Sibling species and host races are well known to persons involved in the screening of insecticidal biological control agents. Such species and races have usually been revealed through very detailed morphological and, in some cases, cytological analyses. Where divergence of these characters is slight it is often necessary to perform hybridization and behavioral tests which may be very time consuming and which may give ambiguous results. During recent years, however, techniques have been developed which make it possible to determine rather rapidly whether gene flow exists between populations.

These techniques involve the separation of specific gene products, normally enzymes, by the gel electrophoresis of single animals (Hubby and Lewontin, 1966). These enzymes tend to be polymorphic, being produced by several alleles which occur at single (or in some cases multiple) genetic loci, and are called "allozymes" (Prakash et al., 1969).

Most of the allelic allozyme forms of an enzyme may be separated by their variable mobilities in starch gel electrophoresis and stained by enzyme-specific stains. In most systems allelic variants are inherited in a simple Mendelian fashion, and their distribution in natural populations is normally in Hardy-Weinberg equilibrium. It is therefore rather simple to compare the frequencies of alleles, for a number of enzyme systems, in various populations to determine the extent of dissimilarity between them.

The data presented here are a representative portion of the results of a broad study of allozyme variation in a sibling species pair of *P. aridicola* made over the past four years. Both of these parasites are closely related to *P. triticus* widely used in the biological control of *Phacodon eucalyptorum* (Smith). The first member of the pair is *P. australis* (Diptera: Tephritidae) which parasitizes *Heterotheca subaxillaris* (Lam.) Brit. and Bur. (Compositae: Asteraceae) along the Texas gulf coast and also in Florida (Nicholson, 1929-1930). A second host, *H. latifolia* Buckl., is also parasitized in central and eastern for release as biological control agents. These species are very closely related and have been considered a single, ecologically variable entity (Harm, 1965).

The second species is undescribed and will be referred to here as Sp. A. It parasitizes the aster *Nasapeperther phyllolocophala* (DC.) Shinn. This host species follows closely the distribution of *H. subaxillaris* along the Texas coast. It also occurs in Florida but is not parasitized there.

Both *H. subaxillaris* and *P. aridicola* occur in close sympathy over much of their distribution in Texas, although the latter prefers somewhat more moist microhabitats. It is from several of these sympatric populations that the samples discussed below were taken. Both host species are annuals which flower in late fall and produce overwintering seedlings. The fall parasite generations emerge from galls, usually in the flower heads, and oviposit in the young seedlings. Here they produce typical leafy rosette galls at the stem spines which continue to develop throughout the winter and from which the next generation emerges in the spring. These fall and spring generations are the largest of the year for both species, although both continue to produce new generations throughout the remainder of the year.

From laboratory studies it was known that the parasites from different host species would readily intermated and to a limited extent produce fertile hybrids even though there are minor cytological differences between them. It was therefore the purpose of this investigation to determine whether significant gene flow occurs in nature between populations of *P. australis* parasitizing *H. subaxillaris* and populations of what is now called Sp. A parasitizing *P. phyllophaga*.

1 Presented at the 2nd International Symposium on Biological Control of Weeds, Rome, Italy Oct. 4-7, 1971
Methods and Materials

Flies to be assayed were collected as pupae from galls in early spring (March 1-5, 1971) from the numbered collection sites in Figure 1. After emergence, the adults were reared for 24 hours and then frozen on dry ice for storage.

Individual files were homogenized in a drop of tria-citric acid buffer (Shaw and Prasad, 1970) and the homogenate absorbed into two rectangular (4 x 9 mm) strips of Whatman No. 5 filter paper. In this way each fly could be entered on two gels. A total of 24 flies could be run on each gel. One of the gels was run with a continuous tria-citric acid pH 8.0 buffer system and the other with a discontinuous (Poulak) buffer system modified from Shaw and Koem (1968). After electrophoresis each gel was sliced into three layers and each layer stained for a different enzyme system. Phosphoglucomutase (PGM) and alcohol dehydrogenase (ADH) were stained on the continuous buffer gel; isocitrate dehydrogenase (IDH) and aldehyde (ALD) were stained on the discontinuous buffer gel. PGM was stained by a modification of the method in Shaw and Koem (1968). The remaining three systems were stained by modifications of the methods in Shaw and Prasad (1970). Flies from both host plants were generally run on the same gel in various combinations of localities so that alleles present in each species and in different localities could be directly compared.

Results

Four enzyme systems were studied during this investigation. Below are described the general characteristics of each system, the alleles segregating at each of the four loci, and their distribution between the sibling species pair. All of the systems to be discussed are inherited as single autosomal Mendelian characters. All migrate anodally and the alleles are lettered according to decreasing anodal mobility, i.e., Ald-1B migrates faster than Ald-1D. Where two or more enzyme systems stain with the same staining procedure, they are considered to be functionally the same and are labelled in order of increasing electrophoretic mobility, e.g., PGM-1 migrates more slowly than PGM-2. When only one locus produces a stain-specific enzyme then that locus is designated as -1, e.g., ADH-1.

Aldolase. Two aldolase alleles (Ald-1A and Ald-1B) are known and are present in both species. Heterozygotes are single banded and migrate at a distance intermediate between the extremes of the homozygotes.

Alcohol dehydrogenase. Alcohol dehydrogenase is represented by three alleles which are shared by both species. These are designated Ald-1A, Ald-1B, and Ald-1C. A rare fourth allele is known from populations of Sp. A and is designated Ald-1D. Heterozygotes are three-banded.

Phosphoglucomutase. A single phosphoglucomutase system will be analyzed here and is designated PGM-1. Four alleles designated in order of decreasing anodal mobility, Pgm-1B, Pgm-1C, Pgm-1D and Pgm-1E are known at this locus. Pgm-1B, Pgm-1C and Pgm-1D are present in
both species, Pmg-1b has not been found in any population of Sp. A. Heterozygotes are double banded. A second system (Pgm-2) is also present but does not stain consistently well to be used.

Isocitrate dehydrogenase. At least three alleles occur at the IDH-1 locus in *P. australis*. Adh-1b is the most slowly migrating and, in heterozygous combination with either Adh-1a or Adh-1c, forms three-banded heterozygotes. The latter two alleles however have similar mobilities, and heterozygotes between them often appear as elongate spots. In Sp. A the pattern appears to be more complex. A larger number of alleles are present and none of the heterozygotes resolve into discrete, multiple-banded patterns, so that phenotypically IDH appears as a series of elongate spots of highly variable mobility in population samples. We therefore have not been able to score allele frequencies for this system in Sp. A.

Table 1 gives the allele frequencies for three loci in two sympatric populations (designated 1 and 2 in figure 1) of flies taken as pupae from galls on their host plants. Variation between the two populations in the ALD-1 system is only quantitative. At the Galveston Island (1) locality the Adh-1a allele is present in highest frequency in the Sp. A population infecting *M. phylocochaeta*, while Adh-1b is the more common allele in the *P. australis* population infecting *H. subaxillaris*. The same situation occurs at the Padre Island (2) locality, although the frequency of the Adh-1b allele in both species is slightly increased.

<table>
<thead>
<tr>
<th>Locality and No.</th>
<th>Species</th>
<th>Total</th>
<th>Ald-1a</th>
<th>Ald-1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galveston Is. (1)</td>
<td><em>P. australis</em></td>
<td>29</td>
<td>0.172</td>
<td>0.828</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>62</td>
<td>0.718</td>
<td>0.282</td>
</tr>
<tr>
<td>Padre Is. (2)</td>
<td><em>P. australis</em></td>
<td>22</td>
<td>0.045</td>
<td>0.955</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>43</td>
<td>0.593</td>
<td>0.407</td>
</tr>
</tbody>
</table>

**Table 1**

Allele frequencies for samples of *Procestodes australis* and Sp. A reared from galls

<table>
<thead>
<tr>
<th>Locality and No.</th>
<th>Species</th>
<th>Total</th>
<th>Adh-1a</th>
<th>Adh-1b</th>
<th>Adh-1c</th>
<th>Adh-1d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galveston Is. (1)</td>
<td><em>P. australis</em></td>
<td>36</td>
<td>0.118</td>
<td>0.816</td>
<td>0.066</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>83</td>
<td>0.000</td>
<td>0.958</td>
<td>0.042</td>
<td>0.000</td>
</tr>
<tr>
<td>Padre Is. (2)</td>
<td><em>P. australis</em></td>
<td>31</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>50</td>
<td>0.026</td>
<td>0.842</td>
<td>0.195</td>
<td>0.026</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locality and No.</th>
<th>Species</th>
<th>Total</th>
<th>Pgm-1a</th>
<th>Pgm-1b</th>
<th>Pgm-1c</th>
<th>Pgm-1d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galveston Is. (1)</td>
<td><em>P. australis</em></td>
<td>30</td>
<td>0.450</td>
<td>------</td>
<td>0.463</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>81</td>
<td>0.000</td>
<td>0.173</td>
<td>0.327</td>
<td>0.500</td>
</tr>
<tr>
<td>Padre Is. (2)</td>
<td><em>P. australis</em></td>
<td>23</td>
<td>0.304</td>
<td>------</td>
<td>0.696</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Sp. A</td>
<td>35</td>
<td>0.029</td>
<td>0.243</td>
<td>0.671</td>
<td>0.057</td>
</tr>
</tbody>
</table>

The Adh-1 locus at Galveston Is. shows only minor quantitative variation in frequency of the major allele, Adh-1a, but qualitative variation at several of the minor alleles. In the Sp. A population the *Pgm-1a* and *Adh-1d* alleles are absent. *Adh-1b* is also absent in the
F. australis sample, but the Adh-1β allele is present in rather high frequency. At the
Padre Is. locality the Adh-1α and Adh-1β alleles are present in the Sp. A population in low
frequency. In contrast, the population of F. australis is fixed for the Adh-1β allele, that
is the allele is homozygous in all individuals.

At the Pgm locus a more highly variable pattern of variation occurs. At Galveston Is,
both qualitative and highly quantitative variation occurs. Allele Pgm-1β is least common in
Sp. A while it is most common in F. australis. In Sp. A however, its absolute frequency is
low and it appears to be in good balance with Pgm-1α and Pgm-1δ. The F. australis popula
tion, on the other hand, is balanced between Pgm-1δ and Pgm-1β in an allele which does not
occur in the Galveston Is. population of Sp. A. At the Padre Is. locality the Pgm-1δ allele
drops to a rather low frequency in Sp. A and disappears altogether in the F. australis
population. The Pgm-1δ allele becomes by far the most frequent in both populations. A low
frequency of the Pgm-1δ allele, not present in the Galveston Is. population, occurs in the
Sp. A population at this locality.

Discussion

The polymorphic alleles in the four enzyme systems represent four levels of differentia
tion. The first is represented by ALD-1 which shows only quantitative variation in two
alleles. Such variation might be ascribed to differential selective regimes and reduced
gene flow between populations of a single species (Schnick and Raven, 1969) and therefore
does not provide solid information about the breeding patterns of these populations.

The second level of differentiation is illustrated by ADH-1. In this system quantita
tive differences between alleles held in common are minor while qualitative differences are
expressed in the presence or absence of certain other alleles of low frequency. Such vari
ation provides evidence for a lack of panmixis, or even frequent hybridization between pop
ulations on different hosts. If either was occurring, the only way the Adh-1β allele could
remain fixed in the Padre Is. population of F. australis would be for selection to elim
inate consistently the Adh-1α, Adh-1δ, and Adh-1β alleles received from the Sp. A popula
tion. This is hardly possible.

The third level of differentiation occurs in the PGM-1. Not only is there very signifi
cant qualitative variation in the high frequency alleles, but also some of the alleles
held in common are very different quantitatively. Such data would seem to argue against
both panmixis and significant hybridization between the two populations.

A fourth level occurs at the IDE-1 locus in which the phenotypes are recognizably
different in most cases. However, some overlap may occur between the heterozygotes of the
faster migrating alleles of F. australis and the alleles of Sp. A which do not show dis	inct banding. Because of the high frequency of the Adh-1δ allele in F. australis, it is
difficult to differentiate visually between populations of that species and Sp. A. It is
thus quite clear from our results that the populations of Prosoephila willistoni on the two host
plants represent two reproductively isolated species between which no gene flow occurs in
nature.

Differentiation of the types seem above now seems to be widespread and has been found
in all groups of organisms which have been investigated. Hubby and Thrueckmorte (1966) in a
study of Prosoephila compared a series of nine triads, each composed of a sibling species
pair and a closely related but morphologically distinct species. They found that the sib
lings shared about 50 per cent of their proteins on the average, while the nonsiblings
shared 17.5 per cent of their alleles. The variation between siblings went from about 86
per cent to as low as 20 per cent of their proteins in common. They concluded that although
a large number of gene substitutions is not a prerequisite for speciation, close morpholog
al similarity does not necessarily mean an absence of genetic diversity.

Ayala et al. (1970) studied four sibling species in the Prosoephila willistoni group. At
least thirty populations of each were sampled and by testing the progeny of the wild
caught females, 14 loci were assayed. Of these, 83 per cent were polymorphic by the criterion that variant alleles be present in at least 1.0 per cent frequency. By the more stringent criterion of 5.0 per cent or higher frequency, 67 per cent of the loci were polymorphic. Over the large collection area (including Brazil, Venezuela, Colombia and Panama) gene frequencies within a species remained fairly constant. However, the most frequent allele in one species might not be the most frequent in another species. This agrees with our own data discussed above and also with data for many populations not mentioned here.

It is now evident that there is an abundance of variation in insect populations which may be used to differentiate between populations of suspected sibling species. In the more difficult cases, such as host races, some index of genetic similarity might be of value, such as that proposed by Ayala et al. (1970) or more recently by Hedrick (1971). These methods require the survey of a large number of enzyme systems.

Acknowledgments

The authors wish to thank Mr. Edward Bennett, Mrs. Cindy Mc Whorter and Miss Mary Jane Andrews for their invaluable assistance during this study. We also wish to acknowledge the financial support of USPHS GM-15769 and PHR-Training Grant GM-00337.

Literature cited


DISCUSSION

HARRIS Can you use this technique for plants and if so can we use it to determine the origin, say, of our West Coast Hypericum population? If we wanted to bring in a Puccinia sp. could we spot the origin of the weed and then go to that location?

HINOTTEL Yes, this could be done. There is just as much genetic variation in plants as there is in animals. There are high levels of heterozygosity, so the information is there in the plant. The only problem would be that gene frequencies tend to be fairly homogeneous over the range of a species. The problem would be to find alleles in the introduced population which are unique to certain populations in the area of origin. This would require a very extensive survey of many populations. It is also difficult to work with plant material because of the difficulty of freeing the enzymes from the cells.

END OF DISCUSSION

* * *