (JOWETT 1959; WILKINS 1960; ANTONOVICS 1966: URQUHART 1971), while ALLEN and SHEPPARD (1971) showed that the direction of dominance changed as the level of copper at which tolerance was tested increased. ANTONOVICS (1968) argued that variable dominance was to be expected, since selection would favor dominance for tolerance on the mine, while away from it recessiveness would be selected if tolerance is, as seems likely (see COOK, LEFEBVRE and MCNEILLY 1972; HICKEY and MCNEILLY 1975), a disadvantage in uncontaminated soil.

In this study, the simple genetic model also showed an apparent shift in the direction of dominance, since the sign of [*h*] changed as the copper concentration increased. However, the goodness-of-fit χ^2 indicated that this simple model was not sufficient, and fitting a fuller one showed that this shift of dominance was caused by the presence of interactions. Once the interactions were taken into account, a much more exact fit was obtained, and a general consistency in the direction and relative magnitudes of the genetic parameters was observed, particularly in the C10 series. Dominance as represented by [*h*] was now always towards nontolerance, while [*j*] and particularly [*l*] were the important interactions.

The negative [*h*] term indicates that the tolerance genes are, one their own, preponderantly recessive. Thus, on the basis of two genes, *Aabb* or *aaBb* would be relatively nontolerant. The positive [*l*] term, however, shows that when both genes are heterozygous, the interaction works in the opposite direction to the individual *h*'s, and the individual is tolerant. Significant [*j*] indicates that one, or both, of the genes, when heterozygous, interacts with the other gene, when it is homozygous, to produce greater tolerance.

This pattern could be produced in two ways. First, if there were a threshold for tolerance, these results might be obtained. If both genes *A* and *B* are of equal magnitude and effect, and at least two copies of the gene are required before any tolerance is observed, then one would find that *AaBb* was equal in tolerance to both *AAbb* and *aaBB*: thus giving a significant positive [*l*] interaction. So long as the addition of more copies of the tolerance genes gives an increase in tolerance, positive [*j*], but no [*i*], interactions would be found. However, this model gives a consistent picture at different concentrations of copper only if the threshold is always at two gene copies: one might expect that as the level of copper

was increased, more gene copies would be required to give the initial tolerance. If three are needed, however, *AaBb* would give as little tolerance as both *Aabb* and *aaBb*, and thus no [*l*] interaction would be obtained.

The second model postulates that one gene is the structural locus producing the protein responsible for giving tolerance, the other a dominant dominance modifier. Thus, if *A* produces the protein, then *AAbb* would be tolerant, but *aaBB* would not. In the absence of *B*, *A* is recessive, so *Aabb* is nontolerant. *AaBb* however would be tolerant, as is *AaBB*. This model would produce both [*l*] and [*j*] type interactions. This latter model would not necessarily predict a change in the overall pattern as the copper concentration increased. This is the model investigated by ANTONOVICS (1968), who showed by computer simulation that it could evolve.

The distinction between the two models is not dependent on the two-locus hypothesis; although it is easier to visualize with two genes, models involving any number of loci, but the same types of interaction, will produce the same pattern in the biometrical analysis. It is obviously not possible to differentiate between them at this stage: if the two-locus model is correct, however, the crosses needed to confirm the presence of two genes should also enable the type of interaction to be deduced.

Either type of gene interaction will help to ensure that the effects of gene flow both onto and off the mine will be minimized. Gene flow onto the mine will result in F_1s, *AaBb*, that will in the main cross with *AABB* plants. The interactions will cause all the progeny of such crosses to be tolerant. Off the mine *AaBb* plants will mainly cross with *aabb* individuals, and their offspring will therefore mainly be nontolerant. Thus, the type of interaction demonstrated here will have the genetic results predicted by ANTONOVICS (1968), and thus for the first time confirms the general accuracy of his prediction.

In the P9 series, while the [*l*] interaction is still significant and positive at both levels of copper, the [*j*] interaction term is not significant at 0.125 ppm, and at 0.5 ppm is negative. This is the only major difference between the results given by C10 and P9. The interpretation of this difference is unclear: while it may be caused by a real difference in the genetic architectures of tolerance in the Copperopolis and Penn populations (and it must be borne in mind that there must be some difference between the two populations in view of the disparity in their tolerances), too much should not be made of this difference in view of the facts that the model still does not fit at 0.5 ppm and the data for the P9 series are not as good as for C10. The overall similarity in the results obtained for the two populations is clear.

One other explanation of the interactions found in this paper must be considered. The populations from this study came from the U.S.A. and Britain, and it is well known that, when distantly related populations are crossed, the breakdown of co-adapted gene complexes could cause nonallelic interactions. Some support for this is given by the fact that there is a partial crossing barrier between Copperopolis and Cerig, in that some F_1 and backcross to Cerig plants died at an early stage. However, crossing barriers of this type are not uncommon in Mimu-

lus, and have been extensively studied by VICKERY (see VICKERY 1974 for a review). He also found that such barriers are not shown just by widely separated populations. *Mimulus guttatus* was introduced into Britain only in about 1830, and on morphological grounds it is apparent that the Cerig population is derived from the Western range of the species. In addition, there was no evidence of barriers between Penn and Cerig, and yet the results from both Copperopolis and Penn are broadly the same. At the time when this experiment was performed, no Californian nontolerant seed was available; I have now collected such material and am performing crosses to determine if the interactions found here could be due solely to unrelatedness of the populations.

The apparent unimportance of unfixed loci in both P9 and C10 indicates that both plants are homozygous for the loci producing the major part of the tolerance of these plants. If there are only two loci of major effect, this is to be expected. It is also expected if the mine populations are relatively isolated; I have no direct evidence on this, but the large size of both the Penn and Copperopolis mines means that the bulk of the tolerant populations will be some distance from uncontaminated soil. However this result should not be interpreted to mean that there were no heterozygous tolerance loci in P9 or C10. The small size of this experiment, and the inaccuracy of tolerance testing, means that any loci of comparatively minor effect would not be detected. It is to be expected that, after the genes producing most of the observed tolerance have become fixed in a mine population, selection would act to favor genes at other loci that enhanced the tolerance in some way. Crosses within both the Penn and Copperopolis populations (MACNAIR 1976) revealed considerable genetic variation, showing that there are other loci involved, which have not become totally fixed in the population.

I am indebted to the late P. M. SHEPPARD, F.R.S., for his advice and guidance while he was my supervisor. I thank M. J. LAWRENCE for some valuable suggestions, and for critically reading the manuscript. During the course of this work I held an N.E.R.C. studentship.

LITERATURE CITED

ALLEN, W. R. and P. M. SHEPPARD, 1971 Copper tolerance in some Californian populations of the monkey flower, *Mimulus guttatus*. Proc. Roy. Soc. Lond. B. **177**: 177–196.

ANTONOVICS, J., 1966 The genetics and evolution of differences between closely adjacent plant populations with special reference to heavy metal tolerance. Ph.D. thesis, University of Wales. ——, 1968 Evolution in closely adjacent plant populations. VI. Manifold effects of gene flow. Heredity **23**: 507–524.

ANTONOVICS, J., A. D. BRADSHAW and R. G. TURNER, 1971 Heavy metal tolerance in plants. Adv. Ecol. Res., **7**: 1–85.

BRÖKER, W., 1963 Genetisch-Physiologische Untersuchungen uber die Zinkvertraeglichkeit von *Silene inflata* Sm. Flora, Jena. **153**: 122–156.

COOK, S. C. A., C. LEFEBVRE and T. MCNEILLY, 1972 Competition between metal tolerant and normal plant populations on normal soil. Evolution **26**: 366–372.

GARTSIDE, D. W. and T. MCNEILLY, 1974a Genetic studies in heavy metal tolerant plants. I. Genetics of zinc tolerance in *Anthoxanthum odoratum*. Heredity **32**: 287–299. ——, 1974b Genetic studies in heavy metal tolerant plants. II. Zinc tolerance in *Agrostis tenuis*. Heredity **33**: 303–308.

HICKEY, D. A. and T. MCNEILLY, 1975 Competition between metal tolerant and normal plant populations. A field experiment on normal soil. Evolution **29**: 458.

JOWETT, D., 1958 Populations of *Agrostis* spp. tolerant of heavy metals. Nature **182**: 816–817. ——, 1959 Genecology of heavy metal tolerance in *Agrostis*. Ph.D. thesis, University of Wales.

MACNAIR, M. R., 1976 The genetics of copper tolerance in *Mimulus guttatus* (Scrophulariaceae). Ph.D. thesis, University of Liverpool. ——, 1977 Major genes for copper tolerance in *Mimulus guttatus*. Nature **268**: 428–430.

MATHER, K. and J. L. JINKS, 1971 *Biometrical genetics* (2nd Edn.) Chapman and Hall, London.

URQUHART, C., 1971 Genetics of lead tolerance in *Festuca ovina*. Heredity **26**: 19–33.

VICKERY, R. K., JR., 1974 Crossing barriers in the Yellow Monkey Flowers of the genus *Mimulus* (Scrophulariaceae). Genetics Lectures, **3**: 33–82. Oregon State University Press.

WILKINS, D. A., 1960 The measurement and genetical analysis of lead tolerance in *Festuca ovina*. Rep. Scott. Pl. Breed. St.: 85–98.

Corresponding editor: B. S. WEIR