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Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species

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ABSTRACT

Acclimation in the thermal tolerance range of insects occurs when they are exposed to novel temperatures in the laboratory. In contrast to the large number of studies that have tested for the ability of insects to acclimate, relatively few have sought to determine the time-course for attainment and reversal of thermal acclimation. In this study the time required for the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, and the Natal fruit fly, *Ceratitis rosa* Karsch, to acclimate to a range of constant temperatures was tested by determining the chill-coma recovery time and heat knock-down time of flies that had been exposed to novel benign temperatures for different durations. The time required for reversal of acclimation for both *Ceratitis* species was also determined after flies had been returned to the control temperature. Acclimation to 31 °C for only one day significantly improved the heat knock-down time of *C. capitata*, but also led to slower recovery from chill-coma. Heat knock-down time indicated that acclimation was achieved after only one day in *C.* rosa, but it took three days for *C. rosa* to exhibit a significant acclimation response to a novel temperature of 33 °C when measured using chill-coma recovery time. Reversal of acclimation after return to initial temperature conditions was achieved after only one day in both *C. capitata* and *C. rosa*. Adult *C. capitata* held at 31.5 °C initially exhibited improved heat knock-down times but after 9 days the heat knock-down time of these flies had declined to levels not significantly different from that of control flies held at the baseline temperature of 24 °C. In both *Ceratitis* species, heat knock-down time declined with age whereas chill-coma recovery time increased with age, indicating an increased susceptibility to high and low temperatures, respectively.

1. Introduction

It has been demonstrated widely that the thermal tolerance of insects is affected by prior thermal experience. Developmental temperature can have enduring morphological and physiological consequences that influence fitness (Gibert et al., 2001; Kingsolver and Huey, 1998). Furthermore, plastic changes in the thermal tolerance range of insects occur when they are exposed to novel temperatures (e.g. Overgaard and Sørensen, 2008; Rako and Hoffmann, 2006; Marais and Chown, 2008). Acclimation is a rapid and reversible change in phenotype (be it physiological, biochemical, or anatomical) in response to chronic exposure to a new environmental condition (Woods and Harrison, 2002), and these plastic changes are often beneficial for the survival of insects in a changing environment (e.g. Fay and Meats, 1987; Terblanche et al., 2006). Across insect taxa, acclimation to temperature is most pronounced at the lower temperature threshold, while there is little flexibility in the upper thermal limit (e.g. Hoffmann and Watson, 1993; Chown, 2001; Chown and Terblanche, 2006).

In contrast to the large number of studies that have tested for the ability of insects to acclimate to changes in temperature, relatively few have sought to determine the time-course for attainment of thermal acclimation. This is surprising given the wide range of temperatures and durations that have been used to induce acclimation, as well as hardening. In the *Drosophila* literature alone, Sinclair and Roberts (2005) identified 27 different published methods to induce changes in thermal tolerance. Hardening, a form of acclimation, has been induced by subjecting animals to rapid changes in temperature in the laboratory (Hoffmann et al., 2003). However, the magnitude of temperature change used in hardening treatments is considerably larger than that of acclimation treatments, and their duration is typically for only 1–3 h followed by a test for change in thermal tolerance (Hoffmann et al., 2003). The physiological changes associated with acclimation and hardening at upper and lower temperatures can be shared, which has led to suggestions that acclimation and hardening represent the same phenomenon, but expressed over different time scales due to the relative levels of stress involved (Bowler, 2005; Loeschcke and Sørensen, 2005). In those species where the time required for complete acclimation

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has been determined, acclimation is relatively rapid and asymptotic (Table 1). In the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) acclimation to low temperatures is very rapid for the cold torpor threshold (Meats, 1973). In *B. tryoni*, the time required to acclimate to a new lower temperature is shorter if the change in temperature is small; for a full acclimation response in cold torpor threshold, only 1 min is required for a drop in temperature of 1 °C (if the new lower temperature is not below torpor temperature; Meats, 1973). Conversely, acclimation to a new higher temperature is fastest at higher temperatures, regardless of the magnitude of change (Meats, 1973). The findings of Meats (1973) suggest that the magnitude of temperature change has important implications for the rate of change in insect tolerance to high and low temperatures. However, data for other species summarised in Table 1 provide inconsistent support for the role of temperature change magnitude in contributing to the rate of acclimation attainment and reversal. Thus, a broader set of information concerning the time course of acclimation would be useful for further understanding of thermal tolerance in this group. Here we provide such information for two species of fruit flies.

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, is one of the world's most destructive horticultural pests and represents an enormous economic burden due to lost productivity, control costs and trade barriers. Molecular genetic evidence suggests that the endemic range of *C. capitata* was central eastern Africa (Baliraine et al., 2004), but in just over 100 years it has spread through human activities and unassisted dispersal throughout Africa, the Mediterranean Basin and Middle East, Central and South America, and parts of North America and Australia (for a review, see Malacrida et al., 2007). In contrast, the Natal fruit fly, *Ceratitis rosa* Karsch, which also originates in central eastern Africa and utilises a similar range of hosts, has a naturalised range restricted to south-eastern Africa and the Mauritius and Réunion islands (Baliraine et al., 2004). The discrepancy between the invaded distribution of *C. capitata* and *C. rosa* poses the question of why the former has so successfully established in regions where it has been introduced? Work to date suggests that differences in the bioclimatic potential of *C. capitata* and *C. rosa* could provide one explanation for their current distribution and changes therein that might follow as a result of climate change. The distribution and abundance of *C. capitata* and *C. rosa* on La Réunion, where they are both introduced and invasive, suggests that each species has a well-defined bioclimatic niche: *C. capitata* is more prevalent at lowland sites where it is warmer and drier, whereas *C. rosa* is found at higher altitudes that experience cooler and wetter conditions (Duyck et al., 2006). Work on the critical thermal limits (the lower or upper temperature at which muscular function is lost) of the two species indicates that the thermal tolerance of *C. capitata* is broader than that of *C. rosa*; the critical thermal minimum (CT_{min}) of both species does not differ (5.4–6.6 °C) but the critical thermal maximum (CT_{max}) of *C. capitata* (42.4–43.0 °C) is significantly higher than that of *C. rosa* (41.8–42.4 °C) (Nyamukondiwa and Terblanche, 2009).

In both *C. capitata* and *C. rosa* it has also been demonstrated that thermal experience during the adult phase can lead to small but significant reversible and irreversible changes in their tolerance of extreme temperatures (Nyamukondiwa and Terblanche, 2010). Acclimation for 7 days to 30 or 20 °C, after being initially held at 25 °C, led to improved heat tolerance and cold resistance, respectively (Nyamukondiwa and Terblanche, 2009). However, the duration of acclimation prior to testing used by Nyamukondiwa and Terblanche (2009) was based only on the few studies that have reported the time required for thermal acclimation to be attained. This current study determined the time required for *C. capitata* and *C. rosa* to acclimate to a range of constant temperatures. Acclimation of each species was tested by determining the chill-coma recovery time and heat knock-down time of flies that had been exposed to novel benign temperatures for different durations. The time required for reversal of acclimation for both *Ceratitis* species was also determined by measuring chill-coma recovery and heat knock-down time after flies had been returned to the control temperature.

Table 1
Time required for attainment and reversal of thermal acclimation in insects. All studies report time of response by adults to novel temperature treatments unless otherwise indicated. Arrows indicate the direction of the temperature change.

Taxon	Attainment		Reversal		Reference
	Treatment	Time	Treatment	Time	
Blattaria					
Blatta orientalis Linnaeus	15 °C↔30 °C	≤20 h	15→30 °C	≤20 h	Mellanby (1939)
			30→15 °C	2–3 days	
Hemiptera					
Cimex lectularius Linnaeus	15 °C↔30 °C	≤20 h	15↔30 °C	≤20 h	Mellanby (1939)
Rhodnius prolixus Stål	15 °C↔30 °C	≤20 h	15↔30 °C	≤20 h	Mellanby (1939)
Diptera					
Bactrocera oleae (Rossi)	25 °C→20 & 30 °C	3–5 days	N/A	N/A	Fletcher and Zervas (1977)
	25 °C→15 °C	5 days			
	25 °C→5 & 10 °C	>11 days			
Bactrocera tryoni (Froggatt)					
Third instar larvae	25 °C→35 °C	8 h	35 °C→25 °C	6–8 h	Beckett and Evans (1997)
Adults	25 °C→8 °C	3 h	8 °C→25 °C	3 min	Meats (1973)
	12 °C→8 °C	2 min	8 °C→12 °C	3 h	
Drosophila melanogaster Meigen	25 °C→29 °C	1 day	29 °C→25 °C	2 days	Levins (1969)
	25 °C→18 °C	7 days	N/A	N/A	Gibert et al. (2001)
Calliphora vicina Robineau-Desvoidy	15 °C↔30 °C	≤ 20 h	15 °C↔30 °C	≤20 h	Mellanby (1939)
Lucilia sericata (Meigen)	15 °C↔30 °C	≤ 20 h	15 °C↔30 °C	≤20 h	Mellanby (1939)
Glossina pallidipes Austen	24 °C→19 °C	5 days	19 °C→24 °C	≤9 days	Terblanche et al. (2006)
	24 °C→16 °C	5 days	16 °C→24 °C		
Lepidoptera					
Bicyclus anynana (Butler)	20 °C→27 °C	2 days	27 °C→20 °C	>3 days	Fischer et al. (2010)
Danaus plexippus Kluk	4–5 °C→23–24 °C	4–6 days	23–24 °C→4–5 °C	>4 days	Kammer (1971)
Lycaena tityrus Poda	27 °C→20 °C	3 days	N/A	N/A	Zeilstra and Fischer (2005)

2. Material and methods

2.1. Source and maintenance of insects

Pupae of *C. capitata* and *C. rosa* were obtained from large, outbred cultures maintained at Citrus Research International, Nelspruit, South Africa. The cultures were held indoors under variable though buffered temperatures (annual temperature range: 15–30 °C). Adult females oviposited through a nylon mesh screen (1 mm mesh size). The eggs were collected in water, held for 24 h, and then transferred to a bran-based larval rearing medium. Third instar 'hopping' larvae burrowed to the surface of the larval rearing medium and migrated to a layer of fine vermiculite where they commenced the pupal phase. Pupae were then shifted from the vermiculite before being sent by express courier to Stellenbosch University, Matieland, South Africa. Upon arrival at Stellenbosch University, the pupae of each species were distributed into nine ventilated 5-l plastic cages, with approximately 250 pupae per cage. Cages were furnished with cotton wool soaked in water, granulated sucrose, and yeast extract powder (Atis® YE, Borregaard Ingredients, Sarpsborg, Norway). Each cage was placed in an incubator set to 25 °C with a 12:12 LD photo cycle until adult emergence.

At 2 days after adult emergence, a plastic dish filled with saturated NaCl solution and covered by insect screen mesh was inserted into each of the 5-l plastic cages containing flies to regulate humidity. Each cage of flies was then placed inside a clear plastic bag that was then wrapped around the cage to seal it so that the humidity in the cage could be increased by the saturated salt solution. In a sealed container, saturated NaCl solution maintains a constant relative humidity of 76%, 75.5% and 75.5% at 20, 25 and 30 °C, respectively (Winston and Bates, 1960). Three cages each of *C. capitata* and *C. rosa* were then introduced to incubators set to 20 or 30 °C while the remaining three cages of each species remained in the incubator set to 25 °C. After acclimation for 10 days, each group of flies was then returned to the incubator set to 25 °C to test for the rate of loss of acclimation.

The actual temperature and relative humidity experienced by flies in each incubator was recorded at hourly intervals using data loggers attached to the inside wall of each cage (DS1923, iButton®, Maxim Integrated Products, Sunnyvale CA, USA). Actual acclimation temperatures (mean ± 1 standard error) experienced by *C. capitata* in incubators set to 20, 25 and 30 °C were 20.9 ± 0.0 °C, 23.9 ± 0.0 °C and 31.6 ± 0.4 °C, respectively. The relative humidity maintained within cages using saturated NaCl solutions was 83 ± 0% at 20.9 °C, 80 ± 0% at 23.9 °C, and 77 ± 0% at 31.6 °C. Actual acclimation temperatures experienced by *C. rosa* in incubators set to 20, 25 and 30 °C were 20.8 ± 1.3 °C, 24.4 ± 0.3 °C, and 33.0 ± 1.0 °C, respectively. At each respective actual acclimation temperature, the relative humidity maintained by saturated NaCl solutions in the cages that housed *C. rosa* was 85 ± 0%, 81 ± 2%, and 77 ± 0%. The results are reported and interpreted using the actual temperatures (to the nearest 0.5 °C) experienced by flies.

2.2. Chill-coma recovery time

Chill-coma recovery times (CCRT) for adult *C. capitata* and *C. rosa* were assessed for each acclimation temperature after different durations of exposure and recovery. Flies (n=25–30) of each species were tested after 1, 3, 5, 7 and 9 days of exposure to acclimation temperatures, and after 1 and 3 days of being returned to the incubator set at 25 °C. Flies were transferred to and weighed in individual 7 ml screw-cap plastic vials of known weight with two 1 mm diameter holes pierced through the cap for ventilation. The vials were then placed into a large zip-lock bag that was plunged into a water bath (GP200-R4, Grant Instruments Inc., Cambridge, UK) held at 0 °C for one hour. The plastic vials were then placed on their side on a table in a room maintained at 25 ± 1 °C and the time required for each fly to regain the ability to stand was recorded. A small number of *C. capitata* that did not recover from the cold shock within two hours (n=27 drawn from all acclimation temperatures) were excluded from analysis. Flies were weighed immediately before the assay and their sex was recorded after the assay was completed.

2.3. Heat knock-down time

In this assay, heat knock-down time (HKDT) was determined for both *C. capitata* and *C. rosa* in relation to duration of exposure to a novel thermal environment. Flies were weighed in ventilated 7 ml plastic vials of known weight before being exposed to a test temperature of 43.9 ± 0.3 °C on a thermal stage. A temperature of 43 °C is known to result in loss of motor control in *C. capitata* and *C. rosa* regardless of acclimation temperature, age or heating rate (Nyamukondiwa and Terblanche, 2009, 2010). The thermal stage was a sealed Perspex box with aluminium top through which water was circulated from a programmable water bath (GP200-R4, Grant Instruments Inc., Cambridge, UK) set at 54 °C. Walls and a removable cover of Perspex enclosed the aluminium stage top to stabilise the temperature of the apparatus. The conditions experienced by the flies led to increased locomotor activity followed by uncoordinated flight, spasms, and then loss of activity. The time (in minutes) at which activity was lost by flies after placing vials on the thermal stage was recorded. Flies (n=25–30) of each species were tested after 1, 3, 5, 7 and 9 days of exposure to acclimation temperatures, and after 1 and 3 days of being returned to the incubator set at 25 °C. The sex of each fly was recorded after the assay was completed.

2.4. Data analysis

All analyses were performed in R 2.10.1 (R Development Core Team, 2010). CCRT and HKDT did not meet assumptions of constant variance and normal errors that are implicit in linear models. Due to this and because CCRT and HKDT are both measured as the time until an event, the main effects and two-way interactions of acclimation temperature, time and sex on CCRT and HKDT for each *Ceratitis* species were determined using Cox's proportional hazards survival regression (function "coxph" in the "survival" library) and log-likelihood tests. Acclimation time and temperature were treated as categorical variables. The reference category for acclimation temperature was set to "25 °C" (function "relevel"). Weight of flies was included in the model as a covariate to account for the influence of size on CCRT and HKDT. CCRT and HKDT were known for all individuals in the analysis so a censoring indicator vector was created and included in the analysis to indicate that no data were censored. The proportionality of hazards assumption for a Cox regression fit (i.e. that the effects of model variables were constant over time) was met in all analyses (function "cox.zph" in the "survival" library). Post-hoc pairwise survival analyses were performed to examine significant main effects and interaction terms.

3. Results

3.1. Ceratitis capitata

Heat knock-down time of *C. capitata* was significantly affected by temperature, time, and their interaction (Table 2). After acclimation to 31.5 °C for one day, HKDT of adult *C. capitata* was significantly longer than acclimation to 21 or 24 °C (Fig. 1A). HKDT of 31.5 °C-acclimated *C. capitata* steadily declined but continued to be higher than the

Table 2
Analysis of deviance table for Cox's proportional hazards survival regression of heat knock-down time (HKDT) and chill-coma recovery time (CCRT) of *Ceratitis capitata* with respect to acclimation temperature, acclimation time (days), sex and weight.

Thermal tolerance assay	Effect	χ^2	*d.f.*	*p*
HKDT	Temperature	27.159	2	< 0.0001
	Time	91.545	6	< 0.0001
	Sex	2.227	1	0.1356
	Weight	0.254	1	0.6146
	Temperature × time	28.002	12	0.0055
	Temperature × sex	7.176	2	0.0277
	Time × sex	9.081	6	0.1691
CCRT	Temperature	59.799	2	< 0.0001
	Time	30.684	6	< 0.0001
	Sex	16.014	1	< 0.0001
	Weight	17.573	1	< 0.0001
	Temperature × time	17.568	12	0.1295
	Temperature × sex	2.0285	2	0.3627
	Time × sex	6.3024	6	0.3902

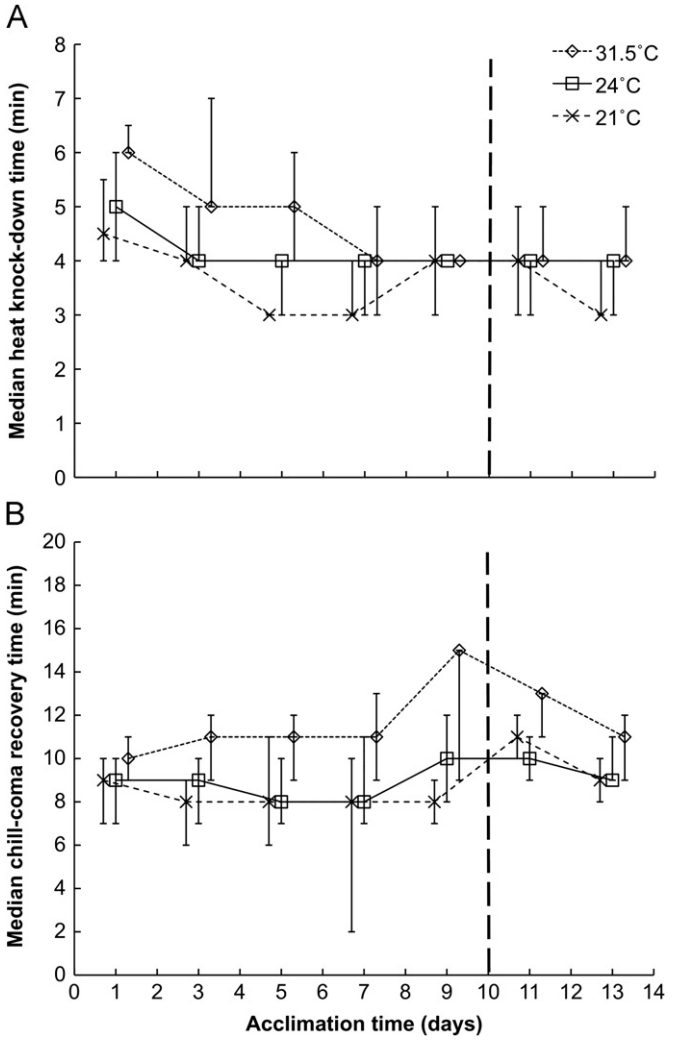

Fig. 1. Change in median heat knock-down time (A) and chill-coma recovery time (B) of adult *Ceratitis capitata* transferred from 24 °C to 31.5, 24 or 21 °C. Error bars indicate the upper and lower 95% confidence interval. The vertical dashed line indicates the return of flies to 24.5 °C after 10 days.

HKDT of flies acclimated to 21 or 24 °C until 7 days after acclimation commenced. However, by 9 days after the commencement of acclimation there was no significant difference in HKDT between *C. capitata* acclimated to 21, 24 or 31.5 °C, and this continued to be the case after all acclimation treatments were returned to 24 °C after 10 days of acclimation (Fig. 1A). The interaction of acclimation temperature and sex also had a significant effect on HKDT of *C. capitata* (Table 2). HKDT of *C. capitata* that were acclimated at 21 °C was marginally shorter in females (median, lower - upper 95% confidence interval = 4 min, 3–4 min) than males (4 min, 4–4 min), but there was no difference between the sexes when acclimated to 24 °C (female: 4 min, 3–4 min; male: 4 min, 4–4 min) or 31.5C (female: 4 min, 4–4 min; male: 5 min, 4–5 min).

CCRT of adult *C. capitata* was significantly affected by acclimation temperature (Table 2). At all ages, including after only 1 day, adult *C. capitata* acclimated to 31.5 °C had a CCRT that was significantly longer than those acclimated to 21 or 24 °C, while CCRT of 21 and 24 °C-acclimated *C. capitata* did not differ (Fig. 1B). CCRT of *C. capitata* also varied significantly with time. After 9 days of acclimation, CCRT was significantly longer than after only one day of acclimation, but following return of flies to 24 °C after 10 days of acclimation, CCRT at 11 and 13 days did not differ significantly from when it was assessed at 1 day of acclimation. Sex of adult *C. capitata* significantly affected CCRT (Table 2), with male CCRT (median = 10 min) being slightly higher than that of females (median = 9 min). There was also a significant effect of weight on adult *C. capitata* CCRT (Table 2); heavier flies were more likely to have a shorter CCRT.

3.2. *Ceratitis rosa*

Acclimation temperature had a significant effect on HKDT of *C. rosa* (Table 3). Overall, adult *C. rosa* acclimated to 24.5 °C had a HKDT that was marginally but significantly longer than that of *C. rosa* acclimated to 21 or 33 °C (Fig. 2A). HKDT of *C. rosa* was also significantly affected by time (Table 3); HKDT of *C. rosa* after acclimation for 3, 5, 7 and 9 days was significantly longer than after only 1 day of acclimation, and after all acclimation treatments were returned to 24.5 °C at 10 days (i.e. 11 and 13 days) (Fig. 2A).

CCRT of *C. rosa* was significantly affected by temperature, time, and their interaction (Table 3). There was no significant difference in CCRT of *C. rosa* between acclimation treatments after one day (Fig. 2B). After three days of acclimation, CCRT of 33 °C-acclimated *C. rosa* was significantly longer than 21 and 24.5 °C-acclimated flies, and this difference continued to be evident at 5, 7 and 9 days. There was no significant difference between CCRT of *C. rosa* acclimated at 21 and 24.5 °C at any tested time. However, the CCRT of 21 and 24.5 °C-acclimated *C. rosa* increased with time: CCRT at 3 days was significantly longer than when

Table 3
Analysis of deviance table for Cox's proportional hazards survival regression of heat knock-down time (HKDT) and chill-coma recovery time (CCRT) of *Ceratitis rosa* with respect to acclimation temperature, acclimation time, sex and weight.

Thermal tolerance assay	Effect	χ^2	*d.f.*	*p*
HKDT	Temperature	6.388	2	0.0410
	Time	18.573	6	0.0050
	Sex	0.530	1	0.4667
	Weight	0.242	1	0.6228
	Temperature × time	18.025	12	0.1149
	Temperature × sex	2.881	2	0.2368
	Time × sex	3.576	6	0.7339
CCRT	Temperature	42.665	2	< 0.0001
	Time	174.869	6	< 0.0001
	Sex	3.003	1	0.0831
	Weight	0.514	1	0.4735
	Temperature × time	27.078	12	0.0075
	Temperature × sex	3.173	2	0.2047
	Time × sex	1.826	6	0.9350

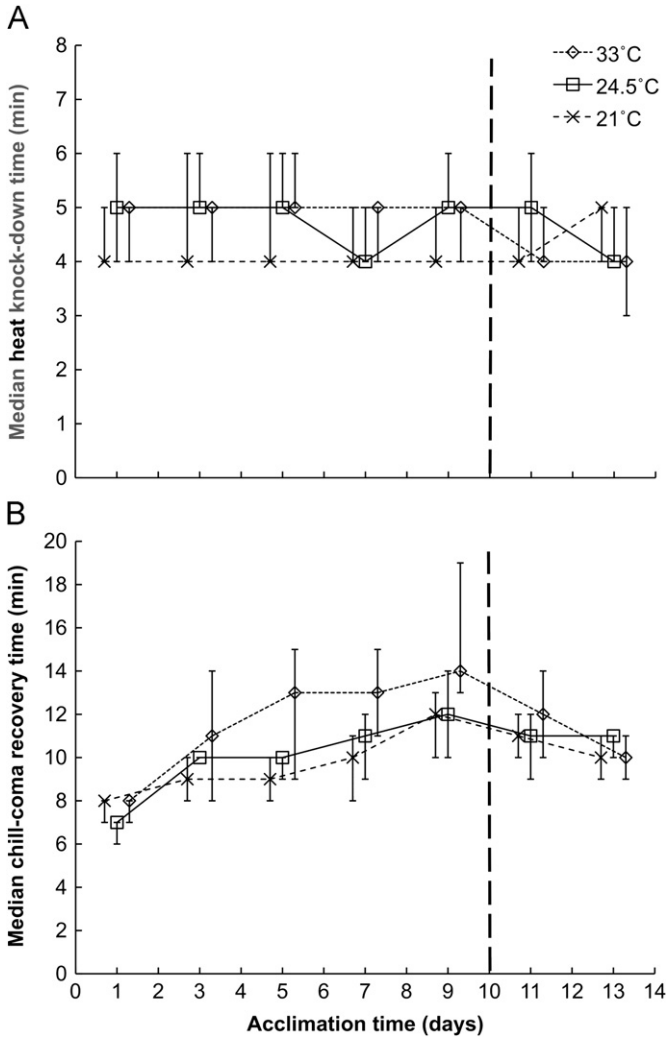

Fig. 2. Change in median heat knock-down time (A) and chill-coma recovery time (B) of adult *Ceratitis rosa* transferred from 24.5 °C to 33, 24.5 or 21 °C. Error bars indicate the upper and lower 95% confidence interval. The vertical dashed line indicates the return of flies to 24.5 °C after 10 days.

initially tested, but was significantly shorter than at 5, 7, 9, 11 and 13 days. Following return to 24.5 °C after 10 days of acclimation, within one day CCRT of 33 °C-acclimated *C. rosa* did not differ significantly from that of 21 and 24.5 °C-acclimated *C. rosa* of the same age (Fig. 2B).

4. Discussion

It appears that acclimation is a relatively rapid process for pest tephritid flies. Only one day was required for *C. capitata* to acclimate to a novel constant temperature. Acclimation to 31 °C for one day significantly improved the heat shock resistance of *C. capitata*, but also led to slower recovery from a cold shock. Rapid acclimation has also been reported in *Bactrocera tryoni* (Meats, 1973). Meats (1973) demonstrated that the mean torpor threshold of adult *B. tryoni* declined to a level commensurate with a novel temperature (from 25 to 8 °C) after only three hours. Results for acclimation by *C. rosa* are mixed. Only 1 day was required for a significant difference in heat knock-down time of 21 °C- and 33 °C-acclimated *C. rosa* from those kept at 24.5 °C whereas a significant difference in chill-coma recovery time was not detected until 3 days. However, the difference between chill-coma recovery time of *C. rosa* acclimated to 33 °C and flies acclimated to other temperatures was greatest after having been exposed for 5 days. Fletcher and Zervas (1977) reported that it took 3–5 days for the cold torpor threshold of adult *Bactrocera oleae* to shift in response to a change in constant temperature of 5 °C. It is possible that delayed acclimation in *C. rosa* relative to *C. capitata* with regard to cool temperature tolerance may have resulted from the 2 °C difference in temperature experienced in incubators set to 30 °C. However, the difference between *C. capitata* and *C. rosa* in the time required for acclimation to novel temperatures is in agreement with results by Nyamukondiwa et al. (2010) that *C. capitata* develops a rapid cold hardening response significantly faster than *C. rosa*.

Reversal of acclimation after return to initial temperature conditions was achieved after only one day in both *C. capitata* and *C. rosa*. When returned to the initial control temperature of 24–24.5 °C, chill-coma recovery time of warm temperature-acclimated flies improved to levels not significantly different from that of flies held constantly at the control temperature. This rapid loss of acclimation reflects the rapid loss of a cold hardening response in *C. capitata* and *C. rosa* within 16 and 0.5 h, respectively (Nyamukondiwa et al., 2010).

For insects, much variation exists in the time reported for reversal of acclimation (Table 1). In general, the magnitude of change seems to have no bearing on the time required for reversal of acclimation. After having initially been acclimated to a higher temperature, reversal of acclimation has been reported to occur in 2 days (adult *D. melanogaster*) and over 3 days (adult *Bicyclus anyana*) where the magnitude of temperature change was similar to that used in this study (Table 1). Similarly, increasing and decreasing temperature changes in excess of 10 °C have led to reversal of acclimation in under 20 h but up to 4 days in a range of taxa (Table 1). Overgaard and Sørensen (2008) demonstrated that thermal adaptation of *Drosophila melanogaster* in response to field temperature variation was a continuous process. In *D. melanogaster*, thermal acclimatisation tracked field temperature so that heat shock survival improved with increasing temperature while cold shock survival declined, with commensurate changes as field temperature declined (Overgaard and Sørensen, 2008). Their results are in accord with previous studies on the same species (Kelty and Lee, 2001; Kelty, 2007).

There are multiple combinations of temperatures that can be used to induce acclimation and test thermal stress. This is evident from the range of temperatures that have been used to test for the time-course for attainment and loss of acclimation (Table 1). While the magnitude of temperature change does not seem to bear on the time required for attainment of acclimation by a population, have these temperature changes been ecologically relevant? In the case of *C. capitata* and *C. rosa*, published data indicate that the temperatures used to induce acclimation in this study are experienced in the geographic range of both species (Duyck and Quilici, 2002; Duyck et al., 2006), exceed the minimum temperature required for their reproductive development (Duyck and Quilici, 2002), and are well below the critical thermal maximum of adults (Nyamukondiwa and Terblanche, 2009). The wide range in the times required for acclimation across species may represent evolutionary responses to different levels of temperature variability and predictability in their environment. Theoretical modelling (Gabriel et al., 2005) and empirical evidence (Kingsolver and Huey, 1998; Deere et al., 2006) suggest that organisms living in environments with predictable temperature fluctuations are more likely to evolve plastic phenotypic traits, while selection does not favour plasticity in those that experience stable or unpredictable temperatures due to an inability to match optimal phenotype with actual conditions. Diurnal temperature fluctuations in part of the geographic range of *C. capitata* and *C. rosa* can exceed 20 °C but are generally predictable from day-to-day (Nyamukondiwa and Terblanche, 2010), so this may contribute to the rapid attainment and loss of acclimation in these species.

In both *Ceratitis* species, age had a significant effect on heat knock-down and chill-coma recovery time. Heat knock-down time declined with age whereas chill-coma recovery time increased with age, indicating an increased susceptibility to heat and cold-shock, respectively. A similar pattern has been found in *D. melanogaster* (reviewed in Bowler and Terblanche, 2008) for both heat knock-down (Pappas et al., 2007) and chill-coma recovery time (David et al., 1998). The results of Pappas et al. (2007) indicated that declines in heat knock-down time in the early adult are likely a developmental rather than an aging phenomenon, and are not strongly associated with levels of inducible heat shock protein expression. For both *Ceratitis* species, the duration of the current study represented a relatively short period of their reported mean life expectancy. At a constant temperature of 25 °C, the life expectancy of *C. capitata* is 65.8 days for females and 75.6 days for males (Carey et al., 2008). The life expectancy of *C. rosa* at a constant temperature of 25 °C exceeds 100 days (Duyck et al., 2010). The results of the current study need to be reconciled with those of Nyamukondiwa and Terblanche (2009) who, measuring the critical thermal maximum and minimum (CT_{max} and CT_{min}, respectively) of both species, found that there was a general improvement in thermal tolerance with age up to 14 days after adult emergence. This apparent conflict between age-related patterns likely arises due to the processes that the two types of assay measure. Both CT_{max} and CT_{min} represent the temperatures at which neuromuscular function is lost, and evidence suggests that this results from disruption of membrane composition, permeability, and ion channel and ATPase activity (e.g. Folk et al., 2007; Macmillan and Sinclair, 2011). On the other hand, heat knock-down and chill-coma recovery times measure the time required for enzyme activity and/or membrane permeability to be lost or return to levels sufficient for neuromuscular function, respectively. Interpreted in this way, it seems that there is progressive increase with age after adult eclosion in the ability for ion regulation to be maintained at warmer and cooler temperatures in *C. capitata* and *C. rosa*. However, as individuals age, neuromuscular function is lost more rapidly when exposed to high temperatures, and recovery of neuromuscular function after exposure to low temperatures is impaired.

Heavier *C. capitata* were more likely to have a shorter chill-coma recovery time. Correlation of clinal variation in size and stress resistance traits of *D. melanogaster* and *D. serrata* also indicates a negative relationship between adult size and chill-coma recovery time (James et al., 1995; Hallas et al., 2002; Hoffmann et al., 2002). Populations of *D. melanogaster* and *D. simulans* with high resistance to cold stress have been shown to possess poor desiccation resistance and low levels of extractable lipids as a proportion of body weight (Hoffmann et al., 2005; Kenny et al., 2008), which suggests an evolutionary trade-off between cold-resistance and starvation resistance that may be controlled by lipid metabolism (Hoffmann et al., 2005). However, the mechanisms that link chill-coma recovery time and body size are yet to be identified with certainty.

Acclimation to benign temperatures by the two *Ceratitis* species investigated in this study occurred rapidly. Earlier studies on thermal acclimation in *C. capitata* and *C. rosa* (Nyamukondiwa et al., 2010) safely assumed that exposure to novel temperatures for 7 days would be sufficient for a full acclimation response; acclimation by *C. capitata* can take as little as one day, while 1–5 days were required for *C. rosa* to acclimate to the novel temperatures experienced in this study. Acclimation and its reversal occurs in many insect taxa, and recent efforts are establishing the processes involved at the organismal, cellular and molecular level of organisation. These advances, as well as data from a wider range of taxa, provide an opportunity to explain the diversity of rates of attainment and loss of acclimation in insects.

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