Hsp90 as a capacitor for morphological evolution

Suzanne L. Rutherford*† & Susan Lindquist*

**Howard Hughes Medical Institute, University of Chicago, 5841 South Maryland Avenue MC1028, Chicago, Illinois 60637, USA*

The heat-shock protein Hsp90 supports diverse but specific signal transducers and lies at the interface of several developmental pathways. We report here that when *Drosophila* Hsp90 is mutant or pharmacologically impaired, phenotypic variation affecting nearly any adult structure is produced, with specific variants depending on the genetic background and occurring both in laboratory strains and in wild populations. Multiple, previously silent, genetic determinants produced these variants and, when enriched by selection, they rapidly became independent of the Hsp90 mutation. Therefore, widespread variation affecting morphogenic pathways exists in nature, but is usually silent; Hsp90 buffers this variation, allowing it to accumulate under neutral conditions. When Hsp90 buffering is compromised, for example by temperature, cryptic variants are expressed and selection can lead to the continued expression of these traits, even when Hsp90 function is restored. This provides a plausible mechanism for promoting evolutionary change in otherwise entrenched developmental processes.

Among the major heat-shock proteins, Hsp90 is unique in its functions[1]. It is not required for the maturation or maintenance of most proteins *in vivo*[2]. Most of its many identified cellular targets are signal transducers—cell-cycle and developmental regulators whose conformational instability is relevant to their roles as molecular switches[3]. Through low-affinity interactions characterized by repeated cycles of binding and release[4], Hsp90 keeps these unstable signalling proteins poised for activation until they are stabilized by conformational changes associated with signal transduction[3,5]. Minor changes in amino-acid sequence can have substantial effects on a protein's conformational stability and Hsp90 recognizes structural features common to unstable proteins rather than specific sequence motifs[2,6,7,37]. Thus, individual members of highly homologous protein families, such as steroid-hormone receptors[8,9] or cyclin-dependent[10] or Src-family[11] kinases, can vary greatly in their dependence on Hsp90.

Studies of yeast illustrate the specificity of Hsp90: at normal temperatures, reductions in Hsp90 levels that have no apparent effects on cell growth or metabolism can completely abolish signalling through Hsp90-dependent pathways[5,8,11]. Conditions that cause general protein damage can divert Hsp90 from its normal targets to other partially denatured proteins[2,6,12,13]. Because of its dual involvement with inherently unstable signal transducers on the one hand, and with the cellular response to stress on the other, Hsp90 may link developmental programs to environmental contingency.

Phenotypic variation in Hsp90 mutants

Mutations in the *Drosophila* Hsp90 gene (*Hsp83*) have been independently isolated in three laboratories[14–16]. The homozygous mutations are lethal[14,16] and the mutants are maintained as heterozygous stocks. We frequently observed flies with several unusual morphological abnormalities in these stocks (up to 5% in some stocks). Abnormalities also arose when *Hsp83* mutants were outcrossed with standard laboratory strains (Tables 1, 2 and Fig. 1); these occurred in 1–2% of the F_1 flies studied and in a high proportion of the crosses. Of 141 crosses with more than 10 different standard laboratory strains, 53 crosses produced flies with observable defects (174 of 10,400 flies scored, 1.7%). Defects ranged from subtle to severe, involved either one or both sides of the animal, and included body-part transformations, disrupted abdominal patterning, bristle duplications, deformed eyes or legs and changes in wing shape or venation (Table 1).

The spontaneous appearance of these developmental abnormalities resulted from altered Hsp90 function. First, similar traits arose with mutant *Hsp83* alleles of independent origin (Table 2). Second, when different heterozygous *Hsp83* stocks were crossed together, producing heteroallelic combinations with even lower Hsp90 function ($Hsp83^1/Hsp83^2$), both the severity and the incidence of some of these abnormal phenotypes were increased (Fig. 1; compare a, c with k, l). Third, when a standard wild-type laboratory strain (*Ore-R*), was raised on food containing a potent, specific inhibitor of Hsp90 (geldanamycin[17]) similar abnormalities were produced (Fig. 1q). Of 271 flies raised on the drug, 21 were abnormal

Table 1 Developmental defects associated with Hsp90 deficit

Body part	Code	Description	No. of observations	Temperature (°C)	F_1?	F_2?
Abdomen	A1	Disorganized tergites	14	25	Yes	–
	A2	External trachea?	7	25	No	n.d.
Bristles	B1	Duplications	36	30, 18	Yes	Yes
	B2	Extra scutellar bristles	48	18	Yes	n.d.
	B3	Split scutellars	8	18	Yes	n.d.
	B4	Forked	5	25	No	n.d.
Eyes	E1	Deformed	22	30	Yes	Yes
	E2	Transformed	7	18	No	–
	E3	Smooth	18	18	Yes	Yes
	E4	Rough	16	25	Yes	Yes
	E5	Black facets	24	18	Yes	Yes
	E6	Eyes absent	3	18	Yes	n.d.
Halteres	H1	Ubx transformations	9	25	Yes	Yes
Legs	L1	Deformed	28	18	Yes	Yes
	L2	Transformed	3	18	No	–
Thorax	T1	Disc eversion	12	25	No	–
	T2	Humeral 'balls'	5	26	Yes	n.d.
	T3	Duplication	6	25	No	n.d.
Wings	W1	Small round	26	18	Yes	Yes
	W2	Notched	6	18	Yes	–
	W3	Wing veins	7	18	No	Yes
	W4	Wing border	5	25	No	–
	W5	Transformed	9	18	Yes	n.d.

Developmental abnormalities produced in *Hsp83* mutants, coded according to the part of the fly affected. The approximate number of observations of, and the temperature most frequently producing, each trait are indicated. The observation of at least one cross producing multiple F_1 flies with a given trait is indicated by 'Yes', as is any instance of transmission of the trait to the F_2 generation. A dash indicates not observed; n.d., not done. Many fewer flies were tested at 30 °C, so this situation is under-represented.

† Present address: Developmental Biology Center, University of California at Irvine, 4205 Biological Science II, Irvine, California 92697-2275, USA.

(7.7%); of 443 controls raised on normal food, only one was affected (mildly—it lacked a bristle).

Effect of genetic background

What disrupted normal development in flies with impaired Hsp90 function? We considered three possibilities of increasing interest. First, the mutants might be more sensitive to the environment: as a stress-response factor, wild-type Hsp90 might simply buffer against 'developmental noise' caused by random micro-environmental effects with little or no genetic basis. Second, Hsp90 mutants might exhibit an increased mutation rate: Hsp90 might be directly or indirectly involved in the fidelity of DNA replication. Third, cryptic genetic variation might be expressed to a greater extent: because it is a chaperone for signal-transduction elements, Hsp90 might normally suppress the expression of genetic variation affecting many developmental pathways.

Further studies supported the third, and most interesting, of these possibilities. Often, when *Hsp83* alleles were crossed to different normal laboratory stocks, several of the F_1 progeny from a given cross shared the same defect, defects distinct from those observed in crosses to other stocks (Table 1 and Fig. 1e–j). Sometimes repeated crosses to the same stocks again produced abnormal flies with the same defects. When affected F_1 flies were crossed together, similarly affected progeny again often appeared in the F_2

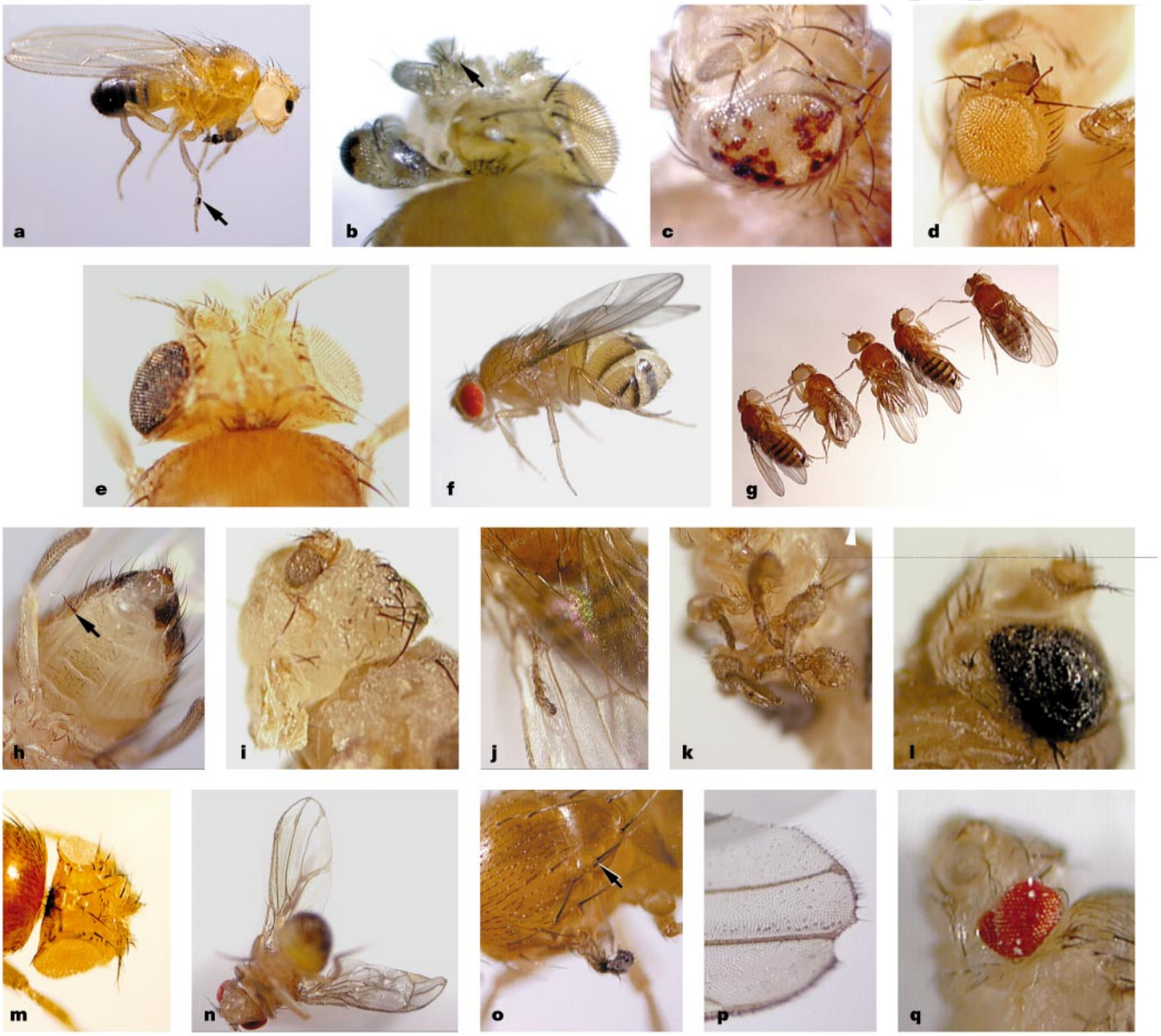

Figure 1 Developmental abnormalities associated with Hsp90 deficits. See Table 1 for coding of traits. Deformities appearing in *Hsp83* mutant stocks: **a**, *13F3/TM6B*, deformed fore-leg (code L1) and transformed 2nd leg (L2) with an ectopic sex-comb (arrow); **b**, *P582/TM6B*, deformed eye (E1) with an extra antennae (arrow); **c**, *e1D/TM6B*, smooth eyes (E3) with black facets (E5); **d**, *P582/TM6B*, eye margin transformed into scutellum (E2). Abnormal F_1 hybrids produced from crosses between between *Hsp83* mutant stocks and marked laboratory strains: **e**, *e6DX582*^{e1}, left eye has black facets (E5); **f**, *e6AXdpp*^{4}, disorganized abdominal tergites (A1); **g**, *e1DXTM3*, *ftz-lacz*, small wings (W1); **h**, *e3AXln(2RH)PL,w*^{m}, extraneous tissue growing out of tracheal pit (A2, arrow), **i**, *19F2XCdc37*^{e4D}, eyes absent (E6); **j**, *13F3XCdc37*^{e1E}, wing margin material growing into wing; **m**, *19F2X582*^{e1}, deformed eye. Heteroallelic *Hsp83* combination *e1D/9J1*: **k**, severely deformed legs (L1), **l**, severe black-facet phenotype (E5). Abnormal F_1 hybrids produced with wild-type laboratory stocks and *Hsp83* mutants: **n**, *e1D* or *9J1XIR-6*, thickened wing veins (W3); **o**, *P582XSamarkind*, transformed wing (W5) and extra scutellar bristle (B2, arrow). Abnormalities in wild-type lines raised on geldanamycin: **p**, *IND-6*, notched wings (W2); **q**, *Ore-R*, deformed eye (E1).

Table 2 Strains yielding abnormal F_1 hybrids when crossed with *Drosophila* Hsp90 mutants or treated with geldanamycin

Category	Stock	Description	*Hsp83* hybrid/treated with geldanamycin	F_1 traits	Source	Date
Marked lab. strains	*dpp[4]*		*e3A*, *e6A*, *P582*	A1, T1	E. Ferguson	4/96
	Hkgsdf		*e1D*, *9J1*	E1	J. Hall	8.89
	In(1)w[m4], *w[m4]*		*e1D*, *P582*	E1	Mid-America	1/96
	In(2Rh)PL, *w[m]*		*e1D*, *e3A*, *e6A*, *9J1*	A1, A2, E1, H1, W5	P. Dimitri	3/96
	TM3, *ftz-lacz*		*e1D*	W3	N. Patel	8/94
	w[1118]; *582[e1]*	Viable P582 excision	*P582*, *e3A*, *e1D*, *e6A*, *e6D*, *19F2*	A2, E1, E2, E3, E5, H1, L1, W5	L. Yue	4/95
	w[1118]; *582[e4]*	Viable P582 excision	*e1D*	E5	L. Yue	4/95
	Cdc37[e1C]	T(3;3)	*e1D*	E5	G. Rubin[14]	9/95
	Cdc37[e1E]	P26(Δ13 amino acids)	*19F2*, *9J1*	T3, W2, W4, W5	G. Rubin[14]	9/95
	Cd37[e4D]	Null; W7(stop codon)	*e3A*, *e4A*, *e1D*, *9F2*, *9J1*	A1, B2, E6, L1, T2, T3, W1	G. Rubin[14]	9/95
	Cyp-1[317]f	G19D	*e1D*	E5, W1	S. Rutherford	3/93
WT lab. stocks	*CT-1*	South Africa, 1954	*e1D*, *9J1*	W1	Mid-America	5/96
	DmAZ	Arizona, 1990	*e1D*, *P582*, *582[211]*, *582[e13]*	W5	T. Karr	10/95
	IR-6	Isofemale *Ives* derivative	*e1D*, *9J1*	E1, E3, W3	G. Gibson[28]	6/96
	IS-3	Isofemale *Ives* derivative	*e1D*, *e6A*, *9J1*	A1, B1, H1, L1, W5	G. Gibson[28]	6/96
	Ore-R	Population cage stock	Geldanamycin	B1, B2, E1, L1, W1, W2	S. Elgin	6/90
	RI-16	Inbred	*P582*, *9J1*	B1, B3, L1	T. Mackay	8/97
	RI-20	Inbred	*9J1*	L1	T. Mackay	8/97
	RI-25	Inbred	*9J1*	T3	T. Mackay	8/97
	RI-27	Inbred	*P582*, *9J1*	T2	T. Mackay	8/97
	Samarkind	inbred	*P582*	L1, T1, W5	T. Mackay	8/97
	wol-	*Ore-R* derivative	*P582*	H1	T. Karr	10/95
Fresh WT lines	*IND-2*	Isofemale Indiana, 9/97	Geldanamycin	W1	R. Krebs	9/97
	IND-6	Isofemale Indiana, 9/97	Geldanamycin	A1, W1, W2	R. Krebs	9/97
	KYA-1D	Kenya, 1996	*e1D*, *9J1*	E5, W3	M. Ashburner	6/96
	KYA-9C	Kenya, 1996	*e1D*, *9J1*	B1, L1, W1, W2	M. Ashburner	6/96
	P-4	Indiana, 8/96	*e1D*, *9J1*	B1, B2, W3	S. Rutherford	8/96
	P3-C	Indiana, 8/96	*e6A*, *9J1*	A1, B1	S. Rutherford	8/96
Hsp83 alleles	*13F3*	R48C		L1	E. Hafen[15]	12/94
	19F2	R48C		L1	E. Hafen[15,16]	12/94
	9J1	E377K		B2, L1, W1	E. Hafen[15,16]	12/94
	e1D	S38L		B2, E3, E4, E5, L1	G. Rubin[14]	6/94
	e3A	S574C			G. Rubin[14]	6/94
	e4A	S655F			G. Rubin[14]	6/94
	e6A	S592F		A2	G. Rubin[14]	6/94
	e6D	E317K			G. Rubin[14]	6/94
	P582	P-element in 5′ UTS		B2, E1, E2, L1, T1, W1	P. Deak	12/94
	582[e11]	Lethal P582 excision		W5	L. Yue	4/95

Strains producing deformed flies when crossed with Hsp90 mutants or raised on geldanamycin. Fly strains were divided into four categories: marked laboratory strains; wild-type laboratory stocks that had been maintained in the laboratory for many years; recently established (<1 year) wild-type lines; and lines carrying mutant *Hsp83* alleles. Mutant *Hsp83* alleles responsible for specific F_1 traits are indicated, with traits coded as in Table 1. Traits listed with the *Hsp83* alleles were observed in those stocks. UTS, untranslated sequence; WT, wild-type.

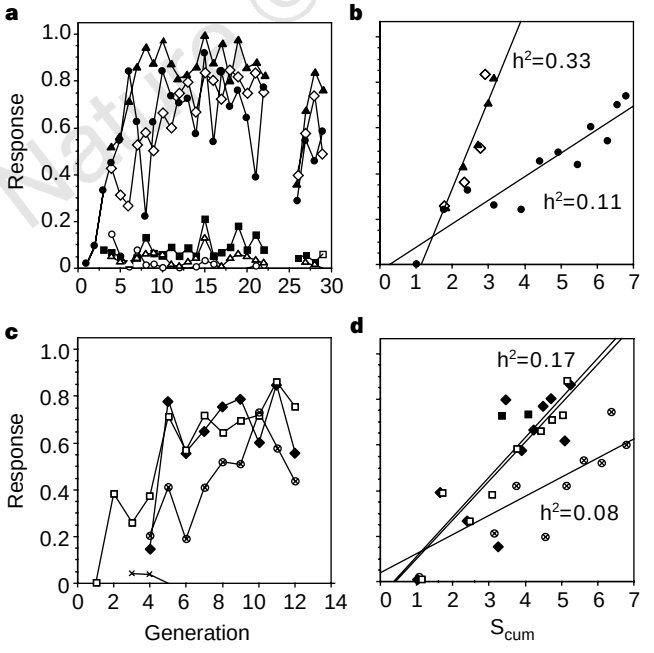

Figure 2 Selection experiments. **a**, **b**, Selection for the deformed-eye trait; **c**, **d**, Selection for the wing-vein trait. Selection lines: open circles, *LE1*; filled squares, *LE2*; open triangles, *LE3*; filled circles, *HE1*; open diamonds, *HE2*; filled triangles, *HE3*; crosses, *LV1*; open squares, *HV1*; circles with crosses, *HV2*; filled diamonds, *HV3*. S_{cum}, cumulative selection differential.

generation (Table 1). This pattern of heritability was unlikely to have been generated by *de novo* mutation and showed that the traits had a genetic basis, correlated to specific genetic backgrounds.

Production of specific traits

To determine whether the abnormal traits could be due to single genetic determinants interacting with the *Hsp83* mutations, we chose a wing and an eye trait for more extensive analysis. Neither trait was observed in any of the original parental stocks, but both appeared among the hybrid F_1 progeny of different crosses between the heterozygous *Hsp83* mutants and other stocks. One of these genetically hybrid F_1 progeny was a male with a deformed eye (Fig. 1m), arising from a cross between an *Hsp83* mutant (*19F2*) and a laboratory strain (w^{1118}) derivative. The other F_1 male had thickened wing veins (Fig. 1n) and was a hybrid resulting from a cross between an *Hsp83* line (*e6D*) and a wild-type strain (*Ives*). In both cases, when these males were crossed to a few (~5) unaffected F_1 females (*Hsp83*/+) from the same parental cross, F_2 flies expressing a phenotype similar to that of the F_1 male were produced. Selection was initiated by crossing affected F_2 progeny to generate high-expression lines (high vein, HV, and high eye, HE). Unaffected progeny were crossed together to generate low-expression lines (LV and LE). We selected either for, or against, the trait in all subsequent generations. By the fourth generation, sufficient numbers of affected flies were produced to split each of the lines into three replicates.

The initial response to this selection regime indicated that the traits were polygenic (Fig. 2a, c). In successive generations, the proportion of affected progeny in the high-expression lines

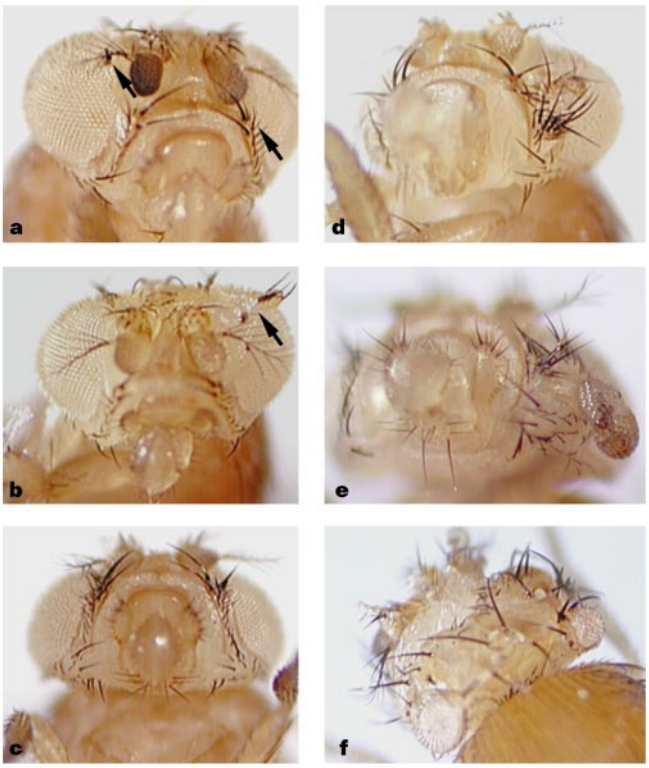

Figure 3 Variation within and between high-expression lines with the deformed-eye trait. **a**–**e**, Range of phenotypes in generation 14 of line *HE2*. **a**, Very mild duplications of wing margin bristles (arrows); **b**, small protuberances (arrow); **c**–**e**, severe bristle duplications and deformities. **f**, A severely affected animal from line *HE3*; in comparison with line *HE2*, many *HE3* mutants had small eyes and bare cuticle rather than overproduction of bristles. *HE3* was the only line in which flies with three antennae were produced.

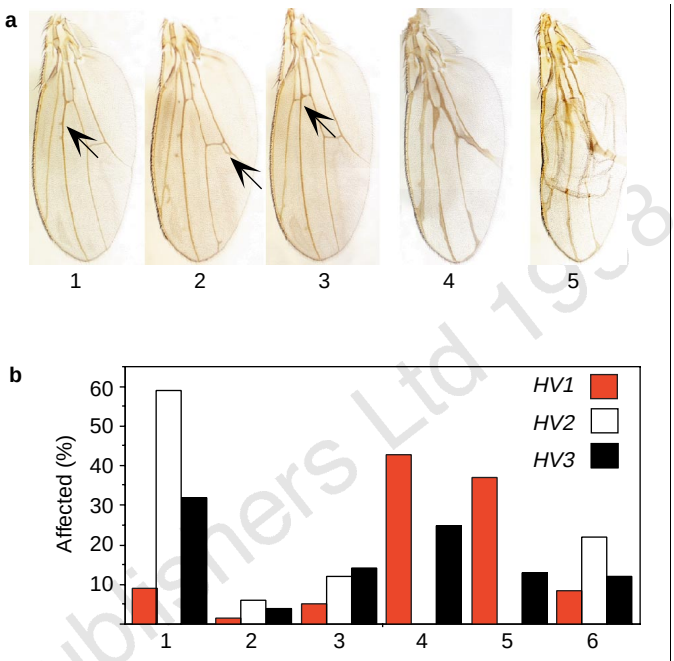

Figure 4 Partitioning of the wing-vein trait between lines. **a**, Wing defects of increasing severity from left to right: bumps on veins, looped veins, double veins, thick veins, and wing blisters. **b**, Per cent of affected individuals within each high-expression line (*HV1–3*) that exhibit traits of these severities (1–5), or other abnormal phenotypes (6).

increased and their phenotypes diverged. Furthermore, both traits exhibited a range of phenotypes within each line (Figs 3, 4). When the wing-vein trait was scored for increasing gradations of severity (Fig. 4), the distribution of affected flies varied markedly between the different high-expression lines (*HV1–3*) and between these lines and the base (parental) populations, which did not express the trait. All of the affected flies in line *HV2* expressed mild to moderate defects, whereas most of the affected flies from lines *HV1* and *HV3* expressed severe defects. This partitioning of the wing-vein phenotype between lines confirmed that more than one genetic determinant affected the trait.

The strong response to selection indicated that even though the founding populations were small, they contained a large amount of previously cryptic genetic variation that was capable of affecting these traits. We quantified this variation for each replicate line by regression of the initial selection response onto the cumulative selection differential[18]; the slope of each regression line is equal to its realized heritability (h^2; Fig. 2b, d). All h^2 values were non-zero, showing that selection had acted on pre-existing genetic factors influencing these traits.

Effect of temperature

Many of the traits seen in *Hsp83* heterozygotes were enhanced at either high (30 °C) or low (18 °C) temperatures (Table 1). For example, the wing-vein trait was produced in the high-expression lines that had been raised at 18 °C but was not observed in replicate cultures grown at 25 °C (results not shown). Conversely, the deformed-eye trait was enhanced at 30 °C and was not observed at 18 °C. To investigate this phenomenon further, we grew the six eye-trait selection lines at different temperatures within the normal growth range of *Drosophila* (Fig. 5). At 18 °C the trait was not expressed. Between 23 °C and 26 °C, the penetrance and the severity of the trait in the three high-expression lines (*HE1–3*) increased sharply, whereas only a few flies from any of the low-expression lines (LE1–3) were affected. However, at 30 °C, 20–30% of the flies in the low-expression lines presented the trait, indicating that some genetic determinants for the trait were still present in a cryptic state at the lower temperatures in these lines. The sigmoidal temperature–response curve (reaction norm; Fig. 5) indicates that

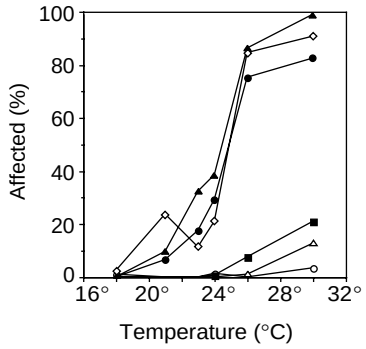

Figure 5 Temperature–response curves (norm of reaction) for the deformed-eye trait. Open circles, *LE1*; filled squares, *LE2*; open triangles, *LE3*; filled circles, *HE1*; *HE2*; filled triangles, *HE3*. Replicate cultures from generations 14–16 were grown at the indicated temperatures.

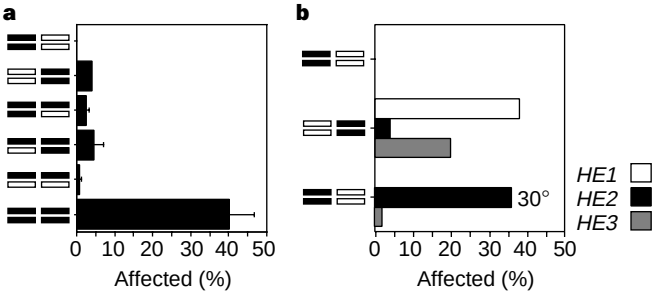

Figure 6 Genetic interactions in the *HE* lines. **a**, The percentage of flies affected (±s.e.m.) by each of the six possible combinations of the two major autosomes (second and third) from line *HE2* (filled rectangles at left of graph) and the unselected control chromosomes (open rectangles). Second chromosomes are shown to the left and third chromosomes to the right. Similar results were obtained with *HE1* and *HE3* chromosomes. **b**, Second and third chromosomes from different HE lines differed in their ability to produce the trait at 18 °C or at 30 °C.

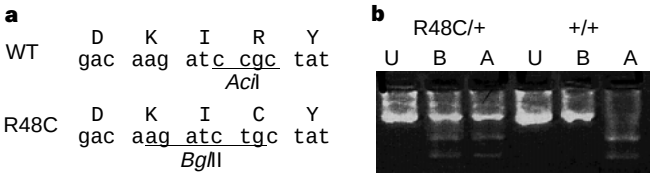

Figure 7 Genotyping of the *19F2* (R48C) *Hsp83* mutation. **a**, The base change causing the R48C mutation destroys an *Aci*I restriction site present in the wild-type sequence (WT) and creates a *Bgl*II site. **b**, Uncut controls (U) are 575 base pairs long. Characteristic *Bgl*II (B) or *Aci*I (A) digests, producing 335- and 240-base-pair products, are shown for R48C heterozygotes (R48C/+) and wild-type flies (+/+).

the eye phenotype is a threshold character[18]; the abrupt appearance of the trait depends on surpassing a threshold set by the number of underlying genetic determinants and their interaction with the environment.

Genetic interactions

To begin to characterize the genetic determinants underlying a particular morphological variant, we isolated chromosomes from each of the high-expression lines (*HE1–3*) in the context of a control genetic background. The effect of each chromosome in producing the deformed-eye phenotype was then tested in isolation and in combination with the other selected chromosomes. Figure 6 shows the complex relationship between the expression of the trait and the genotypes producing it. When either the second or the third chromosome from any of the high lines was heterozygous with the control chromosome in the new genetic background, the penetrance of the eye trait was markedly reduced (Fig. 6a). Homozygosity of any isolated second chromosome had no effect by itself at 25 °C, but appeared to affect the expression of genetic determinants on the third chromosome (Fig. 6). At 30 °C the isolated second chromosomes from line *HE2*, but not those from *HE1* or *HE3*, produced many affected flies (Fig. 6b). Thus, the second chromosomes in the three high-expression lines contain at least two different variants affecting the trait. When these determinants were separated from determinants present on the third chromosome (Fig. 6a), the expression of the trait disappeared.

Role of Hsp90

The deformed-eye lines were founded with *Hsp83* heterozygotes but selected only for the deviant trait, not the *Hsp83*-mutant chromosome (*19F2*). Indeed, two observations indicated that the trait might have become independent of the *Hsp83* mutation. First, even two extra copies of the wild-type *Hsp83* gene[14] did not affect the expression of the trait when crossed into the high-expression lines (results not shown). Second, by the sixth and seventh generations, the trait was expressed in >80% of the flies in the *HE1* and *HE3* lines (Fig. 3a). If the trait continued to depend on the *Hsp83* mutation, as a result of mendelian segregation, at most two-thirds of the viable offspring of each generation would be *Hsp83* heterozygotes and potentially have deformed eyes. To determine directly whether the affected flies contained the *19F2* mutation (Arg 48 → Cys), we used polymerase chain reaction (PCR) genotyping (Fig. 7). In fact, none of the flies tested retained the mutation (generations 16–20; 0 out of 10 for each of lines *LE1–3*, *HE1* and *HE2*, and 0 out of 40 for line *HE3*). Therefore, selection had enriched the genetic determinants to a threshold at which the expression of the trait became independent of the Hsp90 mutation.

When the high-expression lines were outcrossed with normal laboratory strains, the deformed-eye trait was expressed only at very low levels. We took advantage of this fact to re-examine the role of Hsp90 in buffering these now cryptic determinants. The high-expression lines were backcrossed to the parental *Hsp83*-mutant stock (*19F2/TM6B*), which contained one mutant and one wild-type copy of *Hsp83*. At all temperatures the trait exhibited low penetrance in the progeny receiving the control chromosome (*TM6B*; Fig. 8, filled diamonds). The progeny receiving the *Hsp83*-mutant (*19F2*) expressed the trait at a significantly higher level (Fig. 8, open squares). At lower temperatures, or when supplied with normally functioning Hsp90, determinants for the trait were kept in a cryptic state, but there was a dramatic increase in expression of the trait at temperatures above 25 °C. Similar temperature–response curves were produced in crosses between flies containing this mutant *Hsp83* allele and the other high-expression lines (*HE1* and *HE3*). Moreover, another independently isolated *Hsp83* allele, *13F3*, also enhanced the expression of the trait. Thus, wild-type Hsp90 buffered the penetrance and temperature sensitivity of the trait, coupling the appearance of this morphogenetic variant to the environment.

Natural variation in wild flies

Laboratory stocks might differ from wild flies in many respects: genetic drift, inbreeding, relaxed selection and marker mutations might decrease the developmental stability of typical laboratory strains. To determine whether wild flies also carry the same types of morphogenic variation, we obtained previously established wild-type lines from several geographic locations and collected wild flies from the field. When these flies were crossed to *Hsp83*-mutant heterozygotes, morphological abnormalities appeared in F_1 hybrids, both in the recently established wild-type lines and in wild-type laboratory stocks originating from globally diverse locations (Table 1). Nearly 25% of the crosses with wild-type laboratory stocks (37 of 163 crosses, using at least 20 different wild-type laboratory stocks) produced abnormal flies, numbering 120 out of 16,080 scored (0.8%). Two of eleven recently established lines from a wild population represented by independent isofemale lines (*IND-1–12*) produced 8% deformed flies when treated with geldanamycin (45 of 559 flies studied; Table 1). In fact, several flies from line *IND-6* had notched wings (Fig. 1p), while many flies with small wings on one or both sides appeared in line *IND-2*, although neither defect was found in the untreated stocks. Therefore, silent polymorphisms buffered by Hsp90 must also exist in the wild.

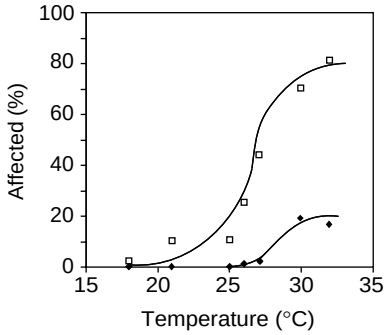

Figure 8 Wild-type Hsp90 buffered the deformed-eye trait. An *Hsp83* mutant stock (*19F2/TM6B*) was crossed to the *HE2* high-expression line and replicate cultures were grown at different temperatures. Results from progeny containing the *19F2* chromosome (open squares) and from controls containing wild-type *Hsp83* on chromosome *TM6B* (filled diamonds) are shown.

A mechanism for 'evolvability'?

We have provided what is, to our knowledge, the first evidence for an explicit molecular mechanism that assists the process of evolutionary change in response to the environment. We suggest that in nature, transient decreases in Hsp90 levels resulting from its titration by stress-damaged proteins could uncover morphological variants for selection to act upon. Consider a simple model for a threshold trait requiring at least six genetic determinants (with no dominance). In a population containing ten independent and additive determinants affecting the trait, each present at a frequency of 0.1, the probability of an individual having at least six of these determinants and thus the trait, is about 1 in 7,000. However, if compromising Hsp90 function were to lower the trait's threshold by just one or two determinants, the probability of the appearance of the trait increases to 1 in 600 or 1 in 78. Once the frequency of a trait is increased in this manner, given a moderate fitness advantage, selection could increase the frequency of genetic polymorphisms affecting the trait to a point at which it no longer depends on reduced Hsp90 function to be expressed in the population.

Evolutionary models must encompass a dichotomy of stasis and change. Evolution exploits genetic differences between individuals in order to remodel developmental programs, yet development is generally robust to individual genetic differences and environmental perturbations. Theoretical models describe how developmental homeostasis is developed and why it is maintained, as well as how it could be disrupted so that evolutionary change can occur[19–23]. Experiments disrupting developmental homeostasis by specific mutations[24–26], by particular stresses during precise windows of development (phenocopy)[27,28], or in species hybrids[29], have shown that populations contain a surprising amount of unexpressed genetic variation that is capable of affecting certain typically invariant traits. Sometimes very specific conditions can uncover this previously silent variation[30–32]. But both the wide variety and unusual character of the morphological variation uncovered when Hsp90 is impaired, and the prevalence of natural stresses that might disrupt it, are unprecedented.

Drosophila Hsp83 mutants have been isolated repeatedly in near-saturation genetic screens for genes that interact in signal transduction[14–16,33]. Mutations in other components of the protein-folding and translation machinery have not been identified in such screens. Moreover, known mutations that generally affect protein biogenesis in *Drosophila*, such as mutations in ribosomal subunits, do not produce such abnormal morphologies[34]. Hsp90 is special because its participation in the stress response is coupled with its critical integrative position in the genetic architecture of development. For example, it seems likely that many variant morphologies could be produced only by changing the output of several developmental processes simultaneously. Genetically 'sensitized' pathways, engineered to destabilize certain phenotypes, reveal potent natural variation affecting these phenotypes[26]. By altering the activities of multiple signal transducers and thereby simultaneously weakening several developmental pathways, Hsp90 can expose such variation, allowing selection to remodel many different processes at once.

In some cases, such as cell-cycle control[10,14,35], Hsp90 supports both the activators and the inhibitors of the same function and alteration of Hsp90 function could uncover variation that would allow selection to sculpt the output of these processes either upwards or downwards. Furthermore, this mechanism is flexible. When Hsp90 function is disturbed, developmental pathways are sensitized to a degree determined by their specific dependence on Hsp90 (which is dictated by the functional significance and inherent stabilities of the relevant targets) and by Hsp90 availability (which is dictated in nature by the severity of the stress). The use of Hsp90 as a capacitor for the conditional release of stores of hidden morphogenic variation may have been adaptive for particular lineages, perhaps allowing the rapid morphological radiations that are found in the fossil record. □

Methods

***Drosophila* strains and culture.** Strains used in this study are listed in Table 2. The *Hsp83* alleles *19F2* and *13F3* are independent isolates of the same point mutation (E. Hafen, personal communication). *IND* isofemale lines were established from larvae found in separate pieces of fruit to decrease the probability that the lines were started from closely related flies. Flies were cultured on standard cornmeal:molasses:agar *Drosophila* medium, except in inhibitor experiments, in which 3 $\mu g\,ml^{-1}$ geldanamycin (National Cancer Institute) was made up in a 1% bakers yeast slurry before an equal volume of Formula 4-24 Instant *Drosophila* Medium (North Carolina Biological Supply) was added.

Quantitative analyses. Responses to selection (Fig. 2) were measured as the fraction of affected progeny in each generation; each fly was given a trait value of 0 (not affected) or 1 (affected). We used the difference in the selection response between successive generations to determine the selection differential. Realized heritability (h^2; Fig. 2b, d) was obtained from the slope of the least-squares regression of selection response plotted against the cumulative selection differential (S_{cum})[18]. Genotype–environment interactions (G × E) were confirmed for the data shown in Fig. 5 by analysis of variance (SAS Inst.). There were highly significant interaction effects (G × E) for the high- and low-expression lines ($P < 0.0001$); and for these lines compared with wild-type lines, which are unaffected at all temperatures ($P < 0.0004$). Genic interaction effects due to epistasis are suggested by the data in Fig. 6. The difference apparent from comparison of the sum of the genotypic values for the isolated second and third chromosomes compared with their combined genotypic value is the interaction deviance due to epistasis between these chromosomes[18]. Epistasis can arise from different underlying causes, including an expression threshold for additive determinants[36]; however, when these data are transformed to a different scale suggested for use with threshold traits (deviation from mean liability in units of standard deviation)[18] a non-additive interaction, indicating more general epistasis, is still apparent (results not shown).

PCR genotyping. Convergent primers framing a 575-base-pair region surrounding the mutation were used to amplify by PCR genomic DNA isolated from single flies by proteinase-K digestion. Digestion products were resolved by electrophoresis through 2% NuSieve agarose (FMC Bioproducts) and were visualized with ethidium bromide.

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Correspondence and requests for materials should be addressed to S.L.