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Effect of heat shock, pretreatment and *hsp70* copy number on wing development in *Drosophila melanogaster*

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Abstract

Naturally occurring heat shock (HS) during pupation induces abnormal wing development in *Drosophila*; we examined factors affecting the severity of this induction. The proportion of HS-surviving adults with abnormal wings varied with HS duration and intensity, and with the pupal age or stage at HS administration. Pretreatment (PT), mild hyperthermia delivered before HS, usually protected development against HS. Gradual heating resembling natural thermal regimes also protected wing development against thermal disruption. Because of the roles of the wings in flight and courtship and in view of natural thermal regimes that *Drosophila* experience, both HS-induction of wing abnormalities and its abatement by PT may have marked effects on *Drosophila* fitness in nature. Because PT is associated with expression of heat-inducible molecular chaperones such as Hsp70 in *Drosophila*, we compared thermal disruption of wing development among *hsp70* mutants as well as among strains naturally varying in Hsp70 levels. Contrary to expectations, lines or strains with increased Hsp70 levels were no more resistant to HS-disruption of wing development than counterparts with lower Hsp70 levels. In fact, wing development was more resistant to HS in *hsp70* deletion strains than control strains. We suggest that, while high Hsp70 levels may aid cells in surviving hyperthermia, high levels may also overly stimulate or inhibit numerous signalling pathways involved in cell proliferation, maturation and programmed death, resulting in developmental failure.

Keywords: *Drosophilia*, heat shock, Hsp70, phenocopy, wing development

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Introduction

Successful development is the outcome of multiple intersecting and coordinated programmes of gene expression. Environmental stresses such as heat, which can disrupt this coordination in developing organisms, can harm the form and function of surviving adults. In wild populations of *Drosophila melanogaster*, for example, up to 15% of individuals surviving peak summer temperatures in nature exhibit substantial wing and abdominal abnormalities (Roberts & Feder 1999). Evaluating the impact of such phenomena on fitness and evolution requires knowledge of: (i) the thermal sensitivity of normal development, and related environmental and genetic factors that may affect this sensitivity; (ii) thermal regimes prevailing in natural environments, including the frequency of natural temperatures sufficient to disrupt normal development; and (iii) the impact of naturally occurring abnormalities on fitness. Here we address the first of these components with a model organism, *Drosophila*, and a model developmental process, wing development during metamorphosis of larva to adult.

Each *Drosophila* wing develops from a wing imaginal disk whose basic patterning is established before and during early metamorphosis (Bier 2000; Klein 2001). During later metamorphosis, the wing disk everts and the two surfaces that become the wing appose. These events are the

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outcome of the coordinated expression of nearly 200 known genes encoding transcription factors, members of the JAK-STAT and EGF-R pathways, receptors and cell surface proteins, neurogenic proteins and other proteins (Brody 1999; Society for Developmental Biology 2002). In the normal adult wing, 'veins' are a structural framework for the wing, enclose tracheae and nerves and are responsible for wing expansion after eclosion from the puparium; during development, the correct placement and growth of veins and suppression of intervein development are principal motifs (Stark *et al*. 1999). While mutations of specific genes for wing disk or wing vein morphogenesis can result in highly reproducible morphological defects, so too can physical and chemical stress that disrupts gene expression or protein function (Welte *et al*. 1995). Indeed, for many years investigators have reported that specific heat or chemical regimes administered at an appropriate developmental stage phenocopy diverse mutations. For example, heat administered to puparia yields adults with aberrant cross-veins similar to the mutant *crossveinless* (Milkman 1961; Milkman 1962), and Mitchell *et al*. described diverse phenocopies from heat shocks during both embryogenesis and pupariation (Mitchell & Petersen 1982; Petersen & Mitchell 1991). The phenocopies resulting from heat shock are highly stage-specific; for example, at the culture temperatures employed in this work, heat shock (HS) at 20–24 h after puparium formation (APF) phenocopies *crossveinless* and HS at 34 h APF results in hooked bristles instead.

For many aspects of cellular and organismal function including outright survival, thermal pretreatment (PT; i.e. exposure to a mild heat shock) enhances tolerance of a severe heat shock (Feder & Hofmann 1999). Few studies, however, have examined inducible thermotolerance of development *per se*. In *Drosophila*, PT reduces the incidence of forked and hooked bristle phenocopies (Petersen & Mitchell 1991), and also enhances resistance of development to the teratogenic effects of the mitotic poisons vinblastine and colchicine (Isaenko *et al*. 2002). One candidate mechanism for heat-inducible protection of development is the expression of molecular chaperones, many of which are inducible (i.e. heat-shock proteins, Hsps). The *Drosophila* genome encodes a full complement of the major eukaryotic Hsps and other molecular chaperones, of which Hsp70 is the most abundant after PT (Feder & Krebs 1998). Hsps clearly underlie PT-inducible survival of heat shock and protection of other processes, presumably by minimizing aggregation of heat-damaged proteins, targeting them for degradation and removal from the cell and/or in facilitating their refolding to their native conformation (Feder & Hofmann 1999). Even in the absence of heat and other stresses, regulated expression of molecular chaperones is essential for normal development (for *Drosophila*, see Arrigo & Tanguay 1991; Rutherford & Lindquist 1998; Elefant & Palter 1999; Yue *et al*. 1999) and reduction in the constitutively present chaperone Hsp90 can uncover cryptic developmental defects (Rutherford & Lindquist 1998). In an *hsp70* allelic series, *hsp70* copy number and Hsp70 protein levels are related inversely to the frequency of wing and eye abnormalities in adults exposed to heat shock while pupae or the teratogen vinblastine while larvae (Roberts & Feder 1999; Isaenko *et al*. 2002). Hence we hypothesized that both PT and the level of Hsp70 after PT should be consequential for the resistance of wing development against heat shock.

These patterns and mechanisms, moreover, have their counterpart in nature, where nonadult *Drosophila* infest necrotic fruit. Temperatures of such fruit, when sunlit, encompass the entire range of PT and heat stress regimes used in laboratory experimentation (Feder 1997; Feder *et al*. 1997; Roberts & Feder 1999; Roberts & Feder 2000). In the wild these conditions can induce both Hsp expression and developmental abnormalities of the wing and abdomen (Feder 1997; Feder *et al*. 1997; Roberts & Feder 1999; Roberts & Feder 2000). Thus, the thermal sensitivity of development, inducible tolerance and Hsps can have a direct bearing on natural selection in the wild. Hsp expression levels in particular are consistent with ongoing selection. Hsp70 levels vary among individuals within natural populations and among populations along natural stress gradients, undergoing laboratory evolution at different temperatures, and undergoing artificial selection. For example, in strains descended from a common ancestor (Bettencourt *et al*. 1999), strains evolving at 25° (OR25A and OR25B) express more Hsp70 than strains evolving at 28° (OR28A and OR28B), which in turn express more than a strain from Africa with low inducible stress tolerance (Zatsepina *et al*. 2001). This variation is heritable and linked to fitness (Krebs *et al*. 1998). In terms of developmental sensitivity to heat, *Drosophila* naturally varies in both susceptibility in phenocopy induction (Mohler 1965; Varma & Sinha 1977) and the specific abnormalities that arise when Hsp90 is suppressed (Rutherford & Lindquist 1998). Susceptibility to induction of developmental abnormalities can itself undergo laboratory selection (Waddington 1940, 1961; Rutherford & Lindquist 1998).

Here we examine how a natural environmental phenomenon (heat stress) affects a well-characterized ontogenetic program (wing development), and how PT and Hsp70 may alter these effects. We incorporate two sources of intraspecific variation: variation among strains with different evolutionary histories and engineered variation. The latter includes (i) an 'extra copy' strain with six transgenic and five wild-type *hsp70* copies and an otherwise isogenic 'excision' strain from which the transgenes have been excised (Welte *et al*. 1993); and (ii) crosses with a deficiency strain lacking the 87C1 chromosomal region, which includes three of the five wild-type *hsp70* copies.

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Materials and methods

Fly stocks and crosses

Extra copy and excision strains were the Chromosome II *hsp70* allelic series, whose creation, Hsp70 levels after PT, development and inducible thermotolerance have been described previously (Welte *et al*. 1993; Feder *et al*. 1996; Tatar *et al*. 1997; Krebs & Feder 1998; Krebs *et al*. 1998; Silbermann & Tatar 2000). Strain Df(3R)T-33 + Df(3R)Kar3^{FE}/TM3 lacks the 87C1 region, including three of the five *hsp70* genes (Gausz *et al*. 1981; J. Gausz, pers. comm.).

Wild-type (i.e. nonmutant) strains were as follows: (i) four lines derived from a single population of the laboratory strain Oregon R that have been maintained at 25 °C (ORA25 and ORB25) or 28 °C (ORA28 and ORB28) for more than 25 years; Bettencourt *et al*. (1999) describes inducible thermotolerance and Hsp70 levels in these lines. (ii) The T32 strain, which has been maintained at 31–32 °C for more than 20 years; Zatsepina *et al*. (2001) describes inducible thermotolerance and Hsp70 levels in this line.

Crosses to the deficiency Df(3R)T-33 + Df(3R)Kar3^{FE}/TM3 were carried out in bottles or vials and maintained at 25 °C except for crosses between T32 and the deficiency which were maintained at 28 °C. In all crosses the males came from the deficiency strain.

Pretreatment and heat shock of pupae

Pupae of known developmental stage and/or age underwent HS of defined duration and temperature, with or without a PT of defined duration and temperature. Table 1 and the Results section contain the HS and PT regimes, which varied by experiment. HS and PT were administered by immersing glass vials (8.5 × 2 cm) containing approximately 30 pupae in water baths regulated at the specified temperature. Vials were maintained at 25 °C after heat shock. These procedures varied slightly according to experimental objectives:

In most experiments with pupae of known age (i.e. time APF), wandering third-instar larvae were allowed to pupate on the walls of vials containing 7 mL of medium. Pupariation occurred within several hours and age was approximated as the time since transfer to the vial. Vials were then heat-shocked as described above. For developmental staging (Bainbridge & Bownes 1981), wandering third-instar larvae were allowed to pupariate spontaneously on the walls of glass vials containing 7 mL of medium, and pupae were inspected periodically with the dissecting microscope until they first exhibited bright yellow eyes (signifying stage P8) or grey/black wings (signifying stage P12). Pupae not satisfying these criteria were discarded. Vials were then heat-shocked as described above. To determine the effect of fine gradations in age APF on induction of wing abnormalities, newly formed puparia were transferred gently to water and buoyant pupae were collected at 2-h intervals (Mitchell & Lipps 1978). Each 2-h collection was aged for a specified interval in humidified air at 25 °C and then placed in 4 mL of 40.5 °C water within a glass vial, which was then immersed for 35 min in a water bath regulated at 40.5 °C. The vial was then removed from the water bath and 5 mL of water immediately added to cool the temperature to 28 °C. Buoyant puparia were then transferred to the walls of a fresh vial containing 7 mL medium, and maintained at 25 °C.

Laboratory simulation of natural heating regimes

We surveyed natural heating regimes previously reported for necrotic fruit (Feder 1997; Feder *et al*. 1997; Roberts & Feder 1999; Roberts & Feder 2000), and immersed vials containing pupae in a thermostatted water bath whose temperature was adjusted periodically to resemble natural heating regimes. The thermostat setting was increased by 2 °C every 15 min until 36 °C, then by 1.5 °C to 37.5 °C, and then by 0.5 °C every 15 min until 40.5 °C. Stage 12 pupae of two strains, ORB25 and T32, underwent these heating regimes. Adult flies that eclosed were scored for wing abnormalities.

Developmental abnormalities

Eclosing adults were anaesthetized with CO_2, examined with a dissecting microscope, sexed and scored for wing abnormalities by comparing both wings with wild-type wings. In addition to the specific abnormalities described below and by Mitchell & Petersen (1982), we noted extra or absent vein material and blistered wings. Images of wings were acquired with a Zeiss Axiocam and processed with Zeiss Axiovision and Photoshop (Adobe) software.

Determination of Hsp70 protein levels

Adult males were collected 1 d ± 4 h after eclosion and placed individually in cryotubes (humidified with 10 µL PBS), pretreated at 36 °C for 60 min by submersion in a thermostatted water bath, allowed to recover at 25 °C for 60 min, immediately frozen in liquid nitrogen and stored at −80 °C. Hsp70 levels in each fly were be determined by ELISA (Welte *et al*. 1993; Feder *et al*. 1996). Samples were lysed by homogenization with a motorized pestle in ice-cold phosphate-buffered saline with Complete Protease Inhibitor (Roche) and centrifuged at 20 000 *g* and 4 °C for 30 min. Total protein concentration was determined by BCA (Pierce) analysis, and Pro-Bind 96-well assay plates (Falcon) were coated with 20 µg protein overnight at 4 °C. After blocking and washing, bound Hsp70 was detected

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Table 1 Effect of age after puparium formation (APF), developmental stage, heat-shock (HS) temperature and duration, and pretreatment (PT) in *Drosophila* populations or strains varying in Hsp70 levels upon heat shock. PTs, when administered, included a 1-h recovery period at 25 °C before administration of HS. Extra-copy *Drosophila* had $2n = 22$ *hsp70* copies, excision and all wild-type *Drosophila* had $2n = 10$ copies, and the deficiency strain had $2n$ = four copies

Stage or h APF	Strain	Heat shock °C and duration (min)	% abnormal without pretreatment (*n*)	*P* of difference among bracketed strains	Pretreatment, PT (min)	% abnormal with PT (*n*)	*P* of difference among bracketed strains	*P* of difference with vs. without PT
48	Excision	40°35′	13.8 (29)	0.41	—			
48	Extra-copy	40°35′	22.7 (22)		—			
48	Excision	40°40′	61.3 (93)	0.73	—			
48	Extra-copy	40°40′	64.7 (34)		—			
48	Excision	40°45′	51.4 (74)	0.75	—			
48	Extra-copy	40°45′	47.1 (17)		—			
48	Excision	40°50′	100 (24)	0.04	—			
48	Extra-copy	40°50′	84.0 (81)		—			
48	Excision	40°60′	100 (42)	0.47	60	8.3 (12)	< 0.001	< 0.001
48	Extra-copy	40°60′	100 (11)		60	76.1 (71)		0.08
48	Excision	40°75′	100 (1)		—			
48	Extra-copy	40°75′	—* (15)		—			
48	Excision	40.5°15′	0 (50)		—			
48	Excision	40.5°25′	39.4 (33)	< 0.001	—			
48	Extra-copy	40.5°25′	4.3 (46)		—			
48	Excision	40.5°30′	33.3 (27)	0.43	—			
48	Extra-copy	40.5°30′	21.4 (14)		—			
48	Extra-copy	40.5°35′	100 (27)		—			
48	Excision	40.5°45′	100 (6)	0.92	—			
48	Extra-copy	40.5°45′	100 (5)		—			
48	Excision	41°10′	0 (14)	0.90	—			
48	Extra-copy	41°10′	0 (18)		—			
48	Excision	41°20′	42.9 (42)	0.05	—			
48	Extra-copy	41°20′	23.3 (43)		—			
48	Excision	41°25′	90.9 (33)	0.21	15	7.7 (13)	0.09	< 0.001
48	Extra-copy	41°25′	100 (16)		15	0 (36)		< 0.001
48	Excision	41°25′	90.9 (33)	0.21	60	0 (30)	< 0.001	< 0.001
48	Extra-copy	41°25′	100 (16)		60	69 (29)		0.01
48	Excision	41°30′	96.8 (31)	0.42	—			
48	Extra-copy	41°30′	100 (20)		—			
48	Excision	41°35′	100 (3)	1.0	15	14.3 (28)	0.22	< 0.001
48	Extra-copy	41°35′	100 (3)		15	4.2 (24)		< 0.001
48	Excision	41°35′	100 (3)	1.0	60	16.7 (12)	0.06	0.006
48	Extra-copy	41°35′	100 (3)		60	50 (20)		0.10
48	Excision	41°45′			15	13.0 (23)	0.37	
48	Extra-copy	41°45′			15	5.0 (20)		
48	Excision	41°45′			60	4.2 (24)	0.17	
48	Extra-copy	41°45′			60	18.2 (11)		
48	Excision	41°55′			15	11.5 (26)	0.63	
48	Extra-copy	41°55′			15	16.7 (18)		
48	Excision	41°65′			15	52.2 (23)	0.06	
48	Extra-copy	41°65′			15	25.0 (24)		
48	Excision	41°90′			15	—* (200)		
48	Extra-copy	41°90′			15	—* (200)		
48	Excision	41.5°5′	0 (25)	0.21	—			
48	Extra-copy	41.5°5′	6.3 (16)		—			
48	Excision	41.5°15′	71.4 (28)	0.44	—			
48	Extra-copy	41.5°15′	85.7 (7)		—			
48	Excision	41.5°20′	100 (17)	0.34	—			
48	Extra-copy	41.5°20′	94.7 (19)		—			

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Table 1 *Continued*

Stage or h APF	Strain	Heat shock °C and duration (min)	% abnormal without pretreatment (*n*)	*P* of difference among bracketed strains	Pretreatment, PT (min)	% abnormal with PT (*n*)	*P* of difference among bracketed strains	*P* of difference with vs. without PT
48	Excision	41.5°25′			15	6.5 (31)	0.57	
48	Extra-copy	41.5°25′			15	3.3 (30)		
48	Excision	41.5°35′			15	10.0 (10)	0.16	
48	Extra-copy	41.5°35′			15	33.3 (24)		
48	Excision	42°5′	0 (37)	0.21	—			
48	Extra-copy	42°5′	4.2 (24)		—			
48	Excision	42°10′	28.9 (45)	0.33	—			
48	Extra-copy	42°10′	12.5 (8)		—			
48	Excision	42°15′	100 (35)	0.38	15	16.7 (18)	0.42	< 0.001
48	Extra-copy	42°15′	97.8 (46)		15	26.9 (26)		< 0.001
48	Excision	42.5°15′			15	16.5 (224)	0.91	
48	Extra-copy	42.5°15′			15	17.0 (94)		
P8	Excision	40.6°35′	58.2 (55)	0.25	60	6.2 (48)	0.27	< 0.001
P8	Extra-copy		48.4 (93)		60	12.3 (73)		< 0.001
P8	ORB25	40.6°35′	70.7 (75)		60	43.4 (122)		0.02
P8	ORA25	40.6°35′	88 (25)		60	59.6 (52)		0.23
P8	A28	40.6°35′	28.8 (59)		60	7.9 (76)		0.12
P8	B28	40.6°35′	83.7 (98)		60	37.5 (96)		< 0.001
P8	T32	40.6°35′	7.9 (76)		60	14.6 (48)		0.96
P8	Df(3R)/TM3†	40.6°35′	79.4 (34)		60	48.4 (31)		0.01
P8	ORB25/TM3	40.6°35′	69.4 (111)		60	47.8 (138)		0.41
P8	ORB25/Df		51.1 (135)	strain	60	11.2 (134)	strain	0.001
P8	ORA25/TM3	40.6°35′	78.8 (33)	> 0.05	60	39.6 (53)	> 0.05	0.07
P8	ORA25/Df		80.5 (41)	TM3 vs. Df	60	6.5 (46)	TM3 vs. Df	< 0.001
P8	T32/TM3	40.6°35′	81.1 (53)	> 0.05	60	46.5 (43)	> 0.05	0.14
P8	T32/Df		46.5 (43)		60	3.1 (32)		0.11
72	Excision	39.5°35′	4.3 (114)	0.48	60	—*	—*	
72	Extra-copy		3.4 (113)			—*	—*	
72	Excision	40.5°35′	57.2 (239)	< 0.001	60	0.1 (266)	(a) 0.81	< 0.001
72	Extra-copy		20.9 (276)			0 (260)		< 0.001
78	Excision	40.5°35′	73.4 (127)	(a) 0.001	60	0.2 (264)	(a) 0.55	< 0.001
78	Extra-copy		37 (121)			0.1 (267)		< 0.001
P12	Excision	40.5°35′	100 (39)	0.95	15	0.0 (22)	0.96	< 0.001
P12	Extra-copy	40.5°35′	100 (34)		15	0.0 (20)		< 0.001
P12	Excision	40.5°40′			15	3.4 (29)	0.94	
P12	Extra-copy	40.5°40′			15	3.8 (26)		
P12	Excision	40.5°45′			15	0.0 (44)	0.16	
P12	Extra-copy	40.5°45′			15	4.3 (23)		
P12	Excision	40.5°50′			15	0.0 (4)	0.77	
P12	Extra-copy	40.5°50′			15	0.0 (7)		
P12	Excision	40.5°55′			15	10.0 (20)	0.13	
P12	Extra-copy	40.5°55′			15	0.0 (22)		
P12	Excision	40.5°60′			15	3.3 (30)	0.30	
P12	Extra-copy	40.5°60′			15	0.0 (32)		
P12	Excision	41°65′			15	—* (12)		
P12	Extra-copy	41°65′			15	—* (8)		
P12	Excision	42°15′			15	2.0 (98)	0.96	
P12	Extra-copy	42°15′			15	2.2 (46)		
P12	Excision	42°20′			15	2.6 (76)	0.25	
P12	Extra-copy	42°20′			15	0.0 (49)		
P12	Excision	42.5°15′			15	14.7 (34)	0.54	
P12	Extra-copy	42.5°15′			15	9.7 (31)		

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Table 1 *Continued*

Stage or h APF	Strain	Heat shock °C and duration (min)	% abnormal without pretreatment (*n*)	*P* of difference among bracketed strains	Pretreatment, PT (min)	% abnormal with PT (*n*)	*P* of difference among bracketed strains	*P* of difference with vs. without PT
P12	Excision	42.5°20′			15	—* (12)		
P12	Extra-copy	42.5°20′			15	—* (8)		
P12	Extra-copy	43°15′			15	—* (5)		
P12	Excision	40.6°35′	22.2 (27)	0.38	60	7.9 (38)	0.55	0.10
P12	Extra-copy		31.8 (44)		60	4.9 (61)		< 0.001
P12	ORB25	40.6°35′	80.4 (46)		60	37.1 (170)		0.005
P12	ORA25	40.6°35′	100 (42)		60	51 (65)		< 0.001
P12	A28	40.6°35′	59 (100)		60	8.7 (69)		0.009
P12	B28	40.6°35′	88 (100)		60	56 (84)		0.002
P12	T32	40.6°35′	94.5 (74)		60	3.7 (135)		< 0.001
P12	Df(3R)/TM3†	40.6°35′	100 (10)		60	47.3 (110)		< 0.001
P12	ORB25/TM3	40.6°35′	87.5 (40)	strain > 0.05 TM3 vs. Df > 0.05	60	25 (148)	strain > 0.05 TM3 vs. Df > 0.05	< 0.001
P12	ORB25/Df		86.7 (30)		60	0 (120)		< 0.001
P12	ORA25/TM3	40.6°35′	100 (13)		60	30.3 (76)		< 0.001
P12	ORA25/Df		100 (13)		60	0 (134)		< 0.001
P12	T32/TM3	40.6°35′	100 (22)		60	50 (24)		0.12
P12	T32/Df		96.7 (30)		60	5.3 (19)		< 0.001

*No survivors; frequency of abnormal survivors could not be determined. †Df/TM3 = Df(3R)T-33 + Df(3R)KarFe/TM3.

with the *Drosophila* inducible Hsp70-specific antibody 7FB (Velazquez *et al*. 1980) coupled to alkaline phosphatase (AP) via secondary [rabbit antirat IgG (Cappel)] and tertiary [AP-conjugated goat antirabbit IgG (Sigma)] antibodies. Incubation with the phosphatase substrate yields a coloured product that was quantified with a microplate reader. Hsp70 concentration is expressed as a percentage of an Hsp70 standard included on each plate.

Statistics

Significance of differences in numbers of adults with abnormal wings was tested by χ^2 (single samples for each contrasting strain or treatment) or analysis of variance (multiple samples). Tests were performed with StatView.

Results

Effects of pretreatment and heat shock of pupae on survival to adulthood

Heat shock (HS) of pupae reduced their survival to adulthood (Fig. 1). In Fig. 1, the curves relating mortality to HS duration are consistent with little mortality after brief HS, increasing mortality with more lengthy heat shock, and complete mortality at a critical HS duration. As HS temperature increased, each of these transition points occurred at lesser HS durations.

Pretreatment (PT) of pupae before HS almost always reduced mortality due to HS (Fig. 1). Pretreatment in advance of prolonged intense HS (e.g. 90 min at 41 °C) had no effect, however.

Puparial age at HS (40.5 °C for 35 min) affected both survival and the impact of PT (Table 1). For example, pupae with HS at 72 h APF survived better (91%) than pupae exposed at 78 h APF (45%). PT (36° and 25 °C for 60 min each) improved the survival of HS at 78 h APF old (to 91%) but not at 72 h APF (to 95%). These effects all were highly significant (ANOVA, $P < 0.001$).

Effects of heat shock of pupae on wing morphology in flies surviving to adulthood

Flies eclosing from control pupae that underwent neither PT nor HS had normal or near-normal wings. By contrast, in adults emerging from pupae that underwent HS, wing abnormalities were often more frequent (Fig. 1, Table 1). These abnormalities (Fig. 2) resembled wing mutations such as *arched*, *blistered*, *balloon*, *curled*, *crossveinless*, *spread* and *wrinkled* (Lindsley & Zimm 1992). At 48 h APF, the fraction of flies eclosing with abnormal wings increased with HS temperature, HS duration, and their product

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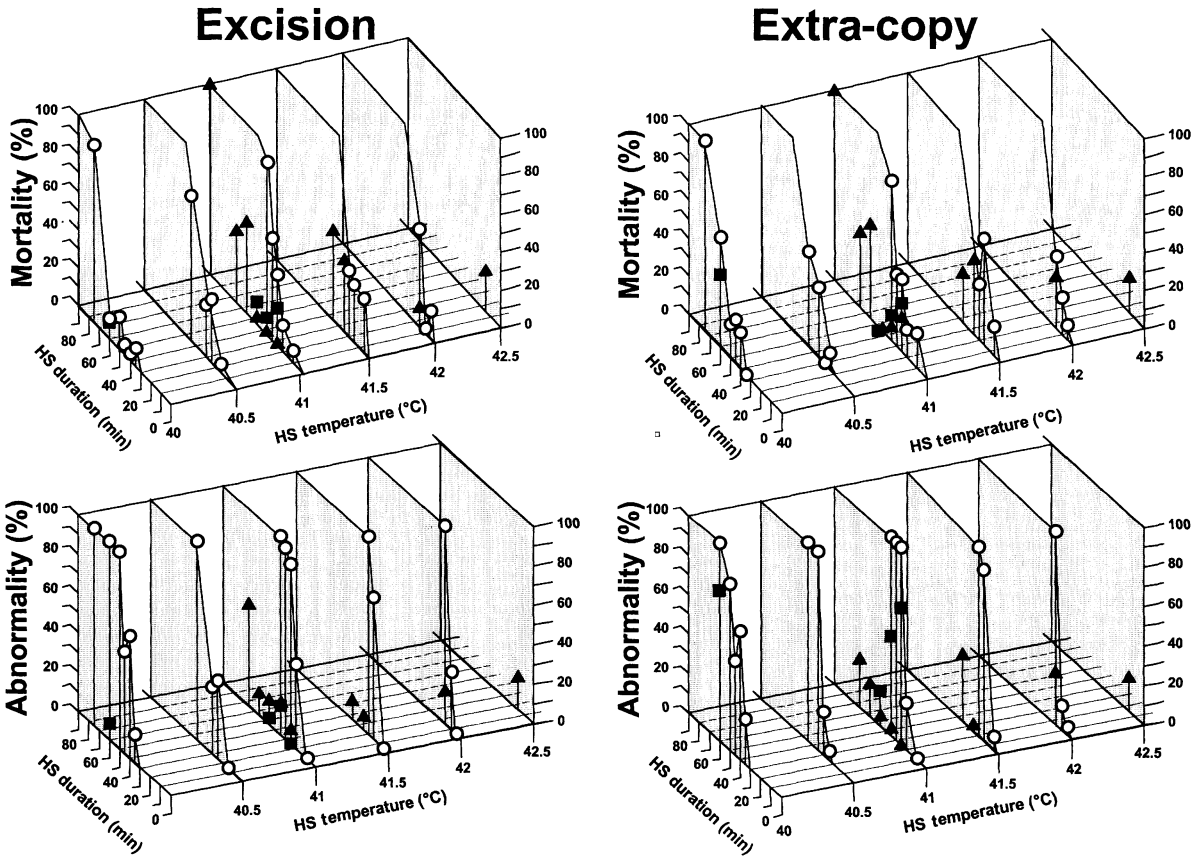

Fig. 1 Effect of *hsp70* genotype pretreatment (PT) at 36 °C, before heat shock, heat shock (HS) duration, temperature on mortality (top), on wing development in surviving flies (bottom). Values are also given in Table 1. ○, no PT; ▲, 15 min PT; ■, 60 min PT.

(Fig. 1). Thus, HS duration and temperature affected the incidence of abnormality in the same way as they did mortality (see above). For many HS temperatures and durations, the majority of pupae survived to adulthood but nonetheless had abnormal wings (Table 1, Fig. 1).

Effects of developmental time on the induction of wing abnormalities by heat shock

As with mortality, the age at HS affected the frequency of abnormalities in surviving adults (Fig. 3), which eclose approximately 5 days APF. Abnormality frequency was 80–100% with HS 40–48 h APF, with lower frequencies preceding and after this interval. Abnormality frequency also increased with age of HS administration between 73 and 86 h APF. This variation was significant in both the extra-copy and excision strains (χ^2, $P < 0.001$).

Pretreatment protects against heat-induced wing abnormalities

As with mortality, PT of pupae before HS typically (but not always) reduced the incidence of wing abnormalities in surviving adults. Figure 1 depicts the foregoing patterns for two exemplary genotypes heat-shocked 48 h APF, but similar results occurred for diverse genotypes, strains, developmental stages at which HS was applied, HS temperatures and HS durations (Table 1). We performed 45 HS experiments in which mortality was > 0% in puparia without PT and < 100% in puparia with PT. Of these, PT significantly reduced the incidence of wing abnormality in 36 and had no significant effect in 5. For HS treatments that induced markedly abnormal blistered wings, PT always reduced the proportion of flies with blistered wings. Below we discuss how several variables result in departure from this typical pattern.

Stage and age. Because *Drosophila* may develop at variable rates, pupae may reach defined developmental stages at variable times (e.g. hours APF). We used both developmental and chronological staging as indicated (Table 1; Fig. 4). For pupae 48 h APF or at P8, PT reduced the incidence of wing abnormalities. This effect of PT was significant in the extra-copy, excision, ORB25, ORB28 strains but not the ORA25, ORA28 or T32 strains. At stage 12, 72 h APF and 78 h APF, PT reduced wing abnormalities among

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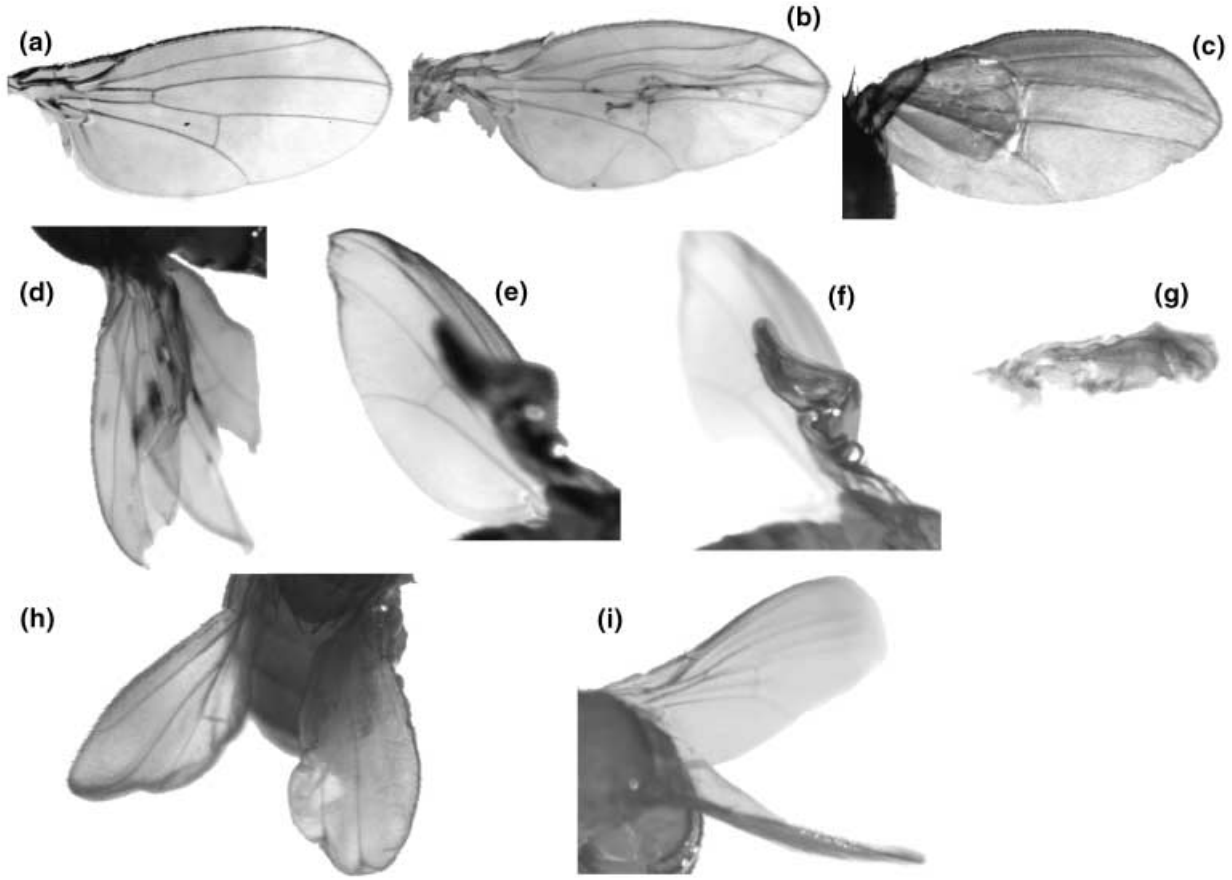

Fig. 2 Wing abnormalities induced by heat shock. The specific strain, age or stage and pupal thermal regime of the flies depicted are indicated in parentheses, but these images were chosen to typify abnormalities evident in many flies. (a) Wild-type wing; (b) ectopic vein material associated with vein or intervein region (excision 48 H APF, 41.5 °C for 15 min); (c) blister at anterior end of wing (ORB25, P12, 41 °C for 35 min). Blisters of various sizes also occurred in other positions; (d) damaged wing with ectopic vein material associated with vein or intervein region (extra-copy, 48 H APF, 42 °C for 15 min); (e) curved wing resembling the phenotype of *arc* mutants (ORB25, P8, 40.5 °C for 35 min); (f) wing in the foreground is crumpled and not expanded [same individual as in (e)]; (g) crumpled fluid-filled wing (extra-copy, 48 h APF, 40.5 °C for 35 min); (h) completely inflated, ballooned wings (excision, 48 h APF, 41 °C for 35 min); (i) concave, scooped wing (ORB25, P8, 40.5 °C for 35 min).

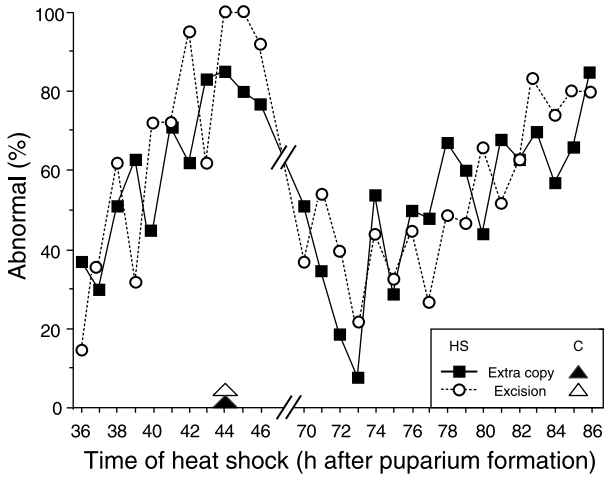

Fig. 3 Proportion of extra-copy and excision adults with abnormal wings as a function of pupal age at heat shock.

eclosing flies in all strains examined. The reduction ranged from 14 to 100% in excision and extra-copy strains.

Duration. When PT protected wing development against HS, PT at 36 °C for 15 min was sufficient for protection (Fig. 1). In comparisons of age-matched pupae, 60 min PT was slightly less protective than 15 min PT. For example, wing abnormalities induced by HS at 48 h APF were more prevalent in extra-copy flies given 60 min PT than in those given 15 min PT (χ^2, $P < 0.01$) (Table 1).

Simulated natural pretreatment

Drosophila pupae in nature undergo gradual warming rather than step changes in PT and HS temperature. To examine if gradual warming was as protective as PT, we exposed P12 pupae to HS (40.6° for 35 min) preceded by no

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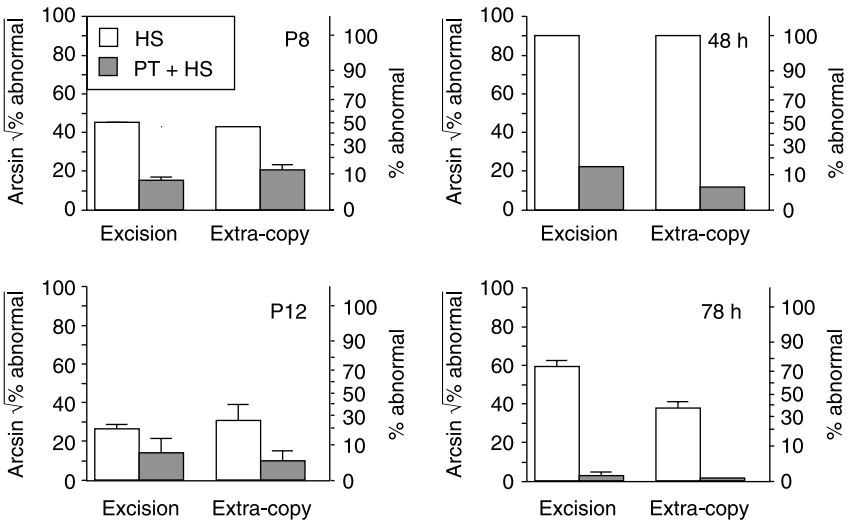

Fig. 4 Pretreatment reduces the percent abnormalities induced by heat shock despite differing effects of stage (P8 or P12), time of development (48 or 78 h after puparium formation).

PT, PT, or gradual warming (Fig. 5). Both PT and gradual warming reduced the incidence of wing abnormalities ($F_{(2,22)} = 52.79$, $P < 0.0001$), and did not differ in their protective effect ($F_{(1,14)} = 0.41$, $P < 0.53$).

Wing abnormalities among strains that differ in Hsp70

A principal objective of this study was to examine the impact of Hsp70 level in heat-shocked pupae on the incidence of wing abnormality in surviving adults. Thus we compared sister strains in an *hsp70* allelic series, deficiency strains with some *hsp70* genes deleted and strains with evolved differences in Hsp70 level. We hypothesized that Hsp70 level should be inversely related to the incidence of abnormalities. The data only occasionally are consistent with this hypothesis.

Excision and extra-copy flies. In pupae undergoing HS without PT, extra-copy flies had fewer abnormalities than excision flies in only 12 of 27 comparisons; of these 12, only three (48 h APF, HS 40.0° for 50 min; 72 h APF, HS 40.5 °C for 35 min; 78 h APF, HS 40.5 °C for 35 min) were statistically significant (Table 1, Figs 1 and 3). In pupae undergoing HS with PT, extra-copy flies had fewer abnormalities than excision flies in only 10 of 22 comparisons; of these 10, none were statistically significant (Table 1, Figs 1 and 3). In fact, in two cases (60 min PT and 40° for 60 min HS at 48 h APF, 60 min PT and 41° for 25 min HS at 48 h APF), extra-copy flies had significantly more wing abnormalities than did excision flies (Table 1). These outcomes may reflect an interaction of developmental rate and chronological age. For example, when pupae are heat-shocked 78 h APF, extra-copy survivors have fewer abnormalities than excision survivors (Fig. 4, Table 1). When this experiment was repeated at the comparable

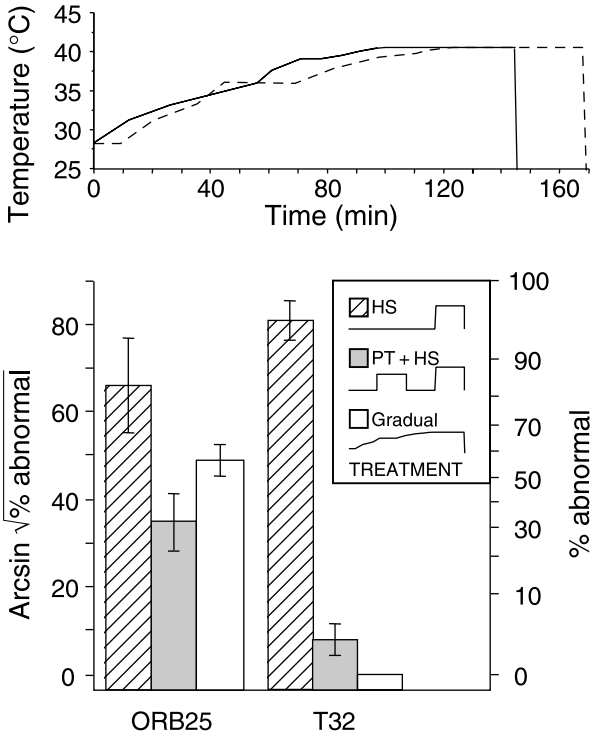

Fig. 5 Effects of PT and gradual warming on HS-induction of wing abnormalities in ORB25 and T32 pupae. HS was 40.6 °C for 35 min. Top: temperature during gradual warming and HS. Experimentation included two gradual warming regimes (solid and broken lines, respectively). Both regimes, when applied to ORB25 and T32 pupae, similarly affected wing development; accordingly, results for the two regimes are pooled in the bottom figure. Bottom: incidence of wing abnormalities. Means are plotted ± 1 standard error are for four vials of approximately 30 pupae heat-shocked at stage P12. Pretreatment or ramp treatment significantly reduced the percentage of abnormal flies after heat shock ($F_{(2,22)} = 52.79$; $P < 0.0001$). The effects of strain and strain × treatment interaction were also significant ($P < 0.05$).

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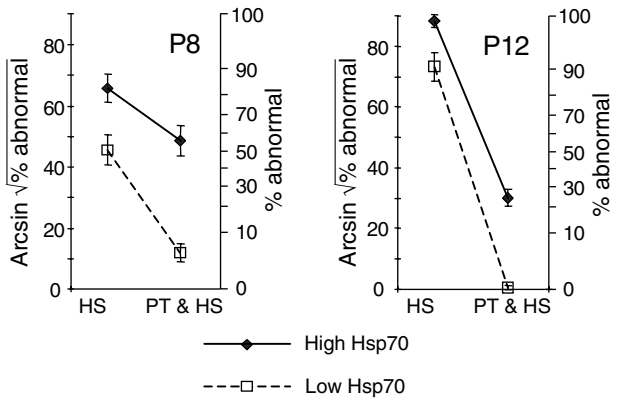

Fig. 6 Interaction of *hsp70* deficiency (see text for description), thermal treatment (HS or HS + PT at the P8 or P12 stage of pupal development) on the induction of wing abnormalities. Adults of three wild-type strains (ORA25 ORB25 and T32) were each crossed to strain Df(3R)T-33 + Df(3R)Kar3^{FE}/TM3 yielding F_1 progeny bearing either a visible marker (and high Hsp70 levels) or the deficiency (with reduced Hsp70 levels). Parental wild-type strain did not significantly affect the proportion of abnormal flies; thus results for the three wild-type strains are pooled in this figure. The interaction between deficiency, treatment was significant among pretreated flies at both stages (P8: $F = 29.941$, $P < 0.0001$; P12: $F = 61.775$, $P < 0.0001$).

developmental stage (P12), the strains did not differ in incidence of abnormalities (Fig. 4). Because induction of abnormalities is sensitive to the time at which pupae undergo heat-shock (Fig. 3), even the occasional instances in which the extra-copy strain exhibits fewer abnormalities than the excision strain could represent differences in developmental rates rather than susceptibility to developmental abnormalities.

Evolved differences in Hsp70 level. Previous work showed that strains ORA28, ORB28 and T32 accumulated lesser amounts of Hsp70 after a standard HS than did strains ORA25 and ORB25. After HS at P8, wing abnormalities were less frequent in ORB25 and ORB28 survivors than in ORA25, ORA28 or T32 survivors, both with and without PT (Table 1). After HS at P12, one of the low Hsp70 strains (A28) had the lowest incidence of wing abnormalities without PT, and two low Hsp70 strains (A28 and T32) had the lowest incidence of wing abnormalities with PT (Table 1). Thus, these strains do not support an inverse relationship between Hsp70 level and incidence of abnormalities.

Reduction of hsp70 *gene dosage.* As expected, the *hsp70* deficiency strains (OR/Df) accumulated less Hsp70 after heat shock than did controls (OR/TM3) (39.4 ± 2.2 vs. 48.6 ± 2.7% of standard, respectively, $F_{(1,20)} = 7.05$, $P = 0.015$). Among the progeny of the strains crossed to the deficiency strain, results were similar at both P8 and P12 (Fig. 6): HS of pupae resulted in fewer wing abnormalities in surviving flies with an *hsp70* deficiency than in controls ($F_{(1,143)} = 50.7$, $P < 0.001$). This outcome was evident in flies both with and without PT. Indeed, PT reduced the incidence of wing abnormalities more in progeny bearing the deficiency than in the control ($F_{(1,143)} = 13.0$, $P < 0.001$).

Discussion

Effects of heat shock and pretreatment on development

As stated in the Introduction, the evolutionary impact of stress induction of developmental abnormalities is a joint function of the intrinsic sensitivity of development to stress, the prevalence of stress in natural microclimates, and the impact of the abnormalities on fitness. Here we have shown that in the absence of PT, the intrinsic sensitivity of development to stress can be considerable. For many HSs, nearly all surviving flies had abnormal wings (Table 1). This finding, moreover, is consistent with a considerable older literature (Goldschmidt 1945; Goldschmidt 1949; Milkman 1962; Petersen & Mitchell 1991) and implies that lethality alone can be an imperfect metric of thermal sensitivity. Indeed, if the net effect of HS on fitness is the product of the probabilities of dying outright (e.g. Fig. 1, top) and developing abnormally (e.g. Fig. 1, bottom), HS is a profoundly effective selective agent. The specific developmental failures responsible for these abnormalities are unknown. Indeed, given that more than 100 proteins participate in wing morphogenesis, candidate genes abound (Birdsall *et al*. 2000; Palsson & Gibson 2000; Zimmerman *et al*. 2000). Alternatively, or in addition, the abnormalities could result from the impact of heat shock on the eclosion process.

Pretreatment, which induces expression of heat-shock proteins and other responses, mitigates the impact of HS on development, as also shown previously (Milkman & Hille 1966; Mitchell *et al*. 1979; Petersen & Mitchell 1982; Isaenko *et al*. 2002). Even 15 min of PT was sufficient to reduce HS-induced wing abnormalities. Its protective effect, however, is not the same for all abnormalities and at all ages or stages of development. For example, PT was especially effective in reducing the incidence of blistered wings. As for age and stage, all cases in which PT did not reduce heat-induced wing abnormalities occurred at 48 h APF or at the P8 stage; in general, PT reduced wing abnormalities more when administered at the P12 stage than at 48 h APF or P8. Either the 72 h to P12 stage is more susceptible to PT, or induction of wing abnormalities is more resistant to PT at 48 h APF and P8. This pattern accords with some key developmental time points in the *Drosophila* wing, but not with others. As an example of the former, the proportion of abnormalities induced by HS is correlated

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with ecdysone titres at the time of HS (Bainbridge & Bownes 1981). The ecdysone regulatory pathway controls wing morphogenesis, and D'Avino & Thummel (2000) propose that the ecdysone regulation of integrin expression is crucial for wing morphogenesis. Other developmental events, by contrast, are not correlated with developmental variation in sensitivity to HS and PT. For example, vein/intervein differentiation is complete by 36 h APF, wing sensory neurones are in place by 18–20 h APF, and the nervous system of the pupa is completely formed and resembles that of the adult at P12 (Murray *et al*. 1984; Garcia-Bellido & de Celis 1992; Tissot & Stocker 2000). Nonetheless, HS and PT occurring after these events have dramatic impacts on wing development.

In nature, organisms seldom undergo a stereotyped HS only or PT + HS regime such as in the present study. Nonetheless, the thermal kinetics of natural objects and microhabitats are such that in nature ectotherms typically undergo gradual warming before experiencing harmful or peak temperatures (Feder 1997; Feder *et al*. 1997; Roberts & Feder 1999; Roberts & Feder 2000). In nature, such gradual warming regimes are sufficient to induce a heat-shock response (Feder 1997; Feder *et al*. 1997; Roberts & Feder 1999; Roberts & Feder 2000). In the present study we imposed a gradual warming regime in the laboratory that resembled those experienced by *Drosophila* in nature; this gradual warming reduced HS-induction of wing abnormalities to an extent similar to that by standard PT (Fig. 5). Neither PT nor natural warming, however, is sufficient to mitigate all HS-induction of wing abnormalities in the laboratory and in nature. Detailed assessments of the schedule of natural HS and PT, which are necessary to estimate the demographic impact of natural hyperthermia on development, are not yet available.

Role of heat-shock proteins in PT effects

Abundant evidence establishes that Hsps are responsible for at least some of PT-induced protection of survival (Feder & Hofmann 1999). Indeed, the PTs of the present study induce Hsp expression readily in *Drosophila*, and either these Hsps or related proteins play key roles in development (see Introduction). Thus, that Hsps are responsible for at least some of PT-induced protection of development is an obvious hypothesis. Our findings, however, either do not support this hypothesis or indicate its opposite: that expression of Hsps (or at least Hsp70) sensitizes development to thermal damage. The latter indication emerges from comparisons of strains with or without an *hsp70* deficiency, in which PT reduced HS-induced abnormalities more in deficiency strains (Fig. 6), and is consistent with prior studies of both cells in culture and whole *Drosophila* (Feder *et al*. 1992; Krebs & Feder 1997; Krebs & Feder 1998).

We suggest that the mixed or negative impact of Hsp70 on HS-induction of wing abnormalities may stem from the roles of Hsp70 family members in signalling pathways that are critical for normal development (Nollen & Morimoto 2002). For example, the nuclear receptor of ecdysone, the major steroid hormone coordinating *Drosophila* metamorphosis, requires Hsp70 for its activation (Arbeitman & Hogness 2000). In mammalian cells, Hsp72 affects the dephosphorylation of c-Jun amino (N) terminal kinases (JNK) and hence suppresses their activation (Mosser *et al*. 1997; Gabai *et al*. 1998). In *Drosophila*, the JNK signalling pathway has been implicated in crossvein formation (Adachi-Yamada *et al*. 1999; Boutros *et al*. 1998; Li *et al*. 1999; Marcus 2001). Suppression of JNK activation during wing development might therefore interfere with wing vein formation. Ectopic wing vein phenotypes are also characteristic of mutations in the EGFR and JAK-STAT pathways (Yan *et al*. 1996), with which Hsp70 family members also interact (Doong *et al*. 2000; Sarkar *et al*. 2001). Thus, while induction of Hsp70 may help keep hyperthermic cells alive, it may also overstimulate or inhibit numerous signalling pathways involved in cell proliferation, maturation and death, resulting in developmental failure (Gabai & Sherman 2002).

By contrast, both Roberts & Feder (1999) and Isaenko *et al*. (2002) reported that increased PT-induction of Hsp70 protected development against disruption. Both studies involved larvae, and in the latter the protection was against mitotic poisons. Different developmental events occur in larvae and pupae, and those in larvae may interact positively with Hsp70 whereas those in pupae do not. Alternatively, induction of Hsp70 expression is greater in pupae than in larvae, and negative impact of such high levels may exceed their benefits, as is the case for inducible thermotolerance (Krebs & Feder 1998).

Conclusion

A recent review entitled 'Ecological developmental biology: developmental biology meets the real world' (Gilbert 2001) emphasizes that, while most developmental research occurs in laboratories under controlled conditions, developmental programmes evolved and are presently executed in natural environments where conditions such as temperature, photoperiod, nutrition, competition and predation are highly variable. Gilbert (2001) also emphasizes, however, that a principal outcome of evolution under natural environmental regimes has been developmental plasticity resulting in phenotypes that are adaptive for each regime. For example, the larval/pupal environment of the African butterfly *Bicyclus* induces either cryptic or predator-deterring wing colouration, each appropriate for a different season of adult activity (Beldade & Brakefield 2002). Gilbert views disruptions of development as principally an outcome

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of anthropogenic stress. Ecological developmental biology needs also to acknowledge that natural stress is real and that development can go awry in the natural setting even without anthropogenic inputs. As this and related studies show (Roberts & Feder 1999; Roberts *et al*. unpublished data); significant fractions of natural populations can develop significant morphological defects when the developmental environment undergoes thermal stress. Such defects affect locomotor ability (Roberts & Feder 1999; Roberts *et al*. unpublished data) and male courtship success (J. Posluszny, unpublished data). The severity of these defects, moreover, can vary with the kinetics of the thermal regime and the expression of a heat-inducible molecular chaperone, which interacts with development in numerous ways. Finally, we emphasize that the *Drosophila* wing is but a representative structure in a single species; environmentally induced developmental abnormalities are present in multiple structures in multiple species (Roberts & Feder 1999; Polak 2002). Both induction of developmental abnormalities in natural environments and the genetic mechanisms that prevent or exacerbate it are ripe for future study.

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References

Adachi-Yamada T, Fujimura-Kamada K, Nishida Y, Matsumoto K (1999) Distortion of proximodistal information causes JNK-dependent apoptosis in *Drosophila* wing. *Nature*, **400**, 166–169.

Arbeitman MN, Hogness DS (2000) Molecular chaperones activate the *Drosophila* ecdysone receptor, an RXR heterodimer. *Cell*, **101**, 67–77.

Arrigo AP, Tanguay RM (1991) Expression of heat shock proteins during development in *Drosophila*. *Results and Problems in Cell Differentiation*, **17**, 106–119.

Bainbridge SP, Bownes M (1981) Staging the metamorphosis of *Drosophila melanogaster*. *Journal of Embryology and Experimental Morphology*, **66**, 57–80.

Beldade P, Brakefield PM (2002) The genetics and evo-devo of butterfly wing patterns. *Nature Reviews Genetics*, **3**, 442–452.

Bettencourt BR, Feder ME, Cavicchi S (1999) Experimental evolution of Hsp70 expression and thermotolerance in *Drosophila melanogaster*. *Evolution*, **53**, 484–492.

Bier E (2000) Drawing lines in the *Drosophila* wing: initiation of wing vein development. *Current Opinion in Genetics and Development*, **10**, 393–398.

Birdsall K, Zimmerman E, Teeter K, Gibson G (2000) Genetic variation for the positioning of wing veins in *Drosophila melanogaster*. *Evolution and Development*, **2**, 16–24.

Boutros M, Paricio N, Strutt DI, Mlodzik M (1998) Dishevelled activates JNK and disk riminates between JNK pathways in planar polarity and wingless signaling. *Cell*, **94**, 109–118.

Brody T (1999) The interactive fly: gene networks, development and the Internet. *Trends in Genetics*, **15**, 333–334.

D'Avino PP, Thummel CS (2000) The ecdysone regulatory pathway controls wing morphogenesis and integrin expression during *Drosophila* metamorphosis. *Developmental Biology*, **220**, 211–224.

Doong H, Price J, Kim YS *et al.* (2000) CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. *Oncogene*, **19**, 4385–4395.

Elefant F, Palter KB (1999) Tissue-specific expression of dominant negative mutant *Drosophila* HSC70 causes developmental defects and lethality. *Molecular Biology of the Cell*, **10**, 2101–2117.

Feder ME (1997) Necrotic fruit: a novel model system for thermal ecologists. *Journal of Thermal Biology*, **22**, 1–9.

Feder ME, Blair N, Figueras H (1997) Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Functional Ecology*, **11**, 90–100.

Feder ME, Cartaño NV, Milos L, Krebs RA, Lindquist SL (1996) Effect of engineering *hsp70* copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. *Journal of Experimental Biology*, **199**, 1837–1844.

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology*, **61**, 243–282.

Feder ME, Krebs RA (1998) Natural and genetic engineering of thermotolerance in *Drosophila melanogaster*. *American Zoologist*, **38**, 503–517.

Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S (1992) The consequences of expressing Hsp70 in *Drosophila* cells at normal temperatures. *Genes and Development*, **6**, 1402–1413.

Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, Sherman MY (1998) Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Letters*, **438**, 1–4.

Gabai VL, Sherman MY (2002) Interplay between molecular chaperones and signaling pathways in survival of heat shock. *Journal of Applied Physiology*, **92**, 1743–1748.

Garcia-Bellido A, de Celis JF (1992) Developmental genetics of the venation pattern of *Drosophila*. *Annual Review of Genetics*, **26**, 277–304.

Gausz J, Gyurkovics H, Bencze G *et al.* (1981) Genetic characterization of the region between 86f1,2 and 87b15 on chromosome 3 of *Drosophila melanogaster*. *Genetics*, **98**, 775–789.

Gilbert SF (2001) Ecological developmental biology: developmental biology meets the real world. *Developmental Biology*, **233**, 1–12.

Goldschmidt RB (1945) Additional data on phenocopies and genic action. *Journal of Experimental Zoology*, **100**, 193–201.

Goldschmidt RB (1949) Phenocopies. *Scientific American*, **181**, 46–49.

Isaenko OA, Karr TL, Feder ME (2002) Hsp70 and thermal pretreatment mitigate developmental damage due to mitotic poisons in *Drosophila*. *Cell Stress and Chaperones*, **7**, 297–308.

Klein T (2001) Wing disc development in the fly: the early stages. *Current Opinion in Genetics and Development*, **11**, 470–475.

Krebs RA, Feder ME (1997) Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. *Cell Stress and Chaperones*, **2**, 60–71.

Krebs RA, Feder ME (1998) Hsp70 and larval thermotolerance in *Drosophila melanogaster*: how much is enough and when is more too much? *Journal of Insect Physiology*, **44**, 1091–1101.

Krebs RA, Feder ME, Lee J (1998) Heritability of expression of the 70-kD heat-shock protein in *Drosophila melanogaster* and its

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relevance to the evolution of thermotolerance. *Evolution*, **52**, 841–847.

Li L, Yuan H, Xie W *et al.* (1999) Dishevelled proteins lead to two signaling pathways Regulation of LEF-1 and c-Jun N-terminal kinase in mammalian cells. *Journal of Biological Chemistry*, **274**, 129–134.

Lindsley DL, Zimm GG (1992) *The Genome of Drosophila Melanogaster*. Academic Press, San Diego.

Marcus J, M (2001) The development and evolution of crossveins in insect wings. *Journal of Anatomy*, **199**, 211–216.

Milkman RD (1961) Temperature adaptation in *Drosophila melanogaster* pupae. *Biological Bulletin*, **121**, 367.

Milkman RD (1962) Temperature effects on day old *Drosophila* pupae. *Journal of General Physiology*, **45**, 777–799.

Milkman RD, Hille B (1966) Analysis of some temperature effects on *Drosophila* pupae. *Biological Bulletin*, **131**, 331–345.

Mitchell HK, Lipps LS (1978) Heat shock and phenocopy induction in *Drosophila*. *Cell*, **15**, 907–918.

Mitchell HK, Moller G, Petersen NS, Lipps-Sarmiento L (1979) Specific protection from phenocopy induction by heat shock. *Developmental Genetics*, **1**, 181–192.

Mitchell HK, Petersen NS (1982) Developmental abnormalities in *Drosophila* induced by heat shock. *Developmental Genetics*, **3**, 91–102.

Mohler JD (1965) The influence of some *crossveinless*-like genes on the *crossveinless* phenocopy sensitivity in *Drosophila melanogaster*. *Genetics*, **51**, 329–340.

Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B (1997) Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Molecular and Cellular Biology*, **17**, 5317–5327.

Murray MA, Schubiger M, Palka J (1984) Neuron differentiation and axon growth in the developing wing of *Drosophila melanogaster*. *Developmental Biology*, **104**, 259–273.

Nollen EA, Morimoto RI (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *Journal of Cell Science*, **115**, 2809–2816.

Palsson A, Gibson G (2000) Quantitative developmental genetic analysis reveals that the ancestral dipteran wing vein prepattern is conserved in *Drosophila melanogaster*. *Development, Genes and Evolution*, **210**, 617–622.

Petersen NS, Mitchell HK (1982) Effects of heat shock on gene expression during development: induction and prevention of the multihair phenocopy in *Drosophila*. In: *Heat Shock: from Bacteria to Man* (eds Schlesinger MJ Ashburner M Tissieres A), pp. 345–352. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Petersen NS, Mitchell HK (1991) Environmentally induced development defects in *Drosophila*. *Results and Problems in Cell Differentiation*, **17**, 29–43.

Polak M (2002) *Developmental Instability: Causes and Consequences*. Oxford University Press, New York.

Roberts SP, Feder ME (1999) Natural hyperthermia and expression of the heat-shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*. *Oecologia*, **121**, 323–329.

Roberts SP, Feder ME (2000) Changing fitness consequences of *hsp70* copy number in transgenic *Drosophila* larvae undergoing natural thermal stress. *Functional Ecology*, **14**, 353–357.

Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.

Sarkar S, Pollack BP, Lin KT *et al.* (2001) hTid-1, a human DnaJ protein, modulates the interferon signaling pathway. *Journal of Biological Chemistry*, **276**, 49034–49042.

Silbermann R, Tatar M (2000) Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. *Evolution*, **54**, 2038–2045.

Society for Developmental Biology (2002) *Wing; Genes Involved in Organ and Tissue Development; The Interactive Fly*. http://sdb.bio.purdue.edu/fly/aimorph/wing.htm

Stark J, Bonacum J, Remsen J, DeSalle R (1999) The evolution and development of Dipteran wing veins: a systematic approach. *Annual Review of Entomology*, **44**, 97–129.

Tatar M, Khazaeli AA, Curtsinger JW (1997) Chaperoning extended life. *Nature*, **390**, 30.

Tissot M, Stocker RF (2000) Metamorphosis in *Drosophila* and other insects: the fate of neruons throughout the stages. *Progress in Neurobiology*, **61**, 89–111.

Varma N, Sinha SP (1977) Genotypic control of spontaneous phenocopies in *Drosophila melanogaster*. *Egyptian Journal of Genetics and Cytolology*, **6**, 1–5.

Velazquez JM, DiDomenico BJ, Lindquist S (1980) Intracellular localization of heat shock proteins in *Drosophila*. *Cell*, **20**, 679–689.

Waddington CH (1940) The genetic control of wing development in *Drosophila*. *Journal of Genetics*, **41**, 75–139.

Waddington CH (1961) Genetic assimilation. *Advances in Genetics*, **10**, 257–293.

Welte MA, Duncan I, Lindquist S (1995) The basis for a heat-induced developmental defect: defining crucial lesions. *Genes and Development*, **9**, 2240–2250.

Welte MA, Tetrault JM, Dellavalle RP, Lindquist SL (1993) A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. *Current Biology*, **3**, 842–853.

Yan R, Luo H, Darnell JEJ, Dearolf C (1996) A JAK-STAT pathway regulates wing vein formation in *Drosophila*. *Proceedings of the National Academy of Sciences USA*, **93**, 5842–5847.

Yue L, Karr TL, Nathan DF *et al.* (1999) Genetic analysis of viable Hsp90 alleles reveals a critical role in *Drosophila* spermatogenesis. *Genetics*, **151**, 1065–1079.

Zatsepina OG, Velikodvorskaia VV, Molodtsov VB *et al.* (2001) A *Drosophila melanogaster* strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. *Journal of Experimental Biology*, **204**, 1869–1881.

Zimmerman E, Palsson A, Gibson G (2000) Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics*, **155**, 671–683.

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