

Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the *hsr-omega* gene in *Drosophila melanogaster*

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Drosophila melanogaster occurs in diverse climatic regions and shows opposing clinal changes in resistance to heat and resistance to cold along a 3000 km latitudinal transect on the eastern coast of Australia. We report here on variation at a polymorphic 8bp-indel site in the heat shock *hsr-omega* gene that maps to the right arm of chromosome 3. The frequency of the genetic element marked by the L form of the gene was strongly and positively associated with latitude along this transect, and latitudinal differences in L frequency were robustly associated with latitudinal differences in maximum temperature for the hottest month. On a genetic background mixed for genes from each end of the cline a set of 10 lines was derived, five of which were fixed for the L marker, the absence of *In(3R)P* and 12 kb of repeats at a

second polymorphic site at the 3' end of *hsr-omega*, and five that were fixed for the S marker, *In(3R)P* and 15 kb of *hsr-omega* repeats. For two different measures of heat tolerance S lines outperformed L lines, and for two different measures of cold tolerance L lines outperformed S lines. These data suggest that an element on the right arm of chromosome 3, possibly *In(3R)P*, confers heat resistance but carries the trade-off of also conferring susceptibility to cold. This element occurs at high frequency near the equator. The alternate element on the other hand, at high frequency at temperate latitudes, confers cold resistance at the cost of heat susceptibility.

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Introduction

Research in *Drosophila* is providing a focus for understanding the physiological and genetic basis of thermal tolerance variation in ectotherms (Huey and Kingsolver, 1993; Duncker *et al.*, 1995; Krebs and Loeschke, 1995; Guerra *et al.*, 1997; Ohtsu *et al.*, 1998). Significant variation in resistance to temperature extremes are known among several groups of closely related *Drosophila* species and these are likely to be adaptive since they are related to the climatic zones where the species are found (Kimura, 1988; Jenkins and Hoffmann, 1994; Gibert *et al.*, 2001). While thermal tolerance is an important factor limiting the distribution of species, some species such as the cosmopolitan *Drosophila melanogaster* successfully survive and reproduce over a broad range of climatic zones that cover a wide spectrum of thermal extremes. Heritable strain variation and regional variation in thermal tolerance have been well documented for this species (Parsons, 1977; Stalker, 1980); however, only recently has a firm basis been established for believing that an adaptive cause underlies this variation. Opposing latitudinal clines in resistance to both heat and cold temperature extremes occurred along a north–south transect of samples from the eastern coast of Australia

(Hoffmann *et al.*, 2002). In samples not confounded by variation in altitude, knockdown time when exposed to heat shock increased towards the tropics whereas recovery time following cold shock decreased towards temperate high latitude regions, as might be predicted under an adaptive scenario.

This Australian eastern coast transect for *D. melanogaster* has recently been shown to vary in a clinal fashion for a number of different quantitative traits, in addition to the heat and cold tolerance clines. Traits that change with latitude include body size (Partridge *et al.*, 1999), egg size (Azevedo *et al.*, 1996) and life-history traits (Mitrovski and Hoffmann, 2001). Clinal variation also occurs for numerous genetic markers, including the frequency of four common cosmopolitan inversions (Knibb *et al.*, 1981), and allele frequencies of numerous polymorphic markers (Oakeshott *et al.*, 1984) including five microsatellite markers (Gockel *et al.*, 2001). Many of these variations are also 'generic', occurring in a latitudinally consistent manner on other continents (Knibb, 1982; Mettler *et al.*, 1977; Inoue *et al.*, 1994), and in related species (Oakeshott *et al.*, 1984). A major question of interest is the relation between the quantitative traits and the genetic markers, and the extent to which we can understand the genetic and physiological/selective basis of the differential adaptation to climate along these latitudinal clines.

Towards this end, we have recently focused on thermal tolerance variation and its relation to polymorphic candidate gene variation (McColl *et al.*, 1996). In

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particular, strong clinal variation in allele frequency of a polymorphic heat shock gene, *hsr-omega*, parallels the thermal tolerance clines down the Australian eastern coast (McCull and McKechnie, 1999). Further, in laboratory studies heat-tolerance variation has been associated with molecular variation in *hsr-omega* and with variation in two other heat shock genes, *hsp70* and *hsp68*, all of which map to the right arm of chromosome 3 (Figure 1) (McCull *et al*, 1996; Feder and Hofmann, 1999). The right arm of chromosome 3 harbours the large cosmopolitan inversion *In(3R)P* that also varies latitudinally (Knibb *et al*, 1981). Both *hsr-omega* and *hsp68* map within the bounds of *In(3R)P*. This linkage and latitudinal association of the heat-shock-protein markers and an inversion hints at a possible causal role of chromosome 3R in clinal thermal-tolerance variation. Intriguingly, heat shock genes may also play a role in recovery from cold stress (Guy *et al*, 1986; Chen *et al*, 1987; Burton *et al*, 1988), and *hsr-omega* in particular has been implicated in expression changes following cold exposure (Singh and Lakhota, 1984; Lakhota and Singh, 1985). A major effect of chromosome 3 on population variation in cold tolerance has been previously indicated (Tucic, 1979).

In this report, we use polymorphic variation in the *hsr-omega* gene as a marker for variation that occurs on the right arm of chromosome 3 and we ask about the relation between variation in this marker and both heat and cold tolerance variation. We chose two different ways of testing tolerance to these extremes, to see if the associations are of general significance. One of these

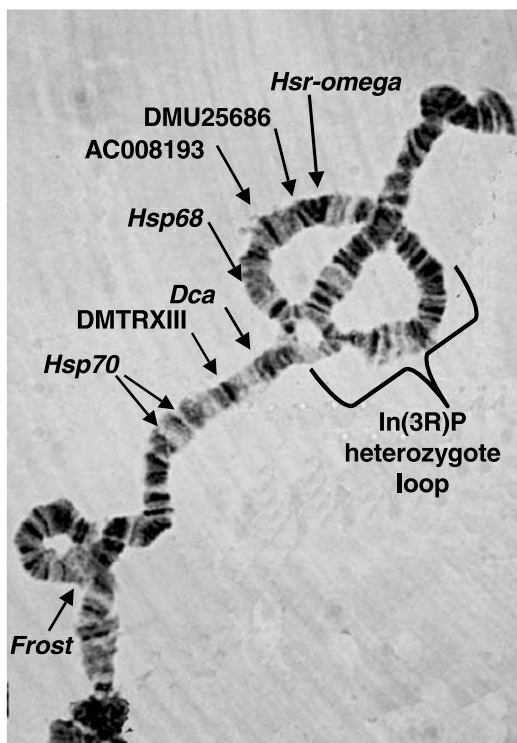


Figure 1 *Drosophila melanogaster* right arm of chromosome three. Depicted are cytological locations of *In(3R)P* (89C2-C3 – 96A18), *hsr-omega* (93D), *Dca* (88D), *Frost* (85E2), *hsp63* (95D), *hsp70a,b* (87A,C), microsatellites DMU25686 (93F), AC008193 (94D) and DMTRX3 (88B3) on a polytene chromosome spread of the right arm of chromosome 3 from an individual heterozygous for *In(3R)P*.

heat-tolerance tests and one of the cold-tolerance tests are identical to those used to detect clinal variation along the eastern Australian transect (Hoffmann *et al*, 2002). Our data strongly suggest that the right arm of chromosome 3 is involved with genetic adaptation to both heat and cold stress and that fitness trade-offs are involved; heat resistance comes at the cost of cold susceptibility, and the cost of being cold tolerant is susceptibility to heat.

Materials and methods

Drosophila lines

A total of 160 *D. melanogaster* isofemale lines were collected from December 1999 to February 2000 along the east-coast of Australia. A minimum of four and maximum of 15 isofemale lines were collected from 19 sites and were reared in vials on treacle medium at 18°C with constant light in the laboratory. Two isofemale lines from the east-coast collection were used for the hybrid study. Line 11T was fixed for the *hsr-omega*^L allele (L) and was collected from Huon, Tasmania. Line 13Q was fixed for the *hsr-omega*^S (S) allele and was collected from Weipa, in far northern Queensland. To produce hybrid lines 25 virgin females from each line were mated *en masse* with 25 males from the other line. Progeny from the two crosses were mixed at eclosion and allowed to interbreed *en masse* for five discrete generations in a large population cage at 25°C. More than 100 single-pair matings were set up from this cage and parents were genotyped for *hsr-omega*^{L/S}. A subset of 15 of these lines were selected for testing according to genotype; five lines fixed for the L allele, five for S and five H lines (50% for each allele). Hybrid lines were reared on treacle medium at 25°C with constant light.

Genotyping – *In(3R)P* cytology

Males from the derived 'hybrid' lines that were fixed for L and S were crossed to a line known to have the standard arrangement of chromosome 3R (Celera, virgin females) and larval progeny were genotyped for the heterozygous loop. Polytene squashes were carried out as described by Yoon *et al* (1973). Briefly, salivary glands were dissected from third instar larvae in Becker Ringer's solution and placed in 45% acetic acid for 10 s, then in 1 M HCl for 30 s, before staining in lacto-acetoorcein for 40 min. After staining, glands were transferred to lactoacetic acid to visualize the glands. The preparation was covered with a cover slip and squashed and examined with × 100 microscopy. The third chromosome was recognized by landmark banding patterns and the presence/absence of an inversion heterozygous loop was recorded.

Genotyping – *hsr-omega*

Single fly DNA extraction was performed using a modified proteinase K method (Saiki *et al*, 1987). Single flies were ground in 100 µl 10 mM Tris-HCl pH 8.2, 1 mM EDTA, 25 mM NaCl and 200 µg/ml Proteinase K. Homogenates were left at room temperature for 25–30 min and then heated to 95°C for 2 min to inactivate proteinase K. DNA (10 µl) was used as template in the PCR amplification (25 µl final volume). Two primers, 5'-GCA GTC TGA GGC AGT TAT CC-3' and 5'-CAA TCT

TTC AAA ATC CGC AG-3', were designed using the EMBL database (DM18307) to give fragments of 86 and 94 bp for the *hsr-omega^s* and the *hsr-omega^t* alleles, respectively. The PCR products were electrophoresed through horizontal 10% thin-layer polyacrylamide gels or for large-scale genotyping, through 3.5% agarose-1000. For Southern blots, DNA extractions were carried out on 25 adults that were glass-pestle ground using a standard proteinase K/phenol/chloroform protocol. The DNA (~3.5 µg/µl) was digested with *Pst*I and *Hind*III (that cut outside but not within the tandem repeat segment) overnight at 37°C. A probe was produced from wildtype genomic DNA (RNase treated) using primers (5'-CGA AAA GGC TTA TCC TCT TGG, 5'-ATA GTG ATT GGG GTA ATC GGG) that bind to each end of the *hsr-omega* 280 bp tandem repeat unit. PCR amplification entailed 36 cycles of 94°C (5 min), 47°C (30 s) and 72°C (10 s). The product was purified using Wizard PCR Preps (Promega) and used in conjunction with a GIGAprime DNA Labelling Kit (Geneworks) to make the ³²P-labelled probe for hybridization.

Thermal tolerance

Thermal tolerance has been variously measured using mortality assays, knockdown assays or recovery assays (Parsons, 1977; McColl *et al*, 1996; Gibert *et al*, 2001). Often, for any one type of stress (eg heat) different physiological and genetic mechanisms have been implicated (Hoffmann *et al*, 1997), at least in part. In order to maximize our chances of detecting effects of mechanisms that may be relevant to natural populations, we have used two different knockdown assays to measure heat resistance, and both a mortality assay and recovery assay for cold tolerance.

Heat tolerance

Two measures of heat tolerance were applied. First, the set of 15 hybrid lines and their two parental isofemale lines were tested for heat knockdown as single flies (females) in small, capped, vials (5 mm by 20 mm) emerged in a circulating waterbath tank at 39°C with a clear glass side for scoring. Adults were reared at 25°C and aged in fresh-medium bottles for 3–4 days prior to testing. Knockdown time was scored in batches of six lines of 20 flies per line (10 nonhardened and 10 hardened). For hardening, flies were placed in a 37°C incubator for 1 h and aged for a further 6 h at 25°C before being tested. Two lines (one parental and one H line; both nonhardened) were included in all batches with the four test lines as internal standard to control for variation between batches. For analyses individual knockdown times were divided by the mean knockdown times of all control flies from the batch, and for ANOVA the data were square-root transformed.

Second, heat-knockdown was carried out in a large knockdown tube, 80 cm long, with an internal diameter of 9.5 cm. Adults were reared and hardened as for the small-vial heat-knockdown test. The tube was surrounded by a water jacket attached to a Ratek circulating bath set at 39°C. The temperature within the tube was 38.5–39°C. Each knockdown run involved a comparison of four lines (150 flies each; sexes mixed): three test and one control line. Lines in the same knockdown run were distinguished by marking them with different coloured (pink, orange, yellow, no dust) fluorescent dusts (Hele-

con, Hackettstown, NJ, USA). Flies were placed in empty vials with about 3.5 mg of fluorescent dust and allowed to recover from dusting for 24 h in fresh bottles, before testing. Flies were not anaesthetized at any time. After collection from heat knockdown in a chronological series of vials, flies were frozen and later sorted and counted according to dust colour under a dissecting microscope.

Tolerance for each line was recorded as average time taken for individual flies to knockdown (as a ratio of the control line) based on 150 flies in each of three replicate runs.

Cold tolerance

This was measured using a chill coma assay (Gibert *et al*, 2001) and a mortality assay (Hoffmann and Parsons, 1993). Briefly, for the chill coma assay single adult flies were exposed to 0°C for 16 h in glass vials. Flies were then transferred to 25°C and recovery time measured every minute. A fly was considered as having recovered when able to stand on its legs. For the mortality assay, cold tolerance was determined by exposing vials of 20 females to -2°C for 2 h and survivors were scored after 24 h. Tests on the hybrid-line set involved preculture at 25°C in constant light. Number of survivors were analysed by nested ANOVA.

Statistics

Climatic data, downloaded from the Australian Bureau of Meteorology website (www.bom.gov.au), were obtained from neighbouring weather stations closest to the collection site. The climatic variables were: average daily maximum temperature (°C) for the hottest calendar month; average daily minimum temperature (°C) for the coldest calendar month; mean monthly rainfall for the wettest month; mean monthly rainfall for the driest month; mean humidity levels at 9 am for the most humid month; and mean humidity levels at 9 am for the driest month. Temperature, rainfall and humidity averages were based on all the available data for the particular weather station usually exceeding 100 years. Associations between latitude were initially tested using linear regression (SPSS 7.0 for Windows). To test for associations between allele frequency, latitude and climatic variables, Mantel matrix comparison tests (and partial mantel tests using matrices of residuals based on standardized differences) were carried out (Fortin and Gurevitch, 1993). These tests minimize the complications of spatial autocorrelation (nonindependence of data points). Matrix comparisons utilized GENETOP, version 3.1 (<http://www.cefe.cnts.mop.ft/> Raymond and Rousset, 1995).

Results

The *hsr-omega* association with heat tolerance in laboratory studies and with latitude, previously reported (McColl *et al*, 1996), is for an A/T variation at the 5' end of the gene that is detected by denaturing gradient gel electrophoresis (DGGE; Figure 2a, b). Since scoring large samples by DGGE is time consuming, and since linkage disequilibrium across many polymorphic sites has been indicated for this region (McKechnie *et al*, 1998), to expedite scoring of allelic variation, we developed a simple gel technique for an adjacent polymorphic site,

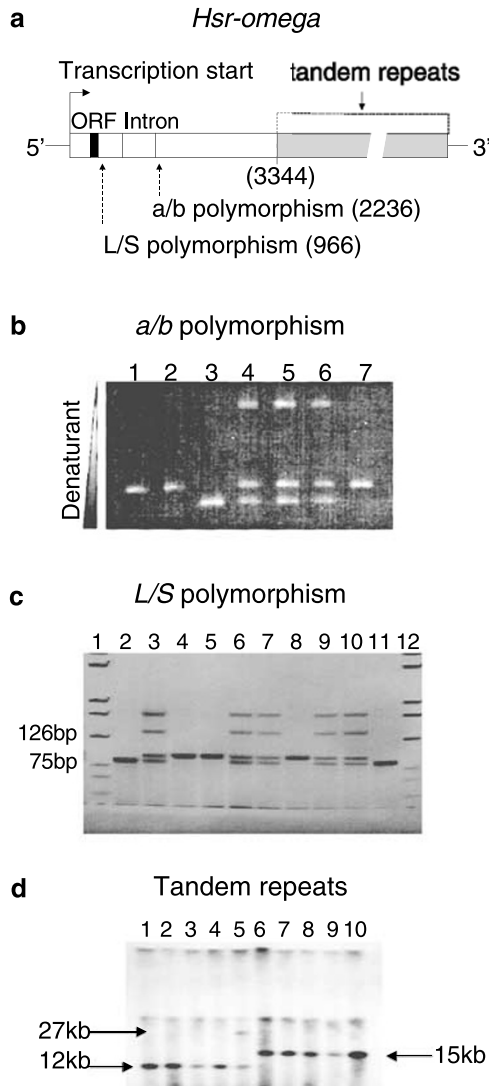


Figure 2 (a) *hsr-omega* structure and polymorphic sites. ORF marks a potential open reading frame. (b) Separation of *hsr-omega^a* and *hsr-omega^b* alleles by denaturing gradient gel electrophoresis. Lanes 1, 2 and 7: *hsr-omega^a* homozygotes, lane 3: *hsr-omega^b* homozygotes and lanes 4–6: *hsr-omega^{a/b}* heterozygotes. Top bands in lanes 4–6 are heteroduplex bands. (c) Separation of *hsr-omega^L* and *hsr-omega^S* alleles by horizontal thin-layer polyacrylamide gels. Lanes 1, 12: pGem DNA marker (Promega); lanes 2, 11: *hsr-omega^S* homozygotes; lanes 3, 6, 7, 9, 10: S/L heterozygotes; lanes 4, 5, 8: *hsr-omega^L* homozygotes. (d) Southern blot of hybrid lines. Lanes 1–5: L1–L5 lines, respectively, lanes 6–10: S1–S5 lines, respectively.

an 8 bp indel polymorphism (Figure 2a, c). The insertion-bearing allele, L, occurred within chromosome sequences that encoded *hsr-omega^a* (McKechnie et al, 1998). We refer to the indel variants as the *hsr-omega* long (*hsr-omega^L* or L) and *hsr-omega* short (*hsr-omega^S* or S) alleles, respectively, indicating the presence (L) or absence (S) of the indel. More distally, at a 3' position in the gene, extensive variation in the number of tandem repeats occur. Some laboratory strains have as few as 5 kb of a 280 bp repeat unit and others as many as 16 kb of these repeats (Hogan et al, 1995). We also detected the repeat-length variation at the 3' end of the gene using Southern blots (Figure 2a, d).

To look for thermal phenotype differences associated with the chromosome 3 variation, we created a set of lines that were controlled for the chromosomal region tightly linked to the L/S variation but that had randomized genetic backgrounds for other genetic differences that occur between high and low latitude populations. We examined the set of five L and five S lines for the presence of *In(3R)P* by crossing each line to a stock known to be homozygous for the standard chromosome 3 arrangement and cytologically inspected the F1 larval salivary gland squashes. Without exception the derivation process resulted in fixation of the L lines for the standard arrangement and fixation of the S lines for the inverted arrangement. We also examined these 10 lines for the 3' repeat-length variation of *hsr-omega* and found that the different sets were virtually fixed for different repeat-length alleles (Figure 2d). The S lines were each homogeneous for repeats totalling about 15 kb in length and the L lines for repeats with a total length of about 12 kb (except for one variable line that contained both 12 and 27 kb repeats). The data suggest that the original two north and south lines chosen for hybridization had been fixed for either the noninverted right arm of chromosome 3 (the southern L line) or the inverted (*In(3R)P*) right arm of chromosome 3 (the northern S line). L and S have acted as markers for linked blocks of genes in this region of chromosome 3. In this instance, S marked both the presence of *In(3R)P* and an allele of *hsr-omega* that contained about 15 kb of 3' repeats and no 8 bp indel at the L/S site.

Using a small-vial tolerance test, the hybrid lines and their parental strains were tested for heat resistance, both with and without a prior mild-heat hardening treatment. The relative heat resistance, for both unhardened and hardened flies, as measured by average knockdown time is depicted in Figure 3a. No significant difference between the parental strains was detected. However, a significant effect of genotype was indicated by nested ANOVA when the analysis was carried out on the 10 lines marked by L and S ($F_{1,8} = 6.60$, $P < 0.05$), with the L lines being more heat susceptible than the S lines (and no suggestion of any interactions). Effects of hardening ($F_{1,8} = 1.029.2$, $P < 0.001$) and of line within genotype ($F_{8,180} = 3.81$, $P < 0.001$) were detected. A genotype by hardening interaction effect ($F_{2,12} = 5.51$, $P < 0.05$) was detected when the five H lines, which were each polymorphic for the L/S markers, were included in the analysis. This was because of the H lines displaying a relatively high, unhardened heat resistance like S lines, but a hardened heat resistance that was relatively low like the L lines. These data support the hypothesis that the chromosome 3 segment marked by the L/S variation is part of the genetics that underlies an individual's susceptibility to heat stress.

The different ways that an organism experiences thermal stress may challenge different physiological and genetic mechanisms. To examine the generality of any effect of this variation on heat resistance, a large-tube heat knockdown test was also carried out on batches of adults from four of the hybrid lines, two L lines and two S lines, both with and without prior hardening. Relative knockdown times for nonhardened lines did not indicate a significant genotype, or line within genotype, effect. However, relative knockdown times for the heat-hardened lines indicated a significant difference between

genotypes ($F_{1,2} = 55.33$, $P < 0.05$), with no effect of line (Figure 3b). This difference in sensitivity between hardened and nonhardened heat tolerance tests for detecting variation between L and S lines is consistent with previous results indicating that *hsr-omega a/b* variation has a larger differential effect for hardened resistance (McCull *et al*, 1996). The L lines, carrying the chromosome 3 genotypes that occur at higher frequency in cool-temperate Australia, were also the more heat

susceptible in this alternate test of heat stress, having shorter relative knockdown times after hardening than the S lines. Thus, for two different measures of heat-resistance S lines outperformed L lines.

We also measured cold tolerance on the set of hybrid lines and their two parental strains using a mortality assay after a short exposure to -2°C . Significant variation occurred among genotypes ($F_{2,14} = 21.40$, $P < 0.001$) with no effect of line within genotype. L lines had a higher cold tolerance (Figure 3c), with a Tukey *post hoc* test indicating that only this genotype was clearly different to the S and H line groups. The parental line that originated from the southern cool-temperate region (that carried the L marker) clearly had a higher cold tolerance than the northern-derived parental line (that carried the S marker). We also used a chill-coma recovery measure of cold tolerance to see if the hybrid-derived L lines were more cold tolerant than the S lines in this different measure of cold sensitivity (Figure 3d). Again, the L lines were more resistant, recovering faster from the chill coma in both females ($F_{1,121} = 24.73$, $P < 0.01$) and males ($F_{1,116} = 5.70$, $P < 0.05$). The chromosome 3 element marked by L therefore provided elevated cold resistance over S-marked elements for two different types of cold stress. The data suggest that the alternate genetic elements fixed in the S and L lines each provide a positive boost to fitness under one type of thermal stress at the cost of conferring a negative fitness effect at the opposite temperature extreme.

We asked if the *hsr-omega L/S* variation marked a region of chromosome 3 that varied clinally as did the previously reported *hsr-omega a/b* variation. Frequency estimates of the L allele along a latitudinal transect of Australian east-coast sites (Figure 4a), all taken at low altitudes of < 200 m, are plotted in Figure 4b and indicate a positive association with latitude. This geographical L/S variation is spatially autocorrelated, since a Mantel difference-matrix comparison between all sites considered pairwise for L allele frequency and linear distance indicated a highly significant association ($P < 0.0001$). Almost exactly the same level of association occurred if latitudinal differences are substituted for linear distance in the matrix comparison. Further, when population differences in L frequency are aligned with geographic separation distances in a partial Mantel test (Fortin and Gurevitch, 1993) that controls for latitudinal differences,

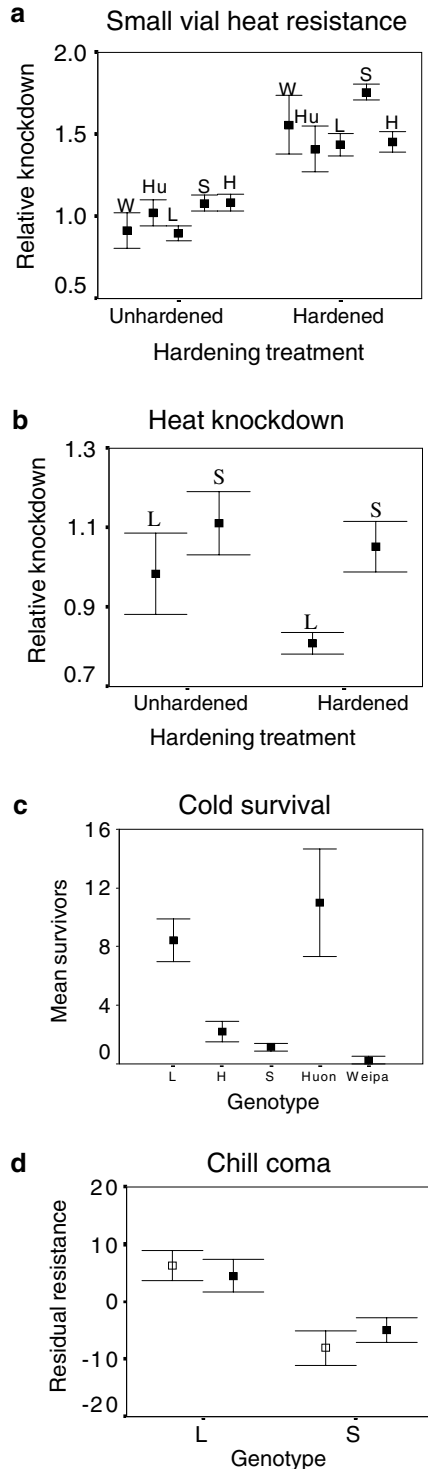


Figure 3 Thermal tolerance measurements. (a) Small-vial heat resistance of hardened and unhardened parental (W: Weipa, Hu: Huon) and hybrid lines (L: fixed for *hsr-omega^L*, H: 50% each allele, S: fixed for *hsr-omega^S*). ■: means based on 10 replicates for parental lines, 10 replicates for each of five lines for hybrid lines. Error bars represent standard error of the mean. (b) Large tube hardened and unhardened knockdown of two L lines and two S lines. ■: mean relative knockdown when compared to a control line, based on three replicates. Error bars represent standard error of the mean. (c) Mean survival after exposure to -2°C for 2 h of parental (Huon and Weipa) and hybrid (L, H, S) lines. ■: mean number of flies surviving based on four replicates for each of five lines in the hybrid lines, and four replicates for parental lines. Error bars represent standard error of the mean. (d) Estimates of chill coma recovery time based on measurements of 15 flies for each of four lines (S) and five lines (L). □: mean female residual recovery time based on measurements of 15 flies for each of four lines (S) and five lines (L). ■: mean male residual recovery time based on measurements of 15 flies for each of four lines (S) and five lines (L).

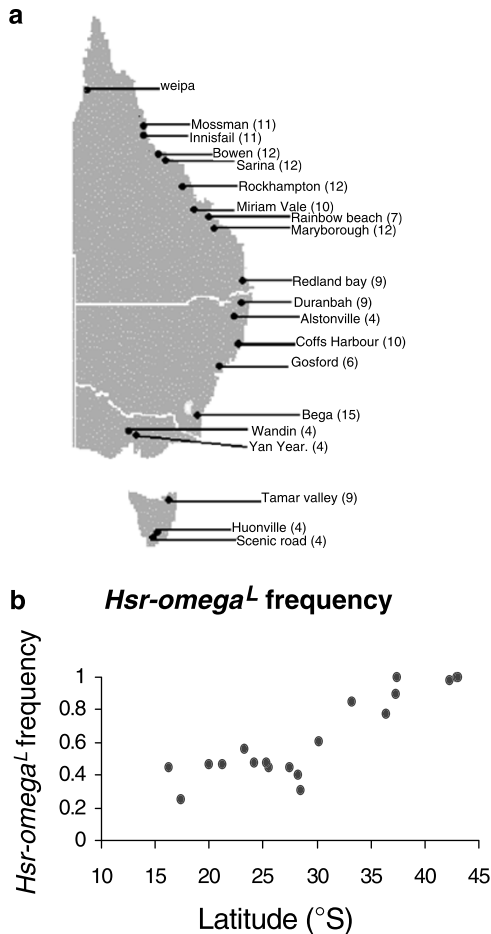


Figure 4 Clines. (a) Map of collection sites from the 3000 km transect on the eastern coast of Australia. Numbers in brackets represent number of isofemale lines genotyped for *hsr-omega*^{L/S} variation (Weipa excluded from latitudinal analyses). Latitudes of sites: Weipa 12.40°S, Mossman 16.27°S, Innisfail 17.32°S, Bowen 20.01°S, Sarina 21.25°S, Rockhampton 23.22°S, Miriam Vale 24.19°S, Rainbow Beach 25.54°S, Maryborough 25.31°S, Redland Bay 27.39°S, Duranbah 28.18°S, Alstonville 28.5°S, Coffs Harbour 30.19°S, Gosford 33.25°S, Bega 36.41°S, Yan Yean 37.34°S, Wandin 37.45°S, Tamar Valley 42.24°S, Huon 43.01°S, Scenic Rd 43.03°S. (b) Estimates of *hsr-omega*^L frequency of 19 collection sites.

or aligned with latitudinal differences in a partial Mantel test that controls for separation distance, the significant associations are completely lost. Since the sample sites were chosen to lie along a near linear, north–south transect, these latitude and linear distance comparisons are behaving as one-and-the-same variable in the Mantel tests. The significant associations confirm the presence of a latitudinal/spatial cline in frequency of that region of chromosome 3 marked by the L allele among Australian east-coast populations, as previously indicated for the *hsr-omega*^{a/b} variation (McColl and McKechnie, 1999). If strong population disequilibrium occurs between the L/S variation and *In(3R)P*, clinal variation of *In(3R)P* could also underlie the clinal variation in this gene. Ongoing investigations should resolve these issues.

Using these latitudinal-transect data we examine L frequency associations with six climatic variables (temperature, rainfall and humidity statistics) in simple difference-matrix Mantel tests. Three of these, average

maximum temperature for the hottest month, average minimum temperature for the coldest month and monthly rainfall for the wettest month, show significant associations (all $P < 0.0001$). Since population differences for each of these three climatic variables are significantly associated with latitude differences (all $P < 0.01$), partial Mantel tests that controlled for latitude were undertaken. Only one of these, the partial Mantel test for maximum temperature differences, retained a significant association with L differences ($P < 0.01$). The association between L and latitude differences is completely removed ($P = 0.12$) in the partial Mantel test that controls for maximum temperature. Therefore, the L frequency differences between populations are robustly associated with population differences in maximum temperature in the hottest month, but not with latitude or with the other climatic variables. These data support the hypothesis that environmental temperature directly or indirectly acts as a proximate selective factor for the region of chromosome 3 marked by the L/S variation.

Discussion

Our field collection suggests that we have identified in *D. melanogaster* a major genetic element that occurs at high frequency in tropical populations from coastal eastern Australia and decreases in frequency at higher latitudes, being replaced clinally by the alternate element. The tolerance data support the idea that the element can have substantial effects on both heat and cold tolerance. These results are consistent with earlier data indicating that allelic variation of *hsr-omega* can underlie heat-tolerance variation (McKechnie *et al*, 1998). They are also consistent with an earlier study indicating that cold-tolerance variation in both adult and larval *D. melanogaster* is largely attributable to a chromosome 3 effect (Tucic, 1979).

What is the nature of the element marked by *hsr-omega* L/S? It seems likely that for our hybrid line data this element is the common cosmopolitan inversion *In(3R)P*, a large inversion covering about six major cytological units and therefore about 6% of the genome (perhaps 1000 genes). In an earlier study, the frequency of *In(3R)P* varied clinally in Australia, along almost the same east coast transect, being present at frequencies up to 60% in the north and almost absent in temperate populations (Knibb, 1982). The frequency of *In(3R)P* has been associated with latitude in several other continents, along with the other common cosmopolitan inversions (Knibb, 1982). What factors other than thermal tolerance may influence the *In(3R)P* cline? *In(3R)P* has also been postulated to be at a disadvantage at high latitudes because it is associated with reduced flight ability under cooler conditions, possibly related to decreased body size (Stalker, 1980). Body size increases with latitude (Partridge *et al*, 1999) and two microsatellite markers that associate with latitude and map within *In(3R)P* (Gockel *et al*, 2001) have also been related to body size in a QTL study (Gockel *et al*, 2002). Clearly, some fine dissection of variable clinal markers and trait associations in this chromosomal region is required to tease apart the chromosomal regions and selective processes at work. However, if the *In(3R)P* cline is still present in Australia, which seems likely, it may be this element that contributes to the opposing clines in tolerance to both temperature extremes (Hoffmann *et al*, 2002).

While an effect of inversions is to maintain coadapted blocks of genes that are inherited as a group (Lewontin, 1974; Krimbas and Powell, 1992), recombination at reduced frequency occurs for the common inversions and linkage disequilibrium is not always strong, especially for polymorphic sites centrally placed in the inversion (Hasson and Eanes, 1996; Andolfatto and Kreitman, 2000; Verrelli and Eanes, 2000). A number of single gene polymorphisms in *Drosophila*, that lie within or in close proximity to inversions, have shown various levels of population disequilibrium with the inversion, yet display independent clinal variation or climatic associations (Knibb, 1983; Van't Land et al, 2000). Thus, the genetic element responsible for these temperature tolerance effects might be smaller than that covered by *In(3R)P*. A group of tightly linked genes surrounding the *hsr-omega* alleles that were isolated in these lines could be the causal elements. If a genetic element smaller than the inversion were to underlie the L/S tolerance associations, we still need to be aware that these fitness associations might not be typical of all *hsr-omega* L and S alleles, given that our crosses started with two isofemale lines. Likewise, our data does not exclude the possibility that other *In(3R)P* chromosomes fail to show these thermal tolerance effects. Nonetheless, our data indicate that large thermal tolerance fitness effects, with potential to be ecologically relevant, are caused by a genetic element marked by *hsr-omega* L/S variation.

What are the candidate genes on the right arm of chromosome 3 that causally affect thermal tolerance and may be part of a coadapted genetic element? Four obvious candidates are *hsr-omega*, *hsp68*, *dca* and *frost*. *Frost* and *Dca* are two genes that are upregulated by cold treatment and map to chromosome 3R at 85E2 and 88D, respectively (Goto, 2000, 2001) (Figure 1). Of these four genes, the expression patterns and potential phenotype affects for *hsr-omega* are the best understood. The evidence is now substantial that allelic variation in *hsr-omega* underlies a significant fraction of the heat sensitivity variation in *D. melanogaster* (McColl et al, 1996; McKechnie et al, 1998). *Hsr-omega* is both constitutively expressed in most tissues and upregulated by environmental factors. The relative and total levels of two major *hsr-omega* RNA transcripts are labile and fast to change in response to many environmental signals. The gene is thought to play a role in the dynamic coordination of nuclear and cytoplasmic transcript processing under various stress and growth conditions (Garbe et al, 1989; Pardue et al, 1990; Lakhotia et al, 1999; Lakhotia et al, 2001). However, we need to be cautious about assigning any genotype effect on thermal sensitivity directly to the differential function of *hsr-omega* alleles. If thermal selection in the field is responsible for the *hsr-omega*^L-cline, the target of such selection may be groups of genes in linkage disequilibrium with *hsr-omega* variation. Transgenic studies with molecular constructs of *hsr-omega* that vary only at the relevant polymorphic sites are required to provide unambiguous evidence of effects of any single gene on these thermal fitness components.

In summary, our data suggest that polymorphic variation of *hsr-omega* is a marker for a genetic element on the right arm of chromosome 3, quite possibly *In(3R)P*, that provides insight into the genetics of adaptation to thermal extremes in a species that is widespread across diverse climatic regions. Variation of

this element, at least in part, explains the known opposing thermal tolerance clines that occur along the eastern coast of Australia. The element found at high frequency in the tropics provides high adult heat tolerance at this end of the cline but incurs a trade-off in also causing cold sensitivity. The element is clinally replaced in cool-temperate southern populations by an alternate element with reciprocal effects. Such clinal polymorphic variation may help the species have a broad climatic range and be a thermal specialist at each end of the range where perhaps only one thermal extreme is regularly encountered; each element, in the right climate, may provide the appropriate thermal resistance benefits with little cost. Limited gene flow along the transect could serve to smooth any 'bumps' in the cline if thermal selection-intensity thresholds happen to change abruptly over space. Also, the polymorphism at any particular latitude might provide seasonal population flexibility where temperature extremes in one generation are interspersed with the opposite extreme several generations later. In this simplistic model, the fitness trade-offs would facilitate selection that leads to seasonal changes in frequency, as has been reported for *In(3R)P* (Stalker, 1980). These data represent one of the few cases where opposing clinal selection on alternative genetic elements has been demonstrated and linked to geographic patterns and adaptive phenotypes.

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