A Potential Regulatory Polymorphism Upstream of hairy Is Not Associated With Bristle Number Variation in Wild-Caught Drosophila

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Manuscript received January 24, 2004
Accepted for publication April 21, 2004

ABSTRACT

To extend results from laboratory genetic mapping experiments to natural populations it is necessary to estimate the phenotypic effects attributable to laboratory-identified genetic factors in nature. We retested a polymorphism found to be strongly associated with an increase of 0.35 sternopleural bristles in laboratory strains in two large samples of wild-caught Drosophila melanogaster. Despite >90% power to detect effects as low as 0.27 bristles (<1% of the total variation in bristle number) we did not replicate the association in nature. Potential explanations for this result are explored.

The community is accumulating a set of reports identifying quantitative trait loci (QTL) for various traits in Drosophila and other model organisms (MacKay 2001). In these investigations, the study organism is generally cultured under standardized laboratory conditions and often variously genetically manipulated prior to genetic analysis (e.g., by inbreeding, chromosome extraction, homogenizing the genetic background, and so on). This serves to improve the signal-to-noise ratio and allow detection of small- to moderate-effect genetic factors.

Conclusions about the evolutionary and ecological relevance of variants identified in such studies are predicated on the assumption that effects detected in the laboratory are similar to those present in natural populations. To address the question of how well laboratory associations hold up in the wild, we retested a particularly strong association between a polymorphism upstream of the transcription start site of the developmental gene hairy (h) and sternopleural bristle number (SBN) in laboratory-reared Drosophila melanogaster (Robin et al. 2002), in outbred Drosophila sampled from nature.

Robin et al. (2002) surveyed 39 variants in a 29-kb region encompassing the h locus in a panel of 57 natural alleles of Drosophila sampled from Raleigh, North Carolina. A single polymorphism, del2187in, was associated with SBN (F = 15.84, P = 0.000081) and survived Bonferroni correction for multiple testing (P-values < 0.05/32 = 0.001563 are significant). del2187in is a complex insertion/deletion polymorphism, and the presence of the allele 2187in was associated with an increase in SBN across four genetic backgrounds, regardless of sex. The estimated effect of an allelic substitution at this locus is between 0.27 and 0.42 bristles (mean is 0.35 bristles; see legend to Figure 1).

We genotyped this candidate polymorphism, with seven other variants across the region (Table 1), in a sample of 2000 D. melanogaster collected in 2001 from a single locality in Napa Valley, California (the nv2001 population). To eliminate any possibility of sample-specific effects, we also genotyped del2187in in a second similarly large sample collected in 1996 in Sonoma Valley, California (the sv1996 population). All markers were in Hardy-Weinberg equilibrium, and abdominal and sternopleural bristle numbers, scored for each individual as previously described (Lyman and Mackay 1998), appeared normally distributed (cf. Genissel et al. 2004).

Bristle number means (phenotypic variance) are 16.7 (4.64), 17.3 (4.79), 15.8 (5.63), and 18.2 (7.63), for male and female sternopleural and male and female abdominal bristle number, respectively, within the wild-caught nv2001 flies.

The following ANOVA models were applied separately to each population to assess the contribution of each polymorphism to the bristle number phenotypes: (1) additive model, which corresponds to a regression of the phenotypic data on the number of major alleles present in each individual and provides an estimate of the effect, a, of an allelic substitution; (2) additive by
sex model, which applies a factorial ANOVA to generate estimates of $a$, the effect of sex, $s$, and an estimate of the genotype-by-sex interaction, $a^s s$; and (3) arbitrary dominance model, whose F-ratio statistic is mathematically equivalent to a one-way ANOVA with three levels, but provides estimates of $a$ and the dominance deviation, $d$.

The candidate polymorphism del2187in showed no association with either bristle trait for any sex or population combination. Indeed, no polymorphism showed a significant effect of $a$ at $P < 0.05$ for any test (Table 1). The arbitrary dominance model for variant AG646-7GC is significant for male abdominal bristle number (ABN) in nv2001 ($F = 4.49$, $P = 0.011$), largely because $d$ is significant ($F = 8.72$, $P = 0.003$). We find no effect of this variant on female ABN and, in common with Robin et al. (2002), find no effect of AG646-7GC on SBN in either sex. Linkage disequilibrium (LD) is low between AG646-7GC and del2187in in both studies. Further work is required to determine if AG646-7GC represents a true bristle number QTL.

Figure 1 plots the estimated additive effect ($a$) of del2187in on SBN from the additive model for each sex and population, and the 95% confidence limits ($a_{\text{mean}}$) on the estimated effects. The number of genotyped individuals used in the regression.

The upper bound on the effect of an allelic substitution at del2187in ($a_{\text{mean}}$) depends on the total observed phenotypic variation, such that as the sum of the variation due to other loci and the environment increases, $a$ is estimated with less accuracy, and $a_{\text{mean}}$ shows a corresponding increase. Since such variance in nature appears higher than that under controlled laboratory environments and genetic backgrounds, we employed very high sample sizes ($N \sim 2000$) to counteract its negative effect on our confidence in $a$ and to obtain a narrow confidence interval. It is noteworthy that our estimate of $a$ is not in any way conditional on the heritability of bristle number in nature. Irrespective of heritability of bristle number (in the laboratory or nature) we can accurately estimate $a$ and place an upper bound on the effect of del2187in. Due to our large sample size the confidence interval on our estimate of $a$ is smaller than previously reported laboratory estimates.

The primary motivation behind efforts to identify QTL under laboratory conditions is to improve the signal-to-noise ratio: reducing variation due to environmental and other loci segregating bristle number QTL will increase power to detect any desired genetic factor. Hence, one might suspect that power to detect moder-

### Table 1

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Common allele frequency</th>
<th>Assay/population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$N^c$</td>
<td>$F^d$</td>
<td>$a (SE)^e$</td>
</tr>
<tr>
<td>A127G</td>
<td>0.75</td>
<td>OLA/nv2001</td>
<td>940</td>
<td>0.172 (NS)</td>
</tr>
<tr>
<td>AG646-7GC</td>
<td>0.69</td>
<td>OLA/nv2001</td>
<td>953</td>
<td>0.583 (NS)</td>
</tr>
<tr>
<td>G688A</td>
<td>0.71</td>
<td>OLA/nv2001</td>
<td>953</td>
<td>0.146 (NS)</td>
</tr>
<tr>
<td>A775C</td>
<td>0.77</td>
<td>OLA/nv2001</td>
<td>957</td>
<td>1.419 (NS)</td>
</tr>
<tr>
<td>A864T</td>
<td>0.70</td>
<td>OLA/nv2001</td>
<td>961</td>
<td>0.284 (NS)</td>
</tr>
<tr>
<td>C2138T</td>
<td>0.57</td>
<td>OLA/nv2001</td>
<td>953</td>
<td>3.167 (NS)</td>
</tr>
<tr>
<td>del2187in</td>
<td>0.74</td>
<td>PCR/nv2001</td>
<td>923</td>
<td>0.227 (NS)</td>
</tr>
<tr>
<td>del2187in</td>
<td>0.71</td>
<td>PCR/nv2001</td>
<td>792</td>
<td>0.487 (NS)</td>
</tr>
<tr>
<td>G2759T</td>
<td>0.97</td>
<td>OLA/nv2001</td>
<td>919</td>
<td>0.489 (NS)</td>
</tr>
</tbody>
</table>

* Polymorphisms are labeled such that the common allele is followed by the position in the hairy alignment of Robin et al. (2002) and then by the rarer allele.

1 Genotypes for most polymorphisms were collected using an oligonucleotide ligation assay (OLA) approach as described previously (Genisiel et al. 2004). del2187in is a complex insertion/deletion polymorphism (common allele ATAAAAAAA; rare allele TATACATAGTATAGTATA). As such is not amenable to this method. Instead we developed a fluorescent fragment size-based assay for an ABI 377 automated sequencer. Briefly, from gDNA we PCR amplified a short fragment about the polymorphism using the oligos del2187in.F, 5’-GACGTTGTTAAGACG[N]CGCTGTTAAGACGACCG-3’, and del2187in.R, 5’-GACGTTGTTAAGACGACCG-3’, where the underlined sequences represent 14-nucleotide (nt) M13-specific tails for second-round amplification, and the region in square brackets a variable-length spacer (0, 2, or 8 nt for del2187in.F, and 0 or 4 nt for del2187in.R), allowing samples to be pooled. Second-round PCR with the oligos M13F.BRL, 5’-GACGTTGTTAAGACGACCG-3’, and M13R.BRL, 5’-GACGTTGTTAAGACGACCG-3’, served to simultaneously amplify and fluorescently label the fragments.

2 Number of genotyped individuals used in the regression.

3 F-ratio computed from a linear regression of genotype on phenotype (NS, $P > 0.05$).

4 Effect (standard error) on bristle number of substituting a common allele with a rare allele (i.e., AA to Aa, or Aa to aa).
Falconer independent estimates of the effect of del2187in from Robin et al. (2002), measured as the difference in mean SBN between 2187in and del2187 marker genotypes among natural h alleles in three genetic backgrounds (four backgrounds were tested but only three showed a significant effect of del2187in on SBN). (1) Among homozygous \( h \) near-isogenic lines (NIL) the effect of del2187in was 0.54. With no dominance, \( a = 0.54/2 = 0.27 \) (as plotted), but if 2187in is dominant to del2187, as suggested by Robin et al. (2002), \( 0.27 < a \leq 0.54 \). (2) For the set of \( h \) NIL made heterozygous against wild-type \( h \) from the Sam homozygous genetic background, \( a = 0.42 \). (3) The effect of del2187in was estimated as \( a = 0.51 \) from the set of \( h \) NIL heterozygous against an \( h^i \) null allele introgressed into the Sam background. However, since the natural \( h \) alleles are combined with a null allele it is difficult to estimate the true value of \( a \) in this background.

Tests, was robust to genetic background, was based on a moderate number of natural alleles, and was consistent with previous quantitative complementation results showing that variation at \( h \) influenced SBN but not ABN (Long et al. 1996; Gurganus et al. 1999). Below we outline some alternative explanations and highlight some testable predictions. Note that these hypotheses are not mutually exclusive and could all play a role.

**Larval competition:** It has been shown that with severe competition among larvae for resources there is stabilizing selection for bristle number, such that the bristle number variance is reduced as larval competition is increased, resulting from a selective elimination of the extreme phenotypes (Kearsey and Barnes 1970). If the populations from which we sampled were subject to stabilizing selection due to larval competition, the power of the Fratio tests may have been reduced. This hypothesis predicts that heterozygotes would have a se-

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**Figure 1.**—Estimated additive genetic effect from a linear regression of genotype at the candidate polymorphism del2187in on SBN. \( a \) is the effect of substituting the common deletion allele with the rare insertion allele, and \( b_{\text{max}} \) is the 95\% confidence limit on this estimate. The dashed lines represent independent estimates of the effect of del2187in from Robin et al. (2002), measured as the difference in mean SBN between 2187in and del2187 marker genotypes among natural \( h \) alleles in three genetic backgrounds (four backgrounds were tested but only three showed a significant effect of del2187in on SBN). (1) Among homozygous \( h \) near-isogenic lines (NIL) the effect of del2187in was 0.54. With no dominance, \( a = 0.54/2 = 0.27 \) (as plotted), but if 2187in is dominant to del2187, as suggested by Robin et al. (2002), \( 0.27 < a \leq 0.54 \). (2) For the set of \( h \) NIL made heterozygous against wild-type \( h \) from the Sam homozygous genetic background, \( a = 0.42 \). (3) The effect of del2187in was estimated as \( a = 0.51 \) from the set of \( h \) NIL heterozygous against an \( h^i \) null allele introgressed into the Sam background. However, since the natural \( h \) alleles are combined with a null allele it is difficult to estimate the true value of \( a \) in this background.

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**Figure 2.**—Power to detect association between del2187in and SBN. Using parameters derived from our tests of association between del2187in and SBN in nv2001 and sv1996 (allele frequency, sample size, and Fratio test error mean square), we calculated the power to detect arbitrary allelic effects, \( a \) (at \( \alpha = 0.05 \)). For the single-sex additive model Fratio test, power is defined as \( 1 - F_{\alpha_{(1)}} \). Using the general noncentrality parameter \( \phi^2 = \left( \sum n_i (\mu_i - \mu)^2 / (\sigma_i^2) \right) \), where \( n_i \) is size of the genotypic class \( i \), \( \mu \) is grand mean, \( \mu_i \) is mean of class \( i \), \( i \) indexes the three genotypes, AA, Aa, and aa, and \( \sigma_i^2 \) is the error mean square. Very similar estimates of power (at \( \alpha = 0.05 \)) are obtained from a Monte Carlo simulation using the observed genotypic class frequencies and sample sizes, additive gene action, and scaling the allelic and Gaussian residual variation so that the site accounts for \( x \%) \% of the total phenotypic variation. We carried out 1000 Monte Carlo replicates for each value of \( x \), with a linear regression of phenotype on genotype performed on each replicate, using the statistical computing language R (http://www.R-project.org). Triangles represent the same estimates of the effect of del2187in on SBN from Robin et al. (2002) described in the legend to Figure 1.
lective advantage and thus be in excess of their expecta-
tion under Hardy-Weinberg equilibrium. However, nei-
ther of our populations show deviation from Hardy-
Weinberg equilibrium with respect to the genotype at
del2187in \((\chi^2 P > 0.05)\), and the observed genotypic
counts are just 12 (43) individuals from the expected
counts under Hardy-Weinberg for the nv2001 (sv1996)
population, with both populations showing a very slight
dearth of heterozygotes.

**Population structure:** If a species is genetically divided
into subpopulations one may not expect to find quanti-
tatively or qualitatively similar associations among
demes. The 10 East Coast North American chromo-
somes sequenced for *hairy* by Robin et al. (2002) appear
similar to a short region about del2187in sequenced in
16 West Coast alleles (accession nos. AY587211–AY58726);
however, neither sample is sufficiently large to detect
small differences in allele frequency.

A better data set is provided by 196 biallelic single
nucleotide polymorphisms (SNPs) in the *Epidermal
growth factor receptor* (Egfr) gene region in 140 lines from
Davis, California, and 86 lines from West End, North
Carolina (Dworkin et al. 2003). Using Fisher’s exact
tests only one SNP showed a significant frequency differ-
ence between populations at the 5\% level after applying
a permutation test (Churchill and Doerge 1994). The
genetic homogeneity between East and West Coast sam-
ple of *Drosophila* for the h and Egfr loci matches previ-
sious observations for the alcohol dehydrogenase region
(Kreitman and Aguadé 1986), suggesting that source
population differences are unlikely to explain the dis-
cordance in association.

**Linkage disequilibrium:** As Robin et al. (2002) point
out, since they did not genotype all polymorphisms in the
h region it is possible that del2187in is not causal and
instead is in strong LD with an ungenotyped causal
QTL. In this case the phenotypic effect at del2187in
would be lower than the effect of the causal QTL by a
factor proportional to the amount of LD between the sites
(cf. Lai et al. 1994). Under such a scenario any reduc-
tion in the level of LD between del2187in and the
true QTL in our outbred populations would reduce the
ability to detect del2187in.

Since the set of SNPs genotyped in this study are
different from those typed by Robin et al. (2002), we
are unable to assess any population-specific differences
in LD structure at the h locus. Instead, to gauge the
level of any such differences, we used data provided in
Kreitman and Aguadé (1986) and Dworkin et al.
(2003) to assess the homogeneity of LD estimates be-
tween populations (Weir 1996, p. 137). These two stud-
ies provide 53 and 14,143, respectively, informative esti-
mates of LD (considering only SNPs showing >5% minor
allele frequency within both populations), of
which only 2 showed a significant difference between
populations after Bonferroni correction.

This implies that patterns of LD are similar among
East and West Coast North American populations. As-
suming that this result also applies to the h gene region,
even if del2187in is not itself the causal QTL, it should
have maintained similar LD with the actual QTL in our
population as it did in the lines used by Robin et al.
(2002).

**Genotype-by-environment interaction:** Environmental
conditions encountered by flies sampled directly
from nature cannot be controlled, and a measured al-
leric effect represents a weighted average of the effect
over environments. Therefore, to the extent that a geno-
typic effect is modulated by environmental factors, the
ability to detect a variant in a natural population may
be diminished.

The magnitude and generality of genotype-by-envi-
ronment interaction (GEI) at the level of individual
QTL is unclear. In one of the best studies Gurganus et
al. (1998) identified significant heterogeneity in bristle
number QTL effect across thermal and sexual environ-
ments in *Drosophila*. However, when we consider errors
in measuring the effects of QTL in different environ-
ments (simulation data not shown), although there is
significant GEI (as observed by Gurganus et al. 1998),
it is difficult to precisely gauge its magnitude. Unfortu-
nately, the pattern of GEI is still largely unknown for
quantitative traits, especially when the environments of
interest are laboratory vs. nature. Thus, we cannot reli-
ably discount or support the possibility that GEI contrib-
utes to the discrepancy between the del2187in associa-
tion in the laboratory and in nature.

**Laboratory effects:** Under some forms of epistasis
the use of isogenic laboratory strains could result in
estimates of effects in the laboratory that would be larger
than those observed in outbred populations. Under a
79) with no epistasis, where both a genotyped locus (B)
and an ungenotyped unknown locus (U) have pheno-
typic effects, estimates of the phenotypic effect of B are
identical in outbred and inbred populations regardless
of frequency or the degree of dominance at either locus.
As an example, however, in a population where the rare
double homozygote *bbuu* has effect \(-\epsilon\) and the other
eight genotypes *+e*, the phenotypic effect associated
with locus B will always be inflated in the laboratory
(isogenic) population.

Although this is a fairly extreme case of synergistic or
reinforcing epistasis, it is the type of epistasis expected
under models of mutation-selection balance in which
only the rare double homozygote genotypes produce
extreme phenotypic effects visible to purifying selection.
The evidence for synergistic epistasis (SE) is equivocal—
compare Whitlock and Bourgouet (2000) and Peters
and Keightley (2000)—however, such experiments have
tended to look at SE between randomly induced
mutations on fitness-related traits, and it is conceivable
that SE is a more general phenomenon within genes,
or pathways of genes, or on traits with small pleiotropic
effects on fitness. For example, Shepard et al. (1989)
demonstrated extensive interactions between the neuro-
genetic loci Notch, Delta, and Enhancer of split on Drosophila eye morphogenesis, while DWORKIN et al. (2003) detected a synergistic interaction of photoreceptor determination between two sites within the same Egfr exon in Drosophila.

Choice of isogenic background may be particularly important if del2187 in epistatically interacts with other loci. ROBIN et al. (2002) generated a set of nearly isogenic lines each having a small section of natural chromosome about h, but otherwise a completely isogenic Samarkand (Sam) genetic background. If a genotyped putative causal SNP epistatically interacts with an allele rare in natural populations but fixed in Sam, the estimated effect at the genotyped SNP will not necessarily be indicative of its effect in nature.

**Future work:** The contrast between the laboratory identification of del2187in as a strong bristle number QTL and the findings of this study suggests that laboratory associations may not always translate directly to average effects (sensu stricto FALCONER and MACKAY 1996, p. 112) in nature. The most viable hypotheses discussed above make testable predictions regarding the relationship between QTL in the laboratory and in nature, and while the experiments are certainly not trivial, the D. melanogaster/bristle number model will allow eventual resolution of the observed difference. To test the hypothesis that uncontrolled environmental variation prevents detection of the effect in our large natural population samples, associations could be examined in a large sample of wild-caught females and in their laboratory-reared progeny. Alternatively, if the laboratory association were conditional on an epistatic interaction with an unidentified homoygous site in the Sam isogenic background, repeating the chromosome substitution scheme of ROBIN et al. (2002) in different genetic backgrounds would eliminate the effect.

It is possible that the discrepancy reported here represents an isolated case, where perhaps the initial result was due to a highly significant epistatic interaction with a particular genetic background or where GEI effects are more important than we suggest. Fortunately, the potential explanations for the observed differences yield testable predictions, and it is within our reach to understand the architecture of a complex model character in terms of the individual nucleotides governing the trait in nature.

We thank A. Genissel and B. P. Lazzaro for help with bristle counting and C. H. Langley, T. F. C. Mackay, P. Beldade, J. D. Gruber, and two anonymous reviewers for helpful comments and suggestions on the manuscript. All data are available at http://estern.bio.ucf.edu/pubs.htm. This work was supported by National Institutes of Health grant GM-58564 to A.D.L.

**LITERATURE CITED**


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Communicating editor: M. AGUADE