RESEARCH ARTICLE

Effects of within-generation thermal history on the flight performance of Ceratitis capitata: colder is better

Nanike Esterhuizen¹, Susana Clusella-Trullas², Corne E. van Daalen³, Ruben E. Schoombie¹, Leigh Boardman¹ and John S. Terblanche¹,*

ABSTRACT

The influence of thermal history on temperature-dependent flight performance was investigated in an invasive agricultural pest insect, Ceratitis capitata (Diptera: Tephritidae). Flies were exposed to one of four developmental acclimation temperatures (Tacc: 15, 20, 25, 30°C) during their pupal stage and tested at these temperatures (Ttest) as adults using a full-factorial study design. Major factors influencing flight performance included sex, body mass, Ttest and the interaction between Ttest and Tacc. Successful flight performance increased with increasing Tacc across all acclimation groups (from 10% at 15°C to 77% at 30°C). Although Tacc did not affect flight performance independently, it did have a significant interaction effect with Ttest. Multiple comparisons showed that flies which had been acclimated to 15°C and 20°C performed better than those acclimated to 25°C and 30°C when tested at cold temperatures, but warm-acclimated flies did not outperform cold-acclimated flies at warmer temperatures. This provides partial support for the ‘colder is better’ hypothesis. To explain these results, several flight-related traits were examined to determine whether Tacc influenced flight performance as a consequence of changes in body or wing morphology, whole-animal metabolic rate or cytochrome c oxidase enzyme activity. Although significant effects of Tacc could be detected in several of the traits examined, with an emphasis on sex-related differences, increased flight performance could not be explained solely on the basis of changes in any of these traits. Overall, these results are important for understanding dispersal physiology despite the fact that the mechanisms of acclimation-related changes in flight performance remain unresolved.

KEY WORDS: Beneficial acclimation hypothesis, Phenotypic plasticity, Developmental variation, Mediterranean fruit fly

INTRODUCTION

The thermal environment experienced by ectothermic organisms has widespread effects on their physiological performance and survival, with ambient temperatures directly influencing their physical ability to perform various activities, including locomotion (Kaufmann and Bennett, 1989; Dillon and Frazier, 2006; Clusella-Trullas et al., 2010). Such thermal effects can be caused by temperature-induced changes in energy availability via alteration in mitochondrial functioning (e.g. O’Brien et al., 1991; reviewed in Hochachka and Somero, 2002; Seebacher and James, 2008), which, in some cases, directly influences the mechanical power output of muscles (Bennett, 1985; Swoap et al., 1993; Lehmann, 1999; reviewed in James, 2013). Furthermore, several other important physiological processes (e.g. metabolic and development rates) are also strongly influenced by the ambient temperature (e.g. Frederich and Pörtner, 2000; Chown and Nicolson, 2004; Irlich et al., 2009; Dell et al., 2011) and may in turn have indirect effects on locomotor capacity. The resulting changes in performance can affect the short- and long-term dispersal capacity of arthropods with significant implications for ecology and evolution (reviewed recently in e.g. Feder et al., 2010; Bonte et al., 2012; Clobert et al., 2012; San Martín y Gomez and van Dyck, 2012).

Performance varies as a function of ambient temperature, and this relationship is dependent on thermal history at several timescales. Indeed, it is well documented that performance is flexible both within and between generations in ectothermic animals (Hoffmann et al., 2003; Rako and Hoffmann, 2006; Chown and Terblanche, 2007; Kingsolver, 2009). The conditions experienced either over the short term within a single life-stage (hardening responses, e.g. Kellett et al., 2005; Basson et al., 2012), throughout a developmental or adult stage (e.g. Kristensen et al., 2008; Fischer et al., 2010; Waagner et al., 2013), or over evolutionary timescales (among populations or between species, for examples see Gibert et al., 2001; Kelty and Lee, 2001; Steigenga and Fischer, 2007) can radically alter tolerance and performance under a given set of environmental conditions. Moreover, it is increasingly clear that the different timescales of thermal exposure may result in different underlying responses and mechanisms (e.g. Colinet and Hoffmann, 2012; Teets and Denlinger, 2013; Waagner et al., 2013).

Within-generation changes in performance phenotypes may reflect responses to environmental conditions, referred to as phenotypic plasticity, and are defined as genotype-by-environment interactions (DeWitt and Scheiner, 2004; Ghahramib et al., 2007; Whitman and Agrawal, 2009). Acclimation is defined by Wilson and Franklin (Wilson and Franklin, 2002) as ‘any facultative modification in a physiological trait in response to changes in an environmental variable in the laboratory. Changes can be in response to the developmental environment or long-term environmental shifts during the later stages of the life history of an organism’. Acclimation has, however, been used to refer to the outcome as well as the treatment of an exposure (for example, see Bowler and Terblanche, 2008). In this study, acclimation is used to define the developmental acclimation temperature (Tacc), which may result in reversible or irreversible phenotypic plasticity (Piersma and Drent, 2003; Terblanche and Chown, 2006).

Several main hypotheses have been proposed to describe the form and nature of the variation in performance after exposure to different thermal conditions (Huey and Berrigan, 1996; Huey et al., 1999; Deere and Chown, 2006). Notable among these hypotheses is the beneficial acclimation hypothesis (BAH), which states that
The immediate effects of ambient temperature on insect flight performance have been well documented (Chown and Nicolson, 2004; Dillon and Frazier, 2006; Samejima and Tsubaki, 2010), but in contrast, the effects of developmental or rearing temperature on flight performance have been less extensively studied. Two notable recent exceptions, however, include work by Frazier et al. (Frazier et al., 2008) on Drosophila melanogaster and by Ferrer et al. (Ferrer et al., 2013) on Grapholita molesta. Both of these studies focus on laboratory responses and show marked effects of developmental temperature on traits of adult flight performance. These studies are, however, limited in their ability to interpret the acclimation hypotheses, mainly because they do not acclimate and test individuals in conditions that are both above and below the optimal rearing temperature. For example, Frazier et al. (Frazier et al., 2008) only focus on low temperature flight ability in Drosophila melanogaster (14–18°C), meaning that a crucial knowledge gap exists across a wider, more benign range of thermal conditions that insects are also likely to encounter in the field. Field studies have also examined the impact of the rearing temperature on dispersal, and by implication, indirectly assessed flight performance (e.g. Loeschcke and Hoffmann, 2007; Kristensen et al., 2008; Chidawanyika and Terblanche, 2011). Given that the studies by Frazier et al. (Frazier et al., 2008) and Ferrer et al. (Ferrer et al., 2013) could not fully assess potential trade-offs between elevated low temperature flight performance and high temperatures, this leaves the information from field assessments of acclimation responses by recapture at bait stations (e.g. Kristensen et al., 2008), which are not especially well linked to laboratory responses (discussed further in Sørensen et al., 2009; Chidawanyika and Terblanche, 2011).

Here, we therefore address the knowledge gap of acclimation effects on flight performance by examining the temperature-dependence of flight ability and its response to rearing temperature in the pupal stage of Ceratitis capitata (Wiedemann 1824), a global agricultural pest, in a full-factorial experimental design. This design aimed to cover the thermo-biological range of typical flight activity in C. capitata and to fully consider the major alternative acclimation hypotheses outlined above. We also specifically aimed to minimize the duration of temperature exposure in development by only exposing the pupal stage. Given that an acclimation response was expected, based largely on the aforementioned literature, factors that may have resulted in flight ability and performance variation were also investigated. To this end, a range of morphological variables [body mass (Mb), wing width, wing length, wing area, wing loading (WL) and aspect ratio (AR)] were examined, as all have previously been implicated as influencing flight performance and manoeuvrability in the field and in the laboratory (e.g. Bartholomew and Casey, 1978; reviewed in Dudley, 2000; Harrison and Roberts, 2000; Berwaerts et al., 2002; San Martin y Gomez and van Dyck, 2012). As proximate explanations for variation in flight performance, whole-animal metabolic rate and the activity of a key aerobic energy pathway enzyme (CCO) were also assessed.

RESULTS

Flight performance

The odds of changing between flight categories ‘failure’ and ‘lift’ or between categories ‘lift’ and ‘flight’ are hereafter considered

List of symbols and abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>aspect ratio</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BAH</td>
<td>beneficial acclimation theory</td>
</tr>
<tr>
<td>CCO</td>
<td>cytochrome c oxidase</td>
</tr>
<tr>
<td>CIB</td>
<td>colder is better</td>
</tr>
<tr>
<td>DAH</td>
<td>deleterious acclimation theory</td>
</tr>
<tr>
<td>HIB</td>
<td>hotter is better</td>
</tr>
<tr>
<td>M B</td>
<td>body mass</td>
</tr>
<tr>
<td>OAH</td>
<td>optimal acclimation theory</td>
</tr>
<tr>
<td>RMR</td>
<td>resting metabolic rate</td>
</tr>
<tr>
<td>T acc</td>
<td>acclimation temperature</td>
</tr>
<tr>
<td>T test</td>
<td>test temperature</td>
</tr>
<tr>
<td>WL</td>
<td>wing loading</td>
</tr>
</tbody>
</table>
equivalent to flight performance (Fig. 1). Flight performance was significantly influenced by test temperature ($T_{\text{test}}$) and the interaction term $T_{\text{test}} \times T_{\text{acc}}$ (developmental acclimation temperature), but not by $T_{\text{acc}}$ alone (Table 1).

An increase in $T_{\text{test}}$ resulted in increased flight performance across all $T_{\text{acc}}$ groups, e.g. at $T_{\text{test}}=15^\circ$C only 10% of flies flew successfully, whereas at $T_{\text{test}}=30^\circ$C, 76.5% did so (Fig. 1A). A significant negative interaction between $T_{\text{test}}$ and $T_{\text{acc}}$ was detected (Table 1; $P<0.0001$). When multiple pair-wise comparisons of flight performance outcomes were undertaken for each $T_{\text{acc}}$ group at each $T_{\text{test}}$, it was found that the only significant differences in performance occurred at $T_{\text{test}}=15^\circ$C and $20^\circ$C (Table 2). Here, flies that had been reared at $T_{\text{acc}}=15$ and $20^\circ$C performed better than those reared at $T_{\text{acc}}=25$ and $30^\circ$C at $T_{\text{test}}=15^\circ$C, but flies reared at $T_{\text{acc}}=15$, $20$ and $25^\circ$C

![Fig. 1. The effect of developmental acclimation temperature ($T_{\text{acc}}$) on flight performance of *C. capitata* flies at different test temperatures ($T_{\text{test}}$). A total of 30 flies (shown in panel A), of which 15 were males (shown in panel B) and 15 were females (shown in panel C), from each $T_{\text{acc}}$ were tested at each $T_{\text{test}}$. The black bars indicate failure to fly, the white bars indicate the ability to obtain lift (but no flight) and the grey bars show the proportion of individuals that were able to perform successful flight.](image)

### Table 1. Results of the best-fit, ordinal logistic regression assessing the effects of sex (males coded as 0, females coded as 1), body mass ($M_b$), test temperature ($T_{\text{test}}$), developmental acclimation temperature ($T_{\text{acc}}$) and the interaction between $T_{\text{test}}$ and $T_{\text{acc}}$ on *C. capitata* flight performance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>s.e.m.</th>
<th>$t$-value</th>
<th>$P$-value</th>
<th>Odds ratio</th>
<th>LCI</th>
<th>UCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>–0.516</td>
<td>0.243</td>
<td>–2.121</td>
<td>0.0339</td>
<td>0.597</td>
<td>0.371</td>
<td>0.962</td>
</tr>
<tr>
<td>$M_b$</td>
<td>0.448</td>
<td>0.134</td>
<td>3.352</td>
<td>&lt;0.0001</td>
<td>1.565</td>
<td>1.204</td>
<td>2.034</td>
</tr>
<tr>
<td>$T_{\text{test}}$</td>
<td>0.474</td>
<td>0.040</td>
<td>11.741</td>
<td>&lt;0.0001</td>
<td>1.606</td>
<td>1.484</td>
<td>1.738</td>
</tr>
<tr>
<td>$T_{\text{acc}}$</td>
<td>0.012</td>
<td>0.035</td>
<td>0.347</td>
<td>0.7286</td>
<td>1.012</td>
<td>0.946</td>
<td>1.083</td>
</tr>
<tr>
<td>$T_{\text{test}} \times T_{\text{acc}}$</td>
<td>–0.008</td>
<td>0.002</td>
<td>–4.744</td>
<td>&lt;0.0001</td>
<td>0.992</td>
<td>0.989</td>
<td>0.995</td>
</tr>
</tbody>
</table>

The variables in this model were selected using the minimal adequate model approach as described previously in Crawley (Crawley, 2007). $T_{\text{acc}}$ was retained because of the presence of a higher order interaction term. Significant effects are highlighted in bold font. s.e.m., standard error of the mean; LCI, lower 95% confidence interval; UCI, upper 95% confidence interval.
performed better than those reared at \( T_{\text{acc}} = 30^\circ \text{C} \) at \( T_{\text{test}} = 20^\circ \text{C} \). No significant differences in flight performance outcomes were detected between the \( T_{\text{acc}} \) groups at \( T_{\text{test}} = 25^\circ \text{C} \) and \( 30^\circ \text{C} \) (Table 2; Fig. 1A).

According to the best-fit model, sex and \( M_b \) also had a significant effect on flight performance with males generally performing better than females for a given body size (Table 1; Fig 1B,C) and an overall positive relationship existed between an increasing flight score (i.e. performance) and \( M_b \).

### Morphology

The scaling relationship between body mass (\( M_b \)) and wing length, wing width and area varied with \( T_{\text{acc}} \) for males but not for females (Table 3A; \( T_{\text{acc}} \times M_b \) effects). There were no effects of \( T_{\text{acc}} \) on aspect ratio (AR) for either sex. Overall, morphological variation (wing length, width, area and WL) changed in relation to \( M_b \) only in cold-acclimated males (Fig 2). In all four of these morphological variables, the slope with \( M_b \) tended to be larger for males than females for a given body size (Table 1; Fig 1B,C). Generally, the intermediate acclimation groups did not differ significantly from each other.

Sex significantly influenced all the wing traits measured but \( T_{\text{acc}} \) only affected \( M_b \), wing length, aspect ratio and wing loading (Table 3B; Fig 3). The significant \( T_{\text{acc}} \times \text{sex} \) interaction indicates that male and female morphology responded differently to \( T_{\text{acc}} \) treatments with regards to \( M_b \), wing width and wing area (Table 3B). However, it is difficult to identify general patterns (cf. Fig. 3A,E). Females tended to be larger and to have greater WL and AR values, whereas males tended to have wider wings. Males appeared to have a stronger response to \( T_{\text{acc}} \) in terms of wing width, but females seemed to respond more strongly to \( T_{\text{acc}} \) in terms of \( M_b \) and wing area. Nonetheless, the main differences in morphology across both sexes appear to be due to the colder \( T_{\text{acc}} \) treatment.

Models that predict fly morphology using flight performance suggest that male scores predict wing length, whereas female flight scores predict \( M_b \), wing length, wing width and area. However, there was not necessarily a simple relationship between a particular morphological trait and a flight score category. For

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**Table 2.** Multiple comparisons of flight performance outcomes between developmental acclimation groups (\( T_{\text{acc}} \)) at each test temperature (\( T_{\text{test}} \)) for both sexes of \( C. \) capitata flies pooled

<table>
<thead>
<tr>
<th>( T_{\text{test}} ) (°C)</th>
<th>( T_{\text{acc}} ) (°C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>1.0000</td>
<td>0.0321</td>
<td>0.0153</td>
<td>1.0000</td>
<td>0.1160</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>1.0000</td>
<td>0.0321</td>
<td>0.0153</td>
<td>1.0000</td>
<td>0.1715</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.0360</td>
<td>1.0000</td>
<td>0.1160</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.2709</td>
<td>1.0000</td>
<td>0.1160</td>
</tr>
</tbody>
</table>

The table shows \( P \)-values resulting from a Kruskal–Wallis test with significant flight score differences between \( T_{\text{acc}} \) groups shown in bold.

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**Table 3.** Summary of results from generalized linear models (GLM) testing effects of acclimation and morphology on flight performance

<table>
<thead>
<tr>
<th>Effect</th>
<th>Body mass (mg)</th>
<th>Wing length (mm)</th>
<th>Wing width (mm)</th>
<th>Wing area (mm²)</th>
<th>Aspect ratio</th>
<th>Wing loading (mg mm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_{\text{acc}} )</td>
<td>( T_{\text{test}} )</td>
<td>( M_b )</td>
<td>Sex</td>
<td>d.f.</td>
<td>Wald</td>
</tr>
<tr>
<td>Intercept</td>
<td>32.1</td>
<td>11.5</td>
<td>4.8</td>
<td>1.0</td>
<td>15.5</td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td>2.8</td>
<td>1.1</td>
<td>0.182</td>
<td>0.8</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>( M_b )</td>
<td>3.8</td>
<td>3.6</td>
<td>2.4</td>
<td>0.5</td>
<td>4.4</td>
<td>1</td>
</tr>
<tr>
<td>( T_{\text{acc}} )×Sex</td>
<td>2.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.2</td>
<td>2.2</td>
<td>1</td>
</tr>
<tr>
<td>( M_b )×Sex</td>
<td>5.5</td>
<td>4.9</td>
<td>0.5</td>
<td>0.2</td>
<td>5.7</td>
<td>1</td>
</tr>
</tbody>
</table>

The effect of (A) acclimation (\( T_{\text{acc}} \)) and body mass (\( M_b \)) on wing length, wing width and area, aspect ratio and wing loading in \( C. \) capitata flies. Significant effects are highlighted in bold. d.f., degrees of freedom; F, females; M, males.
example, flight failure (score=0) seemed to be associated with low wing width, but high wing width was not necessarily indicative of complete flight success (score=2) (flies with a high wing width had similar scores of 0, 1 and 2) in females (Fig. 4F; and see supplementary material Fig. S1 for flight scores pooled across $T_{acc}$).

Overall, no interaction was found between the $T_{acc}$ and flight score with regards to any of the morphological traits (Table 3C; Fig. 4). Female morphology was generally more strongly associated with a particular flight score ($M_{b}$, wing width, wing area; $P<0.05$) and influenced by $T_{acc}$ ($M_{b}$, wing length, AR, WL; $P<0.05$), whereas in...
males, only wing length was significantly different among flight performance scores ($P=0.04$) and wing width, wing area and AR were influenced by $T_{\text{acc}} (P<0.05)$. Using an alternative statistical approach (i.e. log transformation of input variables prior to estimation of WL and AR), the generalized linear models (GLZ) were rerun for these two variables, which showed that the main qualitative conclusions reached with AR and WL values from the traditional equations for WL and AR did not change, with the only exception being the effect of $T_{\text{acc}}$ and $M_b$ on WL in females. Here $T_{\text{acc}}, M_b$ and $T_{\text{acc}} \times M_b$ were significant ($P<0.05$), where previously only $M_b$ was significant (supplementary material Table S1).

**CCO activity and metabolic rate**

The CCO assay revealed that the activity of the enzyme measured at 25°C increased with an increase in $T_{\text{acc}} (P=0.021)$; however, the only significant differences in CCO activity occurred between $T_{\text{acc}}=15°C$ and 30°C (Fig. 5A). In general, metabolic rates increased as $T_{\text{test}}$ increased (Fig. 5B–D); however, the relationship between $V_{\text{CO}_2}$ and $T_{\text{test}}$ differed across acclimation groups, i.e. flies that had been acclimated to 30°C did not respond as strongly to $T_{\text{test}}$ (i.e. they had a shallower rate–temperature slope) (Fig. 5B). Log-transformed mass, sex and $T_{\text{acc}}$ all significantly influenced log mean $V_{\text{CO}_2}$ and log resting $V_{\text{CO}_2}$ ($P<0.01$), and the interaction term LogMass×sex significantly influenced only log mean $V_{\text{CO}_2} (P<0.03$, Table 4). $T_{\text{acc}}$ had no effect however on either of these $V_{\text{CO}_2}$ variables. By contrast, $T_{\text{acc}}$ was a significant predictor ($P<0.0001$) of peak $V_{\text{CO}_2}$ together with log-transformed mass, sex, $T_{\text{test}}$, and interactions between $T_{\text{acc}}$ and $T_{\text{test}}$, and LogMass, $T_{\text{acc}}$ and sex (all $P<0.032$).

**DISCUSSION**

This study shows that the flight performance of adult *C. capitata* is affected not only by the immediate thermal surroundings, but also by recent thermal history, i.e. the temperatures experienced during the pupal developing life-stage. Several findings of this work are important for understanding the evolution of phenotypic plasticity and temperature-dependent performance of ectothermic animals more generally, of which three aspects are perhaps most noteworthy. First, we found that in *C. capitata* flight performance increased with increasing test temperature, largely as might be expected based on other previous examinations of insect flight (e.g. Frazier et al., 2008; Kristensen et al., 2008; Chidawanyika and Terblanche, 2011; Ferrer et al., 2013). A substantial influence of thermal history on adult flight performance was also found, with the major result being that cooler developmental temperatures resulted in improved flight ability at cooler test temperatures. This result excludes the ‘hotter is better’, ‘optimal acclimation’ as well as ‘deleterious acclimation’ from being possible acclimation hypotheses for *C. capitata* flight performance. Given that flies that had been acclimated at colder temperatures performed better at low temperatures, compared with flies that were not cold acclimated,
Fig. 4. Summary of the morphological variables by flight score category. Recorded flight scores (0=failure, 1=lift, 2=flight) across the range of test temperatures as a function of body mass (A,B), wing length (C,D), wing width (E,F), wing area (G,H), aspect ratio (I,J) and wing loading (K,L) in C. capitata. Results from males (first column of panels) and females (second column of panels) are presented by developmental acclimation groups (T_{acc}): 15°C (blue squares), 20°C (green diamonds), 25°C (black triangles) and 30°C (red circles).
Models were run on log-transformed CO\textsubscript{2} and log-transformed mass (LogMass) data using a repeated measures ANCOVA (Kenward-Rogers degrees of freedom (d.f) method). Den., denominator; Num., numerator.

Table 4. Minimum adequate models of mean \( V_{\text{CO}_2} \), peak \( V_{\text{CO}_2} \) and resting \( V_{\text{CO}_2} \) in \textit{C. capitata}

<table>
<thead>
<tr>
<th>( V_{\text{CO}_2} ) (( \mu l ) h(^{-1} ))</th>
<th>Effect</th>
<th>Num. d.f.</th>
<th>Den. d.f.</th>
<th>( F )-value</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>LogMass</td>
<td>1</td>
<td>217</td>
<td>42.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>1</td>
<td>217</td>
<td>10.78</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{test}} )</td>
<td>3</td>
<td>217</td>
<td>315.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>LogMass×Sex</td>
<td>1</td>
<td>217</td>
<td>4.87</td>
<td>0.0283</td>
</tr>
<tr>
<td>Peak</td>
<td>LogMass</td>
<td>1</td>
<td>205</td>
<td>4.70</td>
<td>0.0313</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>1</td>
<td>205</td>
<td>11.66</td>
<td>0.0008</td>
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<tr>
<td></td>
<td>( T_{\text{test}} )</td>
<td>3</td>
<td>205</td>
<td>216.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{acc}} )</td>
<td>3</td>
<td>205</td>
<td>7.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{acc}} \times T_{\text{test}} )</td>
<td>3</td>
<td>205</td>
<td>9.39</td>
<td>&lt;0.0001</td>
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<td></td>
<td>LogMass×( T_{\text{acc}} \times \text{Sex} )</td>
<td>7</td>
<td>205</td>
<td>4.92</td>
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</tr>
<tr>
<td>Resting</td>
<td>LogMass</td>
<td>1</td>
<td>218</td>
<td>56.81</td>
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<tr>
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<td>Sex</td>
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<td>218</td>
<td>112.06</td>
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<tr>
<td></td>
<td>( T_{\text{test}} )</td>
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<td>218</td>
<td>249.81</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

and that hotter acclimated flies did not perform better at higher test temperatures, the ‘beneficial acclimation’ hypothesis can also be ruled out. Thus, these results provide support for the CIB acclimation hypothesis, but this is only partial support because performance improved at only the lower test temperatures, and the benefits thereof decreased at warmer test temperatures. This CIB result is striking, given that this is a tropical (although cosmopolitan) insect pest, which is likely to originate from stable, warm environments in East Africa (Gasperi et al., 1991) and shows high dispersal ability (Karsten et al., 2013). To date, no studies have showed support for CIB in the flight ability of insects across a wide range of commonly encountered temperatures. This hypothesis has, however, been supported in locomotor performance of some terrestrial arthropod species from the sub-Antarctic Marion Island, where it is thought to reflect adaptation to this predominantly low temperature environment (Deere and Chown, 2006). One potential evolutionary advantage for a CIB-type acclimation response in this tropical fruit fly is that it could expand the thermal window for activity across seasons, especially because host plants are unlikely to be a limiting feature of population growth given the highly polyphagous nature of \textit{C. capitata} and that it can occupy rather cool, high-elevation environments in East Africa that may experience strong seasonality.

For \textit{Drosophila melanogaster}, field assessments of dispersal ability support the BAH (Kristensen et al., 2008), and laboratory studies of flight performance support a similar conclusion (Frazier et al., 2008), although the latter study focuses mainly on low temperature flight performance and not the fuller, more benign range of thermal conditions. Here, our study is unique because it can, using a different model species, assess potential trade-offs in flight performance across a broader range of thermal conditions, which are still likely to be representative of the field thermal conditions experienced by \textit{C. capitata} (Terblanche et al., 2010b).

The second major finding of our work is that, although thermal development clearly influences morphology (e.g. Berwaerts et al., 2002; San Martin y Gomez and Van Dyck, 2012; Ferrer et al., 2013), these effects are less straightforward than what might have been expected based on prior work from \textit{Drosophila} species, for example. However, we employed a distinctly different approach to typical assessments of rearing temperature on fly morphology. Most importantly, we subjected flies to thermal variation only in the pupal stage, whereas many previous studies subject either the entire life cycle or only the larval stages to different conditions (for example, Partridge et al., 1994; French et al., 1998; Frazier et al., 2008). It is indeed well established that the timing and duration of temperature variability can have marked effects on body size and wing size (French et al., 1998; Frazier et al., 2001), both of which may interact to determine flight ability (Azevedo et al., 1998; Frazier et al., 2008). Previous work has sought to induce changes in morphology and subsequently infer or measure changes in flight performance (Azevedo et al., 1998; Frazier et al., 2008); however, here we aimed to induce physiological performance variation with a short-duration approach and to then examine the potential mechanisms or morphological changes associated with this performance variation. For example, Frazier et al. (Frazier et al., 2008) show that lower developmental temperatures result in flies having a much larger
wing area and in smaller changes in $M_b$ (cooler flies became larger), resulting in a lower overall WL. They concluded that the improved low temperature flight ability observed was largely driven by these morphological changes. The results here for _C. capitata_ are also similar in that respect – morphological responses to rearing temperature were found. Outcomes for _C. capitata_ were, however, in the opposite direction to that found by Frazier et al. (Frazier et al., 2008), resulting generally in lower $T_{sec}$, flies having a lower $M_b$, which resulted in a lower overall WL. This may in part be due to different timing and duration of thermal treatments. The results were also both temperature- and sex-specific, in contrast with Frazier et al. (Frazier et al., 2008) who generally found similar rearing temperature responses in both sexes, with males and females differing relatively consistently in their morphology [compare with fig. 2 in Frazier et al. (Frazier et al., 2008)]. In _C. capitata_ examined here, wing morphology changed readily in low-temperature acclimated males compared with higher $T_{sec}$ groups, but to a far lesser extent in females. In some of the morphological traits measured, there was pronounced sexual dimorphism (e.g. AR), but in others this was less so (e.g. wing area). Under some of the rearing temperature conditions, the sexual dimorphism was abolished (e.g. $T_{sec}$=15°C, $M_b$ and wing length relative to rearing at optimal conditions); however, in other cases, such as $T_{sec}$=20°C ($M_b$ and wing length) and $T_{sec}$=30°C (wing width), the dimorphism became more pronounced. The reasons for this variation are unclear at present, but one possibility is that these involve temperature- and sex-dependent gene expression and protein regulation. In some cases, this relative increase in WL caused by changes in $M_b$ or wing area could be the reason for flight failure in some treatment groups.

The third main finding of this work was to show how these morphological responses to rearing temperature, resulting from exposure during the entire pupal stage, may in turn be associated with locomotor performance in the adult stage. Clearly, the sex of the flies influenced the morphology and this was temperature-dependent with, for example, female morphology generally more strongly associated with a particular flight score. There was not however, a straightforward relationship between a particular morphological trait and flight ability. Instead, the opposite appears to be more broadly true, such that, low WL or high wing area (for example) is not always associated with flight, but having a high WL or small wing area may well be associated with the failure to achieve flight. Thus, although flight failure could be explained as a result of the absence of a particular morphological trait, the presence of that trait does not necessarily confer greater flight performance, and consequently, dispersal potential (in disagreement with Berwaerts et al., 2002; San Martin y Gomez and van Dyck, 2012). Such a result, if it holds more broadly, may have far-reaching implications for predictions of climate change impacts, or temperature variation, on the dispersal ability of insects as it would complicate the prediction of performance and dispersal from the measurement of only morphological features, as is often undertaken. Naturally, further work would be required to better understand the link between a particular flight score and field dispersal abilities, which may not necessarily be a straightforward relationship. However, it is probably reasonable to assume an overall positive relationship between these two measures of dispersal.

The limited association between morphology and flight ability detected here may be due to physiological or biochemical adjustments that are employed to compensate for the thermal conditions experienced during development, and which may override the impacts of morphological variation on acclimation. This could be particularly true under the highly energy-demanding circumstances of flight (Suarez et al., 1996; Harrison and Roberts, 2000; Skandalis et al., 2011). A proximate mechanism for potentially explaining flight performance in _C. capitata_ was, however, not forthcoming. Specifically, it was found that CCO activity increased with acclimation temperature, whereas if physiological or biochemical compensation was the major expectation, one might expect elevated CCO activity in the lowest acclimation temperature group, which could then perhaps explain the increased performance of this group across a broader range of test temperatures later in adult life. In the case of metabolic rates (at rest, on average or peak), although we found a significant acclimation and test temperature interaction, the direction of effects was again not consistent with the elevated physiological rates hypothesis. Instead, we found higher rates of energy consumption under the 30°C test temperature in flies that had been acclimated at 30°C, with no pronounced difference among the four acclimation groups under 15°C test conditions.

Overall, this study shows that the temperature at which flies are reared affects their morphology and performance in various ways. These morphological changes have been found to be sex-specific and cannot necessarily be used to explain the flight performance outcomes in a specific individual. It is nevertheless possible that motivational and behavioural factors, along with other key physiological or morphological traits that have not been examined here – e.g. variation in phosphoglucose isomerase (Rank et al., 2007), flight muscle mass and/or mitochondrial density and fibre type composition (Swank et al., 2006; and see Skandalis et al., 2011) – are playing a significant role in the flight performance outcomes. Further work examining changes in wing beat frequencies and muscle fibre composition and performance, how these factors differ between sexes, and their temperature dependence in _C. capitata_ would thus be useful. However, an alternative explanation is that multiple small biochemical and morphological adjustments at lower hierarchical levels of biological organization interact, perhaps in non-linear ways, to determine the effects of the rearing temperature on flight performance. If this is the case, then being able to establish a direct link between performance and any single sub-organismal measure might always be challenging, and could limit the value of a ‘reductionist’ scientific approach.

**MATERIALS AND METHODS**

**Study organisms**

Individuals of _C. capitata_ were obtained from a large outbred culture reared indoors under variable although buffered temperatures at Citrus Research International (Nelspruit, South Africa). On arrival at Stellenbosch University (Stellenbosch, South Africa), pupae were divided into four developmental acclimation groups ($T_{acl}$=15, 20, 25 and 30°C) and maintained in temperature-controlled incubators (MRC LE-500, Holon, Israel) under a 12 h:12 h light:dark photoperiod. Essentially, flies were kept for almost the entire duration of the pupal stage at different rearing temperatures. This minimized, but did not eliminate, morphological changes (French et al., 1998) but was sufficient to elicit physiological performance variation, the latter of which was the objective of the study. Other studies have reared flies at different temperatures for longer periods (for example, the entire larval or entire life cycle) and then assessed morphology (e.g. French et al., 1998), and either directly or by inference from flight performance (e.g. Azvedo et al., 1998; Frazier et al., 2008). Pupae were kept at their respective acclimation temperatures until peak adult eclosion ($T_{acl}$=30°C for 5 to 6 days, $T_{acl}$=25°C for 6 to 7 days, $T_{acl}$=20°C for 7 to 8 days and $T_{acl}$=15°C for 12 to 13 days), after which the flies were allowed to mature for 7 to 8 days at 25°C with sugar and water available _ad libitum_ to ensure that flight muscles were fully developed (e.g. Skandalis et al., 2011). Flies were kept at 25°C to ensure that developmental effects were in fact due to longer-term alteration of ontogenetic trajectories and not changes associated with...
morphological reorganization upon eclosion [see Bowler and Terblanche (Bowler and Terblanche, 2008) for similar discussion in terms of thermal tolerance]. Flies from the different *T*~acc~ groups were selected at random for trials, and all assays were undertaken on flies at the same adult developmental age, i.e. a week after eclosion.

**Flight performance**

A full-factorial experimental design was used to determine flight performance of the developmental acclimation groups at four different test temperatures (*T*~acc~: 15, 20, 25 and 30°C). Flight experiments were performed on a custom-built 0.36 m² double-jacketed temperature stage, under which 1:1 water:propylene glycol mix was pumped from a programmable water bath (Huber CC-410wl, Huber, Offenburg, Germany). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Corporation, China) was used to verify the stage surface temperature, and insect body temperatures were measured with a handheld infrared thermometer (Fluke 63 IR series, Fluke Corporation, China; accuracy 0.05°C at 5 cm distance) to ensure that this was always at equilibrium with the chamber surface temperature.

A total of *N* = 480 randomly selected flies (120 per *T*~acc~: 15 males and 15 females per *T*~acc~) were each individually introduced to an inverted transparent plastic container (12 cm length × 12 cm width × 7 cm height) and allowed a 2 min thermal equilibration period on the surface of the temperature stage. Each fly was encouraged to fly by gently prodding it with a thermally-equilibrated and inert, thin plastic rod inserted between the plastic container and the thermal stage. Performance in the first minute was either recorded as ‘flight’ (score=2; the ability to stay airborne and travel the length or height of the container, indicating sustained flight), ‘lift’ (score=1; temporary lift, but with insufficient distance travelled) or ‘failure’ (score=0; walking or no activity). In the latter case, prodding continued until a maximum of 5 min had passed or until flight or lift was achieved. According to the flight scores assigned, a flight score of 1 and 2 reflect the ability to take-off and maintain flight, respectively, and thus, represent significant aspects of flight performance, and not simply behavioural propensity to perform activity. Indeed, sustained flight (score=2) undoubtedly contains elements of physiological performance. For this reason, flight scores are used to reflect performance and not simply the propensity or willingness to fly. No flies were re-used at another temperature. A fly could only be scored in one behaviour category and was removed once this behaviour category was determined. All flies were weighed to 0.1 mg using a digital microbalance (Mettler Toledo MS104S, Switzerland) before each trial to determine fresh mass.

To address the main question of whether the flight scores at different test temperatures were influenced by thermal history, the ordinal logistic regression model method adopted by Frazier et al. (Frazier et al., 2008) was followed. This method delivers the odds of changing between flight categories ‘failure’ and ‘lift’ or between categories ‘lift’ and ‘flight’, based on the different parameters in the model. Firstly, to determine which factors to include in the ordinal logistic regression, a minimal adequate model was obtained by initially fitting the maximal model (which included *T*~acc~ and *T*~min~; sex and all possible interaction terms) and then simplifying the model, starting with the highest order interactions (Crawley, 2007). The full model specifically included sex (where males and females were assigned 0 or 1, respectively) and *M*~b~ as separate factors, because both may independently influence flight performance. The full model also included the interaction between *T*~min~ and *T*~acc~ as an indicator of phenotypic plasticity. The ordinal logistic regressions analyses were run in R (version 2.15.2; R Foundation for Statistical Computing, Vienna, Austria) and included the use of the package MASS (Venables and Ripley, 2002).

**Wing morphology**

Flies from the flight performance trials were used for measurement of wing morphology. Flies were thawed from −80°C and their wings were removed with a scalpel, and the right wings were mounted on a microscope slide with clear nail varnish. Wings for two-dimensional image analysis were photographed using a Leica MZ16A automontage microscope fitted with a Leica DFC 290 fixed digital camera (Leica, Wetzlar, Germany). Supplementary material Fig. S2 shows the landmarks used to determine wing length, wing width and wing area of each wing.

The variables were calculated from the digital images using analysis tools that accompany the Leica software [Leica Application Suite (LAS) v4.1]. From these measurements, aspect ratio and wing loading were calculated using the following equations (Dudley, 2000):

\[ AR = \frac{4R^2}{S}, \]

\[ WL = \frac{M_b}{S}, \]

where *AR* is the aspect ratio, *R* is the wing length in mm and *S* is the wing area in mm²; *WL* is the wing loading, *M*~b~ is the body mass in mg and *S* is the wing area in mm².

To assess the effects of *T*~acc~ and fresh *M*~b~ on morphological variables, a full GLZ with a normal distribution of errors and a log link function was run for each main variable separately (wing length, wing width, wing area, AR and WL), and included a *T*~acc~×*M*~b~ interaction term. Male and female flies were investigated separately as sex seemed to be a major factor influencing the phenotypic plasticity at certain *T*~acc~. If the *T*~acc~×*M*~b~ interaction was not significant, this indicated that the slopes of *T*~acc~ groups were homogeneous. If the interaction term was significant, it was used to interpret the slope variation between groups.

Preliminary analyses suggested that some of the variation in morphology might be sex-related. Therefore, sex was also examined as a factor between *T*~acc~ groups, and the interaction between *T*~acc~ and sex was explicitly tested in a separate set of GLZ analyses run for each morphological variable (*M*~b~, wing length, wing width, wing area, AR and WL). We also examined an alternative approach, namely to log10-transform the input variables *M*~b~ and *S* prior to calculation of WL, and the same for AR (supplementary material Table S1).

In order to explore variation in morphological features (e.g. low or high WL) and between flight score categories, within and between *T*~acc~ groups, an alternative approach to the logistic regression was used. Specifically, morphological variables were treated as the dependent variable and plotted as a function of flight score for both sexes separately, and GLZ were used to test whether morphology varied consistently among flight score groups. Linear regression analyses were run in R, whereas GLZ analyses were run in Statistica 11 (Statsoft, Tulsa, OK, USA), with arithmetic means and error bars indicating 95% confidence intervals unless otherwise stated.

**Metabolic rate**

For this part of the study, a new batch of fly pupae was exposed to developmental acclimation temperatures as described above. The *V*~CO2~ production of individual adult fruit flies was then recorded using a multiplexed flow-through respirometry system [similar to Zrubek and Woods (Zrubek and Woods, 2006)]. The airflow was regulated at 200 ml min⁻¹ via a mass control valve (Sidetran, Sierra International, USA) linked to a mass flow control box (Sable Systems, Las Vegas, NV, USA). Next, air was pushed through the first channel of an infrared CO₂/H₂O analyser (LI-7000, Li-Cor, Lincoln, NE, USA) to obtain a baseline reading, passed through the respirometry cuvette, and returned to the LI-7000 for differential recording of insect *V*~CO2~ production. The set-up included an eight-channel multiplexer (Sable Systems) with the temperature of the respirometry cuvettes being regulated by a programmable, circulating water-filled bath (Huber cc410-wl, Offenburg). The first channel of the multiplexer was used as an empty reference channel to determine baseline readings, whereas the remaining seven were used to record the gas exchange of individual flies in the dark. Each of the eight channels of the multiplexer was consecutively recorded for 30 min and this was repeated at every temperature: 15, 20, 25 and 30°C. The temperature was ramped up during the 30 min baseline, i.e. recording of the first channel. A total of 14 randomly selected flies per *T*~acc~ (7 males and 7 females) were measured at each *T*~acc~ and each respirometry run consisted of males and females from one *T*~acc~. The aim of these trials was to measure resting (inactive) metabolic rate (RMR), although clearly some trials contained periods of voluntary activity. Activity metabolic rate could be easily detected from RMR owing to a several-fold increase in *V*~CO2~. Resting MR was taken as the lowest stable 2 min period of each individual’s respirometry recording. A 2 min period
was necessary to standardize across all individuals and trials because, at higher temperatures, flies seldom remained motionless, even in the darkened cuvettes for long periods. Independent pilot trials over longer periods with electronic activity detectors confirmed that these $V_{\text{CO}_2}$ parameters could be extracted reliably and repeatedly by a trained observer. Given that RMR did not show any acclimation effect, we also extracted two additional parameters from the respirometry traces: (i) the average (including non-resting periods) metabolic rate across the central 20 min of the recording per individual at each temperature (following Gefen, 2011), and (ii) the peak metabolic rate during recordings, i.e. the highest, stable 5 s period of $V_{\text{CO}_2}$ as a possible correlate of maximal voluntary activity and its associated cost. Given the maximum estimated time constant (equilibration time) in our setup, the maximum lag is estimated to be 30 s (5±6 s to achieve <1% of $V_{\text{CO}_2}$ in the respirometry cuvette) and is not likely to confound estimates of peak or minimum stable values. All flies were weighed to 0.1 mg using a digital microbalance (Mettler Toledo MS1045, Switzerland) before and after each respirometry trial to determine fresh mass.

Owing to repeated measures on the same individual at different $T_{\text{acc}}$ for $V_{\text{CO}_2}$ estimates, a repeated measures ANCOVA (Kenward-Roger method) was used, and the minimum adequate model was determined [based on lowest number of terms and lowest Bayes’ Information Criterion (BIC) value] using SAS (proc mixed, Version 5.1, SAS Institute, Cary, NC, USA). The log-transformed values for body mass and $V_{\text{CO}_2}$ measurements were used for these analyses because these better satisfied the assumptions of the mixed model (e.g. homogeneity of variances).

**CCO activity**

For the CCO activity assay, a total of six samples per $T_{\text{acc}}$ group were measured. Each sample consisted of 60 whole male flies, totalling ca. 300 mg as required for sufficient mitochondrial extraction. A mitochondrial fraction was prepared from each sample by means of a mitochondrial isolation kit (MITOISO1; Sigma, MO, USA) following the manufacturer’s protocol (and see Lachenicht et al., 2010). Enzyme activity of the isolated mitochondrial fraction was then measured using a CCO assay kit (CYTOCOX1; Sigma, MO, USA) and a temperature controlled spectrophotometer (PowerWave HT; BioTek, Winooski, USA) at 25°C. All differences between frozen and fresh tissue samples (Fig. 1) showed that the data could be pooled, as there were no significant differences in absorbance over a minute of the activated samples to calculate the absolute change in absorbance. CCO activity in units $\text{mmole of ferrocytochrome c min}^{-1}$ was calculated using the following formula: $V_{\text{CO}_2}$ = $\chi$ mmole of ferrocytochrome c min$^{-1}$. The original fresh mass of each sample was used to convert the results into mass specific CCO activity ($\text{mmole of ferrocytochrome c min}^{-1} \text{ mg鲜}$).

The six samples used per $T_{\text{acc}}$ three were frozen in liquid nitrogen and placed in a -80°C freezer before use and three consisted of fresh flies. A generalised linear model with a normal distribution and a log link function showed that the data could be pooled, as there were no significant differences between frozen and fresh tissue samples ($P=0.102$). There was also no indication that CCO activity was influenced by an interaction between $T_{\text{acc}}$ and the tissue state of the samples ($P=0.084$).

For CCO activity, a GLZ (normal distribution and a log link function) was used owing to violation of the assumptions of ANOVA (variances were heterogeneous). In all cases, residual deviations were inspected for potential over-dispersion, which was not evident in any analysis.

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**Compelling interests**

The authors declare no compelling financial interests.

**Author contributions**


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**Supplementary material**

Supplementary material available online at http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.106526/-DC1

**References**


