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Latitudinal clines in heat tolerance, protein synthesis rate and transcript level of a candidate gene in *Drosophila melanogaster*

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ABSTRACT

The occurrence of climatic adaptation in *Drosophila melanogaster* is highlighted by the presence of latitudinal clines in several quantitative traits, particularly clines in adult heat knockdown tolerance that is higher in tropical populations. However the presence of latitudinal patterns in physiological characteristics that may underlie these traits have rarely been assessed. Protein synthesis has been implicated as an important physiological process that influences thermal tolerance, and this has not been examined in a clinal context. Here, we characterise latitudinal variation in *D. melanogaster* from eastern Australia in both adult heat knockdown tolerance and rates of protein synthesis following rearing at both 25 °C, approximating summer conditions, and 18 °C approximating winter development. We also examined clinal variation in the predominant nuclear transcript of the heat-inducible RNA gene *hsr-omega*, which has been implicated in regulating protein synthesis. We find significant clines in heat-hardened tolerance when cultured at both 18 and 25 °C – tolerance increased towards the low latitude tropics. Rates of protein synthesis measured in ovarian tissue also associated negatively with latitude, however the presence of the clines depended on rearing temperature and heat stress conditions. Finally, *omega-n* levels measured without heat stress showed a positive linear cline. When measured after a mild heat stress higher levels of *omega-n* were detected and the clinal pattern became parabolic – mid-latitude populations had lower levels of the transcript. While congruent latitudinal trends were detected for these three traits, only a low level of positive association was detected between protein synthesis and thermal tolerance providing little evidence that these traits are related at the level of cellular physiology. However the new clinal patterns of protein synthesis and *hsr-omega* variation suggest that these variables exert important influences on traits involved with latitudinal climatic adaptation.

1. Introduction

The threat that global warming poses to the continuing existence of many species has heightened our awareness of the importance of adaptation to thermal extremes in plant and animal populations (Parmesan, 1996; Deutsch et al., 2008). Some of the best examples of such thermal adaptation are in single species that are spread latitudinally over a broad range of climatic regions (e.g., Bahrndorff et al., 2006; Kuo and Sanford, 2009; Zani et al., 2005). In one species, *Drosophila melanogaster*, regional differentiation in adult heat knockdown tolerance is well characterised (Guerra et al., 1997; Fallis et al., 2011), in particular the latitudinal cline along about 3000 km of the east Australian coast (Sgrò et al., 2010). The presence of clinal variation in traits has been used as evidence for natural selection, and temperature is often proposed as the main driver of latitudinal clines in traits and allelic frequencies (Endler, 1977). Clinal distributions therefore provide an opportunity to investigate the physiological mechanisms and genetic basis of ongoing thermal stress adaptation (Hoffmann, 2010).

Latitudinal differentiation of *D. melanogaster* occurs for numerous life-history, morphological and stress resistance traits, and for many genetic markers that include the heat stress genes *hsp70*, *hsr-omega*, and small *hsps* (Hoffmann and Weeks, 2007). While the exact nature of thermal selection that underpins latitudinal clines is not fully understood, the thermal stress experienced by *D. melanogaster* populations across the climatic regions is known to be different. Temperatures experienced by tropical populations are on average higher than temperate populations and tropical environments are more stable than the cooler and highly variable temperate environments in which hot and cold extremes are experienced more frequently (Ghalambor et al., 2006; Hoffmann, 2010). Thermal plasticity, the internal physiological changes that improves survival and reproductive performance under stressful temperatures, is the major way that ectothermic species are adapted to periodic temperature stress. Whether stable tropical or fluctuating temperate environments have the greatest capacity for plasticity of thermal resistance traits is still unclear – the question is well

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researched in *Drosophila* (Trotta et al., 2006; Overgaard et al., 2011; Cooper et al., 2012) and of ongoing general interest (Klopfer and MacArthur, 1960; Brattstrom, 1970; West-Eberhard, 1989; Chown and Terblanche, 2007). The plasticity of thermal-tolerance traits means that both culture temperature and assay conditions influence the strength of latitudinal patterns (Hoffmann et al., 2005; Terblanche et al., 2011). In fact latitudinal clines in adult *Drosophila* heat-knockdown tolerance are stronger after a brief hardening heat stress and the cline disappears altogether when dynamic assay conditions using natural rates of temperature increase are used (Sgrò et al., 2010). Certainly, rearing and testing conditions need to be taken into account when assessing clinal changes in thermal tolerance.

Despite the numerous associations of genetic markers with latitude and thermal tolerance in *D. melanogaster* (Hoffmann et al., 2003; Kolaczkowski et al., 2011) we have limited insight into the cellular and physiological mechanisms that underlie such variation. One study suggests that metabolic rate may be an important physiological trait involved with climatic adaptation, particularly under cooler growth conditions (Berrigan and Partridge, 1997). However, recent application of genomic technologies to understanding the genetic basis of thermal tolerance variation indicate that many candidate genes fall into the 'GO'-groupings of *translation* and *regulation of transcription* (Leemans et al., 2000; Sorensen et al., 2005; Laayouni et al., 2007) suggesting that protein synthesis may be a relevant underlying process. During the cellular heat stress response the RNA expression of many hundreds of genes are up- or down-regulated following heat stress (Sorensen et al., 2005; Kültz, 2005). However, in *Drosophila* within the first 2 h following mild heat stress changes in levels of protein synthesis can be attributed largely to the production of new heat shock proteins (Hsps) and the curtailing of synthesis of normal cellular protein (the 25 °C proteins; Lindquist, 1980; Storti et al., 1980). In fact early studies of heat tolerant strains of *D. melanogaster* demonstrate that changes in these processes are important to heat tolerance (Stephanou et al., 1983). Furthermore, a recent association between variation in heat hardening capacity and levels of total protein synthesis following a heat-stimulus support this idea (Johnson et al., 2009a). We know that Hsps are quickly up-regulated following a mild heat stress, that they have diverse modes of action (Feder and Hoffman, 1999), and that they are involved in protecting the cell from damage caused by heat (Parsell and Lindquist, 1993). While numerous mechanisms that connect heat-stimulated protein translation with heat tolerance are possible, the simplest idea is that faster synthesis of Hsps quickly protect from cell damage and increase heat knockdown tolerance.

Both heat tolerance and protein synthesis levels in *D. melanogaster* have been associated with variation in the heat stress RNA gene *hsr-omega* (Rako et al., 2007; Johnson et al., 2011) that, like the Hsps, is quickly up-regulated following heat stress (Pardue et al., 1990). This single copy gene contains two interesting polymorphic sites, at either end of the gene, and allelic variation at these two sites is largely independent in natural populations. Allelic frequencies at both sites cline with latitude, and both are associated with thermal tolerance variation (Anderson et al., 2003; Collinge et al., 2008). Laboratory selection for high hardened heat tolerance each generation produced both heat tolerant populations and large replicated changes in expression levels of the two major *hsr-omega* transcripts (McKechnie et al., 1998). Furthermore, an increase occurred in the frequency of one allele that is more common in tropical populations (McColl et al., 1996; Anderson et al., 2003). The data strongly suggest that *hsr-omega* is a component of the hardened heat tolerance mechanism in this species. We now have some understanding of how this gene functions. *Hsr-omega* has the potential to influence general processing of diverse RNA transcripts and affect many cellular processes (Lakhotia, 2011), particularly levels of total protein synthesis (Johnson et al., 2011). Low levels of the nuclear-located *omega-n* transcript associate with high basal rates of protein synthesis (Johnson et al., 2009b). *Omega-n* that is present in nearly all tissues is quickly up-regulated following heat shock and binds with crucial nuclear processing factors, an action thought to remove them from their normal role of intron processing. As a consequence intron-containing *mRNA* fails to mature and enter the cytoplasm for translation. This would reduce the synthesis of normal 25 °C proteins (that denature and aggregate under heat stress), and as a result more ribosomes would become available for faster production of protective chaperone proteins. Improved thermal performance might be expected because the energy for protein synthesis is conserved, faster production of Hsps would occur, and reduced levels of any heat denatured 25 °C proteins would interfere less with normal cellular activities (Goldberg, 2003).

The accumulated data and theory connecting *Drosophila* thermal tolerance, protein synthesis, and *hsr-omega*, is intriguing and invites further investigation. However the complexity of the cellular heat shock response, and the diverse nature of reported associations, generates numerous hypotheses about underlying cellular and physiological mechanisms. Here, to clarify relationships we take an exploratory approach at the population level and look for latitudinal clinal variation across climatic regions for all three traits in *D. melanogaster*. To increase our chances of detecting clinal patterns we use several testing/culture conditions. We compare clinal patterns for basal and heat-hardened knockdown tolerance in flies reared under two thermal regimes, 18 and 25 °C, approximating winter and summer growth conditions, respectively. We know from previous research that heat resistance shows clinal variation (Hoffmann et al., 2002), but clinal populations have not previously been examined for hardened heat tolerance other than at 25 °C (Sgrò et al., 2010), although latitudinal clines in adult heat tolerance have been found under cooler and fluctuating rearing temperatures (Hoffmann et al., 2005). We also characterise clinal populations for protein synthesis levels both before and following a mild heat stimulus, and after rearing at both 18 and 25 °C. Finally we ask if expression levels of *omega-n*, the predominant transcript of *hsr-omega*, measured before and after a mild heat stimulus shows any latitudinal pattern of variation. Several interesting patterns emerge and we discuss possible ways that the patterns may relate to thermal tolerance and other fitness traits that vary across climatic gradients.

2. Materials and methods

2.1. Collection and maintenance of D. melanogaster

Two sets of populations were used in this study, one collected in 2008 and one in 2009. The 2008 collection was from 18 locations along a latitudinal gradient on the Australian east coast as reported by Sgrò et al. (2010). The 2008 collection was used for the heat tolerance and protein synthesis estimations. Each mass-bred laboratory population was generated from 30 isofemale lines from that location and maintained at population sizes of at least 500 flies per generation on potato-yeast-dextrose-agar medium in a 12:12 light:dark cycle, both at constant 25 °C and separately at constant 18 °C, for at least four generations prior to testing. Heat knockdown and protein synthesis experiments were performed on F_7 individuals.

For *omega-n* transcript level quantification, *D. melanogaster* populations were collected from eight points along a latitudinal gradient along the east coast of Australia (Northern Tasmania 41.24°S, Melbourne 37.78°S, Gosford 33.29°S, Coffs Harbour 30.38°S, Maryborough 25.54°S, Rockhampton 23.45°S, Bowen