Even though only four lines, each homozygous for different *esterase-2* alleles, were tested, significant fitness differences were detected, and there were significant line × treatment (genotype × environment) interactions for pupal viability and per cent survival of emerged flies. In the variable environment of natural populations, fitness differences expressed at the pupal stage may well contribute to a selective milieu that actively maintains polymorphism at the *esterase-2* locus.

Table 1. Mean pupal viability (%) for each line at each temperature treatment.

| 25° C line | Viability* | 35°C line | Viability | Fluctuating line | Viability |
|---|---|---|---|---|---|
| IH13 | 92.4[a] | IT15 | 33.3[a] | IT42 | 13.2[a] |
| IT15 | 88.3[ab] | IH13 | 31.1[a] | IT15 | 7.7[ab] |
| IT42 | 83.3[bc] | IT42 | 29.5[a] | IT46 | 5.5[b] |
| IT46 | 78.8[c] | IT46 | 16.9[b] | IH13 | 4.7[b] |

* Means with the same superscript are not significantly different.

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## *P* element replacement at the *linotte/derailed* locus in *Drosophila*: presence of the wild-type region in the homologous chromosome increases the efficiency.

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**Abstract:** *P* element replacement is now a useful technique in *Drosophila* molecular genetics (Geyer *et al.*, 1988; Gonzy-Tréboul *et al.*, 1995; McCall and Bender, 1996; Moreau-Fauvarque *et al.*, 1998; Sepp and Auld, 1999; Peronnet *et al.*, 2000). This technique will very likely be more and more used in the coming years as many specialized *P* elements are engineered. In this paper the efficiency of the targeted transposition of *PGawB*, a GAL4-bearing enhancer trap *P* element (Brand and Perrimon, 1993) was compared in two different chromosomal situations at the same autosomal locus: with or without the corresponding region in the homologous chromosome. We observe that the presence of the wild-type region in the homologous chromosome increases the efficiency of *P* replacement. We also observe that this efficiency is positively correlated to the extent of homologous sequences between donor and target *P* elements.

**Results**

P *replacement over a deficiency of the region in the homologous chromosome:* The autosomal locus chosen for this *P* replacement is the *linotte/derailed* (*lio/drl*) locus (cytogenic site

37D on chromosome 2L) encoding a putative receptor tyrosine kinase homologous to the human RYK gene product (Dura *et al.*, 1995; Callahan *et al.*, 1995). In the first protocol the target *Ps*, $lio^{exc2}$ and $lio^{exc8}$, are [$w^-$] derivative of the $lio^1$ *PlacW* insertion with intact *P* sequences obtained after transposase action. This step was necessary because *PlacW* and *PGawB* are both marked with the $white^+$ gene. The $lio^1$ allele corresponds to the insertion of a complete *PlacW* element (10.5 kb) at position 459/460 (see Figure 2 in Taillebourg and Dura, 1999), $lio^{exc2}$ being 0.5 kb long and $lio^{exc8}$ 10.5 kb long. In $lio^{exc8}$, it seems therefore that only a very small defect has occured within the *white* minigene of the *PlacW* element. In order to obtain *P* replacement we used the following dysgenic males: $w^{1118}$, *PGawB-760* /Y; $lio^{exc2}$ or $lio^{exc8}$ / *Sp Df(2L)TW130; Sb D2-3/+*, which were crossed with $w^{1118}$ females. *PGawB-760* is an X-linked *PGawB* element (Thomas Préat, personal communication) and *Df(2L)TW130* is a deficiency extending from 37B9-C1 to 38B2-C1.We have recovered 53 $w^+$ autosomal transposition events with $lio^{exc2}$ as target *P* and 33 with $lio^{exc8}$. In each case one single male [$w^+$, $Sp^+$ and $Sb^+$] was crossed with $w^{1118}$ females. These 86 autosomal insertions of *PGawB* were recovered and tested for linkage with the $lio^1$ *PlacW* element. For this, females trans-heterozygous for each of the new *PGawB* insertions and the $lio^1$ *P* element were generated and mated with $w^{1118}$ males. The progeny of the crosses were then screened for [$w^-$] individuals resulting from segregation of the two $w^+$ marked *P* elements which indicate that the *PGawB* insertion tested has not replaced the $lio^{exc2}$ or the $lio^{exc8}$ elements. If no [$w^-$] individual was observed, the *PGawB* strain was considered as a candidate for replacement. The 53 $w^+$ autosomal transposition events with $lio^{exc2}$ as target *P* recombined with $lio^1$ indicating that no replacement has occurred. Six of the 33 autosomal transpositions events with $lio^{exc8}$ as target *P* were kept and molecularly analysed by Southern blots. Two were not inserted into the *linotte/derailed* locus. Two were abortive *P* replacements since no functional GAL4 sequences were present as inferred by their inability to transactivate an UAS-GFP reporter transgene. Finally, 2 were true replacements (see Table 1). This was shown by Southern blot analysis and expression pattern.

Table 1.

| Corresponding region in the homologous chromosome | *Deficiency* | *Deficiency* | + |
|---|---|---|---|
| Target *P* | 0.5kb | 10.5 kb | ≥ 0.4 kb |
| Donor *P* (*PGawB*) | 11.3 kb | 11.3 kb | 11.3 kb |
| Jumps | 53 | 33 | 110 |
| Replacements | 0 | 4 | 19 |
| incomplete | | 2 | 0 |
| true | | 2 | 19 |
| % of useful *P* replacement | 0% | 6% | 17% |

*P replacement over a wild-type region in the homologous chromosome:* In the second protocol the same *linotte/derailed* locus was chosen. The target *P* is a [$w^-$] derivative, obtained after transposase action, of a *P423* element inserted at position 941/942 and called $lio^{P423.24}$ (see Figure 2 in Taillebourg and Dura, 1999). This $lio^{exc22}$ is more than 0.4 kb long with intact *P* sequences. $w^{1118}$, *PGawB-760* /Y; $lio^{exc22}$ / *CyO; Sb D2-3/+* dysgenic males were crossed to $w^{1118}$ females. 110 autosomal insertions of *PGawB* were recovered and tested for linkage with the $lio^1$ *PlacW* element. For this, females trans-heterozygous for each of the new *PGawB* insertions and the $lio^1$ *P* element were generated and mated with $w^{1118}$ males. The progeny of the crosses were then screened for [$w^-$] individuals resulting from segregation of the two

$w^+$ marked *P* elements which indicate that the *PGawB* insertion tested has not replaced the $lio^{P423.24}$ element. If no [$w^-$ ] individual was observed, the *PGawB* strain was considered as a candidate for replacement of the $lio^{P423.24}$. 25 strains showed linkage with $lio^1$ and were tested by PCR with a GAL4 specific primer and two primers flanking the insertion site of $lio^{P423.24}$. Combination of the GAL4 specific primer with each of the flanking primer allows to assay for replacement of $lio^{P423.24}$ by *PGawB* in one orientation or the other. In 6 cases, no amplification was obtained indicating that the *P* replacement did not succeed. In 19 cases, PCR experiments yielded amplification products indicating that GAL4 sequences were present. This was confirmed by expression pattern (see Table 1). Replacements by *PGawB* were obtained either in the same 5' to 3' orientation as $lio^{P423.24}$ (16 cases) or in the other (3 cases).

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# Predominance of two colonizing species of *Drosophila* in Ehime Prefecture, Japan.

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## Introduction

*Drosophila simulans*, subgenus *Sophophora*, is a sibling species closely related to *D. melanogaster* and distributes throughout the world in association with human habitation. This species, however, was absent from Japan before 1972 except in the Ogasawara (Bonin) Islands, 1000 km south of Tokyo (Okada, 1956; Watanabe and Kawanishi, 1976). The sudden colonization of *D. simulans* in the Japanese mainland was recently reported. In 1976, many individuals of *D. simulans* were collected in the southern and central areas of Japan, but this species was not collected in the intervening area (Watanabe and Kawanishi, 1978). Electrophoretic and morphological analyses suggested that these mainland populations of *D. simulans* shared the same origin, but did not derive from the Ogasawara population (Watada *et al.*, 1986a, b). In addition, Watada *et al.* (1986c) showed that *D. simulans* gradually colonized the intervening area and became abundant in or near the large cities of the area.