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Taxa-specific heat shock proteins are over-expressed with crowding in the Australian plague locust

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ABSTRACT

Most heat shock proteins (Hsps) function as molecular chaperones that help organisms to cope with stress. Although the best empirical evidence is related to heat shock, there is evidence that Hsps and their encoding genes are involved in resistance to other ecologically relevant types of stresses such as those imposed by high population density. We quantified density-dependent gene expression of large (i.e. *Hsp40*, *Hsc70* and *Hsp90*) and small (*Hsp20.5*, *Hsp20.6* and *Hsp20.7*) heat shock genes in neural tissue of fifth-instar nymphs of the Australian plague locust, *Chortoicetes terminifera*, using reverse transcription-quantitative PCR. Locusts are of particular interest when studying the influence of stress induced by high population density since they show an extreme form of phenotypic plasticity changing from a cryptic solitarious phase to a swarming gregarious phase. Crowding led to a synchronous and sustained 2–3 fold increase in the expression of only two *Hsp* genes, *Hsp20.5* and *Hsp20.7*, which do not BLAST with any known animal sequences and therefore are likely to be unique to members of the Orthoptera. This study opens a range of experiments to investigate the possibility of specific roles for these two small Hsps in the resistance to stressful conditions imposed by crowded environments and/or the expression of gregarious behavior as well as their evolutionary significance to locusts whose populations are regularly exposed to high density conditions in the field.

1. Introduction

Heat shock proteins (Hsps) are a class of protective agents (chaperones) synthesized in response to various types of stress (e.g. heat shock, metal toxicity, starvation, disease), but several of them are also essential under normal growth conditions (Agashe and Hartl, 2000). Hsps mediate numerous vital cellular functions by modifying the structures of proteins by re-folding them from a denatured to a native structure. In addition to protein folding, they have been shown to assist protein translocation through a number of cellular membrane systems. Heat shock proteins are commonly divided into families on the basis of molecular weight (e.g. Hsp70, Hsp90, etc.) and sequence homologies. Several large gene families (i.e. of sizes above 30 kDa) are highly conserved across all eukaryotes, with chaperone activities that are ATP-dependent and often assisted by co-chaperones. While they have been found in almost all organisms investigated so far, the small heat shock proteins (i.e. of sizes below 30 kDa) are the most divergent, with the number of Hsps varying among species. The chaperone-like activity of these often constitutively expressed and relatively little studied Hsps has only been shown in vitro so far, where they interact with non-native proteins in an ATP-independent manner (Jakob et al., 1993).

The activities of Hsps may play seemingly conflicting roles in the expression of phenotypic variation arising from the effects of different environments on individuals of the same genotype (reviewed in Williams et al., 2009 for the widely-studied Hsp70 and Hsp90). Such phenotypic plasticity (i.e. *sensu* Schlichting and Pigliucci, 1998) is adaptive in many cases and allows organisms to exploit temporally and spatially heterogeneous environments (West-Eberhard, 2003). On the one hand, Hsps may limit phenotypic plasticity through their protective effects of the cellular response to stress (Williams et al., 2009). For example, genetic or pharmacological manipulations showed that Hsp expression buffers against developmental abnormalities in *Drosophila melanogaster* (Rutherford and Lindquist, 1998; Roberts and Feder, 1999; Williams et al., 2009).

Conversely, the protective role of Hsps may actually facilitate phenotypic plasticity by helping to maintain the expression of an

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alternative phenotype associated with stress. One such chaperone is Hsp70, one of a number of classes of conserved heat shock proteins that can be inducibly expressed. Notably upregulated after or during stress, it is generally seen as a largely anti-apoptotic protein indicative of environmentally mediated cellular stresses that promote protein denaturation and aggregation. Once expressed, the inducible Hsp70 appears to play a role in the age-related transition from relatively sedentary hive work to metabolically demanding foraging behavior in the honey bee (Williams et al., 2009). Increased expression of this Hsp in foragers' thoraces may protect heavily-taxed flight muscles against thermogenesis and protein degradation.

While Hsp70 may maintain phenotypic variation by up-regulation in response to stress, other Hsps may promote novel manifestations of alternative phenotypes through impairment of their buffering capacity during periods of stress. One such candidate is Hsp90 which is one of the most abundant molecular chaperones expressed in cells. Because of its essential function in chaperoning diverse regulators of growth and development in the absence of stress (e.g. cellular kinases, transcription factors, etc), Hsp90 acts as a buffering system against the effects of random genetic variation and environmental change to ensure normal development (Picard, 2002; Sangster and Queitsch, 2005). During stressful conditions, however, the ability of Hsp90 to maintain the functional pathway might be compromised and reveal cryptic genetic variation resulting in novel phenotypes, as shown in the fruit fly *Drosophila melanogaster* and the mustard plant *Arabidopsis thaliana* (Rutherford and Lindquist, 1998; Queitsch et al., 2002). Therefore, even in the absence of differential expression, Hsp90 may still play a role as a capacitor for alternative phenotypes (Queitsch et al., 2002; Jarosz and Lindquist, 2010) and has been discussed as possibly being involved in the evolution of polyphenisms, including phase change in locusts (Williams et al., 2009; Pener and Simpson, 2009).

Locust species express an extreme form of density-dependent phenotypic plasticity known as 'phase polyphenism'. Locust population densities tend to fluctuate wildly with locusts at high densities actively forming groups and living gregariously in mobile migratory bands as juveniles and swarms as adults (Uvarov, 1966; Pener and Simpson, 2009). As an example, in the Australian plague locust, *Chortoicetes terminifera*, gregarious 5th instar nymphs aggregate in bands that are commonly 200–2000 m long, march 50–200 m per day and can be as dense as 1000–5000 m^{-2} at the front (Hunter, 2004; Buhl et al., 2011). As a result of living in these groups, locusts experience long periods of environmental stress, such as competition for food and mates (Simpson et al., 2002; Seidelmann et al., 2005), increased exposure to disease (Wilson et al., 2002) and high risk of cannibalism (Sword et al., 2005; Hansen et al., in press), which might be predicted to have detrimental effects on individual physiology, development and survival. Studying the influence of high population density on Hsps expression in locusts is the first step towards an understanding of the possible roles of Hsps in either the evolution or maintenance of behavioral phase polyphenism.

It now seems clear that the inducible Hsp70 is widely up-regulated at the mRNA level in response to stresses imposed by high density conditions in laboratory or stock populations of insects (Sorensen and Loeschcke, 2001), fish (Gornati et al., 2004; Caipang et al., 2008; Salas-Leiton et al., 2010), mammals (Pan et al., 2006) and birds (Beloor et al., 2010). Consequently, it has been hypothesized that increased Hsp70 expression mediates resistance to stress and increases survival under high population density conditions (see Sorensen and Loeschcke, 2001). Whether additional Hsps contribute to stress resistance at high densities is unknown. For instance, there is controversy about the density-dependent regulation of Hsp90 which plays a central role in regulating normal growth and development (Gornati et al., 2004; Wang et al., 2007; Beloor et al., 2010; Salas-Leiton et al., 2010). To our knowledge, there is only a single study by Wang et al. (2007) that directly tested the effect of crowded conditions on the expression of a range of large and small Hsp families. The authors studied the widespread migratory locust, *Locusta migratoria*, and showed constitutive expression and up-regulation with crowding of most of the Hsps, i.e. members of the Hsp70 and Hsp90 gene families and the small heat shock proteins (sHsps). Of note is that *L. migratoria* showed a complex dynamic pattern of regulation of Hsp90 transcript abundance, with levels of mRNA decreasing rather than increasing within days of crowding, suggesting a different function possibly not related to stresses imposed by longer-term crowding.

In the present study, we quantified density-dependent gene expression of the six Hsp genes previously identified in *Locusta migratoria* in fifth-instar nymphs of the Australian plague locust, *Chortoicetes terminifera,* using reverse transcription-quantitative PCR. This species is a member of the same subfamily (Oedipodinae) and one of Australia's most significant agricultural pests. Despite the lack of striking density-dependent phenotypic changes in colour and morphology which are seen so prominently in *L. migratoria*, *C. terminifera* was recently shown to exhibit full density-dependent behavioural gregarization within days of crowding (Gray et al., 2009; Cullen et al., 2010). Whether stress physiology, and particularly Hsp expression, varies in a similar manner to that of the related *L. migratoria* has not yet been investigated. We were specifically interested in the possibility of a global heat shock protein response to high densities, including both large and small Hsp families, and in the time course of induced changes in gene expression.

2. Methods

2.1. cDNA samples

We generated expression profiles of the Hsp gene transcripts from total RNA samples that had been previously prepared by Chapuis et al. (2011) from solitary and crowded Australian plague locusts. Briefly, a *C. terminifera* rearing colony was established in 2006 with approximately 25,000 locusts. The colony had since been maintained under crowded conditions, and retained at the time of our study most of the genetic variation found in the field (Berthier et al., 2010). Fifth instar nymphs for this study had been reared long-term under either isolated or crowded conditions, respectively, as described in Chapuis et al. (2011). A treatment group of locusts that had been crowded for only 24 h was also included to examine expression levels in short vs. long-term gregarious phase insects. To this aim, half of solitarious 5th instar nymphs were removed from their individual rearing cages 24 h post-ecdysis, maintained as for the gregarious culture and dissected after 24 h. The three rearing density treatment groups are hereafter referred as to isolated, long-term crowded and 24h-crowded. All locust neural tissues were dissected the second day of the 5th larval stage. The brain, optic lobes and the three thoracic ganglia of each insect were dissected. RNA extractions were performed on pooled tissues of 2 males and 2 females (see Chapuis et al. (2011) for details on total RNA sample purity and concentration). Five replicates of such pooled samples were analyzed for each of the three treatment groups (long-term crowded, isolated, and 24 h-crowded).

New first strand cDNA samples were specifically synthesized for this study following Chapuis et al. (2011). We pooled together 5 μl of each cDNA sample to determine reaction efficiency of each of the qPCR assays by means of a standard curve consisting of