

Ecology and stress from a population genetics perspective

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Evidence for the existence of genetic variation in resistance to salt stress is reviewed with special emphasis on osmoregulation by intracellular changes in amino acid concentration. Correlational studies between environmental stress and different kinds of genetic variation in wild plants are discussed with a focus on the role of the mating system in structuring this variation. It is argued that in development of a science of restoration ecology, such results from ecological genetics should play an important role.

INTRODUCTION

IN the process of its maturation, the science of conservation biology has required the fusion of inputs from ecology, genetics, geology, hydrology and anthropology. Restoration ecology is a science made necessary by the many instances of the failure of conservation. It too demands tools and information derived from many branches of science. Thus, in the design of procedures to enhance the biota of stressed environments, it is not only the geophysical, spatial and temporal organization of the communities that must be understood, but also their genetic resources and their potential to deal with stress. This convergence of interests is evident in two recent volumes, *Evolution, Ecology and Environmental Stress* (Calow and Berry 1989) and *Evolutionary Genetics and Environmental Stress* (Hoffmann and Parsons 1991). In the former there are significant contributions by Hoffmann and Parsons mainly concerning the genetics of *Drosophila*, and by Bradshaw and Hardwick concerning plants. The latter includes lengthy discussions (e.g., Chapter 2) on ecological stresses and their consequences for community structure.

It is natural, given the location of this conference in Tammin, Western Australia, a wheat-growing area where the level of secondary salinity in the shire is over 9%, to focus on plants and on salinity stress. From the genetic viewpoint, however, this is not an easy task because, as pointed out by Bradshaw and Hardwick (1989), "although in animals . . . selection for (or against) response to stress has been carried out, . . . there does not appear to be any fully

recorded work on plants." Nevertheless, there have been studies on crop plants such as barley (Finlay and Wilkinson 1963; Epstein *et al.* 1980) and tomato (Epstein *et al.* 1980). Successful breeding of commercially valuable strains of tomato and melon that can thrive on heavily salinized water has been carried out by a joint Egyptian-Israeli group associated with the binational programme CALAR (Cooperative Arid Lands Agricultural Research). Parallel studies in non-agricultural material appear not to have been done.

Revegetation of the heavily salinated lands near Tammin promises to be a slow and costly process, and there is little time to develop a research programme that would combine ecology and genetics with the objective of advising people "on the ground" as to what mix of plants and which spatial and temporal arrangements have the best chance of success. Nevertheless, the opportunity should be taken to recognize how much basic science in plant physiology and genetics remains to be done before we can properly understand the consequences of any environmental engineering that might be carried out.

Ecological genetics has traditionally been closer to basic than to applied science. But as more "hard" scientists become interested in problems that affect the planet, it is likely that this gap will close. One could make a list of all the subjects that are vital to either conservation biology or restoration ecology but which demand more basic research. Such a list would include the role of plant mating systems in design of restoration programmes, how

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plant-herbivore interactions are influenced by population structure, how inbreeding influences minimum viable population size, etc. It is not my intention, however, to discuss all of these areas which are rich in research potential. Rather, in what follows, I have chosen to focus on some aspects of physiological and genetic variation in relation to stressful environments. It is important to recognize that the causal relations among genetic variation, population structure and environmental variability are extremely complicated. Their elucidation would have far-reaching consequences for both conservation biology and restoration ecology. It is important that the gap between the basic sciences involved in understanding these causal mechanisms and the applied sciences be narrowed.

SALINITY STRESS

California and Texas share with the wheatbelt areas near Tammin the problem of salinity of agricultural soils. Indeed salinity is one of the most important problems for the world's irrigated agricultural lands. The effects of salinity on plants are closely related to the rate of carbon acquisition. The latter is also linked to the loss of water. Stresses imposed by saline soils, high temperatures and rates of evaporation, and limited water availability must be regarded as a suite (McNaughton 1991). Physiological responses of plants to multiple stresses have not been widely studied, but as stated by Bazzaz and Morse (1991) "the overall response of the plant is not necessarily predictable from the response to each resource or stress." For native ecosystems, as well as agricultural plants, it is therefore unlikely that a single physiological mechanism will determine reactions to saline stress.

Organisms that live in saline environments must regulate intracellular osmotic gradients so that cells can maintain turgor. It is surprising, as pointed out by Cheeseman (1988), that so long after the recognition of its importance in stress tolerance, no single gene or gene product that directly controls osmoregulation has been identified. On the other hand, there are some striking correlations between physiological and/or metabolic processes and water stress in plants. One such process is the accumulation of the free amino acids during either water stress or salt stress (Dreier 1983; Bokhari and Trent 1985). Other organic solutes have also been implicated in response to osmotic stress (Hsiao 1973; Young *et al.* 1987).

Under normally inhibiting levels of osmotic potential, namely NaCl solutions of about double the osmotic strength of sea water, bacteria such as *E. coli* or *Klebsiella* are able to grow in the presence of "osmoprotectant" molecules such as glycine, betaine and proline (Le Rudulier *et al.* 1982, 1984). Some of these bacterial osmoprotectants are also the molecules that accumulate during osmotic stress in plants.

In the intertidal copepod *Tigriopus californicus*, Burton and Feldman (1982) measured the accumulation of specific amino acids in whole body homogenates of single adults and exhibited the trajectory of this accumulation during short-term hyper and hypo-osmotic stress. Proline, alanine and glycine were shown to respond to these stresses. For example, *T. californicus* acclimated to sea water, when transferred to 150 per cent sea water (51 parts per salt per thousand), accumulated nearly double the original concentration of proline after 10 days. Similarly, within five hours of the shift from 200 per cent sea water (68 ppt) to sea water, proline dropped to less than 40 per cent of its initial level, while glycine and alanine dropped to trace levels.

Proline accumulates in some plants subject to salinity stress (Stewart and Lee 1974) or water stress (Bokhari and Trent 1985). In cultured cells of the grass *Distichlis spicata* there is a striking correlation between the concentration of salt in the medium and the amount of proline accumulated (Daines and Gould 1985). Cells of *Nicotiana tabacum* selected by growth in a highly saline medium also accumulated a high concentration of proline compared to those grown under standard conditions (Wataid *et al.* 1983). Taken together with the work described in the previous paragraph, this suggests that free amino acid buildup is a useful indicator of osmotic stress in a large number of organisms. Whether these amino acids are breakdown products or synthesized *de novo* is not known in general. The ubiquitousness of the response suggests that genes responsible for this property would be desirable to have in species that are to be used in revegetation of drought or salt-stressed lands.

GENETIC VARIATION AND SALINITY STRESS

In order for artificial selection for resistance to or tolerance of any stress to succeed, there must be heritable variation in the source population. The same is true of evolution in nature; natural selection will result in increased tolerance only if genetic variation for tolerance is present in the population. In fact, the rate at which either artificial or natural selection can move the population's average tolerance is proportional to the additive genetic variance for this trait in the population (Fisher 1930). The *narrow-sense* heritability is a measure of the proportion of total variance in tolerance that is due to the additive genetic components. A useful measure of this fraction is the parent-offspring correlation, provided that there are no interactions between genotypes and environments, and no correlations between the environment of the parent and that of the offspring. *Broad-sense* heritability is greater than the narrow-sense measure because it includes contributions to the variation in the trait due to interactions between alleles at a locus, between genes at different loci and between genes and environments.

Table 1. Heritabilities of salt tolerance in grasses* (Ashraf *et al.* 1986).

| Species | Site | Narrow-sense heritability (%) | Broad-sense heritability (%) |
|---------------------------|-----------------|-------------------------------|------------------------------|
| <i>Holcus lanatus</i> | Abraham's Bosom | 41 | 88 |
| <i>Dactylis glomerata</i> | Abraham's Bosom | 54 | 93 |
| <i>Dactylis glomerata</i> | Foryd Bay | 56 | 90 |
| <i>Holcus lanatus</i> | Aberdesach | 29 | 96 |
| <i>Festuca rubra</i> | Parkgate | 29 | 84 |

*NaCl test concentration is 150 mol/m³.

Ashraf *et al.* (1986) estimated broad and narrow-sense heritabilities of salt tolerance in different populations of some species of grass. The results for a test concentration of 150 mol/m³ of NaCl are shown in Table 1. The narrow-sense heritabilities are quite variable and non-additive components of variance are obviously important because of the large discrepancy observed between the broad and narrow-sense measures.

If one were to select artificially on these grasses for tolerance to this concentration of salt, these non-additive components would limit the response that could be achieved. It must also be emphasized that other aspects of the environment may play a role in determining either narrow or broad-sense heritability. For example, heritability of salt tolerance in a population of plants exposed to a temperature regime that sees 30 days of the year above 35°C with low water availability may be very different from heritability in the same population exposed to a regime where the temperature never exceeds 25°C and water is abundant. This is precisely the point made by Gupta and Lewontin (1982) in their study of the effect of temperature on the proportion of variation in bristle number in *Drosophila* that can be attributed to genetic causes.

The study of Ashraf *et al.* (1986) imposed a specific selection regime on the plants, namely the experimentally set level of salinity, and revealed genetic variation. This variation was unspecified in the sense that no known genes were implicated in the variation that gave rise to the heritability estimates. King (1991) reviewed the available data on the genetics of resistance to salt stress in plants and concluded that little is known about specific genes that might mediate such resistance. I turn now to two studies where the selection regimes were again very specific but where *specific* genes were involved in the response to salinity stress. The first of these, by Le Rudulier *et al.* (1984), involves construction and characterization of mutants of the bacterium *E. coli* in osmoregulatory functions. An example is a rare proline-overproducing mutation proAB that confers osmotic tolerance by increasing the intracellular concentration of proline by 125 times. This gene has been mapped as have a number of others. The class is called *bet/osm* genes and all are involved in the organism's ability to withstand salt stress.

A second case where a specific gene is implicated in response to osmotic stress is the study of *Tigriopus californicus* by Burton and Feldman (1983). As mentioned above, this organism regulates cell volume during osmotic stress by accumulating alanine, proline and glycine. The enzyme glutamate-pyruvate transaminase (GPT), which catalyses the final step in alanine synthesis, has two common forms which are produced by alleles at the structural locus for GPT. In Table 2 the differences in the abilities of the genotypes at this locus to accumulate these amino acids is shown. Alanine in particular shows a significantly higher concentration in individuals carrying the GPT^F allele than in those of genotype GPT^{S/S}. The evolutionary significance of this genetic difference is seen in Table 3 where mortality of young larvae raised in 50 per cent seawater salinity was assessed after transfer to seawater. The effect of genotype on mortality is highly significant; those genotypes that accumulate more alanine due to their GPT genotypes survive better.

Table 2. FAA concentration of individual adult male *T. californicus* maintained in 17 ppt and subjected to hyperosmotic stress (4 hr at 34 ppt, t = 20°C).^a (From Burton and Feldman 1983).

| Genotype | GPT ^{F/F} | GPT ^{F/S} | GPT ^{S/S} |
|--------------------------------------|--------------------|--------------------|--------------------|
| Maintained in 17 ppt | | | |
| Proline | 2.18 ± 0.64 | 4.99 ± 0.57 | 1.17 ± 1.01 |
| Alanine | 2.80 ± 0.86 | 1.46 ± 0.37 | 3.24 ± 1.28 |
| Glycine | 0.82 ± 1.52 | 3.18 ± 0.08 | 1.19 ± 1.61 |
| Hyperosmotic stress (4 hr at 34 ppt) | | | |
| Proline | 19.31 ± 9.53 | 6.22 ± 2.58 | 8.89 ± 5.33 |
| Alanine | 12.68 ± 4.54 | 9.88 ± 1.24 | 6.57 ± 1.86 |
| Glycine | 5.72 ± 2.98 | 4.27 ± 1.85 | 3.19 ± 2.00 |

^aFive individuals of each genotype were analysed (×10⁻¹¹ mol individual). Reported values are means ± SD.

The studies by Ashraf *et al.* (1986) and Burton and Feldman (1983) involved experimental perturbation of the environment. This perturbation was, however, inspired by the desire to understand a natural phenomenon. In the first case, a number of the grasses were exposed to salt spray from the ocean, and in the second the copepod was subject to extreme fluctuations in salt concentration as the splash pools dry and are refilled. In other studies the nature of the stress is not as precisely known and ecological genetic information comes in the form of correlations.

Table 3. Larval mortality during hyperosmotic stress.^a (From Burton and Feldman 1983).

| Genotype | Line | N | Mortality (%) |
|-------------------|-----------|-----|---------------|
| GPT ^{FP} | S1 | 80 | 11.3 |
| | G8 | 25 | 20.0 |
| GPT ^{FS} | S1/III-18 | 25 | 8.0 |
| GPT ^{SS} | III-18 | 77 | 40.3 |
| | I-2 | 113 | 38.0 |

^aYoung larvae (naupliar stages I-III) hatched and reared for at least 24 hr in 17 ppt were transferred to 34 ppt; mortality was determined after six days. Sample sizes were limited by the availability of nauplii from low-salinity stock cultures. The effect of genotype on mortality is highly significant ($R \times C$ test of independence, $G = 31.1$, $df = 4$, $P < 0.005$). GPT^{FP} and GPT^{FS} lines do not differ significantly ($G = 1.75$, $df = 2$, $P > 0.1$).

CORRELATIONS AND POTENTIAL STRESS

Although the two kinds of studies I will refer to below are not directly related to salinity stress, they do concern environments that are climatically fairly similar to that of Tammin. The first study by Hamrick and Allard (1972) relates allozyme frequencies in the slender wild oat *Avena barbata* to environmental gradients in California. These workers ranked the quality of the environment from mesic, most fertile sites, to xeric, least fertile sites and studied genetic variation at 5 loci in populations that span this range of environments from mesic to xeric. In Table 4, taken from Hamrick (1979), it can be seen that the genetic similarity to the genotype found in the mesic environment is highly negatively correlated with the aridity of the environment. The authors suggest that this correlation reflects differential adaptation to arid stress.

Table 4. Listing of *Avena barbata* populations from a hillside ranked by Nei's genetic identity measure. Numbers or letter refer to populations. (From Hamrick 1979).

| Identity group | | | | |
|----------------|-----------|-----------|-----------|-----------|
| M | IM | I | IX | X |
| F (0.992) | B (0.773) | O (0.532) | D (0.361) | 6 (0.183) |
| P (0.991) | 4 (0.617) | H (0.525) | K (0.343) | 3 (0.177) |
| 1 (0.914) | | G (0.518) | I (0.208) | 2 (0.157) |
| C (0.899) | | J (0.490) | | A (0.056) |
| E (0.851) | | | | 7 (0.038) |
| N (0.818) | | | | 5 (0.036) |
| | | | | M (0.022) |
| | | | | L (0.015) |

M, mesic; IM, intermediate mesic; I, intermediate; IX, intermediate xeric; X, xeric. Genetic identities, determined by comparing each population with the mesic genotype, are given in parentheses. The numbers in parentheses represent the genetic identity of the population (using Nei's measure) with a specific genotype designated as mesic.

In this example, as in many others in the ecological genetic literature, the nature of the stress is difficult to make precise. Further, the relationship of the genes to the putative stress through some physiological mechanism has not been made. Finally, *Avena barbata* is a highly self-pollinated plant and exhibits substantial linkage disequilibrium so that forces that have an evolutionary effect on any

single gene also affect many other genes. This makes it an excellent candidate for genetic hitchhiking. That is, it may be some locus not in the survey that is the most important contributor to the correlation, and the observed electrophoretic markers are carried along by linkage. Such correlational studies are, however, extremely important because they set the stage for more reductionist approaches that would otherwise not take place.

A second correlational study is that of Hillel *et al.* (1973b) and involves two closely related diploid grasses of the wheat group native to the Near East, *Triticum speltoides* (= *Aegilops speltoides*) and *Triticum longissimum* (= *A. longissima*). The former is primarily an outcrosser while the latter is predominantly self-pollinated. In this study, means and variances of 36 quantitative characters were computed for plants taken from the wild and grown in experimental plots at the Hebrew University. The plants represented 12 populations (listed in Hillel *et al.* 1973a) spanning a range from near Haifa (in the north of Israel) to near Beer-Sheva (in the south).

Hillel *et al.* (1973b) found a major difference between the two species in the structure of correlations with environmental variables, namely distance from the Mediterranean coast, elevation, and mean annual rainfall. Thus, for most characters, the mean trait values in the selfer were highly correlated with environment (especially mean annual rainfall), while for *T. speltoides* only one character out of 36 was correlated with rainfall. A qualitatively similar difference between the species was found for correlations between environmental variables and variances in quantitative traits.

In these two sections I have tried to show four kinds of environmental-genetic relations: specific stress with specific genotypes (Le Rudelier *et al.* 1984; Burton and Feldman 1983), specific stress with genotypes not known (Ashraf *et al.* 1986), non-specific stress and specific genotypes (Hamrick and Allard 1972), and non-specific stress with non-specific genotypes (Hillel *et al.* 1973b). I would claim that in considering qualities of plants that are desired in relation to the properties of an area to be revegetated, advantage should be taken of all of this information. In the long term, specific genes may be introduced into varieties that were hitherto only studied in terms of quantitative variation, and these genes may make a species very desirable for a specific kind of revegetation, such as is needed in the highly salinated areas near Tammin.

THE MATING SYSTEM

Given that the traits studied by Hillel *et al.* (1973a) were shown to have some degree of heritability, the question is raised of the role of the mating system in populations under stress. (Here mating system refers to the relative probability of self-fertilization or insect pollination or wind pollination,

in fact the statistical relationship between the pollen and ovule that unite to form the offspring). *T. longissimum* has a wider range and does better under more extreme conditions than does *T. speltoides*. What are the characters of a plant species' mating system that might make it a better candidate for use in revegetation programmes for environmentally stressed areas?

First, it cannot be the mating system alone that determines this choice; the kinds of issues described above in connection with stress tolerance should probably have the highest priority in choosing a set of plants to restore a damaged ecosystem. But the mating system is an important contributor to survival in small patchy communities which are likely to be the result of extreme selection regimes. Perhaps the ability to self-pollinate *and* to withstand high levels of inbreeding depression should be sought in species for revegetation purposes. Strong selection is likely to reduce the amount of available genetic variation, and selfers are more likely to withstand such loss of genetic variation that predominantly outcrossing species.

A further consideration is that the selection regime imposed by the difficult growth conditions in the process of revegetation might have the pleiotropic effect of selecting for self-fertilization in addition to tolerance of or resistance to the stress. Feldman and Christiansen (1984) discuss this issue in relation to the mode of pollination and show that in plants whose pollen availability is greatest, self-fertilization is likely to be selected for.

A REMARK ON ADAPTATION AND STRESS

There has been a tendency in some of the ecological literature to regard natural populations as having been "optimized" to cope with their environments. This has led to ecological genetic theory in which the criterion of optimization has been substituted for a proper understanding of the underlying dynamics of the system under study. It is a shortcut used to get around the complicated mathematics of an exact evolutionary analysis. The notions of optimization and adaptation have become intermixed and each has been obfuscated in the process. Thus, we find statements such as that of Sibly and Calow (1989), "Thus most populations are at a steady state . . . and have to return to this state after stress or they become extinct." From this it appears that there are only two alternatives, optimization or extinction; intermediate transient phases of evolution are relegated to an unimportant role.

Understood in this sense it would follow that optimization and adaptation are the same thing; a population cannot be adapted to its environment unless it has optimized some ecological or physiological characteristic. The difficulties involved in applying such reasoning have been addressed by Gould and Lewontin (1979), but the experimental

study of Coyne *et al.* (1983) re-emphasizes the point. Coyne *et al.* brought samples of *Drosophila pseudoobscura* taken from a number of apparently stressful environments. They then applied temperature and desiccation stresses to these flies and found very little relationship between the ability to survive the stress in the laboratory and the putative stress in the wild. It can be argued, and it has been repeatedly demonstrated, that the wild and laboratory stresses are not exactly comparable. Nevertheless, experiments such as those just mentioned call attention to the need for more work in the field while admonishing against simplistic interpretations.

In the search for appropriate organisms with which to restore a damaged ecosystem, I would argue that hard science has a contribution to make. This is not to say that issues of economics and urgency are not crucial. They are. But as the area on our planet that needs restoration increases, it would be wise to make the maximum possible use of advances in basic plant physiology and genetics. Indeed, the process of restoration might join agricultural improvement as a major contributor to the directions that these basic sciences take.

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