Pupal heat stress and genetic variation at the *esterase-2* locus of *Drosophila buzzatii*.

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Previous studies of *esterase-2* starch electromorphs in *D. buzzatii* have implicated selection affecting electromorph frequencies, namely: significant association of genotype with environment (Mulley, James, and Barker, 1979; Sokal, Oden, and Barker, 1987), temporal and microgeographic variation within one population (Barker, East, and Weir, 1986), significant differences among genotypes in survival following heat shock (Watt, 1981), significant differences among allozymes in biochemical properties (East, 1982), and perturbation studies of natural populations (Barker, East, and Christiansen, 1989). Temperature effects were indicated in a number of these studies. Drosophila buzzatii breeds and feeds in rotting cladodes (rots) of various species of Opuntia, and temperatures up to 48°C have been recorded in rots exposed to the sun in early afternoon (Dahlgaard and Loeschcke, 1997). At these high temperatures, adults are not present in the rots and larvae have been observed to congregate in shaded parts of the surface of the moist rot. Immobile stages (eggs and pupae) have no such defenses to reduce temperature exposure, so temperature mediated selection may be important at these life stages.

Barker (1994), using sequential gel electrophoresis, identified 25 alleles at the *esterase-2* locus. Lines for a number of these alleles were made homozygous and in the same genetic background by 20 generations of backcrossing to the same stock, i.e., these lines differ genetically only for the *esterase-2* locus and a small region around it. Four of these lines were used here to study the effects on fitness (viability and developmental time) of exposure of pupae to high temperatures: alleles in two lines(IH13 and IT15) are at relatively high frequencies, and in the other two (IT42 and IT46) are at low frequencies in the natural populations from which the alleles were isolated.

Three temperature treatments were used: (i) constant 25°C throughout development, (ii) 25°C, except for constant 35°C for two days during the pupal stage, (iii) 25°C, except for fluctuating 25°C/44.2°C for two days during the pupal stage. In (ii) and (iii), vials with pupae were placed in water baths for the high temperature treatments. For the fluctuating treatment, water bath temperature increased over 15-20 min from 25°C to 44.2°C at noon on each of the two days, and then decreased slowly back to 25°C over about 11-12 hours (Figure 1).

About 100 vials were set up for each line, with 5 pairs parents/vial. IT42 was set up one day later than the other lines, as preliminary experiments had shown time to pupation was about one day less for this line. Parents were discarded after two days, and at midnight on day 7 (day 6 for IT42) a stiff paper strip was inserted into each vial as a pupation site. At 6 am the following day, each strip (with pupae and larvae) was transferred to an empty vial with a moist stopper to maintain humidity. The next morning, strips were moved to a new vial after remaining larvae were removed. Vials for each line were divided into three groups – one returned to 25°C, and the other two to the high temperature treatment water baths at 11 am. The age range of the pupae at the beginning of treatment thus was 0-35 hours. After the two day treatment, these vials were returned to 25°C. From first adult emergence, the numbers and sex of flies emerging from each vial were recorded every eight hours. After emergence ceased, the number of pupal cases in each vial was counted, and pupal viability and developmental time (scored from the time of removal of the paper strip from the medium vial) of

males and females calculated. The numbers of pupae/vial ranged from 5 to 67 (mean = 21), and numbers of replicate vials/line/treatment were 11-16, except for higher numbers set up for IT42 and IT46 at 25°C.

In the high temperature treatments (particularly at 35°C) up to 40% of the flies that eclosed failed to expand their wings and died soon after eclosion. These were counted separately and not included in the viability estimates, but per cent survival of emerged flies was calculated. Data were analyzed using GLM procedure of SAS (SAS Institute, 1989), and means were compared using the Tukey test.

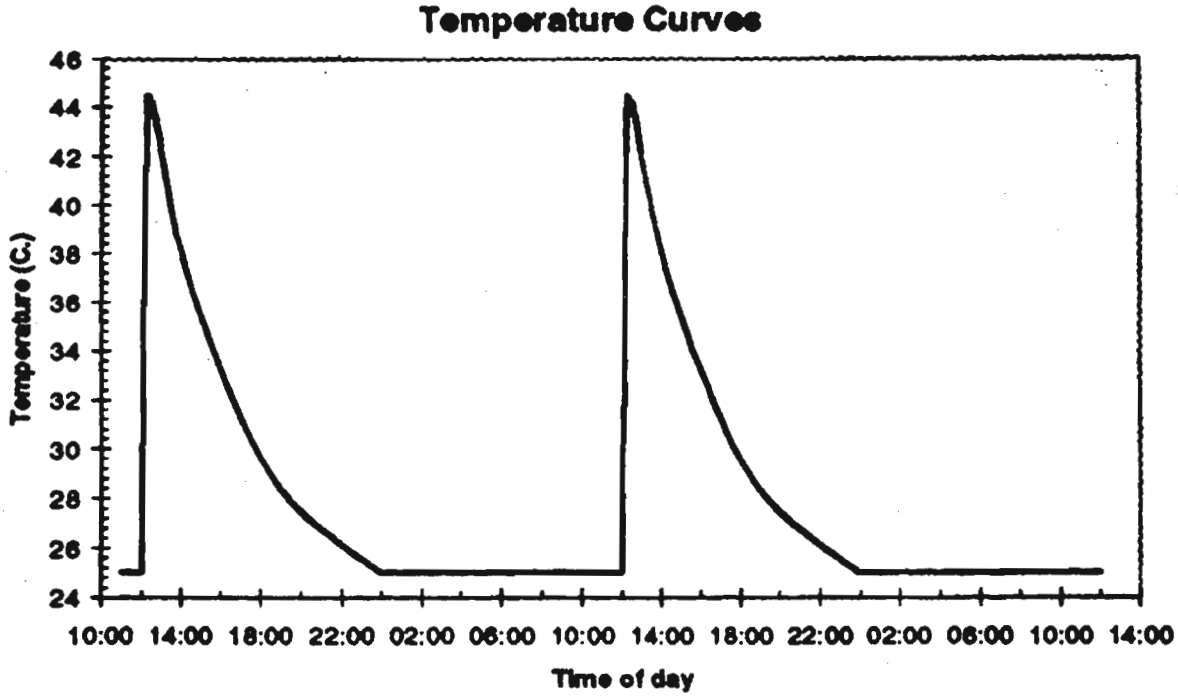

Figure 1. Temperature cycle for the fluctuating temperature treatment.

Pupal viability: Effects of line, treatment, and line × treatment were all highly significant ($P < 0.001$). Treatment means were: 25°C – 83.3, 35°C – 27.2, fluctuating – 7.8. Line means at each temperature (Table 1) show significant changes in the rankings of the lines at different temperatures, with IH13 having highest viability at 25°C and lowest viability in the fluctuating treatment.

Percent survival of emerged flies: Again the main effects were highly significant ($P < 0.001$), while the line × treatment interaction was significant at $P < 0.01$. Treatment means (%) were: 25°C – 99.2, 35°C – 66.5, fluctuating – 87.0, while the only significant line differences were IT42 (77.4) and IH13 (58.6) at 35°C, and IT42 (95.6) and IT46 (76.3) at the fluctuating treatment.

Developmental time: For both males and females, there were significant line ($P < 0.01$) and treatment ($P < 0.001$) effects. For both sexes, developmental time was shorter at 25°C than at the two high temperatures, which were not significantly different. Females had significantly shorter developmental time than males only at 35°C. For both male and female developmental time, IH13 = IT15 > IT42 > IT46.

Both constant 35°C and the fluctuating temperature during the pupal stage imposed substantial stress on flies of all lines – as shown by reduced viability and longer developmental time as compared with constant 25°C.

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