

RESEARCH ARTICLE

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

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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 (T_{acc} : 15, 20, 25, 30°C) during their pupal stage and tested at these temperatures (T_{test}) as adults using a full-factorial study design. Major factors influencing flight performance included sex, body mass, T_{test} and the interaction between T_{test} and T_{acc} . Successful flight performance increased with increasing T_{test} across all acclimation groups (from 10% at 15°C to 77% at 30°C). Although T_{acc} did not affect flight performance independently, it did have a significant interaction effect with T_{test} . 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 T_{acc} 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 T_{acc} 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. Federich 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 Gómez 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; Ghalambor 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 (T_{acc}), 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

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List of symbols and abbreviations

AR	aspect ratio
ATP	adenosine triphosphate
BAH	beneficial acclimation hypothesis
CCO	cytochrome <i>c</i> oxidase
CIB	colder is better
DAH	deleterious acclimation hypothesis
HIB	hotter is better
M_b	body mass
OAH	optimal acclimation hypothesis
RMR	resting metabolic rate
T_{acc}	acclimation temperature
T_{test}	test temperature
WL	wing loading

‘acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment’ (Leroi et al., 1994; and see Wilson and Franklin, 2002). However, the BAH has not received strong support, largely owing to the inability to refute possible alternative hypotheses (Deere and Chown, 2006). Foremost among these alternatives are the colder is better (CIB) (e.g. Frazier et al., 2008) and the hotter is better (HIB) (e.g. Frazier et al., 2006) hypotheses, which have also received some support depending on the traits (e.g. life history, morphology, performance or tolerance traits) and taxa examined. These CIB and HIB hypotheses propose that organisms will perform best after exposure to either colder or hotter conditions at all test temperatures, respectively (see review in Huey et al., 1999). Other potential explanations for acclimation responses include the optimal acclimation hypothesis (OAH) (e.g. Zamudio et al., 1995; Terblanche and Kleynhans, 2009) or deleterious acclimation hypothesis (DAH) (e.g. Loeschcke and Hoffmann, 2002; Terblanche and Kleynhans, 2009), which propose that a particular intermediate environment will result in improved performance, or that the acclimation conditions resulted in damage that led to lower performance upon subsequent testing. The null hypothesis for all of these different acclimation responses is that there will be no phenotypic plasticity response under any environmental conditions (Huey et al., 1999). These major alternatives can be readily differentiated using a full-factorial experimental approach (Huey et al., 1999; and see Deere and Chown, 2006).

Even though phenotypic plasticity has been studied extensively in the context of organism performance, the underlying physiological and biochemical mechanisms driving the performance outcomes are not straightforward (e.g. Sørensen et al., 2009). For insects, some studies have shown a strong thermal acclimation response of resting metabolic rate (e.g. Terblanche et al., 2009; Terblanche et al., 2010a), with, for example, individuals from cool environments having a steeper metabolic rate-temperature reaction norm than those from warmer environments. Even in such cases, however, the underlying mechanisms of acclimation responses remain unclear (Terblanche et al., 2010a; Vorhees et al., 2013). A potential mechanism for thermal acclimation responses in insects may be the direct impact of temperature on metabolic enzymes, but the effects of acclimation temperature on energy production and efficiency (e.g. the activity of cytochrome *c* oxidase, CCO) also vary among ectotherms (Dahlhoff and Somero, 1993; Rogers et al., 2004; Lachenicht et al., 2010). Metabolic pathway enzymes in insects are generally correlated with increased performance at certain temperatures (e.g. Laurie-Ahlberg et al., 1985; McMullen and Storey, 2008), and CCO activity is argued to potentially be a rate-limiting step in ATP production in mitochondria

(Suarez et al., 2000; Hochachka and Somero, 2002). Temperature-related morphological changes, such as variation in wing size and shape (Cavicchi et al., 1991; Zera and Harshman, 2001) and body size (Nunney and Cheung, 1997; French et al., 1998; Frazier et al., 2001), could likewise be driving the outcome of various performance traits and their responses to temperature.

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 (M_b), 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 Martín 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

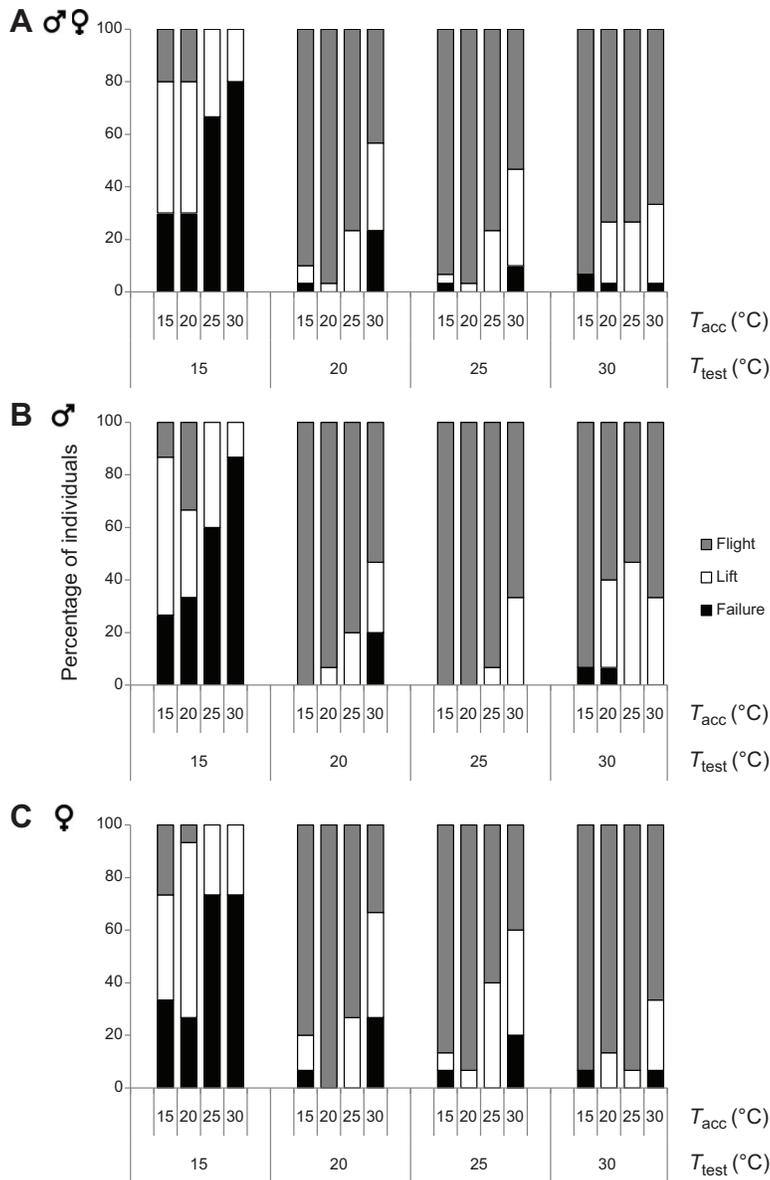


Fig. 1. The effect of developmental acclimation temperature (T_{acc}) on flight performance of *C. capitata* flies at different test temperatures (T_{test}). A total of 30 flies (shown in panel A), of which 15 were males (shown in panel B) and 15 were females (shown in panel C), from each T_{acc} were tested at each T_{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.

equivalent to flight performance (Fig. 1). Flight performance was significantly influenced by test temperature (T_{test}) and the interaction term $T_{test} \times T_{acc}$ (developmental acclimation temperature), but not by T_{acc} alone (Table 1).

An increase in T_{test} resulted in increased flight performance across all T_{acc} groups, e.g. at $T_{test}=15^\circ\text{C}$ only 10% of flies flew successfully, whereas at $T_{test}=30^\circ\text{C}$, 76.5% did so (Fig. 1A). A significant negative

interaction between T_{test} and T_{acc} was detected (Table 1; $P < 0.0001$). When multiple pair-wise comparisons of flight performance outcomes were undertaken for each T_{acc} group at each T_{test} , it was found that the only significant differences in performance occurred at $T_{test}=15^\circ\text{C}$ and 20°C (Table 2). Here, flies that had been reared at $T_{acc}=15$ and 20°C performed better than those reared at $T_{acc}=25$ and 30°C at $T_{test}=15^\circ\text{C}$, but flies reared at $T_{acc}=15$, 20 and 25°C

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_{test}), developmental acclimation temperature (T_{acc}) and the interaction between T_{test} and T_{acc} on *C. capitata* flight performance

Variable	Coefficient	s.e.m.	t-value	P-value	Odds ratio	LCI	UCI
Sex	-0.516	0.243	-2.121	0.0339	0.597	0.371	0.962
M_b	0.448	0.134	3.352	<0.001	1.565	1.204	2.034
T_{test}	0.474	0.040	11.741	<0.0001	1.606	1.484	1.738
T_{acc}	0.012	0.035	0.347	0.7286	1.012	0.946	1.083
$T_{test} \times T_{acc}$	-0.008	0.002	-4.744	<0.0001	0.992	0.989	0.995

The variables in this model were selected using the minimal adequate model approach as described previously in Crawley (Crawley, 2007). T_{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.

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

T_{test} (°C)	T_{acc} (°C)	T_{acc} (°C)			
		15	20	25	30
15	15		1.0000	0.0321	0.0153
	20			0.0321	0.0153
	25				1.0000
	30				
20	15		1.0000	1.0000	0.0360
	20			1.0000	0.0130
	25				0.1715
	30				
25	15		1.0000	1.0000	0.2709
	20			1.0000	0.1106
	25				1.0000
	30				
30	15		1.0000	1.0000	0.5588
	20			1.0000	1.0000
	25				1.0000
	30				

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

performed better than those reared at $T_{acc}=30^{\circ}\text{C}$ at $T_{test}=20^{\circ}\text{C}$. No significant differences in flight performance outcomes were detected between the T_{acc} groups at $T_{test}=25^{\circ}\text{C}$ and 30°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 wing area varied with T_{acc} for males but not for females (Table 3A; $T_{acc}\times M_b$ effects). There were no effects of T_{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 of males reared at $T_{acc}=15^{\circ}\text{C}$ was significantly steeper (in the case of wing length, width and area) or significantly shallower (in the case of WL) than the warmer acclimation groups (Fig. 2; Table 3). Generally, the intermediate acclimation groups did not differ significantly from each other.

Sex significantly influenced all the wing traits measured but T_{acc} only affected M_b , wing length, aspect ratio and wing loading (Table 3B; Fig. 3). The significant $T_{acc}\times\text{sex}$ interaction indicates that male and female morphology responded differently to T_{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_{acc} in terms of wing width, but females seemed to respond more strongly to T_{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_{acc} treatment.

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

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

Effect	Sex	d.f.	Body mass (mg)		Wing length (mm)		Wing width (mm)		Wing area (mm ²)		Aspect ratio		Wing loading (mg mm ⁻²)	
			Wald χ^2	P -value	Wald χ^2	P -value	Wald χ^2	P -value	Wald χ^2	P -value	Wald χ^2	P -value	Wald χ^2	P -value
A. Acclimation temperature (T_{acc}) and body mass (M_b)														
Intercept	M	1			4527.2	<0.0001	872.4	<0.0001	1707.6	<0.0001	29,756.3	<0.0001	367.8	<0.0001
T_{acc}		3			27.1	<0.0001	20.7	<0.0001	20.4	<0.0001	4.3	0.2355	9.1	0.0278
M_b		1			94.4	<0.0001	86.4	<0.0001	107.7	<0.0001	1	0.3287	582.8	<0.0001
$T_{acc}\times M_b$		3			26.3	<0.0001	26.5	<0.0001	23.4	<0.0001	1.3	0.7381	10.5	0.0149
Intercept	F	1			4133.3	<0.0001	466.4	<0.0001	1137.1	<0.0001	24,484.3	<0.0001	141.5	<0.0001
T_{acc}		3			4.8	0.1892	1.1	0.767	1.6	0.6648	3.6	0.3035	1.2	0.7629
M_b		1			195.2	<0.0001	152.2	<0.0001	169.2	<0.0001	0.6	0.4421	446.6	<0.0001
$T_{acc}\times M_b$		3			3.8	0.2888	2.4	0.485	1.8	0.6059	3.1	0.3841	0.9	0.8323
B. T_{acc} and sex														
Intercept	Both	1			253,130.5	<0.0001	59,394.2	<0.0001	104,029	<0.0001	1,912,376	<0.0001	856.8	<0.0001
T_{acc}		3			17.9	<0.001	5	0.1696	6.5	0.0908	53	<0.0001	22.5	<0.0001
Sex		1			31.5	<0.0001	63.3	<0.0001	8.9	<0.001	482	<0.0001	91.1	<0.0001
$T_{acc}\times\text{Sex}$		3			9.5	0.0233	7.8	0.0495	13.9	<0.001	4	0.218	3.9	0.2687
C. T_{acc} and flight score														
Intercept	M	1			12,791.7	<0.0001	28,486.2	<0.0001	49,330.3	<0.0001	814,419.9	<0.0001	119	<0.0001
T_{acc}		3			3.2	0.3641	6.1	0.1056	8.7	0.033	31.6	<0.0001	5.1	0.1649
Score		2			3.5	0.1731	6.5	0.0382	5.5	0.0644	1.1	0.5762	0.8	0.6556
$T_{acc}\times\text{Score}$		6			5.9	0.4371	8.7	0.1939	27.591	0.1101	3	0.8045	9.4	0.1504
Intercept	F	1			11,440	<0.0001	15,505.7	<0.0001	27,591	<0.0001	583,978	<0.0001	524.7	<0.0001
T_{acc}		3			14.6	0.0022	13.5	0.0037	4.3	0.2302	19.7	0.0002	18.5	<0.001
Score		2			6.8	0.0342	13.7	<0.001	13.6	0.0011	0.3	0.8489	3	0.2185
$T_{acc}\times\text{Score}$		6			2.6	0.8596	8	0.2357	7.1	0.3112	12.4	0.054	5.9	0.4296

The effect of (A) acclimation temperature (T_{acc}) and body mass (M_b), (B) T_{acc} and sex, and (C) T_{acc} and flight score (Score: 0, 1 or 2) on M_b , wing length, wing width, wing 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.

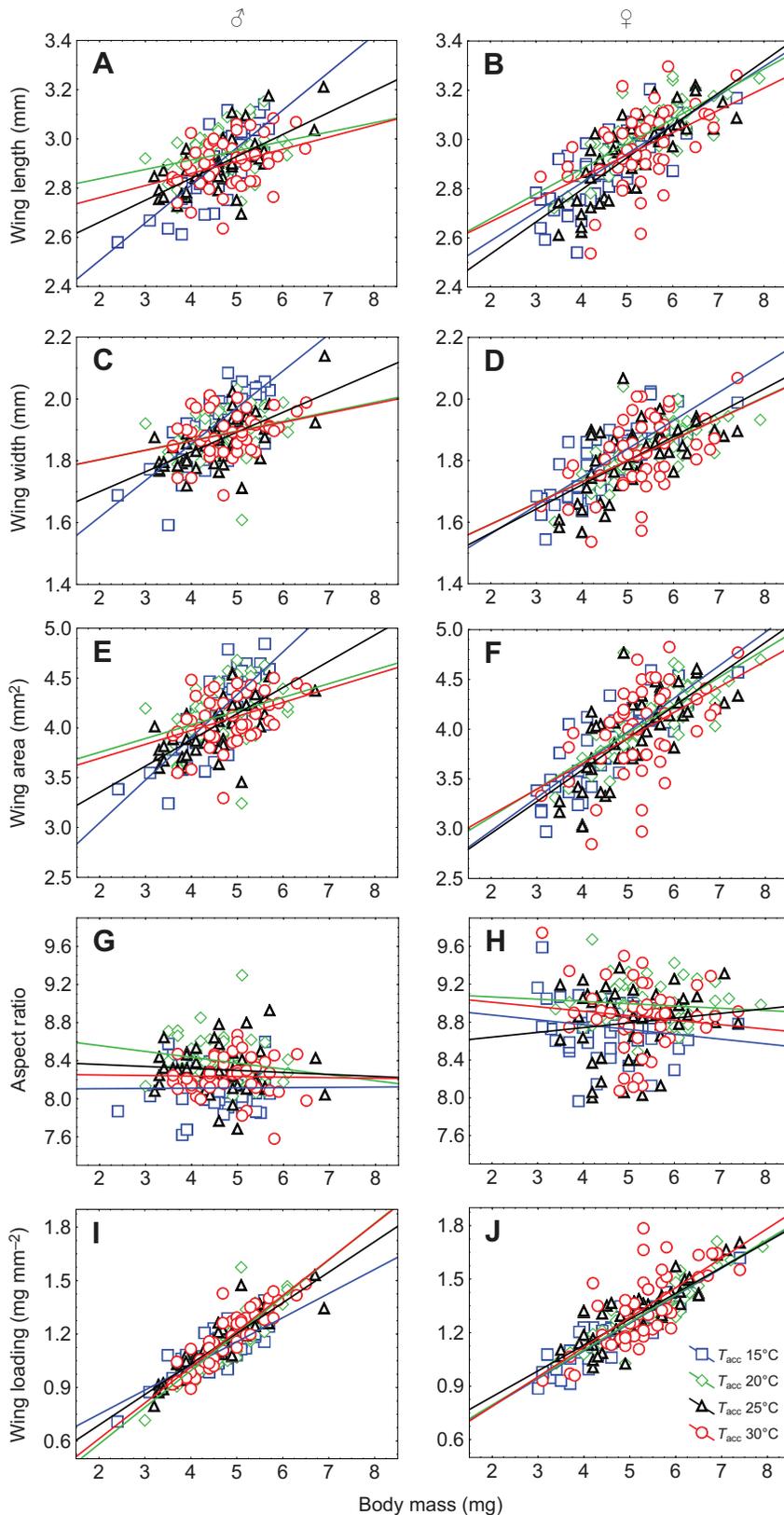


Fig. 2. Scatterplots showing the relationships of morphological variables with body mass. The effect of body mass on wing length (A,B), wing width (C,D), wing area (E,F), aspect ratio (G,H) and wing loading (I,J) in *C. capitata*. Results from males (first column of panels) and females (second column 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). Lines indicate the linear fit through the raw data for each T_{acc} .

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

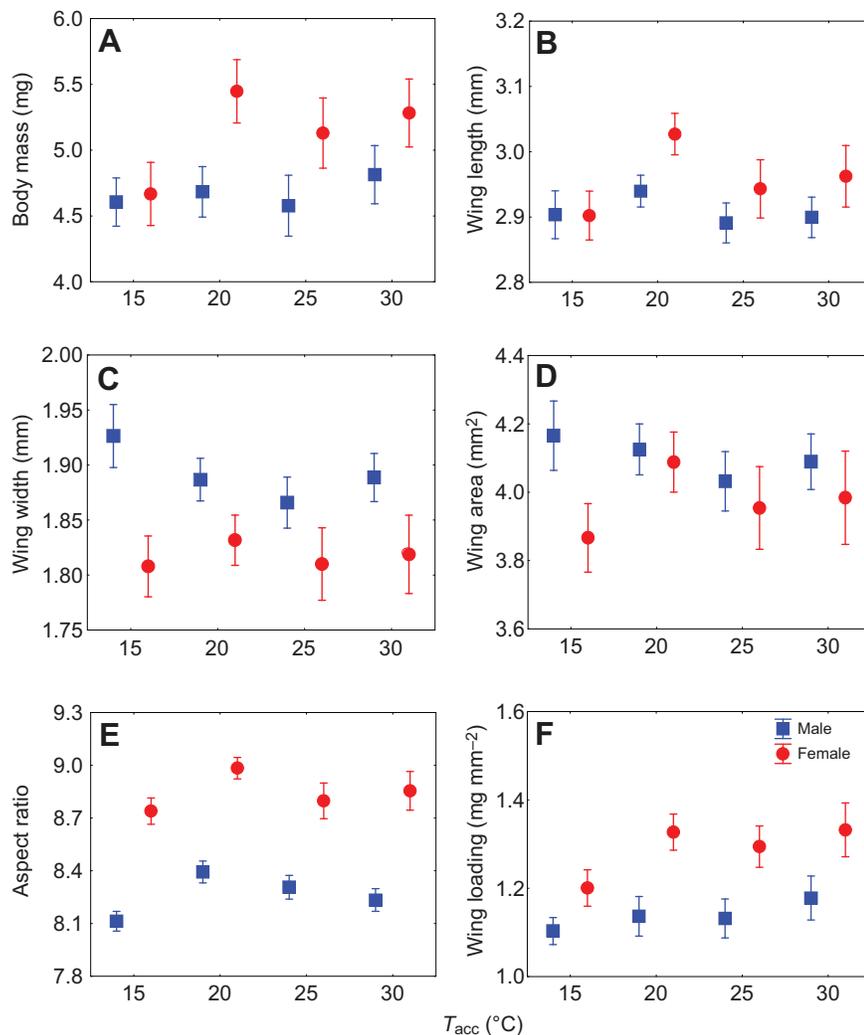


Fig. 3. Summary of effects of acclimation temperature on morphological variables. The effect of acclimation temperature on body mass (A), wing length (B), wing width (C), wing area (D), wing loading (E) and aspect ratio (F) in *C. capitata*. Results are grouped by sex (males, blue squares; females, red circles). Error bars indicate 95% confidence intervals.

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_{acc} ($P<0.05$). Using an alternative statistical approach (i.e. \log_{10} 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_{acc} and M_b on WL in females. Here T_{acc} , M_b and $T_{acc} \times M_b$ 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_{acc} ($P=0.021$); however, the only significant differences in CCO activity occurred between $T_{acc}=15^\circ\text{C}$ and 30°C (Fig. 5A). In general, metabolic rates increased as T_{test} increased (Fig. 5B–D); however, the relationship between \dot{V}_{CO_2} and T_{test} differed across acclimation groups, i.e. flies that had been acclimated to 30°C did not respond as strongly to T_{test} (i.e. they had a shallower rate–temperature slope) (Fig. 5B). Log-transformed mass, sex and T_{test} all significantly influenced log mean \dot{V}_{CO_2} and log resting \dot{V}_{CO_2} ($P<0.01$), and the interaction term $\text{LogMass} \times \text{sex}$ significantly influenced only log mean \dot{V}_{CO_2} ($P<0.03$, Table 4). T_{acc} had no effect however on either of these \dot{V}_{CO_2} variables. By contrast,

T_{acc} was a significant predictor ($P<0.0001$) of peak \dot{V}_{CO_2} together with log-transformed mass, sex, T_{test} and interactions between T_{acc} and T_{test} , and LogMass , T_{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 the 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,

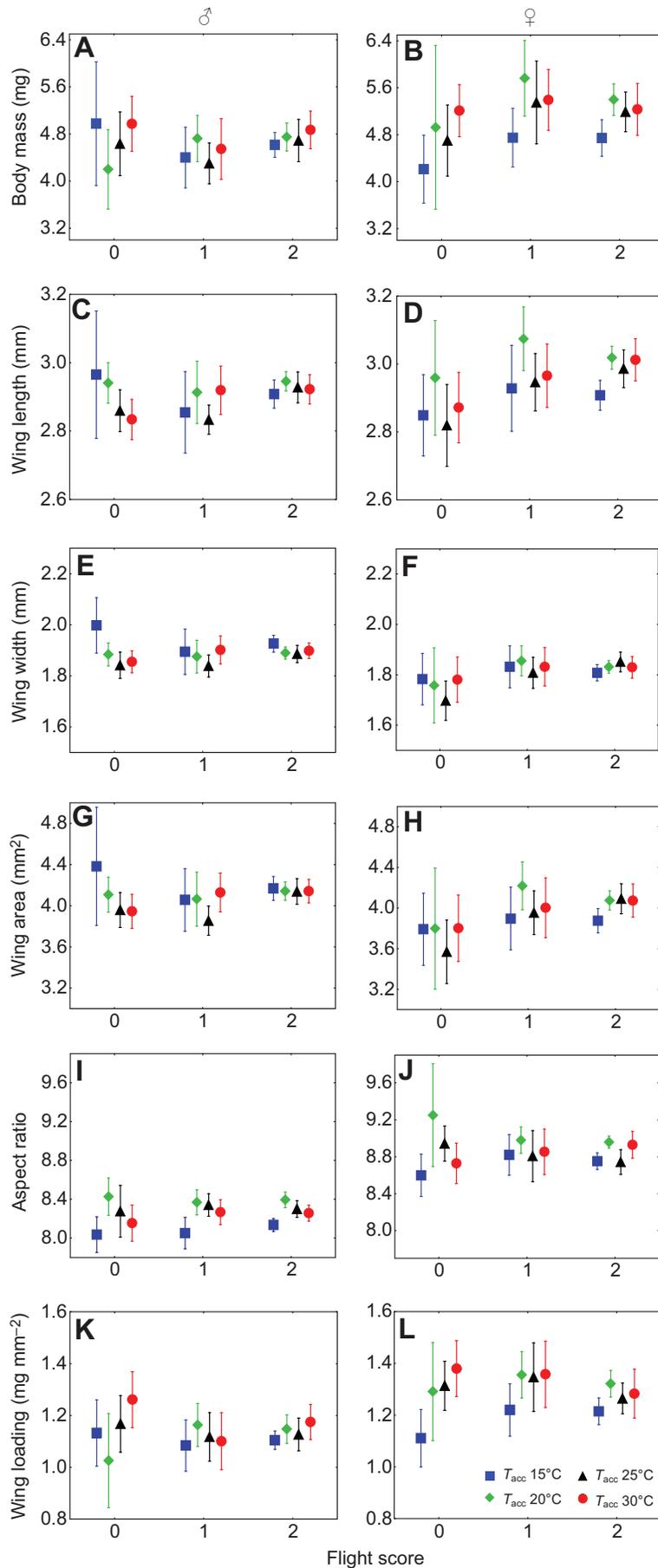


Fig. 4. Summary of the morphological variables by flight score category. Recorded flight scores (0=failure, 1=lift, 2=flight) across the range of test temperatures as a function of body mass (A,B), wing length (C,D), wing width (E,F), wing area (G,H), aspect ratio (I,J) and wing loading (K,L) in *C. 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).

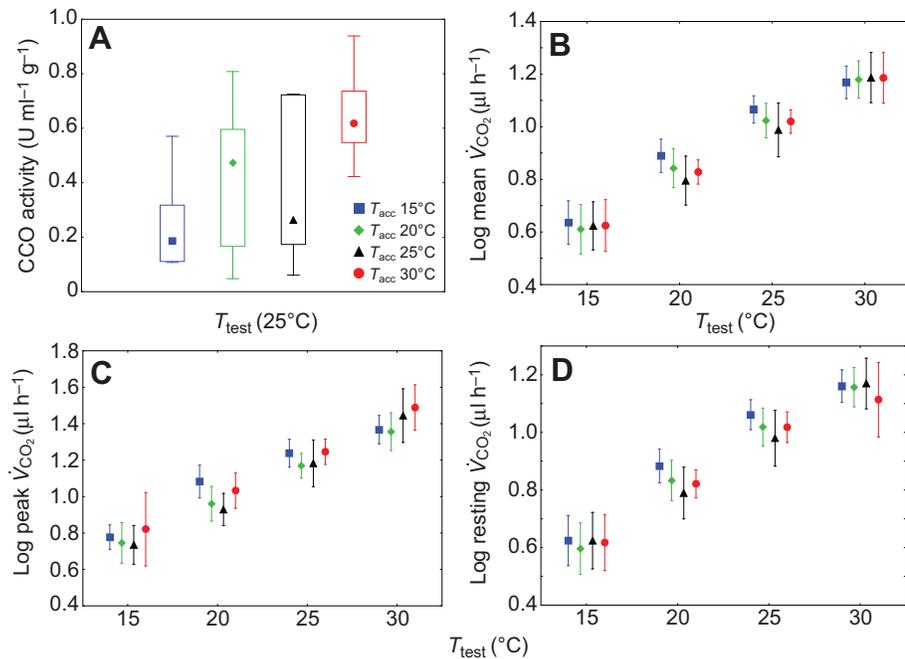


Fig. 5. Summary results for the effects of acclimation temperature on enzyme activity and metabolic rate parameters. The effect of acclimation temperature (T_{acc}) on CCO activity at 25°C (A) and mean \dot{V}_{CO_2} (B), peak \dot{V}_{CO_2} (C) and resting \dot{V}_{CO_2} at different test temperatures (T_{test}) in *C. capitata*. Panel A shows medians (square, circle or triangle as appropriate), quartiles (box) and the non-outlier range (whiskers), whereas panels B–D show the means and 95% confidence intervals.

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 *C. capitata* and that it can occupy rather cool, high-elevation environments in East Africa that may experience strong seasonality.

For *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 *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 Martín 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 *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

Table 4. Minimum adequate models of mean \dot{V}_{CO_2} , peak \dot{V}_{CO_2} and resting \dot{V}_{CO_2} in *C. capitata*

\dot{V}_{CO_2} ($\mu\text{l h}^{-1}$)	Effect	Num. d.f.	Den. d.f.	F-value	P-value
Mean	LogMass	1	217	42.33	<0.0001
	Sex	1	217	10.78	0.0012
	T_{test}	3	217	315.49	<0.0001
	LogMass×Sex	1	217	4.87	0.0283
Peak	LogMass	1	205	4.70	0.0313
	Sex	1	205	11.66	0.0008
	T_{test}	3	205	216.30	<0.0001
	T_{acc}	3	205	7.69	<0.0001
	$T_{acc} \times T_{test}$	3	205	9.39	<0.0001
	LogMass× T_{acc} ×Sex	7	205	4.92	<0.0001
Resting	LogMass	1	218	56.81	<0.0001
	Sex	1	218	112.06	<0.0001
	T_{test}	3	218	249.81	<0.0001

Models were run on log-transformed CO_2 and log-transformed mass (LogMass) data using a repeated measures ANCOVA [Kenward-Rogers degrees of freedom (d.f.) method]. Den., denominator; Num., numerator.

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_{acc} 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_{acc} 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. $T_{acc}=15^\circ\text{C}$, M_b and wing length relative to rearing at optimal conditions); however, in other cases, such as $T_{acc}=20^\circ\text{C}$ (M_b and wing length) and $T_{acc}=30^\circ\text{C}$ (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 Martín 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 features 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_{acc} : 15, 20, 25 and 30°C) and maintained in temperature-controlled incubators (MRC LE-509, 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. Azevedo et al., 1998; Frazier et al., 2008). Pupae were kept at their respective acclimation temperatures until peak adult eclosion ($T_{acc}=30^\circ\text{C}$ for 5 to 6 days, $T_{acc}=25^\circ\text{C}$ for 6 to 7 days, $T_{acc}=20^\circ\text{C}$ for 7 to 8 days and $T_{acc}=15^\circ\text{C}$ 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_{test} : 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_{test}) 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} , T_{test} , M_b , 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_{test} 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):

$$AR = \frac{4R^2}{S}, \quad (1)$$

where AR is the aspect ratio, R is the wing length in mm and S is the wing area in mm²;

$$WL = \frac{M_b}{S}, \quad (2)$$

where 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 $T_{acc} \times M_b$ 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 $T_{acc} \times M_b$ 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) within 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 \dot{V}_{CO_2} 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 (Sidetrak, 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 \dot{V}_{CO_2} 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_{test} , 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 \dot{V}_{CO_2} . 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 temperature, 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 \dot{V}_{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 \dot{V}_{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 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 MS104S, Switzerland) before and after each respirometry trial to determine fresh mass.

Owing to repeated measures on the same individual at different T_{test} for \dot{V}_{CO_2} estimates, a repeated measures ANCOVA (Kenward-Rogers 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 \dot{V}_{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_{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 extraction 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 measurements of sample absorbance were made every 5 s over the course of 1 min. First the inactive samples were measured, after which ferrocyanochrome *c* was added to start the reaction. The resulting absorbance changes were then recorded. The average absorbance over a minute ($\Delta A_{550} \text{ min}^{-1}$) of the inactive sample was subtracted from the average absorbance over a minute of the activated samples to calculate the absolute change in absorbance. CCO activity in units ml^{-1} was calculated using the difference in extinction coefficients ($\Delta \epsilon^{\text{nm}}$) between reduced and oxidised cytochrome *c* at 550 nm. One unit is equal to the amount of CCO that will oxidise 1.0 mmole of ferrocyanochrome *c* min^{-1} (pH 7.0, 25°C). The original fresh mass of each sample was used to convert the results into mass specific CCO activity ($\text{U ml}^{-1} \text{ mg}^{-1}$).

Of the six samples used per T_{acc} , three were frozen in liquid nitrogen and placed in a -80°C freezer before use and three consisted of fresh flies. A generalized 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_{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 deviances were inspected for potential over-dispersion, which was not evident in any analysis.

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Competing interests

The authors declare no competing financial interests.

Author contributions

J.S.T., N.E., S.C.T. and C.V.D. designed the study and performed data analyses with L.B. N.E. and R.S. collected the data. J.S.T., N.E., S.C.T., C.V.D. and L.B. wrote the paper.

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Supplementary material

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References

- Azevedo, R. B. R., James, A. C., McCabe, J. and Partridge, L. (1998). Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. *Evolution* **52**, 1353-1362.
- Bartholomew, G. A. and Casey, T. M. (1978). Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J. Exp. Biol.* **76**, 11-25.
- Basson, C. H., Nyamukondiwa, C. and Terblanche, J. S. (2012). Fitness costs of rapid cold-hardening in *Ceratitis capitata*. *Evol.* **66**, 296-304.
- Bennett, A. F. (1985). Temperature and muscle. *J. Exp. Biol.* **115**, 333-344.
- Berwaerts, K., Van Dyck, H. and Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Funct. Ecol.* **16**, 484-491.
- Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., Lehoucq, V., Matthysen, E., Mustin, K., Saastamoinen, M., et al. (2012). Costs of dispersal. *Biol. Rev.* **87**, 290-312.
- Bowler, K. and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev. Camb. Philos. Soc.* **83**, 339-355.
- Cavicchi, S., Giorgi, G., Natali, V. and Guerra, D. (1991). Temperature related divergence in experimental populations of *Drosophila melanogaster*. III. Fourier and centroid analysis of wing shape and relationship between shape variation and fitness. *J. Evol. Biol.* **4**, 141-159.
- Chidawanyika, F. and Terblanche, J. S. (2011). Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evol. Appl.* **4**, 534-544.
- Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. New York, NY: Oxford University Press.
- Chown, S. L. and Terblanche, J. S. (2007). Physiological diversity in insects: ecological and evolutionary contexts. *Adv. Insect. Phys.* **33**, 50-152.
- Clobert, J., Baguette, M., Benton, T. G. and Bullock, J. M. (2012). *Dispersal Ecology and Evolution*. Oxford: Oxford University Press.
- Clusella-Trullas, S., Terblanche, J. S. and Chown, S. L. (2010). Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiol. Biochem. Zool.* **83**, 519-530.
- Collinet, H. and Hoffmann, A. A. (2012). Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. *Funct. Ecol.* **26**, 84-93.
- Crawley, M. J. (2007). Statistical modelling. In *The R Book*, pp. 323-386. West Sussex: John Wiley and Sons.
- Dahlhoff, E. and Somero, G. N. (1993). Effects of temperature on mitochondria from abalone (Genus *Haliotis*): adaptive plasticity and its limits. *J. Exp. Biol.* **185**, 151-169.
- Deere, J. A. and Chown, S. L. (2006). Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *Am. Nat.* **168**, 630-644.
- Dell, A. I., Pawar, S. and Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. USA* **108**, 10591-10596.
- DeWitt, T. J. and Scheiner, S. M. (2004). *Plasticity: Functional and Conceptual Approaches*. Oxford: Oxford University Press.
- Dillon, M. E. and Frazier, M. R. (2006). *Drosophila melanogaster* locomotion in cold thin air. *J. Exp. Biol.* **209**, 364-371.
- Dudley, R. (2000). *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton, NJ: Princeton University Press.
- Feder, M. E., Garland, T., Jr, Marden, J. H. and Zera, A. J. (2010). Locomotion in response to shifting climate zones: not so fast. *Annu. Rev. Physiol.* **72**, 167-190.
- Ferrer, A., Dorn, S. and Mazzi, D. (2013). Cross-generational effects of temperature on flight performance, and associated life-history traits in an insect. *J. Evol. Biol.* **26**, 2321-2330.
- Fischer, K., Dierks, A., Franke, K., Geister, T. L., Liszka, M., Winter, S. and Pflücke, C. (2010). Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus anynana*. *PLoS ONE* **5**, e15284.
- Frazier, M. R., Woods, H. A. and Harrison, J. F. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **74**, 641-650.
- Frazier, M. R., Huey, R. B. and Berrigan, D. (2006). Thermodynamics constrains the evolution of insect population growth rates: "warmer is better". *Am. Nat.* **168**, 512-520.

- Frazier, M. R., Harrison, J. F., Kirkton, S. D. and Roberts, S. P. (2008). Cold rearing improves cold-flight performance in *Drosophila* via changes in wing morphology. *J. Exp. Biol.* **211**, 2116-2122.
- Frederich, M. and Pörtner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *Am. J. Physiol.* **279**, R1531-R1538.
- French, V., Feast, M. and Partridge, L. (1998). Body size and cell size in *Drosophila*: the developmental response to temperature. *J. Insect Physiol.* **44**, 1081-1089.
- Gasperi, G., Guglielmino, C. R., Malacrida, A. R. and Milani, R. (1991). Genetic variability and gene flow in geographical populations of *Ceratitis capitata* (Wied.) (medfly). *Heredity* **67**, 347-356.
- Gefen, E. (2011). The relative importance of respiratory water loss in scorpions is correlated with species habitat type and activity pattern. *Physiol. Biochem. Zool.* **84**, 68-76.
- Ghalambor, C. K., McKay, J. K., Carroll, S. P. and Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394-407.
- Gibert, P., Huey, R. B. and Gilchrist, G. W. (2001). Locomotor performance of *Drosophila melanogaster*: interactions among developmental and adult temperatures, age, and geography. *Evolution* **55**, 205-209.
- Harrison, J. F. and Roberts, S. P. (2000). Flight respiration and energetics. *Annu. Rev. Physiol.* **62**, 179-205.
- Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York, NY: Oxford University Press.
- Hoffmann, A. A., Scott, M., Partridge, L. and Hallas, R. (2003). Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. *J. Evol. Biol.* **16**, 614-623.
- Huey, R. B. and Berrigan, D. (1996). Testing evolutionary hypotheses of acclimation. In *Animals and Temperature: Phenotypic and Evolutionary Adaptation* (ed. I. A. Johnston and A. F. Bennett), pp. 205-237. Cambridge: Cambridge University Press.
- Huey, R. B., Berrigan, D., Gilchrist, G. W. and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool.* **39**, 323-326.
- Irllich, U. M., Terblanche, J. S., Blackburn, T. M. and Chown, S. L. (2009). Insect rate-temperature relationships: environmental variation and the metabolic theory of ecology. *Am. Nat.* **174**, 819-835.
- James, R. S. (2013). A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. *J. Comp. Physiol. B* **183**, 723-733.
- Karsten, M., van Vuuren, B. J., Barnaud, A. and Terblanche, J. S. (2013). Population genetics of *Ceratitis capitata* in South Africa: implications for dispersal and pest management. *PLoS ONE* **8**, e54281.
- Kaufmann, J. S. and Bennett, A. F. (1989). The effect of temperature and thermal acclimation on locomotor performance in *Xantusia vigilis*, the desert night lizard. *Physiol. Zool.* **62**, 1047-1058.
- Kellett, M., Hoffmann, A. A. and McKechnie, S. W. (2005). Hardening capacity in the *Drosophila melanogaster* species group is constrained by basal thermotolerance. *Funct. Ecol.* **19**, 853-858.
- Kelty, J. D. and Lee, R. E., Jr (2001). Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *J. Exp. Biol.* **204**, 1659-1666.
- Kingsolver, J. G. (2009). The well-temperated biologist. *Am. Nat.* **174**, 755-768.
- Kristensen, T. N., Hoffmann, A. A., Overgaard, J., Sørensen, J. G., Hallas, R. and Loeschcke, V. (2008). Costs and benefits of cold acclimation in field-released *Drosophila*. *Proc. Natl. Acad. Sci. USA* **105**, 216-221.
- Lachenicht, M. W., Clusella-Trullas, S., Boardman, L., Le Roux, C. and Terblanche, J. S. (2010). Effects of acclimation temperature on thermal tolerance, locomotor performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *J. Insect Physiol.* **56**, 822-830.
- Laurie-Ahlberg, C. C., Barnes, P. T., Curtsinger, J. W., Emigh, T. H., Karlin, B., Morris, R., Norman, R. A. and Wilton, A. N. (1985). Genetic variability of flight metabolism in *Drosophila melanogaster*. II. Relationship between power output and enzyme activity levels. *Genetics* **111**, 845-868.
- Lehmann, F.-O. (1999). Ambient temperature affects free-flight performance in the fruit fly *Drosophila melanogaster*. *J. Comp. Physiol. B* **169**, 165-171.
- Leroi, A. M., Bennett, A. F. and Lenski, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. USA* **91**, 1917-1921.
- Loeschcke, V. and Hoffmann, A. A. (2002). The detrimental acclimation hypothesis. *Trends Ecol. Evol.* **17**, 407-408.
- Loeschcke, V. and Hoffmann, A. A. (2007). Consequences of heat hardening on a field fitness component in *Drosophila* depend on environmental temperature. *Am. Nat.* **169**, 175-183.
- McMullen, D. C. and Storey, K. B. (2008). Suppression of Na⁺K⁺-ATPase activity by reversible phosphorylation over the winter in a freeze-tolerant insect. *J. Insect Physiol.* **54**, 1023-1027.
- Nunney, L. and Cheung, W. (1997). The effect of temperature on body size and fecundity in female *Drosophila melanogaster*. Evidence for adaptive plasticity. *Evolution* **51**, 1529-1535.
- O'Brien, J., Dahlhoff, E. and Somero, G. N. (1991). Thermal resistance of mitochondrial respiration: hydrophobic interactions of membrane proteins may limit thermal resistance. *Physiol. Zool.* **64**, 1509-1526.
- Partridge, L., Barrie, B., Fowler, K. and French, V. (1994). Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**, 1269-1276.
- Piersma, T. and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228-233.
- Rako, L. and Hoffmann, A. A. (2006). Complexity of the cold acclimation response in *Drosophila melanogaster*. *J. Insect Physiol.* **52**, 94-104.
- Rank, N. E., Bruce, D. A., McMillan, D. M., Barclay, C. and Dahlhoff, E. P. (2007). Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle. *J. Exp. Biol.* **210**, 750-764.
- Rogers, K. D., Seebacher, F. and Thompson, M. B. (2004). Biochemical acclimation of metabolic enzymes in response to lowered temperature in tadpoles of *Limnodynastes peronii*. *Comp. Biochem. Physiol.* **137A**, 731-738.
- Samejima, Y. and Tsubaki, Y. (2010). Body temperature and body size affect flight performance in a damselfly. *Behav. Ecol. Sociobiol.* **64**, 685-692.
- San Martín y Gomez, G. and Van Dyck, H. (2012). Ecotypic differentiation between urban and rural populations of the grasshopper *Chorthippus brunneus* relative to climate and habitat fragmentation. *Oecologia* **169**, 125-133.
- Seebacher, F. and James, R. E. (2008). Plasticity of muscle function in a thermoregulating ectotherm (*Cordylus porosus*): biomechanics and metabolism. *Am. J. Physiol. Reg. I.* **294**, R1024-R1032.
- Skandalis, D. A., Roy, C. and Darveau, C.-A. (2011). Behavioural, morphological, and metabolic maturation of newly emerged adult workers of the bumblebee, *Bombus impatiens*. *J. Insect Physiol.* **57**, 704-711.
- Sørensen, J. G., Loeschcke, V. and Kristensen, T. N. (2009). Lessons from the use of genetically modified *Drosophila melanogaster* in ecological studies: Hsf mutant lines show highly trait-specific performance in field and laboratory thermal assays. *Funct. Ecol.* **23**, 240-247.
- Steigenga, M. J. and Fischer, K. (2007). Within- and between-generation effects of temperature on life-history traits in a butterfly. *J. Therm. Biol.* **32**, 396-405.
- Suarez, R. K., Lighton, J. R. B., Joos, B., Roberts, S. P. and Harrison, J. F. (1996). Energy metabolism, enzymatic flux capacities, and metabolic flux rates in flying honeybees. *Proc. Natl. Acad. Sci. USA* **93**, 12616-12620.
- Suarez, R. K., Staples, J. F., Lighton, J. R. B. and Mathieu-Costello, O. (2000). Mitochondrial function in flying honeybees (*Apis mellifera*): respiratory chain enzymes and electron flow from complex III to oxygen. *J. Exp. Biol.* **203**, 905-911.
- Swank, D. M., Vishnudasa, V. K. and Maughan, D. W. (2006). An exceptionally fast actomyosin reaction powers insect flight muscle. *Proc. Natl. Acad. Sci. USA* **103**, 17543-17547.
- Swoap, S. J., Johnson, T. P., Josephson, R. K. and Bennett, A. F. (1993). Temperature, muscle power output and limitations on burst locomotor performance of the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **174**, 185-197.
- Teets, N. M. and Denlinger, D. L. (2013). Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiol. Entomol.* **38**, 105-116.
- Terblanche, J. S. and Chown, S. L. (2006). The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). *J. Exp. Biol.* **209**, 1064-1073.
- Terblanche, J. S. and Kleynhans, E. (2009). Phenotypic plasticity of desiccation resistance in *Glossina puparia*: are there ecotype constraints on acclimation responses? *J. Evol. Biol.* **22**, 1636-1648.
- Terblanche, J. S., Clusella-Trullas, S., Deere, J. A., Van Vuuren, B. J. and Chown, S. L. (2009). Directional evolution of the slope of the metabolic rate-temperature relationship is correlated with climate. *Physiol. Biochem. Zool.* **82**, 495-503.
- Terblanche, J. S., Clusella-Trullas, S. and Chown, S. L. (2010a). Phenotypic plasticity of gas exchange pattern and water loss in *Scarabaeus spretus* (Coleoptera: Scarabaeidae): deconstructing the basis for metabolic rate variation. *J. Exp. Biol.* **213**, 2940-2949.
- Terblanche, J. S., Nyamukondiwa, C. and Kleynhans, E. (2010b). Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). *Entomol. Exp. Appl.* **137**, 304-315.
- Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*, 4th edn. New York, NY: Springer.
- Vorhees, A. S., Gray, E. M. and Bradley, T. J. (2013). Thermal resistance and performance correlate with climate in populations of a widespread mosquito. *Physiol. Biochem. Zool.* **86**, 73-81.
- Wagner, D., Holmstrup, M., Bayley, M. and Sørensen, J. G. (2013). Induced cold-tolerance mechanisms depend on duration of acclimation in the chill-sensitive *Folsomia candida* (Collembola). *J. Exp. Biol.* **216**, 1991-2000.
- Whitman, D. W. and Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In *Phenotypic Plasticity of Insects: Mechanisms and Consequences* (ed. D. W. Whitman and T. N. Ananthakrishna), pp. 1-63. Enfield, NH: Science Publishers.
- Wilson, R. S. and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**, 66-70.
- Zamudio, K. R., Huey, R. B. and Grill, W. D. (1995). Bigger isn't always better: body size, developmental and parental temperature and male territorial success in *Drosophila melanogaster*. *Anim. Behav.* **49**, 671-677.
- Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95-126.
- Zrubek, B. and Woods, H. A. (2006). Insect eggs exert rapid control over an oxygen-water tradeoff. *Proc. Biol. Sci.* **273**, 831-834.