PERSPECTIVES ON PLANT POPULATION ECOLOGY

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SINAUER ASSOCIATES INC. • PUBLISHERS
Sunderland, Massachusetts 01375

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For information address
Sinauer Associates Inc.
Sunderland, MA 01375
U.S.A.

Printed in U.S.A.

Library of Congress Cataloging in Publication Data

Main entry under title:

Perspectives on plant population ecology.

Bibliography: p.
Includes index.
1. Plant populations. 2. Botany—Ecology.
I. Dirzo, Rodolfo. II. Sarukhán, Jose.
QK910.P47 1984 581.5'248 83-20182
ISBN 0-87893-142-2
ISBN 0-87893-143-0 (pbk.)

9 8 7 6 5 4 3 2 1

CHAPTER 11

GENETIC VARIATION WITHIN POPULATIONS

Janis Antonovics

HISTORICAL OVERVIEW

The history of the study of genetic variation in populations has been a sequence of paradoxes. Darwin (1859) fully recognized the importance of genetic variation as the raw material for natural selection and went to considerable pains to document its existence, both in domesticated and wild populations. Yet understanding the origin and maintenance of such variation remained for him a thorn in the side of natural selection. Given his assumption of blending inheritance as the most likely pattern of genetic transmission, he was faced with the paradox of how such variation would not disappear in but a few generations (for discussion, see Fisher, 1958). And this paradox could for him only be resolved by implementing, albeit reluctantly, neo-Lamarckian speculations that environmental variations had concomitant effects on genetic variation. The paradox was seemingly resolved with the rediscovery of Mendel's results and the realization that inheritance was particulate. Yet it was precisely the particulate nature of these Mendelian factors (and the distinct mutants that were used to demonstrate their existence) that led to a conflict between those who ascribed to mutation the major directing role in evolution and those who remained convinced that natural selection could result in emergent novelty by the accumulation of small changes. That such a paradox (whether mutation or selection is more important in evolution) should have retarded evolutionary thinking and generated opposing camps of followers is barely understandable in retrospect (Mayr and Provine, 1980), yet it was the reconciliation of these camps that was hailed as one of the major achievements of the Evolutionary Synthesis. Thus,

Huxley (1942) in *Evolution: The Modern Synthesis* reassured us, in a way that now seems almost patronizing, that "Neither mutation or selection alone is creative of anything important in evolution; but the two in conjunction are creative . . . their interplay is as indispensable to evolution as is that of hydrogen and oxygen to water."

A major component of this synthesis was the growth of population genetics, a field of science devoted explicitly to the analysis of genetic variation in populations. As an area of biology, it was unique in having almost from its inception a strong mathematical basis, more analogous to that found in the physical sciences rather than in biology. It therefore remained a relatively small, influential, yet esoteric field of biology, and as it entered the era of The Synthesis, apart from the almost ritualized sparring of the Wright and Fisher schools on many points of emphasis, it presented at last a relatively paradox-free view of genetic variation. Organisms were generally "wild-types," but Mendelian mutations shuffled by recombination and, more often having quantitative rather than qualitative events, provided the raw materials for adaptation. This period and the following decades were marked by detailed studies on the genetics of natural populations of plants (Clausen and Hiesey, 1958a) and animals (Dobzhansky, 1951; Ford, 1964), and by the growth of a rich theory regarding the maintenance of genetic polymorphism (see Williamson, 1958 for an early review). There was a strong focus and interest in clear-cut single gene (or chromosomal) polymorphisms since their genetic basis was easily understood and their frequency readily quantified. But since such polymorphisms were infrequent, there was little appreciation and little thought given to measuring overall levels of genetic variation in populations. During this time quantitative genetics matured into a highly sophisticated science, but while its techniques could partition and identify genetic effects on the phenotype, these techniques were relatively ineffectual in assessing variation at individual loci: at best one could only talk vaguely about "number of effective factors" (Mather, 1949). However, the commonplace of substantial response to artificial selection in a wide range of quantitative traits led Falconer (1960) to hazard that "natural populations probably carry a variety of alleles at a considerable proportion of loci, even perhaps at virtually every locus." While a brilliant guess, it carried only the weight of impression and not that of precise demonstration.

It was not till the advent of electrophoresis and the discovery by Hubby and Lewontin (1966) of large amounts of genetic variation in natural populations that ideas again fell into disarray and new paradoxes arose. Given that populations were so variable genetically, how did such variation arise, and how was it maintained? And given that an almost infinite number of gene combinations were possible with even a modest level of heterozygosity, why is evolution so often

slow and imperceptible. What generates and what constrains this vast reservoir of genes?

Recent discoveries in molecular biology promise, not only to give us more absolute measures in terms of levels of variation at the base-pair level, but to elaborate a classification of variation based on the type of genetic material affected. Variation may occur, not only in structural and regulatory genes, but also in regions of the genome having no overtly functional roles. Structural genes may contain untranslated intervening sequences; they may be interlaced with large noncoding intergenic sequences; there may be "pseudogenes" whose nonfunctional DNA sequences are closely homologous to overtly functional loci; and much of the genetic variation may be caused by insertion, deletion, and modification of transposable elements. Our view of the genome as a string of beads is in its final death throes, as is our view of it as a tightly integrated unit: I myself am tempted to see it not as a sophisticated spaceship but as a temporarily functional drag racer improvised from a junk yard of past efforts.

The discovery of large amounts of variation in populations (for evidence in plants, see Hamrick et al., 1979; Hamrick, 1979), if we are to believe the historical tapestry woven by Lewontin (1974), led to a flurry of explanations which divided us into a "classical school" of neutralists battling with a "balance school" of selectionists. Such controversy not only stimulated further quantification of genetic variation in a large range of organisms but also led to a growth of theories and ideas about its origins, loss, and maintenance. If the appearance of texts is to be used as a criterion, population genetics had finally, in the 1970s, matured into a singular, recognized branch of biology.

Although theoretical population genetics has proffered numerous alternative explanations for the existence of genetic variation, it has also become clear that the application of any particular theory to any particular polymorphism is fraught with difficulties. This has been called "a problem with too many solutions" by Jones et al., (1977) in a review of probably the most extensively studied polymorphism, shell color and pattern in *Cepaea nemoralis*. They suggest that "complex and perhaps unique explanations [of the polymorphism] are needed for almost every *Cepaea* population." Certain electrophoretic variants have now been studied in sufficient detail, so we can clearly understand the causative chain between physiological process, ecological function, and selection differentials. Yet such studies (e.g., Koehn, 1978; Koehn et al., 1980) are probably as remarkable for the time and effort involved as they are for the elegance of results; indeed, it is difficult to see how they can realistically be repeated on numerous,

preferably randomly chosen, allelic variants. In other words, unless we spend the next 100 years gathering more and more information on more and more polymorphisms, it is likely that a balanced perspective on the relative importance of the various forces impinging on gene frequency in populations will elude us. This has been formidably argued by Lewontin and is the pessimistic conclusion of his paradox of variation (Chapter 5; Lewontin, 1974). In order to escape from this dilemma, we need to look more closely at its causes, at inadequacies of past studies, and at different new approaches that might finesse us out of our apparent fate of having to move the mountain one handful at a time.

If we survey past studies of genetic variation within populations, we see that they have been characterized by particular approaches and assumptions. Above all, such studies have been largely correlative and descriptive. Gene frequency has been correlated with geography, environmental factors, history, breeding system, etc. Although such studies may give information about forces acting to differentiate populations, they rarely answer hypotheses about maintenance of variants *within* populations. Only relatively recently (Linhart, 1974; Schaal, 1975; Hamrick and Holden, 1979; Watson quoted in Antonovics, 1978; Turkington and Harper, 1979b) have descriptive approaches been attempted at a within-population level.

Even at a within-population level, the dynamics of the forces changing gene frequency cannot be understood by a static cross-sectional view (cf. the problem of estimating competition from descriptive studies of species niche relationships). For example, if there is frequency-dependent selection, there may be weak differential fitness at equilibrium; such selection may only be detectable by perturbing the system. Experimental studies on genetic variation in natural plant populations are extremely few, and they have either examined differential fitness in transplants among populations (and then only rarely in natural conditions: Antonovics and Primack, 1982), or they have been carried out in agricultural situations (Suneson, 1969). In animals, too, with few exceptions (Kettlewell, 1956; Gaines et al., 1971; Jones et al., 1977), experimental studies have been largely confined to laboratory populations. We are reminded of the naturalist–experimentalist dichotomy that characterized evolutionary arguments before the evolutionary synthesis (Allen, 1978): this dichotomy is clearly a legacy that evolutionary and ecological studies have yet to outgrow.

Another characteristic of past approaches has been that they have assumed, albeit tacitly, that within-population genetic variance is appropriately explained in terms of either properties of entire groups (e.g., effective population size) or properties of groups of particular classes of genotypes (e.g., average selection coefficients of particular genotypes). This has resulted in studies that ignore two important

questions: What is the relevance of genetically variable progeny to the individual? How important is the genetic and environmental context of a genotype to the fitness of the individual? The first question is the paradox of sexual reproduction: Given the large individual disadvantages associated with sexual reproduction, why is such reproduction so common, so persistent, and in many organisms the only reproductive mode? It is a paradox that has stimulated several books and many papers, yet few experiments. Moreover, it is a question that provides an alternative avenue for developing an overview of the mechanisms whereby genetic variance is generated and maintained in natural populations. If we can understand the *raison d'être* of genetic variance for the individual, then do we need to ascribe reasons to each locus? Group properties such as effective population size, gene frequency, and density may be very important factors impinging on genetic variance in particular instances; there may even be group properties that are disadvantageous to the individual yet that maintain genetic variance (e.g., gene flow between adjacent diverging populations); yet answering the question of why individuals have evolved mechanisms for generating highly variable offspring may well help circumvent our current impasse about the source and maintenance of genetic variation within populations. Our recent approaches can be likened to a study of the immune system where we suddenly have access to all the genetic variants that such a system can produce and have decided that a correct approach is to understand the particular significance of each variant to each population of cells. Although the overall significance of the immune system to the individual is clear, this significance would only be dimly seen (if at all) by a detailed study of each variant. As we shall see, this analogy may not be too farfetched. It can also be pursued further. It was not till monoclonal antibodies were developed that precise study of the mechanisms of their individual interactions and origins was possible. Similarly, we have yet to develop techniques and approaches for studying the fitness of particular individuals rather than of genotypic or phenotypic classes.

Lewontin (1974) concluded that "context and interaction [of genes] are not simply second order effects to be superimposed on a primary monadic analysis. Context and interaction are of the essence." The truth of this assertion has never been tested: We have generally attempted to measure selection on a group of individuals with an average genetic background and in an average environment. The effects of the local external environment and the genetic background of a particular individual are rarely considered. Both are likely to be particularly important in plants: first, because plants do not run around

averaging out environmental heterogeneity, and second, because their mixed-mating systems permit both conservation and change of gene interactions. We do not know the answer to many simple questions. How many gene-character combinations lead to equivalent fitness? For any pair of alleles, what is the within-allele genetic variance in fitness due to background, what is the between-allele variance, and what is the interaction effect of allele with background? More plainly, we may wish to ask what is the genetic variance in fitness, either by way of addressing rather grandiose issues such as the applicability of Fisher's fundamental theorem of natural selection, or by way of addressing nuts-and-bolts field biology questions about whether seedling mortality has a genetic component.

GENETIC VARIANCE AND INDIVIDUAL FITNESS

I have just made out my first grass, hurrah! hurrah! I must confess that fortune favours the bold, for, as good luck would have it, it was the easy *Anthoxanthum odoratum*: nevertheless it is a great discovery: I never expected to make out a grass in all my life, so hurrah! . . . It has done my stomach surprising good.

Darwin to Hooker, June 1855

To illustrate how the maintenance of genetic variation in natural populations can be addressed, not as a group property of the population, but as a property of relevance to the ecology of the individual, I will, in this section, outline a few experiments we have been doing with the grass *Anthoxanthum odoratum*. First I will consider briefly some experiments bearing on measuring the fitness gains from producing genetically variable as opposed to uniform progeny. Then I will use some results from these experiments to argue how fitness of individuals can be measured using cloning techniques. And, finally, I will point out how such techniques can be used to map individual phenotypes onto fitness.

Throughout, we have been using as a model system the grass *Anthoxanthum odoratum* (Sweet Vernal Grass) growing in a mown field that has had the same management for over 30 years. Genetically uniform "progeny" have been obtained by cloning tillers from field-collected adults, and genetically variable "progeny" have been obtained as tillers from plants grown from seed produced by those same adults. These tillers are then transplanted as "phytometers" (Clements and Goldsmith, 1924; Antonovics and Primack, 1982) back into the field in formal experimental designs, with a minimum of disturbance to the natural community. The survivorship and fecundity of these transplants is then followed over a number of years and provides an assessment of the fitness of each of the progeny types.

In the first experiment, genotypes were grown both as plots of uniform asexually cloned individuals and as plots of genetically variable half-sib families (tillers from seeds produced by those same individuals). Each plot was planted in a hexagonal fan design (Antonovics and Fowler, in press) of eighty plants to give a range of densities within each plot (Figure 1A). At each of two sites in the field there were four genotypes, represented as variable and uniform "progeny," and each replicated twice to give a total of ca. 2500 tillers. The tillers were preweighed and planted directly into the ground following rooting for four days in water. Individuals were marked with a toothpick and a plastic ring and measured for survival and fecundity for two years, by which time most of them had died. The results (Figure 2) showed that the genetically variable plots outyielded the genetically uniform ones in both sites. The distribution of fitness as estimated by the net reproductive rate ($\Sigma l_x m_x$) over two years was highly skewed, making statistical analysis difficult. However, if we consider the number of plants in each of the inflorescence number

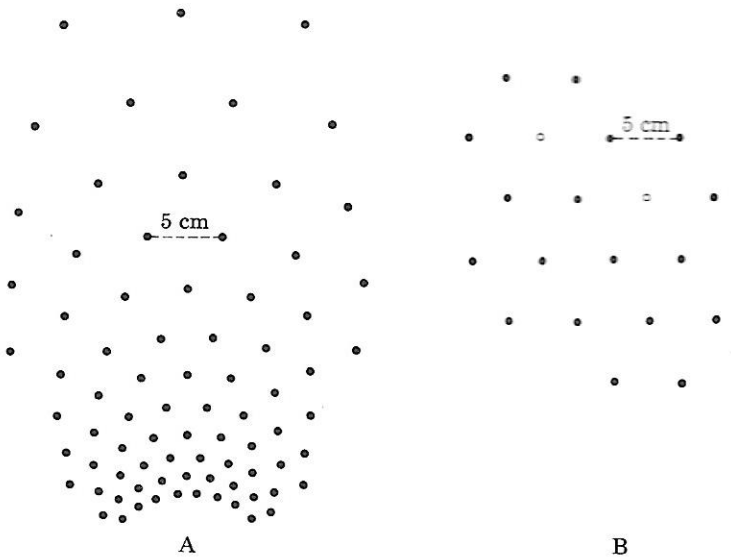

FIGURE 1. Planting designs (A) for experiment to examine effect of density on fitness of genetically variable and nonvariable "progeny," and (B) for experiment to compare fitness of minority and majority genotypes; the minority type is shown in open circles. (See text for further explanation.)

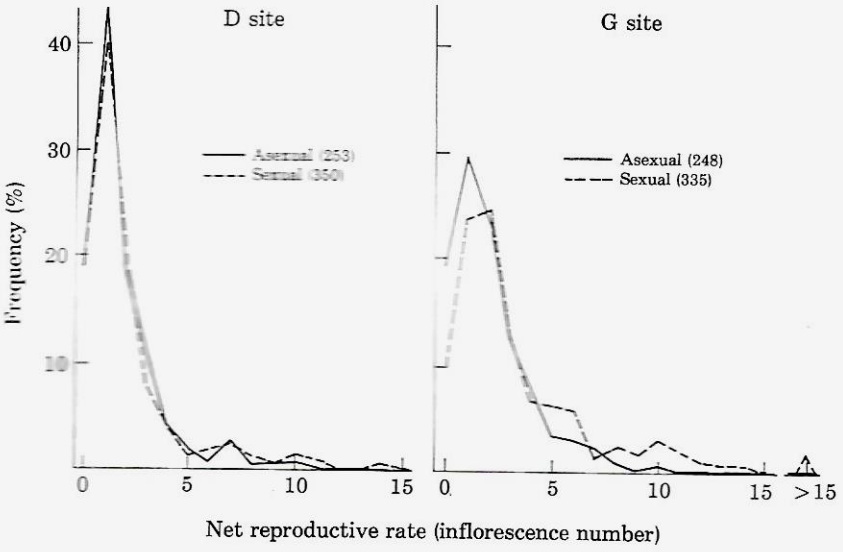

FIGURE 2. Frequency distribution of net reproductive rate over two years for sexually produced half-sibs (genetically variable) and asexual clone (non-variable) progeny arrays, planted at two sites within a field. Sample sizes are in parentheses.

classes of 0, 1, 2, 3, 4, 5–9, and >10, the two-way interaction of inflorescence class and progeny type (sexual or asexual) was significant ($\chi^2_6 = 16.1$; $P < 0.05$). If we consider the second-year reproductive output only, this interaction was highly significant $\chi^2_6 = 17.1$; $P < 0.01$). The overall means gave a relative fitness of the sexual to asexual progeny of 1:0.97 at the D site and of 1:0.80 at the G site. If we consider the second year of the experiment only, these differential fitnesses were much greater (1:0.63 at the D site and 1:0.42 at the G site). Because of the large variance in the data (not surprising in an experiment carried out in situ, in the field), density did not show any consistent pattern in its effects on this relative fitness. This experiment is now being repeated to simulate more closely the seed dispersal profile around a parent individual and so to enable us to develop more realistic estimates of the relative fitness of uniform and variable progeny.

In a second experiment we have looked more closely at the possible cause of this better performance of genetically variable versus genetically uniform progeny. Parents from eight sites were used in the field, and the experiments were planted back into those same sites. At each site, there were 12 plots, and each plot consisted of two genotypes, a "majority type" and a "minority type" within a hexagonal honeycomb of 20 individuals (Figure 1B). There were six pairwise genotype

combinations, each member of a pair being represented as a majority and minority genotype in reciprocal plots. The overall result was that the minority types gave a net reproductive output over three years of 1.89 inflorescences per planted individual, whereas the majority type gave a net reproductive output of 1.26 individuals (for further details, see Antonovics and Ellstrand, in press). Since competitive interaction among individuals were weak, this generalized frequency-dependent selection was probably not mediated by resource partitioning under competition but was probably determined by pathogen effects. Clearly such effects could cause a very large advantage for individuals that are in some sense different from the parent and in a minority around the parent. That frequency dependence is a factor maintaining genetic variance in natural populations is not new; however, its importance as a general mechanism promoting genetically variable progeny has only relatively recently been suggested (e.g., Levin, 1975b; Hamilton, 1980; Lloyd, 1980a; Price and Waser, 1982). Our earlier analogy with the immune system may not be farfetched; genetically variable progeny of an individual may be a pathogen resistance system similar in function to the genetically variable antibody system within individuals of most vertebrate taxa.

However, many other individual advantages of sexual reproduction have been hypothesized (for general discussion, see Williams, 1975; Maynard Smith, 1978a) and this conclusion could well be premature.

Our second issue is the study of the fitness of individual genotypes. Estimating the fitness of an individual requires that we can estimate its contribution to the following generation, that we can ascribe this contribution (in part at least) to a particular phenotype, and that such estimates can be made under natural conditions. To estimate the impact of this contribution on genetic variance, we furthermore need to understand the genetic basis of each phenotype. The problems and difficulties in this process are legion. For example, contribution as measured by schedules of survivorship and fecundity require that we also know male fecundity, that we take into account physiological and genetic quality of the offspring, and that we know how the individual affects the fitness of other related and unrelated individuals in the population. A major problem from a purely methodological standpoint is that a single individual represents an unreplicated unit, whose fitness may be a product of either the local environment or its genotype and whose phenotype and genotype are difficult to determine particularly if, for example, it has died! These problems can be overcome if we use cloned individuals, since then a particular genotype can be replicated and the relative contribution of genotype and en-

vironment to fitness can be assessed. We can illustrate this using data from the minority-advantage experiment discussed earlier. In each experimental plot (Figure 1A), the majority genotype was replicated 18 times as a cloned tiller. Because we know these individuals are genetically identical, the variance in individual fitness (Figures 3 and 4) can now be entirely ascribed to environmental variance: Had these been single individuals of unknown genotype, as in a natural population, the genetic and environmental sources of this variation would be totally confounded. Had different cloned genotypes been randomized within these plots, it also would have been possible to calculate the environmental and genetic contributions to overall fitness and to estimate, with appropriate error variances, the finite rate of increase of each genotype (see Lenski and Service, 1982, for these techniques applied to aphid "clones"). However, in the above studies, the experimental unit was the single genotype plot; and in the case of "home"

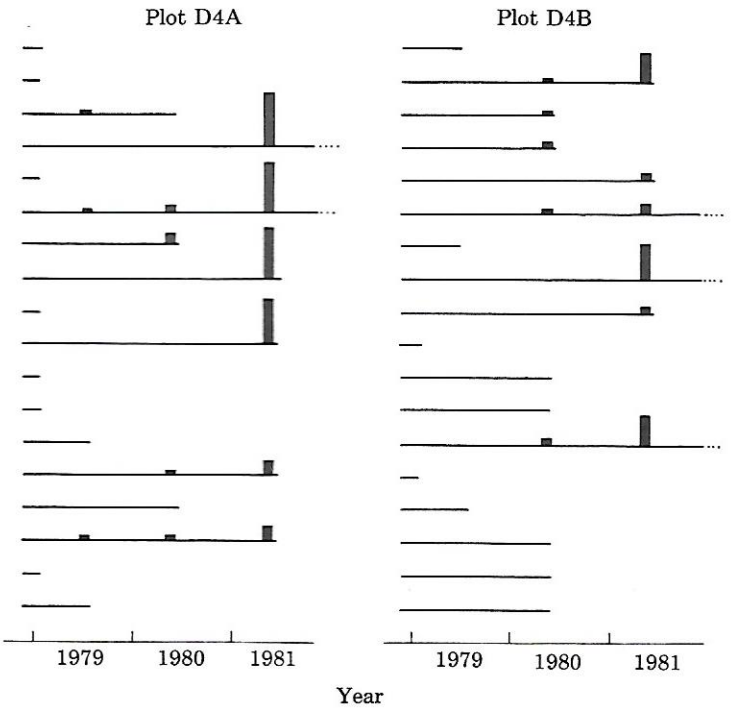

FIGURE 3. Life-histories of genetically identical individuals in two plots (D4A and D4B) of the minority-advantage experiment. Each horizontal line shows the life span of an individual; the vertical bar shows the reproductive output. For scale, the distance between pairs of horizontal lines is equivalent to ten inflorescences.

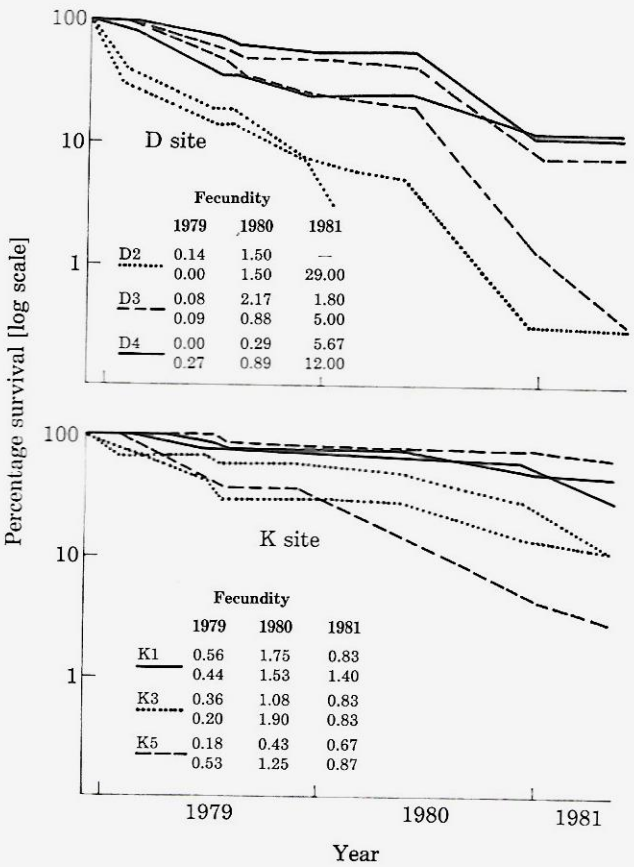

FIGURE 4. Demography of individual genotypes, each cloned as eighteen tillers in two plots of the minority advantage experiment, at two sites (D and K). Similar lines represent replicate plots within each site.

genotypes (i.e., genotypes originating from the same site as that into which the individuals were planted), these were replicated twice. Differences between plots thus also represent environmental variance in fitness, but on a larger (between-plot) scale. Using plot means, it is possible to partition fitness (here measured as net reproductive rate over three years) and its components into variance between sites, between genotypes within site, and between plots within genotype. It can be seen (Table 1) that there are significant differences in survival between genotypes within sites, but that genetic variance in overall fitness with regard to the net reproductive rate is not significant and

TABLE 1. Analysis of variance for differences in survival (arcsin square root transformed), fecundity, and net reproductive rate among genotypes within eight sites (three genotypes per site, each replicated twice).

		Survival to May 1980		Number of inflorescences per flowering plant		Net reproductive rate	
	Degrees of freedom	Mean square[a]	Variance[b] (%)	Mean square	Variance[b] (%)	Mean square	Variance[b] (%)
Site	7	0.553***	60.8	14.16**	25.9	899.6	18.9
Genotypes within sites	16	0.075*	18.3	3.12	0.0	394.1	7.2
Replicate plots within genotypes	24	0.027	20.9	5.25	74.1	329.5	73.9

[a] Significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[b] Percentage of total variation accounted for by each effect was calculated from variance components.

accounts for only 7% of the total variance. Within the subpopulations sampled at different sites in the field, genetic variance in fitness is clearly very low relative to the environmental variance in fitness.

This experiment was not specifically designed to measure genetic variance in fitness and I use it only to illustrate an approach that has not previously been attempted. Moreover, such experiments could be made still more realistic if genetically identical individuals are not vegetative clones but are seed progeny. Genetically identical seeds may be produced by apomixis (as in experiments using parthenogenic strains; e.g., Service and Lenski, 1982) or by special techniques. We are currently exploring techniques using crosses among doubled haploids to generate large amounts of genetically identical, but normally heterozygous, seed. Haploids can be generated in a number of ways. In *A. odoratum* we have used tissue culture of postmeiotic anthers, modifying only slightly techniques used in grass and cereal breeding (Kasperbauer et al., 1980). We have also begun screening (and eventually selection) for production of double embryos. Double embryos may include individuals that develop from an unfertilized synergid cell and thus produce a haploid plant (Riley and Chapman, 1957). In *A. odoratum* we have found such "twins" to occur at a frequency of one per several thousand seedlings, but at present we do not know what fraction are double zygotic, what fraction of these are haploid in origin.

Given such "cloned" individuals, it is possible, not only to place them in natural populations in formal experimental designs, but to assess them for morphological and physiological traits in the greenhouse and laboratory. Character states can thus be "mapped" onto

fitness. Moreover, the characters in question can be analyzed genetically; or conversely, individuals from particular crossing designs can be cloned into the field. Such procedures could, of course, also be carried out with progeny groups rather than with clones; however, in such cases comparisons are less direct and interactions more diffuse. The potential here is tremendous, yet plant population biologists have never exploited the full experimental potential of plants in studying natural selection in wild populations.

Our conclusions from these considerations are naively Darwinian: A powerful approach to understanding the importance of genetic variation in populations is to ask what are the advantages of such variation for the individual and to make direct estimation of individual fitness in natural populations using experimental methods. It is certainly not the only approach and was not a prerequisite for Darwin himself: Cumulative information gained from less intensive, correlative studies will always bear on particular hypotheses and lead deductively to valid conclusions. However, experimental approaches may provide us with new insights and methods of studying variation in natural populations; and in that these approaches are a direct extension and test of Darwin's ideas, it is for the historians to analyze why at least 100 years have elapsed without thorough studies of differential individual fitness within natural populations.