# Potential Variance Affecting Homeotic *Ultrabithorax* and *Antennapedia* Phenotypes in *Drosophila melanogaster*

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ABSTRACT

Introgression of homeotic mutations into wild-type genetic backgrounds results in a wide variety of phenotypes and implies that major effect modifiers of extreme phenotypes are not uncommon in natural populations of Drosophila. A composite interval mapping procedure was used to demonstrate that one major effect locus accounts for three-quarters of the variance for haltere to wing margin transformation in *Ultrabithorax* flies, yet has no obvious effect on wild-type development. Several other genetic backgrounds result in enlargement of the haltere significantly beyond the normal range of haploinsufficient phenotypes, suggesting genetic variation in cofactors that mediate homeotic protein function. Introgression of *Antennapedia* produces lines with heritable phenotypes ranging from almost complete suppression to perfect antennal leg formation, as well as transformations that are restricted to either the distal or proximal portion of the appendage. It is argued that the existence of "potential" variance, which is genetic variation whose effects are not observable in wild-type individuals, is a prerequisite for the uncoupling of genetic from phenotypic divergence.

THE genetic component of quantitative variation is generally attributed to the segregation of a number of alleles of small to moderate effect that tend to interact in an additive manner (Mackay 1995). This standard theory has been augmented in recent years by acceptance of the important contributions of recurrent mutation and rare alleles, as well as complex nonadditive, including epistatic, interactions among loci (Barton and Turelli 1989; Whitlock *et al.* 1995). In most cases, it is also thought that the observed range of variation approximates the possible range, meaning that variation that is of evolutionary significance is observable in natural populations. This perhaps reflects the adaptationist view that the limits of continuous traits are set by the strength of stabilizing selection on each optimum phenotype, with the result that extreme individuals are not seen because natural selection removes the genetic variation that would tend to favor their appearance.

An alternative hypothesis is that the observed variation is just the tip of the iceberg, so that quantitative traits harbor considerably more variation than that which is expressed. According to the concept of canalization (Schmalhausen 1949; Waddington 1957), developmental genetic mechanisms ensure that morphological traits are so well buffered that the effects of alleles that tend to produce abnormal phenotypes are suppressed. A prediction of this hypothesis is that perturbation of a trait by the introduction of a mutation of large effect should give rise either to a significantly expanded range of variation, or to a low correlation between wild-type and mutant phenotypes across the same genetic backgrounds. The classical view by contrast predicts that the observed range of variation simply shifts along with the new trait mean, or, if the mutation creates a novel phenotype, that there should be little or no genetic variation associated with it.

We have shown that introgression of the homeotic mutation *Ultrabithorax* (*Ubx*$^{1}$) into different wild-type backgrounds supports the alternative hypothesis, namely, that there is a low genetic correlation between wild-type and mutant phenotypes (Gibson and van Helden 1997). Here, we extend this analysis by examining in detail some of the more aberrant homeotic *Ubx*$^{1}$ phenotypes and showing that there are alleles with potentially extreme effects that do not obviously affect wild-type haltere development. In addition, we demonstrate that there is considerable "potential variance" available to modify the antenna-to-leg transformation due to the *Antennapedia* (*Antp*$^{73b}$) homeotic mutation. It is argued that an appreciation of the existence of this source of variation has considerable implications for understanding various aspects of evolutionary change.

## MATERIALS AND METHODS

**Fly stocks:** All flies were maintained at 25° (unless 18° is stated) on standard cornmeal with yeast. Wild-type lines were obtained from the Bowling Green Stock Center or from the Ann Arbor fruit markets as listed in Gibson and van Helden (1997). Mutant stocks were obtained from the Bloomington Stock Center: Antp$^{73b}$ (#2259), *Ubx*$^{1}$ (#2866), *Cbx*$^{1}$ (#3433),

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**TABLE 1**

**Primers for PCR amplification of microsatellite markers**

| Marker | Locus | Location | Sequences[a] | Type |
|---|---|---|---|---|
| 1.1 | Notch | 3C | tttgcggcttcgtttgttta<br>gatccgcccacatacacact | GT |
| 1.2 | tenA | 11A | ctcttagtgcgcagggattc<br>gagtcgctcaatggcagg | AT |
| 2.1 | pendulin | 22D | acgtttcgagctcatttcgg<br>acactggtgtgaagggaatg | AAC |
| 2.2 | ninaC | 28A | ctcgcttccaggactttgtc<br>gcatattgatcctctcgaag | AT |
| 2.3 | Sos | 34D | cgagcactgcttgagtgcag<br>gtctgtctgtctgttctacc | AT |
| 2.4 | DHR3 | 46F | cttttgcttcgtagagaaccg<br>tgcggtgtagagaatttcgc | AC |
| 2.6 | Amy | 54A | acgggaacgccatctaac<br>agaagagaccctgcaacaca | GT |
| 2.7 | Distal-less | 60E | gagcactggcaagttaggcg<br>ccttgcttctgtgattgagg | AC |
| 3.1 | trh | 61C | agtacttcgctccgcattcg<br>cctggcttgcactggatc | ACC |
| 3.2 | z600 | 71C | aaatctgttgctcatactgccc<br>aaccggcgaaatgttcag | TTC |
| 3.3 | E74B | 74F | gcatgcaaatgcgcatgtgg<br>ggcaaacaaacgtaatcgcg | AC |
| 3.4 | Antp | 84A | agatccgagatgcgagatgc<br>tgtgcgtgtagattgtcaag | CT |

[a] Sequences are written in the 5′–3′ direction for the forward (top) and reverse (bottom) primers used to amplify ~200-bp fragments of each locus. tenA and z600 were obtained from Goldstein and Clark (1995), Notch and Amy from Schlotterer *et al.* (1997), and the remainder were designed in our lab.

*Df(3L)Aprt-1*/TM3 (#600) with breakpoints at 62A10-B1 and 62D2-5, *Df(3L)M21*/*In(3LR)T33* (#3650) with breakpoints at 62F and 63D, *Df(3L)HR119*/TM6B (#3649) with breakpoints at 63C6 and 63F7, and *Df(3L)GN19*/TM3 (#416) with breakpoints at 63F3 and 64B2.

Lines 27.13 and 17.38 are derivatives of the previously described *Ubx* introgression lines obtained by 10 generations of backcrossing of $Ubx^1$ into two different inbred wild-type lines (Gibson and van Helden 1997) that produce divergent haltere bristle phenotypes. They were further inbred to near-isogenicity by 7 or 8 generations of pair-mating, until two parents were found to be homozygous for each of the microsatellite markers listed in Table 1. Lines without the *Ubx* allele were then bred simply by mating wild-type flies from these lines, while the *Ubx* stocks were maintained by selection of mutant individuals every few generations.

$Antp^{73b}$ introgressions were performed as shown in Figure 2 of Gibson and van Helden (1997), namely by backcrossing 10 virgin female *Antp* flies to 10 males from 1 of 20 wild-type lines, for 10 generations. Phenotypes were scored qualitatively, by assigning numerical values to the degree of transformation in the proximal and distal portions of the appendage: 0, essentially antennal; 1, reduction of the arista or a few large bristles on antennal $S_3$; 2, large growths on $S_3$; 3, some tarsal formation, or elongation of proximal leg-segments; 4, clearly leg-like features; 5, almost perfect leg morphology.

**Genotyping:** Primers for amplification of microsatellites are listed in Table 1, some of which were taken from the studies of Goldstein and Clark (1995) and Schlotterer *et al.* (1997). All PCR reactions were performed in 10-μl volumes using Pfu enzyme (Stratagene, La Jolla, CA) with 40 cycles of 90 sec at 94°, 60 sec at 55 or 60°, and 60 sec at 72°, in an ERICOMP (San Diego) DualBlock thermal cycler. Products were directly labeled with 0.03 μl [$^{32}$P]dATP (New England Nuclear, Boston) spiked in the reaction cocktail, separated by polyacrylamide gel electrophoresis, and imaged using a Bio-Rad molecular imager. Examples of microsatellites on chromosome 2 are shown in Figure 1A to demonstrate the consistent difference between the parental strains 27.13 and 17.38. All genotypes were scored manually by two independent observers, and reactions were repeated in rare cases (<5%) of ambiguity because of poor amplification.

Markers in *Hsp83* and *Dras2* were scored following PCR amplification with the primers Hsp83AF 5′-ACATACAAGGT GAGTAATGC-3′ and Hsp83AR 5′-GGCATCTGCAATGGATT TAC-3′ or Ras2F 5′-TTAGTCATTTGCGTCATCTGC-3′ and Ras2R 5′-TATATGTTGGCTCCTGCTTCC-3′. The line 17.38 allele of *Hsp83* was ~100-bp lower molecular weight than the 1.14-kb allele of line 27.13, and hence heterozygotes were scored by the presence of two PCR bands following 1% agarose gel electrophoresis and ethidium bromide staining. Sequence analysis showed that several single nucleotide polymorphisms distinguish the two *Dras2* alleles in the 0.84-kb amplified fragment. One of these, a synonymous T to C transition in the second exon of the line 27.13 allele at position 516 of the amplified product, was detected by allele-specific oligonucleotide (ASO) hybridization as described by Saiki *et al.* (1986). PCR products from individual flies were dot-blotted directly onto a nylon membrane (Hybond-N+; Amersham, Arlington Heights, IL) under vacuum pressure, fixed by UV-irradiation, and probed with $^{32}$P-end-labeled oligonucleotide 5′-ACTTGT TACCCACCA-3′ to detect heterozygotes carrying the line 17 allele. Stringency washes were performed in 6× SSC at 55°.

**Statistical analysis:** Combined multiple regression/interval mapping was performed using QTL Cartographer software version 1.12 (Basten *et al.* 1997), obtained over the internet

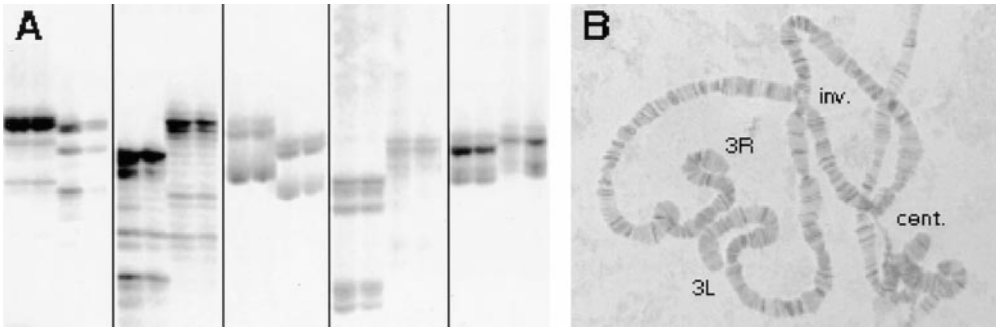


Figure 1.—(A) Examples of microsatellite polymorphisms detected by radioactive labeling and polyacrylamide gel electrophoresis. Each set of four adjacent lanes derives from two homozygous line 17.38 (left) and two homozygous line 27.38 (right) individuals, showing that gel migration differences are reproducible. Heterozygotes in each case appear as the sum of the two parental types. Markers from left to right are 2.2, 2.3, 2.4, 2.6, and 2.7 as listed in Table 1. (B) Polytene chromosome spread of the paracentric inversion on chromosome 3 in line 17.38. The left (3L) and right (3R) arms are indicated, and join at the breakpoints 75E and 88C ("inv.") to form a loop that clearly includes the centromere ("cent.") with the short fourth chromosome visible. The remaining chromosomes, X and 2, are free of inversions.

from the Department of Statistics home page of North Carolina State University (http://statgen.ncsu.edu/). Recombination fractions were first calculated from the genotype data of 111 backcross individuals between the $F_1$ females and line 27.13/*Ubx* males using Mapmaker 3.0 (Lincoln *et al.* 1992) also obtained over the internet from mapmaker@genome.wi.mit.edu. Linkage map positions were in agreement with expectations based on the known location of each marker. These fractions and the phenotypic scores for each individual were then used to generate the plots in Figure 2 using the Zmapqtl option of QTL Cartographer, model 1, which uses all markers to control for genetic background effects, and model 3 (which is equivalent to the original Lander and Botstein (1989) interval mapping method) with estimates at 2-cM intervals. The raw data were plotted using CricketGraph software on a Power Macintosh 8500 computer.

Only two recombination events were detected between markers 3.3 (74F), 3.4 (84A), and a *Ubx* (89D) marker, indicating almost complete suppression of crossing over. Polytene chromosome cytology demonstrated that this can be attributed to the presence of a paracentric inversion in line 17.38 with breakpoints at bands 75E and 88C (Figure 1B). This inversion also caused an increase in recombination on the left arm, so that there was at least one crossover between markers 3.1 and 3.3 in almost all individuals. As a consequence, the QTL peak between markers 3.1 and 3.2 is artificially duplicated (although with reversed sign of the effect) in the QTL analysis when all chromosome 3 markers are included in the analysis. We thus excluded markers 3.3 and 3.4 from the analysis presented in Figure 2.

A simple test for epistasis was performed by two-way analysis of variance of the mean phenotype associated with each of the four genotypes (AABB, AaBB, AABb, or AaBb) for each of the 45 possible two-locus combinations. A significant interaction term was taken as an indication of a nonlinear effect of one locus on the phenotypes because of the homozygote or heterozygote at the other locus.

The least-squares MANOVA routine of Statistica 4.1 (Statsoft 1996) was used to assess the statistical significance of differences among the *Antp* introgressions. The analysis assumed random effects of line ($L$ = 1–15) and replicate (Rep = 1 or 2) and fixed effects of sex (Sex = female or male) and region ($R$ = proximal or distal). Both sides of 10 individuals of each sex, line, and replicate were measured, and the within-individual variance was a small component of the error term, which is mainly due to differences between individuals. Error terms were as follows: MS($R \times L$) for $R$; MS($S \times L$) for $S$; [MS($R \times L$) + MS($S \times L$) − MS($R \times S \times L$)] for $L$; MS($R \times S \times L$) for the $S \times L$ and $S \times R$ pairwise interactions, and $S \times$ Rep($R \times L$) for the Rep($R \times L$) term; and the residual MS for the $R \times S \times L$ interaction (where MS is mean square). Replicates were measured in the second and third generations after completion of introgression. No transformation of the data was performed. The genetic correlation between the regions was determined as $r_{\text{prox,dist}}$ = [MS($L$) − MS($R \times L$)]/[MS($L$) + MS($R \times L$) − 2 × MS(residual)] following Cockerham (1963).

## RESULTS

**Haltere/wing margin transformation is a quantitative trait:** In most genetic backgrounds, *Ubx*$^1$ heterozygotes have just one or two prominent bristles on the anterior margin of each haltere. Artificial selection was applied to increase this number, starting with a genetically diverse population that was assembled from a mixture of 28 *Ubx*$^1$ introgression lines described in Gibson and van Helden (1997). The response to selection of between one-third and one-half of the progeny with high haltere bristle counts in samples of just over 100 individuals per generation was dramatic, as shown in Table 2. Whereas the mean haltere bristle number remained <2 in the unselected population, 14 generations of positive selection resulted in a line with 17 bristles per haltere in females and 14 in males. This response is >10 standard deviation units greater than the expressed variance in the starting population.

The standard deviation of haltere bristle number also increased in response to selection, up to a value of five bristles. This is not strong evidence for canalization, because the variance scaled by the trait mean remained essentially constant. Nevertheless, the genetic variation is not purely additive because for the first seven generations of selection, the distributions of haltere bristle numbers were highly skewed, with two-thirds of the flies having one or two bristles per haltere and the remaining third showing an increasing number of haltere bristles. The simplest interpretation is that there is an approximately normal distribution of genetic factors affecting the trait, but that there is a threshold that must be overcome before any increase in haltere bristle numbers is seen.

Three inbred lines were constructed by repeated pair-

**TABLE 2**

**Response to selection for increased *Ubx* haltere margin transformation**

| Generation | Females | Males |
|---|---|---|
| Parents | 2.0 | 0.7 |
| $F_1$–$F_3$ | 2.0 | 0.8 |
| $S_1$ | 2.2 | 1.1 |
| $S_2$ | 3.6 | 1.1 |
| $S_3$ | 4.4 | 2.7 |
| $S_4$ | 6.3 | 3.6 |
| $S_5$ | 7.1 | 6.3 |
| $S_6$ | 7.0 | 4.3 |
| $S_7$ | 9.7 | 7.8 |
| $S_8$ | 8.2 | 4.2 |
| $S_9$ | 14.1 | 10.1 |
| $S_{10}$ | 14.5 | 10.8 |
| $S_{14}$ | 18.1 | 13.6 |

Mean bristle count per haltere for the 28 lines from which parents were taken; the average of the first three unselected filial generations; and subsequent selected generations ($n >$ 100/generation, selected fraction between 0.3 and 0.5).

mating starting from pairs of flies with very high bristle counts, extracted at selection generation 6. In each case, the proportion of wild-type flies in the progeny dropped steadily to zero. Polytene chromosome squashes revealed that the lines had fixed a chromosome bearing a pericentric inversion on 3R, similar to *In(3R)Payne*, with one breakpoint immediately adjacent to the bithorax-complex (not shown). This chromosome presumably carries a recessive lethal mutation and an enhancer of *Ubx* that is responsible for a significant proportion of the response to selection, but inability to recombine the enhancer from the lethal precluded further mapping of the enhancer. The inbred lines had slightly larger bristle counts than the more outbred selection line at generation 14, but, as the latter had almost the expected 1:2 ratio of wild-type:*Ubx* flies (data not shown), the inversion chromosome cannot have been responsible for the majority of the response to selection.

**A major effect enhancer of the haltere/wing margin transformation:** One of our initial introgression lines that was not included in the establishment of the starting population described above also significantly enhanced *Ubx*, showing a double row of up to 25 bristles along the anterior margin of the haltere [see Figure 3D in Gibson and van Helden (1997)]. Seven generations of pair mating were sufficient to make a further inbred line, 27.13, that was isogenic as assessed by the homozygosity of molecular markers (microsatellites) distributed throughout each of the three major chromosomes. The mean haltere bristle count in this line was 17.3 ($\sigma$ = 4.1, $n$ = 103). An isogenic line fixed for different microsatellite alleles but with halteres devoid of bristles, line 17.38, was similarly constructed from a different introgression stock. These two lines were then used to map quantitative trait loci (QTL) affecting the haltere/wing margin transformation.

Comparison of the distribution of haltere bristle numbers in female $F_2$ and backcross progeny indicated that high bristle counts are recessive to low counts. Backcross of virgin female 17.38/27.13 heterozygotes to *Ubx* 17.38 males resulted in a mean count of 3.4 ($\sigma$ = 4.1, $n$ = 130) haltere bristles in female progeny, compared with 12.6 ($\sigma$ = 5.4, $n$ = 111) for the alternate backcross to *Ubx* 27.13 males. $F_2$ individuals had an intermediate mean of 5.9 ($\sigma$ = 5.2, $n$ = 132), with the distribution highly skewed toward low counts. Thus, in general one or more loci must be homozygous derivatives of line 27.13 to see the strong transformation. These results are also consistent with the notion of a threshold-dependent response to *Ubx* haploinsufficiency. Similar results were seen for males, except that haltere bristle counts were consistently lower than for females.

Each of 10 dimorphic microsatellite markers listed in Table 1 were scored in *Ubx* females derived from the backcross to line 27.13, and combined multiple regression/composite interval mapping (Zeng 1994) was used to map QTL affecting haltere bristle number. Analysis of just 111 individuals provided compelling evidence for a single major effect QTL located toward the end of the left arm of chromosome 3, between cytological bands 62 and 65 (Figure 2). This locus (or cluster of loci) accounts for a difference of 8.2 haltere bristles between 17.38/27.13 heterozygotes and 27.13 homozygotes, or 72% of the total difference between these two genotypes.

There is weak evidence for a second QTL close to the centromere of chromosome 2, although the high LR score may be an artifact of the small sample size as similar significance levels were seen in 5% of 100 bootstrap samplings of the data set. However, this region of the genome also showed a significant epistatic interaction with the major QTL, as double heterozygotes for the markers at cytological positions 46F (*DHR3*) and 71C (*z600*) had lower bristle counts than expected if the interactions were purely additive ($P$ = 0.003, ANOVA). No other epistatic interactions were strongly suggested, and there was no evidence for an X-linked factor. A paracentric inversion in line 17.38 relative to line 27.13 (Figure 1B) prevented mapping of the effects of the right arm of chromosome 3.

Further refinement of the location of the major QTL on 3L, hereafter referred to as *E(Ubx)3L*, failed to identify a particular gene responsible for the effect, but did demonstrate strong dependence of the quantitative effect on genetic backgrounds as indicated in Table 3. Four deficiencies that cover most of the QTL peak from cytological band 62A10 to 64B2 (see materials and methods for *Df* breakpoints) were obtained and tested for complementation of *E(Ubx)3L* on the assumption that if *E(Ubx)3L* is a loss-of-function allele, then hemizygotes ought to show enhancement of *Ubx* at least as

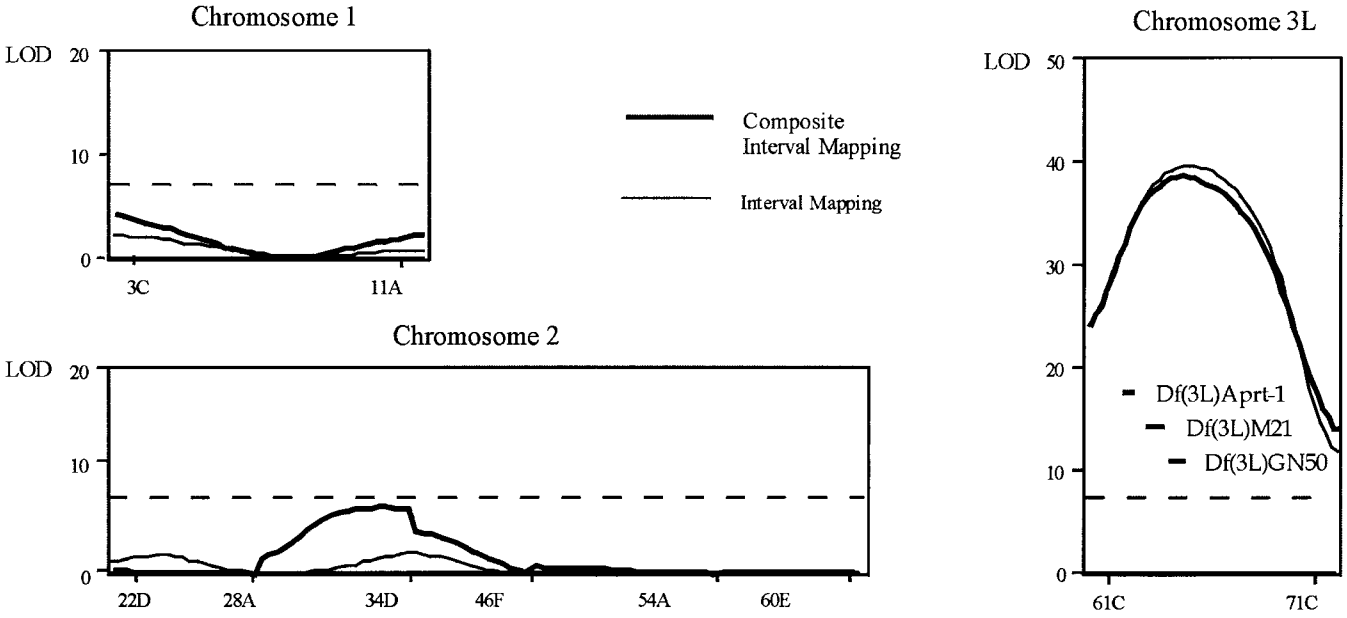


Figure 2.—Composite interval mapping of QTL affecting haltere bristle number in *Ubx* flies. Microsatellite markers at the indicated cytological locations on the three major chromosomes were used to infer the positions of possible QTL affecting the number of bristles on the anterior margin of the halteres of 111 backcross progeny between the inbred lines 27.13 and 17.38. LOD scores indicate calculated likelihood ratio of difference between heterozygotes and line 27.13 homozygotes at each position along the chromosome, for standard interval mapping (Lander and Botstein 1989) and combined multiple regression/interval mapping (Zeng 1994). Dashed horizontal lines indicate the 5% probability cutoff for the combined model, calculated by 100 bootstrap resamplings of the data set. The single QTL on the left arm of chromosome 3 was further mapped to cytological intervals 62–65, and the approximate locations of three deficiencies in the region are indicated. Chromosome 3R was excluded from the analysis due to the absence of crossing over caused by an inversion difference between the lines.

great as that seen in line 27.13. As indicated in Table 3, a strong effect was observed with *Df(3L)Aprt-1*, though one other wild-type chromosome also showed this level of enhancement *in trans* with one copy of *E(Ubx)3L*. *Df(3L)M21*/27.13 heterozygotes have considerably enlarged halteres, but no increase in haltere bristle numbers, while *Df(3L)GN19* and *Df(3L) HR119* heterozygotes do not appear to enhance *Ubx* at all.

*Gap1* mutants also increase the *Ubx* haltere/wing margin transformation (Boube *et al.* 1997), so we considered three components of the *Ras*/*Raf* signalling pathway in the region as candidates for *E(Ubx)3L*. *Dras3* (at 62B) is within *Df(3L)Aprt-1*, but maps too close to the left-hand marker at 61C to be likely to be the modifier (see Figure 2). Line 17.38 has a large (~100-bp) deletion in the 5′ region of *Hsp83* (at 63B), which could theoretically affect the level of expression of the protein. Line 27.13 has a rare haplotype in *Dras2* (at 64B) consisting of six silent polymorphisms (R. Gasperini and G. Gibson, unpublished results) that can be detected by allele-specific oligonucleotide hybridization. Homozygotes for the line 27.13 alleles of these two molecular markers in a second sample of 96 backcross females had significantly higher bristle counts than heterozygotes as expected, and more of these individuals (~85%) had higher than the mean number of bristles (13) per haltere than did homozygotes for either flanking microsatellite marker (~50% for both 3.1 and 3.2). Association with relatively high bristle counts for the *Hsp83* marker (39 of 46 flies) was slightly higher than for *Dras2* (39 of 48 flies), indicating that the QTL is likely to reside distal to cytological interval 64B and hence should be within the region covered by the deficiencies considered above. However, two recombinants of each phase (*Hsp83* homozygotes, *Dras2* heterozygotes; or *Hsp83* heterozygotes, *Dras2* homozygotes) had high bristle counts, so did not provide any information as to the location of the QTL relative to these two genes. These data imply that *E(Ubx)3L* cannot be fully penetrant, and consequently linkage analysis alone will not allow a determination of the precise identity of the QTL in molecular terms. The data also argue against the QTL effect being caused by two linked genes, because in this case recombinants in the region would not be expected to show the high bristle number (greater than 13 bristles) phenotype.

Further evidence that *Dras2* is not *E(Ubx)3L* comes from the finding that the same haplotype is also found in line AA18, which does not complement the enhancing effect of *Ubx* (Table 3). *Hsp83* could be responsible for the QTL effect, but the length polymorphism is probably not involved because most wild-type alleles are the same length as the 27.13 allele. Alternatively, *E(Ubx)3L* could lie within band 62E, which is not covered by the available deficiencies, or in spite of the above linkage

**TABLE 3**

**Effect of genetic background on the *Ubx* haltere margin transformation**

| Cross[a] *Ubx* × wild type | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | Mean[b] | σ | *n* | Mean | σ | *n* |
| 17.38 × 17.38 | 0.0 | 0.0 | 36 | 0.0 | 0.0 | 29 |
| 17.38 × *Df(3L)GN19* | 0.2 | 0.5 | 27 | 0.0 | 0.1 | 30 |
| 17.38 × *Df(3L)Aprt-1* | 2.5 | 1.6 | 14 | 0.6 | 0.6 | 12 |
| 17.38 × AA18 | 0.3 | 0.4 | 68 | 0.1 | 0.2 | 81 |
| 17.38 × 27.13 | 6.0 | 1.0 | 19 | 1.6 | 1.0 | 37 |
| 27.13 × 17.38 | 6.8 | 3.5 | 51 | 2.8 | 1.9 | 37 |
| 27.13 × *Df(3L)GN19* | 6.5 | 2.6 | 37 | 2.4 | 1.4 | 26 |
| 27.13 × *Df(3L)Aprt-1* | 14.5 | 2.5 | 23 | 8.9 | 1.9 | 18 |
| 27.13 × *Df(3L)M21*[c] | 3.0[d] | 1.4 | 9 | 1.5[d] | 0.4 | 4 |
| 27.13 × *Df(3L)HR119* | 3.4 | 1.4 | 14 | 1.3 | 0.6 | 10 |
| 27.13 × AA18 | 5.6 | 2.4 | 45 | 1.4 | 1.0 | 27 |
| 27.13 × 27.13 | 18.3 | 3.7 | 43 | 10.4 | 3.5 | 39 |
| 27.13 × wwwt-1 | 3.5[d] | 4.9 | 5 | 1.4[d] | 2.4 | 7 |
| 27.13 × wwwt-3 | 5.5 | 1.1 | 20 | 4.7 | 1.8 | 20 |
| 27.13 × wwwt-12 | 3.5 | 0.7 | 11 | 0.8 | 0.7 | 9 |
| 27.13 × wwwt-17 | 3.6 | 2.1 | 20 | 0.4 | 0.7 | 22 |
| 27.13 × wwwt-23 | 13.8 | 3.9 | 34 | 10.5 | 4.3 | 17 |
| 27.13 × wwwt-24 | 6.9 | 1.1 | 17 | 2.4 | 1.4 | 16 |
| 27.13 × wwwt-28 | 7.6 | 2.7 | 12 | 2.2 | 1.7 | 22 |
| 27.13 × aawt-7 | 7.0 | 1.6 | 12 | 3.5 | 2.0 | 12 |
| 27.13 × aawt-14 | 8.6 | 2.5 | 11 | 1.3 | 1.3 | 23 |
| 27.13 × aawt-20 | 9.0 | 6.4 | 11 | 2.2 | 2.2 | 27 |

[a] In most crosses, three *Ubx* virgin females were crossed to three males of the wild-type line. The cross to *Df(3L)HR119* was performed 4 mo after the other crosses.

[b] Mean and standard deviation (σ) is indicated for *n* individuals scored (2*n* halteres scored). For the deficiency crosses, hemizygotes were identified by the absence of the dominant marker on the balancer chromosome.

[c] Values are for the minority class, because *Df(3L)M21* has low viability and is maintained over an unmarked chromosome. Mean bristle counts for the majority class were 10.6 for females ($n = 33$) and 5.6 for males ($n = 22$).

[d] These flies had significantly enlarged halteres relative to most *Ubx* animals.

data it may lie proximal to 64C. A more straightforward interpretation is that because the deficiencies in the region do not complement the anomalous phenotype, it is likely that the effect represents a gain rather than loss of function.

**Strong modification of *Ubx* and *Antp* homeotic phenotypes:** Even stronger modification of the *Ubx* hemizygous phenotype was seen in three interactions that resulted in remarkably enlarged halteres reminiscent of recessive viable combinations of mutations that affect the regulatory region of *Ubx* [for example, $bx^{34E}$/*Df(3R)P9* hemizygotes; Kerridge and Morata (1982)]. The mega-haltere phenotypes of wwwt-1/27.13,*Ubx* heterozygotes and of wwwt-7/wwwt-7,*Ubx* flies raised at 18° (Figure 3C), are accompanied by extensive bristle production along the anterior margin as well as flattening of the appendage epithelium characteristic of wing tissue. The wing margin transformation is less pronounced in *Df(3L)Aprt-1*/27.13 hemizygotes. The temperature-sensitive period of the wwwt-7 effect, which has been tentatively mapped to chromosome 3, is in embryogenesis, suggesting that the allele acts in parallel to or upstream of *Ubx* rather than in the late determination of haltere morphology.

To determine whether the modification of homeotic phenotypes is specific to a haploinsufficient allele such as $Ubx^1$, or also applies to dominant, gain-of-function phenotypes, we carried out two further introgressions. *Contrabithorax* ($Cbx^1$) flies have a transformation of wing toward haltere, because of gain of *Ubx* activity in the mesothorax (Lewis 1978), that was not greatly affected by crossing into a panel of wild-type genetic backgrounds. By contrast, the homeotic transformation of antenna to leg due to $Antp^{73b}$ is dramatically enhanced or suppressed in different wild-type backgrounds, and in a region-specific manner.

$Antp^{73b}$ was introgressed into 22 inbred wild-type lines by repeated backcrossing. After 10 generations, the stocks were maintained by sib-mating of *Antp* individuals, but 7 lines were immediately lost, presumably because of low viability and fertility associated with either the mutation or the inversion that causes it. Of the remaining 15 lines, 2 were derived from isofemales trapped at the Ann Arbor fruit market in July 1996, and 13 were

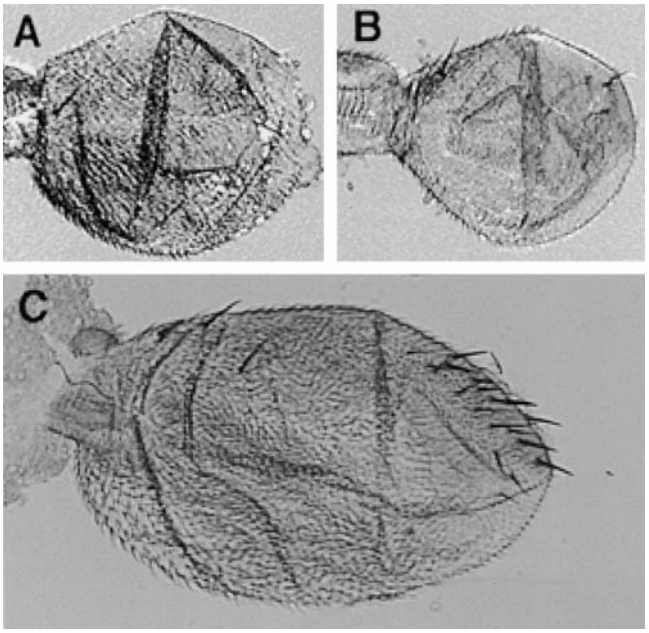


Figure 3.—The mega-*Ultrabithorax* phenotype. A temperature-sensitive allele in line wwwt-7 produces (C) a threefold increase in area of the halteres of *Ubx* flies relative to (A) the phenotype at 25°, even when (B) crossed into the genetic background of a line with minimal *Ubx* transformation, line wwwt-2. Images were captured digitally with NIH-Image as TIFF files, and rearranged using Photoshop 3 software.

from the Wallace collection of near-isofemales from around the world. The extent of antenna-to-leg transformation was scored qualitatively using two arbitrary scales from 0 (almost antenna) to 5 (almost perfect leg), for the distal portion (arista, corresponding to claw, metatarsus, and tarsus) and for the proximal portion (antennal segment 3, corresponding to tibia and femur; Gateff and Schneiderman 1975). Summary values for pooled scores of two successive generations are listed in Table 4 and plotted in Figure 4.

The analysis of variance in Table 5 indicates that (i) there is significant genetic variation affecting the *Antp* phenotype; (ii) the distal and proximal regions are to some extent independently regulated, as shown by the significant line × region effect; and (iii) there is a slight tendency for the degree of difference between the two sexes to vary by line, as shown by the significant sex × line effect. Although the line effect is not significant in the results shown in Table 5, this is because of the high value of the line × region term used as the denominator in the *F*-ratio calculation: when treated separately, line effects were extremely significant. There was also a strong replicate (line × region) effect, which can mostly be attributed to differences in the magnitude of the transformations in the distal and proximal regions between generations, because in no case was the relative effect altered. Though such a strong replicate effect is

**TABLE 4**

**Effect of genetic background on *Antp* transformations**

| | Females | | Males | |
|---|---|---|---|---|
| Line[a] | Proximal[b] | Distal[b] | Proximal | Distal |
| WA3 | 4.0 | 0.5 | 3.8 | 0.8 |
| WA6 | 4.2 | 3.3 | 4.3 | 3.6 |
| WA9 | 2.2 | 2.0 | 2.6 | 2.4 |
| WA10 | 2.2 | 1.3 | 1.9 | 1.0 |
| WA11 | 2.0 | 3.4 | 2.0 | 3.5 |
| WA12 | 3.1 | 2.4 | 3.5 | 3.2 |
| WA15 | 3.7 | 3.6 | 3.4 | 3.4 |
| WA16 | 1.1 | 0.5 | 2.2 | 2.1 |
| WA17 | 1.9 | 1.3 | 2.7 | 1.7 |
| WA19 | 2.9 | 3.4 | 2.8 | 3.9 |
| WA22 | 2.2 | 3.8 | 3.3 | 4.2 |
| WA26 | 1.7 | 1.3 | 2.1 | 1.8 |
| WA29 | 2.9 | 3.4 | 3.4 | 4.1 |
| AA14 | 4.0 | 3.8 | 3.6 | 3.8 |
| AA21 | 3.8 | 3.6 | 3.7 | 3.8 |

[a] Introgression lines obtained by repeated backcrossing of $Antp^{73b}$ for 10 generations to the corresponding wwwt line (WA, world wide wild type/Antp) or aawt line (AA, Ann Arbor wild type/Antp).

[b] Scores were obtained two and three generations after the completion of the introgression, for the distal (claw, metatarsus, and tarsus) and proximal (tibia, femur, and trochanter) transformations, as described in the text.

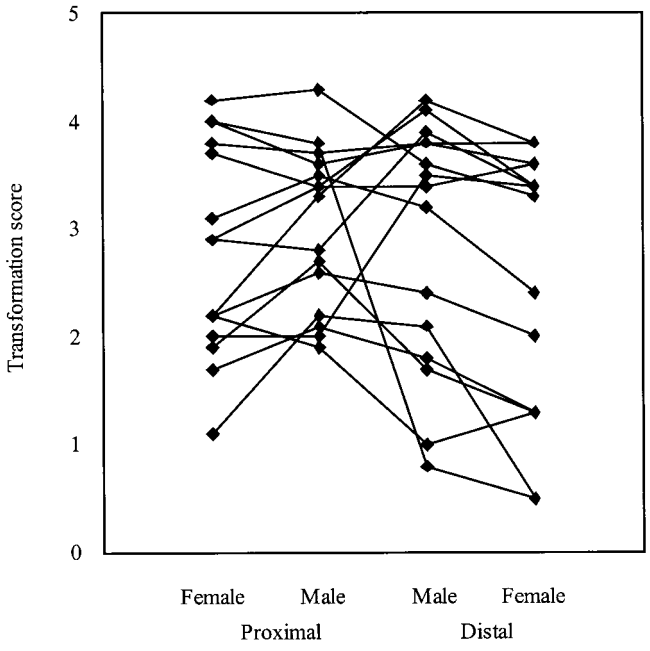


Figure 4.—Genetic correlation of the degree of *Antennapedia* transformation between regions of the antenna. The mean qualitative measures of the degrees of transformation in the distal (claw, metatarsi, and tarsi) and proximal (tibia and femur) portion of each antennal leg are plotted by line and sex. In general, the correlation between males and females is very high for each region, but there is considerable crossing of line means between regions in the center of the plot.

**TABLE 5**

**Analysis of variance of *Antp* introgressions**

| Source[a] | d.f. | MS | Error MS | *F* | *P* Value |
|---|---|---|---|---|---|
| *L* | 14 | 116.9 | 55.9 | 2.1 | 0.092 |
| *S* | 1 | 57.7 | 7.6 | 7.6 | 0.016* |
| *R* | 1 | 26.0 | 49.3 | 0.5 | 0.479* |
| $L \times S$ | 14 | 7.6 | 1.0 | 7.7 | 0.000*** |
| $L \times R$ | 14 | 49.3 | 1.0 | 50.0 | 0.000*** |
| Rep($L \times R$) | 1 | 6.4 | 1.3 | 4.7 | 0.003** |
| $S \times R$ | 1 | 3.5 | 0.9 | 3.6 | 0.080 |
| $L \times S \times R$ | 14 | 1.0 | 0.8 | 1.7 | 0.052 |
| Residual[b] | 1140 | 0.5 | | | |

*$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$.

[a] *L*, line; *S*, sex; *R*, region (proximal *vs.* distal transformations); Rep($L \times R$) is the replicate (line × region) for the comparison of scores obtained in the second and third generations after conclusion of the introgression.

[b] The residual term is the within-line and -treatment variance. Within-individual variance was negligible (MS < 0.1).

uncommon in quantitative genetic analysis, it is not particularly surprising here because the qualitative scales used are somewhat ambiguous. More importantly, the representative phenotypes shown in Figure 5 clearly show the variability of the $Antp^{73b}$ phenotype, and these have persisted in the lines for a further 15 generations.

## DISCUSSION

**Introgression as an alternative to mutagenesis screens:** One of the objectives of this study was to determine whether introgression might be useful as an alternative to mutagenesis screens to identify genes that act in the developmental pathways regulated by *Antp* or *Ubx*. Attempts to identify downstream targets of the homeotic genes as suppressors of mutant phenotypes have met with limited success (Kennison and Tamkun 1988; *cf.*, Gellon *et al.* 1997), possibly because the screens are biased toward detecting cofactors that affect the entire homeotic phenotype. Region-specific modifiers, namely loci that only affect the transformation of part of the leg or haltere, are good candidates for target genes. The variable phenotypes produced by backcrossing of *Antp* and *Ubx* into different wild-type genetic backgrounds are at least consistent with this type of effect, although there also seem to be modifiers that act throughout the appendages. Our identification of different classes of phenotypic effect is consistent with the recent demonstration (Weatherbee *et al.* 1998) that changes in gene expression in the imaginal haltere discs of *Ubx* animals occur at several levels of the downstream hierarchy.

The mega-haltere phenotype in particular is likely to result from reduction in activity of cofactors that mediate UBX function, because it closely resembles the phenotype of flies hemizygous for certain viable recessive

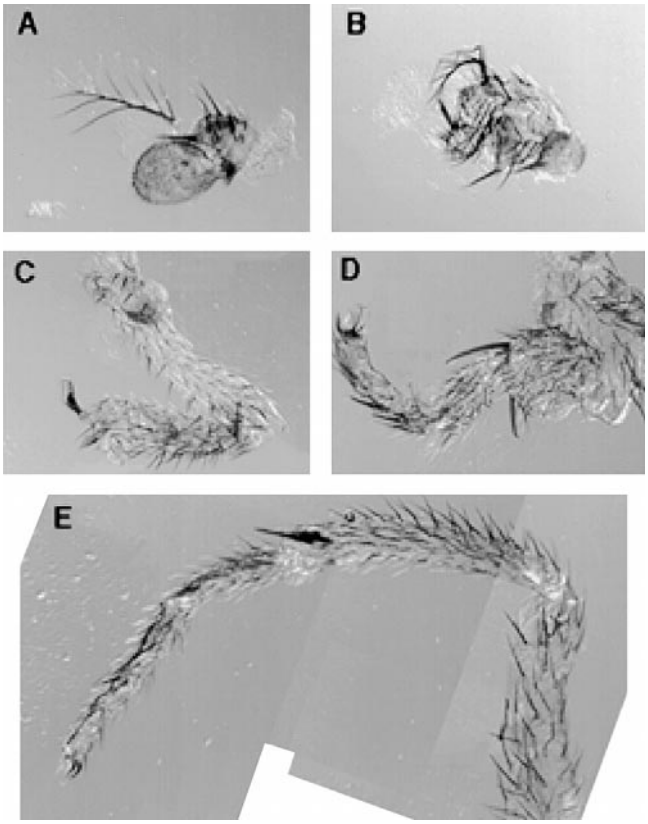


Figure 5.—Diverse *Antennapedia* transformations in different wild-type genetic backgrounds. (A) Wild-type antenna. (B) Weak antenna-to-leg transformation in line wwwt-16. (C) Strong proximal transformation only, line wwwt-3. (D) Strong distal transformation only, line wwwt-22. (E) Almost perfect transformation of antenna to second thoracic leg, line aawt-14. These diverse phenotypes are consistently seen only after at least five generations of introgression of the $Antp^{73b}$ mutation into the respective wild-type inbred lines.

combinations of *Ubx* alleles (Kerridge and Morata 1982). That three independent combinations of genetic backgrounds should produce this phenotype from a relatively small screen involving just 30 different introgressions indicates that there is considerable genetic variation segregating in natural populations affecting the level of UBX activity in the haltere. The variation is likely to act early in development, during the initial specification of the haltere imaginal disc, because the temperature-sensitive period of one of the interactions is during embryogenesis, and because the phenotype covers the entire appendage. It is quite different from clonal transformations such as those produced by mitotic recombination (Struhl 1982), or ether-induced phenocopies (Gibson and Hogness 1996).

The transformation of the haltere margin toward wing, resulting in the development of a pronounced double row of bristles along the anterior margin of the haltere, is good evidence for region-specific modifiers of *Ubx* activity that are likely to act later in development. Boube *et al.* (1997) recently implicated the Ras pathway

in this transformation, noting that *Gap1*; *Ubx* mutants have a relatively high number of bristles at the same location. The sensitivity of this particular phenotype to genetic background (bottom half of Table 3) cautions that their result may have been due to a different polymorphism carried in the *Gap1* stock. Nevertheless, if the identity of *E(Ubx)3L* and other interacting loci could be established, this phenotype would provide a powerful assay for biologically meaningful target genes of *Ubx*. Unfortunately, if *E(Ubx)3L* is a gain-of-function allele, as suggested by the inability of deficiencies covering most of the region (that is, excluding a portion of cytological bands 62E) to complement the mutant haltere bristle phenotype, it will be particularly difficult to identify the gene responsible, especially given the incomplete penetrance of the effect. There are no known neurogenic loci in the region, suggesting that the locus is not acting at the level of bristle determination.

The region-specific modification of the *Antp* transformation does not necessarily imply that the loci responsible are acting downstream of ANTP. It is well known that competence to respond to ANTP protein is temporally regulated (Gateff and Schneiderman 1975), with the distal tip of the appendage being sensitive to heat-shock induced protein some 8–12 hr earlier than proximal portions (Larsen *et al.* 1996). Partial antennal-to-leg transformations can also be produced by mutations in genes such as *spineless-aristapedia* and *distal-less*, both of which act in parallel with the homeotic gene. Further, despite the region-specific character of the transformations observed in different introgressions, there was a strong genetic correlation ($r = 0.63$ calculated from the variance components; Cockerham 1963) between the distal and proximal portions, implying that common loci are regulating transformations in the two regions. Thus, at least some of the variation is likely to affect the level of activity of ectopically expressed ANTP protein, or possibly transcription from the rearranged promoter that turns the gene on in the antennal imaginal disc (Frischer *et al.* 1986). We expect though that detailed characterization of these and other lines will reveal more subtle phenotypes that are likely due to variation in downstream effectors. For example, several of the lines with strong arista-to-metatarsal transformations appear to differ in the robustness of the metatarsi, some being almost leg-like, others very thin with poorly differentiated joints. The major limitation to genetic dissection of these differences is the low viability and fecundity associated with the $Antp^{73b}$ inversion in most genetic backgrounds.

**The notion of potential variance:** Natural populations typically maintain considerably more genetic variation for quantitative traits than classical additive genetic models predict (Dobzhansky 1937). Much of this variation can be attributed to some combination of three basic sources: balanced polymorphisms, rare alleles maintained by mutation-selection balance, and additive effects hidden by dominance and epistatic interactions (Barton and Turelli 1989). Empirical approaches to elucidating the relative importance of these sources rely on the measurement of parameters such as the mutational variance and segregating variance components (for example, Clark *et al.* 1995; Houle *et al.* 1996), all of which assume that alleles have measurable effects on the trait. However, results such as those presented here imply that there may also be extensive pools of "potential variance" that normally do not contribute at all to the variance of a trait, but can readily have an effect if the wild-type state is perturbed (see also Hartl and Dykhuizen 1985).

What mechanisms might maintain such potential variance? The simplest hypothesis is that it is truly neutral: antennal legs essentially never occur in natural populations, so there is no selection pressure to remove alleles that might modify the *Antennapedia* phenotype. However, from a developmental genetic perspective it seems likely that the variance is due to genes that have roles in normal appendage development, and hence that the alleles could have pleiotropic effects that are exposed to selection, however slight these may be. A strong adaptationist might further argue that selection has favored modification of genetic architectures such that legs never partially transform to antennae, or halteres never develop wing-like margins even when the dosage of the homeotic genes is reduced—a process known as canalization (Waddington 1957). In other words, it may be that fundamental genetic pathways are so well buffered that they can completely suppress the effects of polymorphisms that would have a measurable influence if the trait is sufficiently perturbed. We have recently shown, though, that haltere development is not obviously canalized (Gibson and van Helden 1997), and Wagner *et al.* (1997) have used mathematical theory to cast doubt on the efficacy of canalizing selection as a mechanism powerful enough to buffer genetic pathways to completely suppress phenotypic variation. Nevertheless, it does seem that activities of homeotic genes are threshold-dependent, meaning that the effects of modifiers of homeotic gene activity are only seen above or below defined limits.

An obvious question to ask about potential variance is whether or not the "architecture" is similar to that of classical quantitative traits. That is, are there many genes contributing to potential variance, and do they have largely additive effects, with each allele contributing at most a few percent of the total variance (Lai *et al.* 1994; Long *et al.* 1995; Mackay 1995)? The limited evidence presented here and in a related study of photoreceptor determination in Drosophila (Polaczyk *et al.* 1998) suggests that the situation can actually be quite different. For example, *E(Ubx)3L* is a major effect allele by any criterion, contributing up to 72% of the variance for haltere bristle number in some genetic backgrounds, and yet it can be readily suppressed in other back-

grounds. Similarly, a few loci are capable of producing the mega-haltere phenotype that is qualitatively distinct from the standard *Ubx* transformation. By contrast, the complexity of the *Antp* transformations documented here, as well as the response to selection on haltere bristle number in *Ubx* flies, imply that potential variance is not restricted to rare major effect alleles, but seems to be ubiquitous and polygenic. Each different genetic background gives a slightly different response to introgression of a homeotic mutation.

**Implications of potential variance:** What might be the evolutionary significance of potential variance? First, it provides support for the principle that genetic pathways can diverge without any corresponding change at the phenotypic level. As long as the variance has no significant effect on fitness, alleles with potential effects will drift in and out of populations. There is no reason why an allele such as *E(Ubx)3L* should not be fixed in one lineage and lost in another, assuming that it does not have any deleterious pleiotropic effect. Similarly, different ecotypes of *Arabidopsis thaliana* harbor alternate alleles of the *CAULIFLOWER* locus whose effects are seen only in the genetic background of the floral meristem identity gene *APETALA1* (Purugganan and Suddith 1998). A possible signature of this process is the divergence of redundant promoter elements such as those in the *even-skipped* stripe 2 enhancer of the *D. melanogaster* group of species (Ludwig and Kreitman 1995). That is to say, there is accumulating evidence that genetic pathways can differ quite significantly at the molecular level (for example, in terms of the distribution of transcription factor binding sites) yet produce similar functional outputs. The differences can only be seen when the pathway is significantly perturbed, as by the introgression of a major effect mutation.

Though this type of change may not seem very important, over the long term it could become an extremely powerful mechanism of evolution. It is conceivable that phenomena such as the different genetic mechanisms used to generate the nematode vulva without any alteration in cell lineages (Sommer 1997), the profound differences in pattern formation of short- and long-germ band insects, and the astonishing dynamics of sex determination mechanisms among related taxa (Marin and Baker 1998) evolved by a process of genetic drift of the underlying pathway. Stated more strongly, potential variance is a prerequisite for the divergence of genetic mechanisms without concomitant phenotypic change.

Second, potential variance may more directly contribute to hybrid incompatibility. According to the "complementary alleles" model (Dobzhansky 1937; Orr 1995) hybrid breakdown occurs when alleles at two different loci are fixed in two lineages derived from a homogeneous population, where the double heterozygote is subviable as a result of an epistatic interaction. General acceptance of this model awaits direct demonstration of the existence of this type of interaction, as well as theoretical confirmation that mutation rates and effects are sufficient to support the process. Our results, if generalized to a broad range of developmental pathways, suggest that the process need not be limited by the need for new mutations, because there are already alleles of the predicted type segregating, with hidden effects, in natural populations.

To see this, consider the consequence of hybridizing two species, one of which has fixed an allele like *E(Ubx)3L*, and the other a *Ubx* regulatory allele that is suppressed by the genetic background. Many of the progeny would display strongly transformed haltere margins, undoubtedly with maladaptive implications. This is perhaps a far-fetched example, but if there is potential variance to modify a change in homeotic gene function throughout the appendage, then there will also be potential variance affecting more subtle changes in *Hox* expression. The same argument can readily be applied to changes associated with other types of regulatory gene. Summed over many loci, the divergence of genetic backgrounds has the potential to contribute strongly to hybrid incompatibility or to hybrid anomalies. These may occasionally be adaptive, providing a mechanism for the appearance of "bridgeless gaps" (de Vries 1915) that is more palatable than simple selection acting on macromutations. Genetic drift need not be a neutral process if the potential effects of alleles underlying morphological traits are considered.

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