ECOLOGICAL GENETICS AND QUALITY CONTROL

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Ecological genetics is a rapidly emerging field of evolutionary biology dealing largely with mutual relations between the genetic makeup and variation of organisms and their environment. It encompasses aspects of molecular, organismal, and population biology and from these diverse disciplines strives to interpret genetic change in populations and species in terms of various evolutionary and adaptive processes. The renaissance in ecological genetics, to a great extent, is the direct outcome of recent developments in molecular and population biology. Our understanding of the molecular structure and function of the genome is rapidly approaching a level of sophistication at which cause-and-effect relationships between selective forces in the environment and genetic changes at the molecular level can be monitored and tested.

QUALITY CONTROL PROBLEMS AND ECOLOGICAL GENETICS

Quality of mass-reared insects is generally defined and measured in terms of how well the insect population functions in its intended role either in the field or laboratory (Huettel, 1976). Quality control is of greatest concern to those engaged in the mass rearing of arthropods. These programs are usually carried out for five reasons: (1) to provide a substrate on which to rear pathogens, parasites, or predators; (2) for use in testing pesticides and other experimental treatments; (3) for silk and honey production; (4) for use in the sterile and genetic engineering techniques of insect control; (5) for release as a biological control agent. The application of ecological genetic principles is probably more important in the latter two where behavior and response to environmental cues must remain normal if released animals are to compete with their wild counterparts or locate their host.

Phenotypic changes that affect the quality of mass-reared insects fall into two broad categories: those that are environmentally induced and, if recognized, reversible; and those that occur at the genetic level and therefore are difficult to correct without special breeding programs. However, genetic changes that lower the quality of mass-reared arthropods may be too subtle to be readily detected. Frequently a problem may not be noticed until the insect fails to perform satisfactorily in the field.

In some cases, relatively simple methods can be employed to monitor genetically the factory-reared animals for changes that might affect their quality. Some mass-rearing programs now use as a technique gel electrophoresis of enzymatic and nonenzymatic proteins. It is now routine to survey 50 to 100 proteins for genetic variation using gel electrophoresis (c.f., Harris and Hopkinson, 1976).

Since its introduction as a tool of the population geneticist by Lewontin and Hubby (1966) and Harris (1966), gel electrophoresis has generated a vast amount of data on the pattern and level of genetic variation in structural genes of organisms. Yet, although the metabolic functions of these enzymes are known, few studies have dealt directly with the relative fitness of the individuals carrying these allelic variants. Thus, it is not surprising that it is still unresolved what proportion of these variants are maintained by selection (c.f., Lewontin, 1974; Nei, 1975; Dobzhansky et al., 1977).

DEFECTIVE FLIGHT ENZYMES AND LAZY FLIES: AN EXAMPLE

One enzyme about which something is known concerning the adaptive function of its allelic forms is \( \alpha \)-glycerophosphate dehydrogenase (\( \alpha \)-GDH; E.C. 1.1.1.8). This enzyme plays a key role in regulating energy flow in the flight muscle of insects during flight (Figure 1) as it governors the transfer of reducing equivalents from cytoplasmic NADH to the mitochondrial electron-transport chain by way of the glycerol phosphate shuttle (Zebe and McShan, 1957; Zebe et al., 1959; Sacktor and Dick, 1962; Johnson, 1974). The amount of GDH present in flight muscle greatly affects flying ability (Kitto and Briggs, 1962; Brosemer, 1967) and mutants which lack GDH cannot fly (O’Brien and MacIntire, 1972).

Because of its central importance in regulating energy flow, allelic variants of this enzyme are
under tight natural selection in several insects such as Drosophila (Miller et al., 1975; Alahiotis et al., 1977), Celia butterflies (Johnson, in press), and screwworm, Cochliomyia hominivorax (Bush et al., 1976). In these species, variation is maintained by a combination of spacial and temporal patterns of environmental variations in temperature. The screwworm fly, a particularly illuminating example, shows how a gene-enzyme polymorphism maintained by environmental factors can affect the success of a pest management program.

The screwworm "eradication" program is based on the principle that wild females mated to sterile males lay no fertile eggs. By flooding the wild population with sterile flies, the vast majority of wild females will produce no offspring. The success of the sterile insect release method (SIRM) depends on the ability of the released flies to compete with wild flies for mates. Although the screwworm program experienced stunning initial success, it ran into difficulties in 1972 when infestations jumped from 473 to over 95,000 reported cases and remained high in subsequent years.

![Glycerophosphate shunt](image)

**Figure 1.** Glycerophosphate shunt which supplies energy to the cell cytoplasm in the form of NAD⁺. GDH is the enzyme responsible for the production of NAD⁺ in the cytoplasm, while it is the enzyme in the mitochondrion which produces the precursor for the production of NAD⁺.

despite the fact that the number of sterile flies released had risen from less than two billion per year at the start of the program in 1962 to about 10 billion over a much smaller area in 1974 (Bush, 1978).

Scientists at the USDA laboratory at the Mission, Texas, screwworm fly facility suspected that conditions in the factory might be inadvertently selecting a strain of flies with reduced competitive ability. In order to ascertain if genetic drift or selection was occurring in the factory, my laboratory studied genetic variation in factory and wild populations of the fly at the request of the USDA. We wanted to see if the process of colonization and factory rearing had any noticeable genetic effect on the flies.

Because natural populations of the screwworm fly are low, ranging from 100 to 200 per square mile (Hightower et al., 1965), released flies must be able to disperse and remain active at appropriate times if they are to find suitable conditions in which to live and to mate in competition with wild flies. Therefore, the genetic study, using standard gel electrophoresis techniques (Harris and Hopkinson, 1976), concentrated on glycolytic enzymes involved with flight, although others were examined as well.

All the factory samples examined differed significantly in their genetic makeup from wild flies.
This was particularly true of loci controlling flight activity, such as GDH, which showed the most dramatic change (Bush, 1975b). In the screwworm, the GDH enzyme exists in two different forms. Almost all of the factory flies were homozygous for one electromorph, GDH₂, while this form of the enzyme was extremely rare in wild Texas populations, which typically retained the alternate form, GDH₁ (Figure 2).

The difference in GDH between the wild and factory flies, therefore, provided an important clue to the problem. Systematic sampling of a new factory strain was undertaken from the time it was colonized in the laboratory to its eventual introduction and adaptation to the factory mass-rearing conditions. The results were clear. As soon as the new strain was introduced into the factory, GDH₁ began to increase in frequency and GDH₂ to decrease. Within six months, GDH₂ had become about as common as in previous factory-adapted strains (Figure 2).

![Graph](https://example.com/graph.png)

**Figure 2.** Changes in the frequency of the GDH₁ allele during colonization and mass rearing in the factory: open circles, small lab populations; closed circles, large factory populations. (Bush and Neck, 1976)

But does the GDH₂ form of the enzyme in any way affect flight activity? Research on the function of the various forms of GDH in other insects, such as butterflies and fruit flies, suggested that each form of the enzyme functioned satisfactorily only within a specific but different temperature range (summarized by Johnson, 1974).

To test whether the two forms of the GDH enzyme found in the screwworm fly were affected by temperature, Dr. G. Barrie Kitto isolated and purified both forms of the enzyme and established that they did indeed have quite different temperature ranges within which they showed optimal activity (Kitto et al., 1976). The factory-type enzyme (GDH₂) was less active in the temperature range experienced in nature. The high constant temperature used to speed development in the factory was apparently exerting a strong selective force favoring GDH₂ over GDH₁ (Bush et al., 1976). This finding also suggested that the competitive ability of the fly in nature would decrease as the frequency of GDH₁ increased, because factory flies would have to cope with a wide temperature range in nature, and those individuals which lacked the GDH₁ enzyme simply would not be able to fly as well as their wild cousins.

Although the mating behavior of wild flies has never been observed in nature, studies on released
factory-reared flies suggest that at least part of the mating activity of the fly occurs in the air and possibly at specific sites which require normal flight activity (Guillot et al., 1977, 1978). Thus, the factory-reared males with reduced flight capacity would be at a considerable disadvantage in competing for mates. In fact, an ARS team found that wild females were attracted to wounds throughout the day from early morning to late afternoon. Factory-reared females, on the other hand, were not active until early afternoon. They simply could not get their flight muscles operating for lack of sufficient energy.

Mating between wild flies may therefore be completed before the factory flies become active enough for sexual activity. These findings and the results of the genetic and biochemical studies indicate that individuals that are either pure GDH1 or heterozygous may be competitively superior to the factory flies that are homozygous for GDH2 (Bush et al., 1976; Bush and Neck, 1976).

In 1977, the defective factory strain was eliminated and replaced by a new strain with the defective allele at low frequency or absent (Whitten, 1977). Care was taken not to mix the old and new strains. Infestation levels during the summer of 1977 dropped to pre-1972 levels (see LaChance, this volume) and, combined with adverse climatic conditions for the fly during the spring and summer, excellent control was regained in Texas, although difficulty is still being experienced elsewhere.

The problems encountered in 1972 could probably have been averted if the factory had had a sound quality control program to monitor factory populations for harmful genetic changes. Some effort was made to measure the effects of diet and other rearing conditions on fecundity, development time, and longevity, etc., but there were no adequate tests based on known adaptive, ecological, and behavioral traits pertinent to the fly in nature except the performance of the released flies. When they failed to perform, it was too late to act, and because insufficient effort had been devoted to basic ecology and behavior, no one knew what might be causing the problem.

Although the screwworm example serves as model, the effect of selection on several other species during mass rearing or laboratory colonization has been established or inferred for a diverse array of biological traits important to the success of a pest control program. These include phototactic behavior and vision (Markow, 1975; Goodenough et al., 1977), locomotory behavior (Chabora, 1969), oviposition, premating and mating patterns (Rossler, 1975a, b), and larval survival in their host (Hatchett and Gallun, 1970).

The recent work of Homyk (1977) and Homyk and Sheppard (1977) exemplifies the ease with which behavior traits can be selected. They were able to recognize and establish 48 behaviorally different strains of D. melanogaster. Those engaged in mass rearing recognize that colonization of a new strain frequently results in an initial rapid shift in many behavioral traits (Boller, 1972). As these traits influence habitat and mate selection, one might incorporate them into a genetically based quality control system.

LIMITATIONS OF THE GEL ELECTROPHORESIS METHOD

That a simple change in the frequency of a rare allele in a structural gene could reduce flight activity in the fly was a fortunate discovery. The factory can now monitor its flies on a routine basis and replace a strain if the GDH2 allele becomes common. Care must be taken, however, that hidden genetic variation does not give a false sense of security and that geographic variation, if any, is taken into account.

Two problems should be recognized. First, electrophoretic techniques can separate only those genetic variants that differ in total electric charge. Thus, two or more alleles may have identical electrophoretic mobility but very different levels of metabolic activity. Occasionally these alleles may be identified by other special techniques (Singh et al., 1976). A second problem involves changes in gene activity which another locus controls. Selection can operate rapidly on the level of enzyme activity in a population without any variation at the structural gene locus as demonstrated by Ward and Hebert (1972) who selected for both increased and decreased ADM activity in populations homozygous for the Adb2 allele. An allele may therefore have reduced fitness in certain genetic backgrounds and superior fitness in others. Simple electrophoretic surveillance may give a false sense of security if those responsible for quality control are not aware of these factors that affect gene expression activity.
This points up a third problem with the use of ecological genetics in quality control programs. Other genetic changes, such as mutations in control (or regulatory) genes (Wilson et al., 1977) which affect patterns of gene activity, could and undoubtedly will arise. These are not amenable to simple analysis by gel electrophoresis. A subtle change in a chemoreceptor, a modified photoperiodic response, or a change in mating behavior may go unnoticed unless appropriate tests are designed to recognize these changes. Such tests should be based on a comprehensive knowledge of the animal's biology. Environmental and intrinsic biological factors regulating mating behavior, flight activity, dispersal, and feeding behavior as well as the distribution of geographic races must be established and understood sufficiently to undertake a sound quality control program.

Furthermore, the ability to distinguish between genetic and nongenetic induced changes in a factory colony is essential. However, even rearing techniques that induce a nongenetic change, such as reduced body size, shortened life expectancy, lowered fecundity, or altered developmental rates, etc., if disregarded long enough, will exert strong selective pressure on the insect and evoke rapid and possibly undesirable genetic changes. Levels of genetic variation can easily be altered rapidly, for instance, when different numbers of environmental factors are varied (Hedrick et al., 1976).

For this reason, some authors suggest that rearing conditions should approximate as closely as possible conditions normally encountered in nature without adversely sacrificing rearing efficiency (Boller, 1972). In practice, a balance must be reached between quality and quantity. Concentrating only on quantity, a characteristic of many mass-rearing programs, will inevitably lead to problems. In the long run, to release a small number of high-quality insects might be better than to liberate a massive number that are ineffective.

GENETIC EFFECTS OF RELEASED INSECTS ON NATURAL POPULATIONS

The ability of wild insects to rapidly adapt at the genetic level to changing environmental conditions is now well established (Hedrick et al., 1976; Mackauer, 1976). This attribute may also have played a role in altering the behavior of wild screwworm flies. Smith (1973) has suggested that the release of over 10 billion irradiated C. hominivorax every year may have selected a strain of wild flies that avoids its factory-reared counterpart; or if mating occurs with a sterile male, a wild female will mate repeatedly until she encounters a fertile male. Similar responses might be expected for other traits that affect mating behavior such as pheromones and responses to environmental cues. This problem will be difficult to resolve in its early stage of development as population levels of the wild insect may only increase in abundance after the spread of the "resistant" genotype. This aspect of ecological genetics, unnoticed or unappreciated in most mass-rearing programs, warrants investigation.

GENETIC ENGINEERING AND MASS REARING

I have stressed the negative side of mass rearing and its potentially detrimental effects on the quality of the released insects. However, genetic engineering can be employed to enhance certain attributes of the insect that might greatly increase its competitive ability (Chambers, 1977). Again, a genetic improvement can only be made if the function of the trait to be improved in nature and the implications of the change are understood. Increased sensitivity to sex pheromone or increased (or decreased) dispersal or host-seeking behavior might be augmented, for example.

These improvements can be carried out through breeding, but great care must be taken to use appropriate breeding systems so that one trait is not selected for at the expense of others. Selective breeding usually involves inbreeding and drift and the loss of potentially beneficial genetic variation. Appropriate tests must therefore be available to monitor the strain under selection. Hoy and her associates, for instance, are developing pesticide-resistant strains of predatory mites (see Hoy and Roush, this volume).

Another genetic engineering tool now being developed is that of molecular cloning and splicing of genes. In the near future (five to 15 years), it may be possible to modify the genome of mass-reared insects or even the natural pest population itself through the release of an altered mass-reared insect or a nonvirulent carrier virus as has been proposed for use in animals (Vosberg, 1977) and plants (Kleinhofs and Behki, 1977). However, this approach may entail certain hazards and other problems which Levin has discussed elsewhere in this volume.
CONCLUSIONS AND GENERAL RECOMMENDATIONS

The application of ecological genetics to quality control problems encountered in mass rearing must necessarily await the identification of those biological attributes in which genetic alteration could significantly reduce the effectiveness of a released insect. In many plant- and animal-feeding parasitic insects, for instance, host selection is frequently linked to mate selection as mating occurs on the host (Bush, 1975a, b). Host selection, therefore, is a crucial step in the behavioral repertoire of a released parasite. Detailed knowledge of the chemical and physical cues used in host selection provides a basis for designing and implementing a biologically meaningful quality control test. It also suggests ways to implement change in rearing methods that will reduce or eliminate the likelihood of genetic alteration through selection.

As the rational use of ecological genetics in quality control must be based on sound biological knowledge of the organisms, time will be required before its incorporation into a mass-rearing program although, as in the case of the screwworm fly, some genetic monitoring may be initiated immediately if sufficient knowledge exists to interpret genetic variation in terms of biological function.

One way to accelerate adoption of ecological genetics into the repertoire of quality control programs would be to have those involved in mass rearing consult directly with individuals engaged in studies pertinent to various aspects of ecology, behavior, and physiology, and to molecular and population genetics of the insects being reared. Experts in these fields in cooperation with applied biologists from the mass-rearing facility could evaluate effectively the rearing program itself; they could also chart the types of studies most likely to be productive and useful to the rearing program.

In some cases, specific research problems can be undertaken by the rearing-facility staff. Where a study requires special training and facilities, the research may be conducted by individuals outside the rearing program on a contract or grant basis.

The scientists should be drawn from a broad range of disciplines and institutions, since many of the recent advances in areas pertinent to ecological genetics are being made in institutions and academic departments that traditionally have had little contact with agriculture and pest management. Every effort should therefore be made to broaden the input into any panel constituted to advise a mass-rearing program.

The major limitation restricting the implementation of ecological genetics in quality control programs is simply the lack of basic biological information on the life history of pest species (Boller, 1972; Bush, 1975b; Mackauer, 1976; Huettel, 1976; Chambers, 1977). The sequence and cues used in the mating behavior, as well as critical ecological factors, have not been established for many economic pests now being mass reared for SIRM or other purposes. Few mass-rearing facilities have suitably trained personnel to undertake the necessary basic biological studies, and insufficient resources have been devoted to basic research by agencies charged with the responsibility of pest management and control. Emphasis has been placed on putting out brush fires rather than on maintaining a balanced research program. As a result, fundamental biological studies not immediately related to pest control generally rank low in funding priority, a trend that must be reversed.

REFERENCES


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