Both allelic variation and expression of nuclear and cytoplasmic transcripts of *Hsr-omega* are closely associated with thermal phenotype in *Drosophila*

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ABSTRACT   Inducible heat shock genes are considered a major component of the molecular mechanisms that confer cellular protection against a variety of environmental stresses, in particular high temperature extremes. We have tested the association between expression of the heat shock RNA gene *hsr-omega* and thermoresistance by generating thermoresistant lines of *Drosophila melanogaster* after application of two distinct regimes of laboratory selection. One set of lines was selected for resistance to knockdown by heat stress and the other was similarly selected but before selection a mild heat exposure known to increase resistance (heat hardening) was applied. A cross between resistant and susceptible lines confirmed our earlier observation that increased thermal tolerance cosegregates with allelic variation in the *hsr-omega* gene. This cosegregating variation is attributed largely to two haplotype groups. Using quantitative reverse transcription–PCR, we find evidence for divergent phenotypic responses in the two selection regimes, involving both structural and regulatory changes in *hsr-omega*. Lines selected after hardening showed increased levels of the cytoplasmic transcript but decreased levels of the nuclear transcript. Lines selected without hardening showed decreased levels of the cytoplasmic transcript. The allelic frequency changes at *hsr-omega* could not by themselves account for the altered transcription patterns. Our results support the idea that the functional RNA molecules transcribed from *hsr-omega* are an important and polymorphic regulatory component of an insect thermoresistance phenotype.

Heat shock genes are considered an important component of stress responses in insects and other organisms, but data relating the expression of heat shock genes to resistance phenotypes is often associative and inconclusive. Studies on organisms such as lizards (1), fish (2), and limpets (3) show that the expression of these genes differs between related species and that there may be an association between this expression and environmental variation. However, correlation analyses based on species or population comparisons can be criticized for several reasons (e.g., ref. 4). Because organisms are not reared under identical conditions, differences among species may be environmentally modified. Moreover, comparisons of species often cannot be corrected for phylogenetic relatedness so that different species do not represent independent data points. Finally, correlations involving species and populations may arise even when associations are not causal; for instance, increased expression of a heat shock gene and increased resistance may be selected independently by different components of the environment, leading to spurious correlations.

One way of overcoming such problems is to consider associations between heat shock gene expression and resistance phenotypes within populations. In *Drosophila*, several approaches have been used to provide evidence that variation in heat shock genes can be associated with high temperature resistance. One of these has been the examination of resistance in genetically manipulated organisms. For instance, Welte *et al.* (5) found that insertion of extra copies of *hsp70* into the *D. melanogaster* genome increased resistance to heat stress in embryos. Another approach has related variation in resistance to variation in heat shock genes within a population. Krebs and Feder (6) undertook strain comparisons to examine the association between expression of *hsp70* and larval resistance in *D. melanogaster*. They found that lines with increased levels of *hsp70* expression had higher resistance levels. McColl *et al.* (7) used selection to generate variation in heat resistance among replicated lines of *D. melanogaster*. Lines had been selected both after hardening (i.e., rapid acclimation) and without a previous hardening period. They found that allele frequency changes in two heat shock genes (*hsr-omega* and *hsp68*) were associated with the selection response, and that allelic changes differed between the hardened and nonhardened lines.

The association between high temperature resistance and variation in the *hsr-omega* gene is particularly interesting. Characterization of the organization, expression, and function of this gene has been the subject of considerable, and ongoing, research over a number of years, mainly by Pardue and her colleagues (8–12). In adult flies, *hsr-omega* is constitutively expressed in most tissue types; in addition, its two RNA transcripts are stress-induced to higher levels. One transcript is found in the cytoplasm and the other in the nucleus, but no protein product is known (10). Although these transcripts share common heat-stress induction and are alternatively processed products of the same gene, the cytoplasmic transcript (*omega-c*) and the nuclear transcript (*omega-n*) are independently sensitive to different cellular regulatory signals (9). The correlated allelic changes observed by McColl *et al.* (7) suggest that expression levels of one or both transcripts may be associated with thermoresistance and the hardening response.

Here we test if constitutive levels of either or both transcripts of *hsr-omega* have changed as part of the selection response, using competitive reverse transcription–PCR (RT-PCR) with two pairs of unique primers to separately quantify the two transcripts. We also show that heat resistance cosegregates with *hsr-omega* after crossing a heat-resistant line derived by hardening and a susceptible control line. Finally, the molecular nature of allelic changes in the heat-resistant lines is described.

Abbreviations: RT-PCR, reverse transcription–PCR; DGGE, denaturing gradient gel electrophoresis.
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These data confirm that *hsr-omega* variation associates with heat resistance and demonstrate that expression levels have changed consistently in the replicated selection lines. Furthermore, the expression pattern changes depend on whether lines were hardened before selection. These findings indicate that both molecular allelic variation in *hsr-omega* and its expression characteristics are closely associated with knockdown heat resistance and with the hardening response of *D. melanogaster*.

MATERIALS AND METHODS

Lines. The stocks and selection procedure are described by McColl *et al.* (7). Briefly, selection was carried out on a mass-bred population of *D. melanogaster* initiated from the offspring of 62 females collected in February 1994. Flies were released at the top of an 80-cm tube similar to one described by Huey *et al.* (13) surrounded by a water jacket connected to a circulating water bath. Temperature inside the tube is maintained at 38.5–39°C. Flies roll down a series of baffles as they succumb to the heat stress. The last 20% of around 600 flies to be knocked down were used as founders for each generation of each selected line. This procedure was undertaken on two sets of three independently selected lines. The first set (the SH lines) was hardened by placing flies in a 37°C incubator for an hour, 6 hr before testing for knockdown resistance and selection. The second set (the SN lines) was selected without previous hardening. The set of three control lines (CN) were not hardened or selected. As described elsewhere (7, 14), the selection procedure resulted in a marked increase in knockdown resistance. This increase appears to be related at least partly to physiological heat resistance; the selection response does not involve increased desiccation resistance or quiescence because flies from selected lines remain active at a high temperature and do not show correlated changes in desication resistance (14). Lines were selected every generation until generation 20 (hardened lines) or generation 22 (nonhardened lines). They subsequently were maintained by selecting them every second generation until around generation 75, and since then they have been maintained without selection. Although the initial response to selection was fairly even, there is evidence for a selection plateau in some of the lines in the later generations (14).

We tested for thermoresistance after 27 generations. Lines were tested without previous hardening by placing around 600 flies in the knockdown tube. Collecting vials at the bottom of the knockdown tube were replaced every minute to generate a distribution of knockdown times. Mean knockdown times were determined on the basis of three replicate trials for each line.

Cosegregation of Knockdown Resistance. Lines CN-1 and SH-2 (generations 77 and 79, respectively) were crossed. Forty-five lines were established from single pair matings among F_2 individuals, and parental *hsr-omega* genotypes were determined (7) by denaturing gradient gel electrophoresis (DGGE). Thirteen of these lines, representing the full range of *hsr-omega*[a] frequencies (i.e., 0, 25, 50, 75, and 100%), were tested with and without hardening (described above) for heat knockdown resistance at generation F_5. For both treatments flies were cultured at 25° and aged in bottles for 3–4 days before three replicate bottles of ≈150 flies were isolated from each line. Flies were not anaesthetized at any stage. Each knockdown run involved a comparison of four replicate lines (≈150 flies per line), involving three lines with different allele frequencies plus the control line. Lines in the same run were distinguished by marking them with different colors (white, green, pale green, and pink) of fluorescent dust (Helecon, Hackettstown, NJ) as described elsewhere (7). Each of the lines was tested in three different combinations, giving a total of 12 knockdown runs for each treatment. Dust colors were randomized throughout. One line (0% *hsr-omega*[a]) was in-

cluded in all runs as an internal standard to control for variation between knockdown runs. Flies were immediately frozen after knockdown and then sorted by dust color under UV light. Mean knockdown times were determined for each line in each run. These times then were divided by the mean knockdown times of the control line in the same run to provide relative knockdown times (arcsin transformed in the regression analysis).

RNA Extraction. To characterize expression levels of *hsr-omega* transcripts, we isolated total RNA from each replicate line independently three times. RNA was isolated from unstressed whole flies (<1 week old) by using an Rneasy spin column (Qiagen) according to manufacturer's instructions. Between 30 and 50 mg of flies mildly anesthetized with CO_2 were pulverized in 600 μl of chaotropic lysis buffer using a homogenizer probe. Insoluble material was pelleted by two rounds of centrifugation at 20,000 g for 3 min. The lysate was processed immediately or frozen in liquid nitrogen and stored at −70°C.

RT-PCR Quantitation of Transcript Levels. DNase digestion was performed immediately before cDNA synthesis. Incubation was at room temperature for 15 min before chelating Mg^{2+} with 2.5 mM EDTA at pH 8.0. The DNase was inactivated at 65°C for 10 min, then 1 min on ice, followed by brief centrifugation and immediate RT. First-stranded cDNA was synthesized from 1 μg of total RNA by using the Superscript Preamplification System (Life Technologies) and the reverse PCR amplimer to prime RT. One-tenth of the RT reaction was used as source of template in PCR. The primers for *omega-c* (cF, 5′-GGAATTTCGCAATGCAG; cR, 5′-CAGACACAGCTTTCGAATAACG) were designed from published sequence (15) to generate different-sized amplicons from spliced (*omega-c*; 298-bp product; Fig. 1) and unspliced (*omega-n*; and *omega-pre-c*; 1,011-bp products) transcripts for the quantitation of *omega-c*. The primers for *omega-n* (nF, 5′-TGAAACAAGTCGAAAAATCGG; nR, 5′-CAGACA-CAGCTTTCGAATAACG) were designed from unpublished sequence (EMBL file DM18307) to amplify a region of RNA unique to *omega-n* (154-bp product) and allow its quantitation. A constant amount (0.4 pg) of a single-stranded multispecific heterologous RNA competitor fragment containing both the c and n primer binding sites was coamplified by RT-PCR to control for the variable efficiency of both reactions (16, 17). The competitor amplifies with equivalent efficiencies to both the c and n amplicons (M.M.H. and S.W.M., unpublished results). Amplified products were separated by horizontal ultrathin PAGE using a discontinuous buffer system (18), stained with silver (19), and quantitated by densitometry. Correction for competitor size difference from target was performed for precise calculation of target/competitor ratios.

DNA Sequencing. Sequence analysis was performed on 12 lines that were isogenic for chromosome III. These lines were generated from selected (SH-2) and control (CN-2) populations at generations 45 by using the balancer stock BLT: *In(3LR)Ubx*[130]*e/In(3LR)Sb*. Primers were designed from unpublished sequence (EMBL file DM18307), and amplicons were directly sequenced by using a PRISM Dye terminator cycle sequencing kit (Applied Biosystems) according to manufacturer's instructions.

RESULTS

Resistance of Lines at Later Generations. At generation 27, overall means and standard errors were determined from knockdown time (in min) for the replicate lines within each selection and control regime. These values indicate that a strong response to selection was still evident for both the hardened (16.44 ± 2.09) and nonhardened (20.00 ± 4.52) lines; there was a 3- to 4-fold increase compared with the control line (5.82 ± 0.11) in average knockdown time when flies were
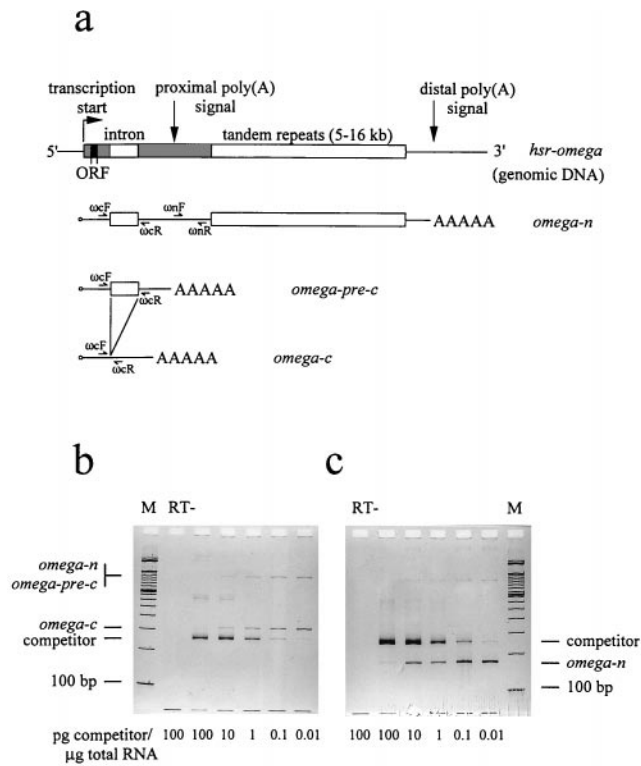
FIG. 1. Competitive RT-PCR assay of constitutive *hsr-omega* RNA abundance in thermoresistant lines. (*a*) RT-PCR strategy. Positions of forward (F) and reverse (R) primers to uniquely detect each transcript (*omega-c* = ωcF, ωcR; *omega-n* = ωnF, ωnR) are indicated adjacent to the transcripts. These underlie the depicted genomic structure of *hsr-omega*. ORF, potential ORF (10). (*b*) Gel showing RT-PCR amplification of *omega-c* over a range of competitor concentrations that allowed estimation of absolute transcript levels (track M, 100-bp ladder size standard; RT-, control lacking RT, see *Materials and Methods*). (*c*) Equivalent gel showing RT-PCR amplification of *omega-n*.

exposed to 39°C. There was almost no overlap between the heat knockdown distributions of the control and selected lines.

Cosegregation of Knockdown Resistance. To test the association between the *hsr-omega*[a] allele and low resistance, a cross was set up between a control line and a hardened selected line at generation 79. A subset of 13 of the derived lines was examined for both knockdown resistance and *hsr-omega*[a] frequency at F$_5$. Resistance, measured after hardening, was tightly associated with the frequency of *hsr-omega*[a] (Fig. 2); lines with a low frequency of *hsr-omega*[a] exhibited greater
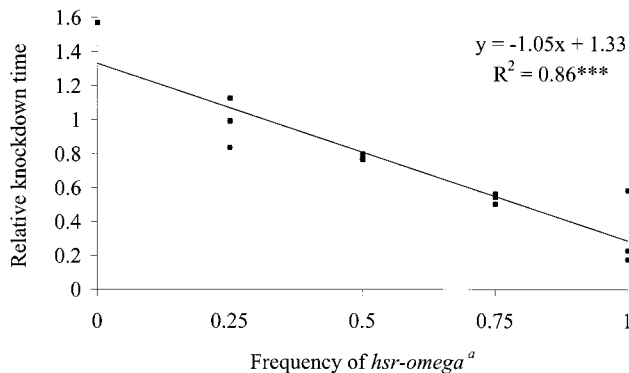

FIG. 2. Distribution of hardened knockdown heat resistance among 13 lines derived from a cross between a susceptible CN line and a resistant SH line, and their regression analysis against *hsr-omega*[a] frequency. ***, $P < 0.001$.

hardened resistance to heat knockdown. Similar results were found when resistance was measured without hardening on the same set of lines ($y = -1.19x + 1.47$, $R^2 = 0.84$, $P < 0.001$). Slopes of the hardened and nonhardened regression lines did not differ significantly ($F_{(1,26)} = 0.51$, $P = 0.48$). Therefore even after a generation of recombination of genetic backgrounds of the susceptible and resistance lines, there was a strong negative association between *hsr-omega*[a] frequency and both hardened and nonhardened resistance to knockdown.

Sequence Variation. To examine the molecular nature of the *hsr-omega*[a/b] polymorphism, a 1,593-bp region of the gene was sequenced in 12 copies of the gene that were separately isolated from control and selected lines. Six of these were *hsr-omega*[a] and six were *hsr-omega*[b], as determined by DGGE (7). The region sequenced included the 432 bp that gave rise to the PCR product that was used for genotype determination. The 3′ end of the 432-bp product terminated at position 2,256 (Fig. 3).

Eighteen polymorphic positions were detected in this 1,593-bp region (and an additional site, 1,575 of the EMBL sequence, that was deleted from all 12 sequenced alleles). The *hsr-omega*[a/b] variation is attributable to only one of these 18 sites, the T to A transversion at position 2,236, because this variation is the only polymorphic position in the 432-bp PCR product. Fifteen of the other polymorphic positions were, in our relatively small sample, in complete linkage disequilibrium with the *hsr-omega*[a/b] polymorphism. This polymorphism included 14 positions involving single base changes within the intron and an 8-bp deletion in the first exon (at position 966), about 60 bp upstream of a conserved region (20) that includes the 5′ intron boundary site. Among the 12 alleles, only four unique sequences were detected and these clearly fell into two haplotype groups. One group, represented by three sequences, encoded *hsr-omega*[a] alleles and all had present the 8-bp "deletion" sequence. The second group, with six identical representatives, encoded *hsr-omega*[b] alleles that were missing the 8-bp deletion sequence.

Quantitation of Transcript Levels. Constitutive levels of nuclear and cytoplasmic transcripts from *hsr-omega* were determined in adults. In separate tubes for each transcript we carried out quantitative RT from each RNA preparation, each mixed with an identical predetermined quantity of a mimic RNA transcript. This RNA provided a standard "competitive"
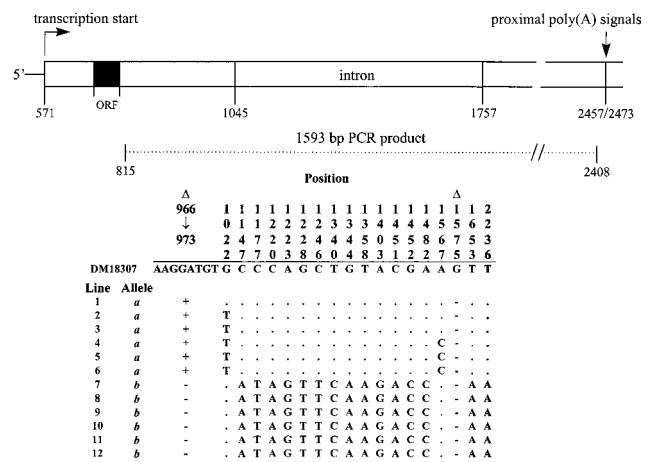
FIG. 3. Nucleotide variation of a 1,593-bp segment of *hsr-omega* among 12 alleles from the heat-resistant and susceptible lines. Dotted line under the block diagram of the 5′ end of the gene indicates the segment sequenced. Depicted are only the variable nucleotide positions (numbered) adjacent to the equivalent EMBL sequence (file DM18307). Dots and positions not included indicate bases are identical to the EMBL entry. Δ indicates a deletion variant. Allele *a* indicates *hsr-omega*[a], and *b* indicates *hsr-omega*[b], as determined by DGGE. Gray shading represents exon sequence. Figure not to scale.

template, and the reaction was followed by quantitative PCR on the cDNA products. Initially, from a thermosensitive Canton S strain, and by spiking replicate RT reactions with a dilution series of RNA competitor, both transcripts were estimated to be at medium-level abundance by electrophoresis, silver staining, and densitometry (Fig. 1). Thus 1 μg of total RNA was estimated to contain 3.87×10^6 *omega-c* transcripts and 3.59×10^6 *omega-n* transcripts. This finding contrasts to the higher levels ($>10^8$ molecules/μg total RNA) for housekeeping genes such as 36-actin (21), and the lower levels (10^4 molecules/μg total RNA) for genes encoding regulatory proteins such as the platelet-derived growth factor receptors (22).

Transcript Expression in Lines. Correlated with the increased resistance to thermal knockdown under both laboratory selection regimes were consistent changes in *hsr-omega* transcript levels. Lines selected for resistance to heat knockdown (the SN lines) had decreased constitutive levels of *omega-c* ($F = 104.9$; df = 1,14; $P < 0.001$), to only 37% that of the thermosensitive control lines, and no difference in constitutive levels of *omega-n* (Fig. 4). In contrast the resistant lines selected only after hardening (SH lines) had increased *omega-c* levels ($F = 73.3$; df = 1,13; $P < 0.001$), to 240% that of the control lines, and a simultaneous decrease of *omega-n* ($F = 47.7$; df = 1,13; $P < 0.001$) to 50% of control lines. The low variance in *omega-c* levels among the SN lines and *omega-n* levels among the SH lines suggest that the low absolute levels of these transcripts have been optimized by the selective environments.

Transcript-Length Polymorphism. The quantitative RT-PCR analysis was able to separate the 8-bp deletion polymorphism that was located in the template region used for the *omega-c* RT-PCR products. Resolved as dual peaks after densitometry, this variation was present in all lines of the selection experiment (Fig. 5), but not in our Canton S strain. Because the technique is quantitative (and if we reasonably assume that expression of the two alleles is quantitatively equal) we have been able to estimate their relative frequency in each line, the control lines having the longer allele (*omega-c^L*) at an average frequency of 0.77. Both selection regimes have decreased the frequency of *omega-c^L*; selection with hardening led to the relatively greater decrease, to about 0.15

after 27 generations (Fig. 5). Thus both selection regimes have led to a similar change in allele frequency at the *hsr-omega* locus, the shorter allele *omega-c^S* being associated with increased resistance.
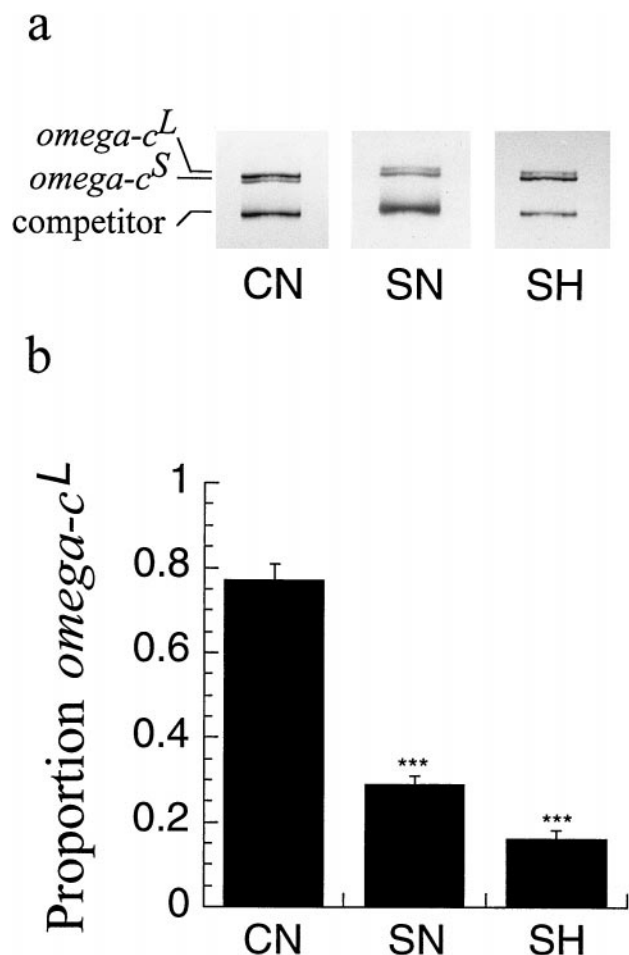
DISCUSSION

By crossing a line selected to have high levels of heat knockdown resistance to a susceptible control line, we have shown that resistance cosegregates with a polymorphic allele of *hsr-omega*. Together with the highly consistent changes that have occurred in *hsr-omega* transcript levels under selection, these data provide evidence that this gene probably is part of the mechanism that provides knockdown heat resistance for adult flies. These findings are consistent with earlier results on allele frequency changes in the selected lines that implicated *hsr-omega* (7).

Our findings highlight the fact that hardening has a marked influence on genetic changes underlying the selection response. Previous results indicated that selection for knockdown resistance after hardening increased the frequency of one of the *hsr-omega* alleles detectable by DGGE, whereas this allele was not significantly altered in lines selected without previous hardening (7). This finding suggested that one of the *hsr-omega* alleles is specifically associated with the hardening process. We have shown that fundamental differences have occurred between nonhardened and hardened selected lines in the nature of changes in specific transcript levels. Individuals with low levels of *omega-c*, regardless of their *omega-n* level, have higher knockdown heat resistance, whereas those with high *omega-c* and low *omega-n* have higher levels of hardened heat resistance. The latter may mount a more effective hardening response. The expression characteristics of *hsr-omega* therefore are linked to the levels of resistance obtained after hardening.

We have found additional polymorphisms in the *hsr-omega* gene, additional to the T/A variation in the second exon detected by DGGE. Within the 1,593-bp sequenced region 18 variable positions were detected (1.1% of sites) among the 12 alleles characterized. Seventeen polymorphic positions each involved a single bp change, and the remaining polymorphism

FIG. 4.   Scattergram illustrating constitutive levels of *hsr-omega* RNAs in thermoresistant and control lines. Line designations are described in the text. Different shading of data points within a treatment refer to different replicate lines and at least two independent RNA isolations from each line are shown. Amplicon levels are shown relative to the 0.4 pg of competitor RNA with which they were all coamplified.

a

b

Fig. 5.   Quantitation of *omega-c*^{L} and *omega-c*^{S} alleles in thermoresistant and control lines (labels as in Fig. 4). (*a*) Examples of *omega-c* RT-PCR products from single RNA isolations from each treatment group. (*b*) Comparison among selected lines of mean ($\pm$ SE) of *omega-c*^{L} transcript level expressed as a proportion of total *omega-c* level in each preparation. Both SN and SH lines differ significantly in *omega-c*^{L} proportion from the CN lines (data arcsin transformed); ∗∗∗, $P < 0.001$.

involved an 8-bp deletion of a repeated sequence. Seven of the eight bases of the deletion sequence were repeated just upstream: 5′ . . . **CAGGATGT**A̲A̲G̲G̲A̲T̲G̲T̲GTG . . . 3′ (the underline indicating the polymorphic deletion). Modeling of RNA secondary structure suggests this deletion could have marked destabilizing effect on the stem-loop structures in this region (G.M., unpublished observation). Our data suggest strong linkage disequilibrium in this region of the gene, with two common allelic groups or lineages that differ on average at 17 positions. One group encodes the *hsr-omega*^{a} alleles and has the 8-bp deletion sequence present, the other encodes *hsr-omega*^{b} alleles and is missing the deletion sequence. Variation present in natural populations is likely to be greater than observed here because our sample size was small. The sample also came from two laboratory lines (isolated from one collecting site), and one of the lines had been selected for heat resistance. We note that these lines did not show an association between the extensive 3′ length polymorphism for repeating units (12) and heat resistance (M.-L. Pardue and K. Travers, personal communication), an observation suggesting that the 3′ variation is not contributing to variation in the heat knock-down phenotype. This finding also implies that the 5′ disequilibrium may not extend to the 3′ end of the gene.

The quantitative RT-PCR analysis led to a second association of *hsr-omega* allelic frequency with heat resistance in these lines. The polymorphic deletion marker (*omega-c*$^{L/S}$) was associated with resistance in both nonhardened (SN) and hardened (SH) selected lines. McColl *et al*. (7) found a decrease in the frequency of *hsr-omega*^{a} marker in the SH lines but not, on average, in the SN lines. This finding contrasts with the decrease in *omega-c*^{L} that was evident in both sets of selection lines after 27 generations (recall that *omega-c*^{L} is a possible marker for *hsr-omega*^{a}, and the other adjacent polymorphic sites, because of the disequilibrium). However, the results presented here still may be consistent with our earlier work with *hsr-omega*^{a}, which reflected allelic changes after only eight generations of selection; the frequency of *hsr-omega*^{a} may have been low in all selected lines at generation 27. Also, there was some heterogeniety at generation 8 in the rate of response to selection, one replicate among the SN lines having both a particularly high resistance level and low frequency of *hsr-omega*^{a}. *Hsr-omega*^{a} and *omega-c*^{L} are likely markers of the same "allele."

It is possible that the allele frequency changes in *hsr-omega* account partially for changes in the transcript levels. However, the similar directional change in frequency of the *hsr-omega-c*^{L} allele under the different (hardened vs. nonhardened) selection regimes argues against a direct causal relationship between the structural allele type and regulation of *omega-c* levels, which varied in opposite directions under the different selective regimes. Instead, changes at other regulatory loci probably also influence transcript levels. Part of the effects of selection may have been to change the transport or distribution of the alternatively processed transcripts between nucleus and cytoplasm.

Hsr-omega is located at polytene locus 93D and is one of the major heat shock-inducible genes. Like the heat shock protein genes, it shares heat shock control elements required for transcriptional up-regulation during heat shock. Under a mild heat shock (31°), at which growth of wild-type flies still can occur, adults that lack the *hsr-omega* heat shock elements do not survive (11). Evidence of this nature in combination with the heat resistance association with divergent changes in *hsr-omega* transcript levels argues for a fundamental role of the gene in determining heat resistance. The possibility that the selection response is largely the consequence of a tightly linked gene in disequilibrium with *hsr-omega* is less likely. Other genes will be involved in determining the resistance response, like the linked *hsp68* located adjacent to *hsr-omega* at position 95D. However, the cosmopolitan inversion In(3R)P, which covers the location of both of these genes, is unlikely to be involved in the selection response because the founder population for the study came from a southern collecting site where In(3R)P is rare (23). Interpreting the phenotypic significance of the specific changes in *hsr-omega* expression patterns will require a more detailed understanding of the gene's function. *Hsr-omega* may be involved with the synchronization of cytoplasmic and nuclear activities that increase metabolic efficiency, especially under changed environmental conditions (11). However, whatever its cellular function(s), the heat inducibility of *hsr-omega* would appear to be an important regulatory component that brings about heat resistance in *D. melanogaster*. It remains to be seen if *hsr-omega* expression is involved with hardening to stresses other than heat, although our selection lines did not show correlated increases in resistance to other stresses (14).

The stress-inducible polytene chromosome puffs of *Drosophila* represent activation of a widely conserved genetic system (24, 25). The 93D puff is unusual among the *D. melanogaster* heat shock puffs (9, 26, 27), and its unique features are shared by only one of the heat shock puffs of other *Drosophila* species (28, 29). Comparative analysis of DNA sequence from such loci confirm that they share a similar organization but divergent sequence (20, 30, 31). Evidently, sequence evolution at *hsr-omega* has been more rapid than at

other heat shock loci. Nevertheless, the *hsr-omega* locus or its functional equivalent may be common to many organisms (11). Understanding the role of *hsr-omega* in the heat shock response in *Drosophila* may reveal molecular mechanisms for stress resistance that are shared with other organisms.

Natural populations appear to be rich in *hsr-omega* length polymorphisms because in addition to *omega*-c$^{L/S}$ described here, and variation in copy number of tandem repeats in the 3′ region (12), we found another length polymorphism within the intronic region of Canton S (M.M.H. and S.W.M., unpublished results). These findings could provide part of the adaptive potential of a population for responding to periods of climatic stress. Our data indicate that trans-acting regulators of *hsr-omega* expression are also a component of this machinery. In general these findings provide a link between our growing understanding of the molecular/cellular biology of the stress-inducible genes of *Drosophila* and a whole organism phenotype of potential ecological significance.

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