Journal of Gerontology: BIOLOGICAL SCIENCES
1997, Vol. 52A, No. 1, B48–B52

Heat-Induced Longevity Extension in *Drosophila*. I. Heat Treatment, Mortality, and Thermotolerance

Aziz A. Khazaeli, Marc Tatar, Scott D. Pletcher, and James W. Curtsinger

Department of Ecology, Evolution, and Behavior, University of Minnesota.

Survival data were collected on a total of 28,000* Drosophila melanogaster *adults in order to investigate mortality patterns and induced physiological responses after a mild thermal stress. A brief, nonlethal heat treatment extends adult life span at normal temperatures by an average of 2 days (64), compared to nontreated controls of the same genotypes. Life expectancy is extended as a demographic consequence of reduced age-specific mortality over a period of up to several weeks after the heat treatment. Heat treatment also increases tolerance to subsequent, more severe thermal stress. Observations on single-sex populations suggest that heat-induced longevity extension is independent of the suppression of reproductive activity.

THERE are two well-known environmental treatments that extend adult longevity in animals reared at normal temperatures: dietary restriction and curtailed reproduction (Bell and Koufopanou, 1986; Holehan and Merry, 1986; Chippendale et al., 1993; Tatar and Carey, 1995). Recently, a new longevity-enhancing environmental treatment has been described: Lithgow et al. (1995) reported that brief exposure to elevated but nonlethal levels of heat increased the average life span of *Caenorhabditis elegans* reared at normal temperatures by 2.7 days, or about 15%, compared to controls that were not heat treated. A connection between heat treatment and life span at normal temperatures is also suggested by the observation that genetic mutations that enhance life span confer enhanced ability to withstand extreme temperatures (Lithgow et al., 1994, 1995).

In many organisms, exposure to high temperatures induces two phenomena: thermotolerance, the ability to survive subsequent exposure to normally lethal levels of heat (Li and Laszlo, 1985; Lindquist and Craig, 1988; Solomon et al., 1991; Parsell and Lindquist, 1993; Krebs and Loeschcke, 1994a,b; Feder et al., 1995), and rapid expression of heat shock proteins (hsps). Some of the hsps are known to protect against thermal stress and act as molecular chaperones (Ellis, 1993; Parsell and Lindquist, 1993). Thermotolerance and hsp induction have been intensively investigated in a wide range of species, but the possible connection between brief thermal treatment and extension of life span at *normal* temperatures is new and potentially of interest to gerontologists.

The present study reports the effect of brief exposure to elevated temperatures on life span at normal temperatures in the fruit fly *Drosophila melanogaster*. A preliminary *Drosophila* experiment in our laboratory suggested a slight but statistically significant heat-induced longevity extension (data collected by A. Khazaeli are reported in Curtsinger et al., 1995). Here we examine the phenomenon on a larger scale, determine whether it is repeatable, and demonstrate its relationship to induced thermotolerance.

Materials and Methods

Stocks. — We used two highly inbred lines of *Drosophila melanogaster* (JWC15 and JWC24) derived from a large, random-mating population established in 1980. The lines have undergone more than 80 generations of full-sib or half-sib mating, giving an inbreeding coefficient very close to 1.0. Flies were cultured under controlled larval density in half-pint bottles with yeasted cornmeal-molasses-agar medium. Stocks were maintained at 24 °C, with constant illumination and 55–65% relative humidity.

Experimental flies. — Unmated flies were collected under light CO_2 anesthesia within 8 hours of eclosion for single-sex cohorts. Flies were collected within 24 h of eclosion for mixed-sex cohorts. Flies were then transferred in groups of 500–700 (counted for single-sex populations; estimated by weight for mixed-sex populations) to cages made from 3.8-liter plastic jars (see Fukui and Kirscher, 1993; Fukui et al., 1996). The mouth of each jar was covered with a screen, and the jars were inverted over an 11-cm-diameter disc of food, which was replaced every other day. Each day dead flies were removed, sexed, and counted until the last death.

Longevity experiments. — To determine whether exposure to nonlethal heat affects longevity at normal temperatures, we heat-treated 4-day-old experimental flies at 36 °C under saturated humidity for 70 min. Prior to and following the heat treatment, the experimental flies were maintained at 24 °C. Control populations were also maintained at 24 °C throughout their lives and were not subjected to any heat treatment. A total of 24 population cages were set up for the longevity experiment: two lines × two treatments (heat-treated and control) × two replicates × three sex compositions (males only, females only, mixed sex). Control and experimental cages were randomly selected and were maintained contemporaneously in the same walk-in incubator.

Thermotolerance experiments. — To confirm that a nonlethal heat treatment increases subsequent tolerance to more extreme thermal stress, we exposed 4-day-old experimental flies to 36 °C under saturated humidity for 70 min. The flies were then returned to 24 °C until day 6, and then tested for tolerance to 36 °C under saturated humidity for 240 min. Experimental flies were maintained at 24 °C at all times when not being heat treated. Control flies were also exposed to 36 °C for 240 min at 6 days but were not exposed to the milder heat treatment at 4 days. Thermotolerance at age 6 days is estimated as the percent of flies dying during the heat treatment and over the next 24 h. A total of 24 contemporaneous population cages were initiated for the thermotolerance experiment: two lines × two treatments (heat-treated and control) × two replicates × three sex compositions (males only, females only, mixed sex).

Data analysis. — Age-specific mortality is calculated by pooling the data from each pair of replicate cages. Initial cohort size (N_0) is found by summing the number of deaths over all days, sorted by sex when appropriate. For each cohort, the number alive at each age x is calculated by deducting the number that died in the previous 24 h, d_{x-1}, from the number alive at age $x-1$, N_{x-1}. The observed age-specific daily mortality rate is $q(x) = d_x/N_x$. The instantaneous mortality rate $u(x)$ is estimated as $u(x) = -\ln[1 - q(x)]$ (Elandt-Johnson and Johnson, 1980). Remaining life expectancy at age 5 days (e_5) is calculated using the BMDP statistical software. Log-linear analysis (Agresti, 1990) is used to test differences in lethality rates in the thermotolerance experiments.

RESULTS

Complete life spans were measured on a total of 13,653 flies in the longevity experiment and a total of 14,213 flies in the thermotolerance experiment. Results of the longevity experiment are shown in Table 1. The critical comparison is remaining life expectancy after the heat treatment in experimental and control populations. In seven of the eight comparisons the remaining life expectancy at day 5 is greater in experimental populations than in controls. The differences in life expectancy range from 0.9 to 3.9 days, and, in all seven cases, are greater in magnitude than two standard errors. In the eighth case (females from mixed-sex cages for line JWC24) the experimental and control life expectancies are not significantly different. Pooling all eight comparisons, the heat treatment in experimental populations extends life span by an average of 1.9 days compared to controls.

Age-specific mortality rates for experimental and control populations are shown in Figures 1 and 2. There was no mortality in the experimental populations during or immediately after heat treatment. In both lines, single-sex cages show a pattern of reduced mortality rates in experimental populations, compared to controls, after the heat treatment. The mortality reduction in experimental populations relative to controls appears to last several weeks after the heat treatment. In mixed-sex cages the pattern is less clear, perhaps because overall mortality rates are higher and life expectancies lower when both sexes are present and reproducing. This is especially true for females. The mortality rate differences can be tested statistically by using nonparametric survival analysis stratified by cage replicate (Elandt-Johnson and Johnson, 1980). Exposure to nonlethal heat significantly decreases mortality rates at normal temperatures (BMDP 1L, Breslow statistic, in each case $\chi^2_{(1)} > 8.3$, $p < .004$) except for females of JWC24 in mixed sex cages ($\chi^2_{(1)} = 0.15$, $p = .69$).

Results of the thermotolerance experiment are shown in Table 2. In seven out of eight comparisons, thermotolerance, as measured by lethality rate during and after severe thermal stress, is greater in experimental populations than in control populations. In most cases thermotolerance is increased by a factor of two. In one case, males of line JWC24, there is no difference between experimental and control lethality levels. On average, control populations suffered 60% mortality whereas experimental populations averaged 32%. Results of the log-linear analysis (Agresti, 1990) show that in all seven cases in which there are differences between experimental and control populations, the difference is statistically significant at the $p < .001$ level.

Table 1. Life Expectancies in Experimental (Heat-Treated) and Control Populations from Two Inbred Lines

	Line JWC15				Line JWC24			
	N_0	N_5	e_5	*SE*	N_0	N_5	e_5	*SE*
Females only								
Experimental	1169	1078	30.9	(0.3)	1004	872	25.2	(0.3)
Control	1429	1375	29.6	(0.2)	1095	972	23.9	(0.3)
Males only								
Experimental	1203	1160	39.2	(0.3)	961	880	31.4	(0.3)
Control	1318	1292	35.3	(0.3)	1007	925	28.6	(0.3)
Female, mixed sex								
Experimental	617	617	28.0	(0.2)	581	579	23.0	(0.3)
Control	648	647	27.1	(0.2)	641	638	23.2	(0.3)
Male, mixed sex								
Experimental	507	503	39.9	(0.4)	418	416	29.8	(0.5)
Control	595	588	37.8	(0.4)	456	456	27.1	(0.5)

Notes. N_x, population size on day x; e_5, remaining life expectancy on day 5.

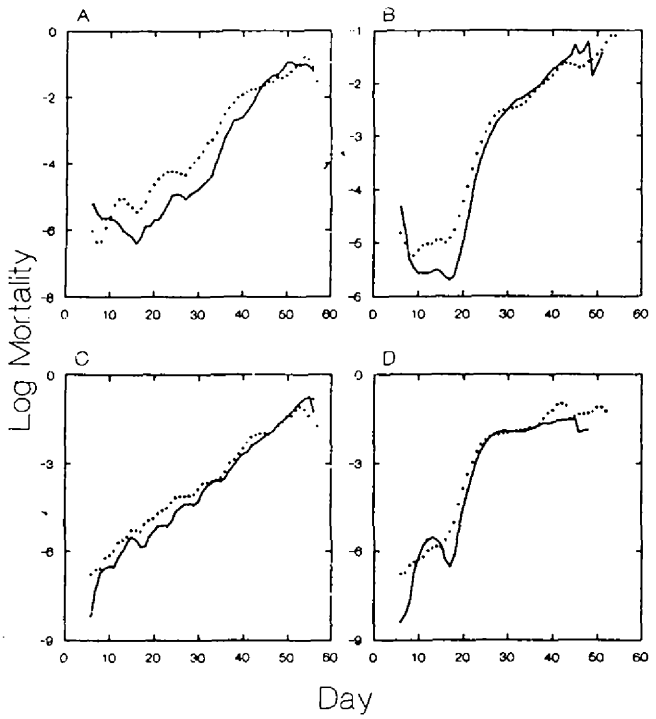

Figure 1. Estimated age-specific mortality rates in contemporaneous experimental (solid lines) and control (dashed lines) populations of *D. melanogaster* of inbred line JWC15. Daily mortality rates are shown in semi-logarithmic plots using natural logarithms. Experimental portions received a 70-min nonlethal heat treatment at age 4 days and were then reared at normal temperatures. Controls received no heat treatment. **(A)** Males, single-sex cages. **(B)** Females, single-sex cages. **(C)** Males, mixed-sex cages. **(D)** Females, mixed-sex cages.

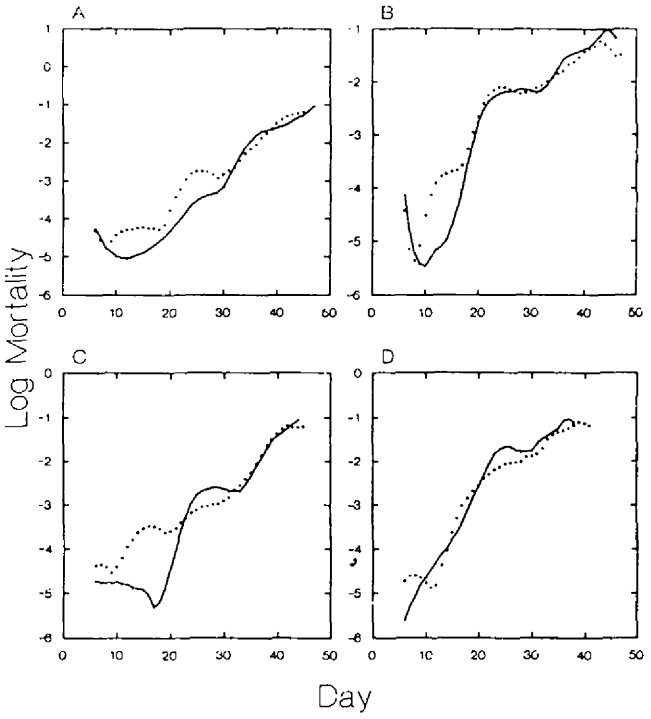

Figure 2. As in Figure 1, for inbred line JWC24.

DISCUSSION

A 70-min heat treatment adds, on average, about 2 days to the life span of inbred *Drosophila* (Table 1). The magnitude of the heat-treatment effect on life span is comparable to that of suppressing reproduction in females. As shown in Table 1, unmated control females from line 15 lived 2.5 days longer than their reproducing counterparts in the mixed-sex cages. The difference was 0.7 day for line 24 females. On the other hand, the magnitude of life span extension reported here is much less than the effect of artificially increasing the number of superoxide dismutase (SOD) and catalyze genes in flies (Orr and Sohal, 1995), where 20% increases were reported.

Heat-induced longevity extension is not merely a consequence of brief, transient decrease in mortality at the time of thermal stress. It is a demographic consequence of reduced mortality rates over a period of several weeks following the heat treatment, as seen in Figures 1 and 2. The heat treatment also increases tolerance to subsequent more extreme thermal stress (Table 2), as observed in many previous studies (Parsell and Lindquist, 1994).

We previously reported that flies that survive a desiccation stress have a reduced rate of subsequent age-specific mortality, relative to unstressed controls (Khazaeli et al., 1995). In that case, the reduced age-specific mortality may have had two nonexclusive causes: population heterogeneity and physiological induction. Heterogeneity reduces poststress mortality rates because frailer individuals are eliminated from the population during and shortly after the period of stress, leaving only more robust individuals in the poststress population. It is proposed that physiological induction reduces poststress mortality rates because the survivors of the stress are altered in such a way that their mortality rates drop, relative to controls. Our previous experiment (Khazaeli et al., 1995) was unable to untangle the possible effects of heterogeneity and induction on poststress mortality because the stresses applied were immediately lethal to 20% or more of the flies. The present experiment involves much milder stresses that cause no immediate mortality, and so the heterogeneity effect is not relevant to the interpretation of the present data. The declines in mortality after mild heat treatment must be attributed to physiological induction.

Our observations suggest that heat-induced reduction of mortality is independent of the well-known phenomenon of longevity extension via suppression of reproduction (Bell, 1986; Partridge et al., 1987; Partridge, 1989; Tatar and Carey, 1995). That is, if heat treatment extended life only by reducing or eliminating reproductive activity, then we would expect to see differences between experimental and control populations in cages where both sexes are present, but no differences where the sexes are collected as virgins and maintained separately. What we see instead is the opposite: a larger difference between experimental and control populations in the single-sex cages, and a smaller difference in the mixed-sex cages.

Aside from the inference regarding reproductive effects, our data provide no information about the mechanism of heat-induced longevity extension. Lithgow et al. (1995;

Table 2. Thermotolerance in Experimental (Heat-Treated) and Control Populations from Two Inbred Lines

	Line JWC15			Line JWC24		
	N_0	N_6	Lethality	N_0	N_6	Lethality
Females only						
Experimental	1231	1108	0.43*	1200	1024	0.29*
Control	1218	1131	0.62	1217	1059	0.56
Males only						
Experimental	1353	1261	0.32*	776	664	0.51
Control	1164	1074	0.63	1090	1014	0.51
Female, mixed sex						
Experimental	694	687	0.32*	601	533	0.29*
Control	761	753	0.63	556	548	0.78
Male, mixed sex						
Experimental	520	512	0.25*	716	648	0.15*
Control	547	541	0.51	569	557	0.51

Notes. N_x, population size on day x.
*$p < .001$, $\chi^2_{[1]}$

Lithgow, 1996) suggest that heat exposure may increase longevity and thermotolerance in *C. elegans* through the induced activity of hsps which affect the ability of cells to cope with the degenerative effects of age and the environment. In light of the association of hsp70 and thermotolerance in *D. melanogaster* (Welte et al., 1993; Feder, 1995), some hsps may also play a role in the extended longevity of adults associated with exposure to nonlethal heat. However, the induced expression of hsp70 in *D. melanogaster* in response to heat is short lived, at least in embryos and larvae, where it returns to normal levels within hours of heat exposure (Feder et al., 1992; Feder, 1995). This seems inconsistent with the observation that increased thermotolerance and decreased age-specific mortality persists for days after heat treatment. The issue is unresolved and requires knowledge about the time course of hsp expression in adults. It is also unknown whether hsp70 or other hsps can cause changes in cells that persist even after the induced hsps are inactivated. It is possible that hsp70 would act concomitantly with other stress response proteins in *D. melanogaster*, as they appear to do in *C. elegans* (Larsen, 1993; Vanfleteron, 1993; Lithgow et al., 1995). In *D. melanogaster*, SOD and catalase increase in response to oxidative stress, and when they are overexpressed in transgenic strains, longevity is increased (Orr and Sohal, 1994). Presently we lack such a direct test of the causal relationship between any hsp and longevity. To further address the potential effect of hsps on longevity, we are currently determining whether extra-copy hsp70 strains of *D. melanogaster* (Welte et al., 1993) have enhanced longevity following heat treatment.

In addition to the issue of the hsps, the observation of heat-induced longevity extension in *Drosophila* raises a number of interesting and unresolved questions: Is heat treatment a new longevity factor, or might it only indirectly affect longevity by causing dietary restriction? If heat treatment improves life span, why are the physiological factors induced by heat treatment not always induced? If flies are subjected to multiple heat treatments, are the effects on longevity cumulative? Will other genotypes exhibit a greater longevity response? Experiments are underway in our laboratory to answer all of these questions.

ACKNOWLEDGMENTS

This research is supported by grants PO1 AG-08761 and RO1 AG-11722 from the National Institutes of Health to Dr. Curtsinger.

We thank G. Lithgow, M. Feder, R. Krebs, and S. Lindquist for discussion and insights on thermal stress. C. Misiak and K. Kelly provided technical assistance.

Address correspondence to Dr. James W. Curtsinger, Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108. E-mail: jwcurt@vx.cis.umn.edu

REFERENCES

Agresti, A. Categorical data analysis. New York: Wiley, 1990.

Bell, G.; Koufopanou, V. The cost of reproduction. In: Dawkins, R.; Ridley, M., eds. Oxford surveys in evolutionary biology. Oxford, U.K.: Oxford University Press, 1986:83–131.

Chippendale, A.K.; Leroi, A.M.; Kim, S.B.; Rose, M.R. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. 6:171–193; 1993.

Curtsinger, J.W.; Fukui, H.H., Khazaeli, A.A.; Kirscher, A.W.; Pletcher, S.; Promislow, D.E.L.; Tatar, M. Genetic variation and aging. Annu. Rev. Genet. 29:553–575; 1995.

Elandt-Johnson, R.; Johnson, N.L. Survival models and data analysis. New York: Wiley, 1980.

Ellis, R.J. The general concept of molecular chaperones. Philos. Trans. R. Soc. London [Biol] 339:257–262; 1993.

Feder, M.E. Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster* model. In: Johnston, I.A.; Bennett, A.F., eds. Phenotypic and evolutionary adaptation to temperature. Cambridge, U.K.: Cambridge University Press, 1995.

Feder, J.H.; Rossi, J.M.; Solomon, J.; Solomon, N.; Lindquist, S. The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. Genes Dev. 6:1402–1413; 1992.

Feder, M.E.; Parsell, D.A.; Lindquist, S.L. The stress response and stress proteins. In: Lemansters, J.J.; Oliver, C., eds. Cell of trauma. Boca Raton, FL: CRC Press, 1995:177–191.

Fukui, H.H.; Kirscher, A.W. Thanatometer II: A chamber designed for large mixed-sex populations of *Drosophila melanogaster*. *Drosophila melanogaster*. Drosoph. Inf. Ser. 72:72–73; 1993.

Fukui, H.H.; Ackert, L.; Curtsinger, J.W. Deceleration of age-specific mortality rates in chromosomal homozygotes and heterozygotes of *Drosophila melanogaster*. Exp. Gerontol. 31:517–531; 1996.

Holehan, A.M.; Merry, B.J. The experimental manipulation of ageing by diet. Biol. Rev. 61:329–368; 1986.

Khazaeli, A.A.; Xiu, L.; Curtsinger, J.W. Stress experiments as a means of investigating age-specific mortality in *Drosophila melanogaster*. Exp. Gerontol. 30:177–184; 1995.

Krebs, R.A.; Loeschcke, V. Costs and benefits of activation of the heat shock response in *Drosophila melanogaster*. Funct. Ecol. 8:730–737; 1994a.

Krebs, R.A.; Loeschcke, V. Effects of exposure to short-term heat stress on fitness components in *Drosophila melanogaster*. J. Evol. Biol. 7:39–49; 1994b.

Larsen, P.A. Aging and resistance to oxidative damage in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 90:8905–8909; 1993.

Li, G.C.; Laszlo, A. Thermotolerance in mammalian cells: A possible role for heat shock proteins. In: Atkinson, B.G.; Walden, D.B., eds. Changes in eukaryotic gene expression in response to environmental Orlando, FL: Academic Press, 1985:277–284.

Lindquist, S.; Craig, E.A. The heat-shock proteins. Annu. Rev. Genet. 22:631–677; 1988.

Lithgow, G.J. The molecular genetics of *Caenorhabditis elegans* aging. In: Schneider, E.L.; Rowe, J.W., eds. Handbook of the biology of aging, 4th ed. San Diego: Academic Press, 1996.

Lithgow, G.L.; White, T.M.; Hinerfeld, D.A.; Johnson, T.E. Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. J. Gerontol. Biol. Sci. 49:B270–B276; 1994.

Lithgow, G.J.; White, T.M.; Melov, S.; Johnson, T.E. Thermotolerance and extended life span conferred by single-gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA 92:7540–7544; 1995.

Orr, W.C.; Sohal, R.S. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science 263:1128–1130; 1994.

Parsell, D.A.; Lindquist, S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27:437–496; 1993.

Parsell, D.A.; Lindquist, S. Heat shock proteins and stress tolerance. In: Morimoto, R.I.; Tissieres, A.; Georgopoulos, C., eds. The biology of heat shock proteins and molecular chaperones. Plainview, NY: Cold Spring Harbor Laboratory Press, 1994:457–494.

Partridge, L. An experimentalist's approach to the role of costs of reproduction in the evolution of life-histories. In: Grubb, P.J.; Whittaker, J.B., eds. Towards a more exact ecology. Oxford, U.K.: Blackwell Scientific, 1989:231–246.

Partridge, L.; Green, A.; Fowler, K. Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. J. Insect Phys. 33:745–749; 1987.

Solomon, J.M.; Rossi, J.M.; Golic, K.; McGarry, T.; Lindquist, S. Changes in hsp70 alter thermotolerance and heat-shock regulation in *Drosophila*. New Biol. 3:1106–1120; 1991.

Tatar, M.; Carey, J.R. Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle *Callosobruchus maculatus*. Ecology 76:2066–2073; 1995.

Vanfleteron, J.R. Oxidative stress and aging in *Caenorhabditis elegans*. Biochem. J. 292:605–608; 1993.

Welte, M.A.; Tetrault, J.M.; Dellavalle, R.P.; Lindquist, S.L. A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. Curr. Biol. 13:842–853; 1993.

Received March 19, 1996
Accepted July 17, 1996