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REVIEW

The evolutionary and ecological role of heat shock proteins

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Abstract

Most heat shock proteins (Hsp) function as molecular chaperones that help organisms to cope with stress of both an internal and external nature. Here, we review the recent evidence of the relationship between stress resistance and inducible Hsp expression, including a characterization of factors that induce the heat shock response and a discussion of the associated costs. We report on studies of stress resistance including mild stress, effects of high larval densities, inbreeding and age on Hsp expression, as well as on natural variation in the expression of Hsps. The relationship between Hsps and life history traits is discussed with special emphasis on the ecological and evolutionary relevance of Hsps. It is known that up-regulation of the Hsps is a common cellular response to increased levels of non-native proteins that facilitates correct protein folding/refolding or degradation of non-functional proteins. However, we also suggest that the expression level of Hsp in each species and population is a balance between benefits and costs, i.e. a negative impact on growth, development rate and fertility as a result of overexpression of Hsps. To date, investigations have focused primarily on the Hsp70 family. There is evidence that representatives of this Hsp family and other molecular chaperones play significant roles in relation to stress resistance. Future studies including genomic and proteonomic analyses will increase our understanding of molecular chaperones in stress research.

Keywords

Adaptation, environmental stress resistance, genetic stress, Hsp, protein quality control.

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INTRODUCTION

Heat shock genes are a subset of a larger group of genes coding for molecular chaperones, i.e. proteins that are involved in 'house-keeping' functions in the cell. The term 'chaperone' is adopted from one of their functions, namely to keep other proteins from getting involved in 'inappropriate' aggregations. Apart from this function, molecular chaperones are involved in transport, folding, unfolding, assembly and disassembly of multi-structured units and degradation of misfolded or aggregated proteins (Fig. 1). These tasks are important under normal cellular conditions, however, the need for molecular chaperones is accelerated under stressful conditions that could potentially damage the cellular and molecular structures in the cells. In this review, we will focus on the ecological and evolutionary roles of stress-inducible heat shock proteins (Hsps), especially on Hsp70, one of the major heat shock proteins that has been intensively studied in model organisms and in naturally occurring populations. Involvement of stress-inducible Hsps in stress resistance has been documented and is reviewed in a number of papers (Lindquist 1986; Feder & Hofmann 1999). The Hsps and other molecular chaperones have been widely studied in many fields of biology and a large number of publications are available on their molecular and physiological functions (see Parsell & Lindquist 1993; Feder & Hofmann 1999; Morimoto *et al.* 1999; Pockley 2003 for reviews). Until recently, the ecological importance of inducible Hsps has been questioned and was rarely addressed. However, Hsps play an important role in the cell's response to a wide range of damaging (stressful) conditions and are important for recovery and survival of organisms (Lindquist 1986). The stress needed to induce Hsps is strongly related to the realized niche of the organism in question (for a review see Feder & Hofmann 1999), e.g. in arctic fish Hsps are induced at around 5 °C and in

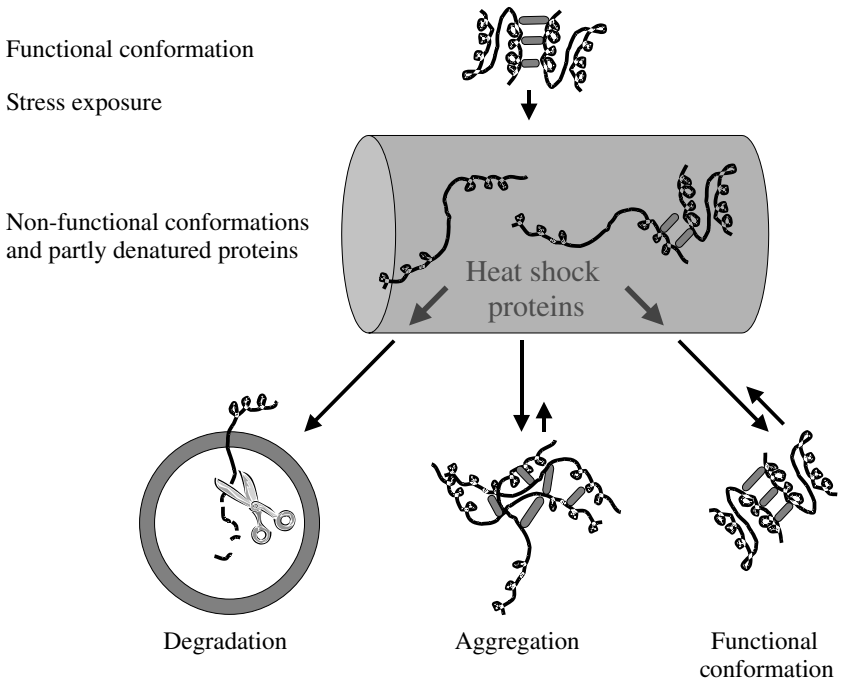

Figure 1 Cellular function of heat shock proteins. The fate of proteins with non-functional conformations after stress exposure may be either to re-obtain the functional conformation, form aggregations with other misfolded proteins or become degraded. Hsps play a helper role in shifting the equilibrium in the direction of more functional proteins or degradation of damaged proteins.

thermophilic bacteria at around 100 °C (Parsell & Lindquist 1993).

Recently, the focus of Hsp research has progressed from laboratory conditions to natural populations and experiments using ecological relevant stress exposures have been performed. This approach has provided data not only on the physiological functions, but also on the ecological and evolutionary roles of Hsps. In the laboratory it has been shown that very small amounts of induced Hsps can have effects on life history traits such as development, stress resistance, life span and fecundity (Rutherford & Lindquist 1998; Sørensen & Loeschcke 2001; Queitsch *et al.* 2002; Rutherford 2003). Therefore, Hsps can be important for natural populations that are exposed to variable environments, including occasional stress exposures and environmental conditions that appear to us as benign. Data and ideas have emerged that suggest that heat shock genes and their products can play an important role in the ecology and evolution of populations. Therefore, a reassessment of the ecological role of Hsps is needed.

The aim of this review is to assemble the increasing evidence of the crucial role of Hsp70 and other stress-inducible heat shock proteins in the stress response system and its likely role in an organisms' immediate and evolutionary response to environmental and genetic stresses. While previous reviews on Hsps have primarily focused on cellular and molecular aspects (Lindquist 1986; Morimoto *et al.* 1999; Pockley 2003), we review the current evidence of the ecological and evolutionary roles of stress-inducible Hsps and touch upon the possible applications. Finally, we suggest future directions of research to increase our understanding of the role of inducible Hsps in ecology and evolution.

BACKGROUND ON HSPS AND STRESS

Defining stress

As stress-inducible Hsps are the focus of this review, it is appropriate to define how the word stress is used in this text. The term has been used in many different fields of biological research and different researchers have proposed different working definitions relevant to their own work (e.g. Selye 1956; Grime 1979; Sibly & Calow 1989; Hoffmann & Parsons 1991; Bijlsma & Loeschcke 1997). In general, stress is defined as a condition that disturbs the normal function of the biological system or a condition that decreases fitness (Hoffmann & Parsons 1991; Bijlsma & Loeschcke 1997). Stress is usually considered to be extrinsic (environmental), however, in this review, we will also include intrinsic stress factors such as genetic stress (e.g. inbreeding and deleterious mutations) and ageing.

The cellular stress response

In the laboratory, different treatments and processes are used to study physiological responses to stress. Acclimation is traditionally a process occurring over long periods of time (days or weeks). Temperatures (or generally stress exposures) used are normally within the threshold for

development and can be applied to any life stage or throughout development (developmental acclimation). Acclimatization has been defined as the same process when it occurs in nature, however, this term has also been used interchangeably with acclimation (Huey & Berrigan 1996). Hardening is typically a much shorter process (or treatment) to a more extreme, but non-lethal stress condition. The changes brought about by hardening are primarily reversible, whereas acclimation and especially developmental acclimation leads to irreversible changes. Hardening or acclimation is known to affect the composition of membrane lipids, energy reserves and (especially for hardening) initiate the stress response including the expression of heat shock proteins (Lindquist 1986; Ohtsu *et al.* 1998; Ohtsu *et al.* 1999). These physiological changes in turn affects many life history and fitness traits such as fecundity, longevity and stress resistance (Krebs & Loeschcke 1994; Loeschcke *et al.* 1994; Dahlgaard *et al.* 1998; Silbermann & Tatar 2000; Hercus *et al.* 2003). Physiological changes in stress resistance and life history traits caused by hardening or acclimation have often been in the direction predicted from an adaptive hypothesis, and acclimation and hardening are generally considered to be adaptive. This expectation is not always met when acclimation is considered. The 'beneficial acclimation hypothesis', which predicts that an organism will perform best under the conditions in which it has been raised (or acclimated) has been the subject of much debate (Hoffmann 1995; Huey & Berrigan 1996; Huey *et al.* 1999; Loeschcke & Hoffmann 2002; Wilson & Franklin 2002). Probably, acclimation (as is the case for hardening) does not optimize performance at the acclimation condition *per se*, but increases performance to future extremes, as benefits particularly seem to exist at the extreme ends of environmental regimes (Levins 1969; Leroi *et al.* 1994; Hoffmann 1995).

Heat shock proteins

The genes coding for Hsps were discovered as chromosome puffs in 1962 in *Drosophila* after exposure to high temperatures, hence the name heat shock (Ritossa 1962). However, it was not until 1973 that the heat shock response was found to coincide with synthesis of a number of new proteins (Tissières *et al.* 1974). The genes and protein products quickly gained much attention and many Hsps have since been characterized. Subsequently, it has been shown that in addition to heat, the heat shock response is induced by a range of stressful conditions (Table 1) (Lindquist 1986; Feder & Hofmann 1999, for reviews). Up-regulation of inducible Hsps is one important part of the cellular stress response, which also includes molecular chaperones (which heat shock proteins are considered as a subset of),

Table 1 Ecological relevant inducers of Hsps. The included stresses individually induce Hsps. However, synergistic interactions among stress types have been shown to lead to even further up-regulation. Combinations of exposure to a range of 'low level stress types' may therefore significantly effect the Hsp expression, and thereby influence on the resistance and fitness of natural populations

Stressor	Reference
Environmental	
High temperature	(Tissières *et al.* 1974; Jenkins *et al.* 1997; Otsuka *et al.* 1997; Li *et al.* 1999; Sonna *et al.* 2002)
Low temperature	(Jenkins *et al.* 1997; Goto *et al.* 1998; Li *et al.* 1999; Martinez *et al.* 2001; Sonna *et al.* 2002; Sejerkilde *et al.* 2003)
Radiation (UV)	(Trautinger *et al.* 1996; Jenkins *et al.* 1997; Kiriyama *et al.* 2001)
Heavy metals	(Steinert & Pickwell 1993; Köhler & Eckwert 1997; Werner & Nagel 1997; Tedengren *et al.* 1999)
Pesticides	(Werner & Nagel 1997; Ait-Aissa *et al.* 2000; Yang *et al.* 2002; Nazir *et al.* 2003)
Hypoxia	(Ma & Haddad 1997)
Salinity	(Diamant *et al.* 2001; Drew *et al.* 2001; Hamilton *et al.* 2001; Spees *et al.* 2002)
High density	(Sørensen & Loeschcke 2001)
Bacterial and viral infection	(Collins & Hightower 1982; Polla 1988; Kaufmann & Schoel 1994; Deitch *et al.* 1995)
Parasitism	(Merino *et al.* 1998; Rinehart *et al.* 2002)
Physical activity	(Skidmore *et al.* 1995; Fehrenbach & Niess 1999)
Desiccation	(Alamillo *et al.* 1995; Tammariello *et al.* 1999)
Oxidative stress	(Ropp *et al.* 1983; Gophna & Ron 2003)
Genetic	
Senescence	(Wheeler *et al.* 1999)
Inbreeding	(Kristensen *et al.* 2002)
Deleterious mutations	(Sherman & Goldberg 2001; Trotter *et al.* 2002; Zhao *et al.* 2002)

antioxidases, proteases and DNA repair systems. A number of investigations have confirmed the importance of Hsps in resistance towards heat and cold and a range of other stresses including insecticides, heavy metals, desiccation, diseases, parasites and inbreeding (Steinert & Pickwell 1993; Matz *et al.* 1996b; Wong *et al.* 1996; Su & Gordon 1997; Kristensen *et al.* 2002).

The heat shock genes are highly conserved and show low between species variation in the coding regions. Among the inducible Hsp70, one of the most conserved Hsps, amino acid similarity between *Escherichia coli* and *Homo sapiens* is around 50%, with some domains being 96% similar (Schlesinger 1990). The amino acid similarity in the same gene between *Drosophila melanogaster* and *H. sapiens* and between *E. coli* and *D. melanogaster* is approximately 70% (Lindquist 1986). The heat shock genes are found in all organisms from bacteria to plants and mammals. The low variation in Hsp genes and their universal presence suggest evolutionary importance and a role in the protection of cells during or after stress (Lindquist 1986; Feder & Hofmann 1999). Increasing evidence points to the function of Hsps as molecular chaperones involved in 'housekeeping' functions in the cell, including prevention of aggregation of damaged proteins, transportation, folding and unfolding, assembly and disassembly of multi-structured units, and in degradation of misfolded or aggregated proteins (Gething & Sambrook 1992; Parsell & Lindquist 1994; Bross *et al.* 1999; Jolly & Morimoto 1999; Gregersen *et al.* 2001). Although some stress specificity exists, it generally seems that the stress response is universal to numerous types of stress. This response is considered to be initiated by the presence of non-native protein conformations in the cell at concentrations above some level (Ananthan *et al.* 1986).

Several families of Hsps have been identified and named according to their molecular weight in kDa. The families consist of one to several closely related genes. Major families are HSP100, HSP90, HSP70, HSP 60, HSP40 and the small HSPs (so-called sHsps of sizes below 30 kDa), and smaller co-factors. The HSP families and their molecular functions are reviewed in detail by Parsell & Lindquist (1993) and Feder & Hofmann (1999). In many organisms Hsp70 is considered to be the major HSP family consisting of solely inducible, constitutive and inducible, and solely constitutive proteins (heat shock cognates).

The protein quality control system

The universal occurrence and important cellular functions of molecular chaperones have led to the idea of a general system involved in protein quality control, operating to maintain homeostasis under normal cellular conditions. The Hsps are a part of this system termed The Protein Quality System (PQC). The importance of the system increases upon exposure to environmental and genetic stresses as a result of increased levels of protein folding disorders (Ananthan *et al.* 1986; Lindquist 1986; Gething & Sambrook 1992; Gregersen *et al.* 2001). The overall function of this system is two-fold – to secure correct folding of proteins and to assist in degradation of denatured or aggregated proteins. The PQC has gained much attention recently with respect to human diseases (Bukau *et al.* 2000; Gregersen *et al.* 2001). The pathology of many mutational human diseases, such as Alzheimer, Creutzfeldt-Jacob (and related animal diseases such as scrapie and BSE), α_1-antitrypsin deficiency, liver diseases and polyglut amino-expansion diseases, such as Huntington's disease, are caused by misfolding of specific proteins (reviewed in Gregersen *et al.* 2003). Additionally, fever caused by infection and ischaemia, hypoxia, oxidative injury, and endotoxemia have been shown to increase the cellular level of misfolded proteins, thereby leading to an up-regulation of the proteins in the quality control system (Favatier *et al.* 1997; Sherman & Goldberg 2001; Snoeckx *et al.* 2001). Variation in the response to missense mutations, pathogens or other environmental stress factors has been shown to correlate with specific variation in the PQC (Favatier *et al.* 1997; Hansen *et al.* 2002). Thus, variation in the efficiency of the quality control system is thought to be an important factor regarding the ability to resist diseases and environmental challenges (Favatier *et al.* 1997; Feder & Hofmann 1999; Gregersen *et al.* 2001; Slavotinek & Biesecker 2001). Therefore, a thorough understanding of this system is required to decipher the complex association between genotype and phenotype.

Due to the high similarity among species of genes coding for some members of the quality control system, e.g. Hsps, one would expect very low variation within species. However, some variation is found in both coding and probably more so in regulatory regions of these genes (Frydenberg *et al.* 1999; Bettencourt *et al.* 2002). The PQC system is therefore likely to be important for maintaining homeostasis in natural populations.

INDUCIBLE HSPS IN AN EVOLUTIONARY AND ECOLOGICAL PERSPECTIVE

Stress as an ecological and evolutionary force

Stress-inducible Hsps are strongly involved in the stress resistance of organisms. Before going into details on this issue, we will discuss stress in an ecological and evolutionary perspective, and argue that exposure to stressful conditions is likely for most populations.

When individuals in a population are exposed to stressful conditions, generally three possibilities exist (Hoffmann & Parsons 1991): (i) The individuals in the population try to

avoid the stress, either by moving to a more favourable habitat, by adjusting their activity patterns or by changing into a physiological state that might be more resistant (e.g. by going into hibernation or diapause). (ii) The population can adapt to the stressful condition through selection or individuals can respond through a plastic response. (iii) Fail in the above and go extinct.

Stressful conditions can thus act as evolutionary forces that populations respond to adaptively. Many environmental factors are likely to affect the distribution and abundance of species and populations in nature. Environmental factors of importance include chemicals and heavy metals (Macnair 1997), competition, predation or parasitism (Davis *et al.* 1998), temperature and humidity (David *et al.* 1983; Cossins & Bowler 1987; Hoffmann & Parsons 1991; Lee & Denlinger 1991; Loeschcke *et al.* 1994; Hoffmann & Parsons 1997b; Huey & Berrigan 2001; Gibbs *et al.* 2003). Genetic stresses include inbreeding and fixation of deleterious mutations (Bijlsma *et al.* 1997; Hoffmann & Parsons 1997b). Although the Hsp genes for some time have been known to be induced by many types of stress, the majority of studies and the best empirical evidence of Hsps and its effects on stress resistance is related to heat stress.

Stresses of an environmental and/or genetic basis are predicted to affect future populations. Due to anthropogenic activity, e.g. global warming, pollution and deforestation (IPCC 2001), environmental changes might even be more drastic and unpredictable in the future than at present. Habitat fragmentation leading to isolation and reduction of population sizes are increasing the degree of inbreeding and genetic drift. However, even without human caused interventions, environmental and genetic factors are changing and will affect the ecology and evolution of species.

There is a wealth of data showing that stressful conditions are an evolutionary force causing adaptation in natural populations. Data on adaptation in natural populations of *Drosophila* to extreme temperatures is reviewed in Hoffmann *et al.* (2003). These data include clinal variation to heat and cold resistance (Hallas *et al.* 2002), adaptive seasonal variation (Chen *et al.* 1991; Grechanyi *et al.* 1997) and adaptive daily variation in temperature resistance (Sørensen & Loeschcke 2002b). Although variation in the coding regions of the *hsp* genes is low, several recent studies have identified clinal variation in *hsp* genes, indicating that natural selection acts on these genes (Otsuka *et al.* 1997; McColl & McKechnie 1999; Bettencourt *et al.* 2002; Anderson *et al.* 2003; Frydenberg *et al.* 2003). Adaptive changes in Hsp expression over days (Nguyen *et al.* 1994; Ferguson *et al.* 1998) and over seasons (Fader *et al.* 1994; Hofmann & Somero 1995; Pyza *et al.* 1997; Minier *et al.* 2000) also support the ecological significance of Hsps in natural populations.

Effects of stress on rates of evolution

One frequently debated topic is the basis for increased phenotypic variance that is often observed during stress (Hoffmann & Parsons 1997a,b; Imasheva *et al.* 1997; Hoffmann & Merilä 1999; Bubliy *et al.* 2000; Kristensen *et al.* 2003). If an increase in phenotypic variance is because of an increased expression of the additive genetic variance component, then increased trait heritability may be observed. This would accelerate natural selection under stressful conditions. Increased genetic variance and heritabilities have been found in some studies, under stressful conditions, for some traits and with some technical approaches used for estimating heritability (Hoffmann & Merilä 1999; Bubliy *et al.* 2001). Increased response to selection was also reported by Clare & Luckinbill (1985) who reported that lines of *D. melanogaster* failed to respond to longevity selection at controlled (low density) conditions, but responded to the selection at uncontrolled (high) density. Service *et al.* (1988) also found that selection for longevity-related characters was successful only under a uncontrolled (high) density (but see Zwaan *et al.* 1995). Therefore, Clare & Luckinbill (1985) concluded that selection must have occurred on genes only expressed at high densities. Such genes could be stress inducible Hsps as these genes are known to affect longevity (Tatar *et al.* 1997).

Some studies involving *Drosophila* (Rutherford & Lindquist 1998) and the self-fertilizing plant *Arabidopsis thaliana* (Queitsch *et al.* 2002) have identified a fundamental role of Hsp90 for the expression of genetic variation. In fly and plant strains with an impaired Hsp90 production, a high frequency of developmental abnormalities occurred. The functional Hsp90 protein appears to buffer or canalise existing variation, which was not phenotypically expressed under normal conditions. Under stressful temperatures, where damaged proteins are competing for available Hsp90, impaired Hsp90 production led to even more severe consequences of the stress (Rutherford & Lindquist 1998; Queitsch *et al.* 2002). A similar result has been obtained for Hsp70 in *Drosophila* (Roberts & Feder 1999). Therefore, the buffering capacity conferred by members of the chaperone system against internal and external stresses seems to be a general phenomenon. Examples of increased expression of genetic variation otherwise unseen when the Hsp-buffering capacity is compromised could be one specific mechanism explaining higher evolutionary rates under stress. Most of the hidden genetic variation revealed in the studies was deleterious. Therefore, the potential of this mechanisms for adaptation to environmental stress it is not known (Lauter & Doebley 2002; Mitchell-Olds & Knight 2002). Nevertheless, the discovery of modifier genes is of extreme interest from a wide range of perspectives.

Cost of expression

Obviously, individuals are likely to suffer if they develop under sub-optimal conditions. However, it is unclear whether a reduced fitness reflects a cost of acclimation or merely of being reared under poor conditions, or a combination of both (Huey & Berrigan 1996; Loeschcke & Hoffmann 2002). It is important to test whether the benefits of acclimation (on stress resistance and longevity) can be separated from costs (e.g. Hoffmann & Hewa-Kapuge 2000). In *D. melanogaster*, Hercus *et al.* (2003) showed that repeated mild stress had a slightly detrimental effect on fertility and fecundity only when flies were exposed to the stress, but not later in life. The time dependency of benefits and potential costs (the expression of high levels of Hsp70) does therefore not necessarily coincide. Along the same lines, it has been shown that when levels of inducible Hsp70 expression after heat hardening return to normal, the beneficial effect of hardening on survival of a heat stress can still be substantial (Fig. 2). Thus, one way of separating costs and benefits is to characterize the time frame of the acclimation response in detail, and then try to separate costs and benefits by altering acclimation treatments (Scott *et al.* 1997; Hoffmann & Hewa-Kapuge 2000; Thomson *et al.* 2001; Wilson & Franklin 2002).

It is important to understand costs, as the ecological importance of inducible Hsps depends on the balance between benefits and costs. Costs of Hsp expression have been shown with regard to fertility/fecundity, energy, development and survival. Costs are thought to arise by the shut-down of normal cell functions during the stress response, the extensive use of energy and the toxic effects of high Hsp concentrations due to interference with normal cell function (Feder & Hofmann 1999).

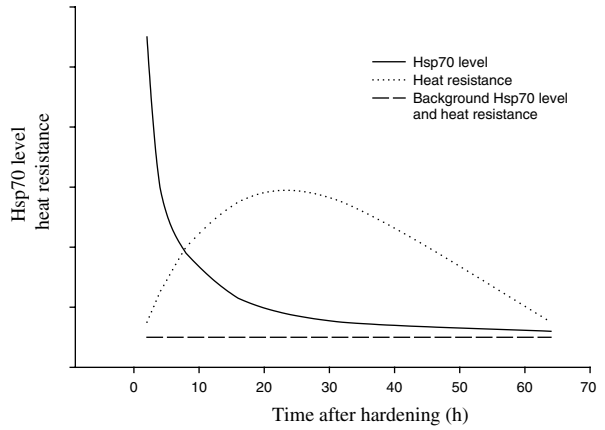

Figure 2 Schematic description of Hsp70 expression level and survival rate in relative units to heat stress after hardening between time 0 and 1 in *Drosophila melanogaster*. The hardening benefits and Hsp70 level were measured at various times after hardening. Hardening increased survival and the Hsp70 level. However, the curves characterizing the two processes do not coincide as Hsp70 level decreases much faster with time than survival. The Hsp70 expression levels therefore only explain a part of the increased thermotolerance obtained by the hardening treatment (Figure from Hoffmann *et al.* 2003).

Direct costs of expressing Hsp70 were investigated by Krebs & Feder (1998), who hardened *D. melanogaster* larvae at different stages (1 h at 36 °C at the 1st, 2nd and 3rd instar stages). Four isofemale lines with an *hsp70–lacZ* fusion transgene expressed beta-galactosidase after heat exposure. This expression was used to estimate the potential energy and nutrient expenditure involved in hardening. Multiple heat exposures reduced survival but did not affect development time. However, beta-galactosidase expression was not correlated with survival, suggesting that the differences in expression cannot explain the survival effects, at least in these four lines. The direct expense of Hsp expression in this study was therefore probably minor.

Nevertheless, other effects are involved in expressing high levels of Hsp70. High levels decrease or even retard growth and cell division (Feder *et al.* 1992; Krebs & Feder 1997) and reduce reproduction (Krebs & Loeschcke 1994; Silbermann & Tatar 2000). Silbermann & Tatar (2000) showed that heat-induced Hsp70 expression in *D. melanogaster* was associated with a reduction in egg hatching among progeny of exposed mothers. Krebs & Loeschcke (1994) found that fecundity of *D. melanogaster*, averaged over the first 2 days after stress treatment, was reduced. However, these fecundity costs are minor under a mild temperature stress. The deleterious effects may explain why cells eliminate Hsp70 in the absence of stress and why the fastest removal occurs in early development, when cell division is most active (Welte *et al.* 1993; Parsell & Lindquist 1994). The tight regulation suggests that a strong trade-off applies to expression of Hsps between the benefits of increased stress resistance on the one hand, and costs to development, fertility or fecundity on the other (Krebs & Loeschcke 1994).

The role of Hsps for adaptation

Inducible Hsps and stressful conditions

The improved heat resistance of insects (Gehring & Wehner 1995; Dahlgaard *et al.* 1998), fish (Basu *et al.* 2002), plants (Sun *et al.* 2002) and mammals (Ulmasov *et al.* 1993; Matz *et al.* 1996a) after Hsp expression is undisputed. However, only rarely have ecologically realistic studies of natural populations been performed (Dyer *et al.* 1993; Fader *et al.* 1994; Gehring & Wehner 1995; Feder *et al.* 1997; Kelty & Lee 1999). Some of the first data on the possible ecological relevance of Hsp expression comes from selection studies. In different species of *Drosophila*, it was shown that (contrary to predictions at that time) expression of Hsp70 was lower in lines frequently, or continuously exposed to severe stress (Bettencourt *et al.* 1999; Sørensen *et al.* 1999; Lansing *et al.*

2000). The interpretation was that the costs of Hsp expression in populations frequently exposed to stress outweighed the benefits and that stress adaptation was achieved through some other means. The same pattern was subsequently found in natural populations of *Drosophila* (Sørensen *et al.* 2001) and in soil invertebrates exposed to heavy metals (Köhler *et al.* 2000). According to these findings, the adaptive role of Hsps in connection to environmental stress resistance seems to occur during periods of relatively rare, unexpected extreme stress exposures and not during every day environmental fluctuations. Other mechanisms of adaptation to stressful conditions are selected for under chronic stressful environmental conditions (Sørensen *et al.* 1999; Sørensen & Loeschcke 2002b).

Inducible Hsps under benign environmental conditions

At the same time as Hsps have been investigated in relation to extreme environmental resistance, other studies suggest that the regulation of inducible Hsps and the stress response is much more fine-tuned than earlier suggested, and not just consists of the states on/off (Fader *et al.* 1994; Sørensen & Loeschcke 2001; Kristensen *et al.* 2002). Sørensen & Loeschcke (2001) showed in *Drosophila* that a moderately high-rearing density during larval stages led to a low, but detectable up-regulation of Hsp70. The authors suggested that either waste product accumulation or food limitation was responsible for the Hsp induction. Adult flies raised under high larval density had increased longevity and heat stress resistance in spite of the stressful developmental conditions (Sørensen & Loeschcke 2001).

Other studies have shown that also age impacts on the expression of inducible Hsps. Mostly, a decrease of Hsp expression level after induction is observed with age. The main reason seems to be a lower capacity to up-regulate expression at an older age (Niedzwiecki *et al.* 1991). However, at senescent ages, a low level of inducible Hsp70 is continuously expressed in *Drosophila* (Wheeler *et al.* 1999). Kristensen *et al.* (2002) showed that inbred flies express more Hsp70 when compared with outbred flies, even during non-stressful environmental conditions. The authors interpreted these data as a mechanistic response, caused by an increase in the levels of misfolded proteins in the cell as a result of an increased frequency of expressed deleterious recessive alleles in inbred lines. It was suggested that the (low) Hsp induction was caused by a more pronounced need for chaperones in order to maintain optimal cell function and homeostasis (Wheeler *et al.* 1999; Kristensen *et al.* 2002).

The results presented above suggest that inducible Hsps are important for fitness also under benign environmental conditions. As part of the protein quality control system Hsps play a major role in the struggle for maintaining a functional cellular machinery upon exposure to intrinsic stress.

Hsps and a long life

Ageing and senescence are subjects that are investigated from both an evolutionary perspective and from a more anthropogenic or medical perspective. Interestingly, there seems to be a general relationship between stress resistance traits and longevity or ageing traits (Mine *et al.* 1990; Rattan 1998; Minois 2000; Parsons 2000; Hercus *et al.* 2003). Several recent papers tie stress exposure, stress resistance and the expression of Hsps to life span (longevity), although the mechanisms and ecological implications are not yet fully understood (Hercus *et al.* 2003). A prevalent theory states that the activation of defense/cleaning systems (Hsps, antioxidases and DNA repair) by stress postpones the deleterious effects that otherwise would occur with age (Minois 2000).

Studies on several organisms have found that various degrees of heat-stress exposure increase longevity (Khazaeli *et al.* 1997; Minois 2000; Hercus *et al.* 2003), and an increased expression level of Hsp70 following heat hardening was found to correlate with increased longevity in transgenic extra-copy *D. melanogaster* lines (Tatar *et al.* 1997). However, truncation selection for longevity led to a decline in heat-induced Hsp70 expression (Norry & Loeschcke 2003), indicating that long-lived flies are more homeotic and need less activation of the above discussed defence or cleaning systems. This down-regulation of inducible Hsp70 expression by longevity selection resembles the outcome of selection for increased heat selection. Additionally, Hercus *et al.* (2003) found long lasting effects on life span in flies that as young adults received mild stress treatments (giving approximately 30% of maximum Hsp70 expression). The severity of the treatment was decreased in order to separate the costs of expression from the benefits, which was partly successful. This indicates that the benefits of Hsp expression can outweigh the costs under these conditions.

One important question is, whether a longer life leads to increased fitness (Rattan 1998; Fonager *et al.* 2002). Data on this topic indicates that increased longevity also increases the lifetime reproductive success, thereby increasing fitness (Norry & Loeschcke 2002). However, these results were obtained in the laboratory, and the ecological relevance should be investigated further.

Ecological relevant life stages

In nature, many species are exposed to high temperatures and high levels of toxicity. A specific example of this is *Drosophila buzzatii* larvae developing in necrotic cactus (Fig. 3). In the juvenile stages mobility is often low and behavioural avoidance is limited. Thus, certain life stages can be particularly vulnerable and exposed to environmental stress (Feder *et al.* 1997; Loeschcke *et al.* 1997). Occupation of different environments for different life stages might select for life-stage-specific Hsp expression and resistance. In *D. buzzatii*, the relationship between heat resistance in

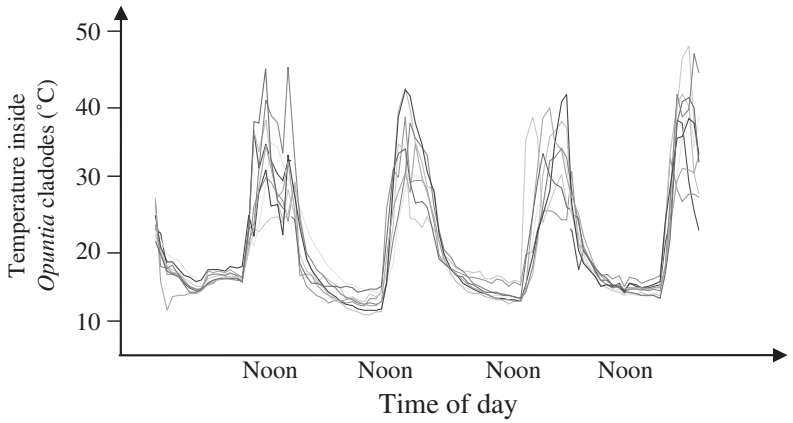

Figure 3 Daily temperatures measured in cladodes of *Opuntia* cacti on Tenerife, Canary Islands. The life cycle of cactophillic *Drosophila* is closely associated with *Opuntia* cacti, with eggs, larvae and pupae inhabiting rotting cladodes. Adult flies emerged from each of the measured rots showing that the preadult life stages are exposed to stressful temperatures under natural conditions.

different life stages was found to be weak (Krebs & Loeschcke 1995; Loeschcke & Krebs 1996). Moreover, Sørensen *et al.* (1999) found no correlation between Hsp70 expression in third instar larvae and adults in laboratory selection lines, indicating at least partly life stage specificity of both stress resistance and Hsp70 expression. However, Krebs *et al.* (1998) found Hsp70 expression to be coupled in two larval stages (first and third instar) and in the adult life stage, indicating that Hsp70 expression is coupled between life stages. Thus, how tightly Hsp70 expression and stress resistance between life stages is coupled remains an unanswered question.

Within the adult life stage, the expression of Hsp70 seems to be down-regulated in concordance with decreasing heat stress resistance during the ecologically relevant life span of *Drosophila* (Sørensen & Loeschcke 2002a). In nature, adult *Drosophila* experience temperatures that induce Hsp70 expression and the expression level is important for resistance and under direct selection at this life stage (Feder *et al.* 2000). Once sexual maturity is reached, the Hsp system might be evolutionarily dispensable, as increased fitness is attained by shifting the balance of energy expenses towards reproduction at the expense of high stress resistance.

In conclusion, available data reveals that Hsps are ecologically relevant for all life stages. However, juveniles could, as a result of their high stress sensitivity and often low mobility, be especially dependent on Hsps for survival. Correlation of Hsp expression levels and stress resistance might or might not exist between life stages. Therefore, knowledge of the ecology of the species in question is vital in order to identify and investigate the relevant life stage when looking for adaptation in the wild.

CONCLUSIONS AND FUTURE PERSPECTIVES

Natural populations are constantly exposed to changing environments and genetic threats. The Hsps buffer this environmental variation and are therefore important factors for the maintenance of homeostasis across environmental regimes. Given the fact that Hsps influence fitness under non-optimal environmental conditions, we argue that the regulation and expression levels of these proteins are of major evolutionary and ecological importance. Here, we have reviewed the recent evidence on the role of Hsp expression from an ecological perspective, including a characterization of factors that induce the heat shock response and a discussion of the costs associated with this response. We suggest that up-regulation of Hsps in addition to being an important part of the response to sudden extreme stress exposures is of ecological relevance on a much wider scale with respect to less severe but regular incidences of stress. We also suggest that the expression level of Hsps, in each species and population, is a balance between benefits to resistance and costs, due to the impacts on growth, developmental rate and fertility that up-regulation of Hsp promotes.

New studies in this field have increased our knowledge on the cost/benefit trade-off of Hsp expression. Until recently, it has been believed that Hsp expression was mainly an emergency, or threshold defence mechanism in relation to short-term stress exposure. However, new results show that Hsp expression is highly fine-tuned (not being only an on–off mechanism) and that Hsps are also continuously expressed after mild chronic stress exposure. Furthermore, results have shown that genetic stress, such as inbreeding and disease-causing mutations lead to an up-regulation of Hsp. This implies that the cells operate to restore homeostasis disrupted by genetic stress (genetic stress $\rightarrow$ disrupted homeostasis $\rightarrow$ increased Hsp expression $\rightarrow$ restored homeostasis).

In conclusion, heat shock proteins are important in relation to stress resistance and adaptation to the environment. The regulation of Hsp is influenced by both environmental and genetic stress factors. In relation to ecological studies, environmental disturbances and genetic stress are topics of large interest and relevance. Understanding the role of Hsps in relation to stress resistance and evolutionary change, and in a more applied perspective as a potential indicator of stress is therefore important. One advantage of Hsps as biomarkers

used for detecting of, e.g. pollutants is that negative consequences of stress disturbances can be detected earlier than with biomarkers based on growth rate, mortality or fertility (Werner & Nagel 1997). Induction of Hsps as markers for cellular stress has mostly been investigated in soil and marine organisms from contaminated sites (Köhler *et al.* 1992; Sanders 1993; Sanders & Dyer 1994; Köhler & Eckwert 1997; Köhler *et al.* 1998). Generally, these results show that Hsps have potential as biomarkers. However, local adaptation and selection for other kinds of adaptive mechanisms may disturb the evaluation of the results. The results by Sørensen *et al.* (1999, 2001) and Köhler *et al.* (2000) showing that there is selection against Hsp expression in populations being exposed to chronic stress clearly demonstrates this problem. As discussed, Hsps can furthermore be induced by non-environmental cues such as genetic stress (Kristensen *et al.* 2002; Trotter *et al.* 2002; Zhao *et al.* 2002). Variation in expression levels is also influenced by the sex- and age-class distribution in the investigated populations. The success of Hsp expression levels as a reliable biomarker, therefore, depends upon the awareness of these aspects in designing experiments and evaluating the results. Other practical applications of research on Hsps are in medicine and animal breeding. An enhanced understanding of the various immunoregulatory mechanisms in which Hsps are involved could help us to harness the power of the molecules in relation to treatment of diseases. Furthermore, research on the potential use of Hsps as a selection criterium in animal breeding is currently being investigated (T.N. Kristensen, unpublished data). These examples show that investigations on Hsps impact on a wide range of scientific disciplines. Although not directly related to ecology and evolution, results from such investigations may contribute to an increased understanding of the ecological role of inducible Hsps.

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 (e.g. quantitative trait loci, microarray and quantitative PCR studies) and proteonomics (e.g. 2-D gel electrophoresis, X-ray crystallography and nuclear magnetic resonance studies) will further elucidate the effects of stress on expression patterns at the DNA, RNA and protein level. In combination with more traditional methods of protein expression analysis (blotting and ELISA techniques), we will in the future obtain a much more detailed understanding of Hsp regulation and expression. Thereby, the role of Hsps, not only in relation to environmental stress in the common sense but also in relation to genetic stress and diseases, will be better understood.

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