Evolution, 36(6), 1982, pp. 1276–1282

APHID GENOTYPES, PLANT PHENOTYPES, AND GENETIC DIVERSITY: A DEMOGRAPHIC ANALYSIS OF EXPERIMENTAL DATA

PHILIP M. SERVICE AND RICHARD E. LENSKI
Department of Zoology, University of North Carolina, Chapel Hill, North Carolina 27514

Received September 18, 1981. Revised February 18, 1982

It is well known that many populations possess substantial amounts of electrophoretically detectable genetic variation. There is accumulating evidence that this variation has adaptive significance, and that environmental heterogeneity is an important factor in the maintenance of this variation (see Hedrick et al., 1976, for a review). In the case of clonal organisms, genetic diversity can be maintained in a population if no clone is relatively most fit in all environments. This argument may be applied to a variety of ecological relationships, including that of an aphid to its host plant. Specifically, we hypothesize that there is an interaction between aphid genotypes and host plant phenotypes such that no aphid clone is relatively most fit on all host phenotypes. The plausibility of this hypothesis is supported by three recent studies of natural populations of phytophagous insects (Edmunds and Alstad, 1978; Mitter et al., 1979; Moran, 1981), and by the vast literature on crop plants and their arthropod pests (e.g., Cartier, 1963; Hatchett and Gallun, 1970; Lowe, 1974; Gould, 1979).

In this paper we report on an experiment designed to test the hypothesis of interaction between aphid genotypes and host plant phenotypes, using a new methodology for the analysis of life history data (Lenski and Service, 1982).

MATERIALS AND METHODS

The Organisms

The host plant of the aphid *Uroleucon rudbeckiae* (Homoptera: Aphididae) is *Rudbeckia laciniata* (Asteraceae), an herbaceous perennial. All plants used in this experiment were of a single clonal lineage derived from a plant collected in Chapel Hill, N.C. Individual plants were propagated by repeated separation and division of root crowns.

Uroleucon rudbeckiae is a large, red aphid. It has a typical aphid annual life cycle without host alternation. The two aphid clones used in the experiment were started from single parthenogenetic individuals collected from sites in Chapel Hill, N.C. All plants used for aphid culture were of the same genotype used in the experiment.

Plant Conditioning

Forty-eight plants were randomly assigned to one of four conditioning treatments. The height and number of stem nodes for each plant were recorded before conditioning began. All plants were conditioned and maintained in a single growth chamber. Half of the plants were subjected to a light intensity of 200–310 microeinsteins $\cdot m^{-2} \cdot sec^{-1}$. The remaining plants were placed under a canopy of fiberglass window screening, and received an illumination of 10–15 $\mu E \cdot m^{-2} \cdot sec^{-1}$. (For comparative purposes, the intensity of full sunlight at midday in June was determined to be 1,125–1,275 $\mu E \cdot m^{-2} \cdot sec^{-1}$, and intensities in heavily shaded regions of a forest floor were 6.4–25 $\mu E \cdot m^{-2} \cdot sec^{-1}$.) Half each of the high and low light plants were watered freely, so that the potting medium remained moist at all times. The remaining plants were watered only when when their leaves were noticeably wilted.

These light intensity and watering regimes were maintained for two months

prior to placing aphids on the plants, and were continued for the duration of the experiment. Conditioning treatments produced four distinct host plant phenotypes. Plants which were freely watered increased more in height than those which were water stressed. Freely watered plants flowered, while stressed plants did not. Plants exposed to high light intensity changed from green to a purplish color, presumably due to production of anthocyanin.

Experimental Procedure

At the end of the plant conditioning period, apterous adults or late instar nymphs were taken from stock cultures and placed in individual cages on plants. Each cage was inspected daily. Because of evidence that first-born nymphs develop differently from later nymphs (Blackman, 1979), the first nymph produced in a cage was discarded. When a subsequent nymph was born, the adult was removed from the cage. The retained nymph was the experimental animal. The cage was inspected daily for the remainder of the life of this aphid. Once reproduction began, births were recorded, and young were removed from the cages daily. When an experimental aphid died, it was preserved in ethanol and later measured.

Experimental Design

The experimental design consisted of three fully crossed factors: aphid clone, light intensity, and water regime; and a fourth factor, individual plant, which was nested within the other three. Aphid clone is considered a random effect. The light intensity and water treatments are considered fixed effects. Data were obtained for 144 aphids grown on 48 plants. These were divided among two aphid clones, two light intensities, and two watering regimes. Thus, there were 18 aphids for each of the eight combinations of crossed effects. Each plant had three aphids.

The Dependent Variable

The notation used here follows our previous usage (Lenski and Service, 1982). S_{xi} is the survivorship of individual i to age class x. S_{xi} must be one or zero. B_{xi} is the number of female offspring born to female i in age class x which survive to enter age class 0 (at which time the mother enters age class $x + 1$).

$\bar{S}_x$ is the proportion of the entire cohort of $N = 144$ experimental aphids which survived to enter age class x. $\bar{B}_x$ is the average number of female offspring born to females in age class x.

$$\bar{S}_x = \frac{1}{N} \sum_{i=1}^{N} S_{xi},$$

and

$$\bar{B}_x = \frac{1}{N\bar{S}_x} \sum_{i=1}^{N} B_{xi}.$$

The finite rate of increase for the entire experimental cohort, F_N, can be obtained from the familiar stable-age equation:

$$1 = \sum_{x=0}^{\infty} F_N{}^{-(x+1)} \bar{S}_x \bar{B}_x.$$

The dependent variable with which we are most concerned is F'_i, where

$$F'_i = \sum_{x=0}^{\infty} F_N{}^{-x} S_{xi} B_{xi}$$

$$= \sum_{x=0}^{\infty} F_N{}^{-x} B_{xi}.$$

It can be shown that the mean of the F'_i is equal to the finite rate of increase of the entire experimental cohort (Lenski and Service, 1982). F'_i is the lifetime contribution of individual i to population growth. It is a measure of the future representation of i's offspring in a population growing at the rate F_N.

Within the context of this experiment, we believe that F'_i is, in fact, an operational definition of individual fitness. In this respect, F'_i has the following important qualities. First, it takes account of the ages at which offspring are produced, not just total fecundity. (We assume that there are no systematic differences in quality of offspring: e.g., females which are more fecund do not tend to have less viable offspring.) Second, by virtue of using the common factor, F_N, in the calculation of F'_i, an individual's reproductive output is

TABLE 1. *Summary data. Numbers presented are means except for* F_n *and number reproducing. Sample sizes are 18 unless otherwise specified. Ages are in days. H and L denote high and low.*

Aphid clone	BC-3				BG-2			
Light	H		L		H		L	
Water	H	L	H	L	H	L	H	L
$\bar{F}'$†	3.01	1.14	0.94	0.69	1.60	0.32	1.11	0.24
F_n	1.202	1.131	1.115	1.105	1.156	1.081	1.121	1.069
Number reproducing	18	10	12	12	11	4	14	10
Age of first reproduction‡	12.17	14.70	14.75	16.92	12.00	15.50	15.29	18.80
Daily reproductive output‡	1.36	1.04	0.71	0.79	1.06	1.11	0.59	0.57
Age of death	37.00	25.06	29.17	25.67	26.17	11.44	35.06	27.39
Length, mm‡	1.86	1.74	1.55	1.52	1.73	1.70	1.53	1.47

† Adjusted for covariable Nodes.
‡ Sample size is number reproducing.

considered in relation to the entire "population" or cohort of which she is a part.

RESULTS

The experimental data are summarized in Table 1. Preliminary analysis indicated that individual plants were responsible for a large amount of variance in F'_i. Because the mean square of the plant effect is used in the denominator of several F ratios (Table 2), we introduced a covariable into the model in order to reduce this mean square. We selected the number of plant stem nodes at the beginning of the conditioning period as the covariable. Number of nodes resulted in a greater reduction in the sum of squares associated with the plant effect than did either plant height or an index based on height and number of nodes.

The analysis of variance for the dependent variable F'_i is presented in Table 2. There are statistically significant ($P < .03$) effects due to aphid clone, watering regime, the aphid clone by light intensity interaction, the number of stem nodes, and to "other" (unexplained) individual plant characteristics.

Aphid Clone × Light Intensity Interaction

The interaction between aphid clone and light intensity is shown graphically in Figure 1. Both clones had a lower mean F'_i (hereafter $\bar{F}'$) under low light intensity than under high light intensity. The interaction results from the fact that the response of one clone to the different light intensities was much more marked than was that of

TABLE 2. *Analysis of variance for dependent variable* F'_i.

Source	*d.f.*	*SS*	*MS*	*F* ratio	*F*	*P*	R^2
Model	47	177.3249	M1	M1/M11	5.3525	<.0001	0.7238
Aphid clone	1	14.2201*	M2	M2/M10	8.4043	.0061	
Light treatment	1	20.8089*	M3	M3/M5	2.4128	.3641	
Water treatment	1	40.1140*	M4	M4/M6	13,764.9099	.0054	
Aphid × light	1	8.6245*	M5	M5/M10	5.0972	.0296	
Aphid × water	1	0.0029*	M6	M6/M10	0.0017	.9671	
Light × water	1	8.9887*	M7	M7/M8	2.6536	.3505	
Aphid × light × water	1	3.3874*	M8	M8/M10	2.0020	.1650	
Plant	40	76.6177					
Nodes	1	10.6300	M9	M9/M10	6.2825	.0165	
Other	39	65.9877	M10	M10/M11	2.1200	.0016	
Error	96	67.6682	M11				
Total	143	244.9931					

* Sums of squares adjusted for covariable Nodes

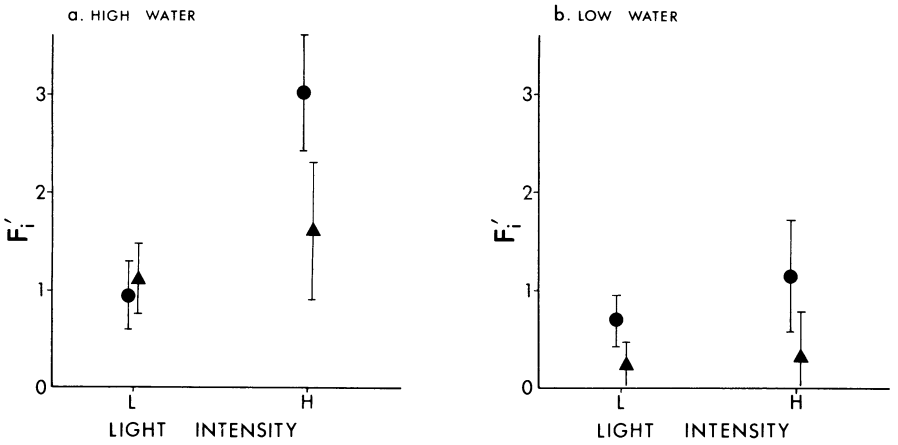

FIG. 1. F'_i (mean ± 2 SE) by light intensity and watering regime. Circles denote one aphid clone, triangles the other. H and L denote high and low.

the other clone. Under the high water treatment (Fig. 1a), this interaction resulted in a reversal in order of $\bar{F}'$ for the two clones. The difference between the two clones under low light intensity (and high water) was not statistically significant, however.

We have assumed that light intensity acts on aphids through modification of plant phenotype, although this assumption is not critical to the existence of a genotype × environment interaction. It is possible, for example, that the interaction is one of aphid genotype × temperature. During the 15 h photoperiod, temperatures in individual aphid cages were unavoidably about 2 C higher on plants under high light intensity than on those under low light intensity.

Determinants of F'_i

The rate of increase of a population depends not only on the lifetime fecundity of females but also on the timing of reproduction, particularly the age at which reproduction begins (Cole, 1954; Lewontin, 1965). Because lifetime fecundity may be confounded with age of first reproduction, as well as with longevity, our analysis considered daily reproductive output rather than lifetime fecundity. Daily reproductive output was obtained by dividing lifetime fecundity by the difference between age of death and age of first reproduction. We performed a stepwise regression of F'_i on age of first reproduction, daily reproductive output, and age of death (Table 3). This regression included only the 91 aphids which reproduced.

As expected, age of first reproduction was the most important of the three factors in explaining variation in F'_i. All three independent variables contributed significantly to the model, however, and this model accounted for more than 87% of the variation in F'_i. These results indicate that reliance on any one life history variable may be misleading when making assessments of relative fitness. It is also noteworthy that F'_i was significantly associated with aphid length ($P\ \{\beta = 0\} < .0001$, $r^2 = .4560$).

The Interpretation of $\bar{F}'$ *and* F_n

F_n (Table 1) is the finite rate of increase calculated separately for each of the eight cohorts defined by the possible combinations of the three crossed factors. That is,

$$1 = \sum_{x=0}^{\infty} F_n^{-(x+1)} \bar{S}_{xk} \bar{B}_{xk},$$

where $\bar{S}_{xk}$ is the proportion of cohort k which survived to enter age class x and $\bar{B}_{xk}$ is the per capita fecundity of females in cohort k while in age class x. In every case, F_n is greater than one, indicating that

TABLE 3. *Stepwise regression of* F'_i *on age of first reproduction, daily reproductive output, and age of death.*

Source	*d.f.*	*SS*	Regression coefficient	*F*	*P*	R^2
Model	3	120.205		201.00	<.0001	0.8739
Age of first reproduction	1	95.532	−0.216	479.23	<.0001	
Daily reproductive output	1	22.785	1.603	114.30	<.0001	
Age of death	1	1.888	0.020	9.47	.0028	
Error	87	17.343				
Total	90	137.548				

both aphid clones could increase in number under all four light and water treatment combinations. This is so in spite of the fact that four of the eight $\bar{F}'$ were less than one. For reasons which we have explained elsewhere (Lenski and Service, 1982), the range in F_n is less than the range in $\bar{F}'$, but the ranking of the F_n over the eight treatment combinations is the same as the ranking of the $\bar{F}'$. Also, the mean of the F_n is not necessarily equal to F_N.

An $\bar{F}'$ less than one in a particular environment does not necessarily indicate that an organism is unable to increase in numbers in that environment. $\bar{F}'$ measures the ability of a cohort to increase proportionally in a larger population which is itself growing at the rate F_N. An $\bar{F}'$ less than F_N indicates that a cohort is declining in size *relative* to the total population. (In this experiment, $F_N = 1.1319$.) F_n is an estimate of adaptedness (sensu Dobzhansky, 1968) to a given environment. $\bar{F}'$ is an estimate of mean individual fitness in an environment.

Assumptions of Analysis of Variance

This paper presents the first use of F'_i in a statistical analysis. We will consider, therefore, at somewhat greater length than usual, the degree to which the data meet the underlying assumptions of the analysis. The discussion in this section refers to the analysis of the mean F'_i per plant. This analysis is identical (in terms of F values and significance levels) to the analysis of the individual F'_i (Table 2), with the exception that there is no explicit "other" (unexplained) plant effect. The use of $\bar{F}'$/plant greatly facilitates the testing of assumptions.

A graphic analysis of residuals using rankits (Sokal and Rohlf, 1969) indicated that the error terms are not normally distributed, although the deviation from normality is not extreme. In general, nonnormality has minor effects with respect to inferences about means in random effects models (Scheffé, 1959). Homogeneity of variance was verified by Bartlett's test and the F-max test ($P > .05$). Both tests, however, assume normality.

For any age x, the individual age-specific fecundities, B_{xi}, are statistically independent, as are the sums of the B_{xi} for individuals. In the calculation of F'_i, however, the B_{xi} are discounted by the factor F_N^{-x}. Since F_N is calculated from the pooled survivorship and fecundity data of the entire experimental cohort, the F'_i are not strictly independent. We have shown (Lenski and Service, 1982) that F_N is a biased estimator of the true finite rate of increase, ϕ, of the statistical population of which the experimental cohort is a sample. When the bias of an estimator is a decreasing function of sample size, as is the case with F_N, jackknifing may be used to further reduce bias (Keyfitz, 1968). Close correspondence between F_N and the jackknifed estimate, $\bar{F}^*$, of ϕ provides evidence that F_N is a good approximation to ϕ. In this experiment, $\bar{F}^* = 1.1325$ and $F_N = 1.1319$. As F_N approaches ϕ, the question of independence among the F'_i becomes moot.

Discussion

We have determined individual lifetime contributions to population growth under minimum density conditions. We believe that the use of minimum density conditions is less objectionable in the case of aphids than for many other organisms. The reproductive adaptations of aphids, particularly parthenogenesis, strongly imply that aphids have been selected for high rates of increase. To the extent that the capacity for rapid increase is associated ecologically with low density conditions, laboratory procedures which minimize density effects are justifiable. Field observations of *U. rudbeckiae* (Service, unpubl. data) support the thesis that aphids are frequently exposed to low densities.

We have assumed that the aphid clone effect (Table 2) is due to genetic differences between the clones rather than to differences in culture conditions of the two clones. We defend this assumption on three grounds: (1) culturing and experimental procedures were designed to minimize any possible culture effects; (2) the culture of each clone was large and apparently healthy at the time of the experiment; and (3) females used to produce the experimental aphids showed no culture related size differences ($t = 0.36$, $P > .70$).

Since aphid clone has been treated statistically as a random effect, we conclude that there is genetic variation within the larger aphid population in ability to survive and reproduce under the environmental conditions established in this experiment. The statistically demonstrated aphid clone × light intensity interaction can also be generalized to the larger aphid population. Therefore, it is highly unlikely that any one aphid clone would be more fit than *all* other aphid clones in both light intensity regimes used in this experiment.

The existence of interactions between aphid genotypes and plant phenotypes suggests that parthenogenesis in aphids may have an advantage in addition to that of rapid population increase. Given appropriate behavior, genotypes may be matched to suitable environments and then replicated rather than destroyed by sexual recombination. A supply of novel genotypes is assured by sexual recombination once each year.

We believe that the quantity F'_i summarizes life history information in a useful and intuitively meaningful way. F'_i can be calculated for a wide variety of organisms, including those with complex life cycles for which it is convenient to observe only a single life stage (e.g., adults in *Drosophila*). It is also possible to observe groups of individuals and to calculate F'_i for each group, thus facilitating investigation of density effects. For some organisms, it may be possible to calculate F'_i from field observations. Lack of strict statistical independence among the F'_i is not a practical impediment to their use in statistical tests in any case where the estimate, F_N, of the population finite rate of increase is based on a reasonably large sample. Any effects due to lack of independence should be negligible and far outweighed by the utility of a quantity which condenses life history data into a single estimate of fitness (see, e.g., Rose and Charlesworth, 1981 p. 193).

Summary

Two clones of the aphid *Uroleucon rudbeckiae* were grown on a single clone of the host plant *Rudbeckia laciniata*. Four different host phenotypes were created by growing plants at two light intensities and under two watering regimes. Age-specific fecundity and survivorship were obtained for individual aphids. These data were used to calculate the lifetime contribution of each aphid, F'_i, to population growth. F'_i is an estimate of individual fitness, and is distinguished from adaptedness.

An analysis of covariance with F'_i as the dependent variable revealed: (1) an effect due to aphid clone; (2) an effect due to water treatment; (3) an effect due to the aphid clone × light intensity interaction; and (4) effects due to uncontrolled phenotypic differences among individual host plants. In a stepwise regression, age of first reproduction, daily reproductive output, and age of death all contributed signifi-

cantly to variation in F'_i. The results are consistent with the hypothesis that genotype × environment interactions maintain clonal diversity in aphid populations.

Acknowledgments

This research was conducted under the direction of Seth Reice. Fred Gould, Nelson Hairston, Charles Mitter, Clifford Parks, Mark Rausher, Sara Via, and Jo Ann White all contributed to this research in various ways and commented upon earlier versions of this manuscript. The experiment could not have been completed without the considerable cooperation of the Botany Department of the University of North Carolina at Chapel Hill. Part of the costs of research were paid for by a grant to P.M.S. from the Smith Fund of the University of North Carolina. R.E.L. was supported by graduate fellowships from the National Science Foundation and the Morehead Foundation at the University of North Carolina.

Literature Cited

Blackman, R. L. 1979. Stability and variation in aphid clonal lineages. Biol. J. Linn. Soc. 11:259–277.

Cartier, J. J. 1963. Varietal resistance of peas to pea aphid biotypes under field and greenhouse conditions. J. Econ. Entomol. 56:205–213.

Cole, L. C. 1954. The population consequences of life history phenomena. Quart. Rev. Biol. 29: 103–137.

Dobzhansky, Th. 1968. Adaptedness and fitness, p. 109–121. *In* R. C. Lewontin (ed.), Population Biology and Evolution. Syracuse Univ. Press, Syracuse.

Edmunds, G. F., Jr., and D. N. Alstad. 1978. Coevolution in insect herbivores and conifers. Science 199:941–945.

Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. Evolution 33:791–802.

Hatchett, J. H., and R. L. Gallun. 1970. Genetics of the ability of the Hessian fly, *Mayetiola destructor,* to survive on wheats having different genes for resistance. Ann. Entomol. Soc. Amer. 63:1400–1407.

Hedrick, P. W., M. E. Ginevan, and E. P. Ewing. 1976. Genetic polymorphism in heterogeneous environments. Ann. Rev. Ecol. Syst. 7: 1–32.

Keyfitz, N. 1968. Introduction to the Mathematics of Population. Addison-Wesley, Reading.

Lenski, R. E., and P. M. Service. 1982. The statistical analysis of population growth rates calculated from schedules of survivorship and fecundity. Ecology 63:655–662.

Lewontin, R. C. 1965. Selection for colonizing ability, p. 77–94. *In* H. G. Baker and G. L. Stebbins (eds.), The Genetics of Colonizing Species. Academic Press, N.Y.

Lowe, H. J. B. 1974. Intraspecific variation of *Myzus persicae* on sugar beet (*Beta vulgaris*). Ann. Appl. Biol. 78:15–26.

Mitter, C., D. J. Futuyma, J. C. Schneider, and J. D. Hare. 1979. Genetic variation and host plant relations in a parthenogenetic moth. Evolution 33:777–790.

Moran, N. 1981. Intraspecific variability in herbivore performance and host quality: a field study of *Uroleucon caligatum* (Homoptera:Aphididae) and *Solidago* hosts (Asteraceae). Ecol. Entomol. 6:301–306.

Rose, M. R., and B. Charlesworth. 1981. Genetics of life history in *Drosophila melanogaster.* II. Exploratory selection experiments. Genetics 97:187–196.

Scheffé, H. 1959. The Analysis of Variance. John Wiley and Sons, N.Y.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman, San Francisco.

Corresponding Editor: D. J. Futuyma