Isozyme Studies of Forest Insect Populations[1]

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Abstract: Data from isozyme analyses are being used to help answer many basic biological questions about forest insect pests and to provide information for a variety of other purposes as well. This paper summarizes the uses of isozymes in quality control of laboratory insect colonies, in studies of insecticide response, as markers of insect parasitoids, and in investigations of species relationships and population differentiation, including patterns of dispersal.

Until recently, genetic studies in forest entomology were handicapped by two rather general assumptions (Wellington 1977). One was that individuals and populations within species are homogeneous units that will behave similarly when treated in a certain way or when subjected to certain environmental conditions. This is an extension of the "if you've seen one, you've seen them all" attitude mentioned earlier in this symposium. If, for example, a particular silvicultural treatment affected an insect population in a certain way in one area, it was often assumed that all other populations of that insect species would respond similarly. A second assumption that hindered initiation of applied biochemical genetics research is a corollary of the first. That is, numerical changes in insect populations (outbreaks, population crashes, and other events) could be explained adequately by understanding external factors affecting the populations.

The life system concept of Clark and others (1967) exemplifies a more realistic approach. According to this scheme, an organism's phenotype and, ultimately, its success in terms of population numbers and persistence, results from the interaction of the genetic makeup of its component individuals with the effective environment.

Since 1974, the importance of intrinsic genetic variation in insect population dynamics has been taken into account in the development of research programs aimed at managing forest insect pests. Data from isozyme analyses are being used to answer basic biological questions and are contributing greatly to our understanding of insects that attack forest trees.

To illustrate the range of isozyme uses in forest entomology, I will summarize some of these research areas.

QUALITY CONTROL OF LABORATORY INSECT COLONIES

Many of the problems associated with conserving genetic resources of forest trees are similar to those dealt with in establishing and maintaining laboratory colonies of insects.

Genetic variation is high in natural populations of insects. This variation is reduced when a limited sample from one area of an insect's range is taken to propagate a laboratory colony. If the number of individuals used to start the colony is small, genetic variation is further reduced in subsequent generations by inbreeding. Colony insects become closely adapted to the laboratory environment and, unless precautions are taken, they may lose the adaptive flexibility characteristic of the wild population. In addition, they may come to differ in important ways from the wild population, making information derived from then of limited value (Bush 1977). After many generations in the laboratory, for example, cultured Lepidoptera, such as budworms and tussock moths, often prefer artificial media to foliage, and groups that diapause (undergo a dormant phase) in the field may become nondiapausing in the laboratory. Although these qualities make the insects easier to rear, their responses in experiments may vary from those of their wild counterparts.

Because responses of colony insects under controlled conditions in the laboratory are often used to estimate behavior of natural populations, it is essential that the degree of overall similarity between the laboratory colony and that of the wild population be known. It is also essential that the colony be monitored over time to detect any further divergence in genetic diversity from wild populations. Quality control—precise, detailed information on differences between laboratory colonies and wild populations, obtained using isozyme analyses—promises to be an integral part of the maintenance of laboratory insect colonies in the future.

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GENETICS OF INSECTICIDE RESPONSE

Because they provide a means of identifying insecticide-resistant genotypes, isozyme analyses have been of great value in relating the presence or absence of specific enzymes to insecticide response. Several studies have shown that changes in esterase sensitivity to inhibition by organophosphorus and carbamate insecticides can confer high levels of resistance (Plapp 1976, Georghiou and Pasteur 1978). Marked differences may occur between esterase zymograms of susceptible and resistant insect groups. For example, the organophosphorus insecticide, dimethoate, acted as a selective agent on esterases of the olive fruit fly; insects that were heterozygous at one of two polymorphic esterase-producing gene loci had a better chance of surviving (Tsakas and Krimbas 1970). Pasteur and Sinegre (1975) found a high correlation between certain gene frequencies at an esterase locus in a mosquito and sensitivity to the toxicant, Dursban.

Among forest insects, inter- and intra-specific differences in response to chemical control agents have been observed repeatedly in the field and, more recently, have been demonstrated in controlled laboratory tests. Robertson and others (1978a) compared the response of six budworm species to selected insecticides and found no single species consistently most or least susceptible to these chemicals. It has been common practice, however, when an insecticide has not been tested on a particular species, to predict its response from results of tests on closely related species. Differential response to insecticides has also been shown in laboratory tests on Douglas-fir tussock moth populations (Robertson and others 1978b).

Effective integrated control of forest pests requires that variations within and between species in insecticide response be more fully assessed and considered. Toward this end, isozyme analyses are now being used on these insects. For example, recent work has shown a relationship between gene frequencies at an esterase locus and insecticide resistance in the tussock moth (Stock and Robertson 1979).

Although, like other areas of isozyme application, this work is in its early phases, we may look ahead to a time when levels of insecticide tolerance can be estimated by simple electrophoretic analyses. From a practical standpoint, adoption of routine genetic assays as part of insect population surveys before implementation of control operations could provide estimates of population response to various insecticides. Ultimately, such information could help in the decisionmaking processes involved in integrated pest management.

MARKERS OF INSECT PARASITOIDS

Parasitoids—parasites that kill the host—are important in natural control of pest insect populations. Techniques to rapidly and accurately assess levels of parasitism are essential in the development of integrated control programs. Parasitism levels are presently assessed through host dissection, which is tedious and often inaccurate, or rearing hosts until parasitoids emerge, a procedure that delays estimates of percent parasitism and requires laboratory space, time, and personnel during the field season. Electrophoresis is emerging as a promising practical alternative to these methods. Because host insects and their parasitoids belong to different taxonomic groups, some isozymes are characteristic enough to be readily associated with one or the other species. On this basis, the presence and identification of parasitoids can be determined (May and others 1977a, Wool and others 1978).

SPECIES RELATIONSHIPS

The value of isozyme data in elucidating systematic relationships is well known (Avise 1974, Ayala 1975). On the basis of electrophoretic differences, for example May and others (1977b) separated the human biting blackfly in Maine from several isomorphic species that do not feed upon humans.

Isozyme information is helping us unravel some taxonomic problems with forest insects, such as the spruce budworm, a species complex described by Freeman in 1967. Most of these recent budworm divisions are based upon traditional morphological comparisons. The large amount of variation present among populations, however, has resulted in considerable uncertainty as to the species status and degree of isolation of many of these groups. In all species, both polymorphic and continuously variable characteristics appear in size and color of larvae, pupae and adults (including wing markings), host plant preferences, cold hardiness, diapause characteristics, and seasonal timing of developmental stages. In addition, varying degrees of reproductive isolation between sympatric species and between allopatric populations within some species occur. To clarify relationships in this group, an integrated approach incorporating pheromone analyses, isozyme analyses, and traditional morphological studies is being used. The electrophoretic method is thus providing a valuable addition to classical approaches to taxonomic studies of forest insect pests.

POPULATION DIFFERENTIATION

Effective control strategies for forest insects are based on a thorough understanding of the population dynamics of each pest. As we learn more about how insect populations are adapted to specific regional host types, we come closer to a management scheme that minimizes disruption of the ecological system of which the pest insect is part. Toward this end, isozyme studies are proving of considerable value. We have begun to answer questions about population differentiation of pest insects as it relates to adaptation to sympatric host plant varieties or species (Bush 1975). The

mountain pine beetle, for example, attacks a diversity of coniferous forest trees over its range, with host species preference varying from place to place. In a mixed stand, the beetles may attack lodgepole pine and apparently ignore other tree species. In a nearby stand, another tree species might be attacked while lodgepole pine goes relatively uninfested. These and other differences revealed in the field and laboratory experiments suggest that stratification of sympatric mountain pine beetle populations may be occuring in many areas. Preliminary work in our laboratory suggests that beetles attacking white pine and lodgepole pine in northern Idaho have different characteristic acid phosphatase frequencies. Similar types of differentiation have been observed in mountain pine beetle populations in Colorado.[3]

Larger differences have been noted between insects in different host tree types when geographic barriers restrict gene flow between them. For example, considerable genetic divergence has occurred between Douglas-fir beetles in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) in coastal Oregon and Douglas-fir var. *glauca* [Beissn.] Franco in northern Idaho (Stock and others 1979). This work supports evidence of behavioral and physiological differences between the two groups of beetles and underlines the necessity for using caution in extrapolating information obtained on one population to the other when control measures are being planned.

Regional population differentiation is also being examined in the southern pine bettle (Namkoong and others 1979), the forest tent caterpillar,[4] the eastern spruce budworm, [5] and several other species of spruce budworm. In the western spruce budworm, we are applying isozyme studies toward clarification of patterns of gene flow among Pacific Northwest populations. It has not been clear, for example whether new budworm outbreaks originate by dispersal from older outbreaks or whether they derive from low-density populations already present in an area. To help answer this question, we have examined genetic characteristics of 20 outbreak populations of western spruce bud worm in Idaho and Montana (Willhite 1979). In this work, one technique that has proven useful is the study of shared rare alleles among the populations (Pashley and Rush 1979). It appears that in some areas of Montana, dispersal plays a more important role in the spread of budworm outbreaks than was previously believed. We have also been able to delineate major patterns of gene flow over the area to help aid prediction of future trends in the spread of budworm outbreaks.

In summary, the union of electrophoretic methodology with applied research in forest entomology is certainly a promising one and we welcome opportunities, such as we have had here today, to exchange ideas and work toward development of productive integrated research programs in forestry.

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