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

R. Frankham [1,*], **A. Torkamanzehi** [2] **and C. Moran** [2]

[1] School of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia
[2] Department of Animal Science, University of Sydney, Sydney, NSW 2006, Australia

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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

* To whom correspondence should be addressed

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 (1988 a, 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 is 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^{+} 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 increased inebriation time.

d) CL. Control lines selected for decreased 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 yeasted ordinary 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 arried 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 1988 a). 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° to 21.0 °C for generations 0 to 8, 22.4 °C for generation 9 and 21.9°–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-

Table 1. Responses to selection in the transposition and control lines expressed as regression coefficients of deviations between high and low inebriation time lines on generations

Deviation	b_1	b_0
TH1–TL1	0.282* ±0.114[a]	0.253** ±0.057[a]
TH2–TL2	0.270** ±0.080	0.316** ±0.042
CH2–CL2	−0.001 ±0.047	−0.049 ±0.026

* $P<0.05$; ** $P<0.01$
b_1, Regression coefficient; b_0, regression coefficient for regression through the origin
[a] Standard error

Table 2. Mean inebriation times of lines in generation 11, cumulative selection differentials, and regressions of deviation from control means on generations for each of the transposition lines

Line	Mean inebriation time (min)	S	b_1	b_0
TH1	10.86	46.8	0.266* ±0.089[a]	0.243** ±0.045[a]
TL1	6.40	48.2	0.035 ±0.108	−0.028 ±0.056
TH2	10.63	52.2	0.245* ±0.109	0.286** ±0.056
TL2	6.34	44.6	−0.025 ±0.105	−0.031 ±0.053
CH2	7.37	48.8		
CL2	7.38	36.7		

* $P<0.05$; ** $P<0.01$
S, Cumulative selection differentials; b_1, regression of deviation from control means on generations; b_0, regression coefficient for regression through the origin
[a] Standard error

tion that responses in the transposition lines had plateaued.

Since there was no significant response in the transposition negative control lines, these can be used to assess the symmetry of response in the transposition lines. Means for the lines in the final generation are given in Table 2. Transposition high lines were well above the control lines, while the transposition low lines were slightly below the controls. Regressions of deviation from control line means (Table 2) show significant response in both transposition high lines, but not in the transposition low lines. Cumulative selection differentials (Table 2) were similar in the high and low transposition lines, and in the control lines.

The realized heritabilities for both pairs of transposition lines were 3%, based on 11 times the regressions of deviations between high and low lines on generations, divided by the cumulative selection differentials.

Discussion

The lines containing mobilised P elements gave significant responses to selection, while the co-isogenic controls lacking P elements gave no significant response. Selection differentials were similar in the two treatments so this cannot account for the difference. Clearly, P element transposition has induced quantitative genetic variation for inebriation time in *Drosophila melanogaster*. This induced variation may have been induced when the 88-4-1 stock was made, during the subsequent evolution of this stock, or as a result of our dysgenic crosses.

Our results extend the range of quantitative characters exhibiting P element-induced genetic variation that is utilisable to give selection response from abdominal bristle number (Moran and Torkamanzehi 1990; Torkamanzehi 1990) to include one that is more convenient to study since it can be scored automatically. In addition, chromosomal contamination with P elements has been shown to induce genetic variation for abdominal bristle number (Mackay 1987; Lai and Mackay 1990), sternopleural bristle number (Mackay 1987; Lai and Mackay 1990) and fitness characters (Mukai and Yukuhiro 1983; Simmons et al. 1984a, b; Yukuhiro et al. 1985; Fitzpatrick and Sved 1986; Mackay 1986; Mackay 1987; Eanes et al. 1988).

How great was the induction of genetic variation? The realized heritability for the transposition lines was 3%, based on deviations between high and low lines. This is lower than realized heritabilities for this character in outbred populations of 10%–14% (Frankham et al. 1988) and 22% (Weber and Diggins 1990).

It might be thought that mutations created by P element insertions would be predominantly loss-of-function mutations that would be of minor significance in plant and animal breeding. However, detailed analyses of transposon induced mutations reveals that they result in both loss of function mutations and excess function mutations (Echalier 1989), the latter class presumably resulting from transposon promoters assuming control of adjacent genes. P element-induced mutations on average reduce the mean for fitness characters (Mukai and Yukuhiro 1983; Simmons et al. 1984a, b; Yukuhiro et al. 1985; Fitzpatrick and Sved 1986; Mackay 1986; Eanes et al. 1988). Conversely, response to selection was predominantly in the direction of increased inebriation time in our transposition lines (this cannot be accounted for by differences in selection differentials). It is likely that inactivating mutations would mainly reduce inebriation time since the ability to resist ethanol vapour and to remain in the inebriometer probably involves the possession of functional alcohol metabolising enzymes, a normal cuticle, the ability to close spiracles, normal energy metabolising enzymes, normal wings, etc. The observed asymmetry could be an artifact due to the selection of some sick and damaged flies (zero heritability) in the low lines (in spite of attempts to avoid such flies). However, a previous preliminary selection study with an outbred population yielded essentially symmetrical responses in

the two directions (R. Frankham unpublished), so it seems likely that at least part of the response was due to gain of function mutations. Consequently, transposon induced mutations affecting quantitative characters should be of value for a wide range of characters in plant and animal breeding. Their ultilisation is likely to involve similar difficulties of screening out desirable from undesirable mutations to that involved in utilising mutations induced by irradiation or chemical mutagens.

In conclusion, P element mobilisation has been shown to induce quantitative genetic variation for inebriation time. This variation has been utilised to give selection response, predominantly in the direction of increased inebriation time.

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