Heredity **83** (1999) 460–468 *Received 21 January 1999, accepted 24 June 1999*

The role of fertility restoration in the maintenance of the inversion *In(2L)t* polymorphism in *Drosophila melanogaster*

ALBERT KAMPING* & WILKE VAN DELDEN

Department of Genetics, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

In order to explain the worldwide latitudinal distribution and seasonal fluctuations in *In(2L)t* frequencies in *Drosophila melanogaster*, fitness differences among *In(2L)t* and *Standard* (*ST*) homo- and heterokaryotypes under high-temperature conditions were determined. Viabilities were measured for high-temperature treatment started at different juvenile stages. The capacity to restore fertility after high-temperature treatment was measured for adults and juveniles. Furthermore, genetic adaptation for increased temperature resistance for these traits was determined for strains which were reared at 33°C for 10 generations. Whereas larva–pupa survival rates were high, highest juvenile mortalities and strongest karyotypic effects were observed during the pupal stage when preceding larval stages were reared at 33°C. *ST* karyotypes showed lowest viabilities. Although mating rate was hardly influenced, sterility was induced for females and males after high-temperature treatment of adults as well as juveniles. Subsequent transfer to 25°C, however, resulted in restored fertility in some of the individuals, depending on the length of the recovery period. Fertility restoration was significantly higher for heterokaryotype males and females. Heterokaryotype superiority for restored fertility as well as for viability was positively correlated with severity of the treatment. Ten generations of selection at 33°C resulted in significant improvement of juvenile survival and fertility restoration for all karyotypes. These fitness components were positively correlated ($r = 0.91$; $P < 0.001$), which might suggest pleiotropic effects. It is concluded that the capacity to restore fertility after heat stress is an important fitness component, especially with respect to the *In(2L)t* polymorphism. The observed heterokaryotypic superiority fits with the idea that the latitudinal distribution of *In(2L)t* frequencies is maintained by balancing selection, with equilibrium values decreasing with latitude.

Keywords: *Drosophila melanogaster*, fertility restoration, genetic adaptation, heat stress, heterokaryotype superiority, inversion *In(2L)t*.

Introduction

Natural habitats of the cosmopolitan species *Drosophila melanogaster* are highly variable with respect to environmental temperature. Extreme temperatures will have direct effects upon behaviour and physiological processes (Prosser, 1986), and will influence fitness components and fitness-related characters like egg production, viability, development time, body weight and longevity (David, 1988; Van Delden & Kamping, 1989, 1991; Van't Land, 1997). Higher environmental temperature is generally accompanied by increased metabolism, faster development, lower body weight and shorter life span. In addition to phenotypic responses related to temperature, genetic adaptation with respect to these traits has been observed at particular temperatures in the laboratory (Huey *et al*., 1991; Partridge *et al*., 1994). Differences in the ability to respond to high temperature may depend on the overall level of genetic variation (e.g. Dahlgaard & Loeschcke, 1997), on genes involved in stress response (e.g. McColl *et al*., 1996) and on specific genes dealing with heat sensitivity (Morrison & Milkman, 1978; Oudman, 1991).

Exposure to high temperature will decrease reproductive activity and will induce complete or partial sterility of both females and males (Cohet & David, 1978; Krebs & Loeschcke, 1994) depending on genetic constitution (e.g. level of inbreeding), temperature and exposure time. Because transcription of many genes is developmentally regulated, particular sensitive periods may occur, and effects of temperature treatment may differ among life stages. When high-temperature damage is restricted,

*Correspondence. E-mail: kampinga@biol.rug.nl

transfer to normal temperatures may result in restoration of fertility (Cohet & David, 1978; Van Delden & Kamping, 1991; Krebs & Loeschcke, 1994), possibly initiated by repair mechanisms and recovery of protein and hormone synthesis. Besides environmental influences on fertility of both females and males, fertility is controlled by many genes located all over the genome (Lindsley & Zimm, 1992), e.g. genes involved in functions for normal oogenesis and spermatogenesis and genes involved in the speciation process.

The inversion *In(2L)t* polymorphism in *D. melanogaster* provides a well documented example of the action of natural selection in relation to environmental temperature. This is illustrated by latitudinal clines in In(*2L*)*t* frequencies on all continents and in both hemispheres (references in Van Delden & Kamping, 1989, 1991 and Van′t Land, 1997) and by seasonal fluctuations in *In(2L)t* frequencies, with *In(2L)t* frequencies positively correlated with environmental temperature (Sanchez-Refusta *et al*., 1990; Kamping & Van Delden, 1999). Laboratory experiments showed a higher survival of karyotypes possessing *In(2L)t*, in relation to high temperature (Van Delden & Kamping, 1989, 1991; Van′t Land, 1997). The presumed specific and conserved allelic content of *In(2L)t* arrangements probably provides a higher fitness to its carriers.

An important aspect of temperature resistance is the ability to maintain or to restore fertility after heat stress, which greatly affects the survival of heat stressed populations. Differences in the degree and speed of restoration of fertility among karyotypes will have considerable influence on relative fitnesses and the genetic constitution in succeeding generations.

In the present paper we examine to what extent restoration of fertility differs among *In(2L)t* and *ST* homo- and heterokaryotypes after high-temperature treatments that induce complete sterility. The effects of exposure to high temperature on fertility are studied after treatments of juveniles and adults, and juvenile survival rates are measured after high-temperature treatment during different developmental stages. Furthermore, genetic adaptations with respect to effects on fertility after heat stress are studied by rearing *D. melanogaster* strains at 33°C for 10 generations. The results obtained will be discussed in the context of other temperature-related traits among *In(2L)t* and *ST* karyotypes and the latitudinal distribution of *In(2L)t* frequencies.

Materials and methods

Strains and culture conditions

Two strains which originated from a tropical greenhouse population (details in Van Delden & Kamping, 1989) were used to measure restoration of fertility among *In(2L)t* and *ST* homo- and heterokaryotypes. Each of the homokaryotype strains was constructed by intercrossing six lines of the appropriate karyotype, which were extracted as described by Van Delden & Kamping (1989). Heterokaryotypes were obtained by crossing the homokaryotype strains. Unless otherwise stated, strains were kept in bottles (125 mL volume with 30 mL food) at 25°C and 50–70% relative humidity under uncrowded conditions. In order to study long-term effects of high-temperature stress, strains were also kept at 33°C for 10 generations. Each generation all emerged flies were first placed at 25°C for a week to recover and then the next generation was started at 33°C. Regular medium consisted of 18 g agar, 54 g sucrose, 32 g dead yeast and 13 mL Nipagin solution (10 g Nipagin per 100 mL ethanol 96%) per 1000 mL water.

Exposure to high temperature

For measuring juvenile survival rates, five replicate vials per karyotype and developmental stage were exposed to 33°C. For 0–1-h-old larvae, 50 individuals per vial, and for treatments started or ended at other developmental stages (indicated in Table 1), 20 individuals per vial were used. Both larva to pupa and pupa to adult survival rates were measured.

The preadult and adult stages were exposed to a high-temperature (33°C) regime to measure effects on fertility. A pilot experiment had shown that the applied treatments led to complete sterility. Preadult treatment included complete juvenile development at 33°C for both strains kept at 25°C as well as at 33°C (see previous section). Adults used for high-temperature treatment were reared at 25°C under uncrowded conditions and exposed to 33°C for periods of either two or four days. Sixty males and 60 females (four days old) of each of the three karyotypes were individually tested in plastic vials (80 × 33 mm, containing 8 mL medium).

To test for restoration of fertility, adults that emerged after preadult treatment and adults exposed to high-temperature treatment were subsequently kept at 25°C for four, eight and 12 days. Then each of the flies (60 individuals per sex, karyotype and treatment) was combined with two 6-day-old untreated virgin flies (reared at 25°C) of the opposite sex and transferred to vials with fresh medium every four days while all vials were scored for progeny.

For the adults of the two-day treatment the number of matings in a 3-h testing period at 25°C was scored after a recovery period of 4 days. After the sexes had been separated the number of matings producing offspring was recorded. For flies without offspring within four days after mating, the mating test was

Table 1 Juvenile survival rates after heat stress among *In(2L)t* and *ST* homo- and heterokaryotypes of *Drosophila melanogaster*. (a) Treatment at 33°C started at different developmental stages. (b) Larval treatments at 33°C started with 0–1 h larvae during two periods followed by transfer to 25°C. Different superscript letters within each row indicate significant differences at the 5% level for larva–pupa and pupa–adult survival separately

	Survival rates					
	Larva – pupa			Pupa – adult		
	In(2L)t/ *In(2L)t*	*In(2L)t*/ *ST*	*ST*/ *ST*	*In(2L)t*/ *In(2L)t*	*In(2L)t*/ *ST*	*ST*/ *ST*
(a) Life stage at which treatment started						
Larvae 0–1 h	0.78^{ab}	0.84^{b}	0.72^{a}	0.37^{b}	0.49^{c}	0.22^{a}
Larvae 48–49 h	0.92^{a}	0.94^{a}	0.95^{a}	0.50^{b}	0.54^{b}	0.13^{a}
Third instar larvae	0.87^{a}	0.95^{a}	0.96^{a}	0.52^{b}	0.55^{b}	0.22^{a}
Early pupa	—	—	—	0.89^{b}	0.92^{b}	0.63^{a}
Late pupa	—	—	—	0.96^{b}	0.87^{a}	0.82^{a}
(b) Treatment period at 33°C						
65 h	0.97^{a}	0.98^{a}	0.97^{a}	0.98^{a}	0.99^{a}	0.98^{a}
Whole larval stage	0.98^{a}	0.96^{a}	0.97^{a}	0.93^{a}	0.93^{a}	0.91^{a}

repeated. Pure model ANOVAS, Tukey tests, contrast tests, chi-squared tests and correlation tests were performed by using Statistix 4.0 analytical software.

Results

Juvenile treatment at 33°C caused considerable reduction in survival, indicating that this temperature was highly stressful. Survival rates are presented in Table 1. Larva–pupa survival rates were significantly ($P < 0.005$) lower for the treatment started with 0–1 h larvae than for the treatments started with older larvae. Highest mortality and strongest karyotypic differences were observed for pupa–adult survival. *ST* homokaryotypes showed lowest pupa–adult survival rates. When temperature stress started at early or late pupal stages, pupal mortality was significantly lower for all karyotypes, compared to temperature treatments during the larval stages. High-temperature treatment starting in different larval stages resulted in large deviations from the expected 1:1 sex ratio. The number of emerging females exceeded the number of males significantly ($P < 0.001$) for all karyotypes. The overall observed fraction of males was 0.30, indicating that high-temperature treatment was less severe for female juveniles. Larvae exposed to 33°C for 65 h and larvae exposed up to late third larval stage showed high survival rates after transfer to 25°C and no significant differences among karyotypes were present (Table 1b). Combining the data of the three karyotypes showed significantly higher overall survival rates for the shorter period of larval treatment ($P < 0.025$).

After 2 days exposure of adults to 33°C, no mortality was observed. However, 4 days exposure to 33°C caused 5.6% mortality in females and 15% in males. Mortality in males was significantly higher than in females ($P < 0.005$). In females no significant mortality among karyotypes was present, whereas in males mortality of *In(2L)t* heterokaryotypes (no mortality) was significantly lower ($P < 0.01$) than for *In(2L)t* homokaryotypes (11.7% mortality), and these two karyotypes possessed significantly lower mortality rates ($P < 0.001$ and $P < 0.005$, respectively) than *ST* homokaryotypes (33.3% mortality). Also during recovery at 25°C of the flies treated for 4 days, additional mortality occurred (2.8% and 5% of the initial number of females and males, respectively), with no significant differences among karyotypes.

Table 2 Percentages of matings (during 3 h at 25°C) and percentages of matings with offspring of females and males of different *Drosophila melanogaster* karyotypes, which had been exposed to 33°C for 2 days followed by a recovery period of 4 days at 25°C. For each sex and karyotype 60 individuals were tested

	% of matings		% of matings with offspring	
Karyotype	Female	Male	Female	Male
In(2L)t/*In(2L)t*	73	87	48	62
In(2L)t/*ST*	87	97	58	53
ST/*ST*	83	87	20	21

Females and males which had been exposed to 33°C for 2 days, were tested for mating ability after a 4-day recovery period at 25°C. The results are presented in Table 2. The mating percentage after 3 h was 81% for females and 90% for males, respectively, which lies within the range of untreated flies under similar test

conditions. No significant differences among karyotypes within each of the sexes were observed; however, the combined data of the two sexes showed a significantly ($P < 0.01$) higher mating rate of heterokaryotypes compared to *In(2L)t* homokaryotypes. The total number of matings was significantly higher ($P < 0.025$) for treated males as compared to treated females. Four days after the first mating test, a second mating test was performed with both mated flies without offspring from the first test, as well as flies without mating during the first test. Mating frequencies were high (>90%) and no significant difference between sexes and karyotypes was observed. Although mating rate was hardly influenced after high-temperature treatment, the percentage of matings producing offspring was highly different among karyotypes (Table 2). Fertility of *ST* homokaryotypes was significantly lower ($P < 0.001$) than the *In(2L)t* homo- and heterokaryotypes for both sexes.

The applied high-temperature treatment of adults as well as treatment during the whole juvenile stage induced complete sterility. Subsequent transfer to 25°C, however, resulted in restored fertility in some of the individuals, depending on the length of the recovery period. Pure model ANOVAS were performed for the effects of the different treatments, recovery periods, sexes and karyotypes on the fraction of adults producing offspring after angular transformation of the data. Analysis of variance for treatment of adults showed significant effects for treatment length ($P < 0.001$), recovery period ($P < 0.001$) and karyotype ($P < 0.005$), whereas sex differences and interactions were not significant. Analysis of variance for treatment during juvenile development of control and selection strains showed highly significant effects for all main factors ($P < 0.001$), and a significant interaction for treatment by sex by recovery period ($P < 0.005$). Differences within each main factor (Tukey tests) are shown in Fig. 1(a) (adult treatment) and Fig. 1(b) (control and selection strains).

The increase in the fraction of adults producing offspring with increasing recovery period at 25°C showed that restoration occurs and that the recovery period is variable among individuals. Fertility was not restored in all individuals after 12 days recovery, indicating that the damage caused by temperature stress was too high for repair for those individuals. After 4 days treatment of adults, restoration of fertility was significantly lower than for the 2-day treatment, indicating greater damage after longer exposure. The significantly higher fraction of adults producing offspring of the strain kept for 10 generations at 33°C, compared to the control strain, indicates selection for increased restoration of fertility after high-temperature stress. Effects of heat stress were significantly lower for females than for males when treatment occurred during the juvenile stage, whereas no sex differences for restored fertility were observed after treatment of adults. The ANOVAS further showed significant differences among karyotypes, with the lowest restored fertility for *ST* homokaryotypes and the highest values for heterokaryotypes.

All main factors were highly significant for each recovery period. Mean values within each of the main factors with indication of significant differences are presented in Table 3. A more detailed analysis of karyotypic differences within each treatment, sex and recovery period is presented in Fig. 2. Heterokaryotype advantage is more pronounced after juvenile treatments, with the strongest effect for one generation treatment of males (juveniles), where only heterokaryotypes showed restored fertility. The observed negative correlation between heterokaryotype superiority and mean overall values for restoration of fertility for treatment lengths as well as for control and selection strains, shows that heterokaryotype superiority is positively correlated with the severity of the treatment (Table 4).

Discussion

Natural *D. melanogaster* populations experience daily and seasonal fluctuations in environmental temperature with varying periods of high-temperature stress, when environmental temperatures exceed those necessary for normal developmental and physiological processes (Feder *et al.*, 1996; Kamping & Van Delden, 1999). Changes in environmental temperature generally occur gradually, which allows appropriate phenotypic responses. These physiological, behavioural and metabolic responses may diminish the effects of thermal stress, such as reduction in viability, reduced reproductive capacity and increased mortality.

Juvenile survival fraction after temperature stress depends on the life stage exposed (Table 1). Larva to pupa survival rates were relatively high. The pupal stage showed highest juvenile mortalities and strongest karyotypic effects when the preceding larval development occurred at 33°C. Besides a reduction in the fraction of emerging adults after exposure of juvenile stages at 33°C, a decrease of adult fitness was observed at normal temperature. It is not possible to rear *D. melanogaster* throughout the whole life cycle at temperatures above 30°C. Though this temperature is not lethal, it induces sterility in all adults and juveniles. Various reasons for sterility induction after thermal stress are possible. Females may show defects during oogenesis and failure of eggs to mature in ovaries, whereas males have to deal with failure to produce mature sperm, developmental arrest after sperm entry

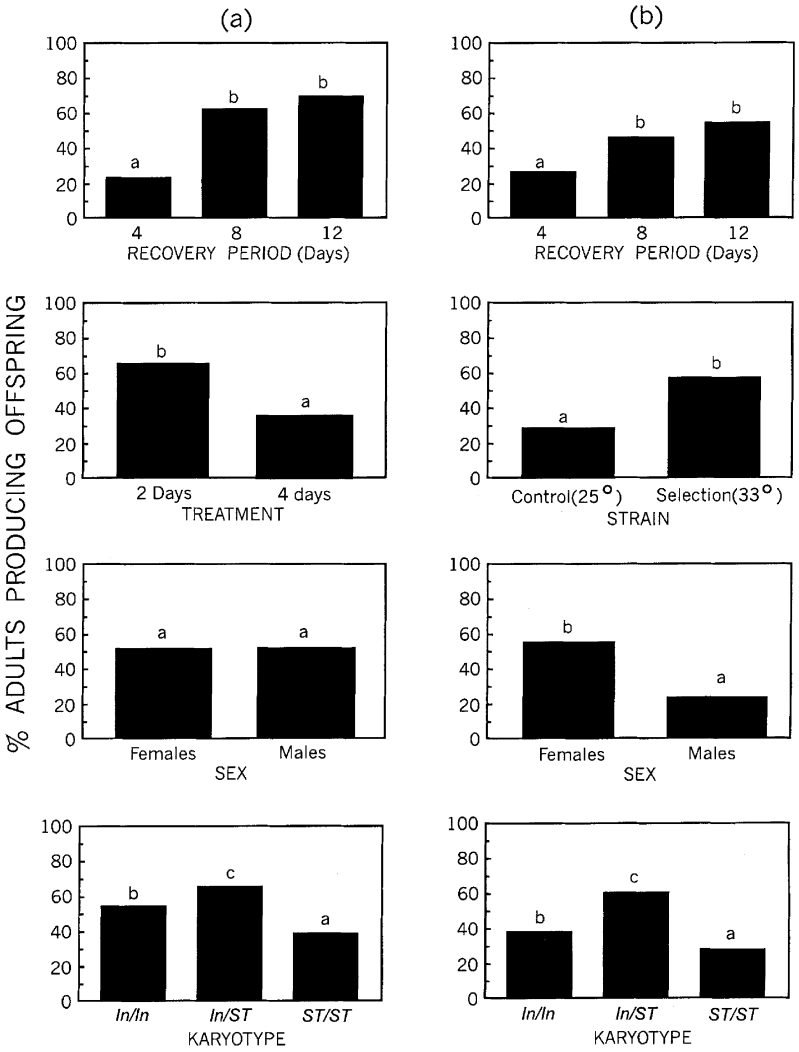

Fig. 1 Percentages of adult *Drosophila melanogaster* producing offspring after high-temperature exposure presented for each main factor. (a) Adult treatment at 33°C for 2 and 4 days; (b) treatment at 33°C during juvenile development for control (25°C) and selection (33°C) strains. Different letters above bars indicate significant differences at the 5% level. In, *In(2L)t*; *ST*, *Standard*.

Table 3 Mean fraction of adult *Drosophila melanogaster* producing offspring after high-temperature stress (33°C), with varying recovery periods at 25°C. (a) Adult treatment for 2 and 4 days; (b) treatment during juvenile development for control (25°C) and selection (33°C) strains. Different superscript letters within each main factor for each recovery period indicate significant differences at the 5% level

	Recovery period (days)				Recovery period (days)		
(a)	4	8	12	(b)	4	8	12
Treatment				Strain			
2 days	0.38^{b}	0.78^{b}	0.84^{b}	Control	0.11^{a}	0.31^{a}	0.39^{a}
4 days	0.09^{a}	0.47^{a}	0.56^{a}	Selection	0.40^{b}	0.60^{b}	0.67^{b}
Sex				Sex			
Female	0.18^{a}	0.63^{a}	0.72^{a}	Female	0.43^{b}	0.63^{b}	0.68^{b}
Male	0.28^{a}	0.61^{a}	0.68^{a}	Male	0.08^{a}	0.29^{a}	0.39^{a}
Karyotype				Karyotype			
In(2L)t/In(2L)t	0.26^{b}	0.63^{b}	0.70^{b}	*In(2L)t/In(2L)t*	0.21^{a}	0.43^{a}	0.49^{a}
In(2L)t/ST	0.33^{b}	0.76^{c}	0.85^{c}	*In(2L)t/ST*	0.43^{b}	0.66^{b}	0.75^{b}
ST/ST	0.11^{a}	0.47^{a}	0.55^{a}	*ST/ST*	0.14^{a}	0.29^{a}	0.37^{a}

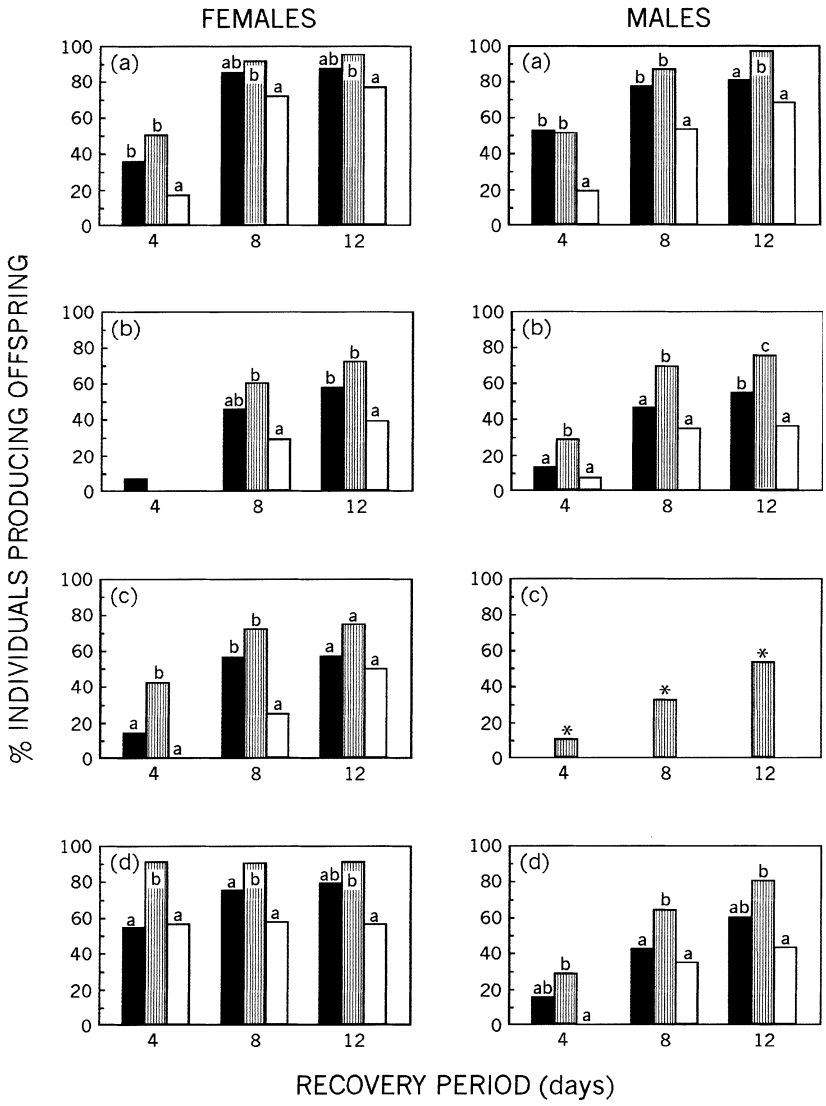

Fig. 2 Percentages of adult *Drosophila melanogaster* producing offspring after treatments at 33°C, followed by recovery periods at 25°C. Adults after (a) 2 days and (b) 4 days exposure at 33°C. Treatment during juvenile development for (c) control and (d) selection strains. Black bars, *In(2L)t* homokaryotypes; dashed bars, heterokaryotypes; open bars, *Standard* homokaryotypes. Values within each recovery period with different superscript letters are significantly different at the 5% level. *Only heterokaryotypes showed restored fertility.

Table 4 Mean restored fertility of *Drosophila melanogaster*, relative values among karyotypes and heterokaryotype superiority for restored fertility after high-temperature stress (33°C). Heterokaryotype superiority is given as the ratio between restored fertility in heterokaryotypes and in *In(2L)t* homokaryotypes. (a) Adult treatment for 2 and 4 days; (b) treatment during juvenile development for control (25°C) and selection (33°C) strains

		Relative values among karyotypes			
	Mean restored fertility	*In(2L)t*/ *In(2L)t*	*In(2L)t*/ *ST*	*ST*/ *ST*	Heterokaryotype superiority
(a) Adult treatment					
2 days	0.66	0.88	1	0.65	1.14
4 days	0.37	0.73	1	0.48	1.37
(b) Strain					
Control (25°C)	0.27	0.45	1	0.26	2.22
Selection (33°C)	0.56	0.72	1	0.55	1.39

into the egg, loss of mobility, and dysfunction during spermatogenesis.

In the present paper we have shown that fertility of both sexes is strongly diminished by high-temperature stress acting on adults as well as juveniles. Subsequent transfer to 25°C resulted in restored fertility in some of the individuals. *In(2L)t* homo- and heterokaryotype females and males have a greater restoration of fertility as well as a higher juvenile survival rate than *ST* homokaryotypes, perhaps suggesting pleiotropic effects of these fitness components ($r = 0.91$; $P < 0.001$). The observed karyotypic differences will have considerable influence on the genetic constitution of succeeding generations.

Ten generations selection at 33°C during juvenile stages resulted in a significantly higher thermal resistance, both for juvenile survival as well as for restoration of fertility, indicating a rapid genetic change. Improvement of thermal resistance is observed for all karyotypes, with a relatively stronger effect for the *ST* karyotype. Heterokaryotypic advantage in this case is less pronounced in comparison to the one-generation juvenile treatment. Evidence for genetic effects of temperature stress has been obtained from laboratory experiments (Huey *et al*., 1991; Partridge *et al*., 1994; Loeschcke & Krebs, 1996) and from natural populations (Coyne *et al*., 1983; Krebs & Loeschcke, 1994; Loeschcke *et al*., 1997) where differences in temperature resistance among geographically distinct populations are associated with their different environmental thermal conditions.

In addition to differences in fertility restoration, reduction in egg-laying rate during and after thermal stress (Cohet & David, 1978), a lower mating frequency of treated females as observed here, and a shorter life span (Economos & Lints, 1986) will also influence reproductive capacity. Although effects of thermal stress on reproductive capacity are generally not permanent, its relative contribution to population fitness and to fitness differences among karyotypes are possibly of greater importance for the maintenance and evolution of genetic variation than the permanent effects of high-temperature stress. Reduction in reproductive capacity, for example, has strong effects on effective population size.

The underlying genetic variation for heat tolerance may be related to metabolic rate (Hoffmann & Parsons, 1989) and genotypes with a lower metabolic rate may exhibit a higher fitness. The longer developmental time for *In(2L)t* karyotypes at various rearing temperatures (Van Delden & Kamping, 1989, 1991; Van ′t Land, 1997) may be related to a lower metabolic rate because of pleiotropic effects of genes associated with *In(2L)t*, controlling developmental time and metabolic rate.

A common phenomenon after thermal stress is the induction of synthesis of several heat shock proteins (HSP), whereas most transcription and translation is suppressed (Morimoto *et al*., 1994). It is assumed that *Hsp* genes will provide protection against the damaging effects of protein denaturation. However, prolonged or severe treatment will inhibit all metabolic activity and individuals will die. The functional significance of *Hsp* genes is illustrated by a higher thermotolerance of individuals with higher heat shock protein levels after high-temperature stress (Feder *et al*., 1996, 1997) and by continued protein synthesis at high temperature by high HSP levels (Gehring & Wehner, 1995). The induction of HSPs depends on temperature and life stage (Feder *et al*., 1997) and is different for the various *Hsp* genes (Gehring & Wehner, 1995). Induction of the small HSPs is maximal at 33°C, whereas synthesis of the large HSPs is continued at higher temperatures.

Heat shock (short exposure to extremely high temperature) and prolonged treatment at moderately high temperature will induce different HSPs at different levels. Therefore induction of the small HSPs seems to be most important during the high-temperature treatments which we used. In contrast to the large HSPs, which are highly conserved (Nagao *et al*., 1990), there is evidence for higher levels of variation for the small HSPs (Gehring & Wehner, 1995). This means that the small HSPs may contribute to the genetic response for thermal resistance after selection for many generations at moderate high temperature (33°C).

The high pupa–adult mortality rates may be caused by toxicity and/or energetic costs of high HSP levels induced by the treatment of the preceding larval stages. Furthermore, through the functional relationships between *Hsp* genes and hormone receptors (e.g. Picard *et al*., 1988), *Hsp* genes may indirectly influence development and survival when juvenile stages are exposed to high temperature. The lower mating activity of treated females may be explained by lower hormone levels resulting from heat stress.

It seems unrealistic to assume that *Hsp* genes are directly responsible for the significant differences among *In(2L)t* and *ST* homo- and heterokaryotypes, because of their chromosomal independence. *Hsp* genes are located on the third chromosome (Lindsley & Zimm, 1992), whereas the chromosome arrangements under investigation are located on the second chromosome. However, transcription of the *Hsp* genes is controlled by genes located on the second chromosome (Parker-Thornburg & Bonner, 1987; Clos *et al*., 1990; Otsuka *et al*., 1997). Therefore we cannot exclude the possibility that *Hsp* modifier genes are associated with the *In(2L)t* region.

The observed positive correlation between heterokaryotype superiority for restoration of fertility and severity of the treatment (Fig. 2 and Table 4) agrees with earlier observations (Van Delden & Kamping, 1991; Kamping & Van Delden, unpubl. obs.), that heterokaryotype advantage, as deduced from karyotype frequencies in laboratory populations, is positively correlated with rearing temperature. These findings are concordant with the idea that the latitudinal distribution of *In(2L)t* frequencies is maintained by balancing selection, with equilibrium frequencies decreasing with latitude. Superiority of inversion heterokaryotypes in *D. melanogaster* has been observed for various fitness components such as productivity, mating ability, sperm productivity (references in Lemeunier & Aulard, 1992), and fitness-related characters such as developmental rate and body weight (Van Delden & Kamping, 1989, 1991, 1997). These traits are affected by environmental temperature and will be involved in the overall fitnesses of karyotypes and consequently lead to an excess of heterokaryotypes under high-temperature conditions. The high thermal resistance of *In(2L)t* homo- and heterokaryotypes probably results from a particular allelic content of the inversion. This agrees with high *In(2L)t* frequencies observed in tropical regions (Anderson *et al*., 1987; Van ′t Land, 1997), *In(2L)t* frequency fluctuations in a tropical greenhouse population (Kamping & Van Delden, 1999), and *In(2L)t* frequencies in laboratory populations kept at various temperatures (Van Delden & Kamping, 1991).

Explanations concerning the latitudinal clines for inversions mostly assume a unique origin and genetic uniformity among inversion karyotypes with identical breakpoints all over the world. Preliminary results from comparisons of *In(2L)t* and *ST* strains by means of RFLP and DNA sequencing show indeed a much greater resemblance among *In(2L)t* strains compared to *ST* strains (Van Delden & Kamping, 1997). Further research on the *In(2L)t* polymorphism will be focused on genetic variability among *In(2L)t* strains from different geographical areas and on the specific genes involved in heat resistance associated with the *In(2L)t* region.

Acknowledgements

We thank H. Mulder for preparing the figures, and A. Rumahloine and L. Hoeksema-du Pui for technical assistance.

References

ANDERSON, P. R., KNIBB, W. R. AND OAKESHOTT, J. G. 1987. Observations on the extent and temporal stability of latitudinal clines for alcohol dehydrogenase allozymes and for chromosome inversions in *Drosophila melanogaster*. *Genetica*, **75**, 81–88.

CLOS, J., WESTWOOD, J. T., BECKER, P. B., WILSON, S., LAMBERT, K. AND WU, C. 1990. Molecular cloning and expression of a hexameric *Drosophila* heat shock factor subject to negative regulation. *Cell*, **63**, 1085–1097.

COHET, Y. AND DAVID, J. 1978. Control of the adult reproductive potential by pre-imaginal thermal conditions. *Oecologia*, **36**, 295–306.

COYNE, J. A., BUNDGAARD, J. AND PROUT, T. 1983. Geographic variation of tolerance to environmental stress in *Drosophila pseudoobscura*. *Am. Nat*., **122**, 474–488.

DAHLGAARD, J. AND LOESCHCKE. V. 1997. Effects of inbreeding in three life stages of *Drosophila buzzatii* after embryos were exposed to a high-temperature stress. *Heredity*, **78**, 410–416.

DAVID, J. R. 1988. Temperature. In: Lints, F. A. and Soliman, M. H. (eds) *Drosophila as a Model Organism for Aging Studies*, pp. 33–45. Blackie, Glasgow.

ECONOMOS, A. C. AND LINTS, F. A. 1986. Developmental temperature and life span in *Drosophila melanogaster*. I. Constant developmental temperature: evidence for physiological adaptation in a wide temperature range. *Gerontology*, **32**, 18–27.

FEDER, M. E., CARTANO, N. V., MILOS, L., KREBS, R. A. AND LINDQUIST, S. L. 1996. Effect of engineering hsp70 copy number on hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. *J. Exp. Biol*., **199**, 1837–1844.

FEDER, M. E., BLAIR, N. AND FIGUERAS, H. 1997. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol*., **11**, 90–100.

GEHRING, W. J. AND WEHNER, R. 1995. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. *Proc. Natl. Acad. Sci. U.S.A*., **92**, 2994–2998.

HOFFMANN, A. A. AND PARSONS, P. A. 1989. Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics*, **122**, 837–845.

HUEY, R. B., PARTRIDGE, L. AND FOWLER, K. 1991. Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution*, **45**, 751–756.

KAMPING, A. AND VAN DELDEN, W. 1999. A long-term study on interactions between the *Adh* and αGpdh allozyme polymorphisms and the chromosomal inversion *In(2L)t* in a seminatural population of *D. melanogaster*. *J. Evol. Biol*., **12**, 809–821.

KREBS, R. A. AND LOESCHCKE, V. 1994. Response to environmental change: Genetic variation and fitness in *Drosophila buzzatii* following temperature stress. In: Loeschcke, V. Tomiuk, J. and Jain, S. K. (eds) *Conservation Genetics*, pp. 309–321. Birkhäuser-Verlag, Basel.

LEMEUNIER, F. AND AULARD, S. 1992. Inversion polymorphism in *Drosophila melanogaster*. In: Krimbas, C. B. and Powell, J. R. (eds) *Drosophila Inversion Polymorphism*, pp. 339–405. CRC Press, Boca Raton, FL.

LINDSLEY, D. L. AND ZIMM, G. G. 1992. *The Genome of Drosophila Melanogaster*. Academic Press, San Diego, CA.

LOESCHCKE, V. AND KREBS, R. A. 1996. Selection for heat-shock resistance in larval and in adult *Drosophila buzzatii*: comparing direct and indirect responses. *Evolution*, **50**, 2354–2359.

LOESCHCKE, V., KREBS, R. A., DAHLGAARD, J. AND MICHALAK, P. 1997. High temperature stress and the evolution of thermal resistance in *Drosophila*. In: Bijlsma, R. and Loeschcke, V. (eds) *Environmental Stress, Adaptation and Evolution*, pp. 175–190. Birkhäuser, Basel.

MCCOLL, G., HOFFMANN, A. A. AND MCKECHNIE, S. W. 1996. Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. *Genetics*, **143**, 1615–1627.

MORIMOTO, R. I., TISSIERES, A. AND GEORGOPOULOS, C. (eds) 1994. *The Biology of Heat-Shock Proteins and Molecular Chaperones*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MORRISON, W. W. AND MILKMAN, R. 1978. Modification of heat resistance in *Drosophila* by selection. *Nature*, **273**, 49–50.

NAGAO, R. T., KIMPEL, J. A. AND KEY, J. L. 1990. Molecular and cellular biology of the heat-shock response. *Adv. Genet.*, **28**, 235–274.

OTSUKA, Y., TAKANO, T. S. AND YAMAZAKI, T. 1997. Genetic variation in the expression of the six hsp genes in the presence of heat shock in *Drosophila melanogaster*. *Genes Genet. Syst.*, **72**, 19–24.

OUDMAN, L. 1991. A locus in *Drosophila melanogaster* affecting heat resistance. *Hereditas*, **114**, 285–287.

PARKER-THORNBURG, J. V. AND BONNER, J. J. 1987. Mutations that induce the heat shock response of *Drosophila*. *Cell*, **51**, 763–772.

PARTRIDGE, L., BARRIE, B., FOWLER, K. AND FRENCH, V. 1994. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. *J. Evol. Biol.*, **7**, 645–663.

PICARD, D., SALSER, S. J. AND YAMAMOTO, K. R. 1988. A movable and regulable inactivation function within the steroid binding domain of the glucocorticoid receptor. *Cell*, **54**, 1073–1080.

PROSSER, C. L. 1986. *Adaptational Biology: Molecules to Organisms*. John Wiley & Sons, New York.

SANCHEZ-REFUSTA, F., SANTIAGO, E. AND RUBIO, J. 1990. Seasonal fluctuations of cosmopolitan inversion frequencies in natural population of *Drosophila melanogaster*. *Génét. Sél. Évol.*, **22**, 47–56.

VAN DELDEN, W. AND KAMPING, A. 1989. The association between the polymorphisms at the alcohol dehydrogenase and α-glycerophosphate dehydrogenase loci and the *In(2L)t* inversion in *Drosophila melanogaster* in relation to temperature. *Evolution*, **43**, 775–793.

VAN DELDEN, W. AND KAMPING, A. 1991. Changes in relative fitness with temperature among second chromosome arrangements in *Drosophila melanogaster*. *Genetics*, **127**, 507–514.

VAN DELDEN, W. AND KAMPING, A. 1997. World-wide latitudinal clines for the alcohol dehydrogenase polymorphism in *Drosophila melanogaster*. What is the unit for selection? In: Bijlsma, R. and Loeschcke, V. (eds) *Environmental Stress, Adaptation and Evolution*, pp. 97–115. Birkhäuser, Basel.

VAN 'T LAND, J. 1997 . *Latitudinal Variation in Wild Populations of* Drosophila melanogaster. Ph.D. Thesis, University of Groningen.