CONTRaints on CHEMICAL COEVOLUTION: WILD PARSNIPS AND THE PARSNIP WEBWORM

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Abstract. — The parsnip webworm (Depressaria pastinacella) and the wild parsnip (Pastinaca sativa) together represent a potentially “coevolved” system in that throughout their ranges the plant has relatively few other herbivores and the insect has virtually no other hosts. Individual wild parsnip plants within a central Illinois population vary in their content and composition of furanocoumarins, secondary compounds with insecticidal properties. Half-sib and parent-offspring regression estimates of the heritability of furanocoumarins demonstrate that this variation is genetically based. Wild parsnip plants also vary in their resistance to damage by the parsnip webworm, which feeds on flowers and developing seeds. In an experimental garden, seed production in the primary umbel ranged from 0 to 1,664 seeds among individuals, and mean seed production of half-sib families ranged from 3.7 seeds to 446.0 seeds. Approximately 75% of the variation in resistance among half-sib families to D. pastinacella was attributable to four furanocoumarin characteristics—resistance is positively related to the proportion of bergapten and the amount of siphonandin in seeds, and negatively related to the amount of bergapten and the proportion of siphonandin in leaves. Each of the four resistance factors had significant heritability. Thus, resistance in wild parsnip to the parsnip webworm is to a large extent chemically based and genetically controlled. Genetic correlations among fitness and resistance characters, however, tend to limit coevolutionary responses between herbivore and plant. In greenhouse plants protected from herbivory, several of the resistance factors have negative genetic correlations with potential seed production. Ossibly, highly resistant plants in the absence of herbivory would be at a competitive disadvantage in the field. The selective impact of the herbivore is also limited in this population by a negative genetic correlation among resistance factors. Selection to increase one resistance factor (e.g., the proportion of bergapten in the seed) would at the same time decrease the amount of a second resistance factor (e.g., the amount of siphonandin in the seed). The wild parsnip and the parsnip webworm, then, appear to have reached an evolutionary “stalemate” in the coevolutionary arms race.

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Though it has long been suspected that intraspecific variation in plant secondary chemistry is associated with variation in susceptibility to insect herbivory in plant populations, the nature of the association is by no means well-defined. While several studies have revealed a correlative association (Dolinger et al., 1973; Edmunds and Alstad, 1978; Chew and Rodman, 1979 [and references therein]; Sturgeon, 1979), it is impossible to determine whether observed differences in insect herbivory are the result of variation in secondary chemistry or the cause of that variation. For example, phenotypically induced alteration in secondary chemistry can occur as the result of insect attack (Green and Ryan, 1972; Rhoades, 1979; Carroll and Hoffman, 1980; Haukoja, 1980; but see Fowler and Lawton, 1985). Virtually all arguments about the evolution of resistance by defensive chemistry presume that variation in secondary chemistry, whether induced or constitutive, is at least partly genetic in origin. While natural selection assuredly acts on phenotypes, change in frequencies of chemical morphs within a population over time, i.e., the type of change implicit in insect-plant coevolution (Janzen, 1980), can occur only if there is either a genetic basis for chemical variation or a genetic basis for differences in the expression or inducibility of secondary substances.

Insect-plant coevolution, as defined by Janzen (1980), also implies that herbivorous insects are capable of exerting sufficient selective pressure to change the frequency of genotypes in a plant population. There is ample evidence to support the contention that insects can reduce growth, reproductive fitness, and survivorship in plants (e.g., Morrow and LaMarche, 1978; Rauscher and Feeny, 1980), and there is also evidence that susceptibility to insect attack is genetically based (Moran, 1981; Lin et al., 1984; Marquis, 1984; Service, 1984). However, there is little or no information on the selective influence of insects on the distribution of chemical phenotypes within a population.
A correlation between plant chemistry and resistance may result not from coevolution sensu stricto but from adaptation by the plant to some other environmental variable and subsequent adjustment on the part of the insect (Jermy, 1984).

For insect-plant coevolutionary arguments to gain validity with respect to secondary plant metabolism, it is essential to demonstrate the genetic variation in both plant secondary metabolism and resistance to herbivory. To examine this question, we selected for study *Pastinaca sativa*, the wild parsnip (Umbelliferae), and *Depressaria pastinacella*, the parsnip webworm (Lepidoptera: Oecophoridae). *P. sativa*, a biennial introduced from Europe and extensively naturalized throughout eastern North America, occurs in great numbers in waste places, open fields, and along railroads (Fernald, 1950; Thompson, 1977). Like many apioid Umbelliferae, *P. sativa* produces linear and angular furanocoumarins (Berenbaum et al., 1984) (Fig. 1). A previous study of variation in furanocoumarin content in a population of *P. sativa* located on Perkins Road approximately 6 km northeast of the University of Illinois at Urbana-Champaign in Champaign County, Illinois, revealed significant differences among individual plants in the quantity and composition of furanocoumarins in the seeds of the primary umbels. The furanocoumarin content of seeds varied from 17.0 to 60.1 μg/seed, and the relative proportions of individual furanocoumarins varied from 2-fold to over 20-fold (Berenbaum et al., 1984).

Furanocoumarins have been shown to be resistance factors against insects. Linear furanocoumarins are toxic or repellent to generalized herbivores (Yajima et al., 1977; Berenbaum, 1978; Muckensturm et al., 1981), yet appear to enhance growth in *Papilio polyxenes* (Lepidoptera: Papilionidae), a specialist on Umbelliferae (Berenbaum, 1981a). Angular furanocoumarins, in contrast, markedly reduce fecundity in *P. polyxenes* (Berenbaum and Feeny, 1981). The furanocoumarins are thus likely candidates for chemical resistance factors even against a specialist such as *D. pastinacella*.

Throughout most of its range, *P. sativa* is attacked heavily by *D. pastinacella* (Hodges, 1974; Thompson, 1978; Hendrix, 1979; Berenbaum, 1981b; Gorder and Mertins, 1984). The caterpillar webs together umbels and feeds on developing flowers and seeds. After it completes larval development, it leaves the umbels to pupate in parsnip stems. Adults emerge within a month and overwinter under bark, in litter or in human habitations (Gorder and Mertins, 1984; pers. observ.). Although it has been reported to occur on *Angelica* and *Heracleum*, also members of the Umbelliferae (Hodges, 1974), *D. pastinacella* is effectively restricted to wild parsnip throughout Champaign County because it is the only host locally abundant (Thompson, 1978). In central Illinois, *D. pastinacella* is the most abundant herbivore in terms of biomass (Thompson, 1977) and can reach infestation levels of 100% of plants in a population (pers. observ.).

*Pastinaca sativa* and *Depressaria pastinacella*, then, together potentially represent a typical “coevolved” association (sensu Ehrlich and Raven, 1964). The plant has few other insect enemies, the majority of which are restricted to parsnip and related plants (Berenbaum, 1981b), and the insect has few other hosts, all of which are closely related and chemically similar to parsnip (Heywood, 1971). The population on Perkins Road is known to have harbored large populations of *D. pastinacella* for several consecutive years. We therefore examined this population in order to address the following questions:

1) How much of the variation in furanocoumarin composition and content in *P. sativa* is genetic in origin?
2) To what degree is resistance to attack by
*D. pastinacella* genetically based and deter-
mined by the chemistry of the indi-
vidual plant?

3) What is the magnitude of the selective
force exerted by the herbivore among
plant individuals? That is, can *D. pas-
tinacella* exert sufficient selection pres-
sure to effect changes in the genetic com-
position of the plant population?

4) If chemical resistance traits are geneti-
cally based, what maintains chemical
variability? In other words, are traits
conferring resistance to *D. pastinacella*
associated with a fitness reduction when
the herbivore is not present?

**MATERIALS AND METHODS**

*Estimation of Genetic Control of
Furanoecoumarin Production*

One widely accepted measure of the ex-
tent to which a trait is under genetic control
is its heritability (Falconer, 1981). The her-
itzability in the narrow sense is the propor-
tion of the phenotypic variance in a trait
that can be attributed to the additive genetic
variance. The heritability is important ev-
olutionarily because the response to selec-
tion is a function of additive genetic vari-
ance (Falconer, 1981). Covariances between
maternal half-sibs and between parent and
offspring were used to estimate heritabilities
of both quantity and composition of furano-
ocoumarin production (Falconer, 1981). As
is the case with many umbellifers (Cruden
and Hermann-Parker, 1977 and references
therein), individual flowers within an umbel
are protandrous; since umbels develop se-
quently from primary to quaternary
(Hendrix and Trapp, 1981), the plant is
temporally dioecious and outbreeding in the
primary umbel is ensured. Moreover, the
unspecialized nature of parsnip pollinators
(Bell, 1971) enhances the probability that
pollen from many sources is involved in
fertilization. All of these characteristics to-
together tend to maximize the proportion of
half-sibs in the primary umbel. For this rea-
son seeds from the primary umbel were used
to estimate genetic parameters. All chemical
analyses were restricted to seeds of primary
umbels and to leaves.

A section of the Perkins Road population
was marked off, and the primary umbels of
all individuals (*N* = 65) were removed and
placed in individual petri dishes. The dishes
were mixed, and the first twenty dishes with
sufficient numbers of seed were chosen for
study. This sampling technique may have
eliminated from the study those plants most
susceptible to insect attack, i.e., those with
too few seed for analysis due to herbivory;
however, accurate estimates of quantita-
tive-genetic parameters require large sam-
ple sizes. Ten individuals from each of these
twenty families were chosen at random and
planted in spring 1983 in an experimental
garden in Phillips Tract, a university-owned
natural area approximately 4 km east of the
Perkins Road population. Individuals were
planted in tilled soil arranged in a com-
pletely randomized design and spaced at 30-
cm intervals. The plot was weeded as ne-
necessary throughout the growing season. Seeds
and leaf samples taken from plants flow-
ering in 1984 in this garden were evaluated
for furanoecoumarin composition and con-
tent. Furanoecoumarins were analyzed by
high-pressure liquid chromatography as de-
scribed in Berenbaum et al. (1984), with the
exception that diethyl ether was used as the
initial extraction solvent. Estimation of
variance components was by standard anal-
ysis of variance of a random model (Fal-
coner, 1981; Model II of Sokal and Rohlf,
1981); observed mean squares were set equal
to their expectations.

Heritability based on half-sib families was
calculated as

\[ h^2 = \frac{4V_F}{V_T} \]

where \( V_F \), the between-family variance (co-
variance between maternal half-sibs), is \( \frac{1}{4} \)
the additive genetic variance and \( V_T \) is
the total phenotypic variance. Since some
individuals died before setting seed, the
family size, \( k \), used to estimate variance
components was calculated as

\[ k = \frac{N^2 - \sum n_i^2}{N(f - 1)} \]

where \( N \) is the total number of plants, \( f \) is
the number of families and \( n_i \) is the number
of individuals in the \( i \)th family. The pos-
sibility that the families may contain some
portion of full-sibs cannot be ruled out,
and therefore the calculated heritabilites
should be considered maximum estimates. We also assume random mating and no in-breeding in the population (but see Mitchell-Olson and Rutledge, 1986). The sampling distributions of heritabilities are unknown (Tallis, 1959; Kendall and Stuart, 1963); standard errors, therefore, were estimated by the jackknife resampling procedure as shown in Efron (1982) but with the variance stabilizing transformation suggested by Arvesen and Schmitz (1970). At each iteration a family is omitted from the analysis of variance and a new heritability is calculated. Pseudovalues, \( \theta_i \), are then calculated as

\[
\theta_i = f h^2 - (f - 1) h^2_{-i},
\]

where \( f \) is the number of families, \( h^2 \) is the heritability calculated with all 20 families, and \( h^2_{-i} \) is the heritability calculated with the \( i \)th family deleted. The average of the pseudovalues yields an estimator that is nearly unbiased and has a standard error of

\[
\text{SE}_\theta = \sqrt{\frac{\sum (\theta - \bar{\theta})^2}{f(f - 1)}}
\]

where \( \theta \) is the average of pseudovalues.

Since seeds borne on the same primary umbel share a common maternal environment, covariances due to additive genetic maternal effects may lead to greater than expected similarities among sibs. Therefore, these effects were isolated and identified. In *Pastinaca sativa*, furanocoumarins are localized in the testa (Ladygina et al., 1970) and thus represent the maternal genotype. For each family, 10 seeds were soaked in distilled water for 24 hours, stripped of their coats, potted, and arranged randomly in a greenhouse on the UIUC campus; germination is not affected by seed coat removal (pers. observ.). The potting soil consisted of a 2:4:2:2 mixture of sand, Drummer silt loam, peat, and perlite. Seed coats were analyzed for furanocoumarins. Plants were left in the greenhouse until minimum flowering size was obtained (approximately four months [Baskin and Baskin, 1979]), then placed outdoors in a sheltered area for three months to satisfy the obligatory preflowering chilling requirement (Baskin and Baskin, 1979), and returned to the greenhouse to flower and set seed. When plants arising from the stripped seeds themselves set seed, they were analyzed for furanocoumarin content of seed coat. This procedure provided data to estimate additive-genetic maternal effects on furanocoumarin production in the offspring by setting the covariances equal to their genetic expectations and solving for the variance components. Parent-offspring and between-family variance have expected genetic composition as follows:

\[
\text{Cov}_{PO} = \frac{1}{2} \sigma_A^2 + \frac{1}{2} \sigma_{Am}^2
\]

\[
\sigma_F^2 = \frac{1}{4} \sigma_A^2 + \sigma_{Am}^2
\]

where \( \sigma_A^2 \) is the additive genetic component and \( \sigma_{Am}^2 \) is the additive genetic maternal effect. These equations ignore variance due to nonadditive effects (Dickerson, 1969) and assume that additive-by-additive maternal effects are negligible. With two equations and two unknowns \((\sigma_A^2, \sigma_{Am}^2)\) we can solve for the unknowns as follows:

\[
\sigma_{Am}^2 = \frac{2(2\sigma_F^2 - \text{Cov}_{PO})}{3}
\]

\[
\sigma_A^2 = \frac{4(2\text{Cov}_{PO} - \sigma_F^2)}{3}
\]

The parent-offspring regression data were also used to provide a second estimate of heritability in furanocoumarin production. The formulae for calculating heritabilities from half-sib offspring on single parent regressions were those shown in Falconer (1981).

**Determination of Resistance Factors and of Genetic Bases for Resistance**

Half-sib families in the Phillips Tract garden were exposed to natural levels of herbivory and were censused regularly throughout 1984 for the presence of *D. pastinacella* larvae and other herbivores. Leaves of overwintering rosettes were inspected for eggs throughout May 1984, and leaf samples were collected for chemical analysis. After larvae vacated the umbels to pupate and seeds had ripened, the entire primary umbel was collected for chemical analysis and counting. Although *P. sativa* has some ability to compensate for floral loss in the primary umbel by increasing seed set in higher-order umbels (Hendrix, 1979), the compensatory ability is limited to certain
size classes of plants, and the largest proportion of viable seeds is borne by the primary umbel (Hendrix, 1979, 1984; pers. observ.). Thus, the reproductive success of the primary umbel is an appropriate indicator of overall plant fitness. Estimates of heritability were obtained from plants grown in the field plot for the following characters:

1) earliest flowering date of the primary umbel;
2) final plant weight—based on oven dry weights of leaves, stems, and reproductive parts;
3) absolute amount of each of six furanocoumarins in leaves at time of oviposition (May 1984);
4) absolute amount of each of six furanocoumarins in mature seeds;
5) relative proportion of each of six furanocoumarins in leaves at time of oviposition;
6) relative proportion of each of six furanocoumarins in mature seeds;
7) total amount of furanocoumarins in leaves at time of oviposition;
8) total amount of furanocoumarins in mature seeds.

Resistance in the field to D. pastinacella was estimated in two ways:

1) proportion of umbellets undamaged in the primary umbel;
2) number of undamaged, filled seeds produced by each individual surviving to set seed.

The number of undamaged seeds produced is the most direct measure of fitness in that, in a biennial species, it represents a plant’s reproductive contribution to the next generation; the proportion of undamaged umbellets in the primary umbel provides an index to the location and timing of insect damage. These variables were compared among individuals to identify phenotypic sources of resistance, and among families (with mean values) to identify between-family sources of resistance.

Stepwise multiple regression (Nie et al., 1975) was used to determine which concentrations or combinations of phytochemicals and other plant characters accounted for variation in seed production in the presence of herbivores; in other words, stepwise multiple regression was used to identify “resistance factors,” plant characteristics (independent or explanatory variables) associated with variation in resistance estimates (the dependent or response variables). A forward stepping procedure based on F-to-enter (4.0) and F-to-remove (3.9) limits was employed. Independent variables with minimum F-to-enter value were added to the model.

Estimate of Directional Selection by D. pastinacella on P. sativa

The change in a character associated with selection, such as by insect herbivory, is the selection differential, the difference between the mean value of a character before and after selection. Lande and Arnold (1983) showed that the covariance between relative fitness and a character is equivalent to the selection differential for that character. Lande and Arnold’s analysis was originally designed to assess the impact of a single episode of selection. In the present context, however, our experiments were designed and carried out prior to the publication of Lande and Arnold’s paper and therefore are not entirely consistent with the optimum design. Thus, the measures of selection represent not one selective event but the net result of all selective events during the life of the plant. We estimated selection differentials for resistance factors in field and greenhouse populations as the covariance between relative fitness and the resistance characters. The characters used were those identified by stepwise regression as the best indicators of insect resistance. Relative fitness was calculated as the number of seeds divided by the mean number of seeds for field plants and by number of secondary rays divided by mean number of secondary rays, a correlate of potential seed production (vide infra), for greenhouse plants. Mean relative fitness in both cases is unity. Chemicals that are environmentally induced, e.g., by insect damage, are not suitable for this type of analysis; however, there is no evidence for damage-induced changes in furanocoumarins in wild parsnip, and attempts to demonstrate such changes in total furanocoumarin production have failed to yield significant effects (unpubl.).

While the selection differential measures
the effect of selection on a character, the selection gradient estimates the intensity of selection. The selection gradient is the regression of relative fitness on a character, and the steepness of the selection gradient is equivalent to the magnitude of the regression coefficient. Selection gradients were estimated by multiple regression of relative fitness on resistance factors. The partial regression coefficients thus obtained estimate the selection gradient for each character. These estimates measure the intensity of selection acting directly upon a character (Lande and Arnold, 1983).

*Estimate of the Phenotypic Costs of Resistance and the Genetic Constraints on Insect Resistance in P. sativa*

To estimate the cost and limits of resistance, we examined phenotypic and genetic correlations between factors associated with resistance in the field and factors providing an index to the reproductive fitness of plant individuals free of herbivory. The genetic correlation measures the degree to which two characters, in this case, furanocoumarin production and fitness, are controlled by the same gene or different linked genes (Falconer, 1981). The sign of the genetic correlation is indicative of the direction of correlated response to selection. For two traits that are positively correlated, for example, selection for an increase in one trait will result in an increase in the correlated trait. The magnitude and sign of the genetic correlation can give an indication of the effectiveness of selection by insect herbivores on plant chemical traits over time.

Genetic correlations between characters $x$ and $y$ were estimated as:

$$ r_A = \frac{\text{Cov}_f(x, y)}{\sqrt{V_f(x) \cdot V_f(y)}} $$

where $r_A$, the correlation due to additive genetic effects, is the between-family covariance of characters $x$ and $y$ divided by the product of the between-family component of variance of $x$ and $y$ as determined by ANOVA (Falconer, 1981). The estimates of genetic correlation and its standard error were calculated by the jackknife procedure as described earlier but without the transformation, since no transformation has been found suitable (Arvesen and Schmitz, 1970).

In the greenhouse, where the 20 half-sib families were maintained free from insect herbivory, the following “cost” parameters, in addition to the furanocoumarin content and composition of leaves and seeds, were monitored over the course of development of the plants:

1) Photosynthetic potential, as measured by leaf conductance to water (cm/sec, measured with a Lambda leaf porometer); conductance is proportional to $CO_2$ flux and thus photosynthetic rate, assuming cell, chloroplast, and chemical resistances are all constant (Nobel, 1974).

2) Number of primary rays in the primary umbel—as a correlate of umbel size and potential seed production.

3) Number of secondary rays in the primary umbel—as an estimate of potential seed production. Each secondary ray bears one schizocarp, which splits at maturity into two mericarps or “seeds.” Using secondary rays as a measure of seed potential obviates problems with inadequate pollination as a factor in seed production in the greenhouse, where plants were manually pollinated.

4) Final plant weight—based on oven dry weights of leaves, stems, and reproductive parts.

A high metabolic cost of production ostensibly would be reflected by a phenotypic correlation between furanocoumarin content and leaf conductance (photosynthetic potential) or by a negative correlation between furanocoumarin production and seed production or plant weight. These relationships were evaluated by examining product-moment correlations among phenotypes. In addition to cost, there may be genetic limitations on resistance. If a single gene controls the quantities of two characters that enhance resistance but increases one character and simultaneously reduces the other, overall improvement of resistance may be impeded. Genetic constraints were evaluated by examination of genetic correlations among resistance characters and fitness characters. For field plants, the only measure of metabolic costs used was plant weight, since destruction of primary umbels by *D. pastinacella* precluded enumerating potential seed or secondary ray number.
WILD PARSNIPS AND PARSNIP WEBWORMS

Table 1. A) List of plant characters studied. B) Key for abbreviations of furanocoumarins.

<table>
<thead>
<tr>
<th>Character</th>
<th>Plants measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering date</td>
<td>Field</td>
</tr>
<tr>
<td>Number of seeds</td>
<td>Field</td>
</tr>
<tr>
<td>Number of primary rays</td>
<td>Greenhouse</td>
</tr>
<tr>
<td>Number of secondary rays</td>
<td>Greenhouse</td>
</tr>
<tr>
<td>Leaf conductance</td>
<td>Greenhouse</td>
</tr>
<tr>
<td>Plant weight</td>
<td>Field and greenhouse</td>
</tr>
<tr>
<td>Furanocoumarins</td>
<td>Field and greenhouse</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Furanocoumarins</th>
<th>Amount</th>
<th>Proportion</th>
<th>Amount</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imperatorin</td>
<td>IMPs</td>
<td>pIMPs</td>
<td>IMPi</td>
<td>pIMPI</td>
</tr>
<tr>
<td>Bergapten</td>
<td>BERs</td>
<td>pBERs</td>
<td>BERi</td>
<td>pBERI</td>
</tr>
<tr>
<td>Isopimpinellin</td>
<td>ISOs</td>
<td>pISOs</td>
<td>ISOi</td>
<td>pISOI</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>XANs</td>
<td>pXANs</td>
<td>XANI</td>
<td>pXANI</td>
</tr>
<tr>
<td>Sphondin</td>
<td>SPHs</td>
<td>pSPHs</td>
<td>SPHI</td>
<td>pSPHI</td>
</tr>
<tr>
<td>Total</td>
<td>TOTS</td>
<td>pSPHs</td>
<td>TOTl</td>
<td></td>
</tr>
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</table>

RESULTS

Estimation of Genetic Control of Furanocoumarin Production

Table 1 contains a key for all abbreviations used in presenting the experimental results. Using data from both field and greenhouse populations, we were able to obtain heritability estimates on many aspects of furanocoumarin production (Table 2). Significant heritability estimates were found for nearly all of the characters, and on occasion some estimates approached unity, indicating complete genetic control over observed variation in certain traits (e.g., sphondin production in seeds) within the study environment. The heritabilities of seed furanocoumarins based on parent-offspring regression were significantly correlated with those obtained by half-sib analysis ($r = 0.657, P < 0.05$), suggesting that the estimates are accurate. Maternal effects on seed furanocoumarin content were negligible compared to total variation in seed content (Table 3).

Determination of Resistance Factors and of Genetic Bases for Resistance

Virtually all insect damage experienced by experimental plants was directly attributable to *D. pastinacella*; regular inspection
TABLE 3. Estimates of maternal effects on furanocoumarin content in seeds of *Pastinaca sativa*.

<table>
<thead>
<tr>
<th>Furanocoumarins</th>
<th>Maternal variance</th>
<th>Proportion of total phenotypic variance contributed by maternal effects1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMPs</td>
<td>−3.323</td>
<td>0</td>
</tr>
<tr>
<td>BERs</td>
<td>−0.424</td>
<td>0</td>
</tr>
<tr>
<td>ISOs</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>XANs</td>
<td>2.291</td>
<td>0.060</td>
</tr>
<tr>
<td>SPHs</td>
<td>0.390</td>
<td>0.100</td>
</tr>
<tr>
<td>TOTs</td>
<td>−1.186</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Negative estimates of maternal variance are treated as zeros.  
* Values not calculated owing to the finding of a negative covariance between offspring and parents.

revealed remarkably few other herbivores. Close examination of leaves of all plants in May 1984 revealed that approximately 99% of 187 surviving individuals in the test plot contained eggs of *D. pastinacella*. Thus, very few plants were rejected outright by ovipositing females.

In the stepwise regression analysis, two estimates of resistance were used separately as dependent variables—percentage of undamaged umbellets and the number of undamaged seeds produced. The principal independent variable accounting for a significant amount of variance in umbellet damage both among individual plants and among half-sib families was the flowering date (Table 4). Plants in the experimental garden that flowered early were able to produce a higher proportion of umbellets that escaped damage. When number of seeds was compared among individuals irrespective of family affiliation, flowering date again accounted for the largest amount of variance (Fig. 2). However, when mean number of seeds per family was examined, four furanocoumarin variables accounted for a significant amount of variance (Table 4): the proportion of bergapten in the seeds (pBERs), the absolute amount of sphonid in the seeds (SPHs), the proportion of sphonid in the leaves (pSPHs) and the absolute amount of bergapten in the leaves (BERI). Collectively, these variables accounted for almost 75% of the variation in seed production in the presence of *D. pastinacella*. All of these resistance components have significant heritabilities in field or greenhouse environments (Table 2).

*Estimate of Directional Selection by D. pastinacella on P. sativa Characters Associated with Resistance*

There were pronounced family differences in seed set; mean seed production for a family in the field varied from 3.7 seeds/primary umbel to 446.0/primary umbel, and individual seed production ranged from 0 to 1,664. In the field, significant selection differentials were found for flowering date and proportion of bergapten in the seeds (Table 5), indicating that selection had shifted the distribution of both characters. The positive and negative selection differentials found for proportion of bergapten in seeds

TABLE 4. Stepwise regressions between webworm resistance estimators and plant characters.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>% of variation explained</th>
<th>Cumulative variation explained</th>
<th>Standardized regression coefficient</th>
<th>Regression F (df)</th>
<th>P</th>
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<tr>
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<td></td>
</tr>
<tr>
<td>Dependent variable: proportion of undamaged umbellets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowering date</td>
<td>14.6</td>
<td>14.6</td>
<td>0.312</td>
<td>11.85 (3,110)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plant weight</td>
<td>5.8</td>
<td>20.3</td>
<td>−0.235</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pBERs</td>
<td>4.1</td>
<td>24.4</td>
<td>−0.212</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Dependent variable: seed number | | | | | |
| Flowering date | 24.5 | 24.5 | −0.471 | 24.9 (3,110) | <0.01 |
| Plant weight   | 11.7 | 36.2 | 0.349  |               |       |
| pISOs          | 4.2  | 40.4 | 0.205  |               |       |

| Dependent variable: mean proportion of undamaged umbellets per family | | | | | |
| Flowering date | 31.8 | 31.8 | −0.564 | 8.4 (1,18) | <0.05 |

| Dependent variable: mean seed number per family | | | | | |
| pBERs     | 36.3 | 36.3 | 0.975  | 10.5 (4,15) | <0.01 |
| SPHs      | 13.9 | 50.2 | 0.508  |               |       |
| pSPHs     | 10.6 | 60.7 | −0.374 |               |       |
| BERI      | 12.9 | 73.6 | −0.370 |               |       |
and flowering date, respectively, indicate that plants with early flowering date and a high proportion of bergapten in the seeds have higher fitness. Since the partial regression coefficient for flowering time was not statistically significant, selection must have acted only indirectly on flowering date. The significant selection differential in flowering date may in part be attributed to selection acting directly on the proportion of bergapten in the seeds, one character for which a significant regression coefficient was found. The phenotypic correlation between flowering date and proportion of bergapten in seeds is negative and significant. Thus, selection for increased proportion of bergapten in seeds automatically results in a negative selection differential in flowering date. Significant partial regression coefficients were found for the other three chemical resistance characters as well. In the greenhouse, a significant selection differential was found for only one of the characters, proportion of bergapten in seeds (Table 6). In this case, none of the partial regression coefficients were significant. The negative selection differential for proportion of bergapten in seeds indicates that the greenhouse plants with higher values of proportion of bergapten in seed had lower fitness.

**Genetic and Phenotypic Correlations between Fitness Parameters and Resistance Factors**

Phenotypic and genetic correlations between statistically significant resistance factors and fitness parameters were measured in the field and greenhouse populations (Tables 7 and 8). None of the genetic correlations in the field were significant, but seven furanocoumarin characters measured in the greenhouse—including the major resistance factors, the proportion of bergapten in seeds and the amount of bergapten in seeds—had significant negative genetic correlations with the number of secondary rays. Photosynthetic potential (leaf conductance) was not genetically correlated with any of the furanocoumarin characteristics, nor was flow-

### Table 5. Directional selection differentials, partial regression coefficients, and phenotypic correlations among resistance characters in field parsnips. FD = flowering date; for other abbreviations, see Table 1.

<table>
<thead>
<tr>
<th>Character</th>
<th>Selection differential</th>
<th>Partial regression coefficient</th>
<th>Standardized regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>-0.1891*</td>
<td>-1.80</td>
<td>-0.148</td>
</tr>
<tr>
<td>pBERs</td>
<td>0.0107*</td>
<td>21.69**</td>
<td>0.883**</td>
</tr>
<tr>
<td>BERI</td>
<td>-0.0027</td>
<td>-11.07*</td>
<td>-0.354*</td>
</tr>
<tr>
<td>pSPHI</td>
<td>-0.0086</td>
<td>-3.38*</td>
<td>-0.351*</td>
</tr>
<tr>
<td>SPHs</td>
<td>0.0106</td>
<td>0.32**</td>
<td>0.500**</td>
</tr>
</tbody>
</table>

**Phenotypic Correlation Matrix**

<table>
<thead>
<tr>
<th></th>
<th>FD</th>
<th>pBERs</th>
<th>BERI</th>
<th>pSPHI</th>
<th>SPHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>1.000</td>
<td>-0.554*</td>
<td>-0.031</td>
<td>0.079</td>
<td>0.263</td>
</tr>
<tr>
<td>pBERs</td>
<td>1.000</td>
<td>0.216</td>
<td>0.092</td>
<td>-0.508*</td>
<td>-0.126</td>
</tr>
<tr>
<td>BERI</td>
<td>1.000</td>
<td>1.000</td>
<td>-0.082</td>
<td>1.000</td>
<td>0.121</td>
</tr>
<tr>
<td>pSPHI</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>SPHs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05 ** P < 0.01.

1 Significance level is for the product-moment correlation of relative fitness with the character.

2 Significance level is for the product-moment correlation among pairs of resistance factors.
TABLE 6. Directional selection differentials, partial regression coefficients, and phenotypic correlations among resistance characters in greenhouse parsnip. FD is flowering date; for other abbreviations, see Table 1.

<table>
<thead>
<tr>
<th>Character</th>
<th>Selection differential¹</th>
<th>Partial regression coefficient</th>
<th>Standardized regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBERs</td>
<td>−0.00142*</td>
<td>−1.61</td>
<td>−0.463</td>
</tr>
<tr>
<td>BERI</td>
<td>−0.00082</td>
<td>−0.16</td>
<td>−0.044</td>
</tr>
<tr>
<td>pSPHI</td>
<td>−0.00091</td>
<td>−0.38</td>
<td>−0.180</td>
</tr>
<tr>
<td>SPHS</td>
<td>0.03104</td>
<td>0.01</td>
<td>0.158</td>
</tr>
</tbody>
</table>

Phenotypic Correlation Matrix²

<table>
<thead>
<tr>
<th></th>
<th>pBERs</th>
<th>BERI</th>
<th>pSPHI</th>
<th>SPHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBERs</td>
<td>1.00</td>
<td>0.321</td>
<td>−0.070</td>
<td>−0.175</td>
</tr>
<tr>
<td>BERI</td>
<td>1.00</td>
<td>0.463*</td>
<td>−0.165</td>
<td></td>
</tr>
<tr>
<td>pSPHI</td>
<td>1.000</td>
<td>1.000</td>
<td>−0.147</td>
<td></td>
</tr>
<tr>
<td>SPHS</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Significance level is for the product-moment correlation of relative fitness with the character.
² Significance level is for the product-moment correlation among pairs of resistance factors.

erating date, a significant correlate of the proportion of umbellets escaping attack by *D. pastinacella*. Potential seed production, as measured by both primary and secondary ray number, was negatively correlated phenotypically with only one furanocoumarin trait in the greenhouse plants (amount of bergapten in seeds), and it was positively correlated with imperatorin in seeds and leaves; two individual furanocoumarins in the leaves (isopimpinellin and xanthotoxin), as well as total furanocoumarin production, were negatively correlated phenotypically with plant weight in field plants exposed to herbivory (Table 7).

**DISCUSSION**

Stepwise multiple regression revealed that four furanocoumarin variables accounted for almost 75% of the among-family variation in seed set in the presence of parsnip webworms. Since all four variables have a significantly high heritability (Table 2), it can be concluded that variation in resistance to parsnip webworm in the wild parsnip is in part genetically based. The opposite signs of the regression coefficients for leaf and seed resistance factors suggest that the mechanism of furanocoumarin action with respect to the insect differs depending on plant part. High amounts of furanocoumarins in seed are associated with increased seed set, consistent with a defensive (allo-mononal) effect. High amounts of furanocoumarins in leaves, which are substantially lower in absolute terms than even low levels in seeds, are associated with reduced seed set, consistent with a host recognition (kairomonal) effect. Physiological and/or behavioral effects of furanocoumarins on *D.

TABLE 7. Significant phenotypic correlations between leaf or seed furanocoumarins and fitness characters, and between fitness characters in greenhouse and field plants.

<table>
<thead>
<tr>
<th></th>
<th><em>r</em></th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse (<em>N</em> = 171)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMPs × number of secondary rays</td>
<td>0.224</td>
<td>0.075</td>
</tr>
<tr>
<td>IMP1 × number of primary rays</td>
<td>0.204</td>
<td>0.075</td>
</tr>
<tr>
<td>BERI × number of secondary rays</td>
<td>−0.167</td>
<td>0.076</td>
</tr>
<tr>
<td>BERI × number of primary rays</td>
<td>−0.161</td>
<td>0.076</td>
</tr>
<tr>
<td>Number of primary rays × number of secondary rays</td>
<td>0.874</td>
<td>0.037</td>
</tr>
<tr>
<td>Field (<em>N</em> = 143)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO1 × plant weight</td>
<td>−0.206</td>
<td>0.082</td>
</tr>
<tr>
<td>XANI × plant weight</td>
<td>−0.217</td>
<td>0.082</td>
</tr>
<tr>
<td>TOTI × plant weight</td>
<td>−0.202</td>
<td>0.082</td>
</tr>
</tbody>
</table>

TABLE 8. Significant genetic correlations between seed or leaf furanocoumarins and fitness characters and between fitness characters in greenhouse plants.

<table>
<thead>
<tr>
<th></th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BERI × number of secondary rays</td>
<td>−0.632</td>
</tr>
<tr>
<td>BERI × number of primary rays</td>
<td>−0.675</td>
</tr>
<tr>
<td>XANI × number of secondary rays</td>
<td>−0.790</td>
</tr>
<tr>
<td>XANI × number of primary rays</td>
<td>−0.643</td>
</tr>
<tr>
<td>SPHI × number of secondary rays</td>
<td>−0.688</td>
</tr>
<tr>
<td>pBERs × number of secondary rays</td>
<td>−1.528</td>
</tr>
<tr>
<td>pISOs × number of secondary rays</td>
<td>−0.888</td>
</tr>
<tr>
<td>Number of primary rays × number of secondary rays</td>
<td>0.809</td>
</tr>
</tbody>
</table>
*pastinacella* may thus be dose-dependent. Undoubtedly, the field environment differs from that of the greenhouse in more ways than the presence or absence of herbivores, but, given the overwhelming impact of the herbivore on seed production, it is instructive to compare field and greenhouse populations as though comparing herbivore-infested and herbivore-free treatments. That furanocoumarin chemistry is important in determining fitness in *P. sativa* in the presence of *D. pastinacella* is suggested by the fact that, while in the greenhouse several furanocoumarins are negatively correlated genetically with the number of secondary rays and hence with fitness, no furanocoumarins are significantly negatively correlated with seed set in the field. In other words, production of furanocoumarins, despite negative genetic correlations with seed production in the greenhouse, increases fitness in the presence of the herbivore in the field.

Both qualitative and quantitative aspects of furanocoumarin chemistry figure in resistance to parsnip webworm herbivory. One possible reason for the dearth of direct evidence that allelochemical variation can account for herbivore variation in other plant-insect associations (see Chew and Rodman, 1979) is that much attention to date has been focused on single-chemical or single-gene resistance factors. This approach, fostered in all probability by early studies demonstrating single gene-for-gene interactions in systems such as Hessian fly and wheat (Hatchett and Gallun, 1970), focused attention on systems in which single chemicals, often controlled at a single locus (e.g., gossypol [Wilson and Shaver, 1973]), were known to function in insect resistance. However, the applicability of the gene-for-gene concept in agricultural situations may simply be an artifact of plant breeding techniques, in which intense artificial selection removes associated protective polygenic effects from oligogenic resistance (Johnson, 1961). At present, no gene-for-gene systems have been documented in nature (Day, 1974). Gould (1983) convincingly argues that, in natural systems, resistance is likely to be quantitative and polygenic. Ostensibly, the involvement of several loci can retard the acquisition of resistance on the part of the herbivore. In the interaction between the wild parsnip and the parsnip webworm, no single furanocoumarin can account for resistance, nor can the total amount of furanocoumarins in leaf or seed. Instead, the content and composition of furanocoumarins throughout the plant are involved in determining resistance. As Feeney (1976) predicted, specialists may vary in sensitivity to representatives of a single class of chemical in their food plants; an adaptation to furanocoumarins as a class in *D. pastinacella* does not confer absolute resistance to all structural types and combinations of furanocoumarins.

While furanocoumarins are undoubtedly associated with resistance to webworms, at least one phenological factor also influences the extent of webworm damage, albeit indirectly. Flowering date is significantly correlated with the percentage of umbels in the primary umbel escaping damage and subsequent seed set (Fig. 2). This phenological trait has, like the furanocoumarin resistance factors, a significant heritability ($h^2 = 0.562 \pm 0.269$). Some measure of “escape in time” (Feeney, 1976) is entirely consistent with what is known of the life histories of both plant and herbivore. Parsnip webworms overwinter as adults and become active very early in spring; females were collected flying at night in March at temperatures near 10°C, and eggs were found on leaves of plants just beginning to leaf out (Nitao, unpubl.). Wild parsnip is primarily restricted to north temperate regions and is a cold-climate plant; indeed, *P. sativa* has an obligate chilling requirement prior to flowering (Baskin and Baskin, 1979). In spring, then, the opportunity exists for the wild parsnip to “outgrow” the webworm by flowering sufficiently early such that at least some umbels of the primary umbel can flower free from insect damage.

Wild parsnip families differed greatly in their susceptibility to parsnip webworm, as shown by the wide range of seed production among families (means ranged from 4 to 446 seeds in the primary umbel). Significant selection differentials were found for several of the resistance traits (Table 5). Further analysis by multiple regression suggests that selection acts directly on all four chemical resistance factors but only indirectly on flowering time. Since flowering time has a
negative phenotypic correlation with proportion of bergapten in seeds, the selection differential for flowering time may be attributed to direct selection for the chemical trait with a resulting decrease in flowering time. Phenotypic correlations may also explain the absence of a selection differential even though a character is under direct selection. Selection acted directly to increase both proportion of bergapten and amount of sphonbidin in seeds, but a significant selection differential was found only for the proportion of bergapten in seeds. In this case, the amount of sphonbidin and proportion of bergapten in seeds have a negative phenotypic correlation, and stronger selection for a higher proportion of bergapten may have nullified the effect of selection for increased sphonbidin. In the greenhouse the only character that displayed a significant selection differential was the proportion of bergapten in seeds. In this case, however, plants with a high proportion of bergapten in seeds had reduced fitness. Since all five resistance characters have significant heritabilities, the mechanism exists to allow herbivores, with sufficient selective pressure, to cause changes in the chemical and hence genetic composition of a population, one principal requirement for demonstrating coevolution (Janzen, 1980).

Genetic correlations among resistance factors shed light on the nature of the coevolutionary relationship between D. pastinacella and P. sativa (Table 9). A favorable genetic correlation is one in which two traits that convey resistance are positively correlated. Hence, selection by herbivores for increased resistance by either trait increases both traits simultaneously. No such favorable genetic correlations were observed in P. sativa. Instead, the only significant genetic correlation is a negative relationship ($r_A = -0.853$) between two traits conveying resistance, proportion of bergapten and amount of sphonbidin in seeds. This type of correlation offers a “no-win” situation, because selection for increased resistance in the form of an increased proportion of bergapten tends to decrease resistance in the form of a decreased amount of sphonbidin in the seeds. The absence of a significant selection differential for the amount of sphonbidin in seeds in the field families is evidence of the ineffectiveness of selection in this context. Moreover, several of the resistance factors accounting for variation in herbivory have negative genetic correlations with total seed production in greenhouse plants protected from insect herbivory (Table 8).

In the presence of the herbivore, this fitness reduction associated with furanocoumarin production is more than offset by increased survivorship of the offspring, so that genotypes that are high in the proportion of bergapten in the seed are favored. In a future generation when the herbivore is absent, however, such genotypes would be at a selective disadvantage because they produce fewer seeds than genotypes susceptible to the herbivore. The finding of a significant negative selection differential for the proportion of bergapten in seed in the greenhouse is consistent with this interpretation.

The nature of reduced competitive ability due to furanocoumarin production may relate to nutrient limitations on furanocoumarin biosynthesis. Light-induced biosyn-
thesis of furanocoumarins, for example, does not take place under conditions of low nutrient availability (Berenbaum and Zangerl, unpubl.). Moreover, there are significant negative correlations between furanocoumarins that share a common biosynthetic precursor. Angular furanocoumarins are negatively genetically correlated with linear furanocoumarins, and 8-substituted furanocoumarins (xanthotoxin and imperatorin) are genetically correlated negatively with 5-substituted furanocoumarins (bergapten, isopimpinellin), suggesting some limitation on the availability of precursor molecules at branch points of a biosynthetic pathway (Berenbaum and Zangerl, unpubl.).

The interaction between the parsnip weevil and the wild parsnip, then, is at an ecological standoff of sorts. The “genetical constraints” (Berry, 1985) on the response of wild parsnip to selection—in the form of high genetic correlations among traits—tend to limit resistance in the plant population when the herbivore is present but also act to reduce resistance when the herbivore is absent. To extend the “evolutionary arms race” concept advanced by Whittaker and Feeny (1971), the interaction between wild parsnip and the parsnip weevil, at least in this central Illinois population, has reached a temporary stalemate. This situation will persist until such time that the environment changes as to favor herbivore or host or until there is a genetic change producing new resistance traits or more variation in existing resistance traits.

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