Negative synergistic epistasis in *Drosophila melanogaster*.

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**Introduction**

Spontaneous deleterious mutations are a constant part of the genomes of all organisms. These genetic changes and their synergistic interactions may in part be responsible for the maintenance and evolution of sexual reproduction and genetic recombination, inbreeding avoidance, senescence, the evolution of mate choice by the good gene mechanism, evolution of degenerate Y chromosomes, DNA repair, genetic control of DNA-element transpositions, extinctions of endangered species by mutational meltdown, and positive correlations between recombination rate and nucleotide diversity (Baer et al., 2007; Charlesworth and Charlesworth, 1998; Desai et al., 2007; Drake et al., 1998; Sanjuan and Elena, 2006). Yet, the role of deleterious mutations in these evolutionary processes depends on the degree of negative synergistic epistasis among the mutations, the average fitness effects of each mutation, including reduction in fitness in homozygotes and dominance in heterozygotes, and the deleterious genomic mutation rate (Garcia-Dorado et al., 2004; Lynch et al., 1999). The estimations of selection coefficients and dominance of deleterious mutations in higher organisms are diverse, and the presence of negative synergistic epistasis for these mutations is controversial. In addition, although there are estimations of the deleterious genomic mutation rate in a number of higher organisms, including nematodes, *Drosophila*, and humans, these estimations are
broad, ranging from about 0.01 to 10 per generation (Eyre-Walker and Keightley, 1999, 2006; Gong et al., 2005). Hence, it is important to determine whether negative synergistic interactions occur among deleterious mutations and to determine the distribution of their fitness effects (see Urwin and Nunn, 2005; Wolf et al., 2000).

**Example of Epistasis**

As a model of the possible influence of negative synergistic epistasis on health and fitness in a diploid organism, let us consider two genes with wild-type alleles $A$ and $B$, deleterious alleles $a$ and $b$, four possible gametic haplotypes ($AB$, $Ab$, $aB$ and $ab$), nine possible genotypes, and fitness values that are dependent upon whether there is or is not negative synergistic epistasis. In this model (shown below), $s$ = selection coefficient against the homozygous mutant alleles, with $s$ for deleterious mutations ranging from almost zero to near one; $h$ = dominance coefficient for heterozygotes, with $hs$ describing the extent to which heterozygotes express the harmful effects of the mutant allele and ranging from zero for completely recessive homozygous mutants to one; and synergistic epistasis $= \varepsilon$, with zero or negative values of $\varepsilon$ for four genotypes ($R$ = recessive genotype and $H$ = heterozygous genotype).

<table>
<thead>
<tr>
<th></th>
<th>$AB$</th>
<th>$Ab$</th>
<th>$aB$</th>
<th>$ab$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AB$</td>
<td>1</td>
<td>$1 - h_b s_b$</td>
<td>$1 - h_a s_a$</td>
<td>$(1 - h_a s_a)(1 - h_b s_b) + \varepsilon_{HH}$</td>
</tr>
<tr>
<td>$Ab$</td>
<td>$1 - s_b$</td>
<td>$(1 - h_a s_a)(1 - h_b s_b) + \varepsilon_{HH}$</td>
<td>$(1 - h_a s_a)(1 - s_b) + \varepsilon_{HR}$</td>
<td></td>
</tr>
<tr>
<td>$aB$</td>
<td></td>
<td>$1 - s_a$</td>
<td>$1 - s_a$</td>
<td>$(1 - s_a)(1 - h_b s_b) + \varepsilon_{RH}$</td>
</tr>
<tr>
<td>$ab$</td>
<td></td>
<td></td>
<td></td>
<td>$(1 - s_a)(1 - s_b) + \varepsilon_{RR}$</td>
</tr>
</tbody>
</table>

For example, for $aabb$ let the fitness of $aa = 1 - s_a = 0.9$ and the fitness of $bb = 1 - s_b = 0.9$. In the absence of negative synergistic epistasis ($\varepsilon_{RR} = 0$) the fitness of $aabb$ will be multiplicative and equal to 0.81($0.9 \times 0.9$), which is below that of $AABB$ individuals (fitness set at one). For negative synergistic epistasis let $\varepsilon_{RR} = -0.1$, and the fitness of $aabb$ will be 0.71 [(0.9 $\times$ 0.9) -0.1]. Since a fitness value of 0.71 is less than 0.81, this is an example of negative synergistic epistasis. How does one identify such negative synergistic interactions, especially when interactions may not be strong?

A hypothetical example of the expected changes in mean fitness over time for the accumulation of new deleterious mutations in the presence and absence of negative synergistic epistasis is as follows:
With synergistic epistasis the decrease in fitness (viability) with an increase in the number of mutants is lower than expected (and non-linear) if the mutants do not interact.

**Crosses Used to Measure Negative Synergistic Epistasis and Results**

The following set of crosses were used to measure negative synergistic epistasis in *Drosophila melanogaster*, using easily identified visible mutants that are also homozygous recessive lethals: Curly (curly wings) that is associated with the CyO balancer second chromosome; Glazed (glazed eyes) that is associated with an inversion of the second chromosome; and Stubble (short bristles) that is associated with the TM3, third chromosome, balancer chromosome (Lindsley and Zimm, 1992). Below the crosses are the number of progeny recovered from the F1 cross and their fitness (viability) in relation to a fitness of one for the wild-type progeny.

<table>
<thead>
<tr>
<th>Possible Progeny</th>
<th>Expected Proportion</th>
<th>Phenotypes</th>
<th>Number Mutants</th>
<th>Number Recovered</th>
<th>Relative Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CyO/Gla;TM3, Sb/+</td>
<td>1</td>
<td>Curly, Glazed, Stubble</td>
<td>3</td>
<td>60</td>
<td>0.02</td>
</tr>
<tr>
<td>CyO/+; TM3, Sb/+</td>
<td>1</td>
<td>Curly, Stubble</td>
<td>2</td>
<td>1096</td>
<td>0.38</td>
</tr>
<tr>
<td>Gla/+; TM3, Sb/+</td>
<td>1</td>
<td>Glazed, Stubble</td>
<td>2</td>
<td>1216</td>
<td>0.42</td>
</tr>
<tr>
<td>CyO/Gla; +/+</td>
<td>1</td>
<td>Curly, Glazed</td>
<td>2</td>
<td>2058</td>
<td>0.72</td>
</tr>
<tr>
<td>CyO/+; +/+</td>
<td>1</td>
<td>Curly</td>
<td>1</td>
<td>2503</td>
<td>0.87</td>
</tr>
<tr>
<td>Gla/+; +/+</td>
<td>1</td>
<td>Glazed</td>
<td>1</td>
<td>2459</td>
<td>0.86</td>
</tr>
<tr>
<td>+/+; Sb/+</td>
<td>1</td>
<td>Stubble</td>
<td>1</td>
<td>2049</td>
<td>0.72</td>
</tr>
<tr>
<td>+/+; +/+</td>
<td>1</td>
<td>wild type</td>
<td>0</td>
<td>2864</td>
<td>1</td>
</tr>
</tbody>
</table>

**Observed Negative Synergistic Epistasis**

Using the relative fitness for each progeny type, the epistasis values for different combination of mutants, using the method of Jasnos *et al.* (2008), are as follows.

\[
\text{Epistasis} = [(\text{fitness} +)(\text{fit Cy Gla})] - [(\text{fit Cy})(\text{fit Gla})] = [(1)(0.72)] - [(0.87)(0.86)] = 0.72 - 0.75 = -0.03
\]
Epistasis = [(fitness +)(fit Cy Sb)] - [(fit Cy)(fit Sb)]
= [(1)(0.38)] - [(0.87)(0.72)] = 0.38 - 0.63 = -0.25

Epistasis = [(fitness +)(fit Gla Sb)] - [(fit Gla)(fit Sb)]
= [(1)(0.42)] - [(0.86)(0.72)] = 0.42 - 0.62 = -0.20

Epistasis = [(fitness +)(fit Cy Gla Sb)] - [(fit Cy)(fit Gla)(fit Sb)]
= [(1)(0.02)] - [(0.87)(0.86)(0.72)] = 0.02 - 0.54 = -0.52

The relationship between fitness and the number of deleterious mutations is shown in the figure below; the expected assumes an average decrease in fitness for a single mutation of 0.82, which is the observed average fitness of the Curly, Glazed and Stubble flies. There is a clear non-linear decrease in number of progeny with the number of mutants. A paired t test, however, did not show a significant difference for the two lines (P = 0.15).

In summary, we observed negative synergistic epistasis among three dominant visible mutations in *Drosophila melanogaster*. A non-linear relationship was observed between the number of mutations and the reduction in viability.


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