
Studying stress responses in the post-genomic era: its ecological and evolutionary role

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Most investigations on the effects of and responses to stress exposures have been performed on a limited number of model organisms in the laboratory. Here much progress has been made in terms of identifying and describing beneficial and detrimental effects of stress, responses to stress and the mechanisms behind stress tolerance. However, to gain further understanding of which genes are involved in stress resistance and how the responses are regulated from an ecological and evolutionary perspective there is a need to combine studies on multiple levels of biological organization from DNA to phenotypes. Furthermore, we emphasize the importance of studying ecologically relevant traits and natural or semi-natural conditions to verify whether the results obtained are representative of the ecological and evolutionary processes in the field. Here, we will review what we currently know about thermal adaptation and the role of different stress responses to thermal challenges in insects, particularly *Drosophila*. Furthermore, we address some key questions that require future attention.

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1. Introduction

All organisms are strongly affected by their surrounding environment, and the environmental factors play an important part in shaping ecology and evolution of biological systems. Environmental stress is especially important at many levels of biological organization (Hoffmann and Parsons 1997; Hoffmann and Hercus 2000). In this context environmental stress is regarded as an “environmental factor causing a change in a biological system, which is potentially injurious” (Hoffmann and Parsons 1991) and which has some fitness consequences (e.g. Bijlsma and Loeschcke 1997) (figure 1). To study and gain further understanding of the nature and consequences of environmental stress from an ecological and evolutionary perspective and to investigate the role of stress response mechanisms, collaboration between evolutionary biologists, physiologists and molecular geneticists is required. These disciplines are concerned with the same questions, asking how organisms adapt to environmental stress. Thus, by integrating these disciplines

there is a considerable potential for major advances in the understanding of stress adaptation.

The multidisciplinary and integrative approach to study mechanisms and constraints of evolution, and to relate variations at different levels of biological organization, from DNA to phenotype, has been termed ‘ecological and evolutionary functional genomics’ (EEFG). The quest is to investigate which genes are accountable for phenotypic traits, and here more specifically, which genes affect acclimation and adaptation on both ecological and evolutionary time scales (Loeschcke *et al* 2004).

We believe that an integrative approach will lead to new and more detailed understanding of biological questions (*see also* Chown 2001) and contribute to the body of knowledge on “how do organisms respond and adapt to the thermal environment” and more specifically “what is the role of the stress response for thermal adaptation in nature”. To address these questions we do not only need to integrate various scientific disciplines as discussed above, but we also need to try to integrate the large body of especially molecular

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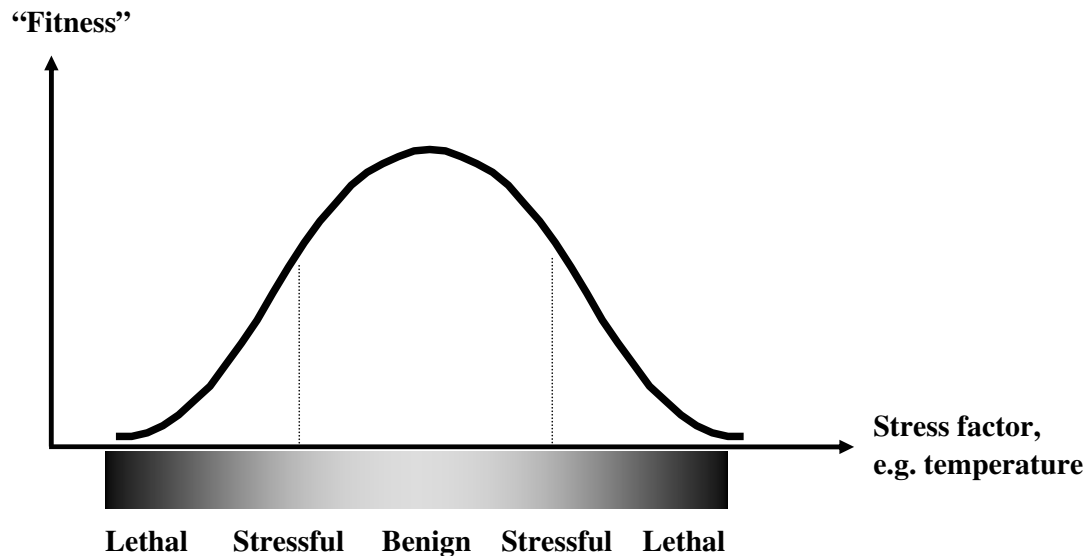


Figure 1. Hypothetical relation between a fitness measure and the amount of stress applied to an organism. When the stress level exceeds a threshold (becomes stressful) the organism becomes stressed. The bar under the x-axis represents the gradual transition from benign, to stressful and lethal conditions. The stress level is a product of stress level (constant or fluctuating) and exposure time, and sensitivity will be different across species, populations and life stages. Thus, the natural situation is much more complex than here in only two dimensions.

knowledge of responses to and effects of stress in the laboratory to a more ecological and evolutionary relevant setting. This idea is not a new one, however. Feder (1996) discussed the current state of evolutionary stress research and pointed out some key questions to be addressed. Over the last 10 years *Drosophila* has developed as a model for the study of adaptation to environmental stress and stress responses and much progress has been achieved. As predicted by Feder (1996) molecular tools have become available that allow the identification of genes important for e.g. resistance and for the study of specific mutant/knock out lines. The ecological relevance of stress resistance and some resistance traits has been investigated as have the costs and benefits of the expression of the stress response, especially Hsp70 level. However, many of the same questions that were asked 10 years ago are still valid and unresolved. The progress of the research and the key questions/obstacles will be discussed throughout this paper.

In this paper we will review the current understanding of how organisms, especially *Drosophila*, respond and adapt to the thermal environment. We will address what role the heat stress response seems to play in nature; we will also address other responses to thermal challenges in insects, some obstacles for this work and some suggestions for future directions of research.

2. Measuring thermal resistance and adaptation

Selection pressures under natural conditions and field fitness of populations are notoriously difficult to assess. Moreover,

even if the natural conditions can be described precisely, different life stages experience different environments and have different sensitivity and ways to deal with these environments. Compared to the usual laboratory settings no environmental factors are constant or even predictable in the field. In the case of thermal adaptation, the information on stress levels, stress types and stress responses for adults and juveniles is largely incomplete. Therefore, we need good correlates for these stress related measures. Measures of temperature resistance obtained in the laboratory should reflect the climatic stress experienced in the field and thereby the selection pressures that populations are exposed to under natural conditions. Otherwise incorrect interpretations and conclusions will be reached regarding thermal adaptation and the importance of stress responses for adaptation of organisms (Hoffmann *et al* 2003). While some easily handled stress measures (usually mortality) might correlate well with more ecologically relevant measures, this link is not always established and might not well represent traits likely to be under selection in the field.

3. Stressors and responses to stress

A large number of cellular responses take place after stress exposures and both resistance and responses to environmental stress are very complex traits (Feder 1996). These traits include the expression of heat shock proteins (Hsps) after exposure to heat and other stressors (Sørensen *et al* 2003). However, while this response probably is the most well studied response to thermal stress in insects, it

is by no means the only one. First of all, exposure to stress might induce behavioral adjustments aiming at avoiding the stress (Dahlgaard *et al* 2001). In addition to Hsps a number of other cellular defences can be activated. These include DNA repair mechanisms, the oxidative stress response, immune and metabolic responses (Pletcher *et al* 2002; Koorsloot *et al* 2004). To investigate known mechanisms behind adaptation and responses to stress and identify new mechanisms an integrative approach is especially important. In some cases candidates have been identified, i.e. processes or genes thought to be involved, in which case these can be investigated directly. However, if none are known, new candidates can be identified in model organisms due to the advances in the field of genomics. Partly or fully sequenced genomes allow studies of gene regulation in response to a treatment or in connection to selection and dense marker maps allow fine scale QTL mapping. This can identify new potential candidate genes or mechanisms for resistance to particular stress types. A microarray study of gene expression changes in *D. melanogaster* identified a few hundred genes immediately upregulated after heat stress, including many *hsp* genes but even more genes not previously associated with the heat stress response (Sørensen *et al* 2005a). While the known *hsp* genes quantitatively seem to constitute the major part of the heat stress response showing generally high fold change induction, numerous genes and pathways not yet investigated in detail seem to be involved. Furthermore, physiological adjustments of metabolism and membrane composition also respond to thermal stress and can play a role for stress tolerance (Ohtsu *et al* 1999; Overgaard *et al* 2005). The physiological changes in turn affect life history and fitness traits such as fecundity, longevity and stress resistance (Feder and Hofmann 1999; Silberman and Tatar 2000; Hercus *et al* 2003). Thus, thermal stress might play a key role as an evolutionary force (Hoffmann and Hercus 2000).

In insects Hsps have been found to be induced by many environmental factors including heat, cold and hypoxia (Sørensen *et al* 2003). Lately, biotic factors like parasitism (Merino *et al* 1998; Rinehart *et al* 2002) and presence of predators (Pauwels *et al* 2005) have been added to the list. Thus, as most if not all natural populations live in environments where these stress factors occur frequently, the stress response has a potential to be very important for all organisms. However, it is often not clear whether an induction of Hsps has any beneficial effect on related traits. For example, does predator induced Hsps enable the prey to hide, escape or otherwise avoid the predator? Or is it a mechanistic side effect of the way the stress response is induced? Thus, the potential beneficial effect of the induction should be examined for a given trait and until that has been done, the evolutionary implications of the expression of Hsps remain unclear. As discussed below this applies even for some thermal resistance traits.

4. Ecological relevance of thermal resistance traits

There are multiple ways to measure thermal tolerance; however, it is not always known whether these measures reflect ecologically relevant conditions and thus whether an evolutionary adaptation should be expected. This applies to survival to short term heat or cold shock that traditionally has been used to assess resistance (e.g. Hoffmann and Watson 1993; Loeschcke *et al* 1994). Moreover, while induction of the stress response is very important for survival following heat shock (Feder and Hofmann 1999), it is less clear whether heat shock is an ecologically relevant trait and gives little insight as to the role of Hsps in natural populations.

The importance of Hsps for other stress types than heat shock survival is supported by a limited number of studies. Heat hardening has been shown to induce cross-tolerance to cold shock resistance (Parsell and Lindquist 1993; Hoffmann *et al* 2003). Burton *et al* (1988) found a protective effect of a mild heat stress on cold shock survival of *Drosophila* larvae. Also, upregulation of Hsp70 expression has been reported following long-term cold exposure (Burton *et al* 1988; Goto and Kimura 1998; Sejerkilde *et al* 2003) suggesting a potential heat hardening effect of this cold treatment. Anoxia, known to induce Hsp70 (Jedlicka *et al* 1997), induces cold tolerance similar to a rapid cold hardening treatment in *Musca domestica* (Coulson and Bale 1991). However, other studies fail to demonstrate cross-resistance (Hoffmann *et al* 2003; Nielsen *et al* 2005) and thus, the protective role of the heat shock response is unclear for some types of stress including cold stress and high-temperature knock-down.

One simplification is that often only a single Hsp is measured, often inducible Hsp70 or the pooled Hsc/Hsp70 level (Sørensen *et al* 2005b; Sarup *et al* 2006). While Hsp70 expression level seems to be a very good indicator of the whole inducible stress response, this is likely to give incomplete information of the stress response of an organism as e.g. Hsp expression may vary over different life stages (Lakhotia and Singh 1989; Krebs *et al* 1998; Lakhotia and Prasanth 2002). Furthermore, it is known that Hsp70 expression level is tightly regulated and influenced by the expression of other Hsps (Morimoto *et al* 1994). Thus, even if no significant change in Hsp70 expression is observed under specific conditions it is possible that changes in other Hsps might occur but have been overlooked.

4.1 Heat shock response and resistance

The molecular function of Hsps, the heat stress response and the mechanism of increased heat shock survival are rather well understood (Lindquist 1986; Parsell and Lindquist 1993; Feder and Hofmann 1999) even though the role of only a minority of the genes responding to heat stress has

been elucidated (Sørensen *et al* 2005a; Nielsen *et al* 2006). Experimental evolution can lead to increased expression levels of Hsp70 if selection targets heat survival after a milder stress inducing Hsps (Sørensen *et al* 1999). Other selection studies of different species of *Drosophila* have shown (contrary to predictions at that time) decreased expression level of Hsp70 in lines frequently, or continuously exposed to stress (Bettencourt *et al* 1999; Sørensen *et al* 1999). This was interpreted as a response to the cost imposed by expression in the frequently exposed populations. The costs of Hsp expression have been investigated and include decreased fecundity (Krebs and Loeschcke 1994) and negatively affected development rate and survival in larvae over-expressing Hsp70 (Krebs and Feder 1998). Thus, the costs outweigh the benefits when expression is frequent and stress resistance is reached by some trait(s) other than inducible Hsp expression. Identical patterns have subsequently been found in soil invertebrates exposed to heavy metals (Köhler *et al* 2000) and in natural populations of species of *Drosophila* (Hoffmann *et al* 2003; Sørensen *et al* 2003; Sørensen *et al* 2005b). According to this, the role of inducible Hsps for adaptation occurs in connection to relatively rare, unexpected extreme stress exposures and not during every day environmental fluctuations.

The roles of Hsps for many other resistance traits like resistance to cold, desiccation and starvation, the mechanisms behind resistance to these stressors and the evolutionary force of environmental factors like cold, desiccation and starvation are less well understood. A better understanding is needed to improve our knowledge about adaptation to the environment and the effects of climatic changes. Given that a wide range of different environmental challenging factors induce the heat stress response and Hsps, this response has the potential to play a central role for the cellular defence against ecologically relevant challenging conditions and constitute an important part of a general stress response.

4.2 Cold responses and resistance

Two main types of responses to cold have been described. A rapid response to acute cold shocks and a classic acclimation response to lowered temperature or decreased day length. Rapid cold hardening (RCH), even within hours, induces increased cold shock survival (Lee *et al* 1987) and decreases the temperature where normal activity can be maintained (Kelty and Lee 1999; Shreve *et al* 2004). Surprisingly, RCH seems not to induce Hsp70 expression even though some mortality is induced (Kelty and Lee 2001; Overgaard *et al* 2005). Nielsen *et al* (2005) studied the effects of HSF deficiency on resistance and hardening ability and found that both cold shock survival and cold hardening ability was unaffected by HSF activation leaving doubt about the role of HSF activation for these traits in *Drosophila*.

Accumulation of cryoprotectants (e.g. glycerol and trehalose) plays a role for long-term cold acclimation and tolerance in many over-wintering insects (Zachariassen 1985; Lee and Denlinger 1991). However, RCH seems not to induce large amounts of cryoprotectants (Chen *et al* 1987; Kelty and Lee 1999). Thus, Hsps and cryoprotectants have yet to be shown to play a large role for the RCH response, at least in *D. melanogaster* (Kelty and Lee 2001; Nielsen *et al* 2005; Qin *et al* 2005). However, in other insects these factors seem to be of some significance (Chen *et al* 1987; Joplin and Denlinger 1990). Moreover, specific Hsps or cryoprotectants not yet investigated might be involved. One supported mechanism behind RCH in flies is homeoviscous membrane adaptation that involves reorganisation of membrane properties (Overgaard *et al* 2005; Lee *et al* 2006; Michaud and Denlinger 2006; Overgaard *et al* 2006).

While no Hsp70 is induced after RCH cold-induction, Hsp70 is found upon re-heating when cold exposures exceeds around 8 h (Burton *et al* 1988; Sejerkilde *et al* 2003; Nielsen *et al* 2005). Then Hsp70 levels induced by cold are considerably lower than those induced by heat hardening (Nielsen *et al* 2005). Thus, the mechanisms induced by rapid cold hardening and cold shock may differ from that of more chronic cold exposures and long term acclimation (Sinclair and Roberts 2005; Overgaard *et al* 2006; Rako and Hoffmann 2006). The basis of these differences is presently not clear and may differ across species.

4.3 Heat knock down responses and resistance

The physiological and molecular mechanisms underlying tolerance and responses to high-temperature knock-down in a knock down tube (Sørensen *et al* 2001) are generally unknown. Even so Hsps are obvious candidates of ensuring a protective effect. The knock-down trait seems to better predict natural thermal adaptation in *Drosophila* and is considered to be an ecologically relevant trait for heat resistance (Hoffmann *et al* 2003; Sørensen *et al* 2005b). However, heat stress survival and high-temperature knock-down resistance have been shown to be genetically different traits that show little cross resistance (Hoffmann *et al* 2003; Bublii and Loeschcke 2005). Resistance to both traits can be improved by heat hardening and there is some evidence that two heat shock genes, *hsp68* and *hsp-omega*, respond to selection for knock-down time (McColl *et al* 1996; McKechnie *et al* 1998). On the other hand the hardening effect on heat knock down seems to be with a smaller benefit to resistance than is observed for heat-shock survival (Sørensen *et al* 2001). Using a QTL approach to investigate the genetic basis of knock down tolerance Norry *et al* (2004) found QTLs in regions with known *hsp* candidate genes. However, in a study of a conditionally expressed HSF deficient mutant Nielsen *et al* (2005) did not find an effect

of HSF deficiency on the knock-down resistance. Given the unclear results on the involvement of Hsps in response to different stress types and potential beneficial effects on resistance towards different stress traits, the idea of Hsps as sole basis for a general stress system is not well founded. Data on costs and benefits of the heat stress response and Hsp70 expression suggest that this system evolves under strong trade offs (Krebs and Loeschcke 1994; Krebs and Feder 1998; Sørensen *et al* 1999). However, much is still to be learned about costs and benefits of different stress response systems in order to understand the evolutionary trade offs involved in adaptation to stressful environments.

4.4 More ecologically relevant traits?

There are some examples of studies where the ecological relevance of thermal stress responses has been considered. Feder *et al* (1997) investigated thermal conditions and the stress response in juvenile *D. melanogaster* in apples in the field. It was found that juvenile *D. melanogaster* was likely to experience lethal temperatures in sunlit necrotic fruits. Feder *et al* (2000) also investigated the activation of Hsp70 in free-ranging adult *D. melanogaster*. They used a transgenic line with a fusion of the structural gene encoding bacterial beta-galactosidase under control of a heat shock promoter. They concluded that most flies behaviourally avoided thermal stress but that a minority of adult *D. melanogaster* might undergo mild thermal stress in nature. Behavioural avoidance was also found in activity patterns of heat adapted flies (Dahlgaard *et al* 2001). Flies avoided exposure to high temperatures by laying eggs late at hot places. The activity pattern was genetically based as the activity was retained after several generations in the laboratory. The same flies showed increased heat knock down resistance without hardening during the afternoon activity periods and this increased heat resistance was tied to the circadian rhythm (Sørensen and Loeschcke 2002).

Recently, studies have focused on effects of temperature that occur at less extreme thermal exposures. These effects are likely to occur regularly under natural conditions and might be ecologically and evolutionarily more relevant. These studies have investigated high- and low temperature effects on reproductive behaviour and ability (Fasolo and Krebs 2004; Sarup *et al* 2004; Shreve *et al* 2004; David *et al* 2005; Jørgensen *et al* 2006) and high temperature effect on flight performance (Krebs and Thompson 2006). Jørgensen *et al* (2006) showed that heat hardening did positively affect some of the reproductive characteristics measured (but not others), suggesting that the heat stress response might be strongly involved for these traits as well as for survival. For example the proportion of males becoming sterile after heat shock was strongly reduced by hardening, while the male fecundity of fertile matings was largely un-affected by the

hardening treatment. However, little information exists on the adaptive responses to less extreme exposures and on the roles of physiological and molecular protection.

A few studies have attempted to test insect adaptation and the effect of stress responses in the field. Thomson *et al* (2001) performed such a test, where the effect of thermal acclimation on parasitism success of *Trichogramma* wasps in the field (a vineyard) was investigated. When released under hot conditions, heat acclimated individuals outperformed control ones and were able to find and parasitize moth eggs, while no difference in success was found under milder conditions. Similarly, Loeschcke and Hoffmann (2007) tested the effect of heat hardening and Kristensen *et al* (2007) examined the effect of selection for thermal resistance on performance in the field at different temperatures in *D. melanogaster*. These studies used a release-recapture design to estimate the ability to locate resources (as a fitness correlate) in the wild under different climatic conditions. The results indicated that laboratory based predictions on relative fitness partly reflected fitness under natural conditions – but also revealed some unexpected outcomes.

Experiments in the field have many limitations. Usually, the trait measured (e.g. ability to locate resources) is complex and a combination of many mechanisms. Thus, it might be less clearly defined how exactly selection or treatment improves performance, however, it might also be more ecologically relevant. As such traits often are a combination of mechanisms and smaller changes in individual traits values might add up to differences more detectable in the field than under standard conditions in the laboratory where single traits are scored individually.

5. Adaptive variation in thermal resistance traits

Support for a protective role of inducible Hsps for acute heat survival comes from many studies on insects (Gehring and Wehner 1995; Dahlgaard *et al* 1998), fish (Basu *et al* 2002), plants (Sun *et al* 2002) and mammals (Ulmasov *et al* 1993). However, only in a few studies have expression of Hsps and stress resistance been linked to ecologically realistic conditions (Fader *et al* 1994; Feder *et al* 1997; Kelty and Lee 1999; Sørensen *et al* 2003).

The ecological role of thermal adaptation and of Hsps is supported by clinal or other geographical variation in candidate genes or stress resistance traits. The results of nature's own experiments can be evaluated by relating variation in altitude, latitude or climatic factors to variation in candidate traits of interest. These can include gene regulation in candidate genes, DNA-sequence variation in candidate genes and variation in resistance phenotype. At the DNA-sequence level there is variation in promoter and coding regions of some of the small heat-shock proteins

(Frydenberg *et al* 2002) and latitudinal variation at two sHsp genes along a north-south gradient in Eastern Australia (Frydenberg *et al* 2003) and in Hsp70 along the same gradient (Bettencourt *et al* 2002).

At the phenotypic level many studies have shown clinal variation in various resistance traits along climatic gradients (Hoffmann *et al* 2003; Sørensen *et al* 2005b; Bahrndorff *et al* 2006; Sarup *et al* 2006). However, for some traits clinal variation is only found in some studies, in one gender or life stage or under specific environmental conditions (*see e.g.* Sørensen *et al* 2005b; Sarup *et al* 2006). This can be caused by several factors. First, the resistance trait measured needs to be relevant for the stress experienced in the field (discussed above). Second, sparse information is usually available about the climatic conditions experienced by the organisms. While annual means in temperatures are usually available and correlate well with latitude, this may have only little effect for the populations. The variation or extreme events during days, seasons and years probably affect populations much more and are thus much more important (Bahrndorff *et al* 2006). Sarup *et al* (2006) made a study of climatic adaptation in different life stages of *D. buzzatii* populations collected in a geographical area where knowledge of many climatic parameters was available. The study identified many non-linear relationships between resistance traits and climatic variables in addition to a low correlation between sexes and different life stages (Krebs and Loeschcke 1995; Krebs and Loeschcke 1999). Recent work has identified changes in clinal variation in allozyme and inversion frequencies that seem to track global climate change (Umina *et al* 2005; Balanya *et al* 2006). Thus, while much evidence suggests that the climate is of importance and a strong evolutionary force, we still have little information as to the exact characteristics of the climatic factors that are of importance. In the controlled laboratory single stress factors can be applied and controlled. This can be used to investigate the physiological responses to this stress factor and determine the effects of these responses on tolerance levels. However, little information exists on how often organisms express and rely on the protection from stress responses under natural fluctuating conditions. To obtain this information we need to sample and measure organisms directly from or in the field. Apart from various practical difficulties in doing this, when organisms are investigated under natural conditions, care has to be taken in interpreting Hsp levels as responses to a single environmental stress factor as the natural environment potentially contains a mix of multiple acute and long term stress factors that can induce stress responses. Thus, these measurements should always be supplemented by additional investigation to account for this caveat. Some examples of natural Hsp measurements are available including McMillan *et al* (2005) and Sagarin and Somero (2006) who measured leaf beetle larvae and

intertidal marine molluscs, respectively, during exposure to different temperatures. However, the environment has to be carefully considered to verify that the stress factors measured really are the responsible stress factors behind the induction.

6. Future directions/perspectives

Natural populations are constantly exposed to challenging environments and it is necessary for the organism to buffer this environmental variation to maintain the cellular homeostasis and high performance across environments. The stress response and heat shock proteins are important for this buffering in relation to stress resistance and adaptation to the environment under some conditions (Sørensen *et al* 2003). However, other responses and mechanisms are expressed under natural conditions and are probably also very important. Local adaptation and selection for other kinds of adaptive mechanisms may disturb the pattern of *e.g.* clinal variation in putative adaptive traits.

The apparent selection for alternative stress resistance mechanisms and, thus selection against Hsp expression in populations being exposed to chronic stress clearly demonstrate this problem (Sørensen *et al* 2003). Actually, two microarray studies report on gene expression changes in *Drosophila* after heat hardening and in lines selected for stress thermal resistance (Nielsen *et al* 2006; Sørensen *et al* 2007). These studies find nearly no overlap among the changes in gene expression induced by heat stress and the changes in lines selected for thermal resistance and, thus, seem to indicate two largely separate mechanisms. Furthermore, incomplete information about ecological relevance of different climatic parameters and measures of stress resistance makes interpretation of the importance and use of mechanisms unclear. Apart from Hsps and other molecular chaperones a suite of other possible stress responses might add to the complexity of the adaptive process. These mechanisms and responses need to be considered. First of all to investigate the relevant stress response and, second, to have a complete understanding that can allow making realistic interpretations regarding adaptation and the role of stress responses.

New technological developments make it possible to investigate the role of genes coding for Hsps (and other candidate genes) in greater detail. A combination of genomics, proteonomics and metabolomics will further elucidate the effects of stress on expression patterns at the DNA, RNA and protein levels and the effect on metabolism (Loeschcke *et al* 2004; Malmendal *et al* 2006). However, such approaches are exploratory and should be considered as a starting point as they only provide information on certain levels of biological organization. Studies of mutant lines might be a very powerful tools to target investigations

on the effect of specific genes (Krebs and Feder 1998; Nielsen *et al* 2005). However, this approach might also have drawbacks. Often mutants behave generally differently from the wild type and show large genotype-environment interactions (Baldal *et al* 2006). Thus, associations or the lack of associations between specific gene mutants and resistance phenotypes might be misleading. Molecular studies should be combined with follow up studies on other levels of biological organization. In order to make solid ecological and evolutionary interpretations relevant stress resistance traits could be tested in selection experiments and natural populations with accurate climatic information. By combining physiological and phenotypic studies of laboratory-selected and naturally-adapted populations, we expect to gain a much more detailed understanding of Hsp regulation and expression and the role of this response for natural populations.

Even though much progress has been made during the last 10 years, several key questions remain relevant (Feder 1996). How much and how often are stress responses induced in natural populations? Do stress proteins and other stress responses actually confer resistance to heat and other environmental factors in the field? Is there a general stress response that improves resistance to many types of stress? In addition to these questions investigations of the regulation of stress responses and identification of candidate genes are needed as an understanding of how, when, under which conditions and in which species different resistance mechanisms and strategies are involved.

We would like to advocate attempts to develop ways to test hypotheses on thermal adaptation under more natural conditions. Although this is often not easy, but will be fruitful and will provide important new information when successful.

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