Epistatic interactions attenuate mutations affecting startle behaviour in *Drosophila melanogaster*

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Summary

Epistasis is an important feature of the genetic architecture of quantitative traits. Previously, we showed that startle-induced locomotor behaviour of *Drosophila melanogaster*, a critical survival trait, is highly polygenic and exhibits epistasis. Here, we characterize epistatic interactions among homozygous P-element insertional mutations affecting startle-induced locomotion in the Canton-S isogenic background and in 21 wild-derived inbred genetic backgrounds. We find pervasive epistasis for pairwise combinations of homozygous P-element insertional mutations as well as for mutations in wild-derived backgrounds. In all cases, the direction of the epistatic effects is to suppress the mutant phenotypes. The magnitude of the epistatic interactions in wild-derived backgrounds is highly correlated with the magnitude of the main effects of mutations, leading to phenotypic robustness of the startle response in the face of deleterious mutations. There is variation in the magnitude of epistasis among the wild-derived genetic backgrounds, indicating evolutionary potential for enhancing or suppressing effects of single mutations. These results provide a partial glimpse of the complex genetic network underlying the genetic architecture of startle behaviour and provide empirical support for the hypothesis that suppressing epistasis is the mechanism underlying genetic canalization of traits under strong stabilizing selection. Widespread suppressing epistasis will lead to underestimates of the main effects of quantitative trait loci (QTLs) in mapping experiments when not explicitly accounted for. In addition, suppressing epistasis could lead to underestimates of mutational variation for quantitative traits and overestimates of the strength of stabilizing selection, which has implications for maintenance of variation of complex traits by mutation–selection balance.

1. Introduction

Epistasis – the dependence of the allelic effects at one locus on the genotype at another locus – is an important feature of the genetic architecture of quantitative traits (Anholt & Mackay, 2004; Flint & Mackay, 2009; Mackay *et al*., 2009). Assessing the magnitude and nature of epistatic interactions is important from several perspectives. First, epistasis causes bias in estimates of allelic effects from quantitative trait locus (QTL) mapping studies when it is present but not accounted for (Carlborg *et al*., 2006; Phillips, 2008). Second, epistatic interactions can be used to infer genetic networks affecting complex traits (Phillips, 2008). Finally, epistasis can affect predictions of long-term response to artificial and natural selection (Wright, 1977; Carlborg *et al*., 2006; Phillips, 2008).

Epistasis is not easy to detect in experiments designed to map QTLs. Genome scans for pairwise epistatic interactions with n markers require m(n − 1)/2 statistical tests, which impose a severe multiple testing penalty and concomitantly low significance thresholds, such that only very large interactions are detectable. Further, all possible two locus genotypes may not be present unless mapping populations are very large; this is a particular problem for closely linked loci.
Nevertheless, epistatic interactions have been reported between QTLs affecting *Drosophila* bristle number (Long *et al.*, 1995; Gurganus *et al.*, 1999; Dilda & Mackay, 2002), wing morphology (Weber *et al.*, 1999, 2001), lifespan (Leips & Mackay, 2000, 2002; Mackay *et al.*, 2006) and startle-induced locomotor behaviour (Jordan *et al.*, 2006). In mice, epistasis has been documented between QTLs affecting many traits related to growth, body weight and morphometry (Brockmann *et al.*, 2000; Cheverud *et al.*, 2001; Klingenberg *et al.*, 2001; 2004; Workman *et al.*, 2002; Yi *et al.*, 2006). Epistasis also features prominently in the genetic architecture of growth rate of chickens (Carlberg *et al.*, 2006), *Arabidopsis* (Kroymann & Mitchell-Olds, 2005) and yeast (Steinmetz *et al.*, 2002; Sinha *et al.*, 2008). Epistatic effects can be as large as main QTL effects, and can occur in opposite directions between different pairs of interacting loci and between loci without significant main effects on the trait. Epistatic effects can also occur between closely linked QTLs (Steinmetz *et al.*, 2002; Kroymann & Mitchell-Olds, 2005; Sinha *et al.*, 2008) and even between polymorphisms at a single locus (Stam & Laurie, 1996).

Epistasis is more readily detectable in crosses among lines in which genetic heterogeneity is reduced and the genetic background is controlled precisely. Diallel crosses have revealed extensive epistasis between introgression lines of tomato for yield-associated traits (Eshed & Zamir, 1996), and between *Drosophila* P-element mutations in a common isoegenic background affecting olfactory function (Fedorowicz *et al.*, 1998; Sambandan *et al.*, 2006), climbing (van Swinderen & Greenspan, 2005) and startle (Yamamoto *et al.*, 2008) behaviour. Clark & Wang (1997) constructed all nine possible two-locus genotypes for several pairs of P-elements and identified extensive epistasis affecting metabolic activity in *Drosophila*.

Other powerful designs for detecting epistatic interactions are to construct a panel of chromosome substitution lines, in which single chromosomes from one strain are each substituted into the homozygous genetic background of a second strain; or even more precisely, to construct segmental introgression lines, in which small genomic segments from one strain are introgressed into the background of a second strain, such that the entire collection of introgression lines tile across the genome. In the presence of epistasis, the sum of the effects of all chromosome substitution lines or introgression lines is greater or less than the difference in the mean value of the trait between the two parental strains. Using these designs, substantial epistasis has been revealed for tomato yield traits (Eshed & Zamir, 1996), a battery of quantitative traits in mice (Shao *et al.*, 2008) and *Drosophila* aggressive behaviour (Edwards & Mackay, 2009).

*Drosophila melanogaster* is an excellent model system for studies of epistasis affecting quantitative traits, due to the ease of constructing the relevant genotypes. Previously, we showed that startle-induced locomotor behaviour is a phenotype that is easily and reproducibly quantified, highly polygenic and exhibits epistasis both at the level of QTLs and P-element mutations in a diallel cross design (Jordan *et al.*, 2006, 2007; Yamamoto *et al.*, 2008). Here, we characterize epistatic interactions among homozygous P-element mutations affecting startle-induced locomotion in the *Canton-S* isogenic background. In addition, we combine mutational analysis with the chromosome substitution design to assess the effects of naturally occurring modifiers of mutations affecting the startle response. We accomplish this by substituting second or third *Canton-S* chromosomes with or without P-element insertion mutations that impair the startle response into 21 wild-derived inbred genetic backgrounds. We show that epistasis is pervasive both for pairwise combinations of homozygous P-element insertion mutations and for mutations in wild-derived backgrounds; that in all cases the direction of the epistatic effects is to suppress the mutant phenotypes; and that there is variation in the magnitude of epistasis among the wild-derived genetic backgrounds. We discuss the implications of these results for genetic networks affecting startle-induced locomotion, phenotypic robustness and maintenance of genetic variation for the startle response in natural populations.

2. Materials and methods

(i) *Drosophila* stocks

The 15 P-element insertion mutants affecting locomotor startle response used in this study have been described previously (Yamamoto *et al.*, 2008). They contain single *P[GT1]* insertions (Lukacsovich *et al.*, 2001) and were generated in the same isogenic *w*¹¹¹B; *Canton-S B* background as part of the Berkeley *Drosophila* Gene Disruption Project (Bellen *et al.*, 2004). All P-elements were inserted within or in close proximity to a well-annotated gene: *trantrak* (*tik*, *BG02461*), *extramacochaetae* (*emc*, *BG00060*), *Hlm7*, *BG02029*, *longitudinalis* lacking (*lola*, *BG02501*), *Calcretulin* (*Crc*, *BG01724*), *neuronalized* (*neur*, *BG02542*), *Laminin A* (*Lana*, *BG01389*), *roundabout* (*robo*, *BG01095*), *Sema-1a* (*BG02570*), *Sema-5c*, *BG01245*, *Darkener of apricot* (*Doa*, *BG02498*), *Cysteine string protein* (*Csp*, *BG01683*), *CG8963* (*BG02470*), *mir-279* (*BG01828*) and *mir-317* (*BG01900*).

Four of the genes tagged by *P[GT1]* insertions were on the second chromosome (*Sema-1a*, *CG8963*, *robo* and *lola*) and 11 were on the third chromosome (*tik*, *emc*, *Hlm7*, *Crc*, *neur*, *Lana*, *Sema-5c*, *Doa*, *Csp*, *mir-279* and *mir-317*). We created all 44 possible
chromosome 2–chromosome 3 double homozygous genotypes of these P-element insertions in the Canton-S background.

Inbred lines were derived by 20 generations of full-sib mating from isofemale lines that were collected from the Raleigh, NC Farmer’s Market in 2003 (Ayroles et al., 2009). We selected 21 Raleigh inbred lines of which males caused no obvious hybrid dysgenesis with Canton-S females when their F_1 hybrids were examined for ovarian development at 28.9 °C. We chose three P[GT1]-tagged genes on the second chromosome (Sema-1a, robo and lola) and seven on the third chromosome (emc, HLHm7, Crc, neur, Sema-5c, Csp and mir-317) for further analyses, and constructed chromosome substitution lines in which a Canton-S chromosome carrying a P[GT1]-element insertion or its co-isogenic P-element free counterpart was substituted in each of the Raleigh inbred line genetic backgrounds, using balancer chromosomes to prevent recombination, and minimizing any remnant P-element activities by using flies with natural population-derived chromosomes as female progenitors (Supplementary Figures 1 and 2). We were able to generate all homozygous genotypes of combinations between each of the 10 P[GT1]-tagged genes and 21 Raleigh inbred lines except in two cases (RAL-765 and mir-317 and RAL-765 and Crc).

All flies were reared on cornmeal–agar–molasses medium at 25 °C under controlled density and aged 3–7 days post-eclosion prior to the behavioural assay.

(ii) Behavioural assay

We measured startle behaviour as described previously (Jordan et al., 2006, 2007; Yamamoto et al., 2008). We placed single flies in a plastic culture vial with medium the day before measurement. The next day, we subjected the flies to a mechanical disturbance by a gentle sudden tap of the vial, which was then immediately placed horizontally under a fluorescent lamp. We quantified startle-induced locomotion as the number of seconds each fly was active in the 45 s period immediately following the disturbance. All tests were done at 25 °C between 8 am and 12 pm. The sample size for all experiments was N = 20 measurements/sex/genotype. For the P-element mutations in the Canton-S background, we measured two males and two females from each of the 44 double homozygous lines, 15 single homozygous parental genotypes and the progenitor Canton-S strain each day for 10 days (N = 2400). We measured two males and two females from each of the second chromosome substitution lines (84 genotypes) each day for 10 days (N = 3360) and one male and one female from each of the third chromosome substitution lines (166 genotypes) each day for 20 days (N = 6640).

(iii) Analysis of epistasis in the Canton-S background

We performed three-way fixed effects analysis of variance (ANOVA) of the startle-induced locomotion scores of the double homozygous genotypes using the model

\[ Y = \mu + C_2 + C_3 + S + C_2 \times C_3 + C_2 \times S + C_3 \times S + C_2 \times C_3 \times S + \epsilon, \]

where \( Y \) is the observed value, \( \mu \) is the overall mean, \( C_2 \) is the effect of the second chromosome insertions, \( C_3 \) is the effect of the third chromosome insertions, \( C_2 \times C_3 \) is the effect of the epistatic interaction between the second and third chromosome insertions, \( C_2 \times S \) and \( C_3 \times S \) represent the sex interaction terms between the second chromosome and third chromosome effects, respectively, \( C_2 \times C_3 \times S \) is the three-way interaction between P-element insertion effects on the second chromosome, the third chromosome and sex, and \( \epsilon \) is the error term.

We estimated 2\( a \), the deviation of the mean startle-induced locomotion score of the homozygous mutations from the mean of the Canton-S strain (Falconer & Mackay, 1996), for each of the 15 homozygous P-element mutations. We then computed the expected startle-induced locomotion score of the double homozygous genotypes from the deviation of \((2a_i + 2a_j)\) from the mean of the Canton-S strain, where the subscripts \( i \) and \( j \) denote the respective homozygous genotypes. The estimate of the epistatic effect (\( I \)) for each double homozygous genotype is then the difference between the expected and observed scores. We assessed the significance of the \( C_2 \times C_3 \) epistatic interactions by performing the same ANOVA models described above to the four relevant genotypes (Canton S, second chromosome homozygote P-element insertion, third chromosome homozygous P-element insertion, and the double chromosome 2–chromosome 3 P-element insertion) for each of the 44 double homozygotes.

(iv) Analysis of epistasis in the Raleigh inbred line backgrounds

We estimated the expected effect of the P-element mutants in each wild-derived inbred line background by subtracting the estimate of 2\( a \) for each mutation in the pure Canton-S background from the observed startle-induced locomotion score of the chromosome substitution lines with wild-type Canton-S chromosomes. The estimate of the epistatic effects (\( I \)) for each inbred line genetic background is thus the difference between the expected effect and the observed startle-induced locomotion score of the line in which the mutant Canton-S chromosome was substituted. We assessed the significance of each of the 208 epistatic
interactions by three-way fixed effect performing ANOVAs using the model:

\[ Y = \mu + G + R + S + G \times R + G \times S + R \times S + \varepsilon, \]

where \( Y \) and \( \mu \) are defined above, \( G \) is the effect of the presence or absence of the \( P \)-element, \( S \) is the effect of sex, \( R \) is the effect of Raleigh inbred line versus Canton-S genetic backgrounds, \( G \times R \), \( G \times S \), \( R \times S \) and \( G \times R \times S \) are the interaction terms and \( \varepsilon \) is the environmental variance. Significance of the \( G \times R \) and/or \( G \times R \times S \) interaction terms indicates significant epistasis. In addition, we determined whether there was variation in epistatic effects among the 21 wild-derived inbred lines by performing the mixed model ANOVAs of the same structure described above across all Raleigh line genotypes, treating the Raleigh genotypes and interactions with Raleigh genotypes as random effects.

3. Results

(i) Epistasis between \( P[GT1] \) mutations affecting startle behaviour in the Canton-S-background

Based on our previous observations of extensive epistasis among double heterozygotes (Yamamoto et al., 2008), we predicted that defects in startle-induced locomotor reactivity should be aggravated in double homozygotes and epistatic interactions would also be prevalent. We generated all 44 possible double homozygous combinations of four second chromosomal and 11 third chromosomal \( P[GT1] \) insertion lines which had been used in the previous double heterozygote analysis, and measured their startle-induced locomotion.

When all 44 genotypes of the combinations of four \( P \)-element-tagged genes on the second chromosome and 11 on the third chromosome were analysed by three-way ANOVA, the main effects of the second and third chromosome insertional mutations were highly significant, as expected (Table 1). The interaction between the second and third chromosome mutations was also highly significant overall (Table 1), which indicates epistatic interactions among these mutations.

The mean startle response scores of homozygous \( P[GT1] \) insertional mutations, estimates of 2\( a \) (the difference between the mean startle response score of the co-isogenic Canton-S B strain and the \( P[GT1] \) mutation), observed and expected double homozygous mutant scores, and estimates of the epistatic interactions between chromosome 2 and chromosome 3 mutations are given in Supplementary Table 1. Since the overall main effect of sex and its interaction terms were not significant (or only marginally significant for the interaction with chromosome 2 mutations, Table 1), we present these data averaged across both sexes. The average startle response of the 15 mutations was 27.59 s, 33% lower than the wild-type Canton-S B score. The average startle response of the 44 double homozygous genotypes was 23.87 s, lower than that of the parental homozygotes from which they were derived, as expected from combining mutations which affect the trait in the same direction, but not as low as predicted from additive combinations of homozygous mutations. Thus, the interactions indicated by the overall ANOVA (Table 1) must predominantly involve suppressing epistasis, such that the double homozygotes are less mutant (i.e. more wild-type) than expected. This trend is clear from the plot of observed versus expected startle response of the 44 double homozygous genotypes (Fig. 1).

Widespread suppressing epistasis was indeed confirmed from estimates of individual epistatic effects for each of the 44 pair wise interactions, of which 35 were at least nominally significant and negative, and 20 had \( P \)-values (assessed from the significance of the \( C2 \times C3 \) interaction term) that survived a
Bonferroni correction for multiple tests (Supplementary Table 1). Graphical depictions of these epistatic interactions are given in Figs 2 and 3. Particularly prominent and abundant epistatic interactions involve robo and Sema-1a on the second chromosome, and emc, Sema-5c, mir-317 and HLHm7 on the third chromosome.

(ii) Epistasis between \( P[GT1] \) mutations affecting startle behaviour and wild-derived genetic backgrounds

We substituted ten Canton-S chromosomes with \( P[GT1] \) mutations that affect startle behaviour as well as the wild-type Canton-S chromosomes into 21 wild-derived Raleigh inbred lines (Supplementary Tables 2 and 3) and measured the startle-induced locomotion behaviour of each of the resulting 250 genotypes. Similarly to the analysis of double homozygous mutations, the observed startle responses of the wild-derived chromosomes in which a \( P \)-element mutation had been substituted was much less than predicted based on the estimate of \( 2 \alpha \) in the Canton-S background (Fig. 4). That is, naturally segregating alleles suppress the mutational effects of the \( P \)-element mutations on startle-induced locomotion. We quantified the epistatic effects of each mutant allele in the
ANOVAs confirmed highly significant variation among the Raleigh inbred line backgrounds for all 10 \(P(GT1)\)-tagged genes; the line term was highly significant in all cases (Table 2). Genotype, i.e. absence or presence of a \(P(GT1)\)-tagged gene, was also significant for most genes. The one exception was \(Sema-1a\), with an average deviation from the control across all Raleigh inbred lines of only 0-19 s (Supplementary Table 2): the large mutant effect observed in the \(Canton-S\) background was eliminated in the wild-derived backgrounds. The Raleigh inbred lines also vary in the extent to which they suppress the effects of mutations (Table 2, Fig. 6), as indicated by the significant \(G \times R\) and/or \(G \times R \times S\) interactions for most of the genes (Table 2). Thus, there is evolutionary potential for enhancing or suppressing effects of single mutations.

4. Discussion

We observed substantial suppressing epistasis among 15 \(P\)-element insertion mutations with large homozygous effects on locomotor startle response in the \(Canton-S\) genetic background. The only previous evidence for genetic interactions among these loci is our diallel cross analysis of locomotor startle response of all 105 double heterozygotes (Yamamoto et al., 2008) among the 15 mutant lines. The diallel cross analysis implicated both suppressing and enhancing epistatic interactions among these loci, rather than the pervasive suppressing epistasis observed in this study. There are two, non-mutually exclusive, explanations for this discrepancy. First, epistasis in the diallel cross analysis was measured relative to the mean startle response of all 105 double heterozygotes and not relative to the startle response of the \(Canton-S\) progenitor strain, as is done in this study. Second, the analysis for double heterozygotes detects dominance by dominance epistasis, and the analysis for double heterozygotes detects additive by additive epistasis: the two sources of epistatic interaction are independent (Falconer & Mackay, 1996).

Epistasis indicates that the interacting loci affect the same genetic network or pathway leading to the quantitative trait phenotype. Suppressing epistasis occurs when the network is buffered against deleterious mutations, such that the trait phenotype resulting from the combination of two mutations is less than additive. An understanding of the mechanism underlying this buffering, or canalization (Waddington, 1957) is required if we are to predict individual phenotypes from knowledge of their genotypes at loci affecting the trait, as well as to predict the response to artificial and natural selection. Can we derive clues about the underlying network affecting locomotor startle response from knowledge of the molecular functions of the 15 interacting loci in this study? The
genes tagged by the P-elements include transcriptional regulators (tik, emc, HLHm7 and lola), a calcium binding chaperone (Crc), an ubiquitin protein ligase (neur), an extracellular matrix protein (LanA), axon guidance molecules (robo and Sema-1a), a protein threonine/tyrosine kinase (Doa) and microRNAs (mir-279 and mir-317), as well as a gene of unknown function (CG8963). However, with the sole exception

Table 2. P values from mixed model ANOVAs of the effects of wild-type Canton-S chromosomes and co-isogenic chromosomes with P-element mutations affecting startle response in 21 wild-derived genetic backgrounds

<table>
<thead>
<tr>
<th>Source</th>
<th>robo</th>
<th>Sema-1a</th>
<th>mir-317</th>
<th>emc</th>
<th>Sema-5c</th>
<th>HLHm7</th>
<th>Csp</th>
<th>Crc</th>
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<tr>
<td>Genotype (G)</td>
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The alleles of the genes are:
- robo<sup>BG01092</sup>, Sema-1a<sup>BG02570</sup>, mir-317<sup>BG01900</sup>, emc<sup>BG00986</sup>, HLHm7<sup>BG00208</sup>, Csp<sup>BG02106</sup>, Crc<sup>BG01724</sup>, and neur<sup>BG02542</sup>. **** P < 0.0001; *** 0.0001 < P < 0.001; ** 0.001 < P < 0.01; * 0.01 < P < 0.05; ns P > 0.05.

Fig. 6. Landscape of epistatic interactions for startle-induced locomotion among (a) second and (b) third chromosome homozygous P-element mutations in Raleigh inbred line genetic backgrounds. The plots depict the estimates of the epistatic effect, I, of the mutations in the different inbred line backgrounds, colour-coded to indicate different values of I. Most estimates of I are negative, indicating widespread suppressing epistasis.
of mir-279, all of these genes have been implicated in the development and function of the central and/or peripheral nervous system (Tweedie et al., 2009), consistent with their role in modulating startle-induced locomotion.

We have shown that suppressing epistasis is prevalent among 15 loci affecting startle response, but have not generated all 105 possible double homozygotes necessary to determine their relationships in the underlying network. Further, locomotor startle response is highly polygenic. Yamamoto et al. (2008) observed significant effects on startle response for 267 of 720 (37%) P-element insertions tested. This analysis does not approach a saturation mutagenesis screen. Even so, a complete analysis of all of these mutations would require synthesis and testing of 35,511 double homozygous genotypes, which ignores the possibility of higher order interactions. An alternative strategy for dealing with the issue of dimensionality when making inferences about epistasis and networks is to take advantage of the multifactorial perturbations of the network from naturally occurring variation. There is substantial naturally occurring variation for locomotor startle response (Jordan et al., 2006, 2007; Ayroles et al., 2009), and comparison of estimates of genetic variation from realized heritability and variation among inbred lines implicates a large contribution of epistasis to the genetic architecture of this trait (Jordan et al., 2007). Thus, we should be able to use single P-element mutations as molecular probes to discover interacting loci without fully exploring the universe of potentially interacting mutations.

We substituted 10 chromosomes containing P-element mutations affecting startle response in the Canton-S background and the wild-type Canton-S chromosomes into each of 21 distinct wild-derived inbred backgrounds. On average, the effects of P-element insertions on startle-induced locomotion were nearly five times greater in the Canton-S background than in the introgression lines (13.17 s compared to 2.76 s). Suppressing epistasis was observed for all 10 mutations, but was very strong for robo, Sema-1a, mir-317, emc, Sema-5c, HLHm7 and lola, and weaker for Csp, Cre and neur. Therefore, wild-derived lines harbour modifiers that attenuate the effects of the homozygous P-element insertions on the startle response. More importantly, there is genetic variation among the lines in the extent to which modifiers suppress the mutant effects. The 21 inbred lines used for this study are a subset of the 192 inbred line Drosophila Genetic Reference Panel (DGRP), for which whole genome sequences are being obtained (Mackay et al., 2008). With the future availability of complete polymorphism data for the DGRP and the ability to construct larger sets of chromosome substitution lines, we will be able to use genome-wide association analysis to map the modifiers suppressing the mutant effects.

Rapid avoidance of sudden and adverse environmental disruptions is essential for survival and, hence, startle-induced locomotion is plausibly associated with fitness. Whereas impairment of startle behaviour may compromise survival, excessive startle behaviour might also be detrimental in that it would interfere with normal behavioural functions, including feeding, mating, and defence of territory; and would be energetically wasteful. Thus, startle behaviour is likely to be under stabilizing selection for an intermediate optimum.

Waddington (1957) suggested that traits under strong stabilizing selection would be strongly genetically canalized; i.e. the phenotype would be robust to deleterious mutations. The mechanism underlying genetic canalization is suppressing epistasis. There has recently been much theoretical attention to the evolution of genetic canalization in terms of developmental and genetic networks (Siegal & Bergman, 2002; Hermisson et al., 2003; Hermisson & Wagner, 2004; Proulx & Phillips, 2005; Gros et al., 2009). Indirect evidence for the evolution of suppressing epistasis comes from experiments demonstrating an increase in genetic variance when mutations are introduced into different wild-derived genotypes (Gibson & Wagner, 2000; Flatt, 2005), as expected if the mutation is in a gene for which the wild-type allele suppresses the effects of segregating variation at other loci, thus releasing previously hidden cryptic genetic variation. Our observations provide direct experimental support for widespread suppressing epistasis in natural populations, as well as natural variation in the magnitude of epistatic modulation. P-element mutations have pleiotropic effects on multiple quantitative traits (Rollmann et al., 2006; Sambandan et al., 2006; Yamamoto et al., 2008); therefore, it will be interesting in the future to evaluate a similar panel of chromosome substitution lines for a suite of quantitative traits to determine whether suppressing epistasis is a general feature of the genetic architecture of most traits; and, if so, whether the same modifiers modulate the effects on multiple traits.

Widespread suppressing epistasis has implications for gene mapping studies of common human diseases and more generally for maintenance of quantitative genetic variation in natural populations. The main effects of individual loci will be underestimated if suppressing epistasis exists but is not accounted for in a mapping study. The collective result of many highly powered whole genome association studies for human common diseases and complex traits is that the main effects of significant loci are small, and together explain very little of the estimated heritable variance for these traits (Altschuler et al., 2008). Suppressing epistasis is one of several explanations...
for this observation. The consequence of genetic variation in suppressing epistasis is that a particular variant affecting the likelihood of developing the disease will have a greater or lesser effect depending on the individual’s genetic background with respect to other loci.

An unresolved problem in evolutionary quantitative genetics is the reconciliation of the twin observations of strong stabilizing selection ($s \sim 0.01$) with large amounts of segregating variation for most quantitative traits, such that heritabilities are of the order of 0.5, and hence genetic variation ($V_G$) is approximately the same as environment variation ($V_E$). Theory predicts that the equilibrium genetic variance maintained by a balance between stabilizing selection and the input of variation from new mutations ($V_M$) is $V_G = V_M/s$. Estimates of $V_M$ are consistently 0.001$V_E$. However, evaluating the equation for $V_G = V_E$ and $V_M = 10^{-4}V_E$, gives $s = 0.001$, which is not a strong stabilizing selection. Alternatively, evaluating the equation for $V_G = V_E$ and $s = 0.01$ gives $V_M = 10^{-2}V_E$, which is too large (Falconer & Mackay, 1996). However, with suppressing epistasis, it is possible that the strength of stabilizing selection is overestimated, and/or the input of mutational variation is underestimated. Support for this hypothesis comes from long-term mutation accumulation experiments on sensory bristle number in which the divergence among inbred lines (Mackay et al., 1995) or among lines selected for divergent trait values from an inbred base population (Mackay et al., 1994) attenuate over time, and the effects of mapped mutations exceed the divergence among lines (Mackay et al., 2005), as would be expected if new mutations exhibit suppressing epistasis.

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