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Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and feeding status

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ABSTRACT

1. Determining the critical thermal limits to activity is a first step towards clarifying how temperature affects population dynamics and geographic distribution of ectothermic insects. However, thermal tolerance may be influenced by a number of factors at the species or population level, including age, gender and feeding status.
2. Here, we report the results of experiments investigating the effects of age, gender and feeding status on adult Mediterranean fruit fly (*Ceratis capitata*) and Natal fruit fly (*Ceratitis rosa*) thermal tolerance. We measured critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) using a dynamic method on different ages (2, 5, 9, 14, 28 days old) and feeding states (recently fed vs. fasted for 48 h) in both genders of adult *C. rosa* and *C. capitata*.
3. Results show that for the adult life-stage of *C. rosa* and *C. capitata* CT_{max} significantly increases with age up to 14 days. Generally, CT_{min} also varied with age and 14-day-old flies were the most low temperature tolerant. However, 28-day-old flies in both species generally had poorer thermal tolerance (i.e. narrower thermal range) than younger flies. Feeding significantly improved both CT_{max} and CT_{min} while gender had little influence.
4. Preliminary comparisons of *C. capitata* and *C. rosa* thermal tolerance suggests that both species have similar CT_{min} (5.4–6.6 °C) but *C. capitata* have significantly higher CT_{max} (42.4–43.0 °C) than *C. rosa* (41.8–42.4 °C). These results support observations that *C. capitata* inhabits warmer geographic areas than *C. rosa*. Furthermore, these data are significant for understanding population dynamics under agro-ecosystem conditions and the potential geographic distribution of these species.

1. Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Medfly), which probably originated from sub-Saharan East Africa (Baliraine et al., 2004), is considered one of the most invasive insect species, having spread and successfully established throughout much of the tropical-temperate parts of the world (Carey, 1991; Vera et al., 2002; Malacrida et al., 2007). Medfly invasion and establishment success is probably facilitated by its highly polyphagous life-history (Malacrida et al., 2007), short generation times (Duyck and Quilici, 2002; Grout and Stoltz, 2007) and possibly rapid evolutionary adaptation (Huey et al., 2005; Malacrida et al., 2007).

Natal fruit fly, *Ceratitis rosa* Karsch, is a highly polyphagous congeneric whose distribution is limited to east and southern Africa (DeMeyer et al., 2008), and has shown alarming invasion potential. For example, on Reunion Island, *C. rosa* was able to rapidly outcompete and competitively exclude Medfly (White et al., 2001) possibly owing to niche segregation (Duyck et al., 2006). However, a major factor which contributes to the successful establishment after introduction of an insect species to a novel environment is its physiological tolerance to environmental stress (e.g. to temperature and water stress) (Huey et al., 2005; Chown et al., 2007).

Temperature affects a range of biochemical and physiological processes and, along with water availability, is probably the major environmental factor affecting insect population dynamics (reviewed in Cossins and Bowler, 1987; Chown and Nicolson, 2004; Angilletta, 2009). Environmental temperature varies temporally and spatially, and insects are typically exposed to some form of thermal stress for much of their life-cycle (Feder et al., 2000; Gibbs et al., 2003; Huey and Pascual, 2009; reviewed in Chown and Terblanche, 2007). Indeed, the ability to withstand thermal stress is significant for the success of insect populations and thus evolutionary fitness in the wild (Loeschcke and Hoffmann, 2007; Sørensen et al., 2009). Furthermore,

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environmental temperatures and insect thermal tolerance may be significantly correlated, and thermal tolerance could therefore be involved in limiting a species' potential geographic distribution (e.g. Kimura, 2004; Bahrndorff et al., 2009; Kellermann et al., 2009).

However, thermal tolerance is not a static trait during an insect's life. While it is well accepted that factors such as thermal history generally affect thermal tolerance of insects (Hoffmann et al., 2003; Chown and Terblanche, 2007), the degree to which other intrinsic factors influence thermal tolerance is not well established for many insect species. Among the most obvious factors which can affect physiological and biochemical processes in insects, including thermal tolerance, are size, age, gender and feeding or nutritional status (reviewed in Spicer and Gaston, 1999; Chown and Nicolson, 2004; Bowler and Terblanche, 2008). Several studies have reported variation in thermal tolerance of insects with age (Bowler, 1967; Krebs and Loeschcke, 1995; Jensen et al., 2007) and nutrition (Hallman and Denlinger, 1998; Shelly and Kennelly, 2003; Shreve et al., 2007). The effects of gender on insect thermal tolerance are less clear, with some studies finding no significant effects (e.g. Terblanche et al., 2007a; Stotter and Terblanche, 2009) and others finding marked variation (e.g. Klose and Robertson, 2004; Newman et al., 2004; Folk et al., 2006). Age-dependent changes in thermal tolerance and the plasticity of thermal tolerance have been relatively well documented for model organisms (e.g. Krebs and Loeschcke, 1995; Jensen et al., 2007) although it is largely unclear what mechanisms cause this variation and if this variation is of evolutionary significance (reviewed in Bowler and Terblanche, 2008).

In this study, we therefore investigate the effects of age, gender and feeding status on thermal tolerance in two fruit fly species of economic concern, *C. capitata* and *C. rosa*, and present a preliminary inter-specific comparison. To date, these factors have been relatively poorly explored in both species and might impact on the way in which climate modelling of either population dynamics or geographic distribution is undertaken.

2. Materials and methods

2.1. Insect culture

Fruit flies (*C. rosa* and *C. capitata*) were reared in square Plexiglass^{TM} cages (800 mm^3) in the laboratory on a 12:12 h dark to light cycle, at room temperature ($25\pm1\,^{\circ}C$) and $65\pm10\%$ relative humidity. Flies were provided with water, sugar and protein for food, and with bananas for oviposition. Colonies were initiated from flies and infested fruit collected in Stellenbosch and the surrounding area (Western Cape Province, South Africa) between December 2006 and March 2007. Fruit fly colonies have been in culture for $\sim$2 years and once every month during summer, wild-caught flies were added to the colony to prevent inbreeding and thus ensure genetic similarity to wild populations. In addition, fruit flies were kept at high numbers to avoid inbreeding depression. All cages were held at similar low densities to avoid stressful crowding effects (Sørensen and Loeschcke, 2001).

2.2. Critical thermal limits

It is increasingly well documented that the methodological approach employed to determine an insect's thermal tolerance can affect the types of insights that can be gained, and ecological relevance, of these thermal limits (e.g. Terblanche et al., 2007b; Chown et al., 2009; reviewed in Chown and Nicolson, 2004). Here, we determined critical thermal limits as a measure of acute temperature tolerance under relatively standard conditions. Individual *C. rosa* or *C. capitata* were placed into a double jacketed chamber ('organ pipes') connected to a programmable water bath (Grant GP200-R4, Grant Instruments, UK) filled with 1:1 water: propylene glycol to allow for subzero temperatures (as in e.g., Terblanche et al., 2007b; reviewed in Lutterschmidt and Hutchison, 1997; Chown and Nicolson, 2004). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05 °C) was inserted into the control chamber to record chamber temperature. We assumed that body temperature of *Ceratitis* individuals is always in equilibrium with chamber temperature under the experimental conditions employed, as has been shown for other, larger fly species (Terblanche et al., 2007b). Both critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) experiments started at a setpoint temperature of 25 °C from which temperature increased for CT_{max} or decreased for CT_{min} at a rate of 0.25 °C/min until all the insects reached their CT_{max}/CT_{min}. While this ramping rate is relatively slow compared to much of the previous work undertaken to date (see discussion in Chown et al., 2009), it was chosen as a compromise between allowing comparisons among studies and being fairly ecologically relevant. The 0.25 °C/min rate used is likely to be 4–5 $\times$ faster than actual heating or cooling rates in the wild although it is largely unclear what impact this methodological variation might have for interpreting effects of acute temperature variation on *Ceratitis* spp. population dynamics. The effect of heating/cooling rate variation relative to the actual microclimatic conditions experienced by these flies is the focus of ongoing investigation. However, the main focus of the present study was comparison among feeding states, genders, and age groups and this is unlikely to be confounded by rate effects since rates were held constant during all experiments. Critical Thermal Limits (CTLs; CT_{min} and CT_{max}) were defined as the temperature at which each individual insect loses co-ordinated muscle function, consequently losing the ability to respond to mild stimuli (e.g. prodding). In the case of CT_{max}, this loss of muscle function coincided with death such that recovery was not possible, while in the case of CT_{min}, recovery occurred, and hence, was not immediately lethal.

Immediately after CTL assays flies were individually weighed to 0.1 mg on a calibrated electronic microbalance (Model BBL 31, Boeco Laboratory Equipment, Germany) to explore the effects of size for gender-related or feeding status-related variation on thermal tolerance.

2.3. Feeding status effects

To determine the effect of feeding status on thermotolerance of *C. rosa* and *C. capitata*, CT_{max} and CT_{min} experiments were undertaken as in Section 2.2. This experiment was conducted using 2- and 9-day-old flies of both species (*C. rosa* and *C. capitata*) and on both genders using fasted and fed flies. 'Fasted' flies were deprived of any food, but had access to water, for 48 h before measuring CTLs. Tephritid fruit flies are typically post-absorptive (the gut is empty) if deprived of access to food for more than 48 h (Jacome et al., 1995; Shelly and Kennelly, 2003). 'Fed' flies were supplied with food (25% sugar water solution) *ad libitum* during their lifetime. However, before measuring CTLs, these flies were temporarily deprived of food for 6 h to induce hunger before reintroducing the food. Flies were then closely monitored and only recently fed flies (i.e. those that had just completed feeding) were removed from cages for thermal tolerance trials. The experimental treatments were undertaken in a random order.