EVOLOUTION OF PLANT RESISTANCE TO MULTIPLE HERBIVORES: QUANTIFYING DIFFUSE COEVOLUTION

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Submitted July 21, 1995; Revised February 12, 1996; Accepted April 9, 1996

Abstract.—Studies of coevolution in plant-herbivore systems have typically focused on tight, pairwise interactions between one herbivore and one host plant species. Diffuse coevolution, by contrast, has received much less empirical attention, presumably because imprecise definitions of diffuse coevolution have hindered the development of experimental approaches for distinguishing between pairwise and diffuse coevolution. Here we provide a definition of diffuse coevolution that leads to three criteria for the operation of pairwise coevolution: susceptibilities (resistances) to different herbivores are genetically uncorrelated, the presence/absence of one herbivore does not affect the amount of damage caused by other herbivores, and the impact of one herbivore on plant fitness does not depend on the presence/absence of other herbivores. All three criteria must be satisfied for coevolution to be pairwise; if any of them fail, coevolution is diffuse. We then describe an experimental design and statistical analysis that permit the partitioning of the total selection imposed on a plant by a set of herbivores into components representing pairwise and diffuse selection, thus allowing determination of whether coevolution is pairwise or diffuse.

Coevolution is commonly believed to be important in the evolution of many plant characters and the ability of herbivores and pathogens to use plants as food and hosts (Ehrlich and Raven 1964; Feeny 1975; Futuyma 1983; Spencer 1988; Thompson 1994). Although the precise nature of coevolution has been described for very few systems, two general types of coevolutionary interactions have been hypothesized (Janzen 1980; Fox 1981; Futuyma 1983). One type is pairwise coevolution, in which a plant and its natural enemy impose reciprocal selection on each other, causing the pair of species to evolve jointly. By contrast, coevolution is often said to be diffuse when more than two species populations are involved (Janzen 1980). Most empirical research has focused on pairwise coevolution, even though it may be rare in nature (Futuyma and Slatkin 1983). Although diffuse coevolution is often invoked to explain various traits, such as the generalized defense mechanisms that are effective to a wide range of parasitic organisms (Fox 1981; Futuyma 1983; May and Anderson 1983), the term has been criticized as being vague or as being a catchall phrase, because most interacting systems involve more than a pair of species (Gould 1988; Thompson 1994).

Such criticism has arisen, we believe, largely from the lack of a precise defini-
tion of diffuse coevolution and an absence of specific criteria that may be used to distinguish the two types of coevolution. However, a general definition of diffuse coevolution and a set of specific criteria that distinguish pairwise from diffuse coevolution have been proposed (Hougen-Eitzman and Rausher 1994). In this view, in a system consisting of several herbivores and one common host species, coevolution is pairwise if susceptibilities to the different herbivores are genetically uncorrelated, and the pattern of selection exerted by each herbivore is independent of whether the other herbivores are present; otherwise, coevolution is diffuse.

This definition is motivated by Lande’s (1979) general equation for multivariate evolutionary change, \( \Delta \bar{z} = G\beta \), where \( \bar{z} \) is the vector of character means (in this case, means of levels of resistance), \( G \) is the genetic variance-covariance matrix, and \( \beta \) is the selection gradient vector. The first portion of the definition relates to \( G \): if susceptibilities are genetically uncorrelated, \( G \) is a diagonal matrix, and the evolutionary response of susceptibility to one enemy is independent of any selection exerted on susceptibilities to other enemies (i.e., the response of one resistance is not genetically constrained by the presence of other herbivores). The second portion of this definition relates to \( \beta \): when susceptibilities are uncorrelated, each element of \( \beta \) corresponds to the selection exerted by a particular natural enemy on host resistance. If each element of \( \beta \) is independent of whether other herbivores are present or absent, then the pattern of selection exerted by one herbivore, and the subsequent evolutionary response of resistance to that herbivore, will not depend on whether the other herbivores are present (i.e., selection will be pairwise). Elements of \( \beta \) may not be independent of whether other herbivores are present in two ways: the presence of one herbivore affects the amount of damage caused by other herbivores (e.g., Krischik et al. 1991; Pilson 1992), or the reduction in plant fitness caused by one herbivore is influenced by the amount of damage caused by other herbivores (i.e., the fitness effects of damage by two herbivores are not additive; see, e.g., Strauss 1991).

These considerations thus lead to three conditions for coevolution to be pairwise rather than diffuse: (1) susceptibilities (resistances) to different herbivores are genetically uncorrelated, (2) the presence/absence of one herbivore does not affect the amount of damage caused by other herbivores, and (3) the impact of one herbivore on plant fitness does not depend on the presence/absence of other herbivores. All three of these conditions must be satisfied for coevolution to be pairwise. If any of them fail, coevolution is diffuse.

Because assessing whether susceptibilities to different herbivores are genetically correlated is relatively straightforward, a number of studies have examined the correlations among susceptibilities to different herbivores. In general, although some examples of both negative and positive correlations exist, in a large majority of pairwise comparisons, resistance to one herbivore or natural enemy is statistically uncorrelated to resistance to the other (Rausher 1992b; M. D. Rausher, unpublished manuscript). These results suggest that condition 1 may be satisfied in many cases and that therefore coevolution will be diffuse only if conditions 2 or 3 are violated. However, few attempts have been made to assess whether these conditions are violated—that is, whether selection is diffuse
(M. D. Rausher, unpublished manuscript)—largely because there are, to our knowledge, no descriptions of general methods that may be used to evaluate these conditions.

In this report, we provide a description of such a method. Because this method also involves estimating the genetic covariance between susceptibilities to two herbivores, it also allows assessment of condition 1 and thus provides a comprehensive methodology for determining whether coevolution is pairwise or diffuse. We also describe an experimental design that permits the partitioning of the total selection imposed on a plant by a set of herbivores into components representing pairwise and diffuse selection. This partitioning allows us not only to distinguish between pairwise and diffuse selection but also to quantify the relative contributions of diffuse and pairwise components of the overall selection imposed by herbivores. We concentrate here on characterizing the pattern of selection that herbivores and pathogens impose on their host plants. In principle, however, the same approach could be used to characterize the nature of selection imposed by plants on herbivores and pathogens.

COMPONENTS OF DAMAGE

As a prelude to developing a model for analyzing selection imposed by herbivores, we first describe a statistical model for the amount of damage imposed by different herbivores on a plant population. We consider explicitly a situation in which a plant population serves as host for two different herbivore species, though generalization to a larger number of herbivores is straightforward (see app. A). In this model, we treat damage as a quantitative trait of a plant, representing the degree of susceptibility (e.g., Simms and Rausher 1987, 1989; Rausher and Simms 1989).

Consider a plant population that is genetically variable for resistance to each of two herbivores. Let $D_{i\hat{j}}$ and $D_{2\hat{j}}$ be the amount of damage to the $j$th plant of the $i$th genotype caused by herbivores 1 and 2, respectively, when both herbivores are present in the environment. Let $R_{1, i}$ and $R_{2, i}$ be the additive genotypic (breeding) values, for genotype $i$, of the amount of damage caused by herbivores 1 and 2, respectively, when both herbivores are present, so that

$$D_{1\hat{j}} = R_{1, i} + \epsilon_{1\hat{j}}$$

and

$$D_{2\hat{j}} = R_{2, i} + \epsilon_{2\hat{j}},$$

where the $\epsilon$'s represent the environmental and nonadditive components of damage.

Next consider the amount of damage imposed on a plant by each herbivore in the same environment as previously but in the absence of the other herbivore. This may be represented by

$$D_{1\hat{j}}' = S_{1, i} + \epsilon_{1\hat{j}}'$$
and

\[ D2_{ij}' = S2_i + \epsilon2_{ij}', \]

where \( S1_i \) and \( S2_i \) are the additive genotypic values, for genotype \( i \), of damage caused by herbivores 1 and 2, respectively, in the absence of the other herbivore.

The values \( R1_i \) and \( S1_i \) reflect the susceptibility of genotype \( i \) to herbivore 1 in environments with and without herbivore 2. Consequently, the difference, \( I1_i = R1_i - S1_i \), represents the change in susceptibility to herbivore 1 due to the presence of herbivore 2. We term this effect the interaction effect on damage. Incorporating this and a similar effect for herbivore 2 into equation (1) yields

\[ D1_{ij} = S1_i + I1_i + \epsilon1_{ij} \]

and

\[ D2_{ij} = S2_i + I2_i + \epsilon2_{ij}. \]

This set of equations indicates that the damage caused to a particular plant by a particular herbivore is the sum of three components: a genotype-specific intrinsic damage reflecting the inherent susceptibility of that genotype in the absence of the other herbivore, a genotype-specific interaction effect representing the modification of the intrinsic damage caused by the presence of the other herbivore, and an individual-specific environmental deviation and nonadditive genetic components.

Interaction effects may arise in a variety of ways, including but not limited to the following. Through direct competitive effects (e.g., Fritz 1992), the presence of herbivore 1 can reduce the average number of individuals of herbivore 2 on a plant and thus reduce the total damage to that plant by herbivore 2. Indirect effects, modulated by induced changes in host quality (Karban and Carey 1984; Karban et al. 1987; Krischik et al. 1991), can also reduce or increase damage by one herbivore in the presence of another. Finally, survival of, and hence damage caused by, herbivore 1 can be reduced in the presence of herbivore 2 if the latter species increases the attractiveness of the host plant to predators or parasitoids (Faeth 1986).

**PARTITIONING SELECTION INTO PAIRWISE AND DIFFUSE COMPONENTS**

In this section, we describe how the total selection pressure exerted by two herbivores on a plant population can be partitioned into a pairwise and a diffuse component. Two approaches to this problem and, more generally, to measuring selection on a character are possible: phenotypic and genetic. The phenotypic approach, based on the methodology first described by Lande and Arnold (1983), quantifies directional selection on a suite of characters in terms of the phenotypic selection gradient, \( \beta \). Each element \( \beta_i \) of \( \beta \), which is estimated by the partial
phenotypic regression coefficients of the trait \( i \) on fitness, in theory reflects the magnitude of selection acting directly on that trait. By contrast, the genetic approach, derived from the covariance approach to measuring selection (Robertson 1968; Price 1970; Crow and Nagylaki 1976), quantifies directional selection on a suite of characters in terms of the genetic selection gradient, \( \mathbf{B} \). Each element \( B_{ij} \) of \( \mathbf{B} \) is estimated by the partial additive genetic regression coefficient of trait \( i \) on fitness and, like the \( \beta_{ij} \), reflects the magnitude of selection acting directly on that trait (Rausher 1992a). In fact, when there are no environmental correlations between the traits and fitness, the two selection gradients should be identical, as would be expected from the two equations for evolutionary change in a suite of characters:

\[
\Delta \bar{z} = \mathbf{GP}^{-1}s = \mathbf{GB}
\]

(Lande 1979) and

\[
\Delta \bar{z} = \mathbf{GG}^{-1}\mathbf{C} = \mathbf{GB},
\]

(Rausher 1992a), where \( \mathbf{P} \) is the phenotypic variance-covariance matrix, and \( \mathbf{C} \) is the vector of genetic selection differentials (measured as the additive genetic covariance between a character and fitness).

Although the phenotypic approach to characterizing selection is experimentally and computationally easier, this approach is more likely to lead to biased estimates of the true pattern of selection for a variety of reasons (Rausher 1992a; van Tienderen and de Jong 1994). In particular, variation in environmental factors that simultaneously affect both a set of traits and fitness will cause estimates of elements of the phenotypic selection gradient, \( \beta_{ij} \), to differ in magnitude, and even in sign, from their true values. This problem is likely to be particularly acute for characters underlying resistance to herbivores (e.g., plant secondary compounds), since substantial evidence indicates that many environmental factors that affect fitness (e.g., soil water and nutrient content) also affect resistance characters (Gershenzon 1984). By contrast, environmentally induced correlations of this type between characters and fitness do not bias estimates of the genetic selection gradient, \( \mathbf{B} \). For this reason, we adopt the genetic approach to characterizing the pairwise and diffuse components of selection exerted by herbivores on their host plants. Nevertheless, for workers interested in adopting a phenotypic approach, we present in appendix B a brief description of how this approach may be implemented.

To partition \( \mathbf{B} \), which is equal to \( \mathbf{G}^{-1}\mathbf{C} \), into pairwise and diffuse components, we first partition \( \mathbf{G}^{-1} \) and \( \mathbf{C} \) separately. Consider first \( \mathbf{G}^{-1} \). When both herbivores are present, the additive genetic variance-covariance matrix for damage is

\[
\mathbf{G} = \begin{bmatrix}
\text{var}_a(D1) & \text{cov}_a(D1, D2) \\
\text{cov}_a(D1, D2) & \text{var}_a(D2)
\end{bmatrix}.
\]

(We will omit subscript \( a \), which indicates that genetic variances and covariances are additive, in the following equations for notational simplicity.)
QUANTIFYING DIFFUSE COEVOLUTION

Taking the inverse of both sides and using equation (3), one obtains

\[
G^{-1} = \frac{1}{\det(G)} \begin{bmatrix}
\text{var}(D2) & -\text{cov}(D1, D2) \\
-\text{cov}(D1, D2) & \text{var}(D1)
\end{bmatrix}
\]

\[= \frac{\det(G_s)}{\det(G)} G_s^{-1} + \frac{\det(G_1)}{\det(G)} G_1^{-1}, \tag{4a}\]

where

\[
G_s = \begin{bmatrix}
\text{var}(S1) & 0 \\
0 & \text{var}(S2)
\end{bmatrix} \tag{4b}
\]

and

\[
G_1 = \begin{bmatrix}
\text{cov}(S1, S2) + 2\text{cov}(S1, I1) & \text{cov}(S1, S2) + \text{cov}(S1, I2) + \text{cov}(S2, I1) + \text{cov}(I1, I2) \\
\text{cov}(S1, S2) + \text{cov}(S1, I2) + \text{cov}(S2, I1) + \text{cov}(I1, I2) & \text{var}(I2) + 2\text{cov}(S2, I2)
\end{bmatrix}. \tag{4c}\]

Note that \( G = G_s + G_1 \). Apart from the scaling constant \( \det(G) \), the first term in equation (4a) involves only the variances of \( S1 \) and \( S2 \). For reasons that will be apparent later, we refer to this term as the “pairwise” component of \( G^{-1} \). By contrast, the second term involves primarily the interaction effects; except for \( \text{cov}(S1, S2) \), \( S1 \) and \( S2 \) appear only in combination with \( I1 \) and \( I2 \). Because the interaction effects represent the influence of one species on the amount of damage caused by the other, the second term represents the “diffuse” component of \( G^{-1} \). The covariance between \( S1 \) and \( S2 \) is included in the diffuse component because this covariance contributes to a diffuse response to selection. Note also that if there is no additive genetic variation for the interaction effects (i.e., \( \text{var}(I1) = \text{var}(I2) = 0 \)), then \( G_1 \) reduces to

\[
G_1 = \begin{bmatrix}
0 & \text{cov}(S1, S2) \\
\text{cov}(S1, S2) & 0
\end{bmatrix},
\]

that is, to a matrix reflecting the genetic constraint producing a diffuse response.

To partition the overall selection differential, \( C \), we first define the pairwise selection differential as

\[
C_s = \begin{bmatrix}
\text{cov}(w1', D1') \\
\text{cov}(w2', D2')
\end{bmatrix} = \begin{bmatrix}
\text{cov}(w1', S1) \\
\text{cov}(w2', S2)
\end{bmatrix}. \tag{5}
\]

Here \( w' \) represents the plant fitness measured in the absence of the other herbivore. For example, \( \text{cov}(w1', D1') \) represents the additive covariance between fitness and damage by herbivore 1 when herbivore 2 is absent. The pairwise selection differential thus represents the selection differential that would be observed if selection exerted by each herbivore were independent of the presence of the other herbivore.
Next we define the diffuse selection gradient, $C_1$, as the difference between the overall selection differential and the pairwise selection differential

$$C_1 = C - C_s$$

$$= \begin{bmatrix} \text{cov}(w, D1) \\ \text{cov}(w, D2) \end{bmatrix} - \begin{bmatrix} \text{cov}(w'1, S1) \\ \text{cov}(w'2, S2) \end{bmatrix}$$

(6)

$$= \begin{bmatrix} \text{cov}(w - w'1, S1) \\ \text{cov}(w - w'2, S2) \end{bmatrix} + \begin{bmatrix} \text{cov}(w, I1) \\ \text{cov}(w, I2) \end{bmatrix},$$

where $w$ represents the fitness measured when both herbivores are present in the environment. Rearrangement yields

$$C = C_s + C_1.$$ (7)

In effect, $C_1$ represents the component of the overall selection differential that arises because selection is not completely pairwise.

If there is no genetic correlation between susceptibilities when both herbivores are present (i.e., $\text{cov}(D1, D2) = 0$), then a nonzero diffuse selection differential, $C_1$, can arise in either of two ways, corresponding to the two terms in equation (6). On the one hand, it may arise if there is additive genetic variation for interaction effects (i.e., $\text{var}(I1) \neq 0$ or $\text{var}(I2) \neq 0$) and if interaction effect co-varies with fitness. This effect, which arises because the presence of one herbivore affects the amount of damage caused by the other, is represented by the second term in equation (6). Consider, for example, a situation in which feeding by herbivore 2, but not by herbivore 1, causes an induced chemical response in the host plant that makes a plant more resistant to both herbivores. If there is genetic variation for the level of induced resistance, then in the presence of herbivore 2 there will be variation in damage caused by herbivore 1. In the absence of herbivore 2, however, all plants will remain uninduced, and there will be no variation among plant genotypes in damage by herbivore 1. Clearly, $\text{var}(S1) = 0$, so the herbivore 1 element of the first term in equation (6) will be zero. However, there is also clearly variation for interaction effects, and if feeding by herbivore 1 reduces fitness, the herbivore 1 element of the second term in equation (6) will be nonzero.

On the other hand, a nonzero diffuse selection differential may be caused by nonadditivity in the effects of damages by the two herbivores on fitness, as is represented by the first term in equation (6). Consider a situation in which there are no interaction effects; thus, $\text{cov}(w, I1) = \text{cov}(w, I2) = 0$, and the second term in equation (6) is zero. Suppose that damage by herbivore 1 decreases plant fitness when herbivore 2 is present, but the same damage has no effect on plant fitness if herbivore 2 is absent. This type of effect might arise, for example, if herbivore 2 weakens a plant sufficiently that any additional damage by herbivore 1 reduces survival or seed production of the plant. Then in the absence of herbivore 2, $\text{cov}(w'1, S1)$ will be zero, whereas in the presence of herbivore 2, $\text{cov}(w, D1)$ (= $\text{cov}(w, S1)$) will be negative (i.e., the diffuse selection differential $C_1$ for herbivore 1 will be nonzero).
A nonzero diffuse selection differential arises in one additional way if there is a genetic covariance between susceptibilities when both herbivores are present (i.e., \( \text{cov}(D_1, D_2) \neq 0 \)): the genetic covariance means that selection exerted by one herbivore on its own resistance contributes to selection on variation for resistance to the other herbivore. This effect is manifested in the first term in equation (6), since even in the absence of interaction effects \( I_1 \) and \( I_2 \), removal of, say, herbivore 2 will eliminate the component of selection on resistance to herbivore 1 caused by herbivore 2 and hence cause a change in the selection differential.

Combining equations (4a) and (7), one obtains the selection gradient

\[
\mathbf{B} = \mathbf{G}^{-1} \mathbf{C} \\
= \mathbf{B}_s + \mathbf{B}_d,
\]

where

\[
\mathbf{B}_s = \frac{\det(G_s)}{\det(G)} G_s^{-1} C_s
\]

and

\[
\mathbf{B}_d = \frac{1}{\det(G)} \left[ \det(G_s) G_s^{-1} C_s + \det(G_1) G_1^{-1} (C_s + C_1) \right]. \tag{8}
\]

As is the case for \( \mathbf{G}^{-1} \), the first term of \( \mathbf{B} \), \( \mathbf{B}_s \), involves only \( w' \) and the variances of \( S_1 \) and \( S_2 \). Moreover, each element of the portion \( G_s^{-1} C_s \) represents the force of selection exerted by a particular herbivore on susceptibility to that herbivore in the absence of the other herbivore (i.e., the pattern of selection that would arise if resistances were uncorrelated and the presence of one herbivore had no effect on selection exerted by the other). We thus term this component of \( \mathbf{B} \) the "pairwise" component of the selection gradient. The second term, \( \mathbf{B}_d \), represents the "diffuse" component of the selection gradient—the portion of the overall selection gradient that is not explained by selection imposed by each herbivore when the other herbivore is absent. Note that while the diffuse component involves elements of the pairwise and diffuse components of \( \mathbf{G} \) and \( \mathbf{C} \) (i.e., \( G_s \) and \( C_s \), these elements occur in equation (8) only in products with \( G_1 \) and \( C_1 \). Consequently, the diffuse component of selection exists only if the diffuse components of \( \mathbf{G} \) and \( \mathbf{C} \) exist.

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS**

The preceding analysis leads naturally to an experimental design that can be used to distinguish pairwise from diffuse selection imposed by herbivores. In this section, we describe this experimental design and discuss briefly how data generated from it may be analyzed. We consider here the simplest system in which diffuse selection might occur: a single-plant population used by two species of herbivores.

The experimental design is a two-way factorial design equivalent to that com-
commonly used to detect genotype × environment interactions and correlations between the same character expressed in different environments (e.g., Fry 1992). One factor incorporates three treatments: (1) plants exposed to natural levels of both herbivores; (2) plants exposed to herbivore 1, herbivore 2 excluded; and (3) plants exposed to herbivore 2, herbivore 1 excluded.

The second factor is family, genotype, or a similar standard unit of quantitative genetics. The individuals in these units are produced by an appropriate breeding design from which additive genetic variation for damage and for fitness can be calculated using standard methods (e.g., Falconer 1989). In the experiment, some individuals of each family unit are subjected to treatment 1, other individuals to treatment 2, and yet other individuals to treatment 3. Treatment is considered a fixed effect, while family is considered a random effect for analysis. Ideally, the plants in the different treatments should be intermixed to avoid confounding treatment effects with effects due to spatial variation. When this is not possible, a split plot design may be used. Variables measured for each plant in the experiment are amounts of damage by each type of herbivore and fitness (or a surrogate).

Analysis of the data generated by this type of experiment proceeds in three steps. First, one tests for whether \( C_1 = 0 \) and whether \( G_1 = 0 \). If either or both of these quantities are nonzero, there is a diffuse component to selection, and, second, one proceeds to estimate \( B_1 \). Finally, even if both \( C_1 \) and \( G_1 \) are nonzero, it is still possible that their effects may cancel, yielding no significant diffuse component of the selection gradient, \( B_1 \); third, one therefore needs to test whether \( B_1 \) differs from zero. In the following paragraphs, we describe briefly how these analyses may be carried out. (We do not consider the details of the statistical technique we suggest, as they and their potential pitfalls are adequately described in the literature; see, e.g., Mitchell-Olsts and Shaw 1987; Shaw 1987.)

### TESTING WHETHER \( G_1 = 0 \) AND \( C_1 = 0 \)

Testing whether \( G_1 = 0 \) proceeds in two steps. First, because genetic variation for interaction effects \( I_1 \) and \( I_2 \) guarantees that \( G_1 \neq 0 \) (except in the highly unlikely situation in which \( \text{var}(I_1) = 2 \cdot \text{cov}(S_1, I_1) \) and \( \text{var}(I_2) = 2 \cdot \text{cov}(S_2, I_2) \); see eq. [4c]), the first step is to determine whether the interaction effects are genetically variable. To make such a determination, a two-way ANOVA involving just treatments 1 and 2 is performed. The treatment main effect indicates whether the presence of herbivore 2 has an overall effect on the amount of damage caused by herbivore 1, and thus indicates whether \( I_1 \), averaged over families, is nonzero. More importantly, the treatment × family interaction indicates whether the magnitude of \( I_1 \) varies among families and hence indicates whether there is genetic variation for the magnitude of this interaction effect. Damage by herbivore 2 is analyzed in a similar fashion using data from treatments 1 and 3.

If no additive genetic variation for interaction effects is detected, \( G_1 \) may still be nonzero if there is an additive genetic covariance between susceptibilities of the two herbivores (i.e., \( \text{cov}(S_1, S_2) = \text{cov}(D_1, D_2) \neq 0 \)). The most powerful way to determine whether this covariance differs from zero is to test whether the
family effect in an ANOVA involving treatments 2 and 3 is significant, using the family × treatment interaction mean square as the denominator in the F statistic (the SAS model; see Fry 1992). If no evidence for a nonzero covariance is obtained in this step, it is reasonably concluded that $G_1 = 0$.

To determine whether $C_1$ differs from zero, first note that, from equations (5) and (6), one obtains

$$C_1 = \begin{bmatrix} \text{cov}(w, D1) \\ \text{cov}(w, D2) \end{bmatrix} - \begin{bmatrix} \text{cov}(w1', D1') \\ \text{cov}(w2', D2') \end{bmatrix}.$$  \hspace{1cm} (9)

Consequently, testing for whether $C_1$ differs from zero reduces to testing the equality of two pairs of genetic covariances (i.e., testing whether $\text{cov}(w, D1) = \text{cov}(w1', D1')$ and whether $\text{cov}(w, D2) = \text{cov}(w2', D2')$). Maximum-likelihood techniques (e.g., Shaw 1987) are appropriate for such tests. In testing the first equality, for example, maximum-likelihood estimates of $\text{cov}(w, D1)$ and $\text{cov}(w1', D1')$ can be estimated from the data of treatments 1 and 2, respectively. The joint likelihood of these estimates can then be compared to the maximum likelihood under the constraint that the two covariances are equal by applying the standard likelihood ratio test (Edwards 1992). The second equality can be tested similarly using data from treatments 1 and 3. A finding of significance for either test would imply that $C_1$ is not zero.

If $C_1$ is not zero, its cause may be partly revealed by performing a standard two-way ANOVA for each herbivore (e.g., for herbivore 1, a two-way ANOVA with damage by herbivore as the dependent variable is performed on just treatments 1 and 2). As noted earlier, a significant treatment × family interaction indicates the presence of genetic variation for the magnitude of interaction effects. Because such genetic variation is a necessary condition for $\text{cov}(w, I1)$ and $\text{cov}(w, I2)$ to be nonzero, absence of a treatment × family interaction effect in the ANOVA would suggest that the diffuse component of the selection differential is not due to the presence of one herbivore affecting the amount of damage caused by the other (i.e., the right-hand term in eq. [6] would be 0). By contrast, absence of significant family main effects, coupled with a significant treatment × family interaction, in this type of analysis would suggest the absence of genetic variation for $S1$, that is, that all genetic variation for resistance to herbivore 1 is due to genetic variation for interaction effects. Such a result would indicate that a nonzero $C_1$ could not be due to the presence of one herbivore affecting the impact of a given amount of damage by the other herbivore on fitness (i.e., the left-hand term of eq. [6] would be 0).

**ESTIMATING $B_s$ AND $B_1$**

From equation (8) it can be seen that $B_s$ is a function of $C_s$ and $G_s$, whereas $B_1$ is a function of these two variables plus $C_1$ and $G_1$. Consequently, $B_s$ and $B_1$ can be estimated if the $C$'s and $G$'s can be calculated. Equations (5) and (9) indicate that the elements of $C_s$ and $C_1$ can be estimated from the additive genetic covariances between fitness and damages within the various treatments (e.g., the first
element of $C$, $\text{cov}(w'1', D'1')$, is the additive genetic covariance between fitness and damage by herbivore 1 in treatment 2. In this section we show that the pairwise and diffuse components of the additive genetic variance-covariance matrix for damage, $\mathbf{G}$, and of the selection gradient, $\mathbf{B}$, can also be calculated from measurements of just damage and fitness. In particular, measurement of the interaction effects ($I1$ and $I2$) and their variances and covariances are not required.

Combining equations (2), (3), (4b), and (4c) and recognizing that $\text{var}(D1') = \text{var}(S1')$, $\text{var}(D2') = \text{var}(S2')$, and so forth, one finds that the pairwise and diffuse components of $\mathbf{G}$ can be estimated from the data by

$$
\mathbf{G}_s = \begin{bmatrix}
\text{var}(D1') & 0 \\
0 & \text{var}(D2')
\end{bmatrix}
$$

and

$$
\mathbf{G}_1 = \begin{bmatrix}
\text{var}(D1) & \text{cov}(D1, D2) \\
\text{cov}(D1, D2) & \text{var}(D2)
\end{bmatrix} - \begin{bmatrix}
\text{var}(D1') & 0 \\
0 & \text{var}(D2')
\end{bmatrix} = \mathbf{G} - \mathbf{G}_s.
$$

These equations indicate that the elements of these matrices are simple functions of either the additive genetic variances of the damages in the three treatments or the additive genetic covariances between damages caused by the two herbivores. The relevant covariance is that between damages when both herbivores are present, that is, the covariance between damages by the two herbivores in treatment 1. Standard univariate and multivariate ANOVA techniques (Falconer 1989; Fry 1992) or maximum-likelihood approaches (Shaw 1987) may be used to estimate these variances and covariances, and the resulting matrices may used to calculate $\mathbf{B}_s$ and $\mathbf{B}_1$ as shown in equation (8).

**TESTING WHETHER $\mathbf{B}_1$ DIFFERS FROM ZERO**

As noted, if both $\mathbf{C}_1$ and $\mathbf{G}_1$ differ from 0, it is possible that their effects may cancel, yielding no significant diffuse component of the selection gradient, $\mathbf{B}_1$. It may therefore be desirable to test explicitly whether $\mathbf{B}_1$ differs from zero, especially if the estimate of $\mathbf{B}_1$ (derived as described in the previous section) is close to zero. Because $\mathbf{B}_1$ is a complex function of genetic variances and covariances from several treatments and its sampling distribution is unknown, deriving a parametric test of whether an estimate of this parameter differs from zero is problematic. We therefore recommend applying a resampling scheme such as bootstrapping or jackknifing (Efron 1982) to the data to calculate a standard error and confidence interval for the estimated $\mathbf{B}_1$. Failure of the confidence interval to overlap zero would constitute reasonably strong evidence that a diffuse component to the selection gradient exists.

**DISCUSSION**

In a plant-herbivore system consisting of one plant and two or more species of herbivores or pathogens, pairwise coevolution occurs when selection imposed
reciprocally by the plant and each herbivore and the response to that selection are not influenced by the presence or absence of the other herbivore species (Gould 1988). As mentioned earlier, three conditions for coevolution to be pairwise have been described (Hougen-Eitzman and Rausher 1994). First, susceptibilities to different herbivores are genetically uncorrelated. Failure of this condition would cause selection imposed by one herbivore to alter the level of resistance to other herbivores as a correlated response (Lande 1979). The evolutionary trajectory of resistance to some herbivores would thus depend on whether others were present. Genetic correlations between resistances to different herbivores are common, but not universal, in plant species that have been examined (Rausher and Simms 1989; Fritz 1992; Rausher 1992b). Second, the presence or absence of one herbivore does not affect the amount of damage caused by other herbivores. In terms of the framework presented here, this condition is analogous to there being no genetic variation for interaction effects. Third, the reduction in plant fitness caused by a given amount of damage by one herbivore is not influenced by the amount of damage caused by other herbivores. A model demonstrating that violation of this condition can render selection diffuse has been presented earlier (Hougen-Eitzman and Rausher 1994). Although we cannot suggest a precise physiological mechanism that would cause this condition to be violated, we note that, in general, combinations of stresses on plants often do not have additive effects on fitness (Harper and McNaughton 1962; Lee and Bazzaz 1980; Fowler and Rausher 1985). Moreover, Strauss (1991) demonstrated nonadditivity of the effects of herbivore damage on fitness in a natural plant-herbivore system.

The experimental design and statistical analysis described here permit a determination of whether these conditions are satisfied and thus whether selection imposed by natural enemies and the consequent evolutionary response is pairwise or diffuse. This analysis consists of three steps. First, test whether \( \mathbf{C}_1 = 0 \) and \( \mathbf{G}_1 = 0 \) (i.e., whether the interaction components of the selection differential and genetic variance-covariance matrix exist). Second, test whether the genetic covariance between susceptibilities to the two herbivores differs from zero when both herbivores are present. Finally, estimate the pairwise and diffuse components of the selection gradient, \( \mathbf{B} \). Interpretation of the results of these tests depends on whether there is genetic covariance between susceptibilities. When the covariance is zero, then there are no genetic constraints that would cause a diffuse response to selection. This is the situation that motivated the development of the methods described here, since in this situation the genetic architecture of resistance does not rule out pairwise coevolution, and it is thus desirable to determine whether the pattern of selection is pairwise or diffuse. To determine whether selection is indeed pairwise, however, requires determining whether criteria 2 and 3 are satisfied. Detection of a significant \( C_1 \) implies that either or both of criteria 2 and 3 are not met and that selection is diffuse. As already described, in some cases it may be possible to determine which of these two criteria has failed. If it can be shown that there is no detectable genetic variation for interaction effects (criterion 2 is satisfied, which implies that criterion 3 has failed) or that there is no detectable genetic variation for damage by the herbivores when they exist alone (criterion 3 is satisfied, which implies criterion 2 has failed). In
cases in which there is genetic variation for both interaction effects and damage in the single-harbivore treatments, further experiments will be necessary to determine which criteria are satisfied. For example, to determine whether the effects of damage on fitness are nonadditive, one may experimentally manipulate the amount of damage caused by one herbivore in both the presence and absence of the other herbivores (e.g., Strauss 1991; Hougen-Eitzman and Rausher 1994). Once it has been established that there is a significant interaction component to C, the relative magnitude of \( B_2 \) and \( B_1 \) can be calculated to determine the relative importance of pairwise and diffuse selection on resistance.

If there is genetic covariance between susceptibilities to the two herbivores when both are present, coevolution is automatically diffuse because genetic constraints result in a diffuse response to selection. Even in this case, however, it may be of interest to determine whether the pattern of selection is also diffuse. Unfortunately, interpretation in this case is complicated by the fact that selection on susceptibility to one herbivore includes a component caused by the other herbivore because of the genetic covariance between susceptibilities. Consequently, removal of, say, herbivore 2 will cause a change in the pattern of selection on inherent susceptibility to herbivore 1, giving rise to an interaction component of C (i.e., \( C_1 \neq 0 \)). This effect arises even though conditions 2 and 3 may be satisfied. Consequently, under these conditions, detection of a significant \( C_1 \) does not necessarily imply that these conditions are violated, and further experiments will be needed to test these condition. Nevertheless, this effect does indicate that the pattern of selection is diffuse, in the sense that the element of the selection gradient corresponding to resistance to one herbivore contains a component caused by selection exerted by the other herbivore. The overall degree of diffuseness, due to this correlated selection and violation of conditions 2 and 3, may be quantified as in the case in which susceptibilities are uncorrelated.

To obtain an intuitive feeling for how interaction effects could lead to a nonzero diffuse selection gradient, consider the following hypothetical example. In this example, a population of a \emph{Brassica} (mustard) species is fed on by the larvae of two species of lepidoptera, one a generalist and the other a specialist. Suppose that this plant population is genetically variable for glucosinolate production and that, although the specialist is not affected by glucosinolate, the level of damage by the generalist decreases as glucosinolate content increases because females are less likely to oviposit on plants with high levels of glucosinolates (e.g., Feeny 1975; Blau et al. 1978). Finally, suppose that feeding damage caused by the specialist, but not that caused by the generalist, releases a volatile chemical that attracts a predator that feeds on both types of larvae (e.g., Dicke and Sabelis 1988) and that the predator limits the amount of damage to an individual plant caused by the generalist. This type of situation could then produce a pattern of damages like that portrayed schematically in table 1, where for simplicity we assume there are only three genotypes of plants, which arise from variation at a single diallelic locus: high-, medium-, and low-glucosinolate genotypes. In the presence of the specialist herbivore, predators will be attracted to all plants, regardless of glucosinolate genotype, and hence damage by the generalist will be limited to the same amount. There will thus be no genetic variation for damage
TABLE 1
A HYPOTHETICAL EXAMPLE OF DIFFUSE HERBIVORY

<table>
<thead>
<tr>
<th>Plant Genotypes*</th>
<th>AA</th>
<th>Aa</th>
<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean damage by generalist:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>When specialist is absent ($D'$)</td>
<td>100</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>When specialist is present ($D$)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Inherent susceptibility ($S$)</td>
<td>100</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>Interaction effect ($I$)</td>
<td>-50</td>
<td>-25</td>
<td>0</td>
</tr>
<tr>
<td>Relative fitness:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>When specialist is absent ($w'$)</td>
<td>.5</td>
<td>.75</td>
<td>1.0</td>
</tr>
<tr>
<td>When specialist is present ($w$)</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
</tr>
</tbody>
</table>

Note.—Three genotypes of *Brassica* are attacked by a specialist and a generalist herbivore. Relative fitness is assumed to be determined by the relationship $w = 1.5 - 0.01D - 0.5H$, where $H = 1$ when the specialist is present and $H = 0$ when absent.

* AA, Aa, and aa indicate low-, medium-, and high-glucosinolate genotypes, respectively.

carried by the generalist, which also means that there will be no additive genetic covariance between susceptibilities to the two herbivores. By contrast, in the absence of the specialist species, predators will not be attracted to the host plants (no volatiles will be released), and the genetic variation for glucosinolate levels will in turn cause genetic variation for damage. If fitness is inversely related to damage, there will also be genetic variation for fitness.

Because there is no genetic variation for damage caused by the specialist, the only relevant selection is that on variation for damage by the generalist, which is due to genetic variation in glucosinolate levels. Therefore, $G$, $C$, and $B$ are $1 \times 1$ matrices or vectors. The values of these matrices and vectors are portrayed in table 2, under the assumptions that the frequencies of high-, medium-, and low-glucosinolate genotypes are 1:2:1. Relative fitness within a “treatment” (specialist present or specialist absent) is assumed to decline linearly with the amount of damage caused by the generalist.

In this admittedly contrived example, there is clearly genetic variation for interaction effects. The value for $I$ is 0 for the high-glucosinolate genotype and $-50$ for the low-glucosinolate genotype. There is also selection for increased resistance to the generalist (increased glucosinolate levels) when the specialist is absent, as indicated by the negative value for $B_s$. This pairwise component of selection arises essentially because damage reduces fitness. By contrast, when the specialist is present, there is no direct selection on glucosinolate level, as indicated by $B = 0$. This is because there is an additional diffuse component of selection, $B_I$, that is positive and equal in magnitude to $B_s$. Negative selection, corresponding to $B_s$, occurs at the stage of oviposition, since low-glucosinolate plants have more eggs deposited on them. At a later stage, however, positive selection, corresponding to $B_I$, occurs because plants with lower levels of glucosinolates have proportionately more larvae removed by predators. The reduction
in damage, and hence incremental increase in fitness caused indirectly by the specialist's presence, is thus greater for low-glucosinolate plants. This effect counteracts the earlier disadvantage to low-glucosinolate plants at the oviposition stage and results in no net selection on glucosinolate level. This example not only illustrates how interaction effects can produce a diffuse component of selection that counteracts the pairwise component but also demonstrates that when interaction effects occur, pairwise selection imposed by one herbivore may be masked by those effects.

The experimental design and analysis presented here have limitations that should be explicitly recognized. One limitation is that it measures only current selection pressures acting on extant genetic variation for resistance. In particular, the amount of damage, and presumably therefore the intensity of selection, imposed by a population of herbivores will often depend on the population density of those herbivores relative to that of the host plant. If, as resistance evolves, the population densities of the herbivores change in response, the pairwise and diffuse components of the selection gradient may also change. It is thus inadvisable to use selection gradients described here in making long-term predictions about evolutionary change. Ideally, the robustness of conclusions about the existence of diffuse selection should be evaluated by measuring the selection gradient at different combinations of herbivore densities, but this will often not be practical.

A second limitation is that the technique requires selective elimination of some herbivores. Although this is doubtless unfeasible in many systems, the development of specifically targeted insecticides is likely to reduce this difficulty in the future.

Whether multispecies plant-herbivore (pathogen) coevolution can be understood by examining just the pairwise interactions involved is a question that has intrigued evolutionary biologists for over a decade (Futuyma and Slatkin 1983),
and more researchers have become aware of the potentially complex interactions among herbivores and/or pathogens (Marquis 1990; Simms 1990; Linhart 1991; Marquis and Alexander 1992). While some authors have expressed opinions regarding the relative commonness of pairwise and diffuse coevolution (see Futuyma and Slatkin 1983), few empirical data exist to substantiate or refute these opinions. Despite the limitations described earlier, we believe that the approach presented here can prove useful in detecting and estimating the magnitude of diffuse selection in a variety of plant-herbivore and plant-pathogen interactions and thus in determining the roles of diffuse and pairwise coevolution in molding plant-herbivore interactions.

ACKNOWLEDGMENTS

We thank J. Antonovics, F. Nijhout, D. Roach, F. Gould, and two anonymous reviewers for helpful comments on this or earlier versions of the manuscript. This work was partly supported by National Science Foundation grants BSR 8817919 and DEB 9318919 to M.D.R.

APPENDIX A

THE CASE OF MORE THAN TWO HERBIVORES

In this appendix, we show how the approach described in the text may be generalized to systems composed of a host plant species and more than two herbivores. In general, with more than two herbivores, G and C cannot be simply partitioned, as in equations (4a) and (7), into components, one of which involves only the variances of the S_{xy} (where S_{xy} here is the expected amount of damage to genotype y caused by herbivore x in the absence of all other herbivores). Nevertheless, it is possible, using the approach described in the text, to address two questions: Is selection imposed by a particular herbivore pairwise or diffuse with respect to any subset of the other herbivores? Is selection imposed by a particular subset k of herbivores pairwise or diffuse with respect to any subset of the remaining herbivores? (Note that the first question is actually a special case of the second in which the subset k consists of just one herbivore species.)

To address the second question, let D(k)_{ij} be the amount of damage to the jth plant of the ith genotype caused by subset k of the herbivores in the presence of all other herbivores, and let D(k)_{ij} be the damage caused by subset k of the herbivores in the absence of subset l but in the presence of the remaining herbivores. Let R(k)_{i} and S(k)_{i} be the additive genetic values for genotype i of the amount of damage caused by subset k in the presence and absence, respectively, of subset l. These values reflect the susceptibility of genotype i to herbivores of subset k with and without subset l present. Consequently, the difference

\[ I(k)_{i} = R(k)_{i} - S(k)_{i} \]

represents the change in susceptibility to subset k due to the presence of subset l. Then

\[ D(k)_{ij} = D(k)_{ij} + I(k)_{i} + \epsilon_{ij}, \]

where \( \epsilon_{ij} \) represents the nonadditive and environmental components of variation in damage. Similarly,

\[ D(k)_{ij} = D(l)_{ij} + I(l)_{i} + \epsilon_{ij}. \]

The last two equations correspond to equation (3) for the case of two herbivores. Experi-
mental analysis precedes as described in the text, except that the three treatments are (1) control (no removal), (2) removal of herbivores of subset \( k \), and (3) removal of herbivores of subset \( l \).

For a system with a small number of herbivore species, additional treatments may permit the decomposition of the effect of subset \( l \) on subset \( k \) into effects of component species of subset \( l \). For example, consider a host plant with three herbivore species, where \( k = \{ \text{species 1} \} \) and \( l = \{ \text{species 2, species 3} \} \). The analysis described previously would permit one to determine whether the joint presence of species 2 and 3 influenced selection exerted by species 1, that is, whether selection due to species 1 is pairwise or diffuse with respect to species 2 and 3 together. If the analysis indicated that selection was diffuse, one might want to determine whether species 2 and/or 3 individually, or the two jointly, contributed to the diffuse selection. This could be determined in principle by adding treatments corresponding to \( l = \{ \text{species 2} \} \) and \( l = \{ \text{species 3} \} \) to the experiment, then applying the analysis separately to \( k = \{ \text{species 1} \} \) and each of these \( l \) subsets. If such an analysis indicated that selection remained diffuse when \( k = \{ \text{species 1} \} \) and \( l = \{ \text{species 2} \} \) but was pairwise for \( k = \{ \text{species 1} \} \) and \( l = \{ \text{species 3} \} \), it would be appropriate to conclude that selection by species 1 was diffuse with respect to species 2 but pairwise with respect to species 3. This type of partitioning of effects will obviously be feasible only when the number of species in subset \( l \) is small, since the number of treatments required for a complete partitioning will increase geometrically with the number of species in that subset.

APPENDIX B

PHENOTYPIC APPROACH TO PARTITIONING SELECTION GRADIENT

In this appendix, we describe how to partition the phenotypic selection gradient into diffuse and pairwise components and how these components may be estimated. One approach to such partitioning is to follow that described in the text, substituting \( \mathbf{P} \) for \( \mathbf{G} \). A simpler approach, however, is possible. Using definitions given in the text, the selection gradient on damages inflicted by two herbivores when both herbivores are present is given by the standard formula \( \mathbf{\beta} = \mathbf{P}^{-1} \mathbf{s} \):

\[
\mathbf{\beta} = \begin{bmatrix}
\text{var}(D1) & \text{cov}(D1, D2) \\
\text{cov}(D1, D2) & \text{var}(D2)
\end{bmatrix}^{-1}
\begin{bmatrix}
\text{cov}(w, D1) \\
\text{cov}(w, D2)
\end{bmatrix},
\]

where the variances and covariances are understood to be phenotypic variances and covariances.

To obtain the pairwise component, \( \mathbf{\beta}_p \), of \( \mathbf{\beta} \), we observe that the pairwise component reflects the pattern of selection on each herbivore that occurs in the absence of the other herbivore. This means that the elements of \( \mathbf{\beta}_p \) are given by

\[
\mathbf{\beta}_p = \begin{bmatrix}
\text{cov}(w_1', D1') \\
\text{var}(D1') \\
\text{cov}(w_2', D2') \\
\text{var}(D2')
\end{bmatrix}.
\]

The diffuse component of the selection gradient, \( \mathbf{\beta}_d \), is then simply the difference between the overall gradient and the pairwise component:

\[
\mathbf{\beta}_d = \mathbf{\beta} - \mathbf{\beta}_p.
\]  

(B1)

Experimentally, \( \mathbf{\beta} \) and its pairwise and diffuse components can be estimated using the same experimental design as described for the genetic analysis in the text. The overall selection gradient is estimated by the coefficients of a multiple regression of relative fitness on damage by each herbivore in the treatment in which both herbivores are present (treat-
ment 1). The element of $\beta$, corresponding to herbivore 1 is estimated by the coefficient of a regression of relative fitness on damage by herbivore 1 in the treatment in which herbivore 2 is absent (treatment 2), while the element of $\beta$, corresponding to herbivore 2 is estimated by the coefficient of a regression of relative fitness on damage by herbivore 2 in the treatment in which herbivore 1 is absent (treatment 3). The diffuse component of selection is then determined from equation (B1).

Statistical assessment of whether a diffuse component of selection is present can be performed by performing a multiple regression of relative fitness on damage in which data for all treatments are used. In such an analysis, a dummy variable for treatment is included, and the significance of the treatment by slope interaction term for damage by a particular herbivore indicates whether the selection gradient for susceptibility to that herbivore (equal to the regression coefficient for that herbivore) differs significantly between the treatment with and without the other herbivore.

This type of analysis can also be used in the context of the genetic approach to provide an approximate test of whether there is a diffuse component to the genetic selection gradient, $\beta$. In particular, a similar analysis can be performed on family means rather than individual phenotypic values to determine whether the slope of the relationship between relative fitness and damage differs among treatments. This approach is only an approximation, however, since the estimated regression coefficients contain components because of nonadditive genetic variance and environmental variance. The tests described in the text are ideally performed using sire or similar components of variance and thus are based primarily on additive components of variance and covariance. They therefore constitute a more exact test for the presence of a diffuse component to the genetic selection gradient. Moreover, the tests described in the text permit a determination of the relative contributions of diffuse components of the genetic variance-covariance matrix and of the selection differential to the diffuse component of the selection gradient.

LITERATURE CITED


*Guest Editor: Arthur E. Weis*