Chapter 4
Heat Shock Proteins and Adaptation to Variable and Extreme Environments

4.1 General Response to Heat Shock and Other Forms of Stress

Eukaryotic organisms evolved sophisticated sensing mechanisms and generic responses at the cellular level to various forms of mild, chronic or acute stresses including HS. Exposure to elevated temperature as well as many other stressful factors disturbs normal folding of proteins while preexisting proteins may undergo denaturation and aggregation which is harmful for the cell (Hightower 1991; Szalay et al. 2007). Gene expression changes are a major component of various stress responses, along with alterations in protein-lipids ratio, rate of metabolism, fragmentation of Golgi complex and endoplasmic reticulum and other multiple disturbances (Kruuv et al. 1983; Vigh et al. 2007; Welch and Suhan 1985). Drastic changes in cytoskeleton structure (Toivola et al. 2010; Welch and Suhan 1986) and drop in ATP level in the cell represent important landmarks of stress response observed in different organisms (Lambowitz et al. 1983; Patriarca and Maresca 1990). Specifically, HS inhibits process of splicing of cellular mRNAs and may lead to complete blockade of cell cycle (Yost and Lindquist 1986). Acute HS usually resulted in the accumulation of high concentration of denatured proteins may also induce cell death by apoptosis or necrosis. Notably, adaptive responses to stress definitely depend on the organism and the natural environments in which it has been evolved and underwent selection.

Live organisms exhibit amazing plasticity in the regulation of genes involved in response to different forms of stress. Up- and down regulation of the expression of multiple genes in response to stress play central role in the adaptation of organisms to both short-term and long-term pronounced changes of environment. The regulation of adaptive response may be realized at the level of transcription induction, translation efficacy and/or stability of resulted mRNAs or proteins.

Stress response usually represents a choice between two antagonistic programs such as induction of adaptogenes including various Hsps or switching on the genes

M.B. Evgen'ev et al., *Heat Shock Proteins and Whole Body Adaptation to Extreme Environments*, DOI 10.1007/978-94-017-9235-6_4

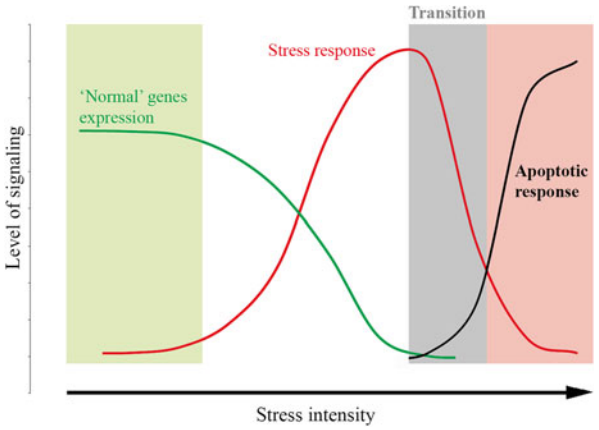

Fig. 4.1 Stress response in terms of "normal" cell genes expression changes and apoptosis process depending on stress severity

underlying cells death by apoptosis (Anckar and Sistonen 2007; López-Maury et al. 2008; He et al. 1998; Chen and Brandizzi 2013; Chu et al. 1996). Moderate heat shock predominantly leads to the induction of heat shock genes playing chaperoning function to prevent proteins misfolding and degradation and, hence, provide adaptive response. More severe heat stress inhibits the expression of *Hsp* genes and activate the expression of genes responsible for apoptosis (Fig. 4.1) (Chen and Brandizzi 2013; Murray et al. 2004).

At the present time during genomic era, new approaches including High-throughput Sequencing and DNA microchip technologies have been developed and used to monitor the consequences of heat shock and other forms of stress at the genome level. These studies performed in yeast, fruit flies and mammals including humans enabled one to describe complete transcriptome after various forms of stress including HS. The large-scale transcriptomic study of many species showed that organisms respond to drastic temperature changes with a multitude of transcriptional changes: up to 40 % of genes showed an altered expression after temperature exposure experiments depending on the species. High temperature has usually stronger effects on gene expression than low temperature, reflected by the higher number of temperature-responsive genes. Such studies help to gain insights into molecular processes underlying induced thermotolerance and acclimation and allowed to describe a lot of genes besides "classical" *Hsp* genes that dramatically changed their expression after temperature elevation in yeast (Gasch et al. 2000; Lyne et al. 2003), *Drosophila* (Girardot et al. 2004), human cells (Murray et al. 2004), *E. coli* (Riehle et al. 2005), fishes (Buckley et al. 2006) etc. Such studies revealed hundreds of stress-related genes and demonstrated that the level of transcription and translation modulation depends on the severity and duration of stress applied. It is of note that phylogenetically distant forms express functionally similar groups of genes including heat shock genes, antioxidative genes, genes involved in the metabolism of hydrocarbons, and genes involved in ubiquitin-dependent degradation of proteins (Buckley et al. 2006). The role of many genes with temperature-dependent expression is not clear yet. For example, it was shown in *Drosophila* that heat shock leads to significant induction of transcription of genes *shark* and *TotA*

(from *turandot* family), however, functions of these genes in the cells after HS were not revealed (Ekengren and Hultmark 2001).

We are well aware that Hsps discussed in this review is not the only protective molecules providing heat-resistance in various organisms. Thus, in other strains and species, mechanisms of basal thermotolerance may include synthesis of thermoprotective osmolites such as trehalose in the yeast (*Saccharomyces cerevisiae*), (Hottiger et al. 1987) and homeoviscious adaptation (i.e. adjustment in the lipid constituents of cells). Besides, thermotolerance may be due to enhanced stability of cellular proteins through modification of their primary structure and regulated depression of normal house-keeping cellular functions to moderate energy requirements under stressful conditions. Thus, in yeast the production of the disaccharide trehalose is induced by temperature elevation more that 20-fold (Hottiger et al. 1987). Transgenic strain of *D. melanogaster,* transformed with overexpressed trehalose-6-phosphate synthase gene (*tps1*), exhibits high tolerance to hypoxia. It was shown that the increase of trehalose concentration in the cells prevents aggregation of various proteins such as Na^+/K^+-ATPase and tubulin and participates in proteins folding in cooperation with chaperones (Chen et al. 2002; Diamant et al. 2001). Generally speaking, adaptive stress responses may be divided into generic responses shared by many stresses and specific responses evolved to cope with a particular challenge (Feder 2007; Lindquist 1986; Nadal et al. 2011). Below we shall discuss in detail the presumptive adaptive roles of heat shock proteins synthesized in response to HS and many other stresses in various eukaryotic organisms, although we clearly understand that stress tolerance results from numerous physiological and molecular mechanisms, of which Hsps are collectively only one.

All proteins and other compounds synthesized in response to temperature elevation may be roughly divided into the following major groups:

1. Molecular chaperones comprising Hsps *per se* and their co-factors involved in restoration of misfolded cellular proteins and, hence, prevention of harmful aggregates formation (Ellis et al. 1989; Lindquist 1986).
2. Components of ubiquitin-proteasome system (UPS) responsible for degradation of irreversibly damaged and denatured proteins (Raboy et al. 1991).
3. RNA- and DNA-modifying enzymes necessary for DNA reparation and restoration of normal RNA-processing mechanisms (Bügl et al. 2000; Jantschitsch and Trautinger 2003).
4. Carbohydrates metabolism enzymes (Buckley et al. 2006; Malmendal et al. 2006; Voit and Radivoyevitch 2000).
5. Regulatory proteins including transcriptional factors and protein kinases.
6. Transport and antioxidant proteins (e.g. glutathion S-transferase) as well as enzymes involved in toxin inactivation (cytochrome p450 etc.).
7. Proteins involved in cellular membranes stabilization (Welker et al. 2010).
8. Non-coding RNAs, e.g. *hsr-ω* (Arya et al. 2007; Jolly and Lakhotia 2006).

Therefore, rapid and abundant synthesis of various Hsps represents the most generic cellular response after temperature elevation, hypoxia, high concentrations of heavy metals, oxidative stress and other multiple forms of stresses. The synthesis of similar but not identical groups of Hsps has been repeatedly demonstrated for

organisms from different unrelated phyla including yeast (*S. cerevisiae*), mollusks (*Mytilus*), insects (*Cataglyphis, Drosophila, Bombyx mori*), fishes (*Gillichthys*), amphibians (*Xenopus*), reptiles (*Phrynocephalus, Gymnodactylus, Lacerta*) etc. (reviewed by Feder and Hofmann 1999). Although it was shown by different groups that Hsps are actively synthesized after stress in the laboratory, it was necessary to prove by field work that their synthesis represents adaptive response evolved in nature to maximize cell and whole organism survival in response to drastic changes in the environment that put them at risk. It was also important to dissect the mechanisms underlying general HS response and define specific roles of individual Hsps families in ecological context in buffering extracellular challenges to minimize intracellular damage.

In general, all organisms may be divided into two groups: stenothermal and eurythermal species. Stenotherms inhabit areas with narrow temperature ranges and may be further subdivided into thermophilic and cold-adapted organisms. Endoparasites of mammalians represent good examples of thermophilic stenothermal organisms. On the other hand, Antarctic animals that have evolved for many millions of years under highly stable, cold temperature represent extreme cold-adapted stenotherms (Somero 2005). Eurythermal species dwell in habitats with highly variable temperature and intertidal organisms that experience every day fluctuations represent an excellent example of this particular group. Notably, from the very beginning Hsp70 was the main object of the investigations on adaptive role of Hsps in aggressive and highly variable environments. Such attention is quite understandable taking into account the key role of Hsp70 family members in proteins folding and transport under normal conditions and after stress (see Chap. 2). Besides, this family of Hsps is highly conserved and abundant synthesis of Hsp70 is easy to detect after HS in various organisms from yeast to humans and flies by Western blotting technique even using heterologous anti-bodies (see Craig et al. 1983; Schlesinger 1990; Feder and Hofmann 1999 for reviews). Therefore, many attempts were performed to correlate the synthesis of Hsp70 with adaptation of various organisms to fluctuating or extreme environmental conditions (Hightower 1991; Hoffmann 2010; Lyashko et al. 1994; Tomanek and Somero 2000; Ulmasov et al. 1993). However, it becomes evident that the unambiguous attribution of stress resistance to Hsp70 or other Hsps groups requires more than correlate evidence (Feder 2007; Jensen et al. 2010; Somero 2005).

4.2 Role of Hsps in Adaptation to Fluctuating Environmental Conditions of Terrestrial Organisms

4.2.1 Interspecific Comparisons (Distant Taxa)

The modes of thermal adaptations certainly greatly depend on the present state of environment and the evolution the species underwent. Many organisms successfully cope with extreme environmental conditions including heat shock by multiple

behavioral adaptations such as hiding and escaping heat stress while others live in habitats with comparatively constant temperature regimes (Feder 2007; Feder and Hofmann 1999; Somero 2005).

Sometimes, different life stages e.g. larvae and adults routinely encounter strikingly different environmental conditions in terms of stress. Thus, while larvae and pupae of *Drosophila* and many other insect species are often heated to stressful temperatures in their natural medium (e.g. rotten fruits) the adults may easily escape high temperature areas and rapidly move to microhabitats with temperatures close to the physiological ones (Feder 1997). Thus, Hamada et al. (2008) identified a thermal sensing pathway in *D. melanogaster* that is tuned on to avoid non-preferred temperatures. The ion channel of the transient receptor potential (TRP) family dTrpA1 functions as a molecular sensor of temperature and activates a small set of anterior cell neurons, the function of which is crucial for selection of preferred temperatures.

Besides, many species from various taxonomic groups including insects and mammals evolved diapauses, hibernation or occasionally desiccation to survive adverse periods of life (Gusev et al. 2011; King et al. 2013; Rinehart et al. 2006).

The comparison of Hsps levels in geographical populations of the same species or different species inhabiting environments with contrasting temperature regimes represents the most widely approach to implicate expression of various Hsps and in particular Hsp70 in adaptation. Such integral ecological approach exploring field studies of non-model organisms may not only shed light on physiological functions of individual Hsps but also permits to evaluate their role in evolution of adaptations to adverse environmental conditions.

Historically, this ecology-based approach with the goal to investigate Hsps specifically in thermally adapted organisms was originally applied in 1980s by several groups including ourselves (Evgen'ev et al. 1987; Feder and Hofmann 1999; Kee and Nobel 1986; Steinert and Pickwell, 1988).

For our large-scale analysis lasted for more than 30 years we used different organisms from leishmania and lizards to mammalian species including camel and human tribes inhabiting thermally contrasting landscapes such as deserts and cold or temperate climatic zones and active at different time during the day.

Thus, we began our studies by comparing cell lines of silk worm (*Bombyx mori*) and gypsy moth (*Lymantria dispar*) (Evgen'ev et al. 1987). It is believed that silk worm (*B. mori*) originated from warm regions of South-East Asia while gypsy moth (*L. dispar*) dwells in cold and moderate climate areas. To our surprise the cells of *B. mori* survive and synthesize all groups of Hsps at extreme temperatures up to 49 °C, while *L. dispar* cells abolish all protein synthesis and die at temperatures exceeding 40 °C. In other words, they behave exactly as *Drosophila* cells and most of the investigated insect species (Dehghani et al. 2011; Feder et al. 1996; Feder and Hofmann 1999; Lindquist 1986; Lozovskaya and Evgen'ev 1984). Subsequently, we performed a comparative analysis of the larval and adult forms of the two above-mentioned moth species and corroborated our results obtained using cell lines. In all such experiments *B. mori* specimens or isolated organs actively synthesized Hsps at higher temperatures and exhibited much higher thermotolerance in comparison with

L. dispar. After this discovery we decided to check whether high thermotolerance coupled with the ability to synthesize Hsps at extreme temperatures represents a common feature of organisms from warm climate zones and deserts.

In the frame of these large-scale studies we compared heat-shock protein expression in several lizard species from Middle Asia sand deserts (Turkmenistan) and common lizard (*Zootoca vivipara* by modern nomenclature or *Lacerta vivipara* in old papers), a species inhabiting temperate areas up to the latitude of the Polar Circle and higher. In the deserts of Turkmenistan the day temperature in summer may exceed 45 °C, while the sand surface is heated up to 70 °C. However, even most resistant lizards (e.g. toad headed sand lizards *Phrynocephalus interscapularis*) cannot survive such extreme temperatures and try to escape over-heating by digging into the sand or by other behavioral adaptations.

We assumed that xeric species inhabiting for a long time extremely hot and dry desert environments should have evolved adaptive behavioral and physiological mechanisms to cope with such adverse conditions and possibly peculiarities of Hsps synthesis represent an essential component of these adaptations. Therefore, we investigated in detail heat shock response (HSR) in nine lizard species from thermally contrasting climate zones including deserts, and these studies in general corroborated our earlier conclusions made when studying Lepidoptera species (Evgen'ev et al. 1987; Ulmasov et al. 1992). Thus, we demonstrated that desert lizard species are able to synthesize Hsps at much higher temperatures in comparison with the species from cold or moderate areas. In our investigation we used an extremely thermotolerant xeric species Chinese toad-headed sand lizard (*P. interscapularis*) active at the day time, a desert species Caspian gecko (*Caspian gecko*) which is active at night, and temperate climate common lizard (*L. vivipara*). The comparison of ^{35}S-methionine incorporation into proteins of the compared species indicated that in highly eurythermal Chinese toad-headed sand lizard (*P. interscapularis*) proteins are still synthesized at 50 °C and higher while in the common lizard *L. vivipara* inhabiting cold and temperate latitudes all protein synthesis is abolished at temperatures higher than 42 °C and the animals rapidly die. Furthermore, there was a clear-cut positive correlation between the constitutive level of Hsp70 in the cells and thermotolerance of all studied species. The analysis of constitutive level of Hsp70 detected in the cells of the above species indicated that toad-headed lizards (*P. interscapularis*) exhibits a maximum concentration of Hsp70, Caspian gecko also exhibits a lower but significant level of Hsp70, while in common lizard the presence of Hsp70 under normal physiological conditions is hardly detectable (Fig. 4.2).

The presence of two isoforms of Hsp70 family that are synthesized at different temperature regimes in *P. interscapularis* represents another important observation made in this study (Fig. 4.3). We designated these proteins Hsp68(+) and Hsp68(−) and demonstrated that while the first protein is synthesized predominantly in response to moderate HS, the latter form Hsp68(−) is constitutively synthesized under normal temperature conditions and after acute HS when the synthesis of all other cellular proteins is completely inhibited (Ulmasov et al. 1992). Subsequently, differential synthesis of various Hsp70 family members depending on the severity of HS was corroborated in many other studies (Feder and Hofmann 1999; Mizrahi et al. 2012; Tomanek and Somero 1999; Tomanek 2005).

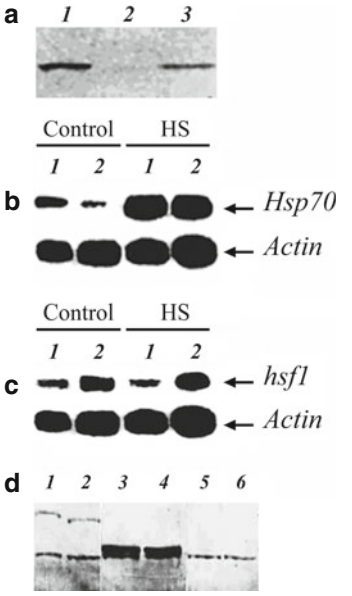

Fig. 4.2 Heat shock response in lizards from contrasting thermal habitats. (**a**) Western blot analysis with antibodies recognizing inducible member of Hsp70 family in different lizard species under normal non-stress conditions. (*1*) *P. intercapsularis*; (*2*) *L. vivipara*; (*3*) *G. caspius*. It is evident that both xeric thermophylic species (*1* and *3*) contain significant amounts of Hsp70 while this protein is not detectable in common lizard from temperate climate (*2*). (**b**, **c**) Northern blot analysis of mRNA present in the cells of *P. interscapularis* (*1*) and *L. vivipara* (*2*) at normal temperature (25 °C) and after HS for 1 h at 42 °C. The membrane was probed with lizard (*L. vivipara*) *hsf1* gene (**b**) and *Xenopus laevis Hsp70* gene (**c**), and reprobed with actin gene to ensure equal loading. (**d**) Western blot analysis of HSF1 levels. *G. caspius* (lanes *1* and *2*); *L. vivipara* (lanes *3* and *4*); and *P. interscapularis* (lanes *5* and *6*) at normal temperature (lanes *1*, *3* and *5*) and after 1 h at 42 °C (lanes *2*, *4* and *6*) (From Zatsepina et al. (2000) with permission)

It is known that all desert animals regularly encounter drastic fluctuations in temperature and, hence, the body temperature of these poikilothermal animals should also change significantly depending on these fluctuations. Along these lines, Cherlin and Muzichenko (1983) demonstrated that daily fluctuations of the body temperature of Middle Asia desert lizard range from 20 to 44 °C.

It was interesting to find out whether the observed fluctuations in the environmental and body temperature, somehow, influence the synthesis of Hsps and specifically Hsp70 in the body of the animal subjected to such drastic changes of the temperature in the field conditions. In the course of these field studies the lizards (*P. interscapularis*) were collected in the desert throughout the day starting from 6 a. m. and *in vivo* injected with ^{35}S-methionine. The labeled proteins were isolated from the livers of injected animals at different time intervals, run on two-dimensional (2D) gels, and labeled Hsp70 members, hsp68(+) and Hsp68(−), were excised and quantified. These experiments demonstrated clear-cut positive correlation between the body (and soil) temperature observed during the day and the levels of Hsp70 synthesis in the cells of *P. interscapularis* (Fig. 4.4). Notably, comparatively

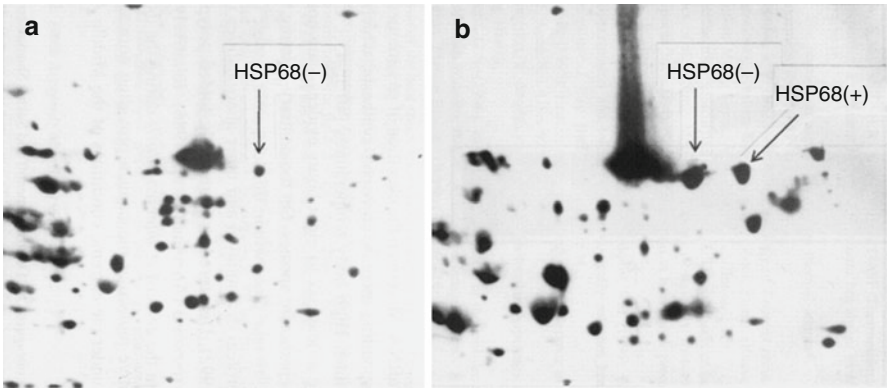

Fig. 4.3 Two dimensional electrophoresis of total proteins labeled *in vivo* by ^{35}S-methionine isolated from toad-headed lizard. (**a**) Normal physiological temperature; (**b**) heat shock. The induced two Hsp70 (Hsp68 in lizards) family isoforms are indicated by arrows (From Ulmasov et al. (1999) with permission)

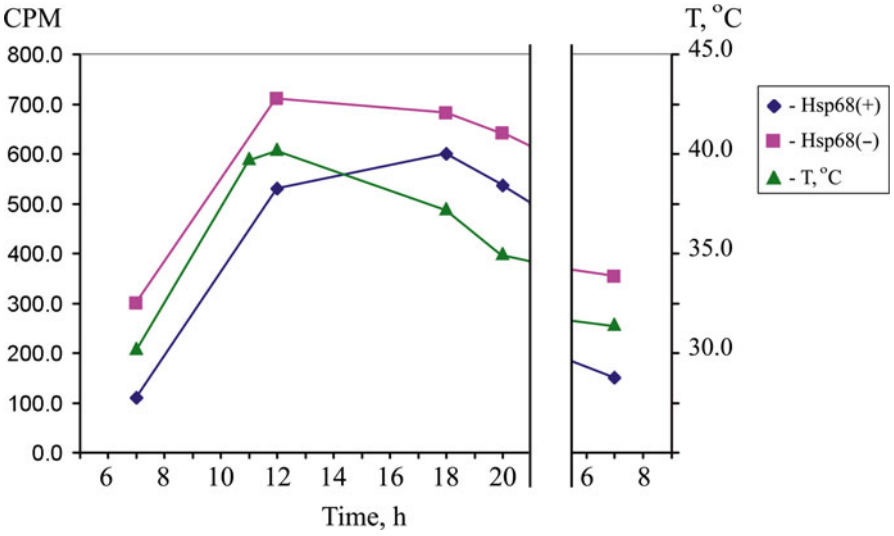

Fig. 4.4 The daily dynamics of body temperature fluctuations and levels of Hsp68(+) and Hsp68(–) in Chinese toad-headed lizard (*P. interscapularis*). Clear-cut correlation is evident (From Ulmasov et al. (1999) with permission)

high level of Hsps synthesis continues for some time after the body temperature drops to 30–35 °C (Ulmasov et al. 1999).

Therefore, it was suggested that high constitutive level of Hsp70 observed in desert lizard species probably represents the major adaptation of xeric organisms in general to environmental challenges such as drastic fluctuation in temperature regime and provides extremely high thermotolerance characteristic for various xeric animals.

High content of Hsp70 and other chaperones in the cells under normal physiological conditions apparently represents "preparative defense strategy" which enables xeric organisms to normally function in the broad range of environmental temperatures without *de novo* induction of stress proteins and switching off the synthesis of normal cellular house-keeping genes.

Thus, when comparing ^{35}S-methionine incorporation into proteins of thermotolerant desert species Caspian gecko and common lizard (*L. vivipara*) we demonstrated that the intensity of proteins synthesis after heat shock (39 and 42 °C) is significantly higher in the latter species. Moreover, the temperature of Hsp70 synthesis induction (T-ON) in *L. vivipara* is significantly lower in comparison with gecko. In other words, as a rule in Northern species the threshold of Hsps induction is lower while the intensity of Hsps synthesis is significantly higher after moderate HS in comparison with desert thermoresistant species with high constitutive levels of Hsps.

The observed high thermoresistance of desert lizard species may be in principle explained by either high stability of Hsps present in their cells after they encounter stressful conditions or by constitutive presence of high concentration of correspondent mRNA. Northern blot analysis demonstrated that the observed differences in the constitutive levels of Hsp70 between *P. interscapularis* and other xeric lizard species and common lizard (*L. vivipara*) are most likely regulated at the level of transcription, and thermoresistant species are usually characterized by significantly higher levels of *Hsp70* mRNAs under non-stress conditions (Ulmasov et al. 1992) (Fig. 4.2).

Later, similar differences in HSR were revealed when the *Cataglyphis* ant, which is one of the most thermotolerant land insect known, was compared with a few other insect species. The two species of *Cataglyphis* used in the study forages at body temperature above 50 °C and their critical thermal maxima are equal to 54–55 °C (Gehring and Wehner 1995). The synthesis and accumulation of heat shock proteins were analyzed in these highly thermotolerant ants and compared with another ant species *Formica polyctena*, an ant living in more temperate climates and to two *Drosophila* species. Similar to *B. mori* and desert lizards (*P. interscapularis*) protein synthesis in *Cataglyphis* ant species continues at temperatures higher than 45 °C as compared to 39 °C determined for *Formica* ants and studied *Drosophila* species. In contrast to *Drosophila* species, desert ant species (*Cataglyphis*) were characterized by significant constitutive levels of Hsps in their cells prior to HS. These findings were interpreted by the authors as preadaptation to extreme temperatures encountered by this species (Gehring and Wehner 1995).

The observed pronounced differences in the constitutive levels of Hsp70 between species from habitats with contrasting thermal regimes may be also explained by different quantity or/and structure of HSF1 in the compared organisms. In order to explore this possibility we investigated by Northern and Western blot analysis the concentration of HSF1 and correspondent mRNA in the cells of *P. interscapularis* and *L. vivipara* (Zatsepina et al. 2000). The experiments clearly demonstrated that the level of HSF1 and correspondent mRNA is significantly higher in the cells of *L. vivipara* in comparison with *P. interscapularis*. Therefore, there is a clear-cut

negative correlation between the concentration of Hsp70 and HSF1 in the cells of the species studied. Southern heat-adapted species are characterized by high level of Hsp70 and low level of HSF1 in the cells under normal conditions while Northern species (*L. vivipara*) exhibits the reverse relationship between these two values (Fig. 4.2). It is plausible to speculate that high constitutive concentration of Hsps in the cells of xeric species allows them to cope with temperature fluctuations without additional induction of Hsps. On the other hand, Northern species rarely exposed to HS normally do not contain high concentration of Hsps in the cells but are able to rapidly respond to temperature increase by intensive synthesis of Hsps due to high constitutive content of HSF1 in the cells. In fact the observed negative correlation between Hsps and HSF1 in the compared lizard species perfectly corresponds to the so called "cellular thermometer" model (see the Chap. 3).

Furthermore, the intensity of *Hsp* genes transcription strongly depends on the efficiency of transcription factors (e.g. HSF1) binding with promoters of stress genes which may include competition between negative and positive regulators of transcription. We performed *in vitro* binding experiments to monitor HSF1 binding efficiency in the studied lizard species and demonstrated that thermotolerant species characteristically differ from the more high-latitude species *L. vivipara* by the presence of certain amount of activated HSF1 bound to *Hsp* genes promoters under normal physiological conditions.

On the other hand, activation (trimerization) of HSF1 and its detectable binding with HSEs in common lizards occurs at 34 °C, and only at 39 °C in *P. interscapularis.* Interestingly, dissociation of HSF1 from DNA in the course of recovery after HS is much longer in *L. vivipara* in comparison with xeric *P. interscapularis* (6 and 1 h, respectively). In addition, competition experiments demonstrated significantly higher binding affinity of HSF1 with *Hsp* promoters of xeric lizard species in comparison with that of *L. vivipara* (Zatsepina et al. 2000).

Therefore, high constitutive level of Hsp70 and other Hsps in the cells of desert lizards is likely controlled at the transcription level and may be due to the presence of certain amount of active HSF1 at their *Hsp* genes promoters providing their "leakage" under normal temperature conditions (Zatsepina et al. 2000). Likewise, subsequently, it was shown in goby (*Gillichthys mirabilis*) that the temperature of HSF1 activation positively correlated with acclimation temperature, which suggests that apparent plasticity in HSF1 activation may play an important role in the heat shock response in various eurythermal organisms and, hence, participate in general environmental control of *Hsps* gene expression (Buckley and Hofmann 2002).

Generally speaking, in the case of phylogenetically distant forms discussed above the observed variability in Hsps levels may resemble their different evolutionary history, while the differences in Hsps constitutive synthesis and induction patterns after HS detected in geographical populations of the same species or phylogenetically close forms from contrasting ecological conditions may rather resemble their adaptation to specific thermal conditions including extreme ones.

In fact, any comparison of phylogenetically distant forms dwelling in contrasting environments and looking for correlations is apt to severe critics because unrelated species should be by definition quite different in many respects due to the divergence

process (Garland and Adolph 1994). On the other hand, the investigation of multiple species from contrasting habitats (e.g. multiple species of lizards from different taxa) may provide some useful information which should be, however, accepted with caution. Therefore, the most direct way to detect correlation between the manifestation of certain trait (e.g. Hsp70 levels) and adaptation to adverse conditions is to study closely related species or, ideally, several geographical populations of the same species from contrasting thermal environments.

4.2.2 The Comparative Analysis of Closely Related Forms

The comparison of close species belonging to the same group or genus but differing by distribution, latitude and consequently average temperature of their habitats represents another and probably more reliable and widely used approach to look for correlation between thermal adaptation and pattern of Hsps synthesis.

Along these lines, during the last two decades thermotolerance was studied in a wide spectrum of *Drosophila* species and strains originating from different climatic zones and considerably differing from one another in the ambient temperature of their habitats. The species that lived in hot climate as expected usually have a higher thermotolerance but exhibited various patterns of HSR (Dehghani et al. 2011; Garbuz et al. 2008; Feder and Hofmann 1999; Feder and Krebs 1997; Lindquist 1986; Somero 2005; Ulmasov et al. 1992).

It was shown, that in *Drosophila Hsp70* genes are practically not active in most tissues under normal temperature conditions but can be rapidly induced by temperature elevation or other forms of stress producing huge amounts of chaperones which can reach about 1 % from total amount of cellular proteins (Velazquez et al. 1983). Notably, under non-stress conditions certain copies of *Hsp70* family are characterized by low tissue-specific expression in spermatogonia of second instar larvae and prepupa (Lakhotia and Prasanth 2002). In *Drosophila*, constitutive members of *Hsp70* family (e.g. Hsc70) apparently serve as major chaperones at normal temperature while the induction of *Hsp70* genes likely serves as last defense line under critic and subcritic conditions.

Therefore, in this respect *Drosophila* species differ drastically from most other insect species studied so far where usually significant constitutive levels of Hsps are observed under normal temperature conditions (Dehghani et al. 2011; Garbuz et al. 2008; Gehring and Wehner 1995).

In our studies we explored several closely related *Drosophila* species belonging to the *virilis* group and several other species of the same genus as outgroups.

D. virilis is the most karyotipically primitive species of the *virilis* phylad and is probably ancestral to it if not to the entire *virilis* group (Patterson and Stone 1952). *D. virilis* is distributed throughout the Northern Hemisphere primarily South to 40°N latitude. *D. lummei,* considered the closest relative of *D. virilis*, occurs from just above 40°N to just above 65°N latitude and from Sweden West to the Pacific coast of Asia (Fig. 4.5). These species are separated by at least five

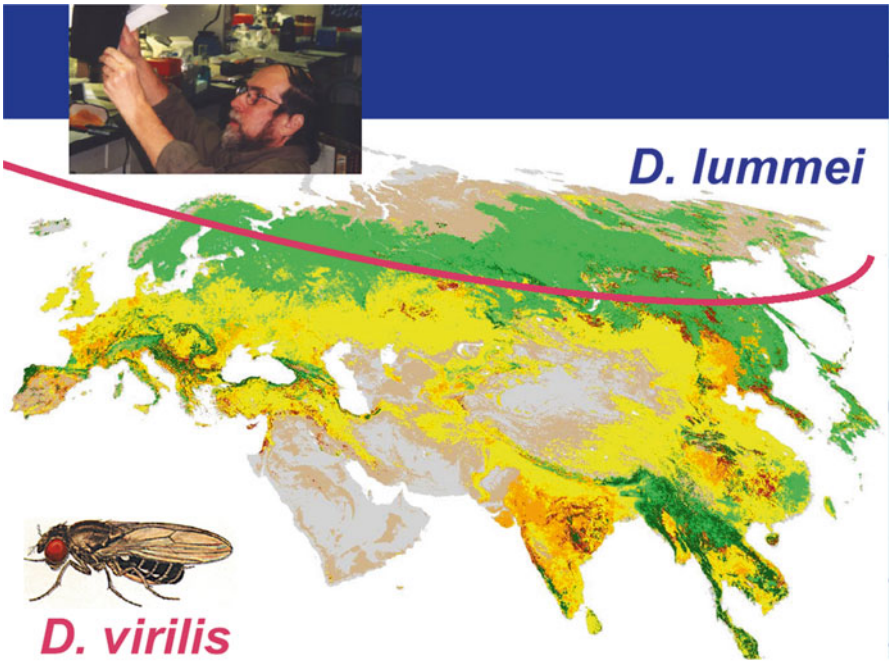

Fig. 4.5 Approximate distribution of *D. lummei* and *D. virilis* throughout Euroasia (*red line*) and one of the authors (M.E.) working with the flies

to six millions years of divergence and can be even crossed in the laboratory to provide fertile progeny (Morales-Hojas et al. 2006; Patterson and Stone 1952; Spicer and Bell 2002). Many aspects of the thermal phenotypes of the species under study exhibit countergradient variation similar to that in other taxa (Feder and Hofmann 1999; Ulmasov et al. 1992; Zatsepina et al. 2001); i.e. the magnitude and threshold for traits correspond to the thermal environments in which the species occur. Thus, the lower-latitude species *D. virilis* exceeds the higher-latitude species *D. lummei* in basal thermotolerance (see also Garbuz et al. 2003), and temperature threshold for HSF activation. Amazingly, we failed to observe induced thermotolerance in *D. lummei* and pretreatment with mild temperatures even decreased the rate of survival after acute HS (Fig. 4.6). As also shown previously (Garbuz et al. 2003), *D. virilis* and *D. lummei* express similar amounts of heat-shock mRNA and protein at low to moderate heat-shock temperatures. On the other hand intense heat shock (e.g. 40–41 °C) almost abolishes heat-shock mRNA and protein expression in *D. lummei*, whereas considerable expression persists after such heat shock in *D. virilis* (Fig. 4.7). For example, Hsp70 concentration is similar in *D. virilis* and *D. lummei* after 37.5 °C heat shock but is markedly greater in *D. virilis* than in *D. lummei* after more severe heat shocks (Fig. 4.7). Notably, in *D. lummei* the reduction in protein level after acute heat shock is even greater for the small Hsps and Hsp40 than for Hsp70. Northern hybridization data

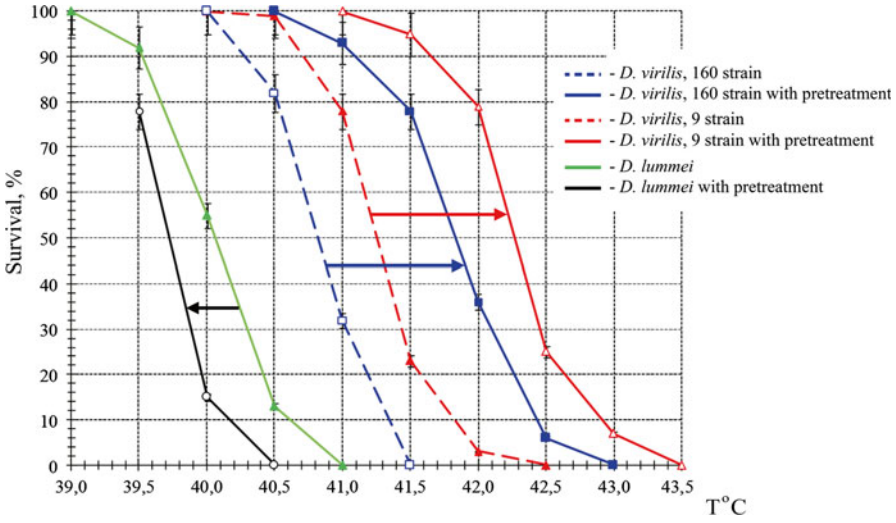

Fig. 4.6 Basal and inducible thermotolerance in *D. virilis* and *D. lummei*

with total RNA demonstrated that in *D. lummei Hsp70* genes transcription is completely inhibited at 40 °C and restored only after 1 h of recovery at normal temperature. In the latter species the Hsp70 accumulation after acute HS is detectable only after 3 h recovery period. On the other hand, HSF in *D. lummei* efficiently binds with promoter DNA as revealed *in vitro* assay even at 41 °C which implies that acute HS (40 °C and higher) does not block HSF activation in this species but apparently interferes with other components of transcription machinery. In contrast, in *D. virilis* significant synthesis of *Hsp70* mRNA efficiently occurs at 40° and even 41 °C. Most species of the *virilis* group studied exhibited positive correlation between the Hsp70 accumulation after heat exposure and thermotolerance. Thus, *D. montana* species from comparatively cold climatic zones was significantly less thermotolerant and was able to synthesized Hsps only after moderate HS in contrast to xeric *D. novamexicana* species belonging to the same species group (Garbuz et al. 2003).

Different levels of Hsp70 after HS in species of the *virilis* group are correlated with the structure of *Hsp70* cluster and *Hsp70* copy number comprising the cluster (see Chap. 5 for the details). Thus, 2D electrophoresis revealed more Hsp70 isoforms in *D. virilis* geographical strains with higher *Hsp70* copy number (Garbuz et al. 2003; Evgen'ev et al. 2004).

However, while the relationship between species thermoresistance and Hsp70 synthesis was evident when species and strains of the *virilis* species group were compared, this correlation was lacking when a species from other *Drosophila* groups were included into the analysis (Fig. 4.7). Thus, adults of *D. melanogaster* (Oregon R strain) found the most thermosensitive strain in our studies synthesize maximal quantity of Hsp70 at 36–37 °C exceeding in this parameters all other *Drosophila* strains and species taken for comparison (Fig. 4.7a) (Krebs 1999).

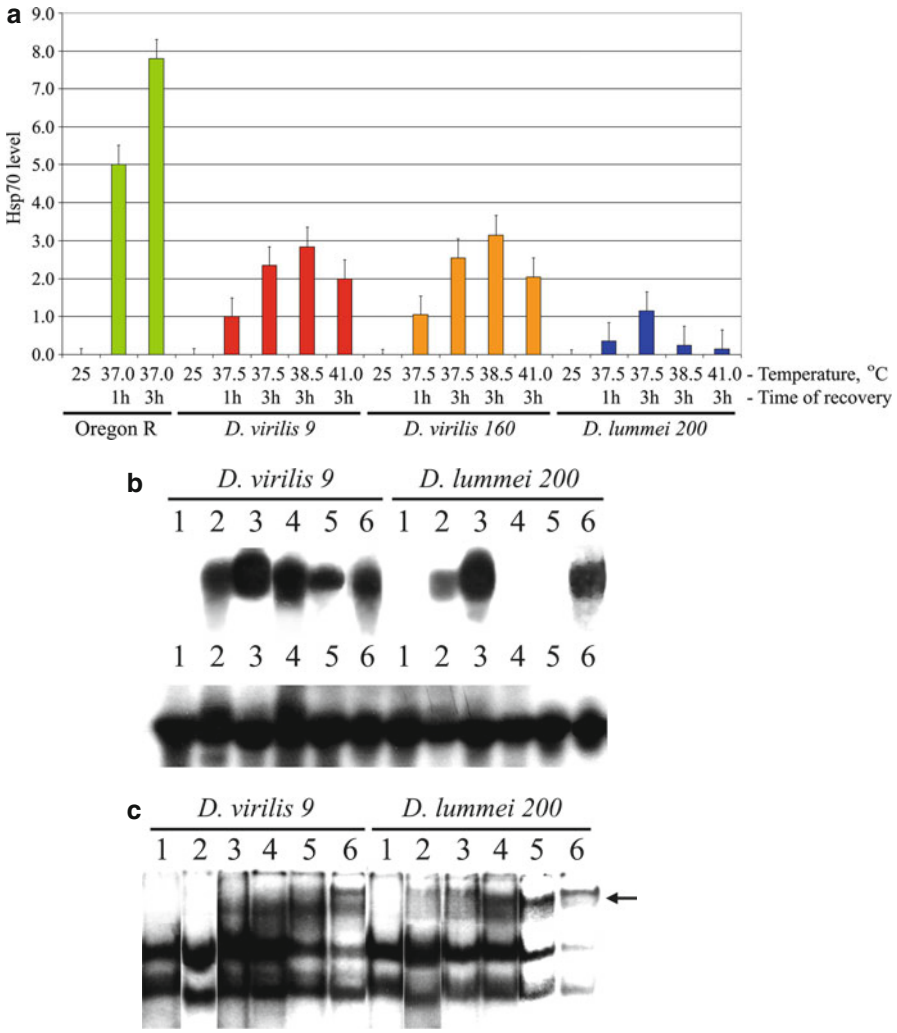

Fig. 4.7 Heat shock response at different levels in *Drosophila* species. (**a**) Hsp70 levels in *Drosophila* strains and species under normal conditions, after HS treatments and after different periods of recovery (adult flies were used). (**b**) Northern blot hybridization of mRNA isolated from *D. virilis* and *D. lummei* subjected to different HS treatments (all 30 min) with labeled *Hsp70* probe. (**c**) HSF-HSE complex in *D. virilis* and *D. lummei* after different heat treatments (marked by *arrow*). 1–25, 2–31.5, 3–37.5, 4–40.0, 5–41.0, 6–40.0 °C and 1 h of recovery at 25 °C (From Garbuz et al. (2003) with permission)

Likewise, *D. mojavensis*, a highly eurythermal species from *repleta* group, with critic temperature equal to 43.5 °C synthesize significantly less Hsp70 after mild HS in comparison with *D. melanogaster* flies (Krebs 1999). Probably in desert flies that often encounter temperature fluctuations induction of intensive Hsps synthesis after mild HS will be deleterious for their growth and development (Feder and Krebs 1997; Zatsepina et al. 2001).

It is of note that all xeric *Drosophila* species used in our studies (e.g. *D. virilis*, *D. novamexicana* and *D. mojavensis*) were characterized by comparatively high temperature for maximal Hsp70 induction (38.5 °C) which was 1 °C higher than that of *D. lummei* and *D. montana* and 1.5 °C higher than in *D. melanogaster* (Garbuz et al. 2002, 2003). Remarkably, at higher temperatures after severe HS all studied thermotolerant species accumulate significantly more Hsp70 in comparison with *D. melanogaster* and other relatively thermosensitive species (Garbuz et al. 2002).

It is noteworthy that according to diverse genetic and biochemical evidence, the inducible Hsp70 proteins play key roles in basic and inducible tolerance specifically to extreme temperatures close to critic ones for the given species. Both the magnitude and threshold of inducible Hsp70 expression are correlated with the natural thermal regime in many species in many taxa (Feder and Hofmann 1999; Norris and Hightower 2000). Our results and data accumulated by other groups corroborated this conclusion showing that in *Drosophila* species inducible members of Hsp70 family play important protective role particularly in the organisms from extreme environments or after acute HS (Garbuz et al. 2003). The investigation of *D. melanogaster* strain with deleted *Hsp70* copies which is not able to survive acute HS (Bettencourt et al. 2008; Gong and Golic 2006) provided independent proof for this statement (see Chap. 8 for details).

Since it is known that Hsp70 is not the only Hsps responsible for thermotolerance the patterns of induction of other Hsps families after heat exposure in a wide spectrum of *Drosophila* species were also compared. Thus, significantly higher thermotolerance observed in *D. virilis* and other xeric species of the group coupled with lower levels of Hsp70 accumulation in comparison with *D. melanogaster* strains after moderate HS suggests possible involvement of other chaperones providing *D. virilis* thermotolerant phenotype. It was demonstrated that *D. virilis* and xeric *Drosophila mojavensis* from *repleta* group are characterized by higher levels of Hsp40 and small Hsps synthesis after moderate and acute HS in comparison to *D. lummei*, and certain strains of *D. melanogaster* (Garbuz et al. 2003; Shilova et al. 2006). It is known that sHsps prevent denatured proteins from aggregation (Morrow et al. 2006), while Hsp40 belonging to J-proteins family serves as a cofactor for Hsp70 (See Chap. 2). Therefore, in principle, significant accumulation of Hsp40 may enhance the Hsp70 efficiency without the increase in Hsp70 concentration. The results obtained suggest that the Hsp40 and small heat shock proteins may play a significant role in thermotolerance and adaptation to temperature fluctuations in thermophilic *Drosophila* species.

It is of note that there are a few exceptions from the general pattern implying the important role of Hsps in thermotolerance in desert animals. Thus, when comparing Hsp expression in two closely related snail species of the genus *Sphincterochila*, a desert species *S. zonata* and a Mediterranean species *S. cariosa*, it was demonstrated that adaptation to different habitats results in the development of distinct strategies of Hsp expression in response to stress, namely the reduced expression of Hsps in desert-inhabiting species (*S. zonata*). The authors speculated that the observed different strategies reflect the difference in heat and humidity encountered in the natural habitats, and that the desert species *S. zonata* relies on

mechanisms and adaptations other than Hsps induction and constitutive Hsp70 synthesis thus avoiding the fitness consequences of continuous Hsp upregulation (Mizrahi et al. 2012).

Differential induction of individual Hsps in snail organs represent another HSR peculiarity described by this group, thus, in *S. cariosa,* heat stress caused rapid induction of Hsp70 and Hsp90 in the foot and kidney tissues, whereas the desert-inhabiting xeric species *S. zonata* displayed delayed induction of Hsp70 proteins in the foot and upregulation of Hsp90 alone in the kidney. Interestingly, similarly to highly eurythermal lizard *P. interscapularis* the investigated snail species contain two isoforms of Hsp70 that are synthesized with different kinetics and in response to different forms of HS (Mizrahi et al. 2012).

Another pertinent investigation has been recently performed using a well-studied model object, migratory locust (*Locusta migratoria*), which is native to the semiarid regions of the World and excellent for study adaptation to fluctuating temperature conditions. In this organism previous exposure to sublethal high temperatures effectively protects neuronal function against future hyperthermia, but unlike many other organisms described above, the deep physiological adaptations are not accompanied by a robust increase of *Hsp70* transcript or protein in the nervous system. It was supposed that the observed lack of Hsp70 induction after HS may be explained by very high level of the constitutive level of Hsps in the tissues and the thermoprotective effect of pre-HS on the nervous system might be mediated not by Hsp70 induction but rather by post-translational modifications or protein trafficking (Dehghani et al. 2011).

In contrast to flies, locusts and other highly mobile organisms that may rapidly escape high temperature zones (Feder and Hofmann 1999), many sessile organisms, as well certain ontogenetic stages such as eggs, larvae and pupae dwelling in periodically heated substrate are subject to daily or seasonal variations in temperature and water availability and, hence, apparently use Hsps synthesis as major component of their survival strategy.

4.2.3 Intraspecific Comparison

Although multiple interspecific comparisons in ecological aspect provided rather reliable correlations between the levels and patterns of Hsps synthesis and species thermotolerance in ecological context (Ulmasov et al. 1992; Feder and Hofmann 1999; Tomanek 2005), in the case of intraspecific studies the situation is not so unequivocal.

In *D. melanogaster*, a number of physiological traits related to thermal extremes including adult cold resistance and heat resistance show clinal patterns (reviewed in Hoffmann et al. 2003). There is also clinal variation in this species for the incidence of reproductive diapause, thorax length and cold and heat resistance (Hoffmann and Weeks 2007; Mitrovski and Hoffmann 2001; Schmidt et al. 2005) that correlates with levels of resistance to thermal extremes (Schmidt and Paaby 2008). In India,

highland populations of *D. melanogaster* have higher levels of desiccation resistance and melanism compared with lowland populations, which matches the hotter and drier conditions experienced in highlands (Parkash et al. 2008a, b).

However, within *D. virilis* and *D. lummei* geographical strains, little if any countergradient in terms of thermotolerance and Hsps synthesis variation is evident (Figs. 4.6 and 4.7). Possibly, the extensive gene flow among populations swamps incipient adaptation to local conditions, a situation that may not be universal in *Drosophila* species (Michalak et al. 2001). Likewise, the investigation of latitudinal clines for stress resistance in *D. simulans* from eastern Australia did not reveal any clinal pattern for desiccation or heat resistance (Arthur et al. 2008).

Furthermore, as in the case of *D. melanogaster* (Krebs et al. 1996), adaptation to laboratory conditions of *D. virilis* wild type strains caught in nature does not seem to significantly contribute to the observed intraspecific HSR patterns. Thus, two strains caught at the same location near Tashkent, Uzbekistan (T53 and T61) exhibit identical patterns of thermotolerance and Hsp70 induction despite the fact that T61 was captured recently and the T53 more than 30 years ago. The only conspicuous departure from this pattern is for *D. virilis* strain 160, a marker strain with at least one known recessive mutation on each autosome. The basal thermotolerance for this strain is considerably lower than for all other *D. virilis* strains studied so far probably due to multiple mutations present (Garbuz et al. 2003).

Likewise, Jensen et al. (2010) were not able to correlate heat-induced Hsp70 synthesis and several measurements of adult heat tolerance in three independent populations of *D. melanogaster,* estimated independently in three laboratories and using slightly different protocols. Although these exceptionally thorough studies revealed substantial within population variation in both Hsp70 and heat tolerance, the authors were not able to detect any significant correlation between these parameters in adults. It was concluded that variation in Hsp70 expression is only likely to explain a small proportion of the observed variation in adult thermoresistance (Jensen et al. 2010).

Probably highly coordinated heat-induced synthesis of Hsp70 encoded by several conserved copies comprising a cluster in *Drosophila* species provides an optimal response to different microclimatic conditions within the species distribution range and the observed intraspecific variations in adult heat tolerance are not directly connected with Hsps system.

However, constant pressure of natural selection is able to modulate HSR within the species.

"Evolution Canyons" intensively studied in recent years represents a perfect example of such natural selection in *D. melanogaster* (Korol et al. 2006; Rashkovetsky et al. 2006). Micropopulations of this species occur on North- and South-facing slopes of the canyon with greatly differing climatic regimes and in the intervening region; the slopes are 400 m apart at the top and 100 m apart at the bottom. Although adult *Drosophila* can traverse several kilometers in a single day, populations on each slope have diverged in body size, heat and desiccation tolerance, thermal preference for oviposition, fluctuating asymmetry in morphological traits, rates of mutation and recombination, and mate preference (Hübner et al. 2013;

Rashkovetsky et al. 2006). The investigators monitored the divergence of the slopes using neutral microsatellite markers and a candidate gene (*Hsp70*) strongly linked to resistance to environmental stress. The frequencies of putatively neutral and non-neutral (*Hsp70Ba*, a heat shock gene) markers in *D. melanogaster* collected from the middle elevation of each slope were monitored. Luckily it was demonstrated that *D. melanogaster* from the Evolution Canyon in Israel are polymorphic for 1.2-kb *P* element insertion that interrupts a regulatory region of one of *Hsp70* genes and, hence, should interfere with transcription and decrease thermotolerance and resistance to other stresses. The analysis performed demonstrated that there are remarkable differences in the frequencies of both microsatellites and *P* element bearing *Hsp70Ba* allele in micropopulations inhabiting the two slopes (Michalak et al. 2001). Characteristically, flies dwelling in the Southern slope and often exposed to heat contain many folds less frequently the *Hsp70Ba* allele with disrupted promoter.

Notably, when the authors directly investigated the role of small Hsps and Hsp40 in thermoadaptation of flies collected from both slopes they were able to demonstrate more pronounced expression of Hsp40 in the flies collected from the Southern slope after mild HS implying a significant contribution of Hsp40 to thermoadaptation under local microclimatic conditions (Carmel et al. 2011). Therefore, it was possible to conclude that the observed divergence in thermoresistance and local adaptation is consistent with certain degree of genetic isolation despite the contiguity of the *D. melanogaster* populations in the canyon (Hübner et al. 2013; Korol et al. 2006; Rashkovetsky et al. 2006).

D. melanogaster flies (T strain) collected in sub-equatorial semiarid tropical zone of Africa in the 1970s (J.R. David, personal communication 1978) represents another example of pronounced differences in heat shock response observed in geographical populations of the same species. T strain was remarkably tolerant of sustained laboratory culture above 30 °C previously considered to be the limit for continuous culture of this species (Parsons 1973). Furthermore, the basal thermotolerance of T strain adults significantly exceeded that of standard wild-type (Oregon R) strain used as a control. In this exceptional ability T strain outperformed other *D. melanogaster* strains and compares favorably with many cactophilic desert *Drosophila* species (Krebs and Loeschcke 1999; Stratman and Markow 1998). However, surprisingly, induced thermotolerance of high temperatures, which in *D. melanogaster* is due in part to the inducible molecular chaperone Hsp70, is only modest in T strain. Expression of Hsp70 protein and *Hsp70* mRNA is likewise reduced and has slower kinetics in this strain (T) than in a standard wild-type Oregon R strain. T strain has higher critic temperature (i.e. the temperature of heat shock when less than 1 % of treated flies survive) in comparison with Oregon R flies (41 °C vs. 40 °C). Notably, maximal level of Hsp70 synthesis was observed in both compared strains at 37 °C but in the case of T strain the level of Hsp70 accumulation was two to threefold lower (Zatsepina et al. 2001). On the other hand, at higher temperatures close to critic value (e.g. 39 °C) flies of T strain still synthesized Hsp70 while in Oregon R flies the synthesis of this chaperone was completely inhibited although *Hsp70* mRNA was detected in both strains. This observation

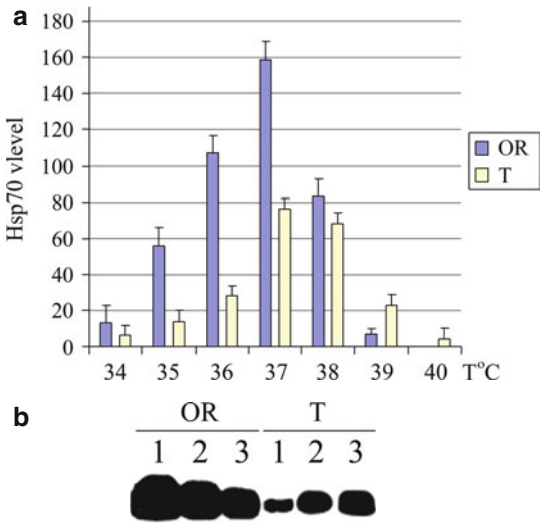

Fig. 4.8 (**a**) Hsp70 levels in Oregon R (OR) and T strains after 30 min HS at different temperatures. (**b**) *Hsp70* mRNA level in both strains after 30 min HS at different temperatures. 1–35, 2–37, 3–39 °C (From Zatsepina et al. (2001) with permission)

suggests higher thermostability of translational machinery in strain T (Fig. 4.8). The compared strains also differ in a similar way in the patterns of constitutive and heat-inducible levels of other molecular chaperones. Thus, T strain is characterized by higher levels of Hsp40 and small heat shock proteins after HS in comparison with *D. melanogaster* Oregon R strain (Zatsepina et al. 2001).

It was shown that the lower Hsps expression in the T strain after moderate HS apparently has no basis in the compromised activation of the heat-shock transcription factor (HSF), which is similar in T and Oregon R flies. Subsequently, it was demonstrated that the observed reduced expression of Hsp70 likely stems from insertion of two transposable elements, *H.M.S. Beagle* in the intergenic region of the 87A7 *Hsp70* gene cluster and *Jockey* in the *Hsp70Ba* gene promoter (Zatsepina et al. 2001) (see Chap. 6 on the role of transposable elements in HSR).

The data accumulated when studying *D. melanogaster* micropopulations from the "Evolution Canyon" and T strain versus Oregon R strain imply strong variation within a single species. Evidently, adaptation via natural selection is sufficiently strong to overcome even the immense phylogenetic inertia of the heat shock response.

4.2.4 Adaptive Role of Hsps in Homothermal Thermophilic Organisms

Most of the studies on the possible role of Hsps in thermoresistance were performed using insects and other poikilothermal organisms, because homothermal animals preserve constant body temperature under various temperature regimes and may only slightly modulate it predominantly in skin or other external tissues subjected to

temperature challenge. However, in some special cases significant changes in external tissues subjected to environmental challenges were observed, thus, in camels often experiencing high temperature in arid areas the skin may reach 40 °C and even more. The camel (*Camelus dromedarius*) represents a homothermal organism perfectly adapted to extreme conditions of arid zones and is capable of tolerating extreme heat accompanied by a significant elevation (up to 41 °C) of the whole body temperature (Schmidt-Nilsen 1972). Western blot analysis demonstrated that camel lymphocytes constitutively express Hsp73 protein which can be significantly induced by heat shock (Ulmasov et al. 1993). Surprisingly, another member of Hsp70 family (Hsp72) is not induced in lymphocytes but can be induced in skin fibroblasts. Probably skin fibroblasts of this animal, which are often exposed to an extremely high temperature challenge, required the synthesis of additional protective protein (Hsp72). Notably, based on labeled methionine incorporation analysis, it was concluded that the general synthesis of proteins in camel cells is more resistant to heat than that seen in human cells (Ulmasov et al. 1993). Recently, inducible and constitutive camel *Hsp70* genes have been sequenced and characteristic differences in the structure of human and camel *Hsp70* regulatory regions were detected (Garbuz et al. 2011a).

Similarly, when two ethnic human groups inhabiting for many generations contrasting thermal environments (Russians vs Turkmens, inhabiting desert Turkmenistan, Middle Asia) were investigated it was demonstrated that while fibroblasts isolated from Turkmens after severe heat shock exhibited intensive synthesis of all Hsps in parallel with synthesis of many other cellular proteins, only trace synthesis of Hsps was observed in the second group, Russians (Fig. 4.9) (Lyashko et al. 1994).

When survival of fibroblasts after severe heat shock treatment was assessed by colony formation assay, the cells of the first group (Russians) exhibited significantly lower survival rates (Lyashko et al. 1994).

Another way to explore the role of Hsps in whole body adaptation in mammals is to monitor the allele frequency of specific SNPs in different heat shock genes in different populations of the same breed. It was shown by several authors that nucleotide changes occurring naturally in the flanking regions of the *HSPA1A* gene might affect the inducibility, level of expression, and stability of *HSP70* mRNA and, hence, contribute to stress tolerance. Thus, in human peripheral mononuclear cells, polymorphisms in the coding region of *HSPA1A* gene were associated with an increased ability to respond to heat stress (Singh et al. 2006). In pigs, an *HSP70* gene polymorphism located in the promoter and 3'-UTR was associated with mRNA stability and stress response (Schwerin et al. 2002). Recently haplotype analysis of the eight SNPs of the *HSPA1A* gene revealed their involvement in heat resistance in Chinese Holstein cattle (heat sensitive breed of cattle that originated in Europe). The analysis demonstrated the presence of significant differences between individuals carrying different haplotypes for most of the heat-tolerance traits studied (Xiong et al. 2013). Such SNPs may be useful in the future as molecular markers to assist selection for heat tolerance in various domestic animals and maybe used as genetic tools to experimentally improve tolerance to high temperature and other forms of stress.

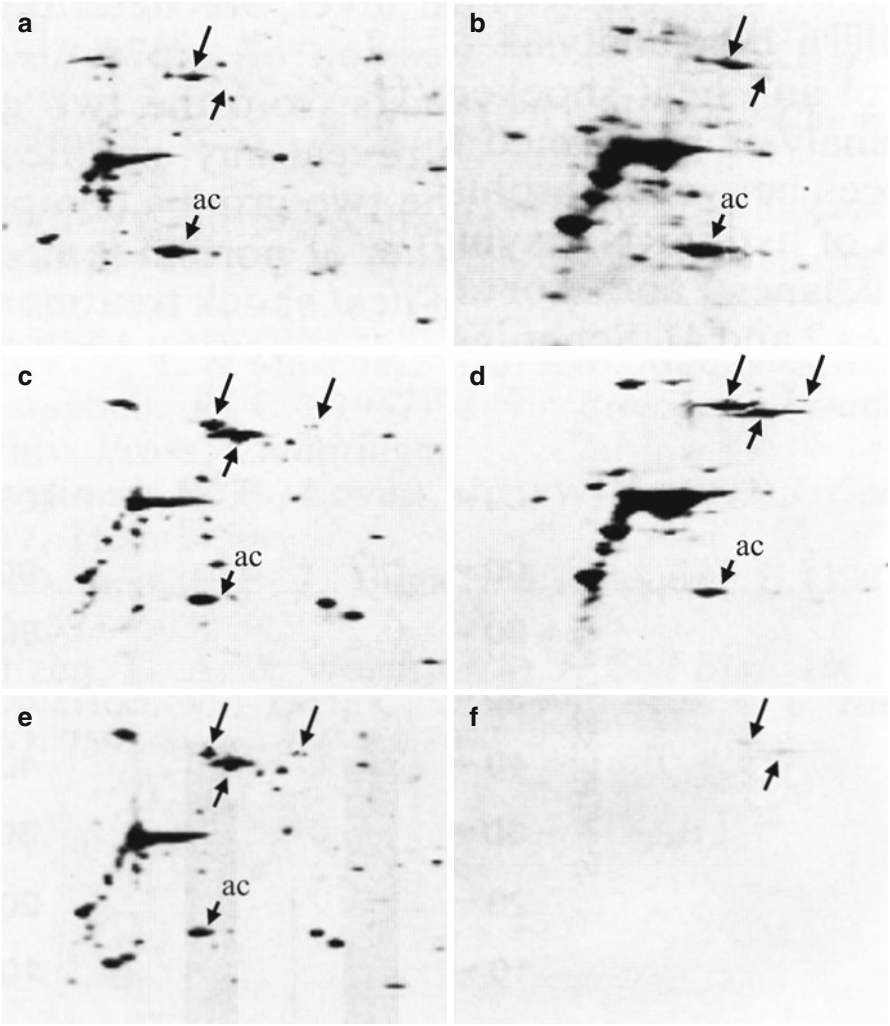

Fig. 4.9 Effect of heat shock duration on protein synthesis in human skin fibroblasts of different origin, observed by two-dimensional electrophoresis of radioactively labeled cellular proteins (**a**, **c**, and **e** cells of Turkmen origin; **b**, **d**, **f** cells of Russian origin). Skin fibroblasts were labeled with ^{14}C-amino acids after incubation at 37 °C (**a**, **b**), after 42.5 °C, 2-h exposure (**c**, **d**), and after 42.5 °C, 6-h exposure (**e**, **f**). Stress proteins of the Hsp70 family are indicated by *arrows*. *Ac* actin (From Lyashko et al. (1994). Copyright (1994) National Academy of Sciences, U.S.A)

4.3 Comparative Data on HSR in Aquatic Organisms

It is evident that aquatic species due to physical properties of water which includes high thermal conductivity should have body temperatures close to that of their surrounding which, however, can change very rapidly in the intertidal zone or in shallow water, which is heated by the sun.

Various intertidal and littoral aquatic (mostly marine) organisms represent another good object for investigation of diverse behavioral and molecular mechanisms, including Hsps synthesis, evolved to cope with fluctuating temperature conditions.

One of the most variable and unpredictable habitats on Earth is the marine rocky intertidal zone located at the boundary between the terrestrial and marine environments. Mussels dominate rocky intertidal habitats throughout the world and, being sessile, endure wide variations in temperature, salinity, oxygen, and food availability due to daily, tidal, seasonal and climatic cycles. Analysis of gene-expression changes in the mussels, fishes and crustaceans species dwelling in different intertidal habitats were summarised in numerous publications (Buckley et al. 2001, 2006; Dong et al. 2008; Podrabsky and Somero 2007; Somero 2005; Stillman and Somero 2000; Tomanek 2008).

In general, when studying various marine organisms from contrasting environments it was demonstrated that similar to terrestrial organisms, threshold of HSF activation and, hence, Hsps induction in the cold-adapted species is significantly lower that in more thermophilic forms. Thus cold-adapted clams *Mytilus trossulus* begin to synthesize Hsp70 at lower temperature than a more Southern species *M. galloprovincialis* (Buckley et al. 2001). In a study of Hsp70 in a genus of outbred subtropical fish, a direct correlation between Hsp70 levels and survival at elevated temperature as a test of phenotypic diversity in acquired thermotolerance was found (Norris and Hightower 2000).

Moreover, the threshold of Hsps induction in snails, mussels, crabs and fishes characteristically varies depending on the climatic cycle and in summer the temperature of Hsps induction is significantly higher than in winter (Buckley and Hofmann 2002; Dong et al. 2008; Tomanek 2008).

Characteristically, as a rule acclimation procedure increases the threshold of Hsps induction in relatively thermophylic or eurythermic species to less extent in comparison with cold-adapted organisms and the latter can usually survive larger temperature increase in comparisons with their physiological range than thermophylic related species. Thus, the investigation of consequences of acclimation process in snails of the genus *Tegula* demonstrated that a more thermoresistant species *T. funebralis* exhibits lower ability to respond to acclimation in terms of modulation of Hsps threshold temperature etc. in comparison with closely-related cold-adapted species *T. brunnei* and *T. montereyi* (Tomanek 2005; Tomanek and Somero 2000). As a rule, species subjected to daily temperature changes are characterized by maximal thermoresistance. Multiple experiments exploring various thermally contrasting intertidal species of mussels and other marine organisms enable one to conclude that acclimation procedure may be highly efficient and can enhance thermoresistance of organisms occupying moderately variable thermal environments (range <10 °C), like the subtidal zone. In such organisms acclimation may activate the HSR at temperatures significantly above those they normally experience in their habitats. In contrast, species from highly variable thermal environments (range <20 °C) or certain eurythermal species have a limited acclimatory plasticity because such species probably “exhausted” genetic components of their thermal adaptation.

Thus, they may live close to their thermal limits and any further increase in temperature is probably going to push them beyond those limits (Tomanek 2005; Tomanek and Somero 1999).

It is evident that the ability to acclimate to variable environmental conditions may affect the biogeographic range of species, their success in colonizing new habitats, and their likelihood of surviving rapid anthropogenic climate change. The responses to temperature acclimation (4 weeks at 7, 13 and 19 °C) in gill tissue of the warm-adapted intertidal blue mussel *Mytilus galloprovincialis*, an invasive species in the Northeastern Pacific, and the cold-adapted *M. trossulus*, the native congener in the region, were compared (Tomanek 2005). Using two-dimensional gel electrophoresis and tandem mass spectrometry, the authors demonstrated that the cold-adapted *M. trossulus* showed increased abundances of molecular chaperones after acclimation at 19 °C, but *M. galloprovincialis* did not, suggesting that the two species differ in their long-term upper thermal limits. In contrast, the warm-adapted *M. galloprovincialis* showed a stronger response to cold acclimation than *M. trossulus*, including changes in abundance in higher number of proteins and differing protein expression profiles between 7 and 13 °C, a pattern absent in *M. trossulus*. It is necessary to keep in mind that consequences of the acclimation process by far are not restricted by the increase of certain Hsps synthesis but lead to profound changes in the general transcription pattern. Thus, acute heat shock exposure (24, 28 and 32 °C) after acclimation to 13 °C for 4 weeks enabled to identify 47 and 61 distinct proteins that changed abundance in *M. galloprovincialis* and *M. trossulus*, respectively. As expected the onset temperatures of greater abundance of some members of the heat shock protein Hsp70 and small Hsps families were lower in the cold-adapted *M. trossulus*.

In the work of Hamer et al. (2004) seasonal changes of the Hsp70 level in mussels were registered. Maximal levels of Hsp72 and Hsp70 were observed in mussels after summer (September), and minimal levels in winter (December). The observed small changes in sea salinity could not cause significant Hsp70 proteins induction.

Overall, these results help to explain why *M. galloprovincialis* has replaced *M. trossulus* in Southern California over the last century, but also suggest that *M. trossulus* may maintain a competitive advantage at colder temperatures. It was also speculated that anthropogenic global warming may reinforce the advantage *M. galloprovincialis* has over *M. trossulus* in the warmer parts of the latter's historical range (Tomanek 2005).

In general, intertidal organisms exhibit patterns of Hsps synthesis similar to those of lizard species inhabiting thermally contrasting biotopes (see above).

Thus, four limpet species of the genus *Lottia*, exhibiting a broad vertical distribution on wave-exposed rocky shores and subjected to dramatic temperature changes during the low tide apparently have distinct strategy of Hsp70 expression depending on the height and orientation in the rocky intertidal zone. In the high-intertidal zone, *L. scabra* typically occupies horizontal surfaces fully exposed to the sun, *L. austrodigitalis* primarily occupies vertical or overhanging surfaces, while *L. pelta* and *L. scutum* are restricted to the low- and mid-intertidal zones. Western blot analysis demonstrated that field-collected as well as acclimated specimens of

the two high-intertidal species had significantly higher constitutive levels of Hsp70 than the low- and mid-intertidal species. Therefore, their cells are preadapted to dramatic temperature changes occurring in these microhabitat conditions. Species dwelling in moderate and low-intertidal zones do not normally experience such temperature gaps and begin to actively synthesize Hsps only after temperature elevation exactly like was observed in *Lacerta* lizard (Dong et al. 2008).

Very similar pattern of Hsps synthesis was demonstrated for three species of snails in the genus *Tegula*. In these species daily temperature fluctuations for relatively thermophylic and eurythermic *T. funebralis* range from 13 to 36 °C, while in cold-adapted species *T. brunnei* and *T. montereyi* inhabiting low-tide zone the temperature varies from 13 to 19 °C and these organisms are rarely subjected to HS in their natural habitats. As expected, the Hsps synthesis in these cold-adapted species is strictly inducible in contrast to *T. funebris* specimens which constitutively synthesize Hsp70 under normal non-stress conditions exhibiting preparative defense strategy (Tomanek 2005; Tomanek and Somero 2000). Interestingly, the development rate of the latter species is significantly lower in comparison with that of congeneric cold-adapted species possibly as a trade off for high constitutive concentration of Hsp70 which was shown to hamper the development in *Drosophila* (Krebs and Feder 1997).

Although most studies of HS and acclimation in aquatic organisms were conducted using marine organisms virtually the same regularities were determined in freshwater species. Thus, the investigation of HSR in two congeneric species of littoral endemic amphipods (*Eulimnogammarus cyaneus* and *E. verrucosus*) from Lake Baikal (Fig. 4.10) that strikingly differ in their vertical distribution and thermal tolerance was performed.

E. verrucosus is a stenobiotic species that preferred temperature 5–6 °C (Timofeyev and Shatilina 2007). *E. cyaneus* is a representative species of the upper littoral zone, the most thermally fluctuating environment in the lake. *E. cyaneus* is significantly more thermoresistant than *E. verrucosus*, and its preferred temperatures are 11–12 °C (Timofeyev and Kirichenko 2004). It was demonstrated that the basal level of Hsp70 synthesis is five times higher in *E. cyaneus* inhabiting the upper littoral compared to the thermosensitive *E. verrucosus* from the more thermally stable lower littoral habitat. The high basal level of Hsp70 likely contributes to the thermotolerant phenotype of *E. cyaneus* and is possibly evolved in response to drastic temperature fluctuations characteristic for the upper littoral zone of Lake Baikal (Votintsev 1961). In addition to the high constitutive expression of Hsp70, *E. cyaneus* also showed a relatively high temperature threshold necessary for Hsp70 induction (27 °C) and relatively low level of Hsp70 accumulation in response to acute HS (only two-fold). Thus, high basal level of Hsp70 obviously allows *E. cyaneus* to overcome temperature fluctuations without additional synthesis, since both short-term acute HS (25 °C) and the mild stress (16 °C) with following recovery does not induce a significant increase in the level of Hsp70 in this species (Bedulina et al. 2013).

In contrast, the more thermosensitive *E. verrucosus* is characterized by several times lower basal level of Hsp70, and much more pronounced induction of Hsp70 concentration in response to the acute HS, HS with recovery, mild HS. Thus, significant Hsp70 induction in *E. verrucosus* already takes place at 13 °C, which is only

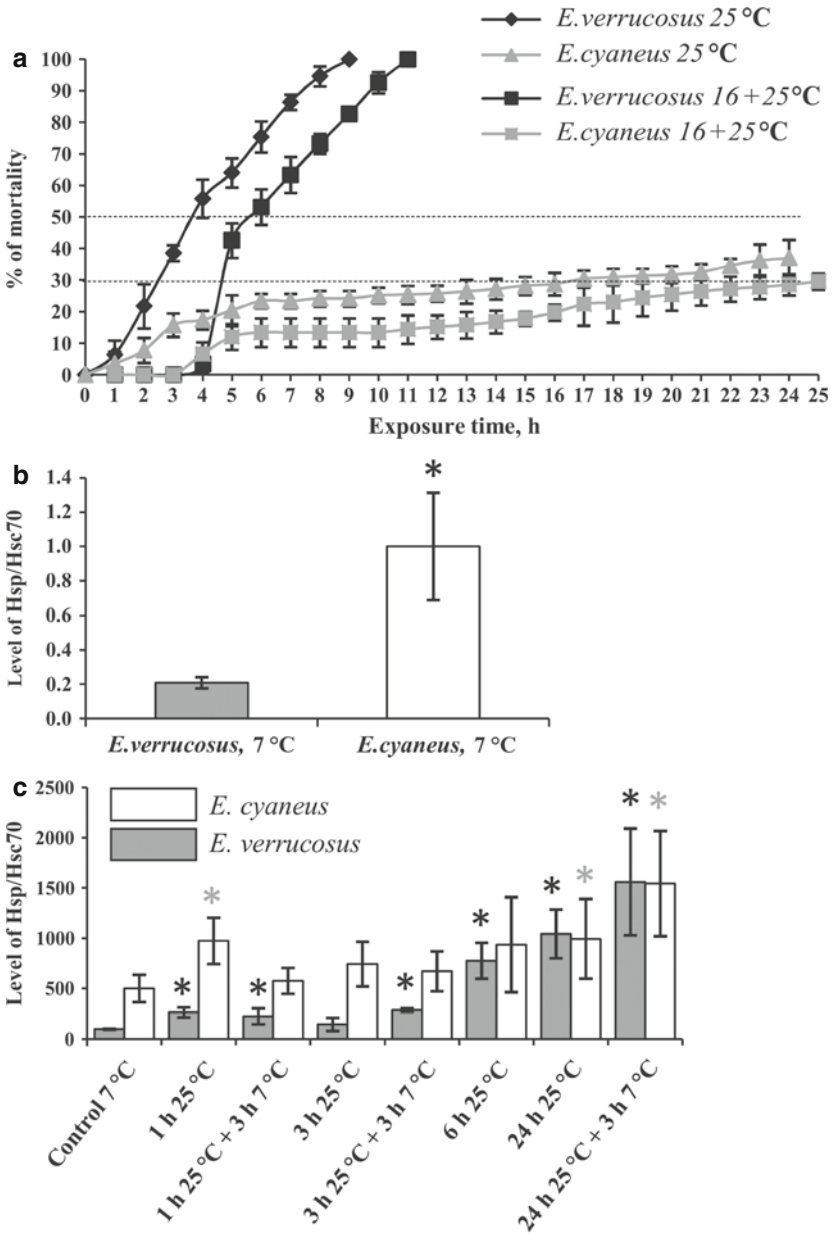

Fig. 4.10 Thermotolerance and Hsp70 synthesis in the two amphipod species. (**a**) Mortality of the amphipods *E. verrucosus* and *E. cyaneus* subjected to 25 °C during 25 h with and without previous exposure to a mild-temperature stress (16 °C for 3 h) followed by 3 h of recovery at 7 °C. The data are expressed as the means and standard deviations. The 50 and 30 % mortality levels are marked by dashed lines. (**b**) The basal levels of Hsp70/Hsc70 normalized to the actin level under control (7 °C) conditions in the studied amphipod species. (**c**) The level of Hsp/Hsc70 in the amphipods under control (7 °C) conditions and under the following heat shock (25 °C) and recovery. The recoveries were 3 h at 7 °C after 3 h of continuous acute HS or after 24 h of continuous HS (From Bedulina et al. (2013))

6 °C above the acclimation temperature, and Hsp70 expression reaches higher levels (eight-fold) within 24 h after the acute HS. Along these lines, studies of the Baltic Sea amphipods also showed that thermally resistant semi-terrestrial intertidal species *Orchestia gammarellus* has a higher basal level of Hsp70 compared to the thermosensitive subtidal *Gammarus oceanicus* as well as less pronounced Hsps induction in response to HS (Bedulina et al. 2010).

4.4 Cold-Adapted Stenothermal Organisms

In the previous section we report about high constitutive concentrations of several Hsps in several aquatic species which often experience dramatic changes in the temperature of their habitats. It is evident that the aquatic environment can be extremely stressful to its sessile inhabitants and to other organisms which cannot rapidly escape the stressful conditions.

In this respect, stenothermal marine high-latitude (in particular, Antarctic) animals that thrive at temperatures down to −1.9 °C are of special interest for understanding molecular mechanisms underlying resistance to stable and extreme conditions. Usually seawater temperatures at 15 m depth range from −1.8 to 1.8 °C. The species dwelling under such conditions are usually highly endemic, they have been geographically isolated in a constantly cold environment for around 25–22 MYA (reviewed in Clarke and Johnston 1996) and are extremely stenothermal (Somero and DeVries 1967) The investigation of heat shock response of Antarctic marine species is very important, because the Southern Ocean has a very stable and narrow temperature range, where sudden temperature rises of 5 °C have not occurred for millions of years. Antarctic species do not survive even modest temperature rise and die even after short temperature exposure at 5–10 °C (Somero and DeVries 1967). In Antarctic notothenioids as well as several invertebrate species the loss of the heat-shock response was discovered (Carpenter and Hofmann 2000; Clark and Peck 2009; Hofmann et al. 2000). Thus, in the notothenioid fish *Trematomus bernacchii*, acute heat stress and cadmium exposure failed to induce Hsp synthesis and increase transcription of any mRNAs for Hsps (except for the cochaperone Hsp40) (Hofmann et al. 2000; Buckley and Somero 2009). Surprisingly, closely related forms sharing the same ancestor and dwelling in cold waters (8–12 °C) of the New Zealand area are able to effectively synthesize Hsps after the temperature elevation (Petricorena and Somero 2007). To elucidate the mechanism responsible for the lack of Hsps induction in *T. bernacchii*, HSF1 activity, Hsp70 mRNA production and protein synthesis patterns, hepatocytes of this species were examined (Buckley et al. 2004). Interestingly, in the course of these studies the presence of activated HSF and Hsp70 mRNA were detected in this organism in non-stress conditions. However, HS and chemical inducers of the heat shock response failed to increase *Hsp70* mRNA levels, HSF1 activity or the levels of any Hsp.

It was also shown that inducible Hsp70 is expressed permanently in the cold-adapted *T. bernacchii* and related Antarctic species, *Pagothenia borchgrevinki* (Nototheniidae), *Harpagifer antarcticus* (Harpagiferidae) and *Lycodichthys*

dearborni (Zoarcidae) (Buckley et al. 2004; Clark et al. 2008; Place and Hofmann 2005). It is likely that certain level of Hsp70 in the cells under non-stress conditions may be necessary to cope with aggregated and misfolded proteins occurring under extremely low temperature (Privalov 1990).

Sequence analysis of the 5′-region of *Hsp70* gene of the Antarctic fish *E. focardii,* which also lack the heat shock response, revealed the presence of potentially functional HSEs and also StREs (Stress response elements), both of which confer the ability to respond to stress (La Terza et al. 2001). One may speculate that because of the presence of permanently active HSF and resulted constitutive Hsps synthesis, the further HSF activation after heat treatment is complicated or requires the induction temperature higher than lethal temperature for the examined Antarctic organisms.

Indeed, investigation of Hsp70 induction in clam (*Laternula elliptica*) revealed up-regulation of two isoforms of *Hsp70* genes at 10–15 °C in digestive gland and gill tissue (Clark et al. 2008). The induction temperature for these genes was between 8 and 10 °C, while permanent expression of Hsp70 and Hsp78 isoforms was detected in this species.

The investigation of the limpet (*Nacella concinna*) also revealed constitutive expression of all members of Hsp70 family. The induction temperature was 15 °C higher than this species can experience in Antarctic waters (seawater temperatures at 15 m depth range from −1.8 to 1.8 °C). Furthermore, three other investigated Antarctic species, a sea star (*Odontaster validus*), a gammarid (*Paraceradocus gibber*) (Clark et al. 2008) and a ciliate (*Euplotes focardii*) (La Terza et al. 2001) completely lost heat shock response. In the case of the sea star and the gammarid several *Hsp70* gene family members were identified in genomes, but they did not show either inducible or significant permanent expression. Larvae of a terrestrial Antarctic flightless midge species *Belgica antarctica* (Diptera, Chironomidae), are also not able to synthesize Hsps after temperature elevation (Rinehart et al. 2006). Interestingly, this midge species also exhibits high level of constitutive Hsps which can be further increased by UV-radiation. Conversely, adults of this species exhibit no constitutive up-regulation of their Hsps synthesis while Hsps induction can be thermally activated (Rinehart et al. 2006). Another well-known model species *Hydra oligactis*, dwelling in extremely cold and stable aquatic conditions also lost the ability to synthesize Hsps in response to temperature elevation despite the presence of correspondent genes in the genome. However, in this particular case the absence of Hsp70 after HS results from very low stability of correspondent mRNA (Brennecke et al. 1998). Surprisingly, close Hydra species (*H. vulgaris*) characterized by the virtual absence of senescence is able in response to HS synthesize Hsps that are implicated in this extraordinary phenomenon (Martínez and Bridge 2012).

In the previous section we described a few species which are not able to respond to temperature elevation by Hsps synthesis induction. Therefore, stenothermal organisms (mostly aquatic) inhabiting stable cold environments for many million years which practically never encounter any significant fluctuations in temperature, salinity and other challenges may lose to different degree their ability to respond to various forms of stress by Hsps induction. The enhanced lipid membrane densities (e.g. higher concentrations of mitochondria), characteristic changes in enzyme

kinetic properties and, even more so, loss of genetic information (e.g. myoglobin and hemoglobin in notothenoid fishes) reflect the specialization of Antarctic organisms to constant low temperature conditions (Pörtner et al. 2007).

Generally speaking, at all levels analyzed, the functional specialization to permanently low temperatures evolved in aquatic organisms implies reduced tolerance to high temperatures, as a trade-off (Petricorena and Somero 2007; Pörtner et al. 2007). Probably high constitutive synthesis of Hsp70 and other chaperones common for such stenothermal forms represents the major compensatory molecular adaptation to deal with elevated levels of protein damage constantly occurring at low temperature conditions which are far from optimum for normal protein folding (Fraser et al. 2007; Fraser and Rogers 2007; Privalov 1990; Place et al. 2004; Todgham et al. 2007). The upregulation of Hsps demonstrated in many Antarctic organisms suggested that low-temperature conditions may be significantly denaturing to cellular proteins, an observation that was supported by elevated levels of ubiquitin-conjugated protein detected in Antarctic notothenoid fish (Place et al. 2004).

4.5 Organisms from High Temperature and Salinity Areas (Extremophiles) and Related Forms

Along with described above desert organisms and aquatic (mostly marine) species that evolved to cope with thermally highly variable environments, there are animal species thriving in highly aggressive extreme areas under conditions of constant high temperature and/or salinity.

During the expedition to Kunashir (Kuril Islands) in 2005 insect larvae of different sizes were collected in the hot mineralized sulphur volcanic spring Fig. 4.11.

The larvae were determined to belong to Diptera species *Stratiomys japonica* van der Wulp (Diptera: Stratiomyidae), common name "soldier flies". The collected larvae were consequently compared with a few other species belonging to the same family (Stratiomyidae). The recent phylogenetic analysis of Stratiomyidae based on molecular characters was performed (Brammer and von Dohlen 2007).

The larvae of Stratiomyomorpha thriving in the hot springs are collectors-gatherers of fine organic particles. They have strong larval cuticle characteristically shagreened and encrusted with plates or "warts" of $CaCO_3$, which is unique among the Diptera (Rozkošný 1982, 1997; Woodley 1989). These features might facilitate colonization of extreme semiaquatic habitats such as hot volcanic springs of Kunashir Island (the Kuril Islands).

Stratiomyid larvae belonging to different lineages are known to inhabit extreme aquatic conditions, such as hypersaline and thermal waters, and demonstrate a remarkable resistance to different chemicals (reviewed in McFadden 1967 and Rozkošný 1982, 1997).

The aim of our study was to compare and try to correlate thermotolerance and the patterns of Hsps expression in (semi)aquatic larvae of several stratiomyid species

Fig. 4.11 (**a**) Major outlet of Stolbovskoi spring on Kunashir Island, general view; (**b**) last instar larva in water; (**c**) adult *S. japonica* (male); (**d**) dead larvae of various instars in an area with local temperature exceeding 50 °C. (**e**) One of the authors (D.G.) during collection trip to Kunashir Island in 2006 (The photos were taken by D. Garbuz and A. Przhiboro)

belonging to different evolutionary lineages and inhabiting extreme habitats with contrasting temperatures and chemistry.

In this work we have investigated four stratiomyids: *Stratiomys japonica* from Stolbovskoi hot spring on Kunashir Island; two species, *Stratiomys singularior* and *Nemotelus bipunctatus* from hypersaline lakes in the Crimea; and *Oxycera pardalina* from a cold spring near St.-Petersburg.

S. singularior is a very common and widely-distributed Transpalaearctic typical eurythermal species. In the Crimea, the larvae of this species were abundant in shallow pools saturated with H_2S, with mineralization approximately 80 g/l. *Nemotelus bipunctatus* was also collected in Crimea in the water margin zone of coastal lagoon-derived Lake Koyashskoe where mineralization of water near the shore was about 280 g/l (Golubkov et al. 2007). The measured temperature in the habitats of the two Crimean stratiomyids used in our studies varied from 15 to 30 °C, but apparently it can be higher in summer and much lower in winter.

For comparison with the above described three thermoresistant species we took cold-adapted species *O. pardalina* from the same family which is widely distributed in Western and Central Europe. The larvae of *O. pardalina* are confined to clear-water cold carbonate springs near St.-Petersburg with a year-round stable low water temperature (5.5–7.5 °C) and low level of total mineralization (0.38–0.42 g/l).

The larvae of the four species were exposed to different temperatures and assessed for survival and Hsps synthesis. The LT_{50} and critical temperature (i.e. the temperature of HS treatment resulting in less than 1 % of survival) were also determined in their larvae.

The LT_{50} and critical temperatures of *S. japonica* larvae did not depend on the temperature of maintaining in the laboratory (31 or 37 °C). Apparently, maintenance of these larvae at different temperatures before HS did not significantly affect their thermotolerance. Therefore, the acclimation procedure was not effective in the studied species. This observation is not surprising because it was previously demonstrated in other highly eurythermal organisms which also did not respond to acclimation procedure (Tomanek 2005; Somero 2005). It was speculated that such forms exhausted genetic components of their thermal adaptation and, hence, live close to their thermal limit. Due to this characteristic feature in the HSR, species from extreme and highly variable environments are likely to be more affected by climate change than species from moderately variable environments.

It is of note, that *D. lummei* flies (see above) were also characterized by the absence of induced thermotolerance probably because in this case the representatives of this cold-adapted species having a diapause rarely (if ever) encounter acute HS in nature.

In general, our experimental data corroborate our field observations on Kunashir Island, in which *S. japonica* larvae died within a few minutes after being swept or transferred into areas of the hot spring where water temperature exceeded 50 °C (Fig. 4.11). For the two Crimean species, LT_{50} for a 30-min heat shock were 45.5 °C for *S. singularior* 46.5 °C for *N. bipunctatus*, while critical temperatures for these species were 47 and 48 °C, respectively (Fig. 4.5). For *O. pardalina* larvae from the cold spring, critical temperature was 43 °C, while LT_{50} was 41.2 °C. Although

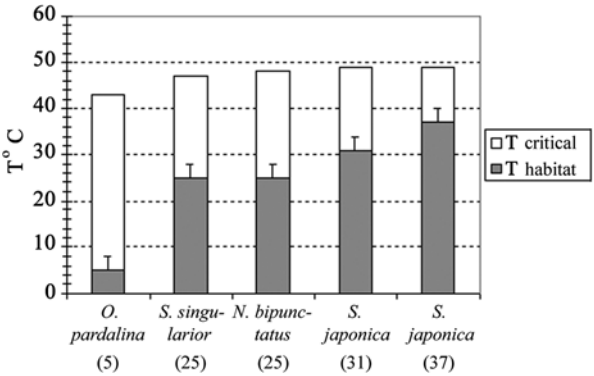

Fig. 4.12 The ratio between control (habitat) temperature and critical temperature for Stratiomyidae species. Numbers in the brackets indicate the temperatures at which the larvae have been maintained before heat-shock treatments. Basically, they are close to the average habitat temperatures of the species studied (From Garbuz et al. (2008) with permission)

heat tolerances were in general correlated with the typical habitat temperatures of these larvae, most surprising is the huge gap (38 °C) between the natural habitat temperatures (6–10 °C) and critical temperatures for *O. pardalina* larvae and in this respect *O. pardalina* apparently greatly exceeds the other three highly eurythermal Stratiomyidae species (Fig. 4.12).

Exploring antibodies recognizing inducible members of Hsp70 family we performed broad-scale analysis of HSR in the Stratiomyidae species studied after different HS treatments. High levels of Hsp70 were present in the larvae of all four species at the temperatures of their habitats, and also at much lower temperatures (ca. 25 °C in the case of *S. japonica* larvae kept several weeks at this temperature in the laboratory). The constitutive concentrations of Hsp70 were approximately the same irrespective of the habitat temperatures of the studied species in nature. A 30-min heat shock treatment increased Hsp70 synthesis two to three-fold in *S. japonica,* maximally after 43 °C treatment. Accompanying the Hsp70 induction in this species are pronounced increases in small Hsps and Hsp68, and a small but significant increase in hsc70 concentration. By contrast, Hsp induction immediately after heat shock treatment is comparatively less in the two Crimean species, and hardly detectable in *O. pardalina* (Fig. 4.13). Notably, the accumulation of Hsp70 continues in the cells of the treated larvae for many hours after heat shock and plateaus approximately 24–36 h after the treatment.

Therefore, the pattern of Hsps in all studied Stratiomyidae species drastically differs from that of *D. melanogaster* and other *Drosophila* species where virtually no inducible Hsp70 is present at normal temperature while even mild HS induced dramatic increase in the synthesis of all groups of Hsps. Furthermore, the synthesis of all Hsps in *Stratiomydae* species in contrast to *Drosophila* species, lasts for many hours and reaches plateaus only after 24–36 h following HS. Intriguely, trypsin proteolysis and subsequent mass spectrometry showed that *Stratiomyidae* Hsp70 is much closer to the correspondent protein from the thermotolerant mosquito species *Aedes aegypti* than to Hsp70 from known species of *Drosophila* (Garbuz et al. 2008). Surprisingly, experiments exploring (^{35}S)L-methionine labeling failed to detect significant synthesis of Hsps in the control untreated larvae of the species studied.

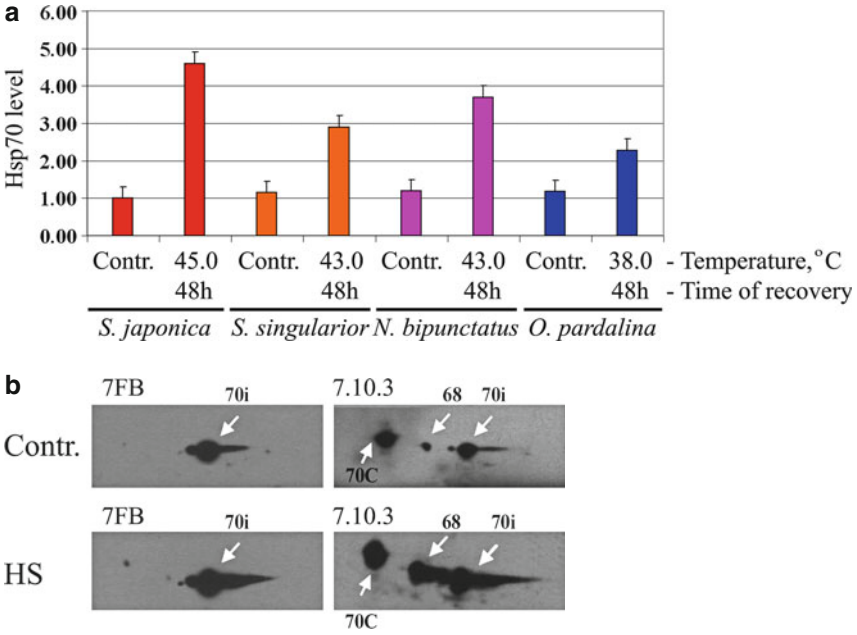

Fig. 4.13 (**a**) The levels of Hsp70 in different representatives of Stratiomyidae family under normal temperature (Contr.) and after heat shock (HS, 30 min). (**b**) The pattern of *Stratiomys japonica* proteins as revealed by two-dimensional electrophoresis and Western blotting of 2D blots sections containing Hsp70 family using 7FB and 7.10.3 antibodies. 7FB recognize predominantly inducible Hsp70 member, while 7.10.3 recognize all Hsp70 family members. Arrows indicate 70i – Hsp70 inducible member; 70C – constitutive member of Hsp70 family; 68 – Hsp68, an inducible member of Hsp70 family (From Garbuz et al. (2008) with permission)

Northern blot hybridization surprisingly failed to reveal any significant levels of either *Hsp70* mRNA or small *Hsps* transcripts under control conditions in any Stratiomyidae species studied. Only RT-PCR studies were able to reveal weak leakage of *Hsp70* genes in *S. japonica* under control conditions (Fig. 4.14). Heat shock, however, resulted in strong and rapid induction of corresponding mRNAs, with a maximum at 43–45 °C and an apparent drop of transcription at 47 °C. The latter temperature represents the LT_{50} for this highly thermoresistant species and probably damages the transcription machinery. The *Hsp70* mRNA synthesized in response to heat shock persists in the cells for many hours and is still detectable in trace amounts even 48 h after HS.

As indicated by electrophoretic mobility-shift assays, in contrast to certain desert lizards mentioned above (e.g. *P. interscapularis*), the active form of HSF is not detectable under control conditions in *Stratiomys* species studied. In response to temperature elevation, however, HSF as expected binds specifically to heat-shock element (HSE) and is able to transactivate the *Hsp* genes. Therefore, the surprisingly high concentration of Hsps observed in the cells of Stratiomyidae species without heat shock is not accompanied by significant transcription of corresponding

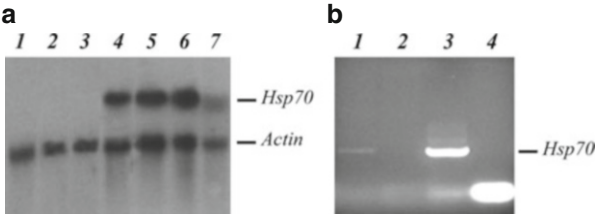

Fig. 4.14 (**a**) Northern blot hybridization of total RNA from *S. japonica* larvae with ^{32}P-labelled *Hsp70* probe in controls and in response to heat-shock treatments. *1*, *2* and *3*–25, 31 and 37 °C respectively (a range of normal conditions in a field), *4*–42, *5*–43, *6*–45 and *7*–47 °C. As a control for equal loading, blots were stripped and rehybridized with actin probe. (**b**) RT-PCR analysis. Lane *1*, *S. japonica* nonheated control larvae kept at 31 °C; lane *2*, *S. japonica*, control reaction mix without reverse transcriptase (larvae kept at 31 °C); lane *3*, *S. japonica* larvae heat-shock treated (43 °C, 30 min); lane *4*, *S. japonica* larvae heat shock treated (43 °C) without reverse transcriptase. The fragment of expected size (661 bps) is indicated. Transfer RNA used as a carrier forms thick bands with lower molecular mass (From Garbuz et al. (2008) with permission)

genes under non-stress conditions. The phenomenon may be probably explained by very low but continuous expression of heat shock genes demonstrated by RT-PCR analysis or, less probably, by the synthesis of Hsps at some earlier stages of larval development when the larvae have been subjected to more severe shock. The last speculation seems less probable since it implies extremely high stability of Hsps in Stratiomyidae species. Furthermore, high concentration of Hsps has been detected in Stratiomyidae larvae kept for a month in the laboratory at 25, 31 or 37 °C.

Apparently, critical temperatures for (semi)aquatic larvae of this family are close to or slightly lower than 50 °C, and the larvae are unable to tolerate higher temperatures. According to Pritchard (1991), no insects were proved to live in hot springs above 50 °C, and very few, mostly Diptera, above 40 °C. Our results confirm this general conclusion.

The data obtained in our studies indicate that the inducible member of the Hsp70 family and other Hsps are continuously present at high concentrations in the larvae of four species of Stratiomyidae family, even in the absence of heat shock.

Characteristically, in contrast to the larvae, in the adult flies of the species investigated, immunoblotting revealed only trace amounts of Hsp70 under non-heat shock conditions.

The magnitude of thermotolerance observed in the investigated Stratiomydae species, in fact, exceeds that of most insect species studied, with *Bombyx mori* being a prominent exception (Evgen'ev et al. 1987). This comparative uniformity in the Stratiomydae species is exhibited despite differing natural thermal regimes, and includes LT_{50} and critical temperatures. Mild heat shock (37 °C) pretreatment did not significantly improve tolerance for extreme temperatures in *S. japonica* larvae which, hence, seem not to have induced thermotolerance.

It is evident that, molecular mechanisms underlying up-regulation of heat shock proteins may differ characteristically depending on the organism. Thus, in desert lizards *Phrynocephalus interscapularis*, high constitutive levels of Hsps and correspondent

mRNA are correlated with activation of HSF under control conditions, and may be further dramatically induced by heat shock treatment (Zatsepina et al. 2000). Apparently animals constantly subject to extreme temperatures have elaborated molecular mechanisms allowing them to maintain a relatively high Hsps level under normal conditions. In this way, they are preadapted for life under the conditions of constant stress without drastic changes in the house-keeping and *Hsp* genes activity.

The observed constitutive expression of Hsps in the cells of the Stratiomyidae larvae may help them to survive the diapause period spent at the larval stage.

Interestingly, the one Stratiomyidae species investigated that naturally occurs in a stable albeit cold thermal environment, *O. pardalina*, although exhibits significant increase in heat shock RNAs synthesis after temperature elevation, synthesized less Hsps than other thermotolerant species after high temperature challenge. The constitutive Hsps synthesis observed in this particular species suggests that extremely cold habitats, may be as challenging for proteins synthesis and homeostasis as in the case of cellular proteins functioning in aggressive extreme conditions of hot volcanic springs (see above Sect. 4.4).

In contrast to the larvae, adults (flies) of all Stratiomyidae species in our study which are able to escape from high temperature areas and, hence, are not subject to the severe conditions of hot or highly mineralized aquatic environments, and their survival apparently does not depend on maintaining high continuous levels of chaperones. The difference in the environment microhabitats probably accounts for the apparent dichotomy between larvae and adults in Hsp70 expression. It is of note, that such dichotomy in the synthesis of chaperones is even more pronounced in the cold-adapted Antarctic animals where larvae in contrast to adults completely lost the ability to activate their Hsps (see Sect. 4.4).

Regardless of the exact mechanism, the functionally extended presence of Hsps in the larvae of all Stratiomyidae species investigated may meet the increased chaperoning needs that these animals experience in response to adverse conditions of hot volcanic streams, highly mineralized Crimean lakes or cold springs in the environs of St.-Petersburg. This maintenance prevents cell injury and irreversible protein aggregation that may occur in response to these types of stress.

Interspecific comparison is a common approach in physiological ecology and other related fields of biology. However, several authors justly underlined many logical and statistical problems associated with using interspecific and in particular two-species comparisons for studying adaptations (Garland and Adolph 1994). In the case of Stratiomyidae species used in the present investigation we cannot state with certainty that exceptionally high thermotolerance and developmental dichotomy in terms of Hsps synthesis represent genetic adaptations in response to natural selection operated in the species studied (high and low temperatures and salt concentration). It is much more likely for this particular group that the observed adaptations to extreme conditions may be a characteristic feature of the ancestor of the whole Stratiomyidae family enabling the descendants successfully colonize various inhospitable environments. There are data suggesting that Mezozoic stratiomyids were already preadapted to colonize different types of extreme habitats, and such

colonization occurred repeatedly in different genera (Rasnitsyn and Quicke 2002; Rozkošný 1997; Whalley and Jarzembowski 1985).

The performed investigation of a broader spectrum of Stratiomyidae and other Diptera species helped to check this preferred hypothesis.

For our analysis Diptera species belonging to different families and inhabiting highly variable environments were taken into analysis. Besides additional Stratiomyidae species i.e. *Oplodonta viridula*, *Beris chalybata* and *Odontomia sp.*, we used two species of horse flies (*Tabanidae*) *Haematopota pluvialis* and *Chrysops relictus* and three species of biting midges belonging to *Ceratopogonidae* family (*Dasyhelea modesta*, *Dasyhelea flavoscutellata* and *Dasyhelea sp. ex. gr. cincta*). The investigation of HSR response at the molecular level including synthesis and stability of Hsps and corresponding mRNAs in several Diptera species enables to describe features probably common to all Diptera as well as molecular adaptations restricted to certain families.

Oplodonta viridula, *Beris chalybata*, *Chrysops relictus* and *Dasyhelea flavoscutellata* belong to typical cold-adapted stenothermal species, *Odontomia sp.* and *Haematopota pluvialis* were collected in the Crimea in the same place as *S. singularior* and *N. bipunctatus*, while biting midges (*Dasyhelea modesta* and *Dasyhelea sp. ex. gr. cincta*) dwell at the littoral area of Northern freshwater lakes. It was shown that all studied species are characterized by significant constitutive level of Hsp70 under physiological conditions of their correspondent niches. Characteristically, the horse flies (*C. relictus*) differ from the investigated representatives of Stratiomyidae and *Drosophila* by significantly higher stability of *Hsp70* mRNA (Garbuz, Przhiboro, personal communication 2014).

Therefore, numerous *Drosophila* species characterized by completely repressed expression of inducible Hsps under normal conditions and strong activation of HSPs synthesis after temperature elevation rather represent exceptions when compared with other Diptera.

Notably, constitutive or chronic expression of Hsps, is not uniformly beneficial. For example, experiments on transgenic *D. melanogaster* strains with extra copies of *Hsp70* genes (see Chap. 8) have demonstrated that overexpression of Hsp70 could be harmful and may increase lethality during development (Krebs and Feder 1997). Evidently the expression level of Hsps in each species and geographical population is a balance between benefits (high thermotolerance) and costs (e.g. negative impact of Hsps overexpression on growth, fertility and other characteristics). It is necessary to keep in mind that *Drosophila* species and other Diptera species used for comparison e.g. Stratiomyidae species greatly (many folds) differ by duration of life-span and this dramatic difference may be the major factor in the observed contrasting patterns of their heat shock response and apparent different sensibility to the constitutive presence of high concentration of Hsp70 in the cells.

In the studies exploring several phylogenetically distant Diptera species we described different patterns of Hsps expression in the families belonging to the order. Thus, while in all *Drosophila* species, the level of Hsps is not detectable under normal physiological conditions and increased dramatically as temperature

Table 4.1 Various levels of *Hsp70* regulation in different Diptera species

	Hsp70 presence under normal conditions	Hsp70 stability	*Hsp70* mRNA presence under normal conditions	*Hsp70* mRNA induction after HS	*Hsp70* mRNA stability
Drosophila	–	–	–	+++	–
Stratiomyidae	+++	+++	–	+++	–
Ceratopogonidae	++	+	+	++	+/–
Tabanidae	+/–	+	–	+++	+++
Chironomidae	+/–	+	+++	+/–	+/–

increased, in the Stratiomyidae species studied we detected high constitutive level of inducible member of Hsp70 in the cells at normal temperature and HS treatment was able to induce only moderate increase in concentration of Hsps including Hsp70. Surprisingly, horse-flies (*C. relictus*) are characterized by high stability of *Hsp70* mRNA after HS.

Finally, larvae of two species of the Chironomidae family dwelling in Antarctic regions and in North Russia exhibit very peculiar pattern of Hsp70 expression. Thus, in contrast to all species of other Diptera families mentioned above, in these species high constitutive level of *Hsp70* mRNA was detected in the larvae under normal physiological conditions and HS treatment was ineffective in the induction of Hsp70 at either transcription or translation levels (Rinehart et al. 2006; Garbuz, Przhiboro personal communication 2014). Therefore, even within the same order (Diptera) different species have strikingly different patterns of heat shock response summarized in Table 4.1.

4.6 Special Cases

4.6.1 Stress Proteins in the Hibernating and Desiccating Organisms

Numerous studies demonstrated important protective role of various Hsps in preserving the integrity of transcription and translation machinery under stressful conditions including extreme ones. Naturally, soon after the discovery of Hsps their concentrations were monitored in "special cases" of adaptations, where organisms enter a diapause, anoxia or severe desiccation. Indeed, various groups of Hsps in many cases dramatically up-regulated in such organisms, at certain stages of their life-cycle associated with long-term anoxia or severe desiccation. Thus, cells of encysted embryos of the brine shrimp (*Artemia franciscana*) survive continuous anoxia for periods of years, during which their metabolic rate is undetectable (King et al. 2013). It was shown that abundant electron-dense granules consisting of the small heat shock/α-crystallin protein (p26) are present in the nuclei of anoxic embryos. Diapause-destined embryos of *Artemia* accumulate massive amounts

of Hsp26 (up to 15 % of total cellular proteins) during the periods of continuous anoxia and this chaperone is not detected or induced in any other life-cycle stages (Beristain et al. 2010). Contrary to expectation, proteins of Hsp70 and Hsp90 families did not undergo anoxia-induced nuclear translocation in the cells of *Artemia* encysted embryos, an unusual result since induction of these Hsps and such translocations were often observed in cells from a variety of organisms experiencing adverse, stressful conditions. For example, Atlantic copepods of genus *Calanus* (*Calanus finmarchicus*) accumulate high quantities of small Hsps during diapause (Aruda et al. 2011). Furthermore, Tardigrade species ("slow steppers") which have unique stress-adaptations that allow them to survive extreme heat, cold and radiation accumulate Hsps including several isoforms of Hsp70 together with glycogen, glycolipids and other specific agents during desiccation (Schill et al. 2004). It is known that low molecular Hsps at high concentration are able to convert the cytoplasm into gel-like matter which effectively preserves water and prevents proteins aggregation.

Furthermore, embryos of annual killifish *Austrofundulus limnaeus*, dwelling in the ephemeral lakes of Northern regions of South America accumulate large quantities of Hsp70 before entering the diapause during the periods of drying of their habitats. These diapausing embryos are highly resistant to a number of environmental insults such as high temperature, dehydration, anoxia, and increased salinity (Podrabsky et al. 2007). Interestingly, in this species embryo-specific form of inducible Hsp70 is expressed during embryonic development and is elevated during diapause. It was suggested that constitutive expression of Hsp70 during development may afford these embryos protection from environmental stresses during development more quickly than relying on the induction of a classic heat shock response.

The sleeping chironomid *Polypedilum vanderplanki*, inhabiting temporary water pools in semi-arid regions of Africa represents another example of possible involvement of Hsps in surviving severe water loss (Fig. 4.15). This chironomid species one of very few insects able to survive prolonged complete desiccation at larval

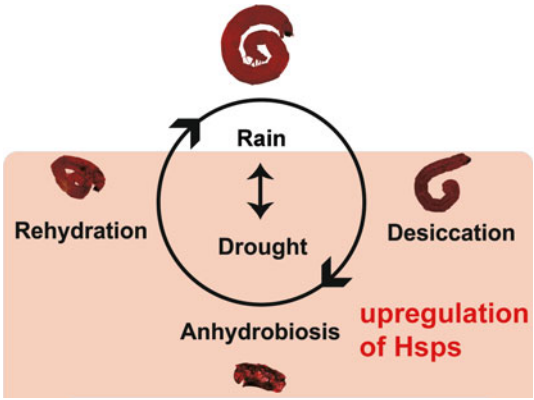

Fig. 4.15 Life cycle of chironomid *Polypedilum vanderplanki* living in Africa droughty regions. Hsp70 elevation takes place at the stage of desiccation and rehydration after dry period (The scheme provided by Oleg Gusev, PhD)

stage, entering a state called anhydrobiosis. Surprisingly, even after years in a dry state, larvae are able to revive within a short period of time, completely restoring metabolism. In order to survive such extreme environments, organisms need to develop special adaptations both at physiological and molecular levels. The analysis performed demonstrated quite different regulation of Hsps in the course of dehydration and rehydration in the larvae. Heat-shock-responsive Hsp70 and Hsp60 genes of this organism showed a two-peak expression in dehydrating and rehydrating larvae. Both small alpha-crystallin heat shock proteins (sHsps) transcripts were accumulated in the desiccated larvae, but showed different expression profiles. Transcripts of *Hsp23* was limited to the late stages of desiccation, suggesting possible involvement of this protein in the glass-state formation observed in anhydrobiotic larvae (Gusev et al. 2011). Furthermore, analysis of the sleeping chironomid genome showed intensive clustering-associated increase in the number of sHsps-coding genes associated with anhydrobiosis. The expression of the sHsps in these anhydrobiotic chironomid-specific clusters are tightly linked to the larvae stage and highly induced by desiccation (Gusev, personal communications).

Similarly, the important role of Hsps in maintaining cellular integrity and enzyme activity during desiccation and rehydration processes has been postulated in the flesh fly (*Sarcophaga crassipalpis*) (Hayward et al. 2004). The patterns of the observed Hsps induction characteristically differed in diapausing and nondiapausing pupae. Thus, in nondiapausing pupae, the expression of inducible Hsp23 and Hsp70 was upregulated by desiccation, while in diapausing pupae, the transcripts of these chaperones are already highly expressed and not further upregulated by desiccation.

The obtained results indicate distinct role for the different Hsps during desiccation stress and rehydration recovery (Hayward et al. 2004).

Up-regulation of various Hsps in response to hypothermy and various forms of oxidative stress usually observed at different stages of hibernation is not restricted to arthropods but was also detected in vertebrates (Sills et al. 1998). The investigation of the heat shock response in anoxia-tolerant turtle (*Trachemys scripta elegans*) demonstrated a strong fivefold increase in the amount of HSF1 under anoxic conditions and upregulation of five Hsps including Hsp70. The data demonstrate organ-specific regulation of Hsps induction during anoxia exposure and aerobic recovery in this turtle species which is probably represents an important aspect of cytoprotection with regard to underwater hibernation of these turtles in cold water (Krivoruchko and Story, 2010).

Mammalian species also explore modulation in the intensity of heat shock proteins synthesis in different phases of hibernation. Thus, compared with summer-active ground squirrels (*Spermophilus tridecemlineatus*), levels of the mitochondrial stress protein GRP75 were consistently higher in certain organs (e.g. intestinal mucosa) of hibernators in each hibernation state (Carey et al. 2000). Furthermore, ground squirrels increase Hsp70 family members as well as ubiquitin-protein conjugates during hibernation (Sills et al. 1998; Feder and Hofmann 1999). Interestingly, different organs of hibernating animal may exhibit a peculiar pattern of Hsps synthesis. Thus it was demonstrated that in the bat (*Murina leucogaster*)

certain muscles did not show any sign of atrophy or tension reduction and this phenomenon correlates with elevated level of Hsp70 in the muscles of the hibernating bats (Lee et al. 2008).

4.6.2 The Role of Hsps in the Life-Cycle of Parasites

Parasites which represent another "special case" may synthesize various Hsps as cellular defense mechanism to survive drastic temperature changes they encounter during their life-cycle. Induction of certain groups of Hsps usually accompanies the infection of mammalian or avian hosts from ectothermal hosts such as insects or by free-living stages. In the case of free-living stages of parasites that do not involve an animal vector sometimes induction of Hsps accompanies the transition from environment to the homothermal host.

The transition from environment with low or temperate temperatures to homothermal host with internal temperature of 37 °C or above represents a characteristic life history feature of many parasitic organisms.

Multiple studies demonstrated developmentally regulated synthesis of Hsps during the life-cycle of parasites which may respond to profound temperature changes by synthesizing different quantities of certain Hsps depending on the variations in their host temperature and life stage. It was shown that different species of intracellular parasites in the genus *Eimeria* (Apicomplexa: Coccidiida) synthesize Hsp70 in the process of infection of mammalian host (mouse) (Clark et al. 1996). The levels of Hsp70 expression in sporozoites of a wild-type parent strain and two precocious lines of *Eimeria tenella*, were compared to investigate the relationship between the heat shock proteins expressed by the parasite and virulence of the strain. The synthesis of Hsp70, which was observed in the entire sporozoites of the wild strain, was drastically reduced to the anterior portion in the precocious lines. The level of Hsps may be dramatically increased in the course of host change, thus in *Trypanosoma brucei* transcription of *Hsp70* and *Hsp83* homologues is enhanced more than 100-fold after the parasite leaves the tsetse fly and enters the mammalian host likely due to cluster organization of *Hsp70* genes providing rapid and high induction of these genes in the species (See Chap. 5) (Muhich and Boothroyd 1989).

Thus, *Schistosoma mansoni* actively synthesized Hsp70 after entering human skin from the water (Neumann et al. 1993). It was also shown that phagocytosis induce Hsp70 expression in *Toxoplasma gondii*. Interestingly, the induction of this chaperone is observed only in virulent strains of this parasite while avirulent forms either do not synthesize Hsp70 in the course of phagocytosis or synthesize low amounts of this protein (Lyons and Johnson 1995).

The malarial organism *Plasmodium falciparum* begins to synthesize Hsp70 at 39 °C, which correlates with periodical body temperature changes usually observed in the malaria patients (Biswas and Sharma 1994). Sometimes the pattern of the Hsps in parasite is organ- or tissue-specific. Thus, when the helminth *Trichinella spiralis* infects rodents, an enhanced expression of Hsp25 and Hsp60

and of these plus Hsp70 was observed at certain, yet different, time-points during infection in rat spleen and rat brain, respectively (Martinez et al. 1999). Multiple examples of developmentally regulated expression of different classes of Hsps in parasitic organisms during the transition to their hosts include parasitic nematodes, protists, cestode parasites and many other species (Biswas and Sharma 1994; Neumann et al. 1993).

It is evident that heat stress severity experienced by the parasites infected poikilothermal organisms such as reptiles or fish or insects should predominantly depend on the temperature of the host body which can vary dramatically.

Along these lines, it was shown that *Leishmania* species dwelling in the blood of thermoresistant desert toad-headed agama actively synthesize Hsps at 40 °C while in the nocturnal gecko all protein synthesis in the parasite cells is completely inhabited at 38 °C (Ul'masov et al. 1988). These experiments exploring different lizard species from thermally contrasting niches for the first time revealed coevolution of parasite-host system with respect to such vital trait as molecular response to temperature elevation (HS).

Notably, large quantities of Hsps usually produced by bacteria and parasites in the host cells after infection often elicit an immune response and may be useful in generating vaccines. Along these lines, it was shown that *Leishmania infantum* deletion mutant, lacking both *Hsp70-II* alleles (*ΔHsp70-II*), provided protection against *Leishmania* infection in the *L. major*-BALB/c infection model and can be a safe live vaccine as immunodeficient SCID mice, and hamsters, infected with mutant parasites did not develop any sign of pathology (Carrión et al. 2011; Folgueira et al. 2008). It was shown that tumour necrosis factor-alpha and phagocytosis induces expression of stress proteins in virulent promastigotes of *Leishmania donovani* (Salotra et al. 1995).

In general, parasites evolved to respond to temperature changes either by high constitutive synthesis of certain Hsps or by their rapid induction following the infection.

Likewise, the heat-induced disaggregase Hsp104 of *Candida albicans* plays a role in biofilm formation and pathogenicity in a worm infection model. Biofilm formation by cells lacking *Hsp104* gene proved to be defective in different *in vitro* models. Thus, biofilms formed by the wild-type strains showed patterns of intertwined hyphae in the extracellular matrix. In contrast, biofilm formed by the *Hsp104* mutant showed multiple structural defects and appeared patchy and loose. Apparently these defects result in decreased virulence of the *Hsp104* mutant observed in the *C. elegans* infection model, hence, providing an indication of a role for Hsp104 in *C. albicans* virulence, in addition to its key role in the thermotolerance (Fiori et al. 2012).

Pathogenic bacteria such as *Salmonella typhimurium* or *Listeria monocytogenes* also actively synthesize various chaperones such as GroEL, GroES, DnaK and HtpG in response to phagocytosis and reactive oxygen species and certain stress proteins e.g. GroEL or DnaK may reach high concentration (Hanawa et al. 1999; Morgan et al. 1986; Wiesgigl and Clos 2001). Similarly, certain bacterial endosymbionts that infect various insect species such as bacterium *Buchnera* constitutively

express a protein, symbionin belonging to Hsp60 (GroEL) family at very high levels as well as several other bacterial chaperones (Sato and Ishikawa 1997).

On the other hand, high conservatism exhibited by Hsps even between very distant phyla sometimes results in cross-reaction of the anti-bodies produced against bacterial Hsps with epitopes of orthologous host's Hsps which may lead to severe autoimmune diseases such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (Tsoulfa et al. 1989; Panchapakesan et al. 1992).

4.6.3 Heat Shock Proteins in Defense

It is known that giant hornets (*Vespa mandarinia japonica*) represent natural enemies of the honeybees and specifically Japanese honeybees (*Apis cerana japonica*). Giant hornets often attack the hives, kill the bees and consume honey stored in the honeycomb. The bees are practically defenseless when attacked by hornets because they cannot pierce their thick chitin. However, the Japanese bees developed an effective defensive strategy for such cases. When the first hornets ("scouts") appear, numerous bees (more than 500) form a ball around the intruder (Fig. 4.16). The temperature inside the bee balls reaches 46 °C and the giant hornets are killed in less than 10 min when they are trapped in a bee ball created by the Japanese honeybees. It was found that the CO_2 concentration inside the bee ball also reaches a maximum in the initial 0–5 min phase after bee ball formation. The lethal temperature of the bees is significantly higher than that of hornets (up to 49–50 °C) and it was concluded that CO_2 produced inside the bee ball by honeybees is a major factor together with the temperature involved in defense against giant hornets (Ono et al. 1995; Sugahara and Sakamoto 2009).

In experimental ecologically relevant episodes of hyperthermia between 33 and 50 °C, Hsp70 expression and Hsp70/Hsc70 activity in brains of nonflying laboratory-held bees increased by only two to three times at temperatures 46–50 °C. In this

Fig. 4.16 Defense heating bee ball around giant hornet attacking the hive (Photo by Masato Ono, Tamagawa University, Tokyo. http://www.hymenoptera.de/html/gallery/Hornissen/exoten/riesenhornisse/Thermal+Defense+by+Acj_Photo+by+Masato+Ono_.JPG.html)

feature honey bee resembles other heat-resistant organisms with low heat shock mediated Hsp70 induction relatively to the basic Hsp70 level. Moreover, Hsp70 induction after such HS was practically undetectable in thoracic-flight muscles. These data suggest that certain honey bee tissues, especially flight muscles, are extremely thermotolerant. Furthermore, Hsp70 expression in the thoraxes of flight-capable bees is probably flight-induced by oxidative and mechanical damage to flight muscle proteins rather than temperature (Elekonich 2009).

A unique case of exploring high temperature for defense was described by Wehner et al. (1992) for desert ants *Cataglyphis* that are able to tolerate body temperature of 50 °C for at least 10 min. These ants presumably voluntarily undergo body temperature of 50 °C and more, probably to escape predators (Wehner et al. 1992).

Furthermore, it was shown in goldfish (*Carassius auratus*) that the expression of Hsp70 can be induced by the presence of a predator. Remarkably, when the fishes were exposed to a potential predator, bluegills (*Lepomos macrochirus*), they experienced an increase in Hsp70 mRNA level in the brain. When goldfish individuals were placed in a transparent tank around which bluegills were swimming, Hsp70 mRNA expression was significantly increased after 6 and 12 h. Notably, goldfish exposed to water circulating through a tank with several bluegills showed no sign of changes in Hsp70 mRNA expression. These results suggest that psychological stress may enhance Hsp70 mRNA expression in the brain just via visual perception and such presumably defensive response may somehow modulate the consequences of the stress (Kagawa and Mugiya 2000).

4.6.4 Hsps as Biomarkers of Environmental Pollution

Large scale field studies of stress proteins in various non model organisms indicated that heat shock proteins may be induced in response to abiotic contamination of the environment, in particular to different chemical pollutants. Basing on these data it was suggested to use Hsps as sensitive natural biomarkers providing information about current state of the environment, which may help to estimate the biological impact of toxic chemicals to live organisms and predict adverse consequences of such exposure. Such biological approach based on quantitative analysis of Hsps is often more sensitive and reproducible than direct monitoring of environmental contamination. There are three aspects of stress proteins that are essential for their use as biomarkers of pollution:

1. Hsps are the important components of the cellular protective and adaptive response;
2. synthesis of Hsps is likely to be induced by a large number of xenobiotics;
3. different Hsps are highly conserved in all organisms from bacteria and plants to man and in this regard the same sets of antibodies (kits) can be used for their detection and measurement in various organisms (Bierkens 2000). The estimation of Hsps concentrations as biomarkers for understandable reasons is applied predominantly in marine and soil toxicology (Feder and Hofmann 1999).

It is of note, that measurements of Hsps level as a biomarker of pollution were already applied in a variety of organisms including: plants (algae) (*Enteromorpha intestinalis*); reef coral *Montastraea franksi*; clamps (*Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus trossulus*); planarian *Dugesia (Girardia) schubarti*; copepods (*Tigriopus japonicas*); amphipods (*Gammarus roeseli*); insect larvae (*Chironomus tentans*); fishes *Cyprinodon nevadensis amargosa*, *Limanda limanda*, *Carassius carassius*, zebrafish; ascidian *Pseudodistoma crucigaster* and many others (Bierkens 2000; Hallare et al. 2005; Lee et al. 2006; Lewis et al. 2001; Mićović et al. 2009; Rios-Sicairos et al. 2010; Venn et al. 2009; Wu et al. 1996).

In several cases, such field studies clearly demonstrated correlation between levels of different pollutants (especially heavy metals) and Hsps levels.

Thus, in *Enteromorpha intestinalis* Hsp70 expression was increased with copper exposure but was not unaltered following exposure to the herbicide Irgarol 1051 (Lewis et al. 2001). Strong induction of Hsp70 occurred in the blue mussels *Mytilus edulis* from Southern Baltic at high concentration of heavy metals. Combination of metals (Cd + Cu) was found to increase the Hsp70 level more intensely than related concentrations of singularly applied metals (Radłowska and Pempkowiak 2002). In *Mytilus trossulus* Hsp70 induction roughly correlated with arsenic and cadmium levels in water (La Porte 2005; Radłowska and Pempkowiak 1998). In the *Mytilus galloprovincialis* Hsp70 and metallothioneins were induced by heavy metals, Pb, Hg and Cd, implying that these stress proteins might be power biomarkers of marine pollution (Mićović et al. 2009).

To identify a sensitive biomarker of freshwater monitoring, pollutant-induced expression of heat shock proteins (Hsps) in the larvae of the aquatic midge *Chironomus tentans* (Diptera, Chironomidae) was evaluated. Different substances, such as nonylphenol, bisphenol-A, 17-alpha-ethynyl estradiol, bis(2-ethylhexyl) phthalate, endosulfan, paraquat dichloride, chloropyriphos, fenitrothion, cadmium chloride, lead nitrate, potassium dichromate, benzo[*a*]pyrene and carbon tetrachloride were used. The response of the Hsps gene expression to chemical exposure was rapid and sensitive to even very low chemical concentrations but it was not stressor specific. Interestingly, an increase in the expression of *Hsps* genes was observed not only for a stress inducible form (Hsp70), but also for a constitutively (HSC70) expressed form (Lee et al. 2006).

Furthermore, zebrafish embryos can be used for water quality assessing with detecting of Hsp70 levels as a biomarker. To evaluate the suitability of these tests for environmental screening, fertilized zebrafish eggs were exposed to water samples collected from five sites of varying levels of pollution from Laguna Lake, Philippines. Reconstituted water was used as laboratory control while water samples from a highly polluted freshwater source were used as a positive control. The levels of Hsp70 in embryos, exposed in water from two sites located closest to Manila, the Philippine capital (Northern West Bay and Central West Bay), showed a pronounced Hsp70 elevation relative to the control probes (Hallare et al. 2005).

Similarly, it was shown that kidney Hsp30 and liver Hsp70 expression can serve as sensitive biomarkers for the presence of field environmental stressors in wild crucian carp populations (An et al. 2014). In field populations of the dab

(*Limanda limanda*) Hsp70 levels correlated with DNA damage level caused by stress conditions (Schröder et al. 2000).

At the present time highly sensitive and cheap RT-PCR approach is commonly used to estimate the levels of *Hsp* genes expression under various environmental conditions. Thus, hepatic expression of Hsp70 and cytochrome P450 1A mRNAs in white mullet (*Mugil curema*) monitored by RT-PCR from July, 2005 until July, 2006 in three coastal lagoons located in the Southern Gulf of California, Mexico, was higher for both genes in the Urias Estuary, which was considered the most polluted among the three systems, especially during the rainy season (summer to fall). These results indicate that fishes *Mugil curema* is a good candidate species to implement biomonitoring programs in tropical coastal environments (Rios-Sicairos et al. 2010).

It is well known that the impacts of marine pollutants on reef corals and their symbiotic algae are an important element of global coral reef decline. It was shown that Hsp70, and to a lesser extent Hsp90 expression increases significantly following exposure to two major marine toxic pollutants (copper and dispertant Corexit 9527) and, hence, their levels may serve as a good indicator of specific coral reef state (Venn et al. 2009).

Hsps as biomarkers in field bioassays were sometimes used in terrestrial invertebrates. Specifically, the Hsp70 response was investigated in isopods *Oniscus asellus* exposed to a heavy metal gradient around smelters near Avonmouth, UK. In the sites closest to the smelter, *O. asellus* showed highest Hsp70 levels. The authors conclude that Hsp70 level in isopod species can be a suitable biomarker of heavy metal pollution in metal-contaminated field sites (Arts et al. 2004).

Likewise, small heat shock protein 20 of the intertidal copepod *Tigriopus japonicus* can be used as a possible biomarker for exposure to endocrine disruptors (Seo et al. 2006).

Robert Tanguay and his group were the first to use the levels of autoantibodies against Hsp27, Hsp60 and Hsp70 for evaluation of deleterious effects of various chemicals and heat on the health state of workers of harmful manufactures, such as chemical industry or steelmaking enterprise (Wu et al. 1996, 1998; Yang et al. 2007).

The goal of these studies was to evaluate lymphocyte and/or plasma Hsp70 levels and levels of autoantibodies against Hsp70 as biomarkers for assessing exposure response of steel workers to complex coke oven emissions (COEs). It was shown that lymphocyte Hsp70 levels increased in the group with intermediate exposure to emissions but decreased in the high-exposure group (Yang et al. 2007). Presence of autoantibodies to heat stress proteins was detected in workers exposed to high temperature and carbon monoxide. Antibodies to Hsp27 and Hsp71 were found more frequently in the high temperature and carbon monoxide-exposed groups than in controls. Anti-Hsp60 antibodies were only detected in workers exposed to high temperature or carbon monoxide (Wu et al. 1996). Exposure to benzene induces autoantibodies to Hsp72 (Wu et al. 1998).

Despite the obvious benefits the wide use of Hsps levels measurements as biomarkers of environmental pollution is by hindered for several reasons. First of all since the induction of Hsps is as a rule is unspecific for different stresses, sometimes

it is difficult or impossible to explain the observed increase to a definite stressor. Second, usually organisms in nature undergo multistress challenges. For example, stress from herbicides may be influenced by the temperature or humidity elevation. Furthermore, sometimes Hsps expression may be modified by certain uncontrolled factors such as parasites contamination. For example, in amphipod *Gammarus roeseli* Hsp70 level increases significantly at response to heavy metals (Pd), but in the case of infection with cystacanths of the acanthocephalan (*Polymorphus minutus*) infected individuals did not exhibit Hsp70 induction (Sures and Radszuweit 2007). Therefore, the monitoring of Hsps levels in field populations should be always carried out in parallel with measurements of many other environmental parameters that may strongly influence the results of such measurements. Specifically, in articles cited above the changes in Hsps concentrations in response to pollution were shown to correlate with: rate of growth (Lewis et al. 2001); cytochrome P450 level (Rios-Sicairos et al. 2010); P-glycoprotein level (Venn et al. 2009); metallothioneins level (Mićović et al. 2009); hemoglobin gene expression (Lee et al. 2006); embryonic lethality (Hallare et al. 2005); alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) activity (Wu et al. 1996).

4.7 Peculiar Structure and Functions of Cellular Proteins in Animals from Thermally Contrasting Environments

Apart from exploring a very ancient and universal system of heat shock genes found in all organisms studied so far, in the species dwelling under extremely cold or xeric conditions many cellular house-keeping proteins underwent adaptive evolution and acquired specific traits combining molecular stability on the one hand and structural flexibility on the other. The investigation of many enzymes from the organisms inhabiting thermally contrasting ecological niches established the correlation between the average temperature of environment and proteins stability. Thus, blue mussel (*Mytilus galloprovincialis*), a native of the Mediterranean Sea, is displacing the native blue mussel (*Mytilus trossulus*) from its habitat in central and Southern California apparently due to physiological adaptations. These adaptations enabling the former species to perform better at high temperatures include the structure and function of the enzyme malate dehydrogenase (cMDH) (Fields et al. 2001). The demonstrated relative warm adaptation of *M. galloprovincialis* cMDH may be one of physiological features that increase the competitive ability of the invasive species in warm habitats (Fields et al. 2001). Interestingly, these differences consistent with warm adaptation result from minor changes in sequence: the *M. trossulus* ortholog differs from *M. galloprovincialis* ortholog by only two substitutions.

Likewise, studies of orthologs of lactate dehydrogenase (LDH) and other vital enzymes performed in different laboratories have revealed the importance of conserving kinetic properties and structural stability during adaptation to temperature, and recently identified the types of amino acid substitutions causing such adaptive variations of proteins in ecological aspect (see below).

There are also a number of examples of qualitative adaptive strategies, when expression of distinctly different isoenzymes contributes to seasonal thermal adaptation by adjusting a particular metabolic node to new environmental conditions. Such isoforms were described for acetylcholine esterase, ferritin, choline esterase, LDH and many other enzymes (see Zakhartsev et al. 2007).

It was also shown that stabilization of proteins tertiary structure preventing their heat denaturation in thermoresistant species was usually achieved by means of specific amino acid substitutions resulting in the formation of additional intramolecular links in the vicinity of active center which stabilize the enzyme structure (Fields 2001).

As a result, in organisms resistant to heat proteins denaturation and misfolding begins at significantly higher temperatures and, hence, the activation of HSF after the disruption of HSF-Hsp90 complexes and beginning of abundant Hsps synthesis occur at much higher temperatures in comparison with related thermosensitive species.

The investigation of various (mostly fungi) thermophiles helps to understand major trend in proteins evolution to cope with constant high temperature conditions. Although proteomes of highly thermophilic prokaryotes provided a lot of valuable information in this respect and were successfully exploited in biotechnology, many proteins required for eukaryotic cell functioning under high temperature conditions are absent from bacteria or archaea. Recently, the comparative analysis of the first genome of the thermophilic fungi (*Chaetomium thermophilum*) with the genomes of two mesophilic species (*Thielavia terrestris* and *Thielavia heterothallica*) revealed consistent amino acid substitutions apparently associated to thermophily. Importantly, the same substitutions were also found in an independent lineage of thermophilic fungi (Van Noort 2013). The most consistent pattern observed in almost all fungal lineages of the thermophilic genomes is the substitutions of lysine by arginine and tryptophane (Van Noort 2013).

Furthermore, whole-genome analysis allowed to conclude that in the case of hyperthermophilic archaea the encoded proteins are characterized by an increase in frequency of charged amino acid residues and a decrease in that of polar uncharged residues as compared to the mesophilic counterparts. However, a discrepancy is noticeable in the overrepresentation of positively and negatively charged residues in hyperthermophilic proteins. The percentage of positively charged amino acids residues is significantly higher in hyperthermophiles compared to mesophiles. Furthermore, there are significant increases in aromaticity and average hydrophobicity and a decrease in the usage of polar uncharged amino acid residues (Ser, Thr, Gln and Asn) in thermophilic proteins. Examination of surface charge distribution reveals a marked increase of positive charge in the surfaces of thermophilic proteins as compared to their mesophilic counterparts (Das et al. 2006; Haney et al. 1999; Nakashima et al. 2003). These features apparently determine the extremely high thermal stability of thermophilic arhaea proteins and their ability to function at high temperatures up to near 100 °C. In general, thermophilic archaea demonstrate many adaptive differences from other organisms living in ordinary conditions, non-connected with protein structure and chaperone functions, in particular

unusual structure of membrane phospholipids, DNA supercoiling and many others (Mirambeau et al. 1984; van de Vossenberg et al. 1998).

Although Hsps and in particular Hsp70 are highly conserved in close species there are data implying that temperature selection may modulate the structure and function of the chaperones depending on specific thermal habitats. It was shown in a few studies that orthologous variants of molecular chaperone from differential thermally adapted species significantly vary in their thermal responses. Thus, comparison of Hsc70 orthologs from polar and temperate notothenoid fishes differ in their ability to prevent thermal aggregation of lactate dehydrogenase (Place and Hofmann 2005). Hsc70 from the Antarctic species lost the ability to completely refold denatured LDH at a lower temperature in comparison with Hsc70 from the temperate species. Likewise, Hsc70 from highly eurythermal marine goby (*Gillichthys mirabilis*) exhibits temperature insensitivity across the range of temperatures that are ecologically relevant for this species during luciferase refolding assays (Place and Hofmann 2001).

These data suggest that structure and functions of chaperones maps onto the thermal history of host species and that temperature selection acted on their molecular structure.

4.8 Conclusions

The broad scale analysis performed herein includes the data obtained in various organisms belonging to different taxa from mollusks and insects to mammalian species that dwell under highly variable and sometimes aggressive habitats. The accumulated evidence enables to conclude that certain chaperone-dependent molecular adaptations may be common for highly phylogenetically diverse organisms while others may be restricted to a specific group or environment. The major established regularities are summarized as follows:

1. Most thermoresistant organisms are characterized by comparatively high constitutive level of Hsp70 and other Hsps in relation to close forms from temperate regions. Such preparative defense strategy provides certain degree of preadaptation at the cellular and organism levels and usually does not require massive Hsps induction under conditions of moderate heat stress in comparison with thermosensitive forms (Fig. 4.17).
2. High constitutive levels of Hsp70 and other chaperones often observed in highly eurythermic and/or thermophilic forms may be regulated at different levels that include transcription efficacy; *Hsp* genes copy number, and stability of correspondent mRNAs or chaperones themselves. Specific mechanisms underlying constitutive and inducible levels of Hsps may vary within the same order (e.g. Diptera).
3. Different organisms explore members of specific Hsps families or their combinations (Hsp70, small Hsps or Hsp40) to respond to stressful conditions and relative role of particular chaperones or their isoforms strongly depends on the severity of the challenge

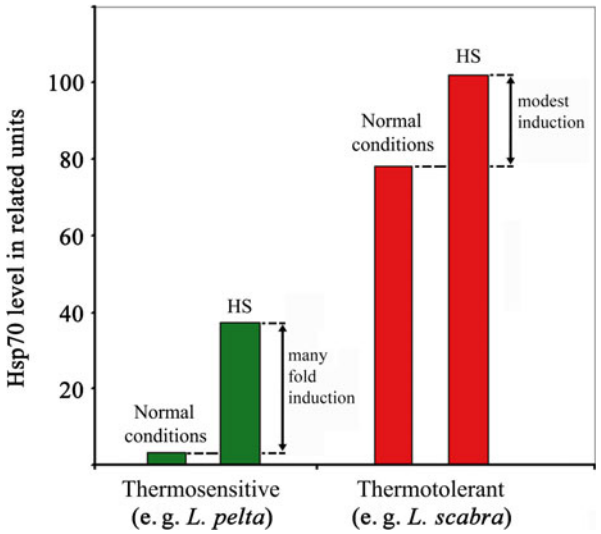

Fig. 4.17 Typical patterns of Hsp70 induction in thermosensitive and thermoresistant organisms. HSR of two thermally contrasting mollusk species are given as example. Two species of limpets (*L. pelta* and *L. scabra*) dwelling in thermally contrasting biotopes were chosen for comparison. Thermoresistant species (i.e. *L. scabra*) usually exhibit high constitutive Hsp70 level and modest induction after HS. In contrast, thermosensitive species (i.e. *L. pelta*) are characterized by low Hsp70 levels and strong induction after temperature elevation (Adapted from Dong et al. 2008 with permission)

4. Activation of HSF and induction of Hsps synthesis (threshold) in heat tolerant organisms occurs at higher temperatures in comparison with related thermosensitive forms, which implies that cellular proteins from thermophilic organisms as well as transcriptional and translational machinery in general are more stable than their mesophilic counterparts.
5. Insect life stages (e.g. larvae versus adults) often differ significantly in the heat shock response and thermoresistance. Thus, larvae of certain stenothermal cold-adapted insect species which constitutively expressing Hsp70, partially or completely lost the ability to activate Hsps synthesis after HS, while their adults preserved this ability.
6. Organisms entering certain life-stages to survive extreme conditions (desiccation, anabiosis, diapause etc.) usually accumulate large amounts of certain Hsps necessary for survival and subsequent recovery.
7. Hsps may be involved in pathogenicity of certain parasites species.
8. Different Hsps may be used as biomarkers of environmental pollution.

References

Anckar J, Sistonen L (2007) Heat shock factor 1 as a coordinator of stress and developmental pathways. Adv Exp Med Biol 594:78–88

An LH, Lei K, Zheng BH (2014) Use of heat shock protein mRNA expressions as biomarkers in wild crucian carp for monitoring water quality. Environ Toxicol Pharmacol 37:248–255

Arthur AL, Weeks AR, Sgrò CM (2008) Investigating latitudinal clines for life history and stress resistance traits in Drosophila simulans from eastern Australia. J Evol Biol 6:1470–1479

Arts MJ, Schill RO, Knigge T, Eckwert H, Kammenga JE, Köhler HR (2004) Stress proteins (hsp70, hsp60) induced in isopods and nematodes by field exposure to metals in a gradient near Avonmouth, UK. Ecotoxicology 13:739–755

Aruda AM, Baumgartner MF, Reitzel AM, Tarrant AM (2011) Heat shock protein expression during stress and diapause in the marine copepod Calanus finmarchicus. J Insect Physiol 57:665–675

Arya R, Mallik M, Lakhotia SC (2007) Heat shock genes – integrating cell survival and death. J Biosci 32:595–610

Bedulina DS, Zimmer M, Timofeyev MA (2010) Sub-littoral and supra-littoral amphipods respond differently to acute thermal stress. Comp Biochem Physiol B Biochem Mol Biol 155:413–418

Bedulina DS, Evgen'ev MB, Timofeyev MA, Protopopova MV, Garbuz DG et al (2013) Expression patterns and organization of the hsp70 genes correlate with thermotolerance in two congener endemic amphipod species (Eulimnogammarus cyaneus and E. verrucosus) from Lake Baikal. Mol Ecol 22:1416–1430

Beristain P, Gajardo G, Bossier P (2010) Species-specific RFLP pattern in the Heat Shock Protein26 gene (Hsp26): a single-locus tool for species identification and experimental testing of habitat-induced isolation in the New World Artemia species. Mol Ecol Resour 10:229–231

Bettencourt BR, Hogan CC, Nimali M, Drohan BW (2008) Inducible and constitutive heat shock gene expression responds to modification of Hsp70 copy number in Drosophila melanogaster but does not compensate for loss of thermotolerance in Hsp70 null flies. BMC Biol 22:5

Bierkens JG (2000) Applications and pitfalls of stress-proteins in biomonitoring. Toxicology 153:61–72

Biswas S, Sharma YD (1994) Enhanced expression of Plasmodium falciparum heat shock protein PFHSP70-I at higher temperatures and parasite survival. FEMS Microbiol Lett 124:425–429

Brammer CA, von Dohlen CD (2007) The evolutionary history of Stratiomyidae (Insecta: Diptera): the molecular phylogeny of a diverse family of flies. Mol Phylogenet Evol 43:660–673

Brennecke T, Gellner K, Bosch TC (1998) The lack of a stress response in Hydra oligactis is due to reduced hsp70 mRNA stability. Eur J Biochem 255:703–709

Buckley BA, Hofmann GE (2002) Thermal acclimation changes DNA-binding activity of heat shock factor 1 (HSF1) in the goby Gillichthys mirabilis: implications for plasticity in the heat-shock response in natural populations. J Exp Biol 205:3231–3240

Buckley BA, Somero GN (2009) cDNA microarray analysis reveals the capacity of the cold-adapted Antarctic fish Trematomus bernacchii to alter gene expression in response to heat stress. Polar Biol 32:403–415

Buckley BA, Owen ME, Hofmann GE (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus Mytilus) changes as a function of thermal history. J Exp Biol 204:3571–3579

Buckley BA, Place SP, Hofmann GE (2004) Regulation of heat shock genes in isolated hepatocytes from an Antarctic fish, Trematomus bernacchii. J Exp Biol 207:3649–3656

Buckley BA, Gracey AY, Somero GN (2006) The cellular response to heat stress in the goby *Gillichthys mirabilis*: a cDNA microarray and protein-level analysis. J Exp Biol 209:2660–2677

Bügl B, Staker BL, Zheng F, Kushner SR, Saper MA et al (2000) RNA methylation under heat shock control. Mol Cell 6:349–360

Carey HV, Frank CL, Seifert JP (2000) Hibernation induces oxidative stress and activation of NK-kappaB in ground squirrel intestine. J Comp Physiol B 170:551–559

Carmel J, Rashkovetsky E, Nevo E, Korol A (2011) Differential expression of small heat shock protein genes Hsp23 and Hsp40, and heat shock gene Hsr-omega in fruit flies (Drosophila melanogaster) along a microclimatic gradient. J Hered 102:593–603

Carpenter CM, Hofmann GE (2000) Expression of 70 kDa heat shock proteins in antarctic and New Zealand notothenioid fish. Comp Biochem Physiol A Mol Integr Physiol 125:229–238

Carrión J, Folgueira C, Soto M, Fresno M, Requena JM (2011) Leishmania infantum HSP70-II null mutant as candidate vaccine against leishmaniasis: a preliminary evaluation. Parasit Vectors 4:150

Chen Y, Brandizzi F (2013) IRE1: ER stress sensor and cell fate executor. Trends Cell Biol 23:547–555

Chen Q, Ma E, Behar KL, Xu T, Haddad GG (2002) Role of trehalose phosphate synthase in anoxia tolerance and development in Drosophila melanogaster. J Biol Chem 277:3274–3279
Cherlin VA, Muzichenko IV (1983) Thermobiology of two lizard species in summer in East Karakums. Zoolog J 62:897–908
Chu B, Soncin F, Price BD, Stevenson MA, Calderwood SK (1996) Sequential phosphorylation by mitogen-activated kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. J Biol Chem 271:30847–30857
Clark MS, Peck LS (2009) HSP70 heat shock proteins and environmental stress in Antarctic marine organisms: a mini-review. Mar Genomics 2:11–18
Clark TG, Abrahamsen MS, White MW (1996) Developmental expression of heat shock protein 90 in Eimeria bovis. Mol Biochem Parasitol 78:259–263
Clark MS, Fraser KP, Peck LS (2008) Antarctic marine molluscs do have an HSP70 heat shock response. Cell Stress Chaperones 13:39–49
Clarke A, Johnston IA (1996) Evolution and adaptive radiation of antarctic fishes. Trends Ecol Evol 11:212–218
Craig EA, Ingolia TD, Manseau LJ (1983) Expression of Drosophila heat-shock cognate genes during heat shock and development. Dev Biol 99:418–426
Das S, Paul S, Bag SK, Dutt C (2006) Analysis of *Nanoarchaeum equitans* genome and proteome composition: indications for hyperthermophilic and parasitic adaptation. BMC Genomics 7:186
Dehghani M, Xiao C, Money TG, Shoemaker KL, Robertson RM (2011) Protein expression following heat shock in the nervous system of Locusta migratoria. J Insect Physiol 57:1480–1488
Diamant S, Eliahu N, Rosenthal D, Goloubinoff P (2001) Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. J Biol Chem 276:39586–39591
Dong Y, Miller LP, Sanders JG, Somero GN (2008) Heat-shock protein 70 (Hsp70) expression in four limpets of the genus Lottia: interspecific variation in constitutive and inducible synthesis correlates with in situ exposure to heat stress. Biol Bull 215:173–181
Ekengren S, Hultmark D (2001) A family of *Turandot*-related genes in the humoral stress response of Drosophila. Biochem Biophys Res Commun 284:998–1103
Elekonich MM (2009) Extreme thermotolerance and behavioral induction of 70-kDa heat shock proteins and their encoding genes in honey bees. Cell Stress Chaperones 14:219–226
Ellis RJ, van der Vies SM, Hemmingsen SM (1989) The molecular chaperone concept. Biochem Soc Symp 55:145–153
Evgen'ev M, Scheiker V, Levin A (1987) Molecular mechanisms of adaptation to hyperthermia in eukaryotic organisms. I. Heat shock proteins synthesis pattern in cell culture and caterpillars of two silk worm species. Mol Biol 21:484–494
Evgen'ev MB, Zatsepina OG, Garbuz D, Lerman DN, Velikodvorskaya V et al (2004) Evolution and arrangement of the hsp70 gene cluster in two closely related species of the virilis group of Drosophila. Chromosoma 113:223–232
Feder M (1997) Necrotic fruit: a novel model system for thermal ecologists. J Therm Biol 22:1–9
Feder ME (2007) Key issues in achieving an integrative perspective on stress. J Biosci 32:433–440
Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response, evolutionary and ecological physiology. Annu Rev Physiol 61:243–282
Feder ME, Krebs RA (1997) Tissue-specific variation in Hsp70 expression and thermal damage in Drosophila melanogaster larvae. J Exp Biol 200:2007–2015
Feder ME, Cartano NV, Milos L, Krebs RA, Lindquist SL (1996) Effect of engineering *hsp*70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of Drosophila melanogaster. J Exp Biol 199:1837–1844
Fields PA (2001) Protein function at thermal extremes: balancing stability and flexibility. Comp Biochem Physiol A Mol Integr Physiol 129:417–431
Fiori A, Kucharíková S, Govaert G, Cammue BP, Thevissen K, Van Dijck P (2012) The heat-induced molecular disaggregase Hsp104 of Candida albicans plays a role in biofilm formation and pathogenicity in a worm infection model. Eukaryot Cell 11:1012–1020

Folgueira C, Carrión J, Moreno J, Saugar JM, Cañavate C, Requena JM (2008) Effects of the disruption of the HSP70-II gene on the growth, morphology, and virulence of Leishmania infantum promastigotes. Int Microbiol 11:81–89

Fraser KP, Rogers AD (2007) Protein metabolism in marine animals: the underlying mechanism of growth. Adv Mar Biol 52:267–362

Fraser KP, Clarke A, Peck LS (2007) Growth in the slow lane: protein metabolism in the Antarctic limpet Nacella concinna (Strebel 1908). J Exp Biol 210:2691–2699

Garbuz D, Evgenev MB, Feder ME, Zatsepina OG (2003) Evolution of thermotolerance and the heat-shock response: evidence from inter/intraspecific comparison and interspecific hybridization in the virilis species group of Drosophila. I. Thermal phenotype. J Exp Biol 206:2399–2408

Garbuz DG, Molodtsov VB, Velikodvorskaia VV, Evgen'ev MB, Zatsepina OG (2002) Evolution of the response to heat shock in genus Drosophila. Genetika 38:1097–1109, Russ

Garbuz DG, Zatsepina OG, Przhiboro AA, Yushenova I, Guzhova IV, Evgen'ev MB (2008) Larvae of related Diptera species from thermally contrasting habitats exhibit continuous up-regulation of heat shock proteins and high thermotolerance. Mol Ecol 17:4763–4777

Garbuz DG, Astakhova LN, Zatsepina OG, Arkhipova IR, Nudler E, Evgen'ev MB (2011a) Functional organization of hsp70 cluster in camel (Camelus dromedarius) and other mammals. PLoS One 6:e27205

Garbuz DG, Yushenova IA, Zatsepina OG, Przhiboro AA, Bettencourt BR, Evgen'ev MB (2011b) Organization and evolution of hsp70 clusters strikingly differ in two species of Stratiomyidae (Diptera) inhabiting thermally contrasting environments. BMC Evol Biol 11:74

Garland T, Adolph S (1994) Why not to do two-species comparative studies: limitations on inferring adaptation. Physiol Zool 67:797–828

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB et al (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 12:4241–4257

Gehring WJ, Wehner R (1995) Heat shock protein synthesis and thermotolerance in Cataglyphis, an ant from the Sahara desert. Proc Natl Acad Sci U S A 92:2994–2998

Girardot F, Monnier V, Tricoire H (2004) Genome wide analysis of common and specific stress responses in adult drosophila melanogaster. BMC Genomics 5:74

Golubkov S, Kemp R, Golubkov M, Balushkina E, Litvinchuk L, Gubelit Yu (2007) Biodiversity and the functioning of hypersaline lake ecosystems from Crimea Peninsula (Black Sea). Archiv für Hydrobiologie 169:79–87

Gong WJ, Golic KG (2006) Loss of Hsp70 in Drosophila is pleiotropic, with effects on thermotolerance, recovery from heat shock and neurodegeneration. Genetics 172:275–286

Gusev O, Cornette R, Kikawada T, Okuda T (2011) Expression of heat shock protein-coding genes associated with anhydrobiosis in an African chironomid Polypedilum vanderplanki. Cell Stress Chaperones 16:81–90

Hallare AV, Pagulayan R, Lacdan N, Köhler HR, Triebskorn R (2005) Assessing water quality in a tropical lake using biomarkers in zebrafish embryos: developmental toxicity and stress protein responses. Environ Monit Assess 104:171–187

Hamada FN, Rosenzweig M, Kang K, Pulver SR, Ghezzi A et al (2008) An internal thermal sensor controlling temperature preference in Drosophila. Nature 454:217–220

Hamer B, Hamer DP, Müller WE, Batel R (2004) Stress-70 proteins in marine mussel Mytilus galloprovincialis as biomarkers of environmental pollution: a field study. Environ Int 30:873–882

Hanawa T, Fukuda M, Kawakami H, Hirano H, Kamiya S, Yamamoto T (1999) The Listeria monocytogenes DnaK chaperone is required for stress tolerance and efficient phagocytosis with macrophages. Cell Stress Chaperones 4:118–128

Haney PJ, Badger JH, Buldak GL, Reich CI, Woese CR, Olsen GJ (1999) Thermal adaptation analyzed by comparison of protein sequences from mesophilic and extremely thermophilic Methanococcus species. Proc Natl Acad Sci U S A 96:3578–3583

Hayward SA, Rinehart JP, Denlinger DL (2004) Desiccation and rehydration elicit distinct heat shock protein transcript responses in flesh fly pupae. J Exp Biol 207:963–971

He B, Meng Y, Mivechi NF (1998) Glycogen synthase kinase 3β and extracellular signal-regulated kinase inactivate heat shock transcription factor 1 by facilitating the disappearance of transcriptionally active granules after heat shock. Mol Cell Biol 18:6624–6633

Hightower LE (1991) Heat shock, stress proteins, chaperones, and proteotoxicity. Cell 66:191–197

Hoffmann AA (2010) Physiological climatic limits in Drosophila: patterns and implications. J Exp Biol 213:870–880

Hoffmann AA, Weeks AR (2007) Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in Drosophila melanogaster from eastern Australia. Genetica 129:133–147

Hoffmann AA, Scott M, Partridge L, Hallas R (2003) Overwintering in Drosophila melanogaster: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. J Evol Biol 16:614–623

Hofmann GE, Buckley BA, Airaksinen S, Keen JE, Somero GN (2000) Heat-shock protein expression is absent in the antarctic fish Trematomus bernacchii (family Nototheniidae). J Exp Biol 203:2331–2339

Hottiger T, Boller T, Wiemken A (1987) Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in Saccharomyces cerevisiae cells subjected to temperature shifts. FEBS Lett 220:113–115

Hübner S, Rashkovetsky E, Kim YB, Oh JH, Michalak K et al (2013) Genome differentiation of Drosophila melanogaster from a microclimate contrast in Evolution Canyon, Israel. Proc Natl Acad Sci U S A 110:21059–21064

Jantschitsch C, Trautinger F (2003) Heat shock and UV-B-induced DNA damage and mutagenesis in skin. Photochem Photobiol Sci 2:899–903

Jensen LT, Cockerell FE, Kristensen TN, Rako L, Loeschcke V et al (2010) Adult heat tolerance variation in *Drosophila melanogaster* is not related to Hsp70 expression. J Exp Zoo A Ecol Genet Physiol 313:35–44

Jolly C, Lakhotia SC (2006) Human sat III and Drosophila hsr omega transcripts: a common paradigm for regulation of nuclear RNA processing in stressed cells. Nucleic Acids Res 34:5508–5514

Kagawa N, Mugiya Y (2000) Exposure of goldfish (Carassius auratus) to bluegills (Lepomis macrochirus) enhances expression of stress protein 70 mRNA in the brains and increases plasma cortisol levels. Zoolog Sci 17:1061–1066

Kee SC, Nobel PS (1986) Concomitant changes in high temperature tolerance and heat-shock proteins in desert succulents. Plant Physiol 80:596–598

King AM, Toxopeus J, MacRae TH (2013) Functional differentiation of small heat shock proteins in diapause-destined Artemia embryos. FEBS J 280:4761–4772

Korol A, Rashkovetsky E, Iliadi K, Nevo E (2006) Drosophila flies in "Evolution Canyon" as a model for incipient sympatric speciation. Proc Natl Acad Sci U S A 103:18184–18189

Krebs RA (1999) A comparison of Hsp70 expression and thermotolerance in adults and larvae of three Drosophila species. Cell Stress Chaperones 4:243–249

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

Krebs RA, Loeschcke V (1999) Genetic analysis of the relationship between life-history variation and heat-shock tolerance in Drosophila buzzatii. Heredity 83:46–53

Krebs RA, La Torre V, Loeschcke V, Cavicchi S (1996) Heat-shock resistance in Drosophila populations: analysis of variation in reciprocal cross progeny. Hereditas 124:47–55

Krivoruchko A, Storey KB (2010) Regulation of the heat shock response under anoxia in the turtle, Trachemys scripta elegans. J Comp Physiol B 180:403–414

Kruuv J, Glofcheski D, Cheng KH, Campbell SD, Al-Qysi HM et al (1983) Factors influencing survival and growth of mammalian cells exposed to hypothermia. I. Effects of temperature and membrane lipid perturbers. J Cell Physiol 115:179–185

La Porte PF (2005) Mytilus trossulus hsp70 as a biomarker for arsenic exposure in the marine environment: laboratory and real-world results. Biomarkers 10:417–428

La Terza A, Papa G, Miceli C, Luporini P (2001) Divergence between two Antarctic species of the ciliate Euplotes, E. focardii and E. nobilii, in the expression of heat-shock protein 70 genes. Mol Ecol 10:1061–1067

Lakhotia SC, Prasanth KV (2002) Tissue- and development-specific induction and turnover of hsp70 transcripts from loci 87A and 87C after heat shock and during recovery in Drosophila melanogaster. J Exp Biol 205:345–358

Lambowitz AM, Kobayashi GS, Painter A, Medoff G (1983) Possible relationship of morphogenesis in pathogenic fungus, Histoplasma capsulatum, to heat shock response. Nature 303:806–808

Lee SM, Lee SB, Park CH, Choi J (2006) Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: a potential biomarker of freshwater monitoring. Chemosphere 65:1074–1081

Lee K, Park JY, Yoo W, Gwag T, Lee JW, Byun MW, Choi I (2008) Overcoming muscle atrophy in a hibernating mammal despite prolonged disuse in dormancy: proteomic and molecular assessment. J Cell Biochem 104:642–656

Lewis S, Donkin ME, Depledge MH (2001) Hsp70 expression in Enteromorpha intestinalis (Chlorophyta) exposed to environmental stressors. Aquat Toxicol 51:277–291

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191

López-Maury L, Marguerat S, Bähler J (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat Rev Genet 9:583–593

Lozovskaya ER, Evgen'ev MB (1984) Heat shock in and regulation of genome activity. Mol Biol 20:142–185

Lyashko VN, Vikulova VK, Chernicov VG, Ivanov VI, Ulmasov KA et al (1994) Comparison of the heat shock response in ethnically and ecologically different human populations. Proc Natl Acad Sci U S A 91:12492–12495

Lyne R, Burns G, Mata J, Penkett CJ, Rustici G et al (2003) Whole-genome microarrays of fission yeast: characteristics, accuracy, reproducibility, and processing of array data. BMC Genomics 4:27

Lyons RE, Johnson AM (1995) Heat shock proteins of Toxoplasma gondii. Parasite Immunol 17:353–359

Malmendal A, Overgaard J, Bundy JG, Sørensen JG, Nielsen NC et al (2006) Metabolomic profiling of heat stress: hardening and recovery of homeostasis in Drosophila. Am J Physiol Regul Integr Comp Physiol 291:205–212

Martínez DE, Bridge D (2012) Hydra, the everlasting embryo, confronts aging. Int J Dev Biol 56:479–487

Martinez J, Perez Serrano J, Bernadina WE, Rodriguez-Caabeiro F (1999) Influence of parasitization by Trichinella spiralis on the levels of heat shock proteins in rat liver and muscle. Parasitology 118:201–209

McFadden MW (1967) Soldier fly larvae in America north of Mexico. Proc US Natl Museum 121:1–72

Michalak P, Minkov I, Helin A, Lerman DN, Bettencourt BR et al (2001) Genetic evidence for adaptation-driven incipient speciation of Drosophila melanogaster along a microclimatic contrast in "Evolution Canyon," Israel. Proc Natl Acad Sci U S A 98:13195–13200

Mićović V, Bulog A, Kučić N, Jakovac H, Radošević-Stašić B (2009) Metallothioneins and heat shock proteins 70 in marine mussels as sensors of environmental pollution in Northern Adriatic Sea. Environ Toxicol Pharmacol 28:439–447

Mirambeau G, Duguet M, Forterre P (1984) ATP-dependent DNA topoisomerase from the archaebacterium Sulfolobus acidocaldarius. Relaxation of supercoiled DNA at high temperature. J Mol Biol 179:559–563

Mitrovski P, Hoffmann AA (2001) Postponed reproduction as an adaptation to winter conditions in Drosophila melanogaster: evidence for clinal variation under semi-natural conditions. Proc Biol Sci 268:2163–2168

Mizrahi T, Heller J, Goldenberg S, Arad Z (2012) Heat shock proteins and survival strategies in congeneric land snails (Sphincterochila) from different habitats. Cell Stress Chaperones 17:523–527

Morales-Hojas R, Vieira CP, Vieira J (2006) The evolutionary history of the transposable element Penelope in the Drosophila virilis group of species. J Mol Evol 63:262–273

Morgan RW, Christman MF, Jacobson FS, Storz G, Ames BN (1986) Hydrogen peroxide-inducible proteins in Salmonella typhimurium overlap with heat shock and other stress proteins. Proc Natl Acad Sci U S A 83:8059–8063

Morrow G, Heikkila JJ, Tanguay RM (2006) Differences in the chaperone-like activities of the four main small heat shock proteins of Drosophila melanogaster. Cell Stress Chaperones 11:51–60

Muhich ML, Boothroyd JC (1989) *Synthesis of Trypanosome hsp70* mRNA is resistant to disruption of trans-splicing by heat shock. J Biol Chem 264:7107–7110

Murray JI, Whitfield ML, Trinklein ND, Myers RM, Brown PO, Botstein D (2004) Diverse and specific gene expression responses to stresses in cultured human cells. Mol Biol Cell 15:2361–2374

Nadal E, Ammerer G, Posas F (2011) Controlling gene expression in response to stress. Nat Rev Genet 12:833–845

Nakashima H, Fukuchi S, Nishikawa K (2003) Compositional changes in RNA, DNA and proteins for bacterial adaptation to higher and lower temperatures. J Biochem 133:507–513

Neumann S, Ziv E, Lantner F, Schechter I (1993) Regulation of *HSP70* gene expression during the life cycle of the parasitic helminth Schistosoma mansoni. Eur J Biochem 212:589–596

Norris CE, Hightower L (2000) The heat shock response in tropical and desert fishes (genus Poeciliopsis). In: Storey KB, Storey J (eds) Environmental stressors and gene responses. Elsevier Science, Amsterdam, p 303

Ono M, Igarashi T, Ohno E, Masami S (1995) Unusual thermal defense by a honeybee against mass attack by hornets (Vespa mandarinia japonica). Nature 377:334–336

Panchapakesan J, Daglis M, Gatenby P (1992) Antibodies to 65 kDa and 70 kDa heat shock proteins in rheumatoid arthritis and systemic lupus erythematosus. Immunol Cell Biol 70:295–300

Parkash R, Kalra B, Sharma V (2008a) Changes in cuticular lipids, water loss and desiccation resistance in a tropical drosophilid: analysis of variation between and within populations. Fly (Austin) 2:189–197

Parkash R, Rajpurohit S, Ramniwas S (2008b) Changes in body melanisation and desiccation resistance in highland vs. lowland populations of D. melanogaster. J Insect Physiol 54:1050–1056

Parsons PA (1973) Genetics of resistance to environmental stresses in Drosophila populations. Annu Rev Genet 7:239–265

Patriarca EJ, Maresca B (1990) Acquired thermotolerance following heat shock protein synthesis prevents impairment of mitochondrial ATPase activity at elevated temperatures in Saccharomyces cerevisiae. Exp Cell Res 190:57–64

Patterson JT, Stone WS (1952) Evolution in the genus Drosophila. The Macmillan Company, New York, p 610

Petricorena ZL, Somero GN (2007) Biochemical adaptations of notothenioid fishes: comparisons between cold temperate South American and New Zealand species and Antarctic species. Comp Biochem Physiol A Mol Integr Physiol 147:799–807

Place SP, Hofmann GE (2001) Temperature interactions of the molecular chaperone Hsc70 from the eurythermal marine goby Gillichthys mirabilis. J Exp Biol 204:2675–2682

Place SP, Hofmann GE (2005) Comparison of Hsc70 orthologs from polar and temperate notothenioid fishes: differences in prevention of aggregation and refolding of denatured proteins. Am J Physiol Regul Integr Comp Physiol 288:1195–1202

Place P, Mackenzie LZ, Hofmann G (2004) Constitutive roles for inducible genes: evidence for the alteration in expression of the inducible *hsp70* gene in Antarctic notothenioid fishes. Am J Physiol Regul Integr Comp Physiol 287:429–436

Podrabsky JE, Somero GN (2007) An inducible 70 kDa-class heat shock protein is constitutively expressed during early development and diapause in the annual killifish Austrofundulus limnaeus. Cell Stress Chaperones 12:199–204

Podrabsky JE, Lopez JP, Fan TW, Higashi R, Somero GN (2007) Extreme anoxia tolerance in embryos of the annual killifish Austrofundulus limnaeus: insights from a metabolomics analysis. J Exp Biol 210:2253–2266

Pörtner HO, Peck L, Somero G (2007) Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Philos Trans R Soc Lond B Biol Sci 362:2233–2258

Pritchard G (1991) Insects in thermal springs. Mem Entomol Soc Can 155:89–106

Privalov PL (1990) Cold denaturation of proteins. Crit Rev Biochem Mol Biol 25:281–305
Raboy B, Sharon G, Parag HA, Shochat Y, Kulka RG (1991) Effect of stress on protein degradation: role of the ubiquitin system. Acta Biol Hung 42:3–20
Radłowska M, Pempkowiak J (1998) Induction of stress proteins in the presence of cadmium in the Baltic blue mussel Mytilus trossulus. Oceanologia 40:153–156
Radłowska M, Pempkowiak J (2002) Stress-70 as indicator of heavy metals accumulation in blue mussel Mytilus edulis. Environ Int 27:605–608
Rashkovetsky E, Iliadi K, Michalak P, Lupu A, Nevo E et al (2006) Adaptive differentiation of thermotolerance in Drosophila along a microclimatic gradient. Heredity 96:353–359
Rasnitsyn AP, Quicke DLJ (eds) (2002) History of insects. Kluwer Publ, Dordrecht
Riehle MM, Bennett AF, Long AD (2005) Changes in gene expression following high-temperature adaptation in experimentally evolved populations of E. coli. Physiol Biochem Zool 78:299–315
Rinehart JP, Hayward SA, Elnitsky MA, Sandro LH, Lee RE Jr, Denlinger DL (2006) Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. Proc Natl Acad Sci U S A 103:14223–14227
Rios-Sicairos J, Betancourt-Lozano M, Leal-Tarin B, Hernandez-Cornejo R, Aguilar-Zarate G et al (2010) Heat-shock protein (Hsp70) and cytochrome P-450 (CYP1A) in the white mullet Mugil curema (Pisces:Mugilidae) as biomarkers to assess environmental quality in coastal lagoons. J Environ Sci Health A Tox Hazard Subst Environ Eng 45:68–74
Rozkošný R (1982) A biosystematic study of the European Stratiomyidae (Diptera). Junk Publishers, The Hague
Rozkošný R (1997) Family stratiomyidae. In: Papp L, Darvas B (eds) Contributions to a manual of palaearctic diptera. Nematocera and lower brachycera, vol 2. Science Herald, Budapest, pp 387–411
Salotra P, Chauhan D, Ralhan R, Bhatnagar R (1995) Tumour necrosis factor-alpha induces preferential expression of stress proteins in virulent promastigotes of Leishmania donovani. Immunol Lett 44:1–5
Sato S, Ishikawa H (1997) Expression and control of an operon from an intracellular symbiont which is homologous to the groE operon. J Bacteriol 179:2300–2304
Schill RO, Steinbruck GH, Kohler HR (2004) Stress gene (hsp70) and quantitative expression in Milnesium tardigradum (Tardigrada) during active and cryptobiotic stages. J Exp Biol 207:1607–1613
Schlesinger MJ (1990) Heat shock proteins. J Biol Chem 265:12111–12114
Schmidt PS, Paaby AB (2008) Reproductive diapause and life-history clines in North American populations of Drosophila melanogaster. Evolution 62:1204–1215
Schmidt PS, Paaby AB, Heschel MS (2005) Genetic variance for diapause expression and associated life histories in Drosophila melanogaster. Evolution 59:2616–2625
Schmidt-Nilsen K (1972) Animals of the deserts. Nauka, Leningrad, p 318
Schröder HC, Batel R, Hassanein HM, Lauenroth S, Jenke H et al (2000) Correlation between the level of the potential biomarker, heat-shock protein, and the occurrence of DNA damage in the dab, Limanda limanda: a field study in the North Sea and the English Channel. Mar Environ Res 49:201–215
Schwerin M, Maak S, Hagendorf A, von Lengerken G, Seyfert HM (2002) A 3′-UTR variant of the inducible porcine hsp70.2 gene affects mRNA stability. Biochim Biophys Acta 1578:90–94
Seo JS, Park TJ, Lee YM, Park HG, Yoon YD, Lee JS (2006) Small heat shock protein 20 gene (*Hsp20*) of the intertidal copepod *Tigriopus japonicus* as a possible biomarker for exposure to endocrine disruptors. Bull Environ Contam Toxicol 76:566–572
Shilova VY, Garbuz DG, Myasyankina EN, Chen B, Evgen'ev MB et al (2006) Remarkable site specificity of local transposition into the Hsp70 promoter of Drosophila melanogaster. Genetics 173:809–820
Sills NS, Gorham DA, Carey HV (1998) Stress protein expression in a mammalian hibernator. FASEB J 12:A379
Singh R, Kølvraa S, Bross P, Jensen UB, Gregersen N et al (2006) Reduced heat shock response in human mononuclear cells during aging and its association with polymorphisms in HSP70 genes. Cell Stress Chaperones 11:208–215

Somero GN (2005) Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Front Zoolog 2:1–9

Somero GN, DeVries AL (1967) Temperature tolerance of some Antarctic fishes. Science 156:257–258

Spicer G, Bell C (2002) Molecular phylogeny of the Drosophila virilis species group (Diptera: Drosophilidae) inferred from mitochondrial 12S and 16S ribosomal RNA genes. Ann Entomol Soc Am 95:156–161

Steinert SA, Pickwell GV (1988) Expression of heat shock protein and metallothionein in mussels exposed to heat stress and metal ion challenge. Mar Environ Res 24:211–214

Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol Biochem Zool 73:200–208

Stratman R, Markow TA (1998) Resistance to thermal stress in desert Drosophila. Funct Ecol 12:965–970

Sugahara M, Sakamoto F (2009) Heat and carbon dioxide generated by honeybees jointly act to kill hornets. Naturwissenschaften 96:1133–1136

Sures B, Radszuweit H (2007) Pollution-induced heat shock protein expression in the amphipod Gammarus roeseli is affected by larvae of Polymorphus minutus (Acanthocephala). J Helminthol 81:191–197

Szalay MS, Kovács IA, Korcsmáros T, Böde C, Csermely P (2007) Stress-induced rearrangements of cellular networks: consequences for protection and drug design. FEBS Lett 581:3675–3680

Timofeyev MA, Kirichenko KA (2004) Experimental estimation of the role of abiotic factors in containment of endemics beyond the bounds of Lake Baikal. Sib Jecol Zh 1:41–50

Timofeyev M, Shatilina Z (2007) Different preference reactions of three lake Baikal endemic amphipods to temperature and oxygen are correlated with symbiotic life. Crustaceana 80:129–138

Todgham AE, Hoaglund EA, Hofmann GE (2007) Is cold the new hot? Elevated ubiquitin-conjugated protein levels in tissues of Antarctic fish as evidence for cold-denaturation of proteins in vivo. J Comp Physiol B 177:857–866

Toivola DM, Strnad P, Habtezion A, Omary MB (2010) Intermediate filaments take the heat as stress proteins. Trends Cell Biol 20:79–91

Tomanek L (2005) Two-dimensional gel analysis of the heat-shock response in marine snails (genus Tegula): interspecific variation in protein expression and acclimation ability. J Exp Biol 208:3133–3143

Tomanek L (2008) The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat-shock response. Physiol Biochem Zool 81:709–717

Tomanek L, Somero GN (1999) Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus Tegula) from different thermal habitats: implications for limits of thermotolerance and biogeography. J Exp Biol 202:2925–2936

Tomanek L, Somero GN (2000) Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (Genus tegula) from different tidal heights. Physiol Biochem Zool 73:249–256

Tsoulfa G, Rook GA, Bahr GM, Sattar MA, Behbehani K et al (1989) Elevated IgG antibody levels to the mycobacterial 65-kDa heat shock protein are characteristic of patients with rheumatoid arthritis. Scand J Immunol 30:519–527

Ul'masov KhA, Ovezmukhammedov A, Karaev KK, Evgen'ev MB (1988) Molecular mechanisms of adaptation to hyperthermia in higher organisms. III. Induction of heat-shock proteins in two Leishmania species. Mol Biol 22:1583–1589

Ulmasov KA, Shammakov S, Karavaev K, Evgen'ev MB (1992) Heat shock proteins and thermoresistance in lizards. Proc Natl Acad Sci U S A 89:1666–1670

Ulmasov HA, Karaev KK, Lyashko VN, Evgen'ev MB (1993) Heat-shock response in camel (*Camelus dromedarius*) blood cells and adaptation to hyperthermia. Comp Biochem Physiol B 106:867–872

Ulmasov K, Zatsepina O, Molodtsov V, Evgen'ev M (1999) Natural body temperature and kinetics of heat-shock protein synthesis in the toad-headed agamid lizard Phrynocephalus interscapularis. Amphibia Reptilia 20:1–9

van de Vossenberg JL, Driessen AJ, Konings WN (1998) The essence of being extremophilic: the role of the unique archaeal membrane lipids. Extremophiles 2:163–170
Van Noort V, Bradatsch B, Arumugam M, Amlacher S, Bange G et al (2013) Consistent mutational paths predict eukaryotic thermostability. BMC Evol Biol 13:7
Velazquez JM, Sonoda S, Bugaisky G, Lindquist S (1983) Is the major Drosophila heat shock protein present in cells that have not been heat shocked? J Cell Biol 96:286–290
Venn AA, Quinn J, Jones R, Bodnar A (2009) P-glycoprotein (multi-xenobiotic resistance) and heat shock protein gene expression in the reef coral Montastraea franksi in response to environmental toxicants. Aquat Toxicol 93:188–195
Vigh L, Nakamoto H, Landry J, Gomez-Munoz A, Harwood JL, Horvath I (2007) Membrane regulation of the stress response from prokaryotic models to mammalian cells. Ann N Y Acad Sci 1113:40–51
Voit EO, Radivoyevitch T (2000) Biochemical systems analysis of genome-wide expression data. Bioinformatics 16:1023–1037
Votintsev KK (1961) The hydrochemistry of Lake Baikal. Trudy Baikalskoj Limnologicheskoj Stancii Akademii Nauk SSSR, Vosochno-Sibirskij Filial 20:1–312
Wehner R, Marsh AC, Wehner S (1992) Desert ants on a thermal tightrope. Nature 357:586–587
Welch WJ, Suhan JP (1985) Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. J Cell Biol 101:1198–1211
Welch WJ, Suhan JP (1986) Cellular and biochemical events in mammalian cells during and after recovery from physiological stress. J Cell Biol 103:2035–2052
Welker S, Rudolph B, Frenzel E, Hagn F, Liebisch G et al (2010) Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function. Mol Cell 39:507–520
Whalley PES, Jarzembowski EA (1985) Fossil insects from the lithographic limestone of Montsech (late Jurassic-early Cretaceous), Lérida Province, Spain. Bull Br Museum Natl Hist (Geology) 38:381–412
Wiesgigl M, Clos J (2001) Heat shock protein 90 homeostasis controls stage differentiation in Leishmania donovani. Mol Biol Cell 12:3307–3316
Woodley NE (2001) A world catalog of the Stratiomyidae (Insecta: Diptera). Myia 11:1–473
Wu TC, Tanguay RM, Wu Y, He HZ, Xu DG et al (1996) Presence of antibodies to heat stress proteins and its possible significance in workers exposed to high temperature and carbon monoxide. Biomed Environ Sci 9:370–379
Wu T, Yuan Y, Wu Y, He H, Zhang G, Tanguay RM (1998) Presence of antibodies to heat stress proteins in workers exposed to benzene and in patients with benzene poisoning. Cell Stress Chaperones 3:161–167
Xiong Q, Chai J, Xiong H, Li W, Huang T et al (2013) Association analysis of HSP70A1A haplotypes with heat tolerance in Chinese Holstein cattle. Cell Stress Chaperones 18:711–718
Yang X, Zheng J, Bai Y, Tian F, Yuan J et al (2007) Using lymphocyte and plasma Hsp70 as biomarkers for assessing coke oven exposure among steel workers. Environ Health Perspect 115:573–577
Yost HJ, Lindquist S (1986) RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell 45:185–193
Zakhartsev M, Lucassen M, Kulishova L, Deigweiher K, Smirnova YA et al (2007) Differential expression of duplicated LDH-A genes during temperature acclimation of weatherfish Misgurnus fossilis. Functional consequences for the enzyme. FEBS J 274:1503–1513
Zatsepina OG, Ulmasov KA, Beresten SF, Molodtsov VB, Rybtsov SA, Evgen'ev MB (2000) Thermotolerant desert lizards characteristically differ in terms of heat-shock system regulation. J Exp Biol 203:1017–1025
Zatsepina OG, Velikodvorskaia VV, Molodtsov VB, Garbuz D, Lerman DN et al (2001) A Drosophila melanogaster strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. J Exp Biol 204:1869–1881