How Genetic Background Masks Single-Gene Heterosis in Drosophila

(polymorphism/selection/isoenzymes/inbreeding/fly)

CHRISTOPHER WILLS AND LOIS NICHOLS

Department of Biology, Wesleyan University, Middletown, Connecticut 06457

Communicated by Theodosius Dobzhansky, November 22, 1971

ABSTRACT

Conditional heterosis associated with two isoallelic forms of octanol dehydrogenase in Drosophila pseudoobscura has been detected in flies that have been (a) inbred for several generations to make the background genotype as homozygous as practicable, and (b) grown on a medium containing a small amount of octanol. The heterosis was first found in inbred males. The possibility that the effect in females was masked by X-linked polymorphic genes affecting the same fitness components as the locus for octanol dehydrogenase was shown to be correct when, on further inbreeding, heterosis was found in the females of one of the lines. The implications of this and other findings for the current understanding of the genetic structure of natural populations is discussed.

We recently reported (1) that single-gene heterosis could be detected in Drosophila pseudoobscura when the rest of the genome had been made sufficiently homozygous by inbreeding. The relative survival of flies segregating for two isoalleles at the octanol dehydrogenase (odh) locus was determined, both in normally outbred flies and those in which the background had been rendered largely homozygous by repeated brother-sister matings. No differential survival was detected in the outbred flies, regardless of culture conditions. Twelfth-generation inbred males grown in cultures containing a small amount of octanol showed, however, an increase in the number of heterozygotes surviving over that expected. This increase was not apparent on any other medium tried. We suggested that the reason this conditional heterosis did not appear in the inbred females was because it was masked by segregating X-chromosome genes modifying the effects of the odh locus. (There was a hint of its appearance in the females of one line, but the deviation was barely significant.)

We show here that our supposition was correct, since further inbreeding has revealed pronounced heterosis in females as well. We also give some less-direct evidence that many polymorphic genes modifying the effects of the odh locus exist on the X chromosome.

MATERIALS AND METHODS

Two loci of Drosophila pseudoobscura that are polymorphic in nature were used. The first, odh, is autosomal, but is not on the third chromosome; therefore, it is not included in naturally occurring inversions. The second, esterase-5 (e-5), is sex-linked (2). The flies were derived from a sample taken in 1964 from a natural population in Berkeley, Calif. (Strawberry Canyon), and have been maintained since in the form of a large number of separate lines by Dr. Satya Prakash. At the start of the experiment, 10 of these lines were obtained from Dr. Prakash, pooled, and the progeny were used to set up a large number of single-pair matings. Once the matings could be seen to be successful, the parents were removed and extracts were examined by vertical acrylamide-gel electrophoresis (3) to determine their odh or e-5 genotypes. Several lines were found in which both parents were homozygous (or hemizygous) for one or the other of the two most common alleles at the odh and e-5 loci. The alleles are named, according to their relative mobilities, odh 1.00 and 1.22, and e-5 1.00 and 1.12 (refs. 2 and 4). Each 1.00 allele is the most common in the populations examined, the odh 1.22 and e-5 1.12 alleles being rarer. For the sake of simplicity, we will refer to the 1.00 alleles as S (for slow) and the other alleles as F (for fast).

All lines in which both parents were homozygous for S or for F were pooled to form the pure-breeding P1 lines. All the flies in a particular P1 line were homozygous for one S or F allele of odh or e-5, but the rest of the genome carried close to the normal amount of heterozygosity. These P1 flies were outbred controls, used to measure the effect of different media on flies with a normal genetic background (1).

In order to examine the contribution to survival of particular isoalleles divorced as much as possible from background, the heterozygosity of the remainder of the genome was reduced. Flies from the P1 F and S lines were crossed en masse and single-pair matings were set up among their progeny. When larvae appeared in these cultures, the parents were removed and their genotypes were determined. Only those matings in which both parents were heterozygous for the S and F odh isoalleles, or in which the female was heterozygous for the S and F e-5 isoalleles, were kept. The flies that emerged from these cultures were the first inbred generation (I1).

The progeny from these matings were in turn brother-sister mated, and again extracts of the parents of the successful cultures were electrophoresed to determine their genotypes. The progeny from crosses segregating for F and S alleles formed the I2 generation. This process was repeated, and the results dealt with in this paper were derived from flies of the I1 generation.

I1 cultures, in which the parents were both F or both S, were chosen from each of three odh and three e-5 inbred lines. These were used to establish pure-breeding pairs of F and S lines within each of the six inbred lines. Each of these pure-breeding lines for F or S differed from its S or F counterpart by the allele in question, a small block of genes extending to either side of the allele, and whatever heterozygous loci remained in the rest of the genome. (A number of other genetic markers

Abbreviations: odh, octanol dehydrogenase locus; e-5, esterase-5 locus; S and F, slow- and fast-migrating isoenzyme alleles, respectively.
were checked in the I\textsubscript{1} generation and were found to be monomorphic; no inversions were seen in salivary glands taken from I\textsubscript{1} larvae.)

The pairs of I\textsubscript{17} F and S lines were crossed, and the progeny from these crosses were allowed to lay eggs for 3 days on the standard cornmeal–molasses–agar medium, to which had been added 3 mM 1-octanol. The flies were subjected to considerable stress on this medium; about one tenth as many survived to adulthood as on the standard medium. The crosses were so arranged that the survivors should segregate I S/S:2 S/F:1 F/F in the case of odh and 1 homozygote: 1 heterozygote in the case of e-5. Those that did survive were examined by electrophoresis to determine any deviations from these Mendelian expectations.

RESULTS

Table 1 shows observed and expected numbers of odh and e-5 flies bred for 17 generations and surviving on octanol medium. The pattern is the same as in our previous report (1). The excess of heterozygotes, when it occurs, is always accompanied by a deficiency of slow homozygotes, while fast homozygotes are found in about their expected numbers. In contrast to the previously published data, however, odh line B now shows pronounced heterosis in the females as well as the males. A reduction of X-linked heterozygosity has apparently occurred in this line since the twelfth generation, while the other two lines have remained relatively unchanged. The e-5 flies grown on the same medium as a control show no deviation from Mendelian expectation in any of the three inbred lines that were tested.

We have collected similar data from three other "stress" media modified in ways unrelated to the activity of odh (not shown in Table 1). No deviations from Mendelian expectation were observed on any of these media.

An unexpected genotype–environment interaction was noted in the process of collection of flies from the stress media. The sex ratio of e-5 flies grown on octanol medium was greatly distorted, with over twice as many females as males emerging from the pupal stage. When the parents of these flies were transferred to standard medium without octanol, they produced progeny with the usual 1:1 sex ratio. No distortion of the sex ratio was detected among the odh flies grown on either medium. The data are given in Table 2.

DISCUSSION

It is, of course, impossible to attribute heterosis to a single locus with certainty unless, as in the case of sickle-cell anemia (5), a direct connection can be made between the function of the gene product and the fitness of the individual. This cannot as yet be done at the odh locus, though we can infer from our data that these odh isoalleles interact in different ways with an excess of an odh substrate in vivo. In vitro studies on the purified gene products may provide clues to these differences.

It can be objected that octanol may not be a natural substrate for odh, and that we are subjecting the flies to a most unnatural situation in the laboratory. We do not pretend to have approximated natural selective factors in our experiment, but say only that it is possible to demonstrate selection coefficients associated with different isoalleles at this locus. Evidence for the localization of odh in the fat bodies (6) has reinforced the likelihood of its being involved in long-chain alcohol metabolism, and has led to a search for odh substrates in the breeding places of D. robusta*. Such studies should provide clues to the true function of the enzyme.

---

* Bremner, T. A., personal communication.

---

**Table 1.** Observed and expected numbers of inbred flies surviving on medium containing octanol

<table>
<thead>
<tr>
<th></th>
<th>S/S</th>
<th>S/F</th>
<th>F/F</th>
<th>x²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>odh</strong>&lt;br&gt;I\textsubscript{17}&lt;br&gt;Line A&lt;br&gt;♂ ♂</td>
<td>22 (34.5)</td>
<td>37 (34.5)</td>
<td>6.16</td>
<td>0.05 &gt; P &gt; 0.025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 (30)</td>
<td>34 (30)</td>
<td>0.72</td>
<td>0.75 &gt; P &gt; 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 (36)</td>
<td>40 (36)</td>
<td>12.2</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 (35.5)</td>
<td>28 (35.5)</td>
<td>15.8</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 (35.5)</td>
<td>38 (35.5)</td>
<td>4.18</td>
<td>0.25 &gt; P &gt; 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (36)</td>
<td>39 (36)</td>
<td>1.37</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Sex ratio of flies surviving on normal and stress medium

<table>
<thead>
<tr>
<th></th>
<th>Number of &lt;br&gt;Medium</th>
<th>Males</th>
<th>Females</th>
<th>M/F</th>
<th>x²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>odh</strong>&lt;br&gt;I\textsubscript{17}&lt;br&gt;Normal</td>
<td>610</td>
<td>650</td>
<td>0.94</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Octanol</td>
<td>638</td>
<td>604</td>
<td>1.06</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Octanol</td>
<td>237</td>
<td>254</td>
<td>0.94</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Octanol</td>
<td>153</td>
<td>380</td>
<td>0.40</td>
<td>96.7*</td>
</tr>
</tbody>
</table>

* = Significant at 0.001 level.
We have not eliminated the possibility that it is not the odh locus, but rather some other closely linked locus or loci, affected by octanol in the medium. There is, however, just as good a chance that such loci would be closely linked to the e-5 locus, yet differential survival of flies segregating at that locus is not seen on octanol medium.

Conditional heterosis cannot be detected at the odh locus in normally outbred flies. The reason appears to be that there are many polymorphic genes modifying the effects of the odh locus scattered throughout the genome. Some of these might be other genes in the pathway of long-chain alcohol metabolism, others might be controlling genes or genes in peripheral pathways. These polymorphs are sufficiently numerous and important to fitness that in an outbred fly the contribution to fitness of the odh locus itself is not measurable by our techniques. The rate of loss of these polymorphisms by inbreeding is slow, since a number of our highly inbred lines show no fitness differences due to odh genotype.

If our explanation is correct, then a number of conclusions follow.

First, in a normally outbred population there is very little genetic load associated with the odh locus. It has been pointed out elsewhere (7, 8) that very large numbers of heterotic polymorphisms can be maintained in a population with small segregational loads per locus if one assumes two things: (a) all these polymorphs are functional, so that a homozygote for any allele can survive and reproduce, and (b) a highly polymorphic population is more fit than a less polymorphic one.

The conditionally heterotic odh locus fits the first of these assumptions, and from the inbreeding experiment we can infer the existence of other polymorphisms in outbred flies that contribute to fitness under our experimental conditions. It seems likely that these outbred flies are better suited to survival in the extreme environment because they are not overly dependent for fitness on one or a few polymorphisms. This illustrates once again the basic tenet of Dobzhansky and others (9) that genetic polymorphism is adaptive!

The second point is that a search for selective differences directly attributable to polymorphisms in outbred organisms, even when selective media tailored to the probable function of the locus in question are used, may be expected to give negative results. We do not imagine that the method used in the present study is precisely applicable to all loci (in fact, all our efforts to discover a selective medium that will affect the e-5 locus have so far been unsuccessful), but we expect that selective conditions directly related to the function of most polymorphic loci can and will be found—and will be found most easily in inbred organisms.

Third, resistance to inbreeding should become more and more marked as the genome becomes more homozygous, since the remaining heterozygous loci play a progressively more important role in the determination of the organisms' fitness.

We have no direct evidence for this, although in two of our seventeenth-generation inbred lines enough heterozygosity was retained on the X chromosome to mask the odh heterosis. This occurred even though theoretically only about \((\sqrt{1/2})^2\) or 0.75% of the heterozygosity that the X chromosome started with would be expected to remain. Direct evidence of resistance to inbreeding in plants has, however, been obtained by Marshall and Allard (10).

Further indications that there are polymorphisms on the X chromosome that are affected by octanol can be gleaned from the fact that inbred flies segregating for the e-5 locus showed a distortion of the sex ratio when raised on octanol medium. The e-5 inbreeding scheme was designed to retain heterozygosity on the X chromosome, and apparently some of this heterozygosity is important to the survival of flies on octanol medium. It is not the same as the residual heterozygosity on the X chromosome that masks the heterotic effect in the odh lines, for a similar distortion of the sex ratio did not occur in the inbred odh lines. Further, it is apparently unlinked to the e-5 locus itself, since the frequencies of e-5 alleles are not affected by growth on octanol medium. (If it were linked, one might expect an excess of e-5 heterozygotes; this was not observed.) This sex-ratio distortion should, therefore, eventually disappear or grow much less pronounced on further inbreeding of the e-5 lines.

More and more evidence is accumulating that the majority of polymorphisms are not selectively neutral, but that each plays a part in the fitness of the organism and that of the overall population. Small selection coefficients in the outbred background and conditional heterosis seem likely mechanisms for the maintenance of many polymorphisms, though undoubtedly each will be found to have its own unique features. Antigen and structural-protein polymorphisms, to name but two, must owe their existence to very different molecular mechanisms from polymorphisms of enzymes.

This work was supported by U.S. Public Health Service Research Grant GM14700. We are grateful to Jay Amberson and Rick Blake for technical assistance.