COMPETITION AND GENOTYPE-BY-ENVIRONMENT INTERACTION IN NATURAL BREEDING SUBSTRATES OF DROSOPHILA

MAURO SANTOS,1,2 KAREL T. EISSES,3,4 AND ANTONIO FONTDEVILA1,5

1Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
2E-mail: m.santos@cc.uab.es
3E-mail: kteisses@dds.nl
4Present address: Department of Plant Ecology and Evolutionary Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
5E-mail: ibgf0@cc.uab.es

Abstract.—Although empirical studies frequently suggest that genotype-by-environment (G × E) interaction can maintain genetic variation, very few data are available to test for the specific conditions necessary for the existence of a protected polymorphism (i.e., the property of an allele even when initially rare). Drosophila species live in patchy environments and their local population structure may be characterized to some extent by Levene's migration pattern, namely by a single pool of individuals that presumably mate at random and breed on discrete and ephemeral resources. We present here a field experiment that links Drosophila ecology and population genetics, which used the alcohol dehydrogenase (Adh) and α-glycerophosphate dehydrogenase (αGpdh) polymorphic loci in D. melanogaster flies raised from Opuntia ficus-indica fruits (prickly pears). The results show that there is density-dependent mortality in those fruits with a relatively high number of larvae (i.e., selection is "soft") and suggest that there is differential viability for αGpdh genotypes. Additionally, a pattern of G × E interaction for fitness values, which is fully compatible with the theoretical conditions required for the existence of a protected polymorphism, was found after weighting the fitness estimates by the relative contribution that each fruit makes to the total adult population. The strong association between Adhβ and αGpdhα alleles suggests that the occurrence of the common cosmopolitan inversion In(2L)it in the population might be responsible for the negative frequency-dependent selection predicted by Levene's model when genetic variation persists in heterogeneous environments.

Key words.—Adh, αGpdh, density-dependent mortality, Drosophila melanogaster, genotype-by-environment interaction, inversions, Levene's model, natural population, protected polymorphism.

Received April 7, 1998. Accepted September 24, 1998.

Multiple-niche-polymorphism models (reviewed by Felsenstein 1976; Hedrick et al. 1976; Karlin 1982; Hedrick 1986; Barton and Clark 1990) consider the effect of habitat heterogeneity on genetic variation within a population. They assume that patches of different selection regimes are small relative to the gene flow distance, which makes the conditions for a protected polymorphism very restrictive (Maynard Smith and Hoekstra 1980; Hoekstra et al. 1985). This is the case because, provided that genetic drift is negligible, gene frequencies in patchy environments are determined by a balance between selection and gene flow (Hedrick et al. 1976; Endler 1977; Hedrick 1986). Maynard Smith and Hoekstra notwithstanding, empirical biologists usually claim that environmental heterogeneity can indeed maintain genetic variation within a population (McDonald and Ayala 1974; Powell and Wistrand 1978; Powell and Taylor 1979; Nevo et al. 1984; Stratton 1994; Cheetham et al. 1995), but results have been somewhat puzzling in various cases (MacKay 1980, 1981). However, the specific conditions necessary to maintain genetic variation in Levene's (1953) or related models have yet to be empirically tested (Roff 1992; Prout and Savolainen 1996).

Levene (1953) considered a one-locus, two-allele model of selection and extreme migration (i.e., there is a single pool of randomly mating individuals) in which at the beginning of each generation juveniles are scattered at random into a number of discrete units (niches) and different genotypes may have different chances of surviving on different niches. An important ingredient of this model is the existence of density-dependent mortality within niches, otherwise environmental heterogeneity in fitness will not by itself maintain any genetic variation. In Levene's scenario there is no drift, and zygotes enter at Hardy-Weinberg proportions in each niche ("constant-fertile-adult-number"; see Roughgarden 1979). This suggests the following naïve empirical approach to test for a genotype-by-environment (G × E) association: (1) choose a species with a patchy population structure, that is, a species that exploits ephemeral resources and is not truly subdivided on the demographic time scale within a given habitat (Harrison and Hastings 1996); (2) focus on a genetic polymorphism for which there are good reasons to think of environment-dependent selection; (3) collect newly emerged adults from breeding sites and score them for the polymorphic locus; and (4) test for genetic differences across patches and try to estimate fitnesses for each patch. If you happen to be a drosophilist, then the choice seems to be rather obvious, owing to the vast amount of information available: pick up Drosophila melanogaster and focus on the alcohol dehydrogenase (Adh) widespread polymorphism (van Delden 1982; McDonald 1983; Chambers 1988; Kreitman and Akashi 1995).

The natural environment of many Drosophila species consists of fermenting fruit, fungi, or cacti on which individuals feed and breed (Shorrock 1982; Reed and Mangan 1986). In fermenting fruit, ethanol is found up to 5% (v/v) (McKechnie and Morgan 1982), and ethanol is known to be a significant selective pressure for Drosophila (David 1988; Mercot et al. 1994). There is a well-known difference between the two common Adh-S and Adh-F allozymes for enzyme activity and capacity to detoxify ethanol in D. melanogaster (Day et al. 1974; McDonald et al. 1980; Maroni et
al. 1982; Anderson and McDonald 1983). These differences could provide the strong selection needed to give a robust polymorphism in heterogenous environments (Maynard Smith and Hoekstra 1980). However, the situation may be complicated by the occurrence of the common cosmopolitan inversion In(2L)R. This inversion is at relatively high frequency on the southern Iberian Peninsula (~16%) and is nearly always associated with alleles Adh and αGpdh (α-glycerophosphate dehydrogenase) (Alonso-Moraga and Muñoz-Serrano 1986; Lemeunier and Aulard 1992). The inversion seems to confer a survival advantage at high temperatures (van Delden and Kamping 1989), to slow down development and decrease body size at a range of temperatures, and to decline in frequency on ethanol-supplemented food (van Delden and Kamping 1991).

A further complication with the naive scenario above is that in Drosophila the number of contributing parental pairs to each natural breeding site is not constant, but females usually aggregate their eggs and larvae over patches (Atkinson and Sharrocks 1984; Rosewell et al. 1990; Kreitman et al. 1992; Sevener and Alphen 1996). Therefore, most patches are expected to be colonized by very few females, and strong competition among larvae is only expected in those patches where a relatively high number of females lay their eggs. We also expect an appreciable chance (FST) that two genes in the same patch will be identical by descent relative to the whole, presumably panmictic, population (reviewed in Santos 1997a). Therefore, zygotes do not enter at Hardy-Weinberg proportions in the patches as assumed by Levene (1953), and we have to approach the empirical scenario by first answering two questions. To what extent does larval crowding affect the survival probability of juveniles? How can we get a reasonable estimate of input zygotic frequencies in the breeding sites? A helpful experiment which is based on Grimaldi and Jaenike (1984; see also Quezada-Díaz et al. 1997), consists of collecting individual resources infested with Drosophila larvae and dividing each resource in two; the larvae in one half are allowed to develop normally, while those in the other half are provided with extra food to relax competition.

We present here an experimental study of variation in gene frequencies at the Adh and αGpdh loci in D. melanogaster flies raised from Opuntia ficus-indica fruits (prickly pears) and their dependence on the spatial distribution of breeding sites and crowding conditions during the larval stage. We will show that there is density-dependent mortality within those fruits with a relatively high density of larvae (i.e., selection is “soft”), and that there is evidence for G \times E interaction when the relative viabilities of αGpdh genotypes are compared across fruits. After weighting the contribution that each fruit makes to the total adult population, it is also found that a protected polymorphism could in theory be maintained. Even though the experiments were motivated to test for the existence of necessary conditions to maintain genetic variation in spatially heterogeneous environments, it should be stressed that a rigorous proof for the existence of a protected polymorphism is practically impossible because of the difficulty of measuring all relevant parameters. This is particularly true in the present situation because D. melanogaster is not restricted to only one fruit species and the occurrence of resource limitation might be strongly dependent on the time scale of fluctuations in the Drosophila population.

**Materials and Methods**

**Description of Collections**

We carried out a field experiment in the late summer of 1995. The experiment was situated under Opuntia ficus-indica, a treelike cactus, in an abandoned plantation at Carboneras on the Mediterranean coast of Spain (Almeria; 37°N, 1°9′W; for details see Ruiz et al. 1986).

A high number of ripe, freshly picked prickly pears were placed in trays at eight different places in the plantation on September 16. These fruits are sweet and fleshy. Their wet weight at the experimental area ranges between 32 g and 92 g. A single rotting fruit may yield up to approximately 1000 Drosophila flies (M. Santos and H. Laayouni, pers. obs.). A small slice at the top of each fruit was cut to allow for oviposition by Drosophila females (for experimental details see Quezada-Díaz et al. 1997). After one week the trays were taken back to the laboratory. Fifty-four fruits were divided in half longitudinally. One half of each fruit was put singly into 250-ml plastic beakers, whereas the other half was added to a whole fresh fruit cut longitudinally and placed into 750-ml jars on a bed of perlite covered with gauze. We have previously shown that: (1) the cutting treatment does not seem to have any disturbing effect on Drosophila; (2) the D. melanogaster flies start emerging after about 10 days, counting from the first day the fruit is left in the field; (3) the number of flies raised is positively related to the number of days the fruits are left in the field (from 1 to 4.5 days); and (4) no effect of crowding is apparent for D. melanogaster after 4.5 days (Quezada-Díaz et al. 1997).

The flies were removed once or twice per day after the first emergence and counted. The Drosophila sampled were divided into four species groups: melanogaster, simulans, buzzatii, and hydei. Care was taken to distinguish D. melanogaster and D. simulans, the males of which can easily be distinguished by their external genitalia (Sturtevant 1919), and the females of which can be distinguished (painstakingly) on the basis of eye size and (easily) by the pigmentation pattern of the sixth tergite (Eisses and Santos 1998). The adults of D. melanogaster were placed into eppendorfs and stored at -29°C until electrophoresis.

**Starch Gel Electrophoresis**

Samples were prepared for electrophoresis by homogenising whole flies with a Teflon stave in a potter plate in 25 μl 10× diluted gel buffer, enough to soak two 3 × 7 mm Whatmann 3MM papers. Approximately 15–80 individuals were scored per gel for alcohol dehydrogenase (ADH, EC 1.1.1.1), glycerol-3-phosphate dehydrogenase (αGPDH, EC 1.1.1.8), and octanol dehydrogenase (ODH, EC 1.1.1.73). Electrophoresis was done in horizontal starch gels with discontinuous Tris-citrate buffer (Poulrik 1957). Staining methods were as described in Eisses et al. (1979) with slight modifications. The Groningen D. melanogaster SSN (Kever and van Delden 1985) and FFF strains were used as reference in electrophoresis. The first strain is homozygous for Adh and αGpdh.
Table 1. Information available for each Opuntia ficus-indica experimental fruit (e.g., locus αGpdh), where $M_j$ is the total number of adults observed with genotype $j$ in fruit section $i$.

<table>
<thead>
<tr>
<th>Half</th>
<th>αGpdh genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>αGpdh&lt;sup&gt;BB&lt;/sup&gt;</td>
<td>αGpdh&lt;sup&gt;BB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Supplemented (“input”)</td>
<td>$M_{11}$</td>
<td>$M_{12}$</td>
</tr>
<tr>
<td>Non-supplemented (“output”)</td>
<td>$M_{21}$</td>
<td>$M_{22}$</td>
</tr>
<tr>
<td>Total</td>
<td>$M_1$</td>
<td>$M_2$</td>
</tr>
</tbody>
</table>

alleles, and the second is homozygous for Adh<sup>h</sup>, αGpdh<sup>F</sup>, and Odh<sup>F</sup> alleles. All three loci segregate for two alleles at Carboneras, but Odh was monomorphic (1% criterion) with Odh<sup>F</sup> at a frequency of 0.991.

Statistical Analyses

The following statistical analyses were carried out for a subset of the O. ficus-indica experimental fruits ($n = 16$; see below). The raw data basically consist of two-way tables for Adh and αGpdh genotypes in each half of a fruit. This leads to a somewhat complex four-way contingency table that can be analyzed by using log-linear analyses (Bishop et al. 1975; Christensen 1990) and involves applying a hierarchical set of increasingly complex models. The resulting log-likelihood statistics ($G$) were used as the basis of an analysis of chi-square.

The saturated log-linear model for the four-way dimensional table is:

$$f_{ijkl} = u + u_{1(i)} + u_{2(j)} + u_{3(k)} + u_{4(l)} + u_{12(ij)} + u_{13(ik)} + u_{14(il)} + u_{23(jk)} + u_{24( jl)} + u_{34(ikl)} + u_{124(ijkl)}$$

where $f_{ijkl}$ is the expected frequency in cell $ijkl$ ($i = 1, 2, \ldots, 16$ [fruits]; $j = 1, 2$ [halves per fruit]; $k = 1, 2, 3$ [Adh genotypes]; $l = 1, 2, 3$ [αGpdh genotypes]), $u$ is the grand mean of the logarithms of the probabilities and the single-numbered $u$s are the main effects of the four variables defined by the subscript letters as above, the double-numbered $u$s are the first-order interaction effects, the triple-numbered $u$s are the second-order effects, and the last term is the third-order interaction effect.

The model above may look formidable, but it allows us to make inferences about what may be biologically relevant phenomena. For instance, take the first-order interaction effect $u_{24}$, which is defined as representing the interaction between fruit halves and αGpdh genotypes averaged over fruits and Adh genotypes (see Table 1). In a $2 \times J$ table, each parameter of the first-order interaction is a function of $J - 1$ cross products (Bishop et al. 1975). Therefore, if we make the reasonable assumption that genotype frequencies in the supplemented (i.e., “nonlimiting” resources) halves estimate the zygotic (input) frequencies in the fruits (see below), then the corresponding cross-product ratios for the first two columns in Table 1,

$$\alpha_{1,2} = \frac{M_{11}M_{22}}{M_{12}M_{21}},$$

and for the first and third column,

estimate the differential larval viabilities of homozygotes αGpdh<sup>FF</sup> and αGpdh<sup>SS</sup>, respectively, relative to that of heterozygotes (Manly 1985). If the first-order interaction effect $u_{24}$ is statistically significant, this would provide some evidence for differential larval viabilities among αGpdh genotypes over all fruits. Additionally, if the second-order interaction effect $u_{24}$ is also statistically significant, the interpretation could be that the larval viabilities are not constant across fruits, namely that there is some indication of G × E interaction for fitness values. However, some caution is necessary because for small expected frequencies the cross-product ratio estimator is liable to be rather biased (Manly 1985).

Additional genetic inferences can also be made from the log-linear model. The effect $u_{24}$ represents the interaction between Adh and αGpdh genotypes over all fruits and halves and relates to the presence of genotypic disequilibrium between the two loci. Haber (1984) advocated the use of log-linear models for characterizing the associations between pairs of genes at two loci, and Weir and Wilson (1986) illustrates the log-linear approach and clarifies the relationships between these models and the two-locus disequilibrium coefficient analysis. In this paper, we will use Weir’s (1979, 1990; see also Weir and Cockerham 1989) additive model to analyze linkage disequilibria whenever the interaction terms involving both loci are statistically significant.

The computer programs used for data analyses were the population genetics software GENEPOL (Raymond and Rousset 1995a) and the statistical software STATISTICA (StatSoft, Inc. 1996). They were run on a 486 (66 Mhz) PC-compatible.

Results

Emergences from Opuntia Fruits

All O. ficus-indica experimental fruits yielded Drosophila adults, and the total number of flies was 9115 (from 30 to 533 flies per fruit). The proportions for the four species emerged were: 27% D. melanogaster, 68% D. simulans, 4% D. buzzatii, and 1% D. hydei. These figures agree relatively well to the previously obtained values in the same population at Carboneras (Quezada-Díaz et al. 1997). In what follows, we will restrict our attention to D. melanogaster.

The average number ($±$ S.D.) of D. melanogaster adults per fruit was 45.11 ($±$ 57.56). The correlation between the numbers in both halves (transformed as $\ln (N + 1)$) was $r = 0.62$ ($P < 0.001$) and that between the numbers of D. melanogaster and D. simulans in each fruit (adding both halves) was $r = 0.38$ ($P = 0.004$). The clumping parameter ($k$) of the negative binomial distribution was estimated after fitting the empirical distributions of emerged flies by means of the GLIM software (Baker and Nelder 1978) with the modified macro NENGIBIN.MAC (Crawley 1993). The maximum likelihood estimates were 0.61 (goodness of fit, $G = 19.83, df = 12, P > 0.05$), and 0.50 ($G = 12.30, df = 9, P > 0.05$) for the supplemented and non-supplemented halves,
Table 2. Genotype frequencies of *Drosophila melanogaster* flies emerged from the high density rotting *Opuntia ficus-indica* fruits at Carboneras (H-fruits). Rows stand for αGpdh genotypes (arranged according to faster migrating alleles: αGpdh<sup>SS</sup>, αGpdh<sup>SB</sup>, and αGpdh<sup>BB</sup>) and columns for Adh genotypes (Adh<sup>SS</sup>, Adh<sup>SB</sup>, and Adh<sup>BB</sup>).

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Number of flies</th>
<th>Rank</th>
<th>Supplemented half</th>
<th>Nonsupplemented half</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1</td>
<td>123</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>13.2</td>
<td>252</td>
<td>11</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>13.3</td>
<td>116</td>
<td>38</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>13.4</td>
<td>344</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>13.6</td>
<td>533</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>13.7</td>
<td>339</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>13.8</td>
<td>145</td>
<td>26</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>13.9</td>
<td>120</td>
<td>34</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13.11</td>
<td>126</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>16.2</td>
<td>311</td>
<td>6</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16.5</td>
<td>311</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>E1.3</td>
<td>310</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>E2.1</td>
<td>515</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>E2.2</td>
<td>334</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>E2.3</td>
<td>213</td>
<td>13</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>E3.1</td>
<td>192</td>
<td>15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>4284</td>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>66</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>108</td>
<td>181</td>
</tr>
</tbody>
</table>

1 Total number of *Drosophila* flies of the four recorded species emerged from each fruit (see text).

2 All 54 experimental fruits were ranked (from highest to lowest) according to the total number of flies emerged.

Table 3. Measures of partial association for the raw data in Table 2.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>G</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit (u&lt;sub&gt;i&lt;/sub&gt;)</td>
<td>15</td>
<td>567.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Half (u&lt;sub&gt;j&lt;/sub&gt;)</td>
<td>1</td>
<td>7.15</td>
<td>0.008</td>
</tr>
<tr>
<td>Adh (u&lt;sub&gt;k&lt;/sub&gt;)</td>
<td>2</td>
<td>176.07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>αGpdh (u&lt;sub&gt;αk&lt;/sub&gt;)</td>
<td>2</td>
<td>321.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruit, half (u&lt;sub&gt;ij&lt;/sub&gt;)</td>
<td>15</td>
<td>48.77</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruit, Adh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>30</td>
<td>65.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruit, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>30</td>
<td>71.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Half, Adh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>2</td>
<td>2.27</td>
<td>0.322</td>
</tr>
<tr>
<td>Half, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>2</td>
<td>7.83</td>
<td>0.019</td>
</tr>
<tr>
<td>Adh, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>4</td>
<td>57.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruit, Half, Adh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>30</td>
<td>41.24</td>
<td>0.083</td>
</tr>
<tr>
<td>Fruit, Half, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>30</td>
<td>46.38</td>
<td>0.029</td>
</tr>
<tr>
<td>Fruit, Adh, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>60</td>
<td>101.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Half, Adh, αGpdh (u&lt;sub&gt;ijk&lt;/sub&gt;)</td>
<td>4</td>
<td>5.38</td>
<td>0.251</td>
</tr>
</tbody>
</table>

Log-Linear Analysis

One potential problem when analyzing contingency-table data is that some cells may contain random zeros because of the relative smallness of the cell probability; in some cases we cannot fit the full spectrum of models to the entire array (Bishop et al. 1975). This problem is particularly acute here given that *D. melanogaster* seems to aggregate their eggs over the fruits (see above), with most fruits containing few or even no eggs (in fact, seven of 54 fruits produced three or fewer *D. melanogaster* flies). We decided, therefore, to discard those fruits that yielded less than 15 *D. melanogaster* flies per half. This left 16 of 54 fruits (hereafter referred to as “high-density fruits” or H-fruits), which globally produced 72% of the *D. melanogaster* adults emerged. Because of the positive correlation between the numbers of *D. melanogaster* and *D. simulans* (see above), the H-fruits also ranked as the most productive when the number of flies emerged was globally considered (Table 2).

A total of 1577 *D. melanogaster* flies were assayed for electrophoresis, a figure that amounts to 92% of the adults collected from the H-fruits. Of these, genotypes at the two polymorphic loci were available for 1550 flies. All results reported here, except where noted, are from analyses based upon these 1550 individuals. Table 2 gives the raw data for the nine genotypic classes at each half of each fruit.

The results from the log-linear analysis are shown in Table 3. This table provides the test for partial association, which depends on the number of factors involved in the term. Stepwise model building by adding or deleting effects found that the best model is that with the second-order interaction effects u<sub>126</sub>, u<sub>136</sub>, and, by implication, the first-order interaction effects u<sub>12</sub>, u<sub>13</sub>, u<sub>14</sub>, u<sub>24</sub>, and u<sub>34</sub>. In other words, Table 3 quite strongly suggests that in our case the best reduced version of the saturated model is

\[
\ln f_{ijkl} = u_{126(ij)} + u_{136(ij)}
\]

The overall findings (and possible conclusions) from the analysis can be summarized as follows: (1) the marginal numbers respectively. These estimates suggest that *D. melanogaster* exhibits marked aggregation in our sampled population.

In f<sub>ijkl</sub> = u<sub>126(ij)</sub> + u<sub>136(ij)</sub>|.
of flies are different between fruit halves (density-dependent mortality); (2) genotype frequencies at both loci are not homogeneous across fruits (correlation of gametes within subpopulations relative to gametes drawn at random from the entire population; i.e., $F_{ST} > 0$); (3) there are nonindependent genotype combinations (genotypic disequilibrium at the two loci); (4) marginal genotype frequencies for $\alpha Gpdh$ genotypes do not seem to be homogeneous for both halves (differential viability); and (5) genotype frequencies for $\alpha Gpdh$ are also heterogeneous between halves across fruits ($G \times E$ interaction). We now further analyze and discuss at some depth each of these findings in turn.

**Density-Dependent Mortality in O. ficus-indica Fruits**

Figure 1 shows the relationship between number of emergent *D. melanogaster* flies in nonsupplemented versus supplemented halves of H-fruits. Differences in the numbers of adult *D. melanogaster* flies emerged from both halves were also tested with Wilcoxon’s matched-pairs signed-ranks test (Siegel and Castellan 1988). Resource limitation apparently had a significant effect on survivorship, and approximately 35% more *D. melanogaster* flies emerged from the supplemented halves ($Z = 2.56, P = 0.010$).

**F-Statistics**

Table 4 shows the $F$-statistics calculated by the methods of Weir (1990). It is worth mentioning that the bootstrap resampling procedure to build a confidence interval would be incorrect here because we have scored the flies for only three loci (Raymond and Rousset 1995b; Rousset and Raymond 1997). Exact tests for population differentiation provided evidence for allelic heterogeneity (i.e., $F_{ST} > 0$) at *Adh* and $\alpha Gpdh$ in both nonsupplemented ($P = 0.004$ and $P < 0.001$, respectively) and supplemented halves ($P = 0.003$ and $P < 0.001$, respectively), but exact Hardy-Weinberg tests for each locus did not detect significant departures from expected binomial proportions in any case (i.e., $F_{IT} = 0$) (Rousset and Raymond 1995). $F_{IS}$-values were negative for both halves, which suggests an excess of heterozygotes within fruits. In summary, this analysis reinforces the previous conclusion of genetic heterogeneity across fruits.

**Linkage Disequilibrium between *Adh* and $\alpha Gpdh$**

Analyses of two-locus linkage disequilibrium coefficients showed 10 significant disequilibria of 32 comparisons. The digenic disequilibrium for all flies emerged, measured with the composite measure $\Delta_{AB}$ (Weir 1990), was estimated to be $-0.0485$ ($P < 0.001$) with allele *Adh* associated to allele $\alpha Gpdh$. An additional sample of 259 flies trapped with banana baits in the same population was also scored for *Adh* and $\alpha Gpdh$, and $\Delta_{AB} = -0.0338$ ($P = 0.007$). Given that both loci were in Hardy-Weinberg proportions and trigenic and quadrigenic coefficients were not statistically significant, the composite measure most likely reflects the linkage disequilibrium (sensu Lewontin and Kojima 1960; i.e., the within-gamete disequilibrium component) between both alleles. This nonrandom association is probably due to the presence of the inversion In(2L)it in the population (see below).

**Relationship between Allelic Frequencies at Both Halves**

The previous conclusion that there may be differential larval viabilities for $\alpha Gpdh$ genotypes (or the chromosome region marked by this locus) is probably open to strong criticisms. It is not at all clear to what extent the two halves of a fruit accurately represent two random samples of eggs that have grown with (nonsupplemented half) or without (supplemented half) competition. Because the number of eggs laid by *D. melanogaster* females in a single prickly pear is finite, sampling genetic differences between both halves could be important even if we assume that eggs and/or larvae are randomly distributed in each fruit. The sampling problem may not be very important when averaging over the whole dataset, but the biological significance of the second-order interaction effect, $u_{1234}$, seems to require particular attention.

If survivorship with respect to *Adh* and $\alpha Gpdh$ genotypes was at random in the high-density fruits (H-fruits), then one might expect allelic frequencies between halves at both loci to be highly correlated. We further assayed most of the *D. melanogaster* adults that had emerged from the fruits for these two loci and had information for gene frequencies in the two halves for 42 fruits in total. Although positive and statistically significant (Fig. 2), the correlations between allelic frequencies in the whole sample of fruits (using the arcsine square
correlation between gene frequencies than that which would be expected by chance, random subsampling was performed (Efron 1982, pp. 69–73). Each bootstrap sample was drawn from the data by sampling (without replacement) 16 points from the 42 pairs of values in Figure 2. There are \(1.67 \times 10^{11}\) ways of doing this, and 6000 random subsamples were generated for each locus. The correlation was estimated directly from the data (arc sine transformed), and also from each of the 6000 bootstrap samples. The bias-corrected percentile method (Efron 1982, pp. 82–84) was used to obtain approximate confidence limits from the bootstrap distribution, and the closest bootstrap value to the bias-corrected 50th percentile was taken as the bootstrap estimate of the parameter. Results are shown in Table 5. The actual values observed are both in the lower 2.5% tail of the distributions, so there is some evidence to reject the hypothesis that they represent a random subsample of the whole dataset. If the same procedure is applied, but now weighting the correlation coefficients by the number of flies emerged in each fruit, the qualitative conclusion is also the same (data not shown). Exact tests of population differentiation (Raymont and Rousset 1995b) comparing both halves from the H-fruits suggest that allele \(\alpha Gpdh^b\) was at a higher frequency in the nonsupplemented halves (\(P = 0.004\)).

**Computer Simulations**

Assuming again that mortality in the fruits was at random with respect to \(Adh\) or \(\alpha Gpdh\) genotypes, we could also ask: what is the probability of having a significant second-order interaction effect (fruit-half-locus; see Table 3) in the log-linear model due to sampling error? In other words, does the sorting out of individuals in two samples within a circumscribed universe (fruit) colonized by a limited number of \(D.\ melanogaster\) females affect the error rate (i.e., the number of Type I errors that we expect to make if the null hypothesis is true) of cross-product ratios? We roughly approached this question by using Monte Carlo simulations.

If we take the actual \(F_{ST}\)-value in the supplemented halves (i.e., "nonlimiting" resources; Table 4) as the expectation from the sampling effect of colonization by laying females, then

\[
F_{ST} = \frac{1}{2N},
\]

where \(N\) is the effective number of locally breeding adults (e.g., Wade and McCauley 1988). We have previously obtained "unbiased" estimates of \(N\) when different numbers of mating pairs contribute to the progeny of each patch (Que-

**Table 5.** Bootstrap analyses of correlation of allelic frequencies (arc sine transformed) between fruit halves (data plotted in Fig. 2). Each bootstrap is based on a random subsampling (without replacement) of 16 fruits (see text for details).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Direct estimate</th>
<th>Bias-corrected percentile method(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All fruits ((n = 42))</td>
<td>High-density fruits ((n = 16))</td>
</tr>
<tr>
<td>(Adh)</td>
<td>0.4220</td>
<td>-0.0401</td>
</tr>
<tr>
<td>(\alpha Gpdh)</td>
<td>0.4722</td>
<td>-0.1215</td>
</tr>
</tbody>
</table>

\(^1\) Bootstrap analyses are each based on 6000 random subsamples.
zada-Díaz et al. 1997), and a figure of at least 10 D. melanogaster females ovipositing on a fruit arises as a reasonable estimate here. (It is important to stress that this value is for an effective number of mating pairs, whereas in the simulations we will assume that females contribute equally to the total number of eggs.) We generated by computer simulation 500 independent samples that matched the situation found in the high-density fruits (H-fruits; Table 2). Namely, 10 mating pairs contributing gametes to each of 16 fruits were randomly selected from a large panmictic population with allelic frequencies equal to those estimated for αGpdh from the row label as “Total” in Table 2, and a number of individuals equal to the actual sample sizes for each fruit in Table 2 were taken at random and sorted out in two samples. For each simulation, we calculated the G-statistic for the second-order interaction effect and the results are shown in Figure 3. The distribution of the G-values did not depart significantly from a χ² distribution with 30 degrees of freedom (i.e., the df for the u₁₂₄ interaction in Table 3; χ²₁₇ goodness-of-fit test = 22.71, P = 0.159), and it is clear that the actual G-value for u₁₂₄ in the tests of partial association (Table 3) is in the upper 5% tail of the distribution. The same can be said from the distribution of G-statistics for the first-order interaction in the simulations (half, locus; data not shown). Therefore, the conclusion is that sampling error does not, by itself, seem to explain the statistically significant results found for the interactions between fruits, halves, and αGpdh genotypes.

Fitness Estimates for αGpdh Genotypes

The previous analyses suggest that the different αGpdh genotypes (or nonrandomly associate genotypes at other linked loci) have different chances of surviving on different fruits. Assuming that there is no selective mortality in the supplemented halves, that is, that they provide a way to estimate the input zygotic frequencies in the Opuntia fruits (a reasonable but obviously untestable assumption), the viability of a particular homozygote relative to the heterozygote in each fruit can be estimated by the corresponding cross-product ratio (Manly 1985; see above and Table 1). Figure 4 shows the fitness estimates for the 16 high-density fruits (H-fruits) used in the log-linear analysis. Confidence intervals were obtained with the percentile method (Efron 1982) by resampling (with replacement) 2000 times using a compiled BASIC program written by E. Rolán-Alvarez (Johannesson et al. 1995; Rolán-Alvarez et al. 1995). A frequency value of 0.01 was assumed whenever the genotype αGpdhS/S was absent in the nonsupplemented halves (Table 2). This does not modify the theoretical expectation nor the statistical tests, but obviously allows to bootstrap genotype frequencies in both halves.

Several findings are relevant from Figure 4. First, only one of 16 fruits (fruit 13.3) shows a marginally significant pattern of overdominance. Second, when pooling over fruits it seems clear that allele αGpdhF is at an overall disadvantage, which is in agreement with the previously reported result that allele αGpdhS was at a higher frequency in the nonsupplemented halves. Third, this does not per se preclude the possibility for a protected polymorphism because in some fruits (fruits 13.6, 13.9, and 16.5) there seems to be directional selection against αGpdhS. It is well known that conditions for a protected polymorphism in Levene’s model require the harmonic means of homozygote’s fitnesses to be lower than that of the heterozygote. The relative contribution that each fruit in Figure 4 makes to the total adult population was directly estimated from the numbers of D. melanogaster flies raised from the nonsupplemented halves. Because the problem of how to get good fitness estimates is quite complicated in the present situation, we have used three different methods to obtain
Table 6. Average fitness values for the three \(\alpha Gpdh\) genotypes raised from the 16 high-density fruits plotted in Figure 4. The relative contribution that each fruit makes to the total adult population was estimated from the numbers of \(Drosophila melanogaster\) flies raised from the nonsupplemented halves. In each case, both the averages of the theoretical and bootstrap values (in parentheses) are given (a value of 0.01 was assumed whenever the theoretical estimate was zero).

<table>
<thead>
<tr>
<th>Method of estimation(^1)</th>
<th>Genotype</th>
<th>(\alpha Gpdh^{GS})</th>
<th>(\alpha Gpdh^{SF})</th>
<th>(\alpha Gpdh^{FF})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arithmetic means (relative to (\alpha Gpdh^{SF}))</td>
<td>Unweighted</td>
<td>1.323 (1.555)</td>
<td>1</td>
<td>0.891 (1.012)</td>
</tr>
<tr>
<td></td>
<td>Weighted</td>
<td>0.161 (0.328)</td>
<td>1</td>
<td>0.542 (0.580)</td>
</tr>
<tr>
<td></td>
<td>Truncated</td>
<td>1.045 (1.126)</td>
<td>1</td>
<td>0.920 (0.942)</td>
</tr>
<tr>
<td></td>
<td>(relative to (\alpha Gpdh^{FF}))</td>
<td>Unweighted</td>
<td>2.645 (3.267)</td>
<td>1.669 (2.014)</td>
</tr>
<tr>
<td></td>
<td>Weighted</td>
<td>0.086 (0.167)</td>
<td>1.113 (1.242)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Truncated</td>
<td>2.467 (2.941)</td>
<td>1.532 (1.740)</td>
<td>1</td>
</tr>
<tr>
<td>Harmonic means (relative to (\alpha Gpdh^{SF}))</td>
<td>Unweighted</td>
<td>0.097 (0.925)</td>
<td>1</td>
<td>0.600 (0.677)</td>
</tr>
<tr>
<td></td>
<td>Weighted</td>
<td>0.012 (0.184)</td>
<td>1</td>
<td>0.383 (0.425)</td>
</tr>
<tr>
<td></td>
<td>Truncated</td>
<td>0.096 (0.834)</td>
<td>1</td>
<td>0.633 (0.685)</td>
</tr>
<tr>
<td></td>
<td>(relative to (\alpha Gpdh^{FF}))</td>
<td>Unweighted</td>
<td>0.097 (0.838)</td>
<td>1.122 (1.255)</td>
</tr>
<tr>
<td></td>
<td>Weighted</td>
<td>0.011 (0.078)</td>
<td>1.002 (1.143)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Truncated</td>
<td>0.097 (0.757)</td>
<td>1.075 (1.088)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\) Unweighted: The observed fitness values were directly used to estimate the average fitnesses in each case. Weighted: Each observed fitness value was weighted by the inverse of its bootstrap variance before estimating the average fitnesses. Truncated: A value of one was assumed whenever the corresponding fitness value was not statistically different from that of the referring genotype (either \(\alpha Gpdh^{SF}\) or \(\alpha Gpdh^{FF}\), i.e., the two most frequent genotypes).

arithmetic and harmonic mean fitnesses from the values plotted in Figure 4 (Table 6). There is, in addition, a potential statistical problem when harmonic mean fitnesses are estimated relative to only the heterozygote because the estimates contain sampling error and the expected value of the harmonic mean is inversely related to the variance of the variable. Therefore, Table 6 also gives the mean fitnesses relative to the most common homozygote.

With the caveats above, the results strongly suggest that a stable genetic polymorphism could in theory be assured. Thus, Figure 5 shows the expected trajectories for allele \(\alpha Gpdh^{GS}\) obtained from the theoretical fitness estimates (relative to \(\alpha Gpdh^{SF}\)) in the 16 H-fruits. There are, however, some fundamental conceptual and methodological problems here that are quite evident when comparing the equilibrium frequency of allele \(\alpha Gpdh^{GS}\) in Figure 5 (~ 0.632) with the actual frequency in Table 2 (0.383). We do not know the relative frequency of each type of fruit in the field, and in their natural environment \(D. melanogaster\) is not restricted to only living in the types of fruits and host plant that we have provided in the experiment. The only possible conclusion we can draw is that the results seem to be compatible with the existence of a protected polymorphism.

**Discussion**

In the 33rd Symposium of the British Ecological Society (Berry et al. 1992), Martin Kreitman (see Kreitman et al. 1992, pp. 307–308), commenting on the \(Adh\) polymorphism in \(Drosophila\), asked: “Are we ready to place the evolutionary story into the ecological theatre? . . . What habitat differences have driven the evolution of \(ADH\)? And do these differences contribute to the patterns of habitat segregation observed for the species? . . . These are the kinds of questions we must answer before we can assess whether scramble competition in ephemeral patches is important for allowing coexistence of the species. They are also the kinds of questions we must answer if ever ecology and evolutionary genetics are to be unified.” These questions relate to some of the deepest problems in population biology, namely the operation of selection in subdivided populations (Barton and Clark 1990; Gillespie 1991; Barton and Whitlock 1997). Because the local population structure of many \(Drosophila\) species may be characterized to some extent by Levene’s migration pattern (with the important additional ingredient that individual patches usually sustain a few sibships), there are clear links between \(Drosophila\) ecology and population genetics (e.g., Santos 1994, 1997a,b).

\(Drosophila melanogaster\) exhibits intraspecific aggregation at Carboneras, and there is evidence of resource limitation due to competition in \(Opuntia\) fruits. This reduces the probability of survival in those fruits with relatively high local

![Fig. 5. Expected trajectories for allele \(\alpha Gpdh^{GS}\) obtained from the theoretical fitness estimates in the 16 high-density fruits (H-fruits) relative to \(\alpha Gpdh^{SF}\) (the “truncated” method of estimation in Table 6 was used). It is assumed that 10 mating pairs randomly sampled from the panmictic population contributed with their offspring to each fruit, and that the relative contribution of each fruit to the total adult population in the next generation is equal to the actual numbers of \(Drosophila melanogaster\) flies emerged from the nonsupplemented halves (see text for details).](image-url)
densities of larvae, and global population density might be
limited by intraspecific competition in these resources (see
Kreitman et al. 1992 and references therein). However, the
positive interspecific correlation coefficient with *D. simulans*
also suggests that these two species are not assorting inde-
pendently. We cannot assess from our data the relative in-
tensities of intra- and interspecific competition; but whatever
the causes of larval mortality, there is clearly the opportu-
nity for selection under natural conditions. Similar conclusions
have been previously reached by a number of authors (Gri-
maldi and Jãinik 1984; Atkinson 1985; Nunney 1990; Jae-
nike and James 1991; Quezada-Díaz et al. 1997), and such
phenomena have probably to be considered as general in wild
populations. Therefore, there is clear evidence that density
regulation occurs within some patches (i.e., selection is
“soft”). The coexistence of low-density patches with no
density-dependent mortality and high-density ones with strong
competition and differential genotype viabilities does not af-
fect the resulting equilibrium arrays in gene frequency. The
reason is that low-density patches might be considered as
“neutral demes” (Karlin 1982).

The results from the log-linear analysis suggest that the
relative viabilities of *αGpdh* genotypes are not equal in the
two halves of a fruit and that the different genotypes have
different chances of surviving on different fruits (Fig. 4). The
additional information provided by the bootstrap analyses
(Table 5) and the computer simulations (Fig. 3) strongly ar-
gues against sampling error. The question arises whether the
observed viability differences may be attributed to the *αGpdh*
locus or to other locus (or loci) associated with it in linkage
disequilibrium. The possibility of the *αGpdh* locus being non-
randomly associated with chromosomal inversions cannot be
rejected, because it is also clear in our data the strong as-
association between *Adh* and *αGpdh*. This association is most
likely due to the presence of the inversion *In(2L)j* in the
population studied because it tends to disappear when only
standard chromosomes are considered (Aguade and Serra
1980; Knibb 1983). The locus *αGpdh* (2-20.5) is included in
the inversion while the locus Adh (2-50.1) is outside, close
to the proximal breakpoint (for patterns of recombination
among *In(2L)j*, *αGpdh*, and Adh see Malpica et al. 1987).
Both loci are in linkage disequilibrium with *In(2L)j*, although
exceptions do occur (Mukai and Voelker 1977; Voelker et al.
1978; Knibb 1983). This inversion has been detected in al-
most every natural population sampled at a frequency that is
known to increase toward the equator (Inoue et al. 1984;
Lemeunier and Aulard 1992). We did not survey the popu-
lation for chromosome arrangements, but *In(2L)j* has been
recorded in southern Spain, where the gamete *Adh*-*αGpdh*
is abundant in the field (Alonso-Moraga and Muñoz-Serrano
1986). Additional evidence for an underlying effect of *In(2L)j*
comes from the fact that the second-order interaction effect
involving fruits, halves, and *Adh* in the log-linear analysis is
also statistically significant when we look at the chi-square
values in the marginal association tests ($\chi^2 = 49.94, P =
0.013$). Conceptually, the tests for marginal association are
equivalent of looking at the simple correlation instead of the
partial correlation.

As discussed above, our results seem to be compatible with
the existence of a protected polymorphism (Table 6). How-
ever, there are a number of difficulties when trying to ex-
trapolate our findings to the whole population. First, the 16
fruits are only a sample from a larger population of fruits.
Second, *D. melanogaster* is not a specialist on *Opuntia*, and
other natural hosts might show a different pattern of fitness
values. Third, the results suggest that the nature and strength
of selection depends on density within fruits; therefore pop-
ulation fluctuations might greatly influence global relative
fitness of the genotypes. As pointed out by Maynard Smith
and Hoekstra (1980), “fitnesses which will maintain poly-
morphism for one set of niche sizes will in general not to do
so if the relative niche sizes are varied.” Therefore, we are
aware that even though the population structure of *D. me-
lanogaster* can be characterized to some extend by the Levene
(1953) migration pattern, there are reasons to doubt about the
appropriateness of the Levene model to the present situa-

What are the reasons for the fitness differences across
*Opuntia* fruits? It may be erroneous to speculate about the
potentially important evolutionary consequences of enzyme
activity variation at *αGpdh* (e.g., Zera et al. 1983) because
of the nonrandom association between *Adh* and *αGpdh* alleles
found in our samples. If, however, we assume that the fitness
differences are related to the presence of *In(2L)j* (see above),
a pattern of G × E interaction could be predicted if we further
assume that there is a recombination load affecting off-
spring’s competitive ability. Recombination load can be de-
defined as the lowering in average fitness of progeny due
to genetic recombination, and there is some empirical evidence
suggesting that *Drosophila* populations do suffer a recom-
bination load (Charlesworth and Barton 1996 and references
therein). The average loss of fitness of an individual because
it has an inversion homozygous mother (there is no recom-
bination in *D. melanogaster* males) can be defined as

$$L_j = \frac{w_1 - w_0}{w_0} = 1 - r,$$

where $w_0$ is the mean fitness of zygotes before selection, $w_1$
is the mean fitness after selection, and $r$ is the recombination
load (Charlesworth and Charlesworth 1973). Provided that
there is random mating in the whole population, each breed-
ing site is colonized by a limited number of females, and
offspring’s fitness is only dependent on $r$, it can be shown
that karyotype frequencies in the whole population after se-
lection are:

$$TX_1 = p_f p_m - r X_f P_m,$$

$$TY_1 = p_f q_m + q_f p_m - r (X_f q_m + Z_f P_m),$$

$$TZ_1 = q_f q_m - r Z_f q_m,$$

where $T$ is the sum of the right-hand expressions; $X$, $Y$, $Z$
denote the frequencies of *ST/ST*, *ST/IN*, and *IN/IN* karyotypes
(*ST* and *IN* stand for two chromosome arrangements); and $p_f$
($= 1 - q_f$) and $p_m$ ($= 1 - q_m$) are, respectively, the
frequencies of *ST* (*IN*) in females and males. It is clear that
karyotype frequencies after selection are a function of the
frequencies of homozygous mothers contributing gametes to
a fruit (see also Wasserman 1968), and that there is a negative
frequency-dependent selection that assures a protected poly-
morphism. In addition, the sampling process of colonization by a relatively small number of females (see Santos 1997a) will cause a wide variation in the frequencies of locally breeding homozygous mothers across breeding sites which, in turn, will make fitness values to vary across these sites. Under this scenario, the negative frequency-dependent selection does not arise as a consequence of G X E interaction (sensu Lev-ene: see Maynard Smith 1970; Bell 1985; Barton and Clark 1990), but it is simply due to the different interactions among genotypes within patches as a function of mother’s founder genotypes. Therefore, it would be valuable to have information about the presence of inversion In(2L)R at Carboneras and its effect on larval crowding as a function of genetic recombination.

ACKNOWLEDGMENTS

We thank I. Hardy for his helpful advice to prepare the trays with O. fico-indica fruits, A. Kamping for providing the Groningen reference strains of D. melanogaster, F. Peris for his help with the GLIM software, E. Rolan-Alvarez for providing the BASIC program to estimate fitnesses, and M. Peiró for technical assistance with electrophoreses. The manuscript benefited from comments and suggestions from D. J. Borash, G. de Jong, L. D. Mueller, the corresponding editor and two anonymous reviewers. We are particularly grateful to one anonymous reviewer for pointing us the potentially important statistical artifact when estimating the harmonic mean fitnesses relative to only the heterozygous genotype. This work was founded by grants PB93-0843 and PB96-1136 from the Dirección General de Investigación Científica y Técnica (DGICYT, Spain) to AF, Contract No. CHRX-CT92-0041 from the Commission of the European Communities, and grant CE93-0019 from the DGICYT.

LITERATURE CITED


EFRON, B. 1982. The jackknife, the bootstrap, and other resampling plans. S.I.A.M., Philadelphia, PA.


HOEKSTRA, R. F., R. BULIMA, and J. DOUGLAS. 1985. Polymorphism from environmental heterogeneity: models are only robust if the heterozygote is close in fitness to the favoured homozygote in each environment. Genet. Res. 45:299–314.


JohANNESSENS, K., E. ROLÁN-ALVAREZ, and A. EKENDAHL. 1995. Incipient reproductive isolation between two sympatric morphs


LEVENE, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331–333.


VAN DELDEN, W., AND A. KAMPING. 1989. The association between the polymorphisms at the Adh and αGpdh loci in In(2L)It inversion in Drosophila melanogaster. Evolution 43:775–793.


Corresponding Editor: E. Brodie III