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Genotypic interactions in an aphid-host plant relationship: *Uroleucon rudbeckiae* and *Rudbeckia laciniata*

Philip Service*
Department of Biology, University of North Carolina, Chapel Hill, NC 27514, USA

Summary. Four clones of the aphid *Uroleucon rudbeckiae* were grown on four clones of the host plant *Rudbeckia laciniata*. Age-specific fecundities were used to determine the fitness, F_i', of each individual aphid. The analysis of variance for F_i' revealed that (1) plant genotype has a significant effect on aphid fitness; (2) there is an interaction between aphid and host plant genotypes with respect to aphid fitness; and (3) aphid fitness is affected by phenotypic differences among individual host plants. Result (2) supports the hypothesis that genotypic interactions between aphid and host may maintain genetic diversity in aphid populations. The results of a preference test, while not significant at customary probability levels, suggested that aphids will choose to feed on plants which confer greater fitness.

Introduction

The significance of genetic variation in natural populations has been a subject of continuing debate. One argument holds that such variation has adaptive significance and is maintained by interactions between genotypes and heterogeneous environments. Evidence in support of this hypothesis is reviewed by Hedrick et al. (1976). Herbivorous arthropods and their food plants should provide useful systems for testing hypotheses about genetic polymorphism and environmental heterogeneity. Food plant quality is an important environmental factor which can vary in both space and time. There have, however, been relatively few studies of the importance of genetic interactions in plant-herbivore systems (Hatchett and Gallun 1970; Edmunds and Alstad 1978; Mitter et al. 1979; Gould 1979; Moran 1981). This scarcity is surprising in view of the considerable research on resistance of crop plants to insect pests (see Maxwell et al. 1972; Gallun et al. 1975) and in view of the widespread interest in plant-herbivore coevolution (Ehrlich and Raven 1964; Feeny 1975). There is evidence of genetic interactions in agricultural aphid-host plant systems (Dunn and Kempton 1972; Lowe 1980). Agricultural workers have, however, generally not designed their experiments to address issues of ecological or population-genetic theory.

This paper reports an experiment designed to test the following hypotheses about genotypic variation in an aphid-host plant system:

1. Host plant genotypes vary in their suitability for aphid growth and reproduction.

2. Aphid genotypes vary in their ability to grow and reproduce on host plants.

3. There is an interaction between aphid genotype and host plant genotype such that the relative fitness of an aphid genotype is a function of plant genotype.

These hypotheses are similar to those proposed by Clarke (1976, 1979) with regard to host-parasite relationships. This experiment is a logical companion to that of Service and Lenski (1982), which examined relationships between aphid genotypes and several *phenotypes* of a single host clone.

Materials and methods

The organisms

The experimental organisms were *Uroleucon rudbeckiae* (Fitch) (Eastop and Hille Ris Lambers 1976) (Homoptera: Aphididae) and its host plant, *Rudbeckia laciniata* L. (Asteraceae). *U. rudbeckiae* has a holocyclic life history consisting of a single sexual generation and numerous parthenogenetic generations per year. *R. laciniata* is an herbaceous perennial. Neither species is economically important. Although other host plant species have been listed (Smith and Parron 1978), such records must be considered tentative in the absence of detailed field evidence. During 2.5 years of observation of a natural population, I never found colonies of *U. rudbeckiae* on other plant species.

The four experimental aphid clones were started from individual parthenogenetic females collected at four locations in Chapel Hill, North Carolina. The aphid clones were maintained for about 1 year prior to the experiment. Each aphid clone was divided into two replicate cultures which were managed so as to minimize intra- and interclonal variation, e.g., all plants used for aphid culture belonged to one clone (R-6). The four experimental plant clones were propagated from individuals collected at three sites in Chapel Hill. The plant clones were maintained in a greenhouse for approximately 1.5 years. Propagation was by repeated division of root crowns. The experimental plants were last divided and repotted 5 months before the experiment, and were conditioned in a growth chamber for 5 weeks immediately preceding the experiment. Aphid culturing and plant conditioning were done under a 15-h light:9-h dark daily

* *Present address:* Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

cycle. Temperatures were approximately 23° C and 17° C during the light and dark periods, respectively. The mean light intensity in the conditioning chamber (at the tops of the plants) was 157.5 μE m^{-2} s^{-1}. At the end of the conditioning period, the height and number of nodes of each plant were recorded.

I have assumed that each aphid and plant clone represents a different genotype. In the case of aphids, this assumption is justified by collection from separate sites early in the growing season: the parents of the clones were either individuals which had developed from sexually produced eggs, or were the immediate descendants of those individuals. In the case of plant clones, the assumption of genotypic difference is based on two criteria. First, the parents of the four clones were taken from three separate sites. Second, when grown in a common environment, the R-6 and R-20 clones produced two readily distinguishable phenotypes, and R-25 and R-26 produced a third phenotype. Despite the fact that clones R-25 and R-26 were taken from the same site and were phenotypically similar, the results of the experiment indicate that they are genotypically distinct.

Experimental design

Four aphid clones and four plant clones were used. There were two replicate cultures of each aphid clone. The experimental design combined crossed and hierarchical features: plant clone was fully crossed with aphid clone and aphid culture; aphid culture was nested within aphid clone; and individual plant was nested within plant clone, aphid clone, and aphid culture. Sixteen individual plants of each plant clone were used.

All terms in the analysis of variance model have been treated as random effects. Conclusions about the effects involving aphid clone (genotype) and plant clone (genotype) may, therefore, be generalized to the larger populations of aphid and plant genotypes from which the experimental genotypes were obtained.

Experimental procedure

Experimental aphids were obtained by taking alate (winged) adults or alatoid nymphs from population cages and placing them in individual stem cages on experimental plants. Each stem cage was inspected at 2-day intervals. The first nymph or nymphs borne by an alate were removed from the cage. When a subsequent nymph was produced, the mother (alate) was removed and the retained nymph became the experimental aphid. If there was more than one subsequent nymph in a cage, the experimental aphid was selected randomly. For 24 of the 64 experimental aphids in clone DF-3, it was necessary to use apterous (wingless) rather than alate mothers: a sufficient number of alates was not available in the cultures. Subsequent analysis has indicated no difference in performance of the progeny of alate and apterous mothers.

The experiment was conducted in a single growth chamber under the light and temperature regimes used for aphid culturing and plant conditioning. Each experimental plant had four individual cages arranged linearly on its stem, and all aphids on a plant were from the same culture. The survivorship and fecundity of experimental aphids were recorded at 2-day intervals for the duration of their lives or until age 25 (50 days). Once an experimental aphid began to reproduce, any nymphs were removed from the cage every 2 days. All ages and rates mentioned subsequently in this paper are based on time intervals of 2 days.

The cohort finite rate of increase, F_N (Lenski and Service 1982), was calculated from the mean age-specific survivorship and fecundity data of the 256 experimental aphids. Then, the lifetime contribution, F_i', of individual i to this cohort rate of increase was obtained from the formula

$$F_i' = \sum_{x=0}^{\infty} F_N^{-x} B_{xi},$$

where B_{xi} is the number of nymphs produced by female i while she is in age class x which survive to enter age class 0 (at which time the mother enters age class $x+1$). The derivation and justification for the use of F_i' are given in detail by Lenski and Service (1982). An important property of F_i' is that it weights the "value" of an individual's progeny according to the ages at which those progeny are produced, and according to the growth rate of the population of which that individual is a part. F_i' is the sum of these values over the lifetime of the individual. The mean of F_i' equals F_N.

Feeding preference tests

Before the end of the experiment, it became apparent that the survivorship, rate of development, and early fecundity of at least some aphid clones were functions of host plant genotype. Therefore, I decided to determine if aphids would choose to feed on plants of a clone which conferred greater fitness. I used aphid clone BG-2 and plant clones R-6 and R-20. Preliminary data indicated that BG-2 aphids had higher fitness on plant clone R-20.

Single individuals of each plant clone were placed together in a population cage. The two plants were arranged so that their stems and leaves were touching in several places. Ten adult aphids were placed on the lower leaves of each plant. The number of aphids on each plant was recorded 12, 24, and 48 h later. The test was replicated ten times. No plant or aphid individual was used more than once. As in the case of all other aphid clones, BG-2 had been maintained on the R-6 plant clone for approximately 1 year prior to the experiment. The aphids used in the tests were taken directly from R-6 plants.

Results

The experimental data are summarized by aphid clone (Table 1) and plant clone (Table 2). The analysis of variance for the mean F_i' per plant (hereafter $\bar{F}'$/plant) is shown in Table 3. I present this analysis (rather than that for F_i') because the assumptions of normality and homogeneity of variance, as well as the additional assumptions implicit in the use of the covariable, are satisfied by using $\bar{F}'$/plant. Qualitatively, the results are the same as for the analysis of F_i'.

In previous experiments, F_i' was found to be positively associated with higher position on a plant stem (Service, unpublished work), and with overall plant size (number of stem nodes) (Service and Lenski 1982). In this analysis, the mean position, in internodes, of the four aphid cages on a plant is used as a covariable. Since aphids were placed as high as practicable on plants, the mean internode value reflects both the size of the plant and the position of aphids

Table 1. Aphid life-history data by aphid clone ($\bar{X} \pm 1$ SE)

	Aphid clone			
	BC-3	BG-1	BG-2	DF-3
$\bar{F}'$/plant	1.14±0.17	1.45±0.28	1.95±0.26	1.57±0.23
$\bar{F}'$/plant[a]	1.23±0.17	1.55±0.22	2.21±0.18	1.12±0.25
Number reproducing	45	43	52	49
Age of first reproduction[b]	5.36±0.18	5.12±0.20	4.62±0.09	5.06±0.14
Fecundity[b]	39.27±2.84	49.72±3.46	48.85±2.60	45.80±3.10
Reproductive rate[b]	2.81±0.17	3.11±0.16	3.47±0.19	3.39±0.19
Age of death	14.00±1.15	14.44±1.20	15.97±1.07	15.00±1.12
Internode	3.55±0.15	3.53±0.17	3.31±0.13	4.30±0.17

[a] Adjusted for covariable: standardized mean internode/plant (see text)
[b] Based on number reproducing

Table 2. Aphid life-history data by plant clone ($\bar{X} \pm 1$ SE)

	Plant clone			
	R-6	R-20	R-25	R-26
$\bar{F}'$/plant	1.17±0.18	2.25±0.24	1.43±0.22	1.26±0.24
$\bar{F}'$/plant[a]	1.17±0.18	2.25±0.21	1.43±0.24	1.26±0.20
Number reproducing	48	51	47	43
Age of first reproduction[b]	5.17±0.12	4.75±0.11	5.15±0.22	5.05±0.16
Fecundity[b]	39.23±3.05	52.25±2.58	45.96±3.09	46.07±3.19
Reproductive rate[b]	2.69±0.14	4.31±0.19	2.84±0.15	2.89±0.14
Age of death	14.72±1.07	14.41±1.00	15.83±1.19	14.45±1.26
Internode	4.02±0.18	3.73±0.13	3.38±0.13	3.56±0.19

[a] Adjusted for covariable: standardized mean internode/plant (see text)
[b] Based on number reproducing

Table 3. Analysis of variance for mean F_i'/plant

Source	*df*	SS	MS	*F*-ratio	*F*	*P*
Aphid clone	3	10.1551[a]	M1	M1/(M2+M4−M5)	2.93	0.0995
Aphid culture	4	2.1507[a]	M2	M2/M5	2.47	0.1011
Plant clone	3	11.6510	M3	M3/M4	4.65	0.0315
Aphid clone × plant clone	9	7.5127[a]	M4	M4/M5	3.83	0.0168
Culture × plant clone	12	2.6134[a]	M5	M5/M7	0.40	0.9534
Mean internode	1	15.4507	M6	M6/M7	28.27	0.0001
Model	32	42.7150			2.44	0.0074
Residual	31	16.9442	M7			
Total	63	59.6592			$R^2=0.7160$	

[a] Adjusted for covariable (see text)

on the plant. Because the plant clones may have differed systematically in size, the mean internode value for each plant was standardized by subtracting the mean value for all experimental plants of the same clone. This procedure ensured that introduction of the covariable did not alter the sum of squares associated with the plant clone effect (Table 3).

In the analysis of $\bar{F}'$/plant (Table 3), there are statistically significant ($P<0.05$) effects due to plant clone, to the aphid clone × plant clone interaction, and to the mean internode position of the aphids on a plant. (The use of a pure random-effects model necessitates an approximate *F*-test for the aphid clone effect.)

The aphid clone × plant clone interactions are illustrated in Fig. 1. The interaction sum of squares can be partitioned among the 36 possible combinations of two aphid clones and two plant clones (Table 4). Two aphid clone pairs, BC-3/BG-2 and BC-3/DF-3, account for about 70% of the interaction sum of squares: and clone BC-3 is involved in nine of the ten largest contributions. Among the plant clone pairs, four contribute about equally to the total, the remaining two contributing very little. The best evidence that plant

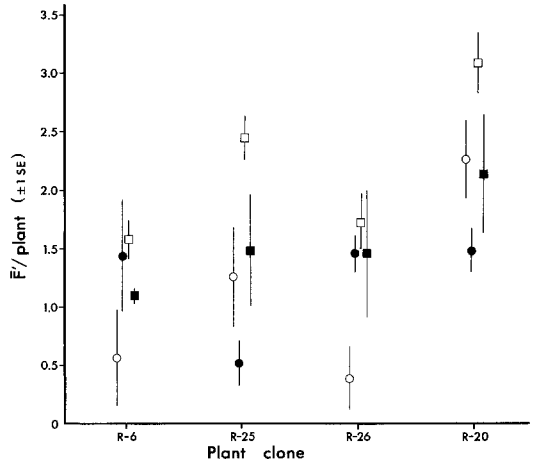

Fig. 1. Mean $\bar{F}'$/plant by aphid clone and plant clone. Values are adjusted for covariable (see text). Aphid clone BC-3 is represented by *solid circles*. DF-3 by *open circles*, BG-1 by *solid squares*, and BG-2 by *open squares*

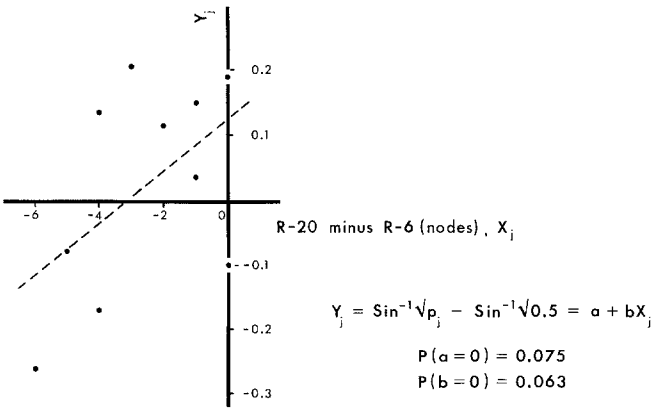

Fig. 2. Analysis of feeding-preference trials. *Dashed line* is weighted least-squares regression. Significance tests are one-tailed

clones R-25 and R-26 represent different genotypes is obtained from Table 4. The R-25/R-26 pair contributes appreciably to the total interaction sum of squares, and the results of pairing the two clones with either R-6 or R-20 are quite different (cf. R-6/R-25 with R-6/R-26).

Feeding preference tests

On the basis of preliminary results (later confirmed, Fig. 1), I hypothesized that aphids of clone BG-2 would choose to feed on plants of clone R-20 in preference to plants of clone R-6. The test for preference is, therefore, one-tailed. In each trial, no attempt was made to match the plants, except to use plant short enough to fit into the population cages used. By the time the preference tests were conducted, however, the mean internode length for R-20 plants was greater than for R-6 plants. As a result, the R-20 plants had significantly fewer nodes than the R-6 plants ($t=3.8806$, $df=9$, $P<0.005$). Since aphid performance is known to be positively associated with number of nodes, the greater number of nodes for R-6 plants represented an undesirable intervening variable.

The feeding preference data were analyzed, therefore, according to the following model:

$$Y_j=(\mathrm{Sin}^{-1}\sqrt{p_j})-(\mathrm{Sin}^{-1}\sqrt{0.5})=a+bX_j,$$

where p_j is the proportion of aphids which were feeding on the R-20 plant at the end of trial j, and X_j is the difference in number of nodes between the R-20 plant and the R-6 plant. The model was tested by weighted least squares regression (Fig. 2), the weights being necessary because of unequal numbers of aphids remaining at the ends of the trials. The negative $\sin^{-1}\sqrt{0.5}$ establishes that $E(Y_j)=a=0$ when $X_j=0$, if the null hypothesis of no preference for R-20 is true.

Under the alternative hypothesis of preference for R-20, the intercept, a, on the ordinate is expected to be positive. Since aphid fitness is positively correlated with number of nodes, the slope, b, is also expected to be positive if aphids choose plants which confer greater fitness. The probability for the observed or greater value of a is 0.075, and the probability for b is 0.063 (one-tailed tests).

Discussion

Genetic variance for fitness in an herbivore-host plant relationship is a necessary condition for evolutionary interaction between the two species. The observed genotypic variability of *R. laciniata* and the observed interaction between

Table 4. Partitioning of aphid clone × plant clone interaction sum of squares. Percentage contributions for pairwise combinations of two aphid clones with two plant clones

Plant clone pairs	Aphid clone pairs						Total
	BC-3/BG-1	BC-3/BG-2	BC-3/DF-3	BG-1/BG-2	BG-1/DF-3	BG-2/DF-3	
R-6/R-20	3.09	6.77[a]	8.62[a]	0.71	1.39	0.11	20.69
R-6/R-25	5.43[a]	10.19[a]	8.29[a]	0.74	0.30	0.10	25.05
R-6/R-26	0.36	0.05	0.12	0.15	0.90	0.32	1.90
R-20/R-25	0.32	0.35	<0.01	<0.01	0.39	0.42	1.48
R-20/R-26	1.35	5.70[a]	10.81[a]	1.50	4.53[a]	0.81	24.70
R-25/R-26	3.00	8.87[a]	10.44[a]	1.55	2.25	0.06	26.17
Total	13.55	31.93	38.28	4.65	9.76	1.82	99.99

[a] Ten largest contributions

aphid and host plant clones, with respect to aphid fitness (Table 3), are properties of the larger populations from which the experimental genotypes were sampled. Although it is not possible to draw conclusions about the maintenance of polymorphisms at individual loci, the results of this experiment do support the hypothesis that genotype × environment interactions can maintain genetic diversity in aphid populations. The apparent superiority of aphid clone BG-2 on all experimental plant clones (Fig. 1) does not weaken this conclusion, that is, the significance of the *random* interaction effect indicates that it is unlikely (1) that BG-2 is superior to BC-3, DF-3, and BG-1 over *all* plant genotypes, or (2) that BG-2 is superior to *all* aphid genotypes on the four experimental plant genotypes. The findings of this experiment extend those of Service and Lenski (1982), which showed an interaction between aphid genotypes and two *phenotypes* of a single host-plant clone.

Aphid fitness was much higher on plant clone R-20 than on the other three plant clones (Table 2). The selective importance of this finding is problematical, however, because the influence of *U. rudbeckiae* on host plant fitness is unknown. The potentially harmful effects of aphid infestation may be outweighed by other selective advantages accruing to the R-20 genotype.

Two points bear on the interpretation of the feeding preference tests. First, aphid fitness is strongly affected by variation among individual plants. This variation exists even when plants are grown under similar conditions, and is only partly accounted for by differences in plant size (Service and Lenski 1982). Thus, the model for analysis of the choice tests can have been only partially successful in controlling non-genotypic influences on host quality. Second, results obtained with other insects (Jermy et al. 1968; Jaenike 1982) raise the possibility that aphids might show increased preference for plant types to which they have been previously exposed, even if those plant types reduce fitness. If host choice was influenced by prior experience, the bias would have been in favor of R-6 plants (i.e., opposite to the direction predicted under the hypothesis of fitness maximization). With these considerations in mind, the near significance of both intercept and slope values in the analytical model lends support to the hypothesis that aphids will choose plants (habitats) which confer higher fitness. The importance of such behavior lies in the possibility that conditions for the maintenance of genetic polymorphism may be less restrictive if genotypes select "optimal" habitats (Taylor 1976).

There are indications that the results of these laboratory experiments are applicable to natural situations. Moran (1981) obtained evidence under semi-natural conditions for the types of fitness differences reported here; and Kennedy et al. (1959) describe behavior which is strongly suggestive of active host selection.

Service and Lenski (1982) argued that F_i' is an operational definition of individual fitness in a population growing at the rate F_N. Giesel has pointed out (personal communication) that the formulation of F_i' is similar to that for the "Wrightian fitness" of a genotype (Charlesworth and Giesel 1972). The conspicuous point of similarity is the weighting of individual or genotypic age-specific fecundities by a function of the *population* growth rate. The mean $\bar{F}'$/plant for an aphid genotype (Table 1) is equivalent to the "Wrightian fitness" of that genotype in the experiment.

It should be emphasized that the values of a fitness measure, including F_i', are specific to the conditions under which they are determined. The absolute fitness of a genotype may vary with temperature, population density, or according to the sample of "competing" genotypes, for example. It is also possible that the relative fitnesses of the aphid genotypes in this experiment might have been different if the genotypes had been allowed to interact directly on the plants; many behavioral components of fitness have been excluded from F_i' by the experimental procedures (see Mueller and Ayala 1981).

Fitness is a phenotypic attribute (Templeton 1982). In principle, therefore, individual fitness (F_i') can be subjected to the same quantitative-genetic analyses as other metric characters. In sexual organisms, however, individuals must be mated before their F_i' can be observed. Whether the contribution of another genome will affect the genetic analysis of F_i' is problematical. If the necessity of mating does not introduce insuperable complications, the quantitative-genetic study of F_i' should be a straightforward exercise. Such analyses would permit the determination of heritability and additive genetic variance for fitness, albeit under restricted conditions, and would be a step toward the solution of problems in testing population-genetic theory (Rose and Charlesworth 1981).

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