1 Introduction

The study of insect pests has changed dramatically in the last two decades. Genetics, and more recently molecular biology, have increased their roles and influence in topics associated with agricultural pests. Until recently most applied biologists would probably have doubted the relevance of these areas of knowledge to problems of pest control in agriculture and forestry (Menken and Ulenberg, 1987; Loxdale and Holland, 1989). However, an ever-increasing number of articles covering biological material about pests and including genetic methods can be found in the specialized literature (Robinson and Hooper, 1989; McPherson and Steck, 1996). Unfortunately, these studies are not numerous enough to cover more than a part of the large range of insect pests.

The olive fruit fly, Bactrocera oleae, is a harmful pest in many temperate countries. However, despite its economic importance, genetic studies are scarce and furthermore, the genetic structure of its populations is completely unknown. Only a few mainly ‘descriptive’ studies of these populations are reported in the literature (Zouros and Krimbas, 1969; Tsakas and Krimbas, 1975; Bush and Kitto, 1979; Tsakas and Zouros, 1980; Loukas et al., 1985; reviews of Zouros and Loukas, 1989; and Loukas, 1989; Ochando et al., 1994). There are two aspects of the biology of B. oleae that make this species of special biological interest, in addition to its status as an important pest; first, it is strictly monophagous, and second, its extended commensalism to man, given its almost exclusive dependence on olive cultivation, which puts it at the mercy of agricultural practices.

Taking these considerations into account, the objective of this study was to investigate the genetic variability present in Spanish populations of Bactrocera oleae. Using gel electrophoresis, we have attempted to obtain information about the inherited inter- and intra-population variation. This could, in turn, make it possible to gain insights into the genetic structure of the populations of this special insect, into the gene flow influence in that structure, as well as into other possible evolutionary processes involved in the maintenance of its genetic variability. Understanding the genetic structure of populations of olive fly can facilitate a more efficient and environmentally safe fight against the pest.

2 Material and methods

2.1 Insects

Four natural populations of B. oleae were studied, namely: QUI collected from central Spain (El Pardo, Madrid province), an area having a continental climate; ATA, from the south-east area (Atajate, Malaga province) with a mild and relatively humid climate; and SER and FEL populations, both from western Spain (Serradilla, Caceres province, and Valle de la Serna, Badajoz province, respectively), which have a dry climate, extreme in the summer, and mild in the winter.

Collections were made by harvesting several hundred infested olive fruits and allowing the larvae to pupate in the laboratory. The flies analysed were always those obtained directly from the fruits.

2.2 Electrophoresis techniques

Standard techniques for horizontal starch gel electrophoresis and assay of enzymes were applied to adult flies, according to Ayala et al. (1972) with minor changes. The buffer system was: gel buffer: 76 mM TRIS, 5 mM citric acid, pH = 8.65; electrode buffer: 300 mM boric acid, 60 mM NaOH, pH = 8.1 (Poulakik, 1957; Ayala et al., 1972).

Nine enzymatic loci (chosen at random) were studied: alde-
hyde-oxidase (Ao), acid phosphatase dehydrogenase (Aph),
two esterase loci (Est-1 and Est-2), fructokinase (Fk), hexok
inase (Hk), hydroxybutyrate-dehydrogenase (Hbdh), malate
dehydrogenase (Mdh) and phosphoglucomutase (Pgm). A
Drosophila melanogaster monomorphic strain (Xanol), was
used as control in all gels. This strain has been used as ref
ence ‘100’ for allele designation in all cases. A sample size
of 100 (or as close as possible) was used in most of the cases.

2.3 Data analysis

Three classical statistics to quantify variability were used:
\( P \), polymorphism, the proportion of polymorphic loci (two
criteria of polymorphism were used, the 95% and the 99%
criterion); \( H \), heterozygosity, the average number of het
rozygous individuals; and \( n \), the average number of alleles
per locus.

Departures from the Hardy–Weinberg equilibrium were
tested by means of chi-square of heterozygote adjustments, in
addition to the calculation of Wright’s (1951; 1978) \( F_{IS} \) and
\( F_{IT} \) statistics.

The heterogeneity among populations of different geo
graphical origin was measured by calculation of the fixation
indices. These parameters \( F_{IS}, F_{IT} \) and \( F_{ST} \) were introduced
by Wright (1943, 1951) as ‘inbreeding coefficients’, but they
have been generalized to describe the genetic structure of
populations (WRIGHT, 1965, 1978; NEI, 1977, 1987; NEI and
CHESSET, 1983) and offer a convenient means of sum
marizing population structure.

Genetic identities and distances through the Nei method
(NEI, 1972, 1978), provide insights into the global divergence
between every two populations, and at the same time, allow
the construction of a dendrogram.

With regard to gene flow Wright’s method was used (der
ived from Wright’s fixation indices, WRIGHT, 1943, 1951,
1969). Thus, gene flow (Nm) was estimated from \( F_{ST} \)
using the relationship \( F_{ST} = (1/(4Nm + 1)) \), that is to say,
\( Nm = (1 – F_{ST})/4F_{ST} \), in which \( N \) is the effective size of the population and \( m \) is the proportion of the population that are
migrants.

3 Results

Table 1 displays the amount of variability found at each
of the nine loci studied in the four populations. The allelic
frequencies, the proportion of heterozygous indi
viduals (both, observed and expected from Hardy–
Weinberg equilibrium), and the number of genomes
sampled, are shown. Several features are noticeable in
table 1. First, variation at loci range from highly poly
morphic, such as Ao, Est-2, and Pgm, to almost com
pletely monomorphic, such as Fk and Hk; second, the
same allele has become fixed or represents the highest
frequency in the four populations, with the exception of
Ao in QUI population. However, the gene frequencies
were not uniform in the different populations studied.
On the other hand, the observed and expected fre
quencies of heterozygotes do not agree in most cases as
the four populations show equilibrium frequencies only
for the Pgm locus and for Ao and Hbdh, two of the
populations do so. In the other cases, there are 18 out
of 26 heterozygous frequencies observed which are far
from the Hardy–Weinberg equilibrium. Moreover, in
all the cases except one (locus Ao in QUI population),
the lack of agreement is due to a deficiency of het
rozygotes.

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1 significant at \( P < 0.05 \) level.
2 significant at \( P < 0.001 \) level.
3 no data.
Table 2. Genetic variation in the Spanish populations of Bactrocera oleae: Heterozygosity ($H$), mean number of alleles per locus ($n$), and polymorphism ($P$), at 95% and 99% criteria

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<td>$I$</td>
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Table 3. Estimates of $F$-statistics on variable loci

Table 4. Nei’s genetic identities ($I$, above the diagonal) and genetic distances ($D$, below the diagonal) in four Spanish populations of Bactrocera oleae

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4 Discussion

4.1 Amount of variability

Although only nine loci have been assayed, these may be considered to be a random sample of the genome. Singh and Rhomberg (1987), who have compiled extensive variability and species data, conclude that doubling the number of studied loci has no significant effect on the proportion of polymorphic loci or on the mean heterozygosity (although they do make reference to a higher number of loci). Therefore, taking this opinion and also the fact that a large sample size (about 100 individuals) was used, it is felt that the present data are representative of the real genetic variation in B. oleae.

The olive fruit fly is an extremely monophagous species. According to some authors (Powell, 1971; Levington, 1973; Gillespie and Langley, 1974; Yong, 1992), one would expect a positive correlation between the genetic variation of a species and the degree of its environmental diversity. However, comparison of the genetic variability levels present in the populations of the olive fruit fly studied (tables 1 and 2), with those present in other fruit flies (Diptera, Tephritidae), provides the evidence that genetic variability in B. oleae does not follow that expectation. Thus, available data on Ceratitis capitata (Wiedeman) (Huettel et al., 1980; Morgante et al., 1981; Gasperi et al., 1986, 1991; Loukas, 1989; Milani et al., 1989; Malacrida et al., 1992; Reyes and Ochando, 1994), which is an extremely polyphagous species, show values of heterozygosity, polymorphism, and number of alleles per locus that are much lower, in general, than the values obtained on B. oleae. Information available on other Tephritidae, such as Anastrepha fraterculus (Wiedeman) and the genus Rhagoletis (Dipt., Tephritidae) (Berlocher and Bush, 1982; Malavasi and Morgante, 1983; McPherson et al., 1988; McPherson, 1990) once again serves to underscore the idea of the high degree of variability shown by the olive fruit fly, especially with reference to polymorphism and number of alleles per locus. Comparison with other species of the group, such as Bactrocera cucurbitae (Coquillet), which has a wide host range, or Bactrocera umbrosa (Fabricius) and Bactrocera albistrigata (de Meijere), both with a host range restricted but higher than that of B. oleae (Yong, 1988, 1990, 1992) also indicates a higher degree of variability in B. oleae.

The question which subsequently arises is whether these high values are typical of the species or, on the
contrary, whether our populations represent an exceptional case. Unfortunately, not much data are available on the genetic variability of natural populations of *B. oleae*. Only a few studies can be found in the specialized literature that can be considered ‘populational’, in which at least two populations or various loci have been studied (TSAKAS and KRIMBAS, 1975; BUSH and KITTO, 1979; TSAKAS and ZOUROS, 1980; LOUKAS et al., 1985; reviews from LOUKAS, 1989; and ZOUROS and LOUKAS, 1989; OCHANDO et al., 1994). Specifically, the mean values given by LOUKAS (1989) in his compilation are \( n = 3.467 \) and \( H = 0.188 \); these values are reduced to \( n = 2.538 \) and \( H = 0.153 \), if the biased high information obtained for Est-A and Est-B loci is eliminated (the author does not give information on polymorphism). Previous data (OCHANDO et al., 1994) on two Spanish populations of *B. oleae* and 12 loci reveal values of: \( H = 0.1339, n = 2.6818 \), and \( P = 0.7727 \) or 0.9545 (according to the criteria of polymorphism used). All these values are clearly similar to those obtained in the four olive fly populations analysed. Therefore, the high rate of genetic variability observed seems to be typical of this species.

In summary, the proposed hypothesis as to the possible correlation between degree of environmental diversity and degree of genetic variability clearly does not hold in the strictly monophagous *B. oleae*. Other possible explanations for the high genetic variation observed need to be explored; but for this purpose, more comprehensive information on the complete range of distribution of the species is needed.

### 4.2 Patterns of variability

The first characteristic of note in the analysis of intra-populational variability in the present data is the lack of agreement with Hardy–Weinberg expectation (table 1). This situation is true of all four populations studied and applies to the majority of the variable loci. Interestingly, the lack of concordance between the values obtained and the expected values in a situation of Hardy–Weinberg equilibrium is due in all cases except one (*Ao* in QUI population) to a deficiency of heterozygotes (positive \( F_i \) values, table 3). Although other authors (SNYDER and LINTON, 1958 in Simulidae; MARINKOVIC et al., 1987; and IZQUIERDO and RUBIO, 1989 in *Drosophila*) have observed this same tendency in their results with other species of Diptera, this does not appear to be a general situation. The heterozygous deficiency detected in these olive fly populations could be due to different causes:

1) Technical problems, leading to lack of detection of electromorph classes. Yet the technique has been used on multiple species of insects along with the fact that some heterozygotes were indeed detected, and finally, the excess of homozygotes for some loci is not always general for all four populations for the same loci.

2) The presence of null alleles is another possibility. The fact that no null homozygotes were detected, most probably discounts this possibility.

3) Assortative mating can also lead to deficiency of heterozygotes. However, it seems unlikely that six different allozyme loci would show effects on characters related to mating selection or that all six are linked to loci of this type.

4) Sampling of groups of individuals belonging to different populations, the Wahlund effect, is also possible. However, the present study involves working with a species with a very specific ecology, and the samples were captured in uniform fields and in limited areas. In addition, it is highly unlikely that in all four analysed populations we would be sampling as a unit a group that comes from various subpopulations.

5) Inbreeding brought about by various causes is another possible explanation. One of these causes is bottleneck effects, which appear to occur in the *B. oleae* populations during the winter, although the almost constant availability of fruit throughout the year probably prevents a drastic effect on the population size. Moreover, at the time of sampling (the months of November and December) there have been several generations of expansion (NEUENSCHWANDER et al., 1986; DE ANDRES, 1991) due to the high availability of fruits. Secondly, the sampling of ‘families’ is another cause of inbreeding, but in the case of this species this does not appear probable because each female tends to lay only one egg in each fruit (NEUENSCHWANDER et al., 1986; FLETCHER, 1989a; DE ANDRES, 1991); this sampling was carried out at a time of high availability of fruits; and, in addition, several hundreds of olives were collected in every case. Furthermore, the present populations show an U-shaped allele frequency distribution corresponding to that expected in large populations (NEI et al., 1975; HUETTEL et al., 1980).

It is true that, as SNYDER and LINTON (1954) point out, significant departures from panmixia for some loci joined with equilibrium distribution of genotypes to some others is not compatible with some of the potential causes discussed above for heterozygous deficiency, such as inbreeding or the Wahlund effect.

With respect to the interpopulational distribution of the variability detected, the basic characteristic observed is that for all loci (except *Ao*, in QUI population) the same allele is the one which shows the maximum frequency or is fixed in all the populations. Moreover, the allele which shows the second highest frequency (except *Ao* and *Mdh* for QUI population) is the same. This similarity cannot be explained by random drift as drift effects should be uncorrelated in the different populations.

Notwithstanding, the existence of gene flow, which has a tendency to make populations uniform, cannot be ignored. Therefore, we attempted to infer the role played by gene flow in relation to the genetic structure of the populations.

In the case of *B. oleae*, available knowledge about migratory capacity comes from ‘direct’ information, through ecological studies, by mark–release–recapture, as well as by laboratory experiments. These studies have shown that *B. oleae* is a species with a capacity for dispersal over great distances, although normal behaviour in the presence of abundant fruit trees is of low vagility (NEUENSCHWANDER et al., 1986; FLETCHER, 1989a, b). Furthermore, obstacles such as a road or a pine forest can stop their dispersal. If the facts that
excess dryness is a limiting element for this stenotrophic species, and that the analysed populations are separated by hundreds of kilometres of dry climate (except SER – FEL), are considered then we would first expect the gene flow to be relatively scarce among the populations. In fact, it has been demonstrated that dispersal of individuals and hence gene flow is over much shorter distances than individuals are capable of moving (EHRlich and RAVEN, 1969; SLATKIN, 1985a, 1987).

We have quantified gene flow between the populations using Wright’s method (WRIGHT, 1943). Surprisingly enough, the resulting value can be considered high, $N_{m} = 8.9$. This data seem to indicate that a high gene flow exists among the olive fly populations studied, in spite of what was suspected from previous ecological knowledge of the species and knowing that ‘potentially’ it is capable of migrating long distances. In spite of using common methods for calculation of the gene flow, such as Slatkin’s ‘private alleles’ (SLATKIN, 1985b, 1987) and that derived from Wright’s ‘fixation indices’ (WRIGHT, 1951), as an initial approach, it is apparent that they unfortunately represent only a rough approximation to reality (SLATKIN, 1981, 1985b; DALY, 1989).

Therefore, even when these limitations are kept in mind, if the gene flow is effectively high, we would expect a clear geographic uniformity of the variability. The QUI population shows two evident exceptions (loci Ao and Mdh, table 1) and in general, among the present populations there are clear quantitative differences in the variables loci (table 1). If the gene flow is, in effect, common, all the loci should experience the same standardizing effect, without exception.

According to SLATKIN (1987), species for which direct methods indicate little current gene flow but indirect methods indicate much higher levels, probably have undergone large-scale demographic changes relatively frequently. Gene-frequency differences may exist even if gene flow is common in species in which population extinctions and recolonizations are occurring. Bactrocera oleae seems to go through a certain bottleneck in the winter, but it is not known to what extent, and, besides, this probably does not imply an exterior recolonization of existing populations, since all of them suffer the bottleneck more or less simultaneously.

Perhaps, and this seems most probable to us and is in accordance with the other data already discussed (deviation from the Hardy–Weinberg equilibrium in intrapopulation variability discussion), gene flow could occur at a relatively high rate, at least among three of the populations (ATA, SER, FEL), but local variation in selection intensity (perhaps caused by differences in insecticide use among farmers) might be strong enough to maintain differentiation among populations.

On the other hand, the mean value of the $F_{st}$ fixation index is 0.0274 (table 3), which can be considered low. This could mean that the interpopulational differentiation is low, on average, and so the gene flow effect is strong. However, if the values for each locus are analysed, great differences, in some cases over 10, are seen; and furthermore, for three of the six variable loci analysed, the differentiation is statistically highly significant. This high degree of heterogeneity among loci is not explainable solely by the effect of such a high gene flow as that indicated by the statistical analysis.

The effects of gene flow should necessarily be consistent among all loci because gene flow, as well as genetic drift, both affect all loci and alleles in the same way. Therefore, neither of these processes can individually explain the present variability. Thus, if there is significant heterogeneity among loci, which is true in the present case, this heterogeneity may be taken as evidence for selection (LEWONTIN and KRKAUER, 1973).

The $F_{st}$ fixation index measures the differentiation of loci among multiple populations considered as a whole. However, it presents two disadvantages when globalizing the data: very different $F_{st}$ can be averaged for different loci (as has been shown), and it considers equally the relationship among all the populations, even when the patterns of variability could not be uniformly distributed over the entire area of distribution of the species. This can cloud the relationships of each population with each of the others. For this reason, genetic distances and identities which take into account the relative weight of the different loci in the differentiation, as well as the possible distinct degree of differentiation between different populations compared two by two, have also been used in the interpopulational comparisons. Even so, genetic distances have the disadvantage of normally high standard errors.

Thus, genetic distances were calculated in order to evaluate the variability differentiation among the populations. The values obtained range from 0.0150 to 0.0520 (table 4), which can be considered normal for the geographic population differentiation in insects in general, and in Tephritidae specifically (BERLOCHER and BUSH, 1982; MILANI et al., 1989; GASPERI et al., 1991). With respect to Bactrocera, the only available data are between two Spanish populations with a distance of 0.0941 (OCHANDO et al., 1994), which is higher than those obtained in the present study. In any case, the information available up to this time is too limited to allow for general conclusions as to the degree of geographic differentiation among local populations of this species.

The phylogenetic tree resulting from these distances, is extraordinarily illustrative (figure). Three clear groups stand out: one represented by the QUI population, another by the ATA population, and a third, consisting of SER and FEL populations. These groups are totally coherent with the climatology and geographical

![Figure. Dendrogram of four Spanish natural populations of Bactrocera oleae constructed from the distance matrix shown in table 4](image)
location of the sampled areas (see Material and methods). Consequently, it appears to add arguments for the already-mentioned action of natural selection as being responsible for the uncovered variability.

Summarizing, three important results emerge from the present study. First, the amount of variability detected in the populations of Bactrocera oleae, is higher than that observed in other Tephritidae species, which disagrees with the expected relationship between amount of variability and degree of environmental diversity. Second, gene flow could be high as the ‘general’ similarity in the most frequent alleles stand up. But, and third, the distribution pattern of the variability shows a clear inhomogeneity among loci. As gene flow (like genetic drift), has the same average effect on all nuclear genes, it would be reasonable to conclude that natural selection, probably due, at least in part, to agricultural practices, in combination with gene flow, are responsible for the geographic patterns of allozyme variation observed in B. oleae. Thus, a more efficient fight against this pest must take into account the high level of gene flow among different geographic areas and a higher coordination of agricultural practices from different administrations.

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