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Pervasive effects of *P* element mutagenesis on body size in *Drosophila melanogaster*

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Summary

A set of *Drosophila melanogaster* was generated, all derived from a common isogenic base stock and each with a single new *P* element insert on the second or third chromosome. The lines were scored for their body size, measured as thorax length. *P* inserts were associated with highly significant effects on body size, although the genotypes of the construct and of the control prevented deduction of the direction of mutant effects. In addition to mutant effects on the thorax length of both sexes, there were also highly significant sex-specific effects. Pleiotropic effects of inserts affecting body size on viability and bristle number, as ascertained in a separate study of these lines (Lyman *et al*., 1996), were weak. Insertional mutagenesis is potentially a powerful tool for investigating the genes involved in size-control in *Drosophila*, but the technique requires fine tuning for use on polygenic and fitness-related traits.

1. Introduction

Body size is of importance in evolution and in agriculture, because of its associations with fitness, production and life history traits. The control of cell proliferation and growth is central to all developmental processes (Lawrence & Struhl, 1996; Edgar & Lehner, 1996). The mechanisms leading to the decision to terminate growth at the end of development will determine the body size of the adult. An important aim, therefore, is to identify the genes responsible for targeted growth in multicellular organisms. In *Drosophila*, the size of adult structures derived from imaginal discs is controlled in part by targeted growth to an approximately fixed maximum number of cells, even under conditions permissive of further growth. The targeted growth is a consequence of local cell interactions intrinsic to the growing structures, as in vertebrates (Simpson *et al*., 1980; Bryant & Simpson, 1984; Bryant & Levison, 1985; Bryant & Schmidt, 1990).

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Natural variation in body size in *Drosophila* has a well-documented quantitative genetic basis, with a narrow-sense heritability of between 0·2 and 0·6 in the laboratory (Robertson & Reeve, 1952; Reeve & Robertson, 1953) and of 0·2–0·3 in nature (Coyne & Beecham, 1987; Riska *et al*., 1989; Prout & Barker, 1989). Genes on all major chromosomes contribute to variation in body size, and are predominantly additive in effect (Robertson, 1954; Wilkinson *et al*., 1990; Partridge & Fowler, 1993). Very few genes with a major effect on body size of *Drosophila*, particularly ones that increase it, have been identified (but see Leevers *et al*., 1996). The existence of standing quantitative variation and mutational variance (Santiago *et al*., 1992) for the trait suggests that mutagenesis could be successful. Insertional mutagenesis has been used for this purpose in studies of a classic quantitative trait, bristle number, in *Drosophila* (Mackay *et al*., 1992; Mackay, 1996; Lyman *et al*., 1996), and also of olfactory behaviour (Anholt *et al*., 1996) and metabolic regulation (Clark *et al*., 1995). The method has the advantages that the presence of the mutation can be determined independently of any effect on the phenotype and the mutations of interest are tagged for further analysis. The main aim of the present study was to determine whether *P* element

insertional mutagenesis could be used to induce mutations for body size in *Drosophila*. An additional aim was to determine appropriate methods for mutagenesis of quantitative traits, since detection of mutant effects and their direction for *P* element insertions has not always been straightforward (e.g. Clark *et al*., 1995; Lyman *et al*., 1996).

An important issue in evolutionary genetics concerns the mechanisms by which genetic variation for quantitative characters is maintained in natural populations (e.g. Barton & Turelli, 1989; Keightley & Hill, 1990; Kondrashov & Turelli, 1992; Caballero & Keightley, 1994; Houle *et al*., 1996). Of particular interest are the genetic correlations between quantitative traits, and their relationships to fitness. For instance, if new mutations affect more than one quantitative character, then the level of standing genetic variance for each will be lowered (Turelli, 1985). If new mutations that affect a quantitative trait also have deleterious pleiotropic effects on fitness that are not mediated through the effect on the quantitative trait, then there will be a spurious appearance of stabilizing selection on quantitative traits (Barton, 1990; Keightley & Hill, 1990). In this study, we examined the correlations between the effects of *P* element inserts on body size and on two other quantitative traits – abdominal and sternopleural bristle number – and on the viability component of fitness, determined in a parallel study (Lyman *et al*., 1996).

2. Materials and methods

(i) Drosophila *stocks*

The gene markers and chromosomes used are described in Lindsley & Zimm (1992). All flies were reared on 7 ml Lewis medium in shell vials at 25 °C. The host strain for the inserts was an inbred *Samarkand* (*Sam*) strain into which a third chromosome from a strain unrelated to *Sam* and bearing ry^{506} had been substituted using balancers, to produce *Sam 1*; *Sam 2*; ry^{506} (*Sam* ry^{506}:). The strain contained neither complete nor defective *P* elements, and was an *I* strain with respect to the *I-R* hybrid dysgenesis system. The method of insertional mutagenesis used has been described in detail elsewhere (Lyman *et al*., 1996). New insertions of *P*[*lArB*], which bears a ry^+ marker, were generated on the second and third chromosomes of the inbred *Sam* ry^{506} strain. New inserts in general lower fitness (Mackay, 1985; Partridge *et al*., 1985; Mackay *et al*., 1992; Lyman *et al*., 1996), and the insert-bearing second and third chromosomes were maintained balanced against *SM5* or *TM3*, respectively. Most (91 %) of these insert lines contained a single *P* insertion (Lyman *et al*., 1996). We worked with 64 second chromosome and 46 third chromosome single *P* insert lines. They were scored within 6 months of their production – about 7 or 8 generations.

A control stock against which to measure the effect of the inserts was needed, for two reasons. First, we wished to deduce the direction of any mutant effects. Second, adult body size is sensitive to the environment during growth. A control stock was therefore required to act as a standard, subject to parallel environmental effects. The base stock into which the inserts were made was homozygous for ry^{506}, while the insert stocks were ry^+. Preliminary measurements on the original *Sam* inbred line and on the base stock showed that the former was considerably larger (increase in thorax length on average 0·056 mm in females, 0·04 mm in males), effects comparable in magnitude to the difference in size between the largest and smallest inset lines. The insert lines also on average showed an increase in size of comparable magnitude in relation to the base stock. Both differences may have occurred because the homozygous ry^{506} mutant interferes with larval growth, and the presence of the ry^+ on the inserts at least partially rescued this. The *rosy* marker also decreased mean bristle number and viability (Lyman *et al*., 1996), and the latter property could affect larval density, which is a problem for the standard rearing environment required for work on body size. Lines that had been put through the mutagenesis procedure but that had not received an insert (e.g. Clark *et al*., 1995; Lyman *et al*., 1996) were therefore not suitable controls, and this complicated the detection of mutant effects. A potential method to circumvent the deleterious effects of *rosy* would be to backcross the ry^+ marker into the base stock, and use this stock to give the control mean value for the trait, but the difference in number of copies of the ry^+ gene between control and insert lines could still be a problem.

Ultimately the solution to this problem must rest either on the use of a visible marker system that does not affect body size or viability, or on the use of molecular methods to detect the presence of the *P* inserts in a wild-type genetic background. In the present study, we used the *Sam* inbred line as a standard yardstick for environmental effects on body size during rearing. We could detect mutant effects by comparison of the insert lines with one another, but we could not determine the direction of the mutant effects.

(ii) *Measurement of effects of* P *insertions on thorax length*

Development at lower experimental temperatures increases body size in *Drosophila* (Alpatov, 1930; Anderson, 1966; Partridge *et al*., 1994), while lower

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levels of nutrition and increased larval crowding cause a decrease (Robertson, 1959*a*, *b*; Atkinson, 1979). The environmental influences on the trait mean that conditions during growth must be controlled as precisely as possible if maximum information about genetic effects is to be obtained. To examine the effects of the *P* inserts on thorax length we therefore set up larvae at standard density. Numbers were expanded in the preceding 2 or 3 generations, using low-density culture in food vials. First instar larvae were then collected from each line, and set up at a standard density of 100 larvae per food vial in each of a minimum of 7 and a maximum of 15 vials per line. Survival rates to adulthood were mostly in the range 80–90%. The frequency of wild-type flies present varied greatly between insert lines, but the presence of the balancer heterozygotes prevented these fitness differences from affecting density in the culture vials. Among the eclosing adults, those homozygous for the insert chromosome were retained and frozen at −20 °C. Five females and 5 males from each vial were later scored for thorax length, measured from the base of the anterior humeral bristle to the tip of the pronotum under an M8 Wild stereomicroscope with camera lucida, using a Summa Sketch II digitizing pad connected to a Macintosh SE30 computer. At the same time as we set up the cultures of the insert lines to be measured, we also set up 20 standard density vials of the inbred *Sam* stock in exactly the same way, and analysed the size of the insert lines relative to *Sam*.

(iii) *Correlations with effects of inserts on bristle number and viability*

These same insert lines had been scored in a separate study for their abdominal and sternopleural bristle number and for their viability (Lyman *et al*., 1996). The data from that study were combined with those on thorax length from the present study, to examine the correlations between them.

(iv) *Statistical analyses*

The effect of the *P* insert on thorax length for each insert line was measured in relation to the *Sam* control stock cultured at the same time. For each insert line and for the *Samarkand* control line, the mean scores for the males and females from each culture vial were used to calculate a line mean and 95% confidence limits. Vial means rather than individual fly scores were used because there was significant variation in means between the vials of a single line.

Analysis of variance was used to test formally for significance of the effects of the inserts on thorax lengths, and for sex-specificity. The vial means for each insert line were expressed as a deviation, positive or negative, from the control *Sam* mean. For the second and third chromosomes separately, these deviations were used as the dependent variable in the analysis, with independent variables sex (fixed effect) crossed with line (random effect) and with vials nested within line. Expected mean squares and variance components were calculated, and used to construct synthetic denominators that accommodated the unbalanced design and allowed significance testing. The analysis was done using the JMP Version 3 statistical package for the Macintosh (Chapter 13; SAS Institute, 1994).

3. Results

(i) *Effects of* P *inserts on thorax length*

The deviations from the *Sam* mean of mean thorax length and their 95% confidence limits for each of the insert lines are shown in Fig. 1, each scaled relative to the corresponding *Sam* standard deviation. On average the *Sam* standard deviation was 0·0145 mm for females and 0·0139 mm for males. This scaling was used to produce a conservative estimate of line differences. The largest and smallest deviations from *Sam* were 0·0095 mm and −0·0465 mm, 0·0460 and −0·0873 mm for chromosome 2 insert males and females respectively; 0·0228 mm and −0·1026 mm, 0·0116 mm and −0·1426 mm for chromosome 3 insert males and females respectively. These differences are comparable to the effects of 19 generations of intense artificial selection on the trait in a random-bred population (Partridge & Fowler, 1993). Most insert lines showed smaller thorax length than the control stock, but this cannot be taken to indicate that most new insertions lowered thorax length because the control stock and the base stock differed both in the origin of their third chromosomes and in their complement of *rosy* alleles (see Section 2).

Variance components are shown in Table 1. There was a highly significant effect of insert line for both chromosomes, indicating mutant effects of the inserts on both sexes. For neither chromosome was there a significant main effect of sex ($P < 0{\cdot}05$, tested as a fixed effect in the analysis). This does not indicate that there was no effect of sex on thorax length, but rather that there was no overall tendency for inserts to affect males more than females or vice versa. There was a highly significant interaction between sex and insert line, indicating that individual inserts had significantly sex-specific effects on thorax length, affecting males more in some lines and females more in others.

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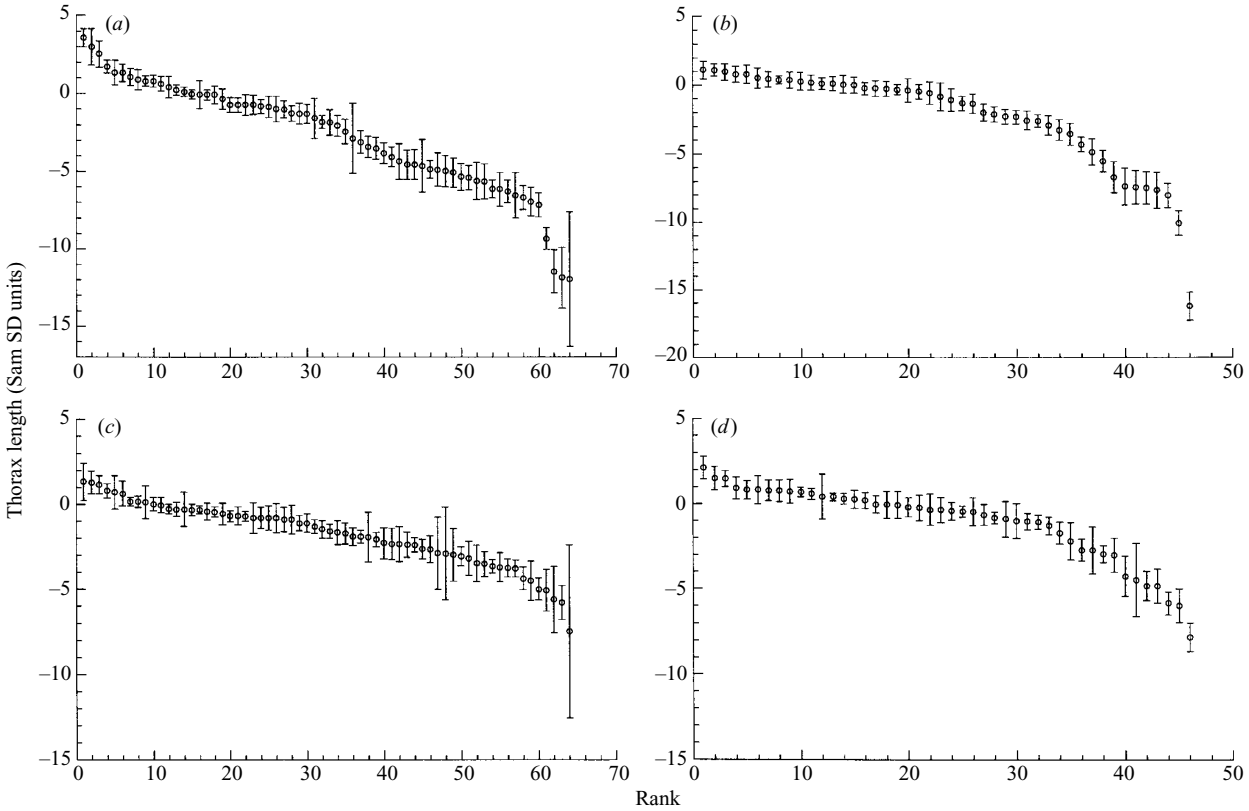

Fig. 1. The means (circles) and 95% confidence intervals (error bars) for the second and third chromosome insert lines ranked by thorax length, scaled by the standard deviation for the *Samarkand* stock (see text). (*a*) Females with an insert on chromosome 2. (*b*) Females with an insert on chromosome 3. (*c*) Males with an insert on chromosome 2. (*d*) Males with an insert on chromosome 3.

Table 1. *Variance components and significance of effects from the analysis of variance on thorax length. See text for further details*

Source of variation	Chromosome 2 d.f		Chromosome 3 d.f.	
Sex	1	Fixed effect (NS)	1	Fixed effect (NS)
Line	63	0·000384**	45	0·000336*
Sex × Line	63	0·000448**	45	0·000860**
Vials	705	0·0000453**	555	0·0000290**

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

(ii) *Correlations of effects of inserts on body size with effects on bristle number and on viability*

Because of unequal variances of the bristle traits and viability for lines with different thorax lengths, Spearman rank correlations were calculated. For inserts on chromosome 2, none of the correlations was significant, and for neither chromosome was the correlation with sternopleural bristle number significant. For chromosome 3 there was significant, although not strong, positive correlation between thorax length and abdominal bristle number ($r_S = 0{\cdot}29$, $P < 0{\cdot}05$) and negative correlation ($r_S = -0{\cdot}31$, $P < 0{\cdot}05$) between thorax length and viability.

4. Discussion

The most important finding was that the *P* insertions caused significant variation between insert lines in thorax length. The direction, and therefore the magnitude, of individual mutant effects could not be deduced, but inspection of confidence limits and the results of the analysis of variance indicated that there were highly significant mutant effects. It will be important in future work to establish whether there are co-ordinate mutant effects on different body parts, and the cellular basis of the change in size. The inserts had strongly correlated mutant effects on the thorax lengths of males and females, and also showed significant sex-specificity, as was found for effects of the inserts on bristle number (Lyman *et al.*, 1996). Standing genetic variance has been reported to induce genetic correlations close to unity between the sexes for various disc-derived morphological traits of adults (Cowley & Atchley, 1988). It would be interesting to

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investigate the mechanisms underlying the sex-specificity of mutational effects found in the present study.

The effects of the *P* inserts on thorax length did not show strong pleiotropic correlations with effects on bristle number; only for chromosome 3 was there a weak negative correlation with abdominal bristle number. The observed genetic correlations between thorax length and the bristle traits were so low that they are unlikely in themselves to constrain the standing genetic variation for these traits. For chromosome 3 inserts there was a weak negative correlation between thorax length and viability. Large adult body size produced by artificial selection is associated with an increase in the duration of pre-adult development and with lowered pre-adult survival (Partridge & Fowler, 1993; Santos *et al*., 1992, 1994), which may explain the correlated effects of the chromosome 3 inserts on thorax length and viability.

P element mutagenesis appears to be a powerful method for inducing mutations in body size, and may in the future help to reveal the mechanisms at work in size-control. For the technique to realize its full potential for polygenic traits with a marked environmental component of variance, and for traits associated with fitness, a marker that does not affect body size or viability is needed. This could be achieved by a suitably validated visible marker in a common genetic background, or by the use of molecular methods to detect the *P* insert in a wild-type genetic background. The direction of mutant effects will then be apparent from comparison with controls. Once loci affecting body size have been identified, it will also become possible to make molecular surveys of genetic variation in natural populations (e.g. Mackay & Langley 1990; Lai *et al*., 1994), and to study how evolution of the trait occurs between different populations of *D. melanogaster* (e.g. Coyne & Beecham, 1987; James *et al*., 1995, 1997) and different *Drosophila* species.

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