P element transposon-induced quantitative genetic variation for inebriation time in *Drosophila melanogaster*

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**Summary.** Bi-directional selection was carried out in co-isogenic stocks with and without mobilised P element transposons to determine whether P elements induce quantitative genetic variation for inebriation time in *Drosophila*. There was significant response to 11 generations of selection in both pairs of replicates of bi-directional selection from an isogenic base stock in which P elements had been mobilised. Conversely, there was no significant response to 11 generations of identical selection in the control lines derived from a relatively inbred line lacking P elements. Thus, P elements have induced quantitative genetic variation for inebriation time.

**Key words:** Transposon mutations – Artificial selection – Ethanol tolerance – Quantitative character – *Drosophila melanogaster*

**Introduction**

Transposons or mobile genetic elements are genetic elements that are capable of autonomous transposition (often replicative) and excision. Transposons were first found in maize by Barbara McClintock in the 1940s (see McClintock 1984), and have subsequently been discovered in bacteria, *Drosophila*, yeast, mice and man. No phylogenetic group has been shown to be free of them, and they are presumed to be ubiquitous.

Transposons show polymorphisms in location and are usually present in multiple copies in dispersed locations. In *Drosophila melanogaster* there are over 30 different families, each with about 30 copies per genome, comprising in all about 10% of the total genome (Rubin 1983; Finnegan 1989).

Transpositions of mobile elements are a major cause of mutations in *Drosophila* and other species. In fact, about 50% of "spontaneous" mutations in *Drosophila* are due to transposon insertions (Rubin 1983; Echalier 1989), and many insertional mutations have been described in maize, yeast and mice.

Mackay (1984) recognised that transposons were likely to cause mutations for quantitative as well as qualitative characters. P elements are among the most easily mobilised and well characterised of *Drosophila* transposons. They can be mobilised in dysgenic crosses between males of stocks containing P elements and females of stocks lacking them (see Engels 1983). Mackay (1984, 1985) reported greater response to bi-directional selection for abdominal bristle number in lines derived from dysgenic crosses (M females × P males) than in lines from non-dysgenic crosses (P females × M males) between the same strains. However, this result was not repeatable for abdominal bristle number in three independent experiments (Torkamanzehi et al. 1988; Torkamanzehi 1990), or for sternopleural bristle number (Pignatelli and Mackay 1989). The experimental approach of hybridising unrelated outbred P and M stocks suffers from three defects, namely that P elements are eventually mobilised in both crosses, that the X chromosome constitutions are not identical in the two crosses, and that responses due to transposon-induced genetic variation must be distinguished from responses from base population polymorphisms and differences between the two stocks.

Unequivocal evidence for generation of novel genetic variation for abdominal bristle number due to P element mobilisation as revealed by selection response has been provided by Torkamanzehi (Moran and Torkamanzehi 1990; Torkamanzehi 1990). They used an inbred M stock
and a co-isogenic transformed P stock derivative to overcome inherent design problems in previous experiments.

It is important to determine whether transposons are capable of inducing quantitative genetic variation that can be utilised to give selection response for other traits. One of the major impediments to quantitative genetics research is the labour involved (see Frankham 1982). Weber (1988a, b, c, d, e, f) has devised a range of automated systems for scoring quantitative characters. Notable among these is the inebriometer that measures the time flies take to become anaesthetised under the influence of ethanol (or other) vapour. Anaesthetised flies can be counted automatically as they fall through an electric eye, and they can be sorted automatically using a fraction collector. Consequently, inebriation time may be ideal for studies on the ability of transposons to induce genetic variation as it offers the potential for carrying out much larger and more sensitive experiments than can be done for bristle number characters. Inebriation time behaves as a typical quantitative character (see Frankham et al. 1988) and has already been used for a range of quantitative genetic studies (Cohan and Graf 1985; Cohan and Hoffmann 1986; Hoffmann and Cohan 1987; Frankham et al. 1988; Weber and Diggins 1990).

The aim of this work was to determine whether mobilised P element transposons are capable of creating quantitative genetic variation for the model quantitative character, inebriation time in *Drosophila melanogaster*. Bi-directional selection was carried out in lines founded from a base population with mobilised P elements (derived from a dysgenic cross between inbred co-isogenic lines, one lacking P elements, and the other containing P elements) and in control lines founded from an isogenic base population lacking P elements. Significant response to selection was obtained in lines with mobilised P elements, but not in lines lacking P elements.

### Materials and methods

#### 1. Base populations

The base population for the control selection lines was the relatively inbred *ry*~506~ M strain supplied by Prof. Margaret Kidwell (Daniels et al. 1987). Daniels et al. (1987) introduced intact P elements and a P element vector marked with *ry*~5~ into this strain by microinjection to yield strain 88-4-1. This latter strain contains approximately 15 intact P elements and approximately 40 degenerate elements per haploid genome.

The transposition positive lines were founded from a dysgenic cross between 88-4-1 and *ry*~506~; while the control lines were founded from the *ry*~506~ strain that lacks P elements. Previous experiments using these same lines and crosses (Moran and Torkamanzehi 1990; Torkamanzehi 1990) confirmed that they had the predicted cytotypes and transposition statuses.

#### 2. Selection experiment

Four treatments were used, namely:

- a) TH. Transposition positive lines (subsequently referred to as transposition lines) selected for increased inebriation time.
- b) TL. Transposition positive lines selected for decreased inebriation time.
- c) CH. Control lines selected for decreased inebriation time.
- d) CL. Control lines selected for increased inebriation time.

Two replicate lines (1, 2) of each treatment were maintained, but one pair of control lines had to be discarded due to contamination.

In all selection lines, the selection intensities for inebriation time were approximately 20%. One hundred virgin females and one hundred virgin males were selected from approximately 1000 flies (females plus males) run each generation. No selection was carried out in generation 3 of line TH1 and in generation 10 of line CL2, due to technical problems in the first case, and low yield of progeny in the second. A generation of no selection was interposed between generations 7 and 8.

Flies were collected as virgins and stored with sexes together at 12°C on papered yeast medium bottles prior to running through the inebriometer. At this temperature, flies raised at normal temperatures will not mate for several weeks. Further, they do not mate while being run through the inebriometer (K. Weber personal communication and unpublished data of R. Frankham), though they subsequently reproduce normally. Thus, selection can be carried out on large virgin populations without the necessity of separating sexes.

The selected character was inebriation time as measured in the inebriometer (Cohan and Graf 1985; Frankham et al. 1988; Weber 1988a). Details of the apparatus and running conditions were similar to those described by Frankham et al. (1988), except that the flies were not pretreated with ethanol vapour.

The ethanol vapour concentration was increased slightly over the course of the experiment by increasing the temperature of the ethanol. This was necessary to avoid problems of increasing phenotypic variances and distributions becoming ragged as the selection lines responded to selection. The temperature of the ethanol was 20.7°C to 21.0°C for generations 0 to 8, 22.4°C for generation 9 and 21.9°C–22.0°C for generations 10 and 11. Runs in generations 6 and 7 had very low means, presumably as a result of ethanol condensation in the apparatus. This problem was solved by fitting a heater stirrer to control the temperature of the water in the water jacket around the fly tube. The temperature in the water jacket was set about 2°C above that of the ethanol to prevent condensation.

Mean inebriation times were based on the mass of flies in the fractions. We have established that mass of flies in the fractions can be used to obtain an accurate measure of means. Selection differentials were recorded in all generations except generation 8. Cumulative selection differentials were obtained by summing selection differentials and scaling them up to account for the missing value.

Flies were raised at 25°C (generations 0–2), 18°C (generation 8) or 22°C (generations 3–7 and 8–11) on PS medium (Frankham et al. 1988) with 20 pairs of parents used per 295 ml bottle.

### Results

Responses to selection, measured as regressions of deviations between pairs of high and low lines on generations (Table 1), were significant for both pairs of transposition lines, but not for the control pair. There was no indica-