Changes in Relative Fitness With Temperature Among Second Chromosome Arrangements in *Drosophila melanogaster*

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ABSTRACT

Development time and body weight of *In(2L)t*, *R* (a putative short inversion on the left arm of the second chromosome) and *ST* (standard) karyotypes of *Drosophila melanogaster* were measured at different temperatures. Frequency changes were followed in populations polymorphic for *In(2L)t* and *ST* and kept under different environmental conditions. These experiments were carried out in order to explain the worldwide latitudinal clines for *In(2L)t* and other inversions. To avoid interactions with the *Adh* and *αGpdh* loci, which also have latitudinal clines, all karyotypes were homozygous $Adh^{S}\alpha Gpdh^{F}$. *In(2L)t* homokaryotypes had a longer development time and a lower weight than the other karyotypes at all temperatures. *R/ST* heterokaryotypes had the shortest development time and *ST/ST* had the smallest weight decrease with increasing temperature. The differences among the *In(2L)t* and *ST* karyotypes in development time were further analyzed in an experiment where the age at which 50% of the larvae were able to become adults, without further food ingestion, was determined. In polymorphic populations at 20° and 25° a significant decline of *In(2L)t* frequencies was observed. At 29.5° and 33° there was no change in *In(2L)t* frequencies but a significant excess of heterokaryotypes occurred. On ethanol-supplemented food the most drastic decline in *In(2L)t* frequency was observed. Populations transferred at 2- and 3-week intervals at 25° exhibited large differences in final *In(2L)t* frequencies. The frequency changes could in part be attributed to the differences in development time and to previously observed differences in high temperature resistance. The experiments prove that the karyotypes are under selection. The results are discussed in relation to the geographic distribution of *In(2L)t*.

SEVERAL common cosmopolitan inversions in *Drosophila melanogaster* show latitudinal clines in North America, Asia, Australia, Europe and Africa (METTLER, VOELKER and MUKAI 1977; VOELKER, MUKAI and JOHNSON 1977; INOUE and WATANABE 1979, 1980; STALKER 1980; KNIBB, OAKESHOTT and GIBSON 1981; KNIBB 1982; INOUE, WATANABE and WATANABE 1984; ANDERSON, KNIBB and OAKESHOTT 1987). In both the Northern and the Southern hemispheres, inversion frequencies decrease with increasing latitude. Such repeatable geographic associations strongly suggest the action of natural selection. Latitudinal clines have also been observed for several allozyme polymorphisms. Among these polymorphisms, the alcohol dehydrogenase (*Adh*) and the α-glycerophosphate dehydrogenase (*αGpdh*) clines are particularly well-documented: the frequencies of the Adh^{S} and $\alpha Gpdh^{F}$ alleles decline with latitude (JOHNSON and SCHAFFER 1973; ANDERSON 1981; OAKESHOTT *et al.* 1982). The clines for these two polymorphisms have been attributed to the latitudinal cline for the common cosmopolitan inversion *In(2L)t*, because the Adh^{S} and the $\alpha Gpdh^{F}$ alleles are nearly always associated with *In(2L)t* (WATANABE and WATANABE 1977; VOELKER *et al.* 1978; INOUE, TSUNO and WATANABE 1981). An analysis of the data from the eastern United States, Australia and Japan by INOUE *et al.* (1984), *e.g.*, showed significant negative correlation between latitude and the frequencies of both *In(2L)t* and Adh^{S} but not between latitude and Adh^{S} frequency in standard (noninversion) chromosomes. This result suggest that the Adh^{S} cline might be attributed to linkage with *In(2L)t*. However, VOELKER *et al.* (1978), KNIBB (1983) and ANDERSON, KNIBB and OAKESHOTT (1987) found that the *Adh* cline was largely independent of *In(2L)t*. VAN DELDEN and KAMPING (1989) argued that, despite the association of *Adh* and *αGpdh* with *In(2L)t*, selection occurred at both loci.

VAN DELDEN and KAMPING (1989) observed linkage disequilibrium between the *Adh* and *αGpdh* loci due to the presence of *In(2L)t* for a prolonged period in a population kept in a tropical greenhouse. The frequency of *In(2L)t* (and also the frequencies of another, short, inversion on the left arm of the second chromosome) increased in laboratory populations kept at 29.5° and 33°. In populations transferred to lower temperatures, the frequencies of *In(2L)t*, Adh^{S} and $\alpha Gpdh^{F}$ decreased and *In(2L)t* was finally lost. Subsequent tests of egg-to-adult survival at different temperatures proved that at high temperature, survival

of individuals possessing *In(2L)t*, either in the homokaryotypic or the heterokaryotypic state, was higher than that of the standard (*ST*) karyotype of identical allozyme constitution (*i.e.*, $Adh^{S}\alpha Gpdh^{F}$). Thus it seemed that *In(2L)t* had a selective advantage at high temperature. It is feasible that the geographic distribution of *In(2L)t* is correlated with temperature resistance. Because in paracentric inversions no effective recombination occurs in heterokaryotypes, the gene content of such inversions may be highly conserved and blocks of genes can be maintained (ANDERSON 1989). Such gene complexes may be involved in adaptation to particular environmental conditions.

In addition to latitudinal clines for inversions and allozymes, several morphological and life history traits in *D. melanogaster* also have clinal distributions. For traits like body size, body weight, number of bristles, number of ovarioles and egg production, lower values were found in tropical African populations than in Europe (DAVID 1979; DAVID, BOCQUET and DE SCHEEMAEKER-LOUIS 1977; COHET and DAVID 1978). A similar relationship was observed for the American continent and for Australia (LEMEUNIER *et al.* 1986).

Besides differences in temperature resistance among *In(2L)t* and *ST* karyotypes (VAN DELDEN and KAMPING 1989) we noticed differences in other traits that may have profound effects on survival and reproduction under particular environmental conditions. The present study is aimed at the analysis of karyotypic differences in body weight and development time. For this purpose we used karyotypes of identical allozyme composition to prevent interactions with allozyme polymorphisms. Populations, polymorphic for *In(2L)t* and *ST*, were started under a number of environmental conditions, including different temperatures and variable levels of crowding. An attempt is made to relate the results with the geographic distribution of *In(2L)t*.

MATERIALS AND METHODS

Strains: Three homokaryotypic strains, which originated from the K-71 tropical greenhouse population were used (details in VAN DELDEN and KAMPING 1989): *In(2L)t/In(2L)t*, homozygous for *In(2L)t*; *ST/ST*, homozygous for the standard sequence of the left arm of the second chromosome, and *R/R*, homozygous for an unidentified short inversion (provisionally named *R*) of the left arm of the second chromosome. Each strain was composed by intercrossing six lines of the appropriate homokaryotypes, which had been extracted from the greenhouse population as described in VAN DELDEN and KAMPING (1989). All strains were homozygous $Adh^{S}\alpha Gpdh^{F}$. In addition, the three heterokaryotypes *In(2L)t/ST*, *In(2L)t/R* and *R/ST* were used, which were obtained by crossing of the appropriate homokaryotypes. Unless otherwise stated, strains and populations were kept in bottles (125-ml volume with 30 ml food) at 25° and 50–70% relative humidity under uncrowded conditions. Regular medium consisted of 1000 ml water, 18 g agar, 54 g sucrose, 32 g dead yeast and 13 ml Nipagin solution.

Weight and development time measurements: For each of the 6 karyotypes, 5 replicate vials with 50 eggs each were set at 4 temperatures: 20°, 25°, 29.5° and 33°. The numbers of pupae were determined and the numbers of emerging flies were counted at 3-hr intervals at 25°, 29.5° and 33° and at 8-hr intervals at 20°. For each karyotype and temperature, 5 replicate samples of 10 males were weighed. Weights were analyzed with Tukey's multiple comparison tests, after ANOVA. Median development times were determined with a probit regression analysis (FINNEY 1947) as this method took care of occasional very early and very late emerging individuals; 95% confidence limits were determined following the method of WARDLAW (1985).

Determination of time needed to reach critical weights: To determine the time at which critical weights for pupation and emergence were reached, *In(2L)t/In(2L)t*, *ST/ST* and *In(2L)t/ST* larvae were collected within 1 hr after hatching. The larvae were transferred to Petri dishes (200 larvae per dish) containing 40 ml regular food with 100 mg/liter streptomycin which were placed at 20°, 25°, 29.5° and 33°. Samples of larvae were transferred to vials containing only agar medium (18 g/liter) after a stay of variable length on regular food (for transfer times see Figure 1). The number of replicate vials, each with 20 larvae, per transfer time was 5 or 10. The numbers of pupae and flies were recorded. The median larval age of transfer at which 50% of the larvae were able to emerge as adults (MTT) was calculated by means of a probit regression analysis (FINNEY 1947); 95% confidence limits were determined following the method of WARDLAW (1985). For particular larval transfer times, five replicate samples consisting of five emerged flies were weighed.

Experimental populations: Experimental populations, polymorphic for *In(2L)t* and *ST*, were started with heterokaryotypes in 7 different environments, thus the initial frequency of *In(2L)t* was 0.50. In each environment 2 replicate populations were started, each consisting of 3 bottles. In each generation the newly emerged flies from the 3 bottles forming a replicate population were thoroughly mixed and thereupon the next generation was started with 3 bottles each with 80 pairs of flies. Four populations were kept at 20°, 25°, 29.5° and 33° respectively. In these populations larval crowding was kept moderate by removing the parents after approximately 300 eggs per bottle had been laid. Generation intervals were 14 days for the 25° and 29.5° populations and 21 days for the 20° and 33° populations. In the latter population, however, all flies emerged at 33° within 14 days after founding were first placed at 25° for 1 week in order to restore male fertility and then transferred to 33°. Three additional populations (HD14, HD21 and Eth) were kept at 25° but under conditions different from those experienced by the previously mentioned 25° control population. Egg-laying time was here 6 days which gave much greater numbers of eggs and high larval densities in populations HD14 and HD21 (both kept on regular food), which differed in generation intervals: 14 and 21 days, respectively. The Eth population was kept on food supplemented with ethanol (12% by volume). Despite the egg-laying period of 6 days, larval densities were moderate; generation interval was 21 days.

Frequencies of *In(2L)t* were determined in generations 6, 12 and in generation 18 (20°, 33°, HD21 and Eth populations) or 24 (25°, 29.5° and HD14 populations). Per replicate population, *In(2L)t* frequencies were determined in 80 males prior to the transfer to new bottles. Males were individually crossed with *dumpy black* females (*dp*: II, 13.0; *b*: II, 48.5); single F_1 females were backcrossed to the *dp b* strain (VAN DELDEN and KAMPING 1989). The F_2 offspring

TABLE 1

Median development times (hr) of six karyotypes at different temperatures under optimal food conditions

Temperature (°C)	Karyotypes		
	ST/ST	*ST/In(2L)t*	*In(2L)t/In(2L)t*
20	366.2^{a} (363.4–369.0)	377.1^{b} (374.9–379.4)	391.8^{c} (389.6–393.9)
25	219.7^{a} (218.0–221.4)	218.6^{a}* (217.1–220.1)	233.3^{b} (232.1–234.5)
29.5	192.8^{a} (190.8–194.7)	191.0^{a}* (189.8–192.1)	196.9^{b} (195.3–198.5)
33	188.5^{a} (186.7–190.3)	190.2^{a}* (188.9–191.4)	213.8^{b} (211.6–215.9)
	ST/ST	*ST/R*	*R/R*
20	366.2^{ab} (363.4–369.0)	364.6^{a}* (362.6–366.6)	369.6^{b} (366.1–373.1)
25	219.7^{b} (218.0–221.4)	211.5^{a}* (209.6–213.5)	223.4^{c} (221.6–225.1)
29.5	192.8^{b} (190.8–194.7)	185.9^{a}* (184.7–187.0)	191.1^{b} (189.5–192.7)
33	188.5^{b} (186.7–190.3)	183.8^{a}* (182.0–185.6)	192.9^{c} (191.1–194.8)
	In(2L)t/In(2L)t	*In(2L)t/R*	*R/R*
20	391.8^{c} (389.6–393.9)	379.3^{b} (377.4–381.3)	369.6^{a} (366.1–373.1)
25	233.3^{b} (232.1–234.5)	221.7^{a}* (220.3–223.1)	223.4^{a} (221.6–225.1)
29.5	196.9^{b} (195.3–198.5)	190.0^{a}* (188.8–191.1)	191.1^{a} (189.5–192.7)
33	213.8^{c} (211.6–215.9)	190.6^{a}* (189.3–191.9)	192.9^{b} (191.1–194.8)

The ninety-five percent confidence limits are given in parentheses. Median development times within each row which share a common superscript letter are not significantly different at the 5% level; * indicates heterokaryotypes significantly different from midparent values.

were checked for recombinants. In the case that no recombinants were found, the extracted parental chromosome was classified as *In(2L)t*; when regular recombination fractions were found, the extracted chromosome had the *ST* sequence. In generation 6 only one F_1 female per parental male was backcrossed, in generations 12, 18 and 24 the F_2 offspring of 2 F_1 females per parental male were assayed. In that case karyotype frequencies could also be estimated, based on the expected ratio of $(p^2 + \frac{1}{2}pq) : pq : (q^2 + \frac{1}{2}pq)$, where p stands for the frequency of *ST* and q for the frequency of *In(2L)t*. The procedure differed for the 33° population where 10 F_1 females per parental male were backcrossed (in generation 6 also), so both second chromosomes of the parental male, could be identified and karyotype frequencies among the original sample of males could be determined.

Selection coefficients (s) for the 20°, 25°, HD14, HD21 and Eth populations were calculated from the changes in *In(2L)t* frequencies (q) over number of generations (t) by means of the expression:

$$s = \frac{1}{t}\left[\frac{q_0 - q_t}{q_0 q_t} + \ln\frac{q_0(1 - q_t)}{q_t(1 - q_0)}\right]$$

assuming selection against the *In(2L)t* homokaryotype only.

RESULTS

Development time and body weight: Development times are given in Table 1. ANOVAs (not presented) showed significant karyotype, temperature and interaction effects both for the whole data set and for each of the three blocks of a particular heterokaryotype with its corresponding homokaryotypes. Degree of dominance varied with karyotype combination and with temperature. Development time of *In(2L)t/In(2L)t* was longer than that of *In(2L)t/ST* and *ST/ST* at all temperatures. *In(2L)t/ST* and *ST/ST* had equal development times, except for 20° where *ST/ST* developed faster than *In(2L)t/ST*. Remarkable is that at 33°, *In(2L)t/In(2L)t* had a significant longer development time than at 29.5°. At 33° the difference in development time between *In(2L)t/In(2L)t* and both *In(2L)t/ST* and *ST/ST* was about 24 hr; at this temperature the relatively greatest difference between these karyotypes was observed. *R/ST* had the shortest development time of all karyotypes at all temperatures.

TABLE 2

Mean weights (mg) of groups of ten males (standard errors in parentheses)

Temperature (°C)	Karyotypes		
	ST/ST	*ST/In(2L)t*	*In(2L)t/In(2L)t*
20	9.40^{b} (0.04)	9.76^{c}* (0.04)	9.20^{a} (0.04)
25	8.44^{b} (0.05)	8.72^{c}* (0.07)	8.14^{a} (0.07)
29.5	8.08^{b} (0.04)	8.16^{b}* (0.05)	7.64^{a} (0.05)
33	7.46^{c} (0.04)	6.96^{b} (0.04)	6.42^{a} (0.04)
	ST/ST	*ST/R*	*R/R*
20	9.40^{a} (0.04)	9.56^{a} (0.05)	9.48^{a} (0.05)
25	8.44^{a} (0.05)	8.36^{a} (0.05)	8.56^{a} (0.13)
29.5	8.08^{b} (0.04)	8.12^{b}* (0.06)	7.82^{a} (0.05)
33	7.46^{b} (0.04)	7.36^{b}* (0.07)	6.66^{a} (0.04)
	In(2L)t/In (2L)t	*In(2L)t/R*	*R/R*
20	9.20^{a} (0.04)	9.68^{c}* (0.04)	9.48^{b} (0.05)
25	8.14^{a} (0.07)	8.44^{b} (0.09)	8.56^{b} (0.13)
29.5	7.64^{a} (0.05)	8.08^{b}* (0.07)	7.82^{a} (0.05)
33	6.42^{a} (0.05)	6.66^{b}* (0.04)	6.66^{b} (0.04)

Means within each column which share a common superscript letter are not significantly different at the 5% level (Tukey's test for multiple comparisons, after ANOVA); * indicates heterokaryotypes significantly different from midparent values.

Mean body weights are given in Table 2. ANOVAs (not presented) showed significant karyotype, temperature and interaction effects. Degree of dominance varied with karyotype combination and with temper-

ature. Body weights decreased with increasing temperature in all karyotypes. *ST/ST* had the smallest weight loss (21%) and *In(2L)t/ST* the greatest (31%) when the weights at 33° and 20° were compared. *In(2L)t/In(2L)t* had consistently the lowest weight at all temperatures, but for the other karyotypes the weight order varied with temperature. The weight of *R/R*, *e.g.*, was low at the highest temperatures, but intermediate at the lower temperatures. Heterokaryotypes could be either heavier than the corresponding homokaryotypes (like *e.g.*, *In(2L)t/ST* at 20° and 25°), intermediate to both karyotypes (like *In(2L)t/ST* at 33°) or equal to both homokaryotypes (like *R/ST* at 25°).

Time needed to reach critical weight: A further analysis of the differences in development time among the *In(2L)t/In(2L)t*, *In(2L)t/ST* and *ST/ST* karyotypes was performed in a separate experiment where the larvae were tested for the time needed to reach their critical weight. Drosophila larvae have to acquire a certain minimum weight before pupation can begin (BAKKER 1961, 1969; ROBERTSON 1963). In this experiment groups of larvae were first kept on regular food for variable periods of time and then transferred to agar medium. When the transfer occurred too early, the larvae had not been able to reach their critical weights and would fail to pupate and become adults. By varying the duration of the stay on regular food, the time needed for attaining critical weight could be determined for the three karyotypes tested. The results are shown in Figure 1, while the MTTs are given in Table 3. At 20° and 25°, *In(2L)t/ST* had the lowest MTT and *In(2L)t/In(2L)t* the highest, while *ST/ST* was about intermediate. At 25° the difference among *In(2L)t/ST* and *In(2L)t/In(2L)t* was about 6 hr, at 20° about 15 hr. At 29.5° and 33°, however, there was no difference between *In(2L)t/ST* and *ST/ST* while the MTT of *In(2L)t/In(2L)t* was longer. At the latest transfer time at 33° (60 hr) only 1% of the *In(2L)t/In(2L)t* larvae were able to pupate in contrast with 95% of the *In(2L)t/ST* and *ST/ST* larvae. Apparently considerable genotype-environment interactions occur for MTT. At the earliest times of transfer (68–88 hr at 20°, 42–54 hr at 25°), the fraction of eggs that became pupae was much higher than the fraction that ultimately gave rise to flies (data not presented), thus considerable pupal mortality occurred. Pupal mortality was as high as 80% at the earliest transfer times and decreased until 5% or less at the latest times of transfer. Pupal mortality of *In(2L)t/ST* was considerably lower than that of *ST/ST* at comparable transfer times.

Weights were determined for males originated from larvae which had been transferred to agar after they had stayed for periods of variable length on regular food (Table 4). The most complete data set was that

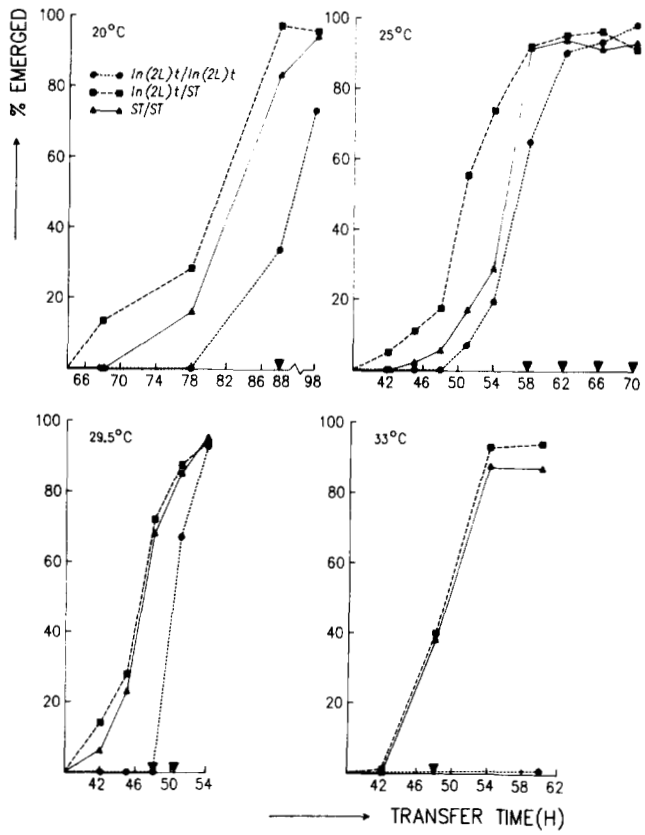

FIGURE 1.—The relation between larva-adult survival of *In(2L)t/In(2L)t*, *In(2L)t/ST* and *ST/ST* and the time of transfer from regular to agar medium at 20°, 25°, 29.5° and 33°. Weights of emerging adults were determined in the transfer time groups indicated by triangles on the *x*-axis.

for 25°. Except for the earliest transfer time (where *In(2L)t/ST* exceeded *ST/ST* in weight), *In(2L)t/ST* and *ST/ST* were equal in weight and exceeded *In(2L)t/In(2L)t*. An identical tendency was observed for the other temperatures where the data were limited to one or two transfer times. A few representative weight measurements of females with respect to karyotypic differences (not shown) gave results identical to those for males.

The results of this experiment were partially in agreement with the previous one in which the larvae could complete their development under optimal conditions on regular food. In both experiments *In(2L)t/In(2L)t* had the longest development time and the lowest weight at all temperatures. The differences in development time between *In(2L)t/ST* and *ST/ST* observed at 20° and 25° in the MTT experiment, however, were not present under optimal conditions. In both experiments *In(2L)t/In(2L)t* combined slower development with lower adult body weight. The slower development of *In(2L)t/In(2L)t* might be brought about by a slower attainment of the critical weight for pupation, *e.g.*, because of less efficient feeding or metabolism (ROBERTSON 1963). The difference between *ST/ST* and *In(2L)t/ST* in reaching

TABLE 3

Median age of transfer (hr): the age at which 50% of the larvae were able to become adults without further food ingestion (95% confidence intervals in parentheses)

Karyotype	Temperature (°C)			
	20	25	29.5	33
In(2L)t/In(2L)t	92.9 (91.9–93.8)	57.1 (56.4–57.7)	51.8 (51.3–52.3)	>62
In(2L)t/ST	77.6 (76.7–78.6)	50.7 (50.0–51.4)	46.3 (45.7–46.9)	50.4 (49.6–51.2)
ST/ST	85.8 (84.7–87.0)	54.1 (53.5–54.6)	47.0 (46.5–47.6)	50.9 (50.2–51.7)

TABLE 4

Mean weights (mg) of groups of five males which had been transferred as larvae from regular food to agar food at various transfer times (standard errors in parentheses)

Temperature (°C)	Transfer time (hr)	Karyotype		
		In(2L)t/In(2L)t	*In(2L)t/ST*	*ST/ST*
20	88	1.83 (0.07)[c]	3.52 (0.07)[a]	2.14 (0.13)[b]
25	58	1.75 (0.03)[b]	2.43 (0.07)[a]	1.83 (0.09)[b]
	62	2.25 (0.04)[b]	2.53 (0.08)[a]	2.45 (0.05)[a]
	66	2.34 (0.04)[b]	2.95 (0.05)[a]	2.89 (0.05)[a]
	70	2.56 (0.03)[b]	3.02 (0.06)[a]	2.98 (0.06)[a]
29.5	48	—†	1.78 (0.10)[a]	1.74 (0.02)[a]
	51	1.40 (0.03)[b]	2.00 (0.07)[a]	2.02 (0.12)[a]
33	48	—†	1.62 (0.04)[a]	1.65 (0.05)[a]

Means within each row which share a common superscript letter are not significantly different at the 5% level (Tukey's test for multiple comparisons).

† Still no flies emerged.

their critical weights was apparently temperature dependent and occurred only at the lower test temperatures.

***In(2L)t* frequency changes in experimental populations:** There were considerable differences in the magnitude of the changes of *In(2L)t* among the populations kept under different conditions (Figure 2). In comparing the four temperature regimes it was found that at 29.5° and 33° there was no decrease in inversion frequencies after 24 and 18 generations, respectively. The frequency of *In(2L)t* was still around 0.50. In all generations where inversion frequencies were assayed in the 29.5° and 33° populations a significant excess of heterokaryotypes was observed, this was more pronounced at 33° than at 29.5°. At the lower temperatures a significant decline of *In(2L)t* frequencies with time was observed, the most prominent decline was found at 20° where the inversion frequency was 0.12 in generation 18. No heterokaryotype excess was found at 20° and 25°.

Among the populations kept at 25° considerable differences occurred among the various regimes. The populations kept at high density which were transferred at 3-week intervals hardly showed changes in inversion frequency. The high density populations transferred at 2-week intervals on the other hand, exhibited a sharp decline in inversion frequency,

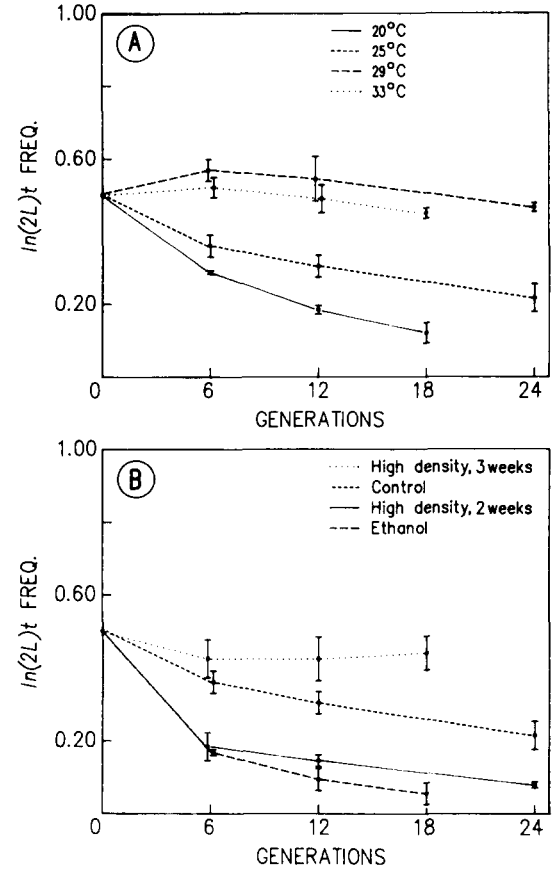

FIGURE 2.—Changes in mean *In(2L)t* frequencies in polymorphic populations kept under different environmental conditions (two replicate populations per environment). A, frequency changes at four temperatures; B, frequency changes at 25° in populations kept on ethanol supplemented food and kept under high larval density conditions with transfer times of 14 days or 21 days. The control population is the 25° population from the A group. Vertical lines indicate standard deviations.

which was more pronounced than the decline in the control 25° population. The population kept on ethanol-supplemented food had the lowest *In(2L)t* frequency: 0.07 after 18 generations.

DISCUSSION

In a previous study (VAN DELDEN and KAMPING 1989) considerable differences in egg-to-adult survival among *In(2L)t*, *R* and *ST* karyotypes were observed at 29.5° and 33°(but not 20° and 25°). In the present study differences in development time as well as in

body weight were found. Under optimal food conditions (excess of food, no larval crowding) the *In(2L)t* homokaryotype is consistently slower at all test temperatures, while *ST/ST* and *In(2L)t/ST* are equal in development time except for development at 20° where *ST/ST* is slightly faster. Karyotypes involving the *R* chromosome also differ from each other and from the *In(2L)t* and *ST* karyotypes. The *R/ST* heterokaryotype, *e.g.*, has the fastest development at all temperatures.

A further analysis of development time for the three karyotypes involving *In(2L)t* and *ST*, by means of determination of the time after which further development can be completed without further food ingestion, reveals a typical difference with the determinations under optimal conditions. In the former case, the time needed for 50% of the larvae to develop into adults at 20° and 25° is shorter for *In(2L)t/ST* than for *ST/ST*, while *In(2L)t* homokaryotypes are invariably slower than the other karyotypes in reaching this point. It further appears that *In(2L)t/In(2L)t* has the lowest weight at all temperatures in both tests where weight and development time were determined. Under unlimited food conditions there is karyotype-temperature interaction: at low temperatures *e.g.*, *In(2L)t/ST* is heavier than *ST/ST*, while at 33° the opposite is true. Also in the transfer experiment *In(2L)t/In(2L)t* has a consistent lower weight, but the difference in weight between *In(2L)t/ST* and *ST/ST* exists only at the earliest transfer times. It thus appears that *In(2L)t/ST* is able, at least at the lower temperatures, to ingest and/or to exploit the food in a more efficient way than *ST/ST* and *In(2L)t/In(2L)t*, while the latter karyotype is inferior to both other karyotypes in this respect. It is not clear why the apparent advantage of *In(2L)t/ST* at the lower temperatures in attaining more rapidly the critical weight for pupation compared to *ST/ST* (a lower critical weight for *In(2L)t/ST* is not likely in view of its higher weight) is not associated with faster development under optimal conditions. It could be that *In(2L)t/ST* has a relatively extended pupal phase, which compensates for the shorter larval phase. It may well be that *In(2L)t/In(2L)t*, in addition to a slower larval development, also has a prolonged pupal stage.

That both survival at high temperature and development time are important fitness components and may lead to drastic changes in inversion frequencies is shown by the outcome of the experiment where populations were kept in different environments and *In(2L)t* frequencies were determined.

A strong disadvantage of *In(2L)t* is observed at lower temperatures, while at 29.5° and 33° no frequency changes occur. A likely explanation for the decline at 20° and 25° is the slower development time of *In(2L)t/In(2L)t* homokaryotypes, compared to *ST/ST*. In the population system with discrete generations the slower developing karyotype is at a relative disadvantage. Our data confirm those of INOUE (1979) and INOUE, WATANABE and WATANABE (1984) who observed a rapid decline and final elimination of *In(2L)t* and other inversions in experimental cage populations at 25°. Our conclusions about the role of development time in the elimination of *In(2L)t* are confirmed by the difference in reaction between the HD14 and HD24 populations. The disadvantage of slower development will be more pronounced under high larval crowding conditions as is demonstrated by a more rapid decrease of *In(2L)t* in the HD14 population compared with the 25° control population with moderate larval crowding. The HD21 population on the contrary does not show a decrease in *In(2L)t* frequency. VAN DELDEN and KAMPING (1979) found that, under conditions as applied here, only 53% of the flies had emerged after 2 weeks, which will lead to overrepresentation of the faster developing genotype in a 2-week scheme compared to a 3-week scheme.

The absence of a decline in *In(2L)t* frequencies at 29.5° and 33° can be attributed to higher survival of inversion karyotypes compensating for the handicap of slower development. Temperature dependent differences in survival among second chromosome karyotypes have been demonstrated by VAN DELDEN and KAMPING (1989). They found no differences in egg-to-adult survival at 20° and 25°, but at 29.5° survival rates were 0.70, 0.82 and 0.64 for *In(2L)t/In(2L)t*, *In(2L)t/ST* and *ST/ST*, respectively (*ST/ST* and *In(2L)t/ST* being significantly different) while at 33° the survival rates were 0.52, 0.68 and 0.28, respectively (egg-to-adult survival of *ST/ST* was significantly lower than of *In(2L)t/In(2L)t* and *In(2L)t/ST*). Also in this case all karyotypes were homozygous for $Adh^{S}\alpha Gpdh^{F}$. These survival rates indicate heterokaryotype advantage leading to stable equilibrium frequencies for *In(2L)t* of 0.59 and 0.71 at 29.5° and 33°, respectively. The *In(2L)t* frequency in the 29.5° and 33° populations, however, stayed at about 0.50. This may indicate that, though high temperature resistance is an important adaptive trait, differences in other fitness components also occur.

Evidence for the selective advantage of *In(2L)t* at high temperature was previously given by VAN DELDEN and KAMPING (1989) who found that in laboratory populations polymorphic for the chromosome arrangements *In(2L)t*, *R* and *ST*, as well as for the allozyme loci *Adh* and *αGpdh*, the initial *In(2L)t* frequency increased from 0.06 to 0.25 and 0.37 at 29.5° and 33°, respectively. In the present experiment the effects of temperature could be studied without the potentially disturbing effects of the presence of other inversions or variation for the two allozymes. Seasonal

TABLE 5

Selection coefficients calculated from the changes in *In(2L)t* frequencies over generations in five populations, assuming a selective disadvantage for *In(2L)t/In(2L)t* only (see text)

Generation interval	Population				
	20°	25°	HD14	HD21	Eth
0–6	0.146	0.232	0.797	0.112	0.763
6–12	0.429	0.148	0.228	0.016	0.722
12–18	0.556			0.000	1.000
12–24		0.144	0.498		
Average	0.467	0.175	0.508	0.043	0.828

fluctuations in natural populations, with highest *In(2L)t* frequencies in the warm seasons, were found by STALKER (1980) and SANCHEZ-REFUSTA, SANTIAGO and RUBIO (1990), which confirms the influence of temperature (or temperature related effects) on inversion frequencies.

It further turns out that *In(2L)t* is highly sensitive to ethanol in view of the more rapid decline in the Eth population, compared to the control 25° population. In populations polymorphic for *Adh* the decline in *In(2L)t* frequency could be ascribed to the association in *In(2L)t* with Adh^S, but in the present case *Adh* was not variable and the effect seems to be caused by a greater susceptibility of *In(2L)t* compared to *ST*. One reason for this could be the lower body weight of *In(2L)t* homokaryotypes as it has been shown that increased tolerance to alcohols is often accompanied by greater body weight (VAN DELDEN and KAMPING 1983).

Table 5 shows calculated selection coefficients for the indicated generation intervals. When curves were drawn for the decrease in inversion frequencies, using the average selection coefficients from Table 5, a reasonable good fit with the observed points was obtained (departures of 0.06 or less in frequency). The assumption that selection is acting exclusively against *In(2L)t/In(2L)t* is based on the observed differences in development time and will hold predominantly for the 20°, 25°, HD14 populations. It is not excluded, however, that the fitness of the heterokaryotype is also reduced in some circumstances. At 20°, *e.g.*, its development time was slower than that of *ST/ST*. The selection coefficients presented here are therefore merely indicators of the differential selective forces acting on karyotypes. Differences in other fitness components, among the karyotypes, appear to be present (W. VAN DELDEN and A. KAMPING, manuscript in preparation) which will certainly influence karyotype frequency changes under particular environmental conditions.

With respect to the geographical distribution of *In(2L)t*, our data are consonant with the universal finding of a decrease in frequency with latitude, a clinal distribution pattern shared with other inversions (METTLER, VOELKER and MUKAI 1977; VOELKER *et al.* 1978; INOUE and WATANABE 1980; STALKER 1980; KNIBB, OAKESHOTT and GIBSON 1981; KNIBB 1982; INOUE, WATANABE and WATANABE 1984; ANDERSON, KNIBB and OAKESHOTT 1987). The high frequencies of *In(2L)t* in tropical regions (up to 0.60) are probably equilibrium frequencies due to overdominance of the heterokaryotype. The reason for the low frequencies of inversions at higher latitudes (less than 1% at 50° latitude N in Europe, *e.g.*) may be superior resistance to low temperatures of *ST* chromosome arrangements. Differences among the karyotypes in body weight and development time as well as in other traits are probably also involved. In this respect it is particularly relevant that body size and associated characters are positively correlated with latitude [see LEMEUNIER *et al.* (1986) for a review], while we find that *In(2L)t/In(2L)t* has a consistently lower body weight. The meaning of this finding is not clear, but it is undoubtedly connected with the differences among *D. melanogaster* populations living in tropical and cool temperature regions. The former kind of populations, *e.g.*, will probably count considerable numbers of flies throughout the years, while in cool regions cycles of reduction and recolonizing will occur. This may lead to distinguished positions in the r-K continuum (PARSONS 1983). Further research is aimed at clarifying this point by an extensive analysis of life history traits.

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