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The effect of developmental temperature fluctuation on wing traits and stressed locomotor performance in *Drosophila melanogaster*, and its dependence on heterozygosity

Anders Kjærsgaard[1,2], Nguyet Le[1], Ditte Demontis[3],
Zorana Kurbalija Novičič[4], Volker Loeschcke[1]
and Cino Pertoldi[1,5,6]

[1]*Department of Bioscience, Integrative Ecology and Evolution, Aarhus University, Aarhus, Denmark,*
[2]*Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich,*
Switzerland, [3]*Institute of Human Genetics, Aarhus University, Aarhus, Denmark,*
[4]*Institute for Biological Research 'Sinisa Stankovic', University of Belgrade, Belgrade, Serbia,*
[5]*Department 18/Section of Environmental Engineering, Aalborg University, Aalborg, Denmark*
and [6]*Aalborg Zoo, Aalborg, Denmark*

ABSTRACT

Background: Natural environments fluctuate and all organisms experience some degree of environmental variance. Global climate models predict increasing environmental variance in the future. Yet we do not fully understand how environmental variation affects performance traits.

Questions: Does temperature fluctuation during development affect adult size and wing shape in *Drosophila melanogaster*? If so, are the effects predictable? Do they depend on heterozygosity? Do fluctuations in developmental temperature affect adult physiological performance at high temperature?

Methods: We tested the effect of one fluctuating (21°C/29°C) and several constant (21°C, 23°C, 25°C, 27°C, 29°C) developmental temperature regimes on three wing morphometric traits (wing length, wing width, and wing shape) in an experiment using three inbred lines of *D. melanogaster* and their first-generation hybrids. We also tested the effect of fluctuating and constant developmental temperature on adult locomotor performance at several high and stressful test temperatures (32°C, 34°C, 36°C, 38°C, 40°C).

Results: Performance mostly declined if the flies were reared under the fluctuating temperature regime versus the constant temperature regime with the same mean (25°C). Heterozygosity level also affected the traits investigated, with crossbreds usually having higher trait values. Crossbred genotypes compared across constant temperatures also showed greater plasticity in wing aspect.

Correspondence: A. Kjærsgaard, Department of Bioscience, Integrative Ecology and Evolution, Aarhus University, Ny Munkegade, Building 1540, 8000 Aarhus C, Denmark. E-mail: anders.kjaersgaard@biology.au.dk

Conclusion: The widespread use of constant developmental temperatures in laboratory experiments may lead to overestimation of performance.

Keywords: climate change, environmental variation, inbreeding depression, locomotor activity, non-linear reaction norm.

INTRODUCTION

Many living organisms experience stressful conditions seasonally, but this happens also on a daily and even shorter basis. Daily ambient temperature amplitudes of 15°C or more are common in nature (e.g. Pétavy *et al.*, 2004). It is well known that temperature variation can have a marked effect on the distribution and abundance of ectothermic organisms (Cossins and Bowler, 1987; Hoffmann and Parsons, 1991). The proximate factors involved in the adaptive process are manifested at every level of biological organization, from molecular complexes to behaviour (Hochachka and Somero, 2002; Podrabsky and Somero, 2004; Garland and Kelly, 2006; Angilletta, 2009).

In the context of climate change, the increasing variance in temperature (Schär *et al.*, 2004; McGregor *et al.*, 2005; Jentsch *et al.*, 2007) has received considerable attention recently (Jentsch *et al.*, 2007). It is becoming clear that extreme temperature events may affect organisms disproportionately compared with what would be expected from the mean temperature increase alone (Bozinovic *et al.*, 2011). Hence, an increase in environmental variance may have negative effects on population size and fitness (Ruel and Ayres, 1999; Easterling *et al.*, 2000; Pertoldi and Bach, 2007; Kubisch and Poethke, 2011).

Most organisms are sufficiently versatile to deal with environmental variance encountered on a regular basis. However, an increase in the environmental variance may lead to a change in conditions that result in negative population growth rate and a concomitant decline in population size. A population may overcome these challenges by evolving, migrating to more suitable habitats, and/or through plastic responses. If none of these responses is possible, the population is likely to face extinction (Bell and Collins, 2008). The possible phenotypic responses to altered climatic regimes may become more limited due to concurrent anthropogenic impacts on habitats. Pollution, the introduction of exotic species, and destruction and fragmentation of habitats are important factors in this regard (McCauley, 1993; Allendorf *et al.*, 2001; Pertoldi and Bach, 2007; Chown *et al.*, 2010). Fragmentation of habitats may, for instance, lead to the isolation of populations and a reduction in size of individual populations. This scenario leads to higher inbreeding rates and possibly inbreeding depression (Lynch and Walsh, 1998; Joubert and Bijlsma, 2010; Bijlsma and Loeschcke, 2012). Indeed, many wild populations have small population sizes, resulting in low genetic variability. This reduces their potential to respond via evolutionary adaptation to environmental changes. In such cases, phenotypic plasticity may be an important way to cope with the changing environment (Pertoldi and Bach, 2007). Translocation has been suggested as a means to reduce inbreeding rates and inbreeding depression in small, inbred populations (Moritz, 1999; Tallmon *et al.*, 2004). Reproduction of individuals from genetically distinct populations is known as heterosis or hybrid vigour (Dobzhansky, 1950; Andersen *et al.*, 2002). The resulting increase in heterozygosity is likely to increase the long-term survival probabilities of the population by increasing the evolutionary potential. The benefits of heterozygosity have been demonstrated on numerous occasions and they are often augmented under stressful conditions (Armbruster and Reed, 2005; Liao and Reed, 2009). The latter includes variable environments

where extreme temperatures and variation in other factors are encountered (e.g. Kristensen *et al.*, 2008a).

In recognition of the influence of environmental variance on the phenotype, a simple and valid method to predict the effects of environmental variance on performance traits would be useful. Jensen's inequality has been suggested as such a tool, enabling researchers to predict the effects of environmental variance on fitness (Ruel and Ayres, 1999). Jensen's inequality is a mathematical property of non-linear functions (Jensen, 1906). It states that, for any non-linear function (as fitness curves often are), variance is predicted to consistently elevate or depress the response variable, i.e. the mean of x does not coincide with the mean of the function $f(x)$. Hence, if the function is linear, the variance around the mean x does not affect the mean $f(x)$ (no second derivative). If the function is decelerating (second derivative is negative), the response variable is predicted to be lower than that of the mean x. If the function is accelerating (second derivative is positive), then the response variable, $f(x)$, is predicted to exceed the value obtained from the mean x (Ruel and Ayres, 1999).

The aim of this study was to examine the effects of different temperature regimes on wing morphometric traits in three highly homozygotic inbred lines of *D. melanogaster* and in crosses between them. They were reared under both a fluctuating temperature regime and several constant ones. We wished to determine whether Jensen's inequality is a valid predictor of the trait responses of three wing morphometric traits following development under a fluctuating temperature regime. Also, whether it is dependent on heterozygosity level, i.e. if inbreeding exacerbates differences in developmental temperature regime.

We also examined whether development under a fluctuating temperature regime is manifested in adult physiological performance at high temperature by assessing locomotor performance at several high temperatures until activity ceased. Locomotor performance has been suggested to be a good estimator of the overall physiological state of an individual (Gibert *et al.*, 2001; Kjærsgaard *et al.*, 2010a). It may, however, also reflect the decision-making ability and current motivational state of the animal (Martin, 2003). Hence, locomotor performance is a composite trait of high complexity. It integrates a range of underlying phenotypic components into behaviour. It is therefore influenced by many factors. Heat stress, for example, has well-documented effects on a range of traits associated with locomotor performance (e.g. Kjærsgaard *et al.*, 2010a, 2010b). There is direct experimental evidence for an effect of preceding heat stress on the locomotor performance of adult *D. melanogaster* (Loeschcke and Hoffmann, 2007; Bettencourt *et al.*, 2009), as well as heat stress in the pre-adult stages on adult locomotor performance (Gilchrist *et al.*, 1997; Gibert *et al.*, 2001; Roberts *et al.*, 2003). However, studies remain relatively sparse given the complexity of locomotor performance (e.g. Gibert *et al.*, 2001) and the large impact temperature has on most phenotypic traits. The same is the case for effects of inbreeding on locomotor performance (but see Kristensen *et al.*, 2008b). We believe that locomotor performance is a highly sensitive trait to unravel even small effects of developmental conditions and genotype.

METHODS

Three inbred lines of *Drosophila melanogaster* Meigen (F ≈ 1) were used. They were created by full sib mating for 20 consecutive generations at 25°C. The flies used to generate these lines were taken from an outbred laboratory population maintained at a census size of approximately 1000 individuals per generation (for further details, see Bubliy and Loeschcke, 2005). Following the inbreeding procedure, they were maintained at 20°C and a 12/12 h light/dark

cycle for approximately 100 generations before performing this experiment. A molecular analysis performed before the experiment began verified that the three lines were in fact still isogenic (see below).

Prior to the initiation of this experiment, two generations were completed at constant 25°C – the grand mean temperature across all developmental temperatures. All experimental matings and egg-laying were performed at this temperature and subsequently eggs were transferred to one of the developmental temperatures as specified below. The flies were reared in 200 mL culture bottles with 36 mL medium. For each line there were eight bottles containing 20 pairs per bottle, laying eggs for 24 h. Offspring from the different bottles were mixed before setting up the next generation.

From the three inbred lines, referred to as line 1, 2, and 3 respectively, we obtained three crossbred lines by crossing two of the respective inbred lines: one between line 1 and line 2 referred to as line 1×2, one between line 1 and line 3 referred to as line 1×3, and one between line 2 and line 3 referred to as line 2×3. The crossbred lines were created by setting up sex-reciprocal crosses of, for example, 20 males of line 1 with 20 females of line 7 and vice versa. The other two crossbred lines were made using the same procedure. In the analyses, equal proportions of each sex reciprocal cross were used to give one pooled crossbred line estimate. We only examined the effect of outbreeding in the F1 generation crossbreds.

Molecular analysis

DNA was extracted from 30 female flies from each of the lines using a modified CTAB extraction procedure (Doyle and Doyle, 1987). Fifteen microsatellites, known to be polymorphic in natural populations of *D. melanogaster*, were amplified using multiplexed PCR. Reactions were performed with Ampliqon Taq Polymerase Master Mix 1.5 mM (Ampliqon, www.ampliqon.com). The PCR settings for all microsatellites consisted of a preheat step at 95°C for 3 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 40 s, amplification at 72°C for 40 s, and a final extension step at 72°C for 7 min. The PCR products were run on an acrylamide gel and loaded into an ALFexpress sequencer. Fragment sizes were determined afterwards using Fragment Analyser (Pharmacia). The markers (with highest number of observed alleles in brackets) were: CAD (3), DM3C (2), SCAB (2), DROYTD3 (2), DMX1 (2), DMX2 (2), DM3B (2), DM3J (2), BIB (2), DM32 (2), DMX3 (1), DMX4 (1), DMDELTEX (1), DMHSP82 (1), and TOR (1). All lines were completely homozygous at these markers. More than one allele therefore indicates that lines were fixed for different alleles. Primer sequences were obtained from three web-based resources that are no longer accessible (courtesy of the Christian Schlötterer laboratory, Charles Aquadro laboratory, and David Goldstein microsatellite page). Information on some of these markers, however, has been published (Schug *et al.*, 1998; Colson *et al.*, 1999). Details for all primers are available from the corresponding author upon request. We used 5′-labelled forward primers obtained from MWG Biotech (www.mwg-biotech.com).

Wing measurements

Wings of flies developed at constant temperatures of 21°C, 23°C, 25°C, 27°C, and 29°C and under a fluctuating temperature regime of 21°C/29°C were used. The fluctuating regime alternated between 21°C (from 20.00 to 08.00 h) and 29°C (from 08.00 to 20.00 h), shifting
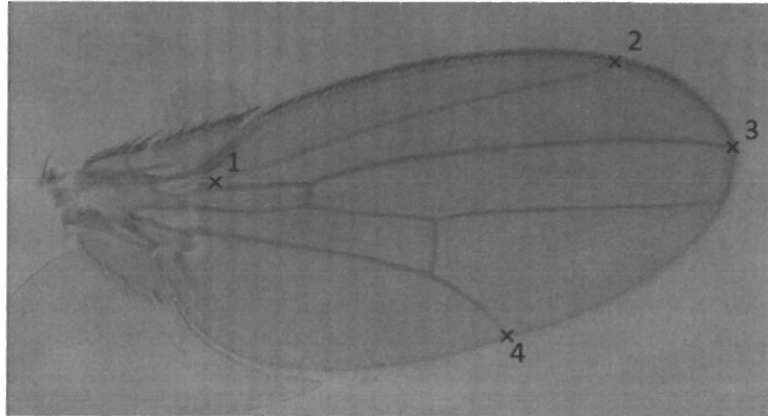
Fig. 1. The wing landmarks used in this study. Wing length was defined as the distance between landmarks 1 and 3, and wing width as the distance between landmarks 2 and 4.

phase together with a 12/12 h light/dark regime. Hence the mean temperature of this treatment was 25°C. Approximately 50 wing pairs from males of the parental and crossbred lines (the latter with equal proportions of each sex reciprocal cross) were removed and placed on microscope slides with a drop of acetic acid. The wings were measured using a camera attached to a dissecting microscope and a computer with the software IM1000 version 1.1. They were measured by the use of the software package ImageJ version 1.33u (Rasband, 2001). We measured four landmarks to estimate wing length and wing width as well as the composite trait wing aspect (wing length/wing width ratio). The distance between landmarks 1 and 3 represents the wing length. The distance between landmarks 2 and 4 represents the wing width (Fig. 1).

Stressed locomotor performance

We used the inbred lines and crossbred lines reared under two temperature regimes: the fluctuating regime 21°C/29°C and the constant 25°C regime as described above. In these flies we investigated adult locomotor performance at five high temperatures: 32°C, 34°C, 36°C, 38°C, and 40°C. Performance was measured as the duration of locomotor activity until the last recorded activity count. Since the flies were not fed during the assay, performance is likely a combination of heat, desiccation, and starvation resistance.

When the flies were 0–7 hours old, they were separated into males and females, and transferred under CO_2-anaesthesia to new vials and maintained at standard laboratory conditions. We only used males in this experiment. As with the wings, we used equal proportions of the sex reciprocal crosses for the crossbred estimates. Stressed locomotor performance was measured in 3-day-old individuals using the 'Drosophila Activity Monitoring System' (Trikinetics Inc., Waltham, MA). We used three monitoring units that could each contain 32 flies, allowing a total of 96 flies to be monitored simultaneously. Trials were conducted between 09.30 and 16.00 h. Each fly was transferred to a capillary glass sealed with parafilm at one end and a small cotton wool stopper at the other to allow gas exchange. The flies were transferred into the capillary glass vials without anaesthesia and inserted randomly among lines into the monitoring units. The units were placed in

incubators at 32°C, 34°C, 36°C, 38°C, and 40°C. The activity monitors registered activity every time the fly passed the middle of the capillary glass vial by disruption of an infrared light beam and summed the interruptions into bins of 30 s.

Statistical analyses

All statistical analyses and graphs were conducted in R (R Development Core Team, 2011). Data were log-transformed to increase homogeneity (Zar, 1999), although the tests applied here are robust to deviations from normality when sample sizes are approximately equal. We used a mixed-models framework with restricted maximum likelihood (REML) implemented in the package 'lme4'. Subsequently, we calculated type-II ANOVA tables for the model objects by performing likelihood-ratio χ^2 using the 'car' package.

To test for an effect of temperature (fluctuating 21°C/29°C and constant 25°C) and heterozygosity level (i.e. effect of inbreeding) and their interaction, we ran a linear mixed model where line was nested in heterozygosity level.

For stressed locomotor performance, test temperature was further included to test for an overall effect of test temperature, developmental temperature regime, heterozygosity level, and their interactions.

RESULTS

Wing traits

Mean wing length decreased with increasing developmental temperature both in the parental and the crossbred flies (Fig. 2). The relative difference between the lowest and highest temperature (constant 21°C and 29°C) was 16.0%, 13.6%, and 13.1% for parental lines 1, 2, and 3 respectively, and 13.8%, 14.0%, and 15.0% for crossbred lines 1×2, 1×3, and 2×3 respectively. In most lines (1, 3, 1×2, 1×3, 2×3), the mean wing length of flies reared at 25°C was significantly greater than that of flies reared at 21°C/29°C (*t*-test with sequential Bonferroni correction, results not shown). In general, we found a significant difference in mean wing length between the parental and crossbred lines, with the crossbreds having longer wings (Table 1; Fig. 2). The interaction between temperature and heterozygosity (inbred or crossbred) was marginally non-significant ($P = 0.07$; Table 1).

Wing width also decreased with increasing developmental temperature both in the parental and the crossbred flies. The relative difference between the lowest and highest temperature was 15.4%, 14.4%, and 13.0% for inbred lines 1, 2, and 3 respectively, and 14.3%, 15.6%, and 14.8% for crossbred lines 1×2, 1×3, and 2×3 respectively. Similar to wing length, for five of six lines reared at 25°C wing width was significantly larger than that of flies reared at 21°C/29°C (1, 3, 1×2, 1×3, 2×3). The interaction between temperature and heterozygosity level was again marginally non-significant ($P = 0.06$; Table 1).

The composite trait wing aspect showed a slight increase with temperature, mainly in the crossbred lines (Fig. 3). Comparatively narrower wings were observed at higher temperatures in the crossbred lines. There were also differences when contrasting the constant 25°C regime and fluctuating 21°C/29°C regime (Table 1). The 21°C/29°C treatment resembled the higher temperatures the most (Fig. 3). Heterozygosity level also had a significant effect on wing aspect, but there was no temperature × heterozygosity interaction.
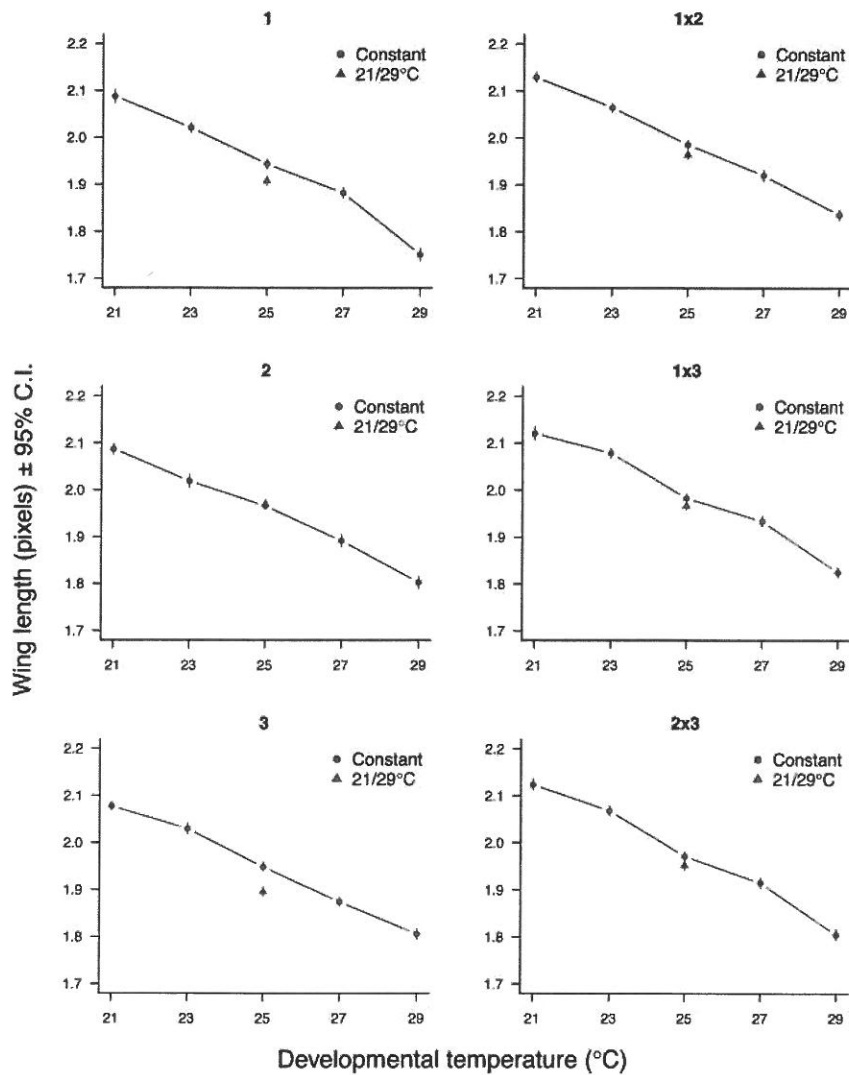
Fig. 2. Mean wing length as a function of developmental temperature.

Stressed locomotor performance

Stressed locomotor performance following development at a constant 25°C or fluctuating 21°C/29°C decreased with increasing test temperature both in the parental and the crossbred flies (Fig. 4; Table 1). However, there was a significant interaction also (Table 1), indicating that heat resistance declined faster with test temperature for the inbred lines than for the crossbreds. Overall, the mean locomotor performance of the flies reared at 25°C was significantly higher than that of the flies reared at 21°C/29°C, as indicated by the significant effect of developmental regime (Table 1). This difference was highest at the intermediate test temperature of 36°C and the treatments converged towards the two

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Table 1. Linear mixed models (see text) for the wing traits with factors Temperature (levels: 25°C constant and 21°C/29°C fluctuating developmental temperature) and Heterozygosity (levels: inbred, hybrid), and Line nested in Heterozygosity. Linear mixed model for heat resistance time with factors Test temperature, Heterozygosity, and Developmental regime (levels: 25°C constant and 21°C/29°C fluctuating developmental temperature), and Line nested in Heterozygosity

Trait	Source	d.f.	χ^2	$Pr(> \chi^2)$
Wing length	Temperature	1	65.73	<0.001***
	Heterozygosity	1	3.92	0.048*
	Temperature × Heterozygosity	1	3.18	0.074[#]
Wing width	Temperature	1	100.5	<0.001***
	Heterozygosity	1	0.57	0.45
	Temperature × Heterozygosity	1	3.57	0.059[#]
Wing aspect	Temperature	1	12.68	<0.001***
	Heterozygosity	1	5.47	0.019*
	Temperature × Heterozygosity	1	0.17	0.68
Heat resistance time	Test temperature	4	9690	<0.001***
	Heterozygosity	1	38.7	<0.001***
	Developmental regime	1	1138	<0.001***
	Test temperature × Heterozygosity	4	106.2	<0.001***
	Test temperature × Developmental regime	4	438.8	<0.001***
	Heterozygosity × Developmental regime	1	28.3	<0.001***
	Test temperature × Heterozygosity × Developmental regime	4	19.4	<0.001***

[#]$P < 0.10$, *$P < 0.05$, ***$P < 0.001$.

highest test temperatures (38°C and 40°C; Fig. 4). All interactions between the factors test temperature, heterozygosity level, and developmental regime were also highly significant (Table 1).

DISCUSSION

Daily thermal cycles are ubiquitous in large parts of the biosphere. The variance seems to be increasing due to climate change (IPCC, 2007). So the question is, what are the consequences of fluctuating temperature regimes for phenotypes in a population? We addressed this question in *D. melanogaster* by examining:

1. The effects of a fluctuating temperature regime (21°C/29°C) versus those of a constant temperature regime on performance traits within lines.
2. Whether Jensen's inequality is a valid tool to predict the effects of temperature variance on the wing morphometric traits investigated here.
3. The effect of inbreeding versus outbreeding on fitness-related traits under fluctuating and constant temperature regimes.
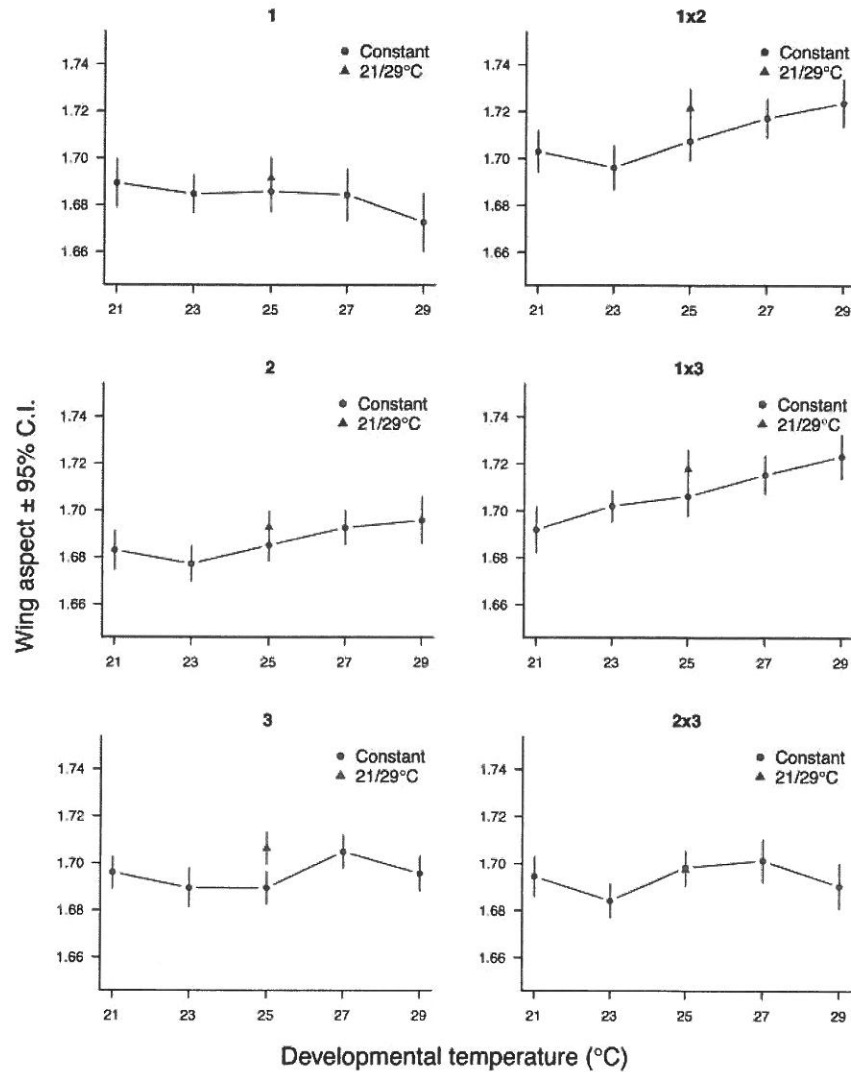
Fig. 3. Mean wing aspect as a function of developmental temperature.

The results show a clear pattern for the fluctuating temperature regime to depress mean trait performance, which has been reported previously (Pétavy et al., 2004; Patterson and Blouin-Demers, 2008; Ragland and Kingsolver, 2008; Kingsolver et al., 2009). Some authors have also reported beneficial effects of fluctuating temperature (Worner, 1992; Kingsolver et al., 2009; Les et al., 2009; Bozinovic et al., 2011; Fischer et al., 2011). Such a discrepancy is to be expected, since experiments have used different mean temperatures and variances as well as different species and populations with different evolutionary histories and thermal niches. It has, for example, been shown that growth rate increases with temperature variation in the lower relative to the higher temperature range. This is in accordance with Jensen's inequality for this performance curve (Worner, 1992; Ragland and Kingsolver, 2008; Bozinovic et al., 2011; Fischer et al., 2011).
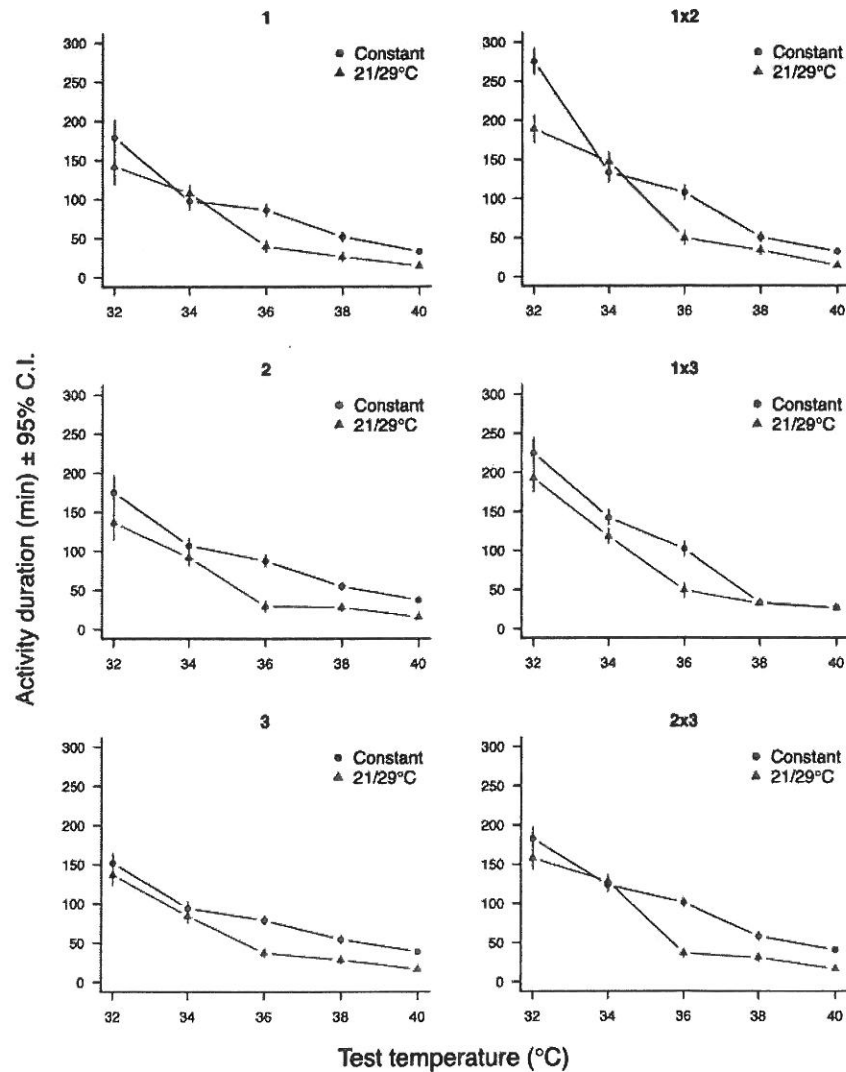
Fig. 4. Mean stressed locomotor performance as a function of test temperature and following development at either constant 25°C or fluctuating 21°C/29°C.

The degree and pattern of temperature variance seems to play an important role in determining the fitness consequences. Folguera *et al.* (2011) showed that intermediate temperature fluctuation had a positive effect on body size and growth rate of the terrestrial woodlouse (*Porcellio laevis*) compared with no or high temperature fluctuation. The upper developmental temperature of the fluctuating regime used in our study borders the stressful realm for *D. melanogaster* (Hoffmann, 2010). This could explain the poorer locomotor performance in this treatment compared with the constant temperature treatment, and also in wing length and width if we assume these are correlated with body size (Wilkinson *et al.*, 1990; Reeve *et al.*, 2000) and that large body size is advantageous (e.g. Stearns, 1992; Blanckenhorn, 2000; Roff, 2002) when the mean developmental temperature is similar. Pétavy *et al.* (2004) reported a similar

pattern for wing length and thorax length as well as viability and fecundity, but the amplitude of fluctuation was even greater than in our experiment (mean 25.5°C, range 18–33°C). All wing traits following development in the fluctuating temperature regime tended to resemble the higher temperatures (27°C and 29°C) more closely than the constant treatment. Hence, it seems that the highest temperature (29°C) of the cycle has the greatest impact on the wing traits. Development at 29°C is more stressful than at the low temperature of 21°C we used (David *et al.*, 1983; Hoffmann, 2010).

This, however, does not explain why the wing traits did not follow the predictions of Jensen's inequality for the fluctuating temperature regime. We would expect from the quasi-linear reaction norms that the variance in temperature would not change the trait mean of the treatments with the same temperature mean but different variance. This discrepancy with Jensen's inequality may relate to the acclimation response or lag phase that followed the temperature shift. It is known that the concentrations of heat shock proteins (HSPs) remain elevated in *D. melanogaster* for extended periods after exposure to heat stress, well beyond the phase duration used in our study (Dahlgaard *et al.*, 1998; Sørensen *et al.*, 2003). Up-regulation due to stressful conditions confers costs in terms of development and reproduction and even heat resistance when Hsp levels become too high (Krebs and Loeschcke, 1994; Feder and Hofmann, 1999). However, recent experiments have shown that intra-population variation in Hsp expression explains only a very small proportion of thermal stress resistance in isofemale lines, which suggests that other mechanisms are involved (Bahrndorff *et al.*, 2010; Jensen *et al.*, 2010). It cannot be excluded that smaller body size at maturity may be advantageous if activity is primarily confined to the high end of the temperature scale. It is well known that temperature drives selection on body size (e.g. Bochdanovits and De Jong, 2003), in addition to the plastic responses seen in the present study, for example. However, this would likely have resulted in superior performance in the stressed locomotor performance assay, so an adaptive explanation is unlikely.

The detrimental effects may become more pronounced when the phase shift is rather abrupt as in this study and not gradual, as is often the case in natural environments. On the other hand, the phase shifts followed a regular pattern, which would tend to be more irregular over a period of days in natural environments. The latter has been shown to have a detrimental effect on fitness compared with a regular pattern of fluctuation (Schaefer and Ryan, 2006). The inbred lines used in this study were inbred under a constant temperature regime. It cannot be excluded that some adaptation to constant temperature has occurred in the process. However, we believe this is unlikely to explain our results because inbreeding was achieved rapidly and consequently with limited opportunity for adaptation.

Acclimation to fluctuating temperature has been found to increase heat resistance in many organisms (e.g. Schaefer and Ryan, 2006; Fischer *et al.*, 2011; Oliver and Palumbi, 2011; van Dooremalen *et al.*, 2011). In this study, we observed the opposite in that stressed locomotor performance was reduced for flies reared in a fluctuating temperature regime (21°C/29°C) compared with flies reared at a constant 25°C (Fig. 4; Table 1). It would appear therefore that any positive effects of daily exposure to moderately high temperature during development were lost when emerging flies were transferred and kept under standard laboratory conditions prior to testing. Stressed locomotor performance here, however, likely constitutes a combination of the factors heat, desiccation, and starvation resistance because food and water were not available. The reaction norms are qualitatively similar to those reported by Kjærsgaard *et al.* (2010a), who also found the largest difference in this type of stressed locomotor performance at the middle range of high test temperatures in lines that differed in levels of

thermal adaptation. It is possible that other factors such as metabolic rate and starvation resistance become more important as test temperature increases. This could explain why differences between inbred and crossbred lines diminish again above 36°C.

One consequence of our findings (smaller wings, altered wing aspect, and reduced physiological performance in stressful environments) is that tropical species with similar responses that are already close to their critical upper temperature limit (Deutsch *et al.*, 2008; Duarte *et al.*, 2012; Kellermann *et al.*, 2012) may be affected. Indeed, population persistence may come into question (Pertoldi and Bach, 2007). On the other hand, an increase in mean temperature has been predicted to give rise to increased population fitness of temperate ectothermic species, as they will move towards higher relative fitness in terms of intrinsic population growth rate (Deutsch *et al.*, 2008). However, if the increased mean temperature is accompanied by a concomitant increase in variance, this effect may be partially counteracted. A reduction in mean performance in the traits investigated here could therefore lead to different evolutionary trajectories than predicted from models that do not incorporate future increases in climatic variance.

The importance of the findings discussed above becomes even more pertinent when we take inbreeding into account. The traits underlying at least some of the components of locomotor activity are influenced by several genes during ontogeny (Jordan *et al.*, 2006, 2007; Long and Rice, 2007). The same is true for body size (e.g. van Heerwaarden and Sgrò, 2011). These traits therefore require that an array of biochemical pathways remains relatively unaffected by environmental perturbations to avoid negative consequences for fitness (Hochachka and Somero, 2002). The genome-wide loss of heterozygosity with inbreeding is therefore likely to influence locomotor performance and wing length ($\approx$ body size). As previously mentioned, the depression of trait values under the fluctuating temperature regime may influence population mean fitness and size and thereby genetic variation. Few laboratory studies have specifically addressed the effects of fluctuating temperatures in relation to inbreeding (but see Kristensen *et al.*, 2008a). We found hybrid vigour for wing length, wing aspect, and stressed locomotor performance. Perhaps more importantly we also found interactions with temperature regime. This was most pronounced for stressed locomotor performance but also marginally non-significant for both wing length and wing width. Hence, the depression of trait means due to temperature regime tends to become more pronounced with inbreeding.

Increased climatic variance can affect migration capacity (Kubisch and Poethke, 2011). It is interesting that wing aspect was higher in at least two of the crossbred lines as this may represent an adaptive response to environmental temperature (Azevedo *et al.*, 1998; Hoffmann and Shirriffs, 2002; Santos *et al.*, 2006; Kjærsgaard *et al.*, 2010b). Narrower wings may facilitate wider dispersal (Carreira *et al.*, 2006), faster flying (Snodgrass, 1993), and better manoeuvrability (Ellington, 1984) at high temperature. At lower temperature, more lift is required to compensate for lower wing beat frequency (Starmer and Wolf, 1989; Azevedo *et al.*, 1998; but see Frazier *et al.*, 2008), in which case the relative size of the wing area to body weight becomes more important. The shorter wing length and consequently lower wing aspect found here for the inbred lines could represent a constraint on resource allocation due to higher maintenance costs arising from inbreeding (e.g. Ketola and Kotiaho, 2009), especially since wing size is highly correlated with body size. When food resources are scarce or absent, starvation may impose constraints on locomotor performance in *Drosophila* (Turelli and Hoffmann, 1988). This could explain, in energetic terms, the difference in performance of the inbred and crossbred offspring developed under the different thermal regimes.

The results of this study and others that have investigated the effects of temperature fluctuation make it clear that there is a need to focus on this source of environmental variation in the future. It seems that Jensen's inequality is not generally applicable, presumably due to acclimation responses. Furthermore, the responses may be contingent on genetic background in terms of heterozygosity. This study has demonstrated that there can be interactive effects between inbred and crossbred flies and different temperature regimes with the same mean values. Stressed locomotor performance showed a high degree of sensitivity in revealing these patterns.

ACKNOWLEDGEMENTS

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