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# Is Function of the Drosophila Homeotic Gene *Ultrabithorax* Canalized?

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Manuscript received December 6, 1996
Accepted for publication July 31, 1997

ABSTRACT

Genetic variation affecting the expressivity of an amorphic allele of the homeotic gene *Ultrabithorax*, (*Ubx*$^{1}$) was characterized after 11 generations of introgression into 29 different isofemale lines. Heterozygotes display a range of haploinsufficient phenotypes, from overlap with wild-type halteres to dramatic transformations such as a 50% increase in area and the presence of over 20 bristles on the anterior margin of each haltere. In both the wild-type and mutant genetic backgrounds, there is moderate genetic variance and low environmental variance/developmental asymmetry, as expected of a trait under stabilizing selection pressure. Surprisingly, there is little evidence that mutant halteres are more variable than wild-type ones, so it is unclear that haltere development is also canalized. The correlation between wild-type and *Ubx* haltere size is very low, indicating that interactions among modifiers of *Ubx* are complex, and in some cases sex-specific. The potential quantitative genetic contributions of homeotic genes to appendage morphology are discussed, noting that population-level effects of variation in key regulatory genes may be prevalent and complex but cannot be readily extrapolated to macroevolutionary diversification.

FROM both evolutionary and developmental perspectives, body plans are highly stable entities. Abnormalities such as a doubling of the number of wings on the body of a fly are rare, indicating that developmental genetic programs seem to be well buffered against environmental variation. The fact that significant changes in body plans usually only occur at the genus level or higher suggests that developmental programs may also be well buffered against genetic variation. Slow rates of morphological evolution have been attributed to persistent stabilizing selection (SPICER 1993), entrenchment of early ontogeny (ARTHUR 1988), and canalization of genetic programs (WADDINGTON 1957). However, it is not clear that ontogenetic and phylogenetic stability are mechanistically linked, and therefore it is important to study how ontogenetic stability is achieved.

One way to address this problem is to study the evolutionary genetics of well-defined developmental pathways. The homeotic genes of Drosophila are responsible for defining the identities of the various body segments (LEWIS 1978). Their contribution to evolution is somewhat paradoxical, in so far as they are clearly important for divergence between higher taxa, but regulate traits that are among the most stable at the species level. For example, homologues of the *Ultrabithorax* (*Ubx*) gene in certain crustacea have variable anterior boundaries in the thoracic region that correlate with changes in appendage morphology (AVEROF and AKAM 1995), and homeotic gene expression patterns are actually evolutionarily quite labile (WARREN *et al.* 1995; ROGERS *et al.* 1997). However, within *D. melanogaster*, homeotic abnormalities attributable to *Ubx*, such as changes in haltere or third leg morphology, are almost nonexistent under normal growth conditions. For homeotic genes, and possibly most key regulatory genes, the extrapolation from variation within populations to macroevolutionary change is not simple and should be understood within the context of the buffering of development.

Observations such as these lead to the hypothesis that homeotic gene function may be "canalized." This term refers to the resistance of developmental traits to perturbation (WADDINGTON 1942) and can be subdivided into two types of effect (WAGNER *et al.* 1997): environmental canalization is the reduction of asymmetry within individuals, whereas genetic canalization is the reduction of variability (the capacity to vary). Genetic canalization is formally defined by low mutational variance, but since this is experimentally difficult to measure, it is more easily documented by observation of an increase in variance when a trait is perturbed. In a recent review, SCHARLOO (1991) discussed five detailed studies of canalization: the vibrissae in mice (DUNN and FRASER 1958), and in Drosophila the scutellar bristles (RENDEL *et al.* 1965), ocelli (MAYNARD-SMITH and SONDHI 1960), anal papillae (STERN 1958), and wing-vein interruption (SCHARLOO 1964). He concluded that canalization is causally inhomogeneous in so far as allelic, background genetic and environmental effects act differently on the various traits (see also ROBERTSON 1965). Furthermore, interpretation of the causes of canalization can be profoundly influenced by the cell biol-

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ogy and in particular the possibility that many traits may be compound. A mechanistic understanding of canalization consequently requires specific genetic dissection of each individual trait.

There are at least two reasons to expect that *Ubx* function in haltere development may be canalized. First, the haltere-to-wing transformation in loss-of-function mutants is strongly recessive. Although *Ubx* does show some haploinsufficiency, the fact is that there is a marked qualitative difference between the dominant enlargement of the haltere seen in hemizygotes and the development of unambiguous wings in viable recessive mutant combinations (see MORATA and KERRIDGE 1980). In general terms, one copy of the gene is sufficient to suppress wing development, resulting in dominance for homeotic identity. Second, the haltere might be expected *a priori* to experience strong stabilizing selection. It is thought to act as a balancing organ during flight, and asymmetry would presumably be undesirable. Stabilizing selection is a precondition for the evolution of canalization (CURNOW 1964), though as pointed out by WAGNER *et al.* (1997) it is not sufficient. The reason is that whereas stabilizing selection acts on the expressed variation, canalizing selection acts on the capacity to produce variation. The stronger the stabilizing selection, the less opportunity there is for variation for this capacity to accumulate. Since selection depends on the existence of variation, strong stabilizing selection can actually prevent the evolution of canalization. Consequently, haltere development may be stabilized, but not canalized.

To determine whether this is the case and more generally to ask whether genetic variation affecting *Ubx* function conforms to the classical additive genetic model, we have examined haltere morphology in a variety of wild-type genetic backgrounds. There are four possible outcomes to the introgression of a mutant allele into isofemale lines. These are shown graphically in Figure 1, a plot of the possible relationships between trait means in wild-type and mutant flies. First, *Ubx* could simply increase the size of the haltere in a uniform manner in all lines (Figure 1A). A prediction of this additive genetic model is that there would be no significant genotype-by-line interaction (that is, wt *vs.* *Ubx* genotype-by-isofemale line interaction) in an analysis of variance. Second, *Ubx* could increase the size of all halteres to a common point, such as a threshold above which true transformation to wing would occur (Figure 1B). A prediction of this model is that there would be a reduction in variance of the trait in the mutant background. Third, *Ubx* could increase the size of the halteres to varying degrees in different lines, without affecting the overall variance (Figure 1C). This model is equivalent to the crossing of the norms of reaction in different environments (FRY *et al.* 1996) and implies high levels of genetic variation at loci that modify the *Ubx* phenotype. Fourth, *Ubx* could increase the overall variance of haltere size among lines, with or without (Figure 1D) the crossing of line means [see MORENO (1995) for an example from the effect of *extramacrochetae* on bristle numbers]. Evidence for this model, after appropriate scaling of the variance by the mean, would imply canalization.

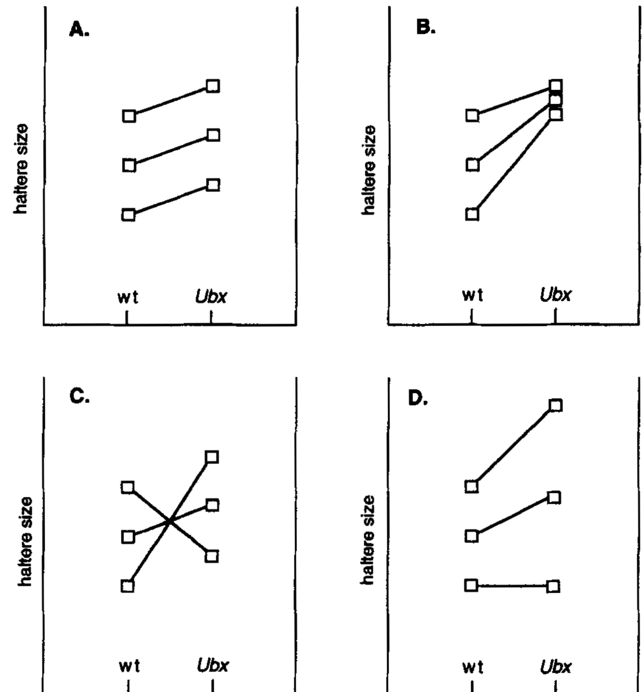


FIGURE 1.—Four models for the effect of the genetic background on *Ubx* function. Haltere size, increasing along the *Y* axis, is plotted for three hypothetical lines in wild-type (left) and *Ubx* (right) flies. (A) Additive model: haltere area is increased by a similar magnitude in all lines. (B) Decrease in variance model: all halteres increase in size toward a similar maximum. (C) Crossing of line means model: due to epistasis, and/or rare alleles, the effect of the genetic background is uncorrelated between wild-type and *Ubx* siblings. (D) Canalization model: there is an overall increase in variance among line means, which in the case drawn is due to a multiplicative genetic effect, in that lines with larger wild-type halteres have even larger *Ubx* halteres. Note that similar plots in Figure 6 compare relative instead of absolute change in haltere size between genotypes.

Here we show, by performing a series of tests on the variance components of haltere morphology following introgression of the $Ubx^1$ allele into 29 different isofemale lines, that the third of these models explains most of the effect of the genetic background on *Ubx* function. Though not providing direct evidence for canalization, we discuss how strong stabilizing selection may affect the structure of variation affecting a complex developmental trait.

## MATERIALS AND METHODS

**Stocks:** $Ubx^1$ is a protein-null mutation due to an insertion of a *doc* element in the untranslated portion of the first exon

**TABLE 1**

**Wild-type stocks**

| Line | No. | Name | Origin | Haltere phenotype |
|---|---|---|---|---|
| 1 | 3607 | BER1 | Bermuda | Shape change in *Ubx* |
| 2 | 3608 | BER2 | Bermuda | Relatively rotund |
| 3 | 3609 | BOG1 | Bogota, Columbia | Relatively elongate |
| 4 | 3610 | BOG2 | Bogota, Columbia | Smallest in *Ubx* |
| 5 | 3614 | CA1 | Capetown, S. Africa | Smallest *Ubx* effect |
| 6 | 3615 | CA2 | Capetown, S. Africa | |
| 7 | 3616 | CO3 | New York, USA | Largest *Ubx* effect |
| 8 | 3617 | CO4 | New York, USA | Large *Ubx* effect |
| 9 | 3623 | M2 | Australia | |
| 10 | 3624 | M4 | Australia | Small *Ubx* effect |
| 11 | 3631 | PYR2 | Pyrenees, Spain | No bristles |
| 12 | 3632 | PYR2 | Pyrenees, Spain | No bristles |
| 13 | 3635 | Reids1 | Madiera, Portugal | Relatively elongate |
| 14 | 3636 | Reids2 | Madiera, Portugal | Large female *Ubx* effect |
| 15 | 3638 | RVC2 | California, USA | |
| 16 | 3639 | RVC3 | California, USA | |
| 17 | 3644 | VAG1 | Athens, Greece | No bristles, small *Ubx* effect |
| 18 | 3645 | VAG2 | Athens, Greece | |
| 19 | 3649 | Wild2A | Ohio, USA | Large *Ubx* effect |
| 22 | 3655 | Wild5B | Georgia, USA | |
| 23 | 3659 | Wild10A | S. Carolina, USA | Large male *Ubx* effect |
| 24 | 3660 | Wild10D | S. Carolina, USA | Shape change in *Ubx* |
| 25 | 3669 | CT1 | Australia | Large wt, small *Ubx* effect |
| 26 | 3670 | CT109 | Australia | Largest wt |
| 27 | 3682 | 7-21-88#b1 | Kakamega, Kenya | Many bristles |
| 28 | 3683 | 7-21-88#b2 | Kakamega, Kenya | |
| 29 | 3693 | 5-17-88#b1 | Makindu, Kenya | Smallest wt |
| 31 | 3703 | 5-15-88#a | Nairobi, Kenya | |
| 32 | 3704 | 5-15-88#b | Nairobi, Kenya | Relatively rotund |

No. and name refer to the designations given by the Bowling Green Stock Center.

(Weinzerl *et al.* 1991) that behaves genetically as an amorph (Morata and Kerridge 1980). Homozygotes die as larvae, and heterozygotes show enlargements of the haltere. The stock (no. 2866: *Ubx*$^{1}$ *e*/TM3) was obtained from the Bloomington Stock Center in August, 1994. The chromosomal region including the mutant allele was introduced into the "outbred" laboratory strain "Ives" (Charlesworth and Charlesworth 1985) by repeated back crossing for 10 generations.

Thirty-two wild-type isofemale lines were obtained from the Bowling Green Stock Center in January, 1996. Table 1 shows the source of 29 of these lines that survived introgression, as well as any distinguishing features of the haltere phenotype. Most of the lines are somewhat inbred, as expected after up to 20 years in culture: the average decline in heterozygosity relative to Hardy Weinberg expectation, *F*, over 10 microsatellite markers spread throughout the genome was 0.69 (data not shown). Inversions are rare in the lines, as determined by polytene chromosome squashes, but at least one line did contain an inversion on chromosome *3R* around *Ubx*.

**Crosses:** The introgression scheme is shown in Figure 2. Briefly, for each wild-type line, one *Ubx*/Ives male was crossed to several virgin females of the line. *Ubx* male progeny were backcrossed to 10 virgins of the wild-type line in the $F_1$, thereby ensuring removal of the Ives *X* chromosome from each introgression stock. Thereafter, for a further 10 generations, 10 *Ubx* virgin females were backcrossed to 10 males of the wild-type line, allowed to lay eggs for 4 days, and transferred to back-up vials. In each generation, the first 10 *Ubx* females scored were taken, such that no conscious selection on haltere morphology was performed. In most cases, *Ubx* halteres possess a diagnostic large bristle adjacent to the stalk (see Figure 3A) and are larger than the wild type. However, in several lines it is difficult to distinguish *Ubx* from wild type, and it is likely that some weak selection for enhancement of the *Ubx* phenotype was performed. This would result in underestimation of the effects of genetic variation affecting the genotype by line interactions among lines.

Ten generations of introgression in *D. melanogaster* is predicted to lead to replacement of over 90% of the original genome of the *Ubx* stock with that of the wild-type line (see discussion in True *et al.* 1996). This calculation is based on complete replacement of the unselected chromosomes (*X* and 2) and retention of 10 cM on either side of the selected locus. Sample scoring of microsatellite loci (data not shown) confirmed that this is approximately the case. *Ubx* is located in cytological interval 89E on the right arm of chromosome *3*, and markers in *Ubx* and in *Antp* (84B) remain heterozygous for the original *Ubx*-linked allele in most lines (whereas markers on the left arm of chromosome *3* tend to be homozygous and to differentiate the lines, indicating that further inbreeding has occurred). Uniformity of haltere phenotypes after the fifth generation of introgression (see Figure 4) suggests that most of the significant genetic background effects had been introduced by this time. We conclude that all introgression lines differ throughout their genomes but for a portion of the right arm of chromosome *3*. Some phenotypes may result from introgression of alleles linked to *Ubx*, and it should be

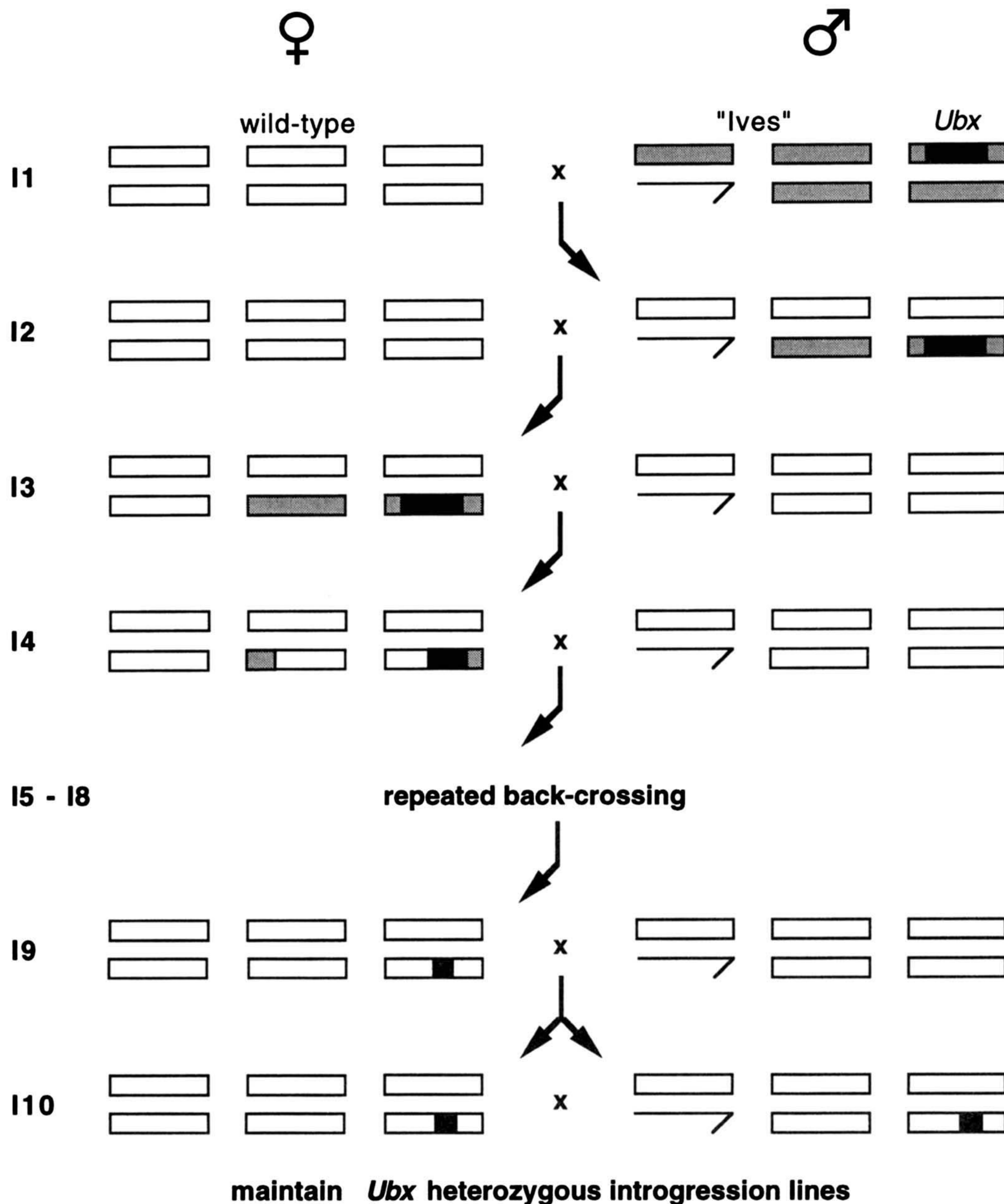


FIGURE 2.—The introgression scheme. Boxes from left to right within a sex represent chromosomes *1* (*X* or *Y* for males on right), *2* and *3* with no shading for wild type, gray for "Ives," and black for genes linked to the original *Ubx*[1] chromosome. I1–I10 refer to the generation of introgression. In each generation after I2, 10 *Ubx* virgin females were crossed to 10 wild-type males.

noted that these alleles could derive from the Ives strain rather than the unique *Ubx e* chromosome.

**Morphological analysis:** Flies were grown under standard conditions at 25° on 10 ml of cornmeal/yeast medium in glass vials up to a density of 150 larvae per vial. Haltere bristle counts were performed directly on the 10 females selected in each generation. For the shape analyses, more care was taken to reduce environmental effects. At generations 8 and 10, 10 *Ubx* females were allowed to lay eggs for 4 days in plastic bottles on agar supplemented with grape juice and sprinkled with dry yeast. Fifty to sixty crawling 1st and 2nd instar larvae were then hand-picked and transferred to glass vials with cornmeal/yeast medium, where they were grown at 25°. This resulted in an average of 10–15 flies of each genotype and sex, of which five were chosen at random for dissection: that is to say, all animals scored for each line were derived from the same cross and vial. Both sides of each animal were scored to provide an independent estimate of environmental effects expressed as developmental asymmetry. The forelegs, wings, and halteres were removed with a pair of micro-scissors, transferred to a drop of Ringers solution on a glass microscope slide, and covered with a cover slip. With practice, this procedure takes ~5 min per animal, taking care to prevent folding of the wings as they are squashed.

Size measures were obtained over the next few hours using NIH Image software (RASBAND 1995) on a PowerMacintosh 7100 computer, after capture of brightfield video images with a Data Translation QuickCapture board (DT2255) from a Zeiss Axioplan microscope to a 15-inch flat screen monitor. Outlines of just the capitellum (the bulbous portion, excluding the stalk) of halteres (200× magnification) and the blade of each wing (50× magnification) were traced manually with between 20 and 30 reference points. The area of the haltere, and the major and minor axes of an ellipse of best fit in raw form as pixels or square pixels were calculated automatically using NIH Image software. Direct measurement of appendage length and breadth gave very similar values as the major and minor axes, but were more variable and more subject to observer bias. Tibia lengths of each foreleg were similarly calculated directly in pixels, with a typical error of <5% between

repeat measures (data not shown). All measurements were taken by the senior author (G.G.).

**Analysis of variance:** Statistical analysis was performed using the least squares MANOVA routine within Statistica 4.1 software (STATSOFT 1996) for the Macintosh. All analyses assumed random effects of line ($L$ = 1, 2, ... 29), but fixed effects of sex ($S$ = female or male) and genotype ($G$ = *Ubx* or wild type). The error term included effects due to individuals within lines as well as variance between left and right sides. Thus, for each phenotype, the mixed model ANOVA compared main effects of sex, genotype and line, as well as the pairwise interaction effects (sex by genotype, sex by line, genotype by line) and the three-way interaction (sex by line by genotype). For the ANOVA presented in Table 3, the sources of error were partitioned as follows (see MACKAY *et al.* 1996): the denominator mean squares (MS) were as follows: MS($G \times L$) for $G$, MS($S \times L$) for $S$, [MS($G \times L$) + MS($S \times L$) − MS($G \times S \times L$)] for $L$, MS($G \times S \times L$) for the pairwise interactions, and the residual MS for the threeway ($G \times S \times L$) interaction. The design was complete, there was little or no correlation between means and variances, and the normal probability plots were essentially linear, so no transformations of the data were required.

For the test of the replicate genotype by line effect, $R(G \times L)$, the generation 8 data was missing 29 of 1160 values (2.5%) spread across all lines. Two separate analyses were performed, with the complete but unbalanced data set as the repeat measure, or with a balanced design derived by random deletion of one value from all treatments in both replicates. Both gave identical $F$ ratios of 0.6, using the Sex × $R(G \times L)$ mean square as the denominator. Inclusion of the generation 8 data did not significantly alter any of the other parameters estimated in this study either.

Simple regression of haltere size on tibia length was marginally significant but accounts for <10% of the variation in haltere size and is of the same order as the regression of haltere size on wing size. Since haltere size is determined largely independently of body size, tibia length was not considered as a covariate in the analyses presented here, but it should be recognized that effects on body size may be a component of the phenotypic variation.

Quantitative genetic parameters were estimated from analyses of variance performed for each sex separately as follows (SOKAL and ROLFF 1981: p. 337; FALCONER and MACKAY 1996). For both genotypes pooled and given an inbreeding coefficient $F$, the genetic variance $V_G$ is equal to $(1/2F)\sigma^2_L + F\sigma^2_{GL}$ where $\sigma^2_{GL}$ equals $(MS_{GL} - \sigma^2)/n$. $n$ = 10, the number of observations per group; $\sigma^2$ is the within group variance (the error mean square); and $\sigma^2_L$ equals $(MS_L - \sigma^2)/na$, with $a$ = 2, the number of sexes compared (note that line is treated here as a random factor). For the two genotypes also analyzed separately, $V_G$ is equal to just $(1/2F)\sigma^2_L$ where $\sigma^2_L$ equals $(MS_L - \sigma^2)/n$. The environmental variance, $V_E$ is then $\sigma^2 - (1 - F)V_G$. The genetic correlation between the genotypes was estimated using Robertson's method for calculating the correlation between family members across treatments in a two-factor analysis of variance as:

$$r_{wt,Ubx} = (MS_L - MS_{G\times L})/(MS_L + MS_{G\times L} - 2.MS_{error}).$$

Within individual variance was calculated as the squared deviation between the measurement for one side and the average of the two sides. $F$-tests for unequal within-individual variances between treatments were then performed on the values for individuals within treatments (genotype and sex).

## RESULTS

**Variation affecting haltere bristle number:** Introgression of the dominant loss-of-function $Ubx^1$ allele into 29 different wild-type lines quickly uncovered remarkable levels of genetic variation affecting haltere morphology. This was most apparent for the number of bristles on the anterior margin of the haltere. In some lines, a clear double row of bristles appeared (Figure 3D), supporting the interpretation that this trait represents a partial transformation of haltere toward wing identity. Wild-type halteres are devoid of bristles, although some lines have a variable number of fine hairs, usually adjacent to the stalk.

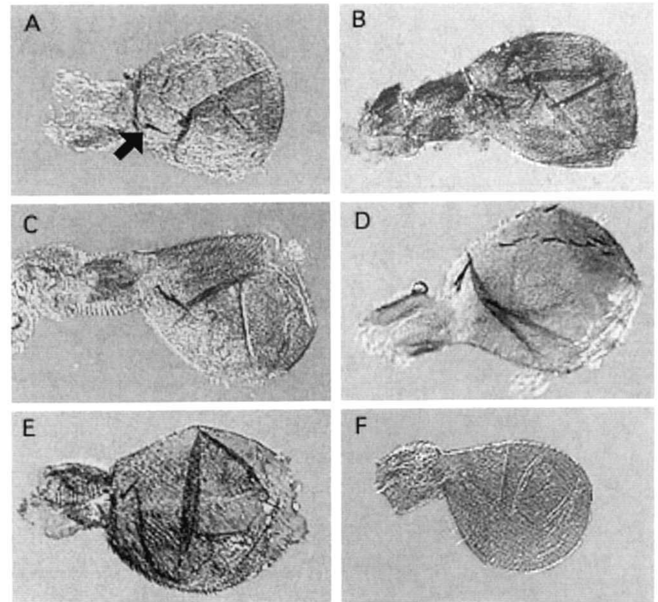


FIGURE 3.—Haltere phenotypes. Representative halteres from *Ubx* females of lines 2 (A), 13 (B), 17 (C), 27 (D) and 7 (E) as well as a wild-type female haltere (F) illustrate differences in shape (A *vs.* B), number of bristles (C *vs.* D, most of the second row is out of the plane of focus in D), and size (E). Arrow in A points to the "diagnostic" *Ubx* bristle at the base of the capitellum. Images were captured at the same magnification (200×) as TIFF files with NIH Image software, and then reduced to scale and rearranged using Photoshop 3.0 software.

The response of haltere bristle number to introgression, without selection, is plotted in Figure 4. Three features of the response stand out. (1) There is a distribution of values for the mean number of bristles per haltere between 0 and 6, with a mode of 2.2, while one line (no. 27) is a clear outlier with between 15 and 20 bristles per haltere. (2) The mean number of bristles per line drops in the first few generations of introgression, then in some lines increases slightly (two examples for individual lines are highlighted in Figure 4) and settles down after the seventh generation. The initial drop may be interpreted as dilution of loci in the selected *Ubx*/Ives parents that promote bristle development, while the few cases of significant increase thereafter could be explained by accumulation of loci from the particular isofemale line that show nonadditive interactions. (3) Regression of the mean number of haltere bristles per line from one generation to the next

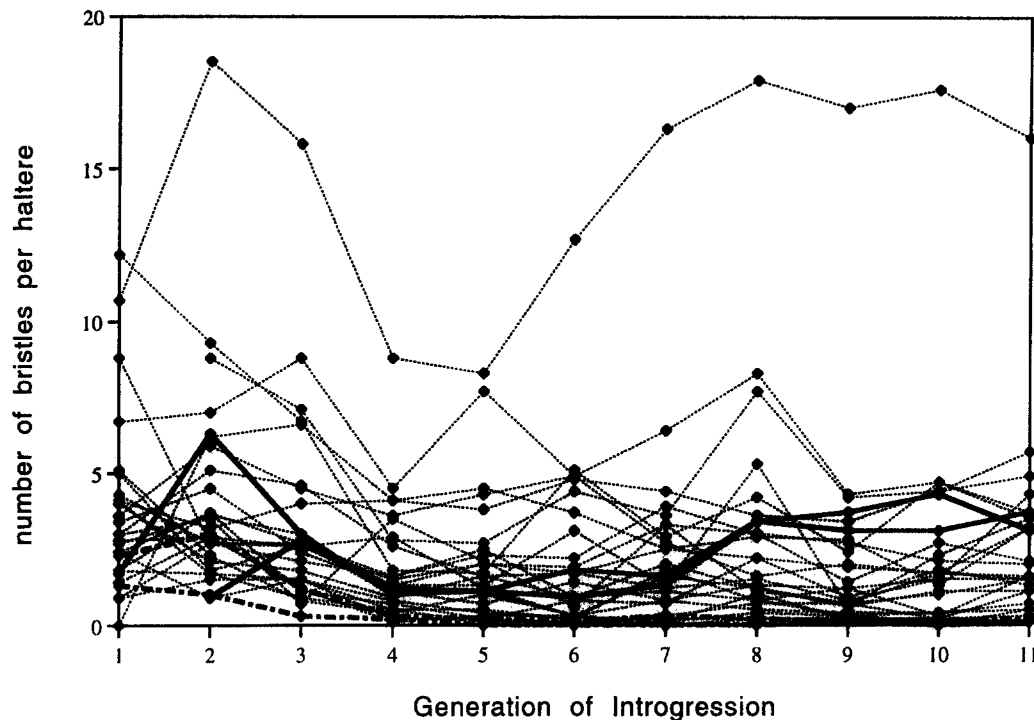


FIGURE 4.—Response to introgression for bristle numbers. Mean number of bristles per haltere for both sides of 10 females per generation are plotted against the generation of introgression along the x-axis. Line 27 is the outlier with a mean of 16 bristles per haltere after I7; lines 11 and 12 (heavy dashes) have almost no bristles after I4; and lines 10 and 31 (bold) are highlighted to indicate typical trajectories of the response.

was significant for all generations, with a regression coefficient above 0.95 for the last three generations, indicating that the majority of the variation is heritable and between lines. The fluctuation from generation to generation is greater for a few of the lines, possibly indicating variable sensitivity to environmental deviations (particularly larval density effects on growth), or possibly due to chance.

The number of bristles in females is significantly greater than in males. Despite a high correlation between males and females for bristle number at generation 11 ($r > 0.95$), the sex by line interaction term in a two-factor analysis of variance (Table 2) is highly significant. That is to say, the degree of sex-specificity of the *Ubx* effect is line-dependent, though much of the specificity is restricted to a few lines. For example, lines 4 and 28 each have greater than three bristles per haltere in females but fewer than one in males, whereas line 6 has between three and five bristles per haltere in both sexes. Sex-specific effects are also a common feature of between-line variation for abdominal and sternopleural bristle numbers (MACKAY 1995), as well as a variety of other traits such as olfactory behavior (MACKAY *et al.* 1996), body size, longevity (M. L. WAYNE, S. NUZHDIN and T. F. C. MACKAY, personal communication), and haltere morphology (see below).

There was no general correlation between haltere bristle number and either abdominal bristle number

TABLE 2

**Analysis of variance of haltere traits after 10th generation of *Ubx* introgression**

| Source | Haltere area | | | | | Bristle number[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d.f. | MS × $10^6$ | DMS × $10^6$ | *F* | *P* | d.f. | MS | DMS | *F* | *P* |
| Genotype (*G*) | 1 | 600.0 | 4.9 | 123.2 | **** | | n.a. | | | |
| Sex (*S*) | 1 | 461.0 | 1.4 | 324.6 | **** | 1 | 86.19 | 1.43 | 60.5 | **** |
| Line (*L*) | 28 | 8.6 | 5.3 | 1.6 | NS | 27 | 12.67 | 0.29 | 43.2 | **** |
| $G \times S$ | 1 | 23.5 | 1.0 | 23.3 | **** | | n.a. | | | |
| $G \times L$ | 28 | 4.9 | 1.0 | 4.8 | **** | | n.a. | | | |
| $S \times L$ | 28 | 1.4 | 1.0 | 1.4 | NS | 27 | 1.43 | 0.29 | 4.9 | **** |
| $G \times S \times L$ | 28 | 1.0 | 0.4 | 2.7 | **** | | n.a. | | | |
| Error | 1044 | 0.4 | | | | 1026 | 0.29 | | | |

NS, $P > 0.05$; **** $P < 0.0001$. MS and DMS (denominator mean square) measures in pixels for haltere area.

[a] Bristle number was transformed by taking the square root to produce a more normal distribution. Since there are no bristles on wild-type halteres, the genotype interaction term is not applicable; analysis excludes line 27. Note that sex × line *P* value for haltere area was highly significant ($P < 0.0001$) when the genotypes were analyzed separately ($F_{28,522} = 4.1$ for *Ubx*; $F_{28,522} = 2.4$ for females).

or sex comb teeth (data not shown). This observation does not, however, necessarily imply that the traits are under control of different genes, as fine structure analysis indicates that the same alleles can have inverse effects on different neurogenic precursors (MACKAY 1995). It is however unlikely that all of the effects can be attributed to neurogenic loci, as the location of haltere bristles varies among lines. Genes that are differentially active along the proximal-distal axis may locally promote wing-like, and hence bristle, development to different extents in the various lines. Interestingly, the most extreme line, 27, has a relatively small number of abdominal bristles and a relatively large number of sex comb teeth. In line 17, which has only rare haltere bristles, these relations are reversed. Noting that mutational screens for modifiers of *Antennapedia* and *Deformed* mainly identified cofactors and regulators of several homeotic genes (KENNISON and TAMKUN 1988; HARDING *et al.* 1995), it is possible that a portion of the genetic variation affecting *Ubx* function also affects the wild-type activity of other homeotic genes, in this case *abdominal B* and *Sex combs reduced.*

**Quantitative variation affecting haltere dimensions:** After as few as five generations of introgression, it was also apparent that there is extensive genetic variation for haltere size and shape. We quantified some of the differences simply by dissecting halteres and measuring their dimensions and area when squashed on a microscope slide. Since the haltere is a simple, bulbous structure without major morphological landmarks, and the capitellum does not always squash in the same orientation, it was not possible to generate meaningful descriptors of shape differences. The analysis below was for just the overall area and lengths of the major and minor axes of both sides of five sibling flies of each sex and genotype for each of the 29 lines in progeny of the 10th generation of introgression.

Representative halteres are shown in Figure 3. Examples of rotund (Figure 3A), elongate (Figure 3B), average (Figure 3C) and large (Figure 3E) *Ubx* halteres, as well as the double row of bristles in line 27 (Figure 3D), are shown by comparison with a wild-type haltere (Figure 3F).

The differences among lines are presented graphically in Figure 5 for females (top) and males (bottom). The cross-hatched bars represent mean wild-type haltere length, while the shaded portion shows the increase in area due to *Ubx*. There is a only a slight tendency for lines with larger wild-type halteres to show smaller transformations. Thus, the wild-type phenotype is not a good predictor of the *Ubx* phenotype, and there is not a uniform *Ubx* phenotype to which all lines gravitate. The effects of modifiers of haltere morphology seem instead to be scrambled by the introgression of the major-effect *Ubx* mutation. To quantify the significance of these effects and distinguish among the four models presented in the introduction, the following analyses were performed.

First, hierarchical analysis of variance of the complete data set after the 10th generation of introgression indicates that there is a highly significant two-way interaction effect between genotype and line (Table 2). This result excludes the simplest interpretation (Figure 1A), that the effect of *Ubx* on haltere size is purely additive.

Second, we tested whether there is a significant change in variance of haltere dimensions in the *Ubx* and wild-type genetic backgrounds. Comparison of the standard deviations of line means in the APPENDIX indicates that, if anything, there is a slight increase in phenotypic variance in *Ubx* flies. Similarly, the estimated genetic variance components, $V_G$ (Table 3) are greater in *Ubx* than wild-type flies, at least in females. These observations exclude the possibility that the *Ubx* mutation increases the size of all halteres toward a common level (Figure 1B). *F* ratio tests were performed to assess the significance of the increase in variance (Table 4). Considering the phenotypic variance of the line means, there is no significant effect of *Ubx* on haltere area, or the lengths of either the major or minor axes. Considering all individuals within a treatment, *Ubx* females have significantly greater variation for haltere area and the length of the major axis than wild-type females, but these effects are nonsignificant when scaled by the treatment means.

Third, we asked whether the interaction was due to crossing of line means. The data for the haltere area data set was reanalyzed after transformation to remove any treatment effects on the variance. Dividing each term by the variance of the relevant treatment (sex and genotype) results in a variance of unity for each treatment. The two- and three-way interaction terms in an ANOVA were again highly significant ($F_{28,28} = 4.9$; $P < 0.0001$) for the genotype by line interaction with the genotype by sex by line term as the source of error. A graphical representation of the significance of the crossing of line means is provided in Figure 6. For this plot, the overall effect of sex and genotype on haltere size was first removed by dividing each value by the mean of the relevant treatment. This results in relative rather than absolute changes in haltere area, but the overall shape of the plot is clearly similar to that in Figure 1C. As check of the possibility that the crossing of line means was simply due to uncontrolled environmental (for example, vial) effects, a similar set of data obtained after the eighth generation of introgression was included in a separate ANOVA. This data set was incomplete, which may bias estimates of variance parameters, but it provides an estimate for a replicate genotype by line interaction term, $R(G \times L)$, which was nonsignificant ($F_{58,58} = 0.6$; $F_{crit} = 1.5$). Survey of the literature also indicates that vial effects for morphological traits are generally negligible relative to treatment

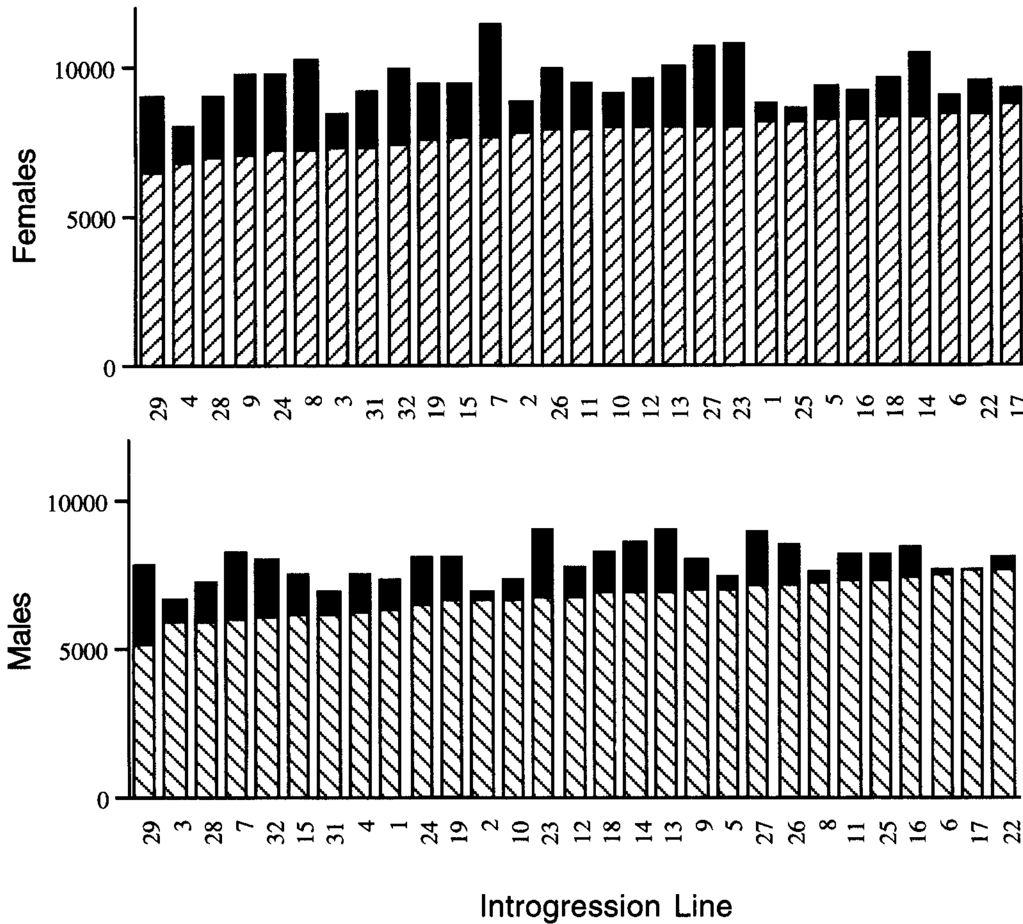


FIGURE 5.—Increases in haltere area by line and sex. Histograms of mean *Ubx* haltere area (in square pixels) for five individuals grown at low density at 25° in black are stacked on the mean haltere area of five wild-type siblings arranged in ascending order by line, cross-hatched upward for females and downward for males. Note that the order of lines is similar but not identical for the two sexes.

effects, and we conclude that most of the crossing of line means has a genetic basis.

Fourth, COCKERHAM's procedure (1963, p. 88) was used to partition the effects of crossing of line means and overall change in genetic variance on haltere area. The two sexes were treated separately, and the additive

**TABLE 3**

**Estimates of quantitative genetic parameters of haltere area in 29 isofemale lines after 10 generations of introgression of *Ubx***

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Both | wt | *Ubx* | Both | wt | *Ubx* |
| *Mean* | 7577 | 6754 | 7909 | 8597 | 7736 | 9458 |
| $MS_L \times 10^5$ | 50.5 | 36.4 | 37.3 | 49.6 | 31.4 | 53.6 |
| $MS_{GL} \times 10^5$ | 23.3 | | | 35.5 | | |
| $MS_{Within} \times 10^5$ | 3.8 | 3.8 | 3.8 | 3.6 | 3.2 | 3.9 |
| $\sigma^2_L \times 10^5$ | 2.34 | 3.26 | 3.36 | 2.30 | 2.83 | 4.94 |
| $\sigma^2_{GxL} \times 10^5$ | 1.95 | | | 3.19 | | |
| $r_{wt,Ubx}$ | 0.41 | | | 0.18 | | |
| $V_G \times 10^5$ | 3.0 | 2.4 | 2.4 | 3.9 | 2.1 | 3.6 |
| $CV_G$ | 7.3 | 7.2 | 6.2 | 7.2 | 5.9 | 6.3 |
| $V_E \times 10^5$ | 2.8 | 3.0 | 3.0 | 2.4 | 2.6 | 2.8 |
| $CV_E$ | 7.0 | 8.2 | 6.9 | 5.7 | 6.6 | 5.6 |
| $V_I \times 10^5$ | 0.8 | 0.8 | 0.8 | 1.0 | 0.9 | 1.1 |
| $h^2$ | 0.52 | 0.44 | 0.45 | 0.62 | 0.44 | 0.56 |

Parameter estimates are for the mean, mean squares from independent ANOVAs, variance components for line ($\sigma^2_L$) and genotype by line interaction ($\sigma^2_{GxL}$), genetic correlation coefficient $r_{wt,Ubx}$, coefficients of genetic ($CV_G$) and environmental variance ($CV_E$), developmental asymmetry ($V_I$), and heritability ($h^2$). See MATERIALS AND METHODS for description of calculations.

TABLE 4

**Tests for increased phenotypic variance in *Ubx* vs. wild-type flies**

| | | Line means | | Individuals | |
|---|---|---|---|---|---|
| | | *F* | *P* | *F* | *P* |
| Haltere area | Female | 1.69 | 0.09 | 1.47 | *** |
| | Male | 1.03 | 0.47 | 1.01 | .46 |
| Haltere major axis | Female | 1.79 | 0.06 | 1.37 | ** |
| | Male | 1.08 | 0.42 | 1.13 | .14 |
| Haltere minor axis | Female | 1.07 | 0.43 | 1.05 | .35 |
| | Male | 1.23 | 0.29 | 1.16 | .10 |

*F*-ratios are ratio of *Ubx* to wild-type variance with associated *P* values (d.f. = 28 for line means; 289 for individuals). *** $P < 0.001$, ** $P < 0.01$. The "Line means" columns give the comparison of the mean phenotypic value for the 10 halteres of each sex and genotype (see Table 2, standard deviations); the "Individuals" columns compare the total variance among all individuals for each treatment.

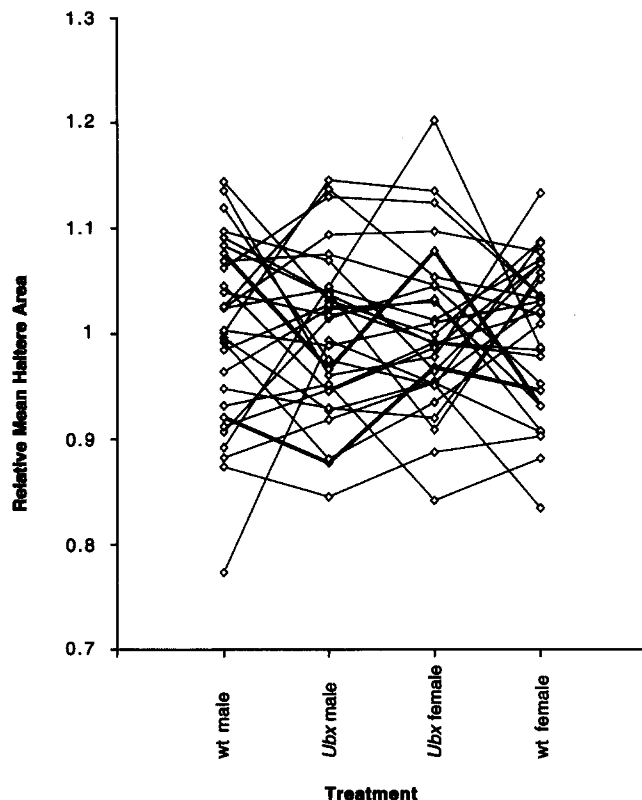


FIGURE 6.—Crossing of line means is responsible for most of the genotype by line interaction. For each of the four treatments (left to right: wild-type male, *Ubx* male, *Ubx* female, wild-type female) the line means for haltere area were divided by the relevant treatment mean to produce the relative mean haltere area. Note the higher density of crossed lines between genotypes for each sex than between the two sexes in the middle. In most cases, the relative change in haltere size was in the same direction for both sexes, but the two cases with a reversal of the relative magnitude of the response in males and females are highlighted in bold.

TABLE 5

**Summary of estimates of coefficients of variation and heritability for three phenotypes in 29 isofemale lines after 10 generations of introgression of *Ubx***

| | Wild type | | *Ubx* | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| $CV_G$ | | | | |
| Haltere area | 7.2 | 5.9 | 6.2 | 6.3 |
| Haltere major axis | 3.5 | 2.9 | 3.4 | 3.6 |
| Haltere minor axis | 4.1 | 3.2 | 3.4 | 3.0 |
| $CV_E$ | | | | |
| Haltere area | 8.2 | 6.6 | 6.9 | 5.6 |
| Haltere major axis | 4.1 | 3.8 | 4.2 | 3.2 |
| Haltere minor axis | 5.4 | 4.7 | 4.8 | 4.3 |
| $h^2$ | | | | |
| Haltere area | 0.44 | 0.44 | 0.45 | 0.56 |
| Haltere major axis | 0.42 | 0.37 | 0.39 | 0.56 |
| Haltere minor axis | 0.37 | 0.32 | 0.33 | 0.33 |

The coefficients of genetic ($CV_G$) and environmental variance ($CV_E$), and the narrow sense heritability ($h^2$) were calculated assuming an inbreeding coefficient of $F = 0.69$, as described in the text.

genetic variance components, $\sigma^2_{wt}$ and $\sigma^2_{Ubx}$ for the two genotypes as well as the genetic correlation between them, $r_{wt,Ubx}$, were calculated from the analysis of variance as described in MATERIALS AND METHODS, following SOKAL and ROLFF (1981: p. 337) and ROBERTSON (1959). Then, for a comparison of just two treatments (genotypes within a sex), the genotype by line interaction term can be partitioned as follows:

$$\sigma^2_{G\times L} = [(\sigma_{wt} \cdot \sigma_{Ubx})(1 - r_{wt,Ubx})] + \sigma_{wt} - \sigma_{Ubx})^2/2,$$

where the term in square brackets is the component due to crossing of line means, and the term on the right side is due to differences in among-line variances. Substituting values from Table 3, for females, $\sigma^2_{G\times L} \sim 3.07 \times 10^5 + 0.15 \times 10^5$, and for males, $\sigma^2_{G\times L} \sim 1.95 \times 10^5 + 0.00 \times 10^5$, whence we conclude that >95% of the interaction term is due to the low correlation between lines across genotypes in both sexes. In summary, the data provides little evidence for an increase in genetic variance in the mutant background, but suggests that there are extensive nonadditive interactions among modifiers of the effect of the $Ubx^1$ allele on haltere development.

**Coefficients of genetic variation:** The levels of genetic and environmental variation scaled by the trait mean provide some indication of the type of selection pressures that may be acting on the trait (HOULE 1992). The coefficients of variation were calculated (see Table 3) as $CV_G$ equals $100\sqrt{(V_G)}/M$, and $CV_E$ equals $100\sqrt{(V_E)}/M$, where $M$ is the trait mean. Values for haltere area and the two haltere axes are listed in Table 5. As expected, the coefficients for the two-dimensional trait (area) are larger than those for one-dimensional length measures. We also calculated these coefficients for wing

length from our data set and obtained values indistinguishable from those averaged from a number of studies presented in Table 1 of HOULE (1992; data not shown). For all three haltere traits, the coefficients of genetic variance are in the low to moderate range, and the coefficients of environmental variance are also relatively low. As a result, the heritability estimates are in the moderate range.

One caveat to this analysis is that the isofemale lines, and hence the introgression lines, are not fully inbred, so the line mean squares include a component due to within-line genetic variance (as well as possible vial effects). The values in Tables 3 and 5 were calculated assuming an inbreeding coefficient of $F = 0.69$, which is an estimate obtained from the average decline in heterozygosity relative to Hardy-Weinberg expectations of 10 microsatellite markers in the isofemale lines (data not shown). Assuming that the true value of $F$ lies between 0.5 and 1, confidence limits can be placed on the estimates of the genetic and environmental variance components, and thence the coefficients of variation. This suggests a maximal error of $\pm 1$ on each $CV_G$ or $CV_E$ estimate. Since the values even for the haltere area measure are slightly lower than those for another trait thought to experience weak stabilizing selection, sternopleural bristle number (HOULE 1992; MACKAY 1995), we conclude that haltere dimensions, particularly the axial measures, do show the expected properties of a trait undergoing stabilizing selection. The same conclusion is implied by the observation of very low developmental asymmetry. Remarkably, the within-individual variance ($V_I$ in Table 3) is almost identical between sexes and genotypes ($P > 0.05$ for all treatment comparisons; $F$-tests), indicating that any environmental canalization is strong enough to buffer against the effects of the *Ubx* mutation.

**Sexual dimorphism of the *Ubx* effect on haltere morphology:** The significant sex by genotype by line interaction term in the ANOVA (Table 2) indicates that not all lines respond to *Ubx* in a similar manner in both sexes. *Post hoc* comparisons suggest that the effect is predominately due to a few lines that show a larger increase in haltere area in females than in males. Two of these lines (numbers 8 and 9) are highlighted in bold in Figure 6. Overall, the genetic correlations between the sexes are much higher than the correlations between the genotypes estimated by the same method ($r_{male,female} = 0.84$ for wild type, 0.71 for *Ubx* flies, compared with $r_{wt,Ubx} = 0.41$ for male, 0.18 for female flies). This can also be seen by the lower density of crossed lines between the *Ubx* means in the middle of Figure 6 relative to the male and female comparisons on either side. This example of sexual dimorphism indicates that the two sexes are genetically distinct not just for visible traits, but also for their response to genetic perturbation.

Finally, it is worth noting that there was, surprisingly, a significant genotype by line effect on wing size. Fourteen of the 29 lines showed at least a 3% reduction in wing area in *Ubx* flies compared with their wild-type siblings, usually in both sexes. This ranged up to a 10% reduction, which is more than the increase in haltere area in some lines. This result may indicate a subtle role for *Ubx* in wing development, perhaps acting early in the definition of the imaginal disc precursors, or could be due to segregation of allele(s) of genes linked to *Ubx*, or may indicate an indirect allometric effect of growth in mutant larvae. Wing size was examined as a naive test of the hypothesis that since the *Ubx* haltere transformation is toward wing identity, haltere and wing may show correlated dimensions between lines, but this was not generally the case.

## DISCUSSION

Different isofemale lines produced such a broad array of haltere phenotypes in the presence of the $Ubx^1$ mutation that it was almost possible to tell which line a particular fly belonged to simply by looking at the halteres. The differences are not so apparent in wild-type siblings, which suggested that there had been an increase in variance in the mutant background, and hence that haltere development is canalized. However, statistical analysis of the data shows that, despite a low correlation between wild-type and *Ubx* haltere phenotypes, there is little if any real increase in variance of haltere dimensions. The overall increase in size has an effect more like increasing the magnification on a microscope: it is easier to see differences on larger specimens. Nevertheless, the results have implications for the architecture of genetic variation affecting developmental gene activity, and the relationship between stabilizing selection and canalization.

**Is *Ubx* activity canalized?** Canalization is classically regarded as a property of polygenic traits. Defining it as a reduction of variability (WAGNER *et al.* 1997) also allows the term to be applied to the effects of single genes. In the extreme case of a gene that is homozygous lethal when deleted and that shows a completely dominant phenotype, that is, the heterozygotes (+/−) and the wild-type homozygotes (+/+) are phenotypically equivalent, there is little scope for a contribution to genetic variation. Even allowing for a class of homozygous-viable loss-of-function alleles with less activity than the hemizygote, and hence a phenotype, it is clear that the evolution of dominance reduces variability and implies some degree of canalization. Given that many key regulatory genes, including *Ubx*, are recessive lethals with little or no haploinsufficiency, this argument provides a simple explanation for some of the buffering of developmental systems. The problem still remains to determine what causes the evolution of dominance (FISHER 1928; WRIGHT 1934) and whether it has more to do with the physiology of gene function or the effects

of modifier alleles on heterozygous mutations (GIBSON 1996; MAYO and BÜRGER 1997).

Canalized genetic systems are expected to show an increase in variance of the perturbed state relative to the wild type. With respect to bristle number, there is a striking difference between the wild-type background (no bristles, no variance) and the 29 *Ubx* introgression backgrounds (an approximately normal distribution between zero and six bristles, with one outlier at over 15 bristles), but this is not a valid statistical comparison. This result can also be explained by supposing that the effect of the *Ubx* mutation is to overcome the threshold-dependent suppression of all bristle development on the haltere, revealing an underlying distribution that determines the number of bristles produced. While there is some evidence from interline crosses for epistasis among loci affecting bristle numbers (G. GIBSON, unpublished data), there is no way of determining whether these effects are reduced in the wild-type state. It is difficult to see how canalizing selection pressure could act directly to reduce the variance of bristle numbers when bristles are normally absent, unless the stabilizing selection pressure were so strong that it produced the threshold in the first place.

With respect to the shape of the haltere, information pertaining to the presence of canalization is somewhat ambiguous. On the one hand, the low environmental variance, especially within individuals, is evidence for environmental buffering. More generally, the genetic variance parameters for each of the appendage measures are close to those that might be expected for a trait experiencing strong stabilizing selection, and it is not difficult to imagine that asymmetry in the size of a balancing organ such as the haltere could be detrimental. MOLLER (1996) has shown that developmental asymmetry for wing length in house flies is subject to sexual selection. On the other hand, the lack of any clearly significant change in variance of haltere dimensions between lines in the mutant background argues against genetic canalization. The increase in size due to *Ubx* in some lines is as great as the entire range of wild-type effects, so it is unlikely that the genetic perturbation is too small relative to the variance to see any effect on buffering. The simplest interpretation is that stabilizing selection induces strong environmental canalization, but that there is no direct genetic canalization of haltere development.

**Indirect canalization:** However, two indirect mechanisms that could restrict the genetic variance of haltere morphology, even in the *Ubx* background, are suggested by the observation that the change in variance of haltere area is less than that of the major axis, at least in females. We propose the term "emergent canalization" to describe the situation where negative covariance among components of a trait acts to restrict the variance of the whole. For meristic and other traits measured on a one-dimensional scale, the variance of the whole is equal to the sum of the variances of the parts plus the covariance between the parts: $\mathrm{Var}(Z) = \mathrm{Var}(X) + \mathrm{Var}(Y) + 2\mathrm{Covar}(X, Y)$. $\mathrm{Var}(Z)$ can only be less than $\mathrm{Var}(X)$ or $\mathrm{Var}(Y)$ if the covariance is negative. This could occur as a result of direct selection on the whole or through antagonistic pleiotropy acting on genes that regulate aspects of the parts (ROSE 1982). Thus, if an increase in length of the haltere tended to be offset by a reduction in breadth, the overall area would remain constant. This certainly seems to be the case in a few lines but cannot be a general explanation since the overall correlation between major and minor axes of the haltere is significantly positive ($r = 0.75$).

It is more likely that the variance of the whole is limited by constraints on the parts, a phenomenon we refer to as "induced canalization." For traits measured in two (or more) dimensions, the variances cannot simply be summed, especially as here where there appears to be extensive epistatic interaction between genotypes. Consequently, a statistically significant effect in one axis need not translate into a significant effect on the area. If the variance of some component of a compound trait is limited by stabilizing selection, then the variance of the whole trait will generally be proportionately smaller. Since the intensity of canalizing selection is proportional to the amount of genetic variance (WAGNER *et al.* 1997) for developmental stability, there may be no opportunity for canalizing selection, despite the appearance of reduced variability. Furthermore, if the restriction of variance affecting the parts is itself due to apparent stabilizing selection as a consequence of pleiotropy (GAVRILETS and DE JONG 1993), the compound trait of interest will appear to be canalized without actually directly experiencing selection.

**Sources of nonadditive effects on haltere morphology:** The highly significant crossing of line means for haltere size between wild-type and *Ubx* animals may be due to at least three types of cause: epistasis, rare alleles, and random environmental deviations. The latter seems to make a relatively small contribution, since the genotype by line interactions in two replicates were not significantly different. With respect to genetic causes, the variation may be attributed to alleles at the wild-type *Ubx* locus (our experimental design is actually similar to a complementation test) and/or extragenic modifiers. The epistasis model postulates that there are a number of genes that are highly polymorphic in natural populations and that interact with one another and with *Ubx* in nonadditive ways. Chance segregation of these alleles in isofemale lines could explain the low correlation between genotypes observed in our introgressions. The rare alleles model, by contrast, postulates that the differences are due to one or a few different alleles in each line that are presumably maintained by mutation-selection balance and by chance segregated and/or arose in each of the lines.

Such rare alleles, possibly including polymorphisms

in *Ubx* itself, would also interact nonadditively with *Ubx*$^1$ and in some cases could have absolutely no effect on wild-type haltere development. In other words, it is possible that haltere morphology is modified by different genes and alleles in the wild-type and *Ubx* genetic backgrounds. For example, if haltere shape is predominately affected by genes downstream of *Ubx* in wild-type animals, but by upstream regulators of *Ubx* in the mutants, there would be no correlation between the phenotypes. In this case, the source of variation in the *Ubx* background would be truly "hidden" in the sense that it makes no contribution to normal development but provides a latent pool of variation that can become significant when the genetic system is perturbed. The sexual dimorphism of the response in some lines provides an extra level of complexity to the genetic architecture. Demonstration that epistasis is the cause of crossing of line means awaits mapping of the actual loci responsible for the effects and measurement of their interactions in different combinations.

**Do homeotic genes contribute to morphological variation?** Despite the prevalence of hidden genetic variation affecting haltere morphology, the range of phenotypes in *Ubx*$^1$ heterozygotes overlaps the wild-type range, and it can be difficult to distinguish these individuals in some lines. This observation, along with the fact that *Ubx* regulatory sequences are spread over 100 kb of DNA and must present a large target for the input of mutational variance, implies that there is strong potential for hypomorphic variants of the *Ubx* gene to contribute quantitative variation to haltere morphogenesis. If so, the quantitative effects of *Ubx* are in strong contrast with the apparent stability of the domain and level of *Ubx* expression not just among dipterans, but throughout the Insecta. The expression of UBX protein in the primordium of the hind-wing of the hawkmoth Manduca has been interpreted as evidence that changes in the downstream targets of the homeotics were most likely responsible for the evolutionary transition from four to two wings (Warren *et al.* 1995). Taken at face value, this further implies that a major source of genetic variation at the species level that could have contributed to the morphological transition was not actually utilized. Statistical descriptions may thus overestimate the amount of additive genetic variance that is available for directional selection, and more importantly, the extrapolation from variation within to variation between species is unjustified.

This conundrum might be resolved in several ways. An intriguing possibility is that quantitative effects due to regulatory genes that act early in a pathway, such as the homeotics, are more likely to disrupt environmental buffering and hence be selected against. However, this hypothesis is not supported by the observation that there is little change in phenotypic variance in *Ubx* flies. Another possibility is that a large component of the *Ubx* contribution to the trait mean is epistatic, and hence not available for directional selection, and/or that the quantitative effects may only be additive within strict thresholds. Alternatively, in natural populations the environmental component of the variance, as well as the contributions of other loci, may swamp the effects due to *Ubx*. Should the molecular variation in *Ubx* be associated with pleiotropic reductions in fitness, the potential contribution of the gene to evolutionary change would be further reduced. Quantitative genetic approaches are now available that should allow fine-structure dissection of the genetic architecture of traits that are under the control of key regulatory genes.

We thank Antonio Garcia-Bellido for pointing out, several years ago, that there is considerable natural variation affecting *Ubx* activity, the Bowling Green Stock Center for the lines, Günter Wagner for discussions on canalization, Trudy Mackay for encouragement and advice on the quantitative genetic analysis, and K. Green and P. Polaczyk for their comments. The advice of two anonymous reviewers was also a great help in revision of the manuscript. This work was supported by a Basil O'Connor Fellowship of the March of Dimes Birth Defects Foundation to G.G.

## LITERATURE CITED

Arthur, W., 1988 *A Theory of the Evolution of Development.* Wiley & Sons, Chichester, UK.

Averof, M., and M. Akam, 1995 Hox genes and the diversification of insect and crustacean body plans. Nature **376:** 420–423.

Carroll, S. B., S. D. Weatherbee and J. A. Langeland, 1995 Homeotic genes and the regulation and evolution of insect wing number. Nature **375:** 58–61.

Charlesworth, B., and D. Charlesworth, 1985 Genetic variation in recombination in Drosophila. I. Responses to selection and preliminary genetic analysis. Heredity **54:** 71–83.

Cockerham, C. C., 1963 Estimation of genetic variances, pp. 53–94 in *Statistical Genetics and Plant Breeding,* edited by W. D. Hansen and H. F. Robertson. NAS-NRD, Washington DC.

Curnow, R. N., 1964 The effect of continued seletion of phenotypic intermediates on gene frequency. Genet. Res. **5:** 341–353.

Dunn, R. B., and A. S. Fraser, 1958 Selection for an invariant character—'vibrissae number'—in the house mouse. Nature **181:** 1018–1019.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics,* Ed. 4. Longman, Harlow, UK.

Fisher, R. A., 1928 The possible modification of the response of the wild type to recurrent mutations. Am. Nat. **62:** 115–126.

Fry, J. D., S. L. Heinsohn and T. F. C. Mackay, 1996 The contribution of new mutations to genotype-environment interaction for fitness in *Drosophila melanogaster.* Evolution **50:** 2316–2327.

Gavrilets, S., and G. DeJong, 1993 Pleiotropic models of polygenic variation, stabilizing selection, and epistasis. Genetics **134:** 609–625.

Gibson, G., 1995 Epistasis and pleiotropy as natural properties of transcriptional regulation. Theoret. Popul. Biol. **49:** 58–89.

Harding, K. W., G. Gellon, N. McGinnis and W. McGinnis, 1995 A screen for modifiers of *Deformed* function in Drosophila. Genetics **140:** 1339–1352.

Houle, D., 1992 Comparing evolvability and variability of quantitative traits. Genetics **130:** 195–204.

Kennison, J. A., and J. W. Tamkun, 1988 Dosage-dependent modifiers of Polycomb and Antennapedia mutations in Drosophila. Proc. Natl Acad. Sci. USA **85:** 8136–8140.

Lewis, E. B., 1978 A gene complex controlling segmentation in Drosophila. Nature **276:** 565–570.

Mackay, T. F. C., 1995 The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. Trends Genet. **11:** 464–470.

Mackay, T. F. C., J. B. Hackett, R. F. Lyman, M. L. Wayne and R. R. H. Anholt, 1996 Quantitative genetic variation of odor-

guided behavior in a natural population of *Drosophila melanogaster*. Genetics **144:** 727–735.

MAYNARD-SMITH, J., and K. C. SONDHI, 1960 The genetics of a pattern. Genetics **45:** 1039–1050.

MAYO, O., and R. BÜRGER, 1997 The evolution of dominance: a theory whose time has passed? Biol. Rev. **72:** 97–110

MOLLER, A. P., 1996 Sexual selection, viability selection, and developmental stability in the domestic fly *Musca domestica*. Evolution **50:** 746–752.

MORATA, G., and S. KERRIDGE, 1980 An analysis of the expressivity of some bithorax transformations, pp. 141–154 in *Development and Neurobiology of Drosophila*, edited by O. SIIDIQQI, P. BABU, C. HALL and J. HALL. Plenum Press, London.

MORENO, G., 1995 Genetic architecture, genetic behavior, and character evolution. Annu. Rev. Ecol. Syst. **25:** 31–44.

RASBAND, W., 1995 *NIH Image*, Version 1.55, available from NTIS, 5285 Port Royal Rd, Springfield, VA 22161, part number PB93-504868.

RENDEL, J. M., B. L. SHELDON and D.E. FINLAY, 1965 Canalization of development of scutellar bristles in Drosophila by control of the *scute* locus. Genetics **52:** 1137–1151.

ROBERTSON, A., 1959 The sampling variance of the genetic correlation coefficient. Biometrics **15:** 469–485.

ROBERTSON, F. W., 1965 Variation in scutellar bristle number—an alternative hypothesis. Am. Nat. **99:** 19–24.

ROGERS, B. T., M. D. PETERSON and T. C. KAUFMAN, 1997 Evolution of the insect body plan as revealed by the *Sex combs reduced* expression pattern. Development **124:** 149–157.

ROSE, M., 1982 Antagonistic pleiotropy, dominance, and genetic variation. Heredity **48:** 103–118.

SCHARLOO, W., 1964 Mutant expression and canalization. Nature **203:** 1095–1096.

SCHARLOO, W., 1991 Canalization: genetic and developmental aspects. Annu. Rev. Ecol. Syst. **22:** 65–93.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry*, Ed. 2. W.H. Freeman, San Francisco.

SPICER, G., 1993 Morphological evolution of the *Drosophila virilis* species group as assessed by rate tests for natural selection on quantitative characters. Evolution **47:** 1240–1254.

STATSOFT, INC, 1996 *Statistica for the Macintosh*, User's Guide, Version 4.1. Tulsa, OK.

STERN, C., 1958 Selection for sub-threshold differences and the origin of pseudo- exogenous adaptations. Am. Nat. **2:** 313–316.

TRUE, J. R., B. S. WIER and C. C. LAURIE, 1996 A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. Genetics **142:** 819–837.

WADDINGTON, C. H., 1942 The canalization of development and the inheritance of acquired characters. Nature **150:** 563.

WADDINGTON, C. H., 1957 *The Strategy of the Genes*. Allen & Unwin, London.

WAGNER, G. P., G. BOOTH and H. BAGHERI-CHAICHIAN, 1997 A population genetic theory of canalization. Evolution **51:** 329–347.

WARREN, R. W., L. NAGY, J. SELEGUE, J. GATES and S. CARROLL, 1995 Evolution of homeotic gene regulation and function in flies and butterflies. Nature **372:** 458–461 [published erratum in Nature **373:** 451 (1996)].

WEINZERL, R. O. J., J. M. AXTON., A. GHYSEN and M. E. AKAM, 1987 *Ultrabithorax* mutations in constant and variable regions of the protein coding sequence. Genes Dev. **1:** 386–397.

WRIGHT, S., 1934 Physiological and evolutionary theories of dominance. Am. Nat. **68:** 25–53.

Communicating editor: T. F. C. MACKAY

## APPENDIX

**Summary values**

| | Haltere Area | | | | Haltere major axis | | | | Haltere minor axis | | | | Bristle no. | | Tibia length | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | Male | | Female | | Male | | Female | | Male | | | | | |
| Line | Ubx | wt | Ubx | wt | Ubx | wt | Ubx | wt | Ubx | wt | Ubx | wt | Female Ubx | Male Ubx | Female pool | Male pool |
| 1 | 8681 | 8118 | 7343 | 6394 | 114 | 116 | 107 | 101 | 97 | 90 | 87 | 80 | 0.0 | 0.0 | 245 | 237 |
| 2 | 8899 | 7893 | 6958 | 6648 | 116 | 111 | 103 | 100 | 98 | 90 | 86 | 85 | 1.0 | 0.2 | 258 | 240 |
| 3 | 8375 | 6972 | 6676 | 5899 | 116 | 105 | 107 | 99 | 92 | 84 | 80 | 76 | 2.7 | 0.6 | 249 | 237 |
| 4 | 7949 | 6804 | 7514 | 6288 | 114 | 104 | 109 | 102 | 89 | 84 | 88 | 78 | 0.7 | 0.3 | 253 | 240 |
| 5 | 9319 | 8228 | 7467 | 7057 | 121 | 112 | 109 | 104 | 99 | 94 | 87 | 86 | 6.3 | 2.4 | 258 | 245 |
| 6 | 8980 | 8386 | 7708 | 7558 | 118 | 116 | 109 | 107 | 97 | 92 | 91 | 90 | 4.9 | 2.3 | 259 | 251 |
| 7 | 11352 | 7616 | 8252 | 6022 | 135 | 110 | 118 | 99 | 107 | 88 | 89 | 77 | 4.3 | 0.8 | 255 | 248 |
| 8 | 10186 | 7191 | 7632 | 7266 | 127 | 107 | 110 | 109 | 102 | 86 | 88 | 85 | 0.5 | 0.3 | 261 | 245 |
| 9 | 9750 | 7010 | 8039 | 7012 | 122 | 107 | 111 | 103 | 102 | 85 | 92 | 87 | 0.8 | 0.0 | 251 | 246 |
| 10 | 9017 | 7943 | 7324 | 6726 | 117 | 112 | 108 | 103 | 98 | 90 | 86 | 83 | 2.9 | 0.8 | 258 | 242 |
| 11 | 9350 | 7877 | 8191 | 7317 | 119 | 112 | 111 | 106 | 100 | 90 | 94 | 88 | 0.2 | 0.0 | 257 | 247 |
| 12 | 9532 | 7957 | 7808 | 6775 | 121 | 113 | 110 | 103 | 100 | 90 | 91 | 84 | 0.1 | 0.2 | 267 | 254 |
| 13 | 9946 | 7972 | 8982 | 6926 | 128 | 115 | 123 | 107 | 99 | 89 | 93 | 83 | 0.6 | 0.3 | 247 | 241 |
| 14 | 10356 | 8318 | 8639 | 6917 | 129 | 115 | 117 | 104 | 102 | 92 | 94 | 85 | 2.9 | 1.2 | 256 | 244 |
| 15 | 9361 | 7595 | 7480 | 6157 | 122 | 109 | 110 | 102 | 98 | 89 | 87 | 77 | 0.5 | 0.3 | 257 | 248 |
| 16 | 9104 | 8232 | 8446 | 7412 | 120 | 112 | 115 | 109 | 96 | 94 | 93 | 87 | 0.6 | 0.4 | 250 | 246 |
| 17 | 9235 | 8747 | 7584 | 7666 | 123 | 116 | 111 | 112 | 96 | 96 | 87 | 87 | 0.1 | 0.0 | 266 | 254 |
| 18 | 9561 | 8256 | 8231 | 6912 | 123 | 113 | 113 | 106 | 99 | 93 | 93 | 83 | 1.4 | 0.3 | 262 | 260 |
| 19 | 9363 | 7547 | 8112 | 6650 | 123 | 110 | 112 | 102 | 97 | 87 | 93 | 83 | 2.5 | 1.3 | 268 | 256 |
| 22 | 9438 | 8391 | 8143 | 7725 | 119 | 114 | 111 | 109 | 101 | 94 | 94 | 91 | 0.5 | 0.2 | 265 | 253 |
| 23 | 10721 | 7988 | 9057 | 6759 | 131 | 111 | 120 | 103 | 104 | 91 | 96 | 83 | 3.1 | 2.5 | 255 | 248 |
| 24 | 9727 | 7188 | 8090 | 6508 | 120 | 108 | 109 | 101 | 103 | 85 | 94 | 82 | 3.5 | 1.1 | 252 | 246 |
| 25 | 8582 | 8164 | 8199 | 7370 | 115 | 112 | 112 | 108 | 95 | 93 | 93 | 87 | 0.7 | 0.2 | 253 | 252 |
| 26 | 9882 | 7858 | 8500 | 7219 | 123 | 112 | 118 | 107 | 102 | 89 | 92 | 86 | 0.4 | 0.1 | 256 | 249 |
| 27 | 10613 | 7987 | 8930 | 7177 | 131 | 109 | 118 | 106 | 103 | 94 | 97 | 86 | 1.6 | 7.3 | 264 | 248 |
| 28 | 8997 | 6999 | 7256 | 5952 | 117 | 104 | 106 | 95 | 97 | 86 | 87 | 80 | 5.8 | 1.3 | 250 | 240 |
| 29 | 8989 | 6444 | 7851 | 5218 | 119 | 101 | 111 | 92 | 96 | 81 | 90 | 72 | 0.6 | 0.1 | 247 | 235 |
| 31 | 9145 | 7304 | 6935 | 6215 | 118 | 106 | 104 | 98 | 99 | 88 | 85 | 80 | 4.7 | 0.2 | 241 | 232 |
| 32 | 9862 | 7354 | 8021 | 6123 | 121 | 106 | 110 | 97 | 103 | 88 | 93 | 80 | 4.1 | 2.8 | 248 | 237 |
| Mean | 9458 | 7736 | 7909 | 6754 | 121 | 110 | 111 | 103 | 99 | 89 | 90 | 83 | 2.0 | 0.7 | 255 | 246 |
| $\sigma$ [X] | 730 | 561 | 611 | 603 | 5.3 | 4.0 | 4.7 | 4.5 | 3.8 | 3.7 | 3.9 | 4.3 | 1.9 | 0.8 | 6.9 | 6.7 |
| $\sigma$ [I] | 337 | 301 | 286 | 279 | 7.1 | 7.1 | 7.5 | 5.6 | 7.6 | 6.5 | 4.7 | 5.9 | 0.6 | 0.3 | 3.9 | 4.2 |

The listed mean is the mean within treatment value; $\sigma$ [X] is the standard deviation of the mean treatment value, and $\sigma$ [I] is the mean square root of the within individual variance ($\sqrt{V_I}$) for the treatment.