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The Effect of Fluctuating Temperatures During Development on Fitness-Related Traits of *Scatophaga stercoraria* (Diptera: Scathophagidae)

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ABSTRACT Development of ectotherms is highly temperature dependent. Studies using variable thermal environments can improve ecological relevance of data because organisms naturally face day-to-day stochastic temperature fluctuations as well as seasonal changes in the amplitude of such daily fluctuations. The objective of this study was to investigate if, and to what extent, the use of constant temperatures is justified in studies of the model species, yellow dung fly, *Scatophaga stercoraria* (L.). We examined the effect of temperature fluctuation on the expression of several life history traits and the effect on subsequent adult longevity. We used two fluctuating temperature treatments with the same mean but different amplitudes (15/21°C, 12/24°C; 12/12 h), and three constant temperature treatments spanning the wide temperature range faced in the wild (12, 18, and 24°C). Large temperature fluctuation was mostly detrimental (lower juvenile survival, slower growth, smaller body size, and longer development), whereas moderate temperature fluctuation usually gave responses similar to the constant regime. When developing in fluctuating temperatures, adult longevity (no effect), body size (lower), and wing shape (narrower wings) deviated from the expectations based on the constant temperature reaction norms, presumably because of acclimation responses. Contrary to some studies no obvious beneficial effects of moderate temperature fluctuation were observed. Instead, yellow dung flies seem to canalize development in the face of temperature fluctuation up to a point when detrimental effects become unavoidable. The relatively greater effects of extreme constant developmental temperatures question their biological relevance in experiments.

KEY WORDS acclimation, Jensen's inequality, climate change, temperature variance

Temperature has a significant effect on all living organisms owing to the temperature dependency of biochemical reactions and the stability of molecules (Hochachka and Somero 2002). Its potency as a selection agent is evident by various macro-ecological patterns explained by temperature, such as species distributions (Cossins and Bowler 1987, Hoffmann and Parsons 1991) or clinal variation within species (Rezende et al. 2010). Effects of temperature are observed at all levels of phenotypic organization from molecules to behavior and life history of an organism. For these reasons the contemporary climate changes have been an increasing concern over the past decades, and many research data show the effect of these changes on various aspects of organism survival and

reproduction of organisms (Walther et al. 2002, Parmesan 2006). While the changes in mean temperature have received considerable attention, the effect of increasing temperature variation (around the mean) has not been addressed adequately until recently. Potentially, it is even more important for population persistence (Easterling et al. 2000, Jentsch et al. 2007, Pertoldi and Bach 2007).

Thermal performance curves (TPCs) are useful for describing and testing the effects of temperature on ectotherm performance (Izem and Kingsolver 2005). TPCs typically have three phases: an accelerating (i.e., convex) phase at low temperatures followed by a nearly linear increase at intermediate temperatures (Fig. 1). This phase is followed by a decelerating phase (with a precipitous concave drop) at high temperatures that are stressful and often lethal to the organism (Schoolfield et al. 1981, Ikemoto 2005, Shi et al. 2011). Small temperature fluctuations around benign intermediate temperatures within the linear phase are not expected to differ much from the corresponding constant mean temperature. Larger fluctuations, in contrast, encompass both the slightly accelerating and the strongly decelerating part of the TPC at low and high

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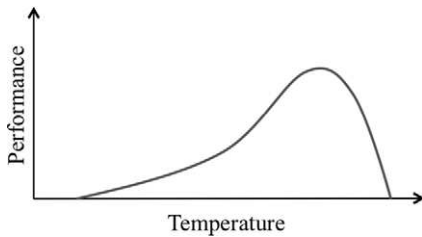


Fig. 1. Hypothetical thermal performance curve depicting the typical nonlinear relationship of insect performance with temperature. Performance accelerates slowly at low temperatures, followed by a linear phase at intermediate temperatures. After maximal performance a sharply decelerating phase follows at high temperature.

temperatures, respectively (Fig. 1). In this case, temperature fluctuations are expected to differentially affect performance of organisms relative to performance at constant temperature with the same mean, a phenomenon known as Jensen's inequality (Ruel and Ayres 1999). This mathematical property of nonlinear functions states that, for any nonlinear function (as fitness curves often are), variance is predicted to consistently elevate or depress the response variable such that the function $f(x)$ of the mean of x does not coincide with the mean of the function: $E(f(X)) \neq f(E(X))$ (Jensen 1906). Equality is only achieved if the function is linear. If the function is decelerating (second derivative is negative; i.e., concave), then the mean of the function is always less than or equal to the function of the mean; if the function is accelerating (second derivative is positive; i.e., convex), then the reverse is true. Jensen's inequality is sometimes also referred to as the rate summation effect or, when used explicitly in the context of temperature variation, the Kaufmann effect (Worner 1992).

The results of experiments investigating thermal variation do not always conform to expectations based on Jensen's inequality of TPCs obtained for constant developmental temperatures. Kingsolver et al. (2009) attributed an opposite effect on development time in *Manduca sexta* (L.) to acclimation associated with changes in temperature. Petavy et al. (2001a) ascribed lower than expected body size in *Drosophila melanogaster* (Meigen) and *Drosophila simulans* (Sturtevant) to stress responses when transient extreme temperatures were involved. Gabriel (1999) modeled performance curves for survival with time delays of acclimation after a shift in environmental state between a stressful and a nonstressful state (e.g., in temperature). The adaptive value of acclimation was, not surprisingly, shown to depend on the interplay between the duration of the time delay, the variance of the environmental cue, and information reliability. Known proximate mechanisms involved in thermal acclimation include changes in expression of heat shock proteins (Sørensen et al. 2003), cell membrane fluidity (Hazel 1995, Overgaard et al. 2006), or the functional capacities of oxygen delivering systems (Pörtner 2002). Different thermal sensitivities of growth and cell differentiation may also influence

development when temperature fluctuates (Kingsolver 2000). Reduced metabolic losses in a colder thermoperiodic phase may be advantageous (Karl and Fischer 2008, Fischer et al. 2011), and organisms may be adapted to temperature fluctuation via genetically fixed (i.e., evolved) diurnal changes in gene expression (Schaefer and Ryan 2006, Fischer et al. 2011). The phenotypic response to temperature variation is therefore contingent on the mean and variance in environmental temperature, the temporal pattern of fluctuation, and the temperature specific performance (i.e., the shape of the reaction norm).

The yellow dung fly, *Scatophaga stercoraria* (L.), is an important decomposer of the dung of livestock (Blanckenhorn et al. 2010). A thorough understanding of how environmental variation affects its abundance is therefore instrumental and economically relevant. The aim of this study is to test whether the use of constant development temperatures in experiments are justified for this species and to increase our general knowledge about how development in fluctuating temperature environments affects fitness-related traits of insects.

Materials and Methods

Population Description. The population used in this experiment was collected in Denmark in September 2010 in the central part of Jutland from an organic farm field with grazing dairy cattle near the town of Give (55.85° N, 9.23° E). The population was established from 25 pairs collected while mating on dung pats. They were brought to the laboratory where further mating was allowed before the males were removed for the females to lay eggs undisturbed.

Experimental Setup. We reared flies at two fluctuating temperature treatments with the same mean but different amplitudes (15/21°C and 12/24°C), in addition to three constant temperature treatments spanning the mean (optimal) as well as the extreme temperatures of the large amplitude fluctuating treatment (12°C, 18°C, 24°C); thus, covering a large part of the temperature range experienced in nature (Demont et al. 2008). Furthermore, 12°C is at the lower limit at which yellow dung flies show direct egg-to-adult development (as opposed to pupal winter diapause), and temperatures beyond 24°C show strong negative effects (Ward and Simmons 1990, Blanckenhorn et al. 2001). Before the experiment flies were propagated for five generations at standard laboratory conditions (constant 18°C, a photoperiod of 16:8 [L:D] h cycle, and 50% relative humidity [RH]) (Blanckenhorn et al. 2010). The experiment was initiated by setting up 11 pairs for mating and subsequent egg laying. Fifty eggs from individual females were transferred with 10 eggs to each of five small dung containers containing excess fresh dung (>2 g per individual) (Amano 1983). They were distributed randomly to one of the five temperature regimes: constant air temperature 12, 18, and 24°C, and fluctuating 15/21 and 12/24°C (step function, Mir-154 incubators, SANYO Electric Co., Ltd., Munich, Germany), with a 12/12 h daily photoperiod.

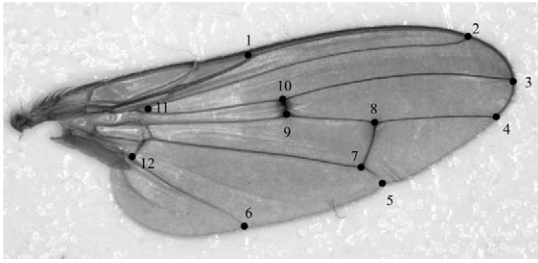


Fig. 2. A yellow dung fly *Scatophaga stercoraria* wing (dorsal view) showing 12 landmarks used to investigate wing morphology as a consequence of development at constant mean temperature treatments (12, 18, and 24°C) and two fluctuating treatments (15/21 and 12/24°C; 12/12 h cycle) with the same mean (18°C).

Two of the 11 females laid fewer than 50 eggs in the clutch. Here we distributed only eight and nine eggs, respectively, to each temperature treatment. The dung was collected at a nearby organic farm, homogenized by thorough mixing, and frozen at -80°C before being used.

Containers were checked daily for emerging flies to obtain estimates of development times and egg-to-adult survival. Flies were transferred to glass vials and fed ad libitum amounts of water saturated with sugar to investigate longevity on this diet at constant 18°C as a function of developmental temperature. We checked daily for dead flies, which were frozen for morphometric measurements. Right and left hind tibia and wings were detached from the individual and placed on a paper sheet with adhesive glue. They were then photographed under magnification using a camera (Leica DFC 490, Leica Microsystems GmbH, Wetzlar, Germany) connected to a computer. Digital measurements of hind tibia length and wing vein landmark positions were obtained with the software tpsDig version 2.16 (Rohlf 2010).

Statistics. The data were analyzed with restricted maximum likelihood methods (REML) using generalized linear mixed-effects models (glmer, lme4 library) in the statistical software R (R Development Core Team 2011). Model simplification was attempted to obtain the best model given the data. We ran analysis of deviance on the full models for each trait to obtain estimates of the significance of the fixed factors, which are calculated with type-II Wald χ^2 tests. Egg-to-adult survival and sex ratio were analyzed assuming a binomial distribution of the data. The sexes were combined for egg-to-adult survival. The remaining data on development time, growth rate, longevity, hind tibia length (average of the left and right), wing centroid size, wing loading, and wing aspect were tested for normality and homogeneity of variance by visual inspection and using Shapiro-Wilk's tests.

The wings were characterized by 12 landmarks (Fig. 2). Wing centroid size was calculated from the right wing by taking the square root of the sum of all the squared interlandmark distances. For this calculation the landmarks five and six, which were often missing, were excluded to increase overall sample size.

In cases where only the left wing was intact it was used instead of the right wing. Conceptually similar to wing loading, that is, the wing area to body size ratio, we calculated wing centroid size of the right wing divided by the hind tibia length. Finally, as a simple measure of wing shape, we also calculated the wing aspect as the wing length (distance 3–11 in Fig. 2) divided by the width (distance 1–6).

Focusing here on the differences between variable and constant temperatures with the same mean, we generally analyzed temperature regime (18°C constant, 15/21 and $12/24^{\circ}\text{C}$) as a fixed effect for egg-to-adult survival and sex ratio. For the remaining variables sex was additionally included. In all analyses family was included as a random factor. For most variables we additionally compared only the three constant temperatures (12, 18, and 24°C), which have been analyzed before (Blanckenhorn 1997a). A full model with all (constant and fluctuating) temperature regimes is provided in Supp. Table 1 (online only) where effect sizes can be compared.

Results

Our analyses showed that the high fluctuation treatment, encompassing both a slightly accelerating (convex) part of the thermal performance curve at low temperatures (12°C) but also a (concave) precipitous drop after the maximum at high temperatures (24°C), generally deteriorated performance (lower egg-to-adult survival, slowed development, and growth rate) relative to the constant temperature with the same mean (18°C) (Table 1; Figs. 3 and 4).

There was no significant effect of fluctuating temperature regime on the sex ratio of the emerging flies (Table 1). The effect of fluctuating temperatures on egg-to-adult survival was significant ($\chi^2_2 = 11.07$; $P = 0.004$), with higher survival at low fluctuation (15/21°C; 83.7%) followed by constant (18°C ; 80.7%) and high fluctuation (12/24°C; 65.9%) treatments (Table 1). Adult longevity on sugar and water did not significantly vary among the constant 18°C and the two fluctuating developmental temperature regimes (Table 1; Fig. 2). When comparing the three constant developmental temperatures, the lowest longevity was recorded at the high temperature (24°C) ($\chi^2_2 = 70.65$; $P < 0.001$). Females longevity was significantly longer than male longevity ($\chi^2_1 = 9.65$; $P < 0.001$), particularly at 12°C (temperature by sex: $\chi^2_2 = 9.62$; $P = 0.008$).

Egg-to-adult development of *S. stercoraria*, took significantly longer duration under fluctuating temperature treatments (12/24 > 15/21 > constant 18°C ; $\chi^2_2 = 382.06$; $P < 0.001$), and durations significantly varied with sex ($\chi^2_1 = 179.27$; $P < 0.001$; males > females) (Table 1; Fig. 3). There was no significant interaction between temperature and sex. An analogous pattern was found in the relationship between temperature and growth rate (Table 1; Fig. 3). The relationship was significantly different between sexes ($\chi^2_1 = 625.43$; $P < 0.001$). In addition, a significant interaction between temperature and growth rate was

Table 1. Life-history traits of the yellow dung fly, *Scatophaga stercoraria*, reared at a constant mean temp treatment (18°C) and two fluctuating treatments (15/21 and 12/24°C; 12/12 h cycle) with the same mean (18°C)

Trait	Treatment (sample size)	Model estimate and \pm contrasts	SE	Test statistic	Test value	Best model and deviance <i>P</i> level	
Sex-ratio ^a	18°C (11)	-0.141	0.220	Z	—	Temperature ^{NS}	
	15/21°C (11)	-0.041	0.303		-0.136		
	12/24°C (11)	+0.424	0.325		+1.306		
Egg-to-adult survival ^a	18°C (11)	1.348	0.281	Z	—	Temperature ^{**}	
	15/21°C (11)	+0.236	0.343		0.688		
	12/24°C (11)	-0.751	0.309		-2.430		
Development time (d)	♀	18°C (45)	22.63	T	—	Temperature ^{***}	
		15/21°C (48)	+2.02		0.20		+9.98
		12/24°C (27)	+3.13		0.24		+13.10
	♂	18°C (40)	+1.80	0.21	—	Temperature \times sex ^{NS}	
		15/21°C (40)	+3.72	0.21	+17.45		
		12/24°C (39)	+4.74	0.21	+22.07		
Growth rate (mm/d)	♀	18°C (45)	0.133	T	—	Temperature ^{***}	
		15/21°C (48)	-0.013		0.0013		-9.58
		12/24°C (27)	-0.020		0.0016		-12.98
	♂	18°C (40)	+0.022	0.0014	—	Temperature \times sex [*]	
		15/21°C (40)	+0.010	0.0014	+15.32		
		12/24°C (39)	+0.010	0.0014	+7.29		
Hind tibia length (mm)	♀	18°C (45)	3.00	T	—	Temperature ^{***}	
		15/21°C (48)	-0.05		0.019		-2.57
		12/24°C (27)	-0.11		0.023		-4.94
	♂	18°C (40)	+0.75	0.020	—	Sex ^{***}	
		15/21°C (40)	+0.76	0.020	+36.94		
		12/24°C (39)	+0.56	0.020	+37.55		
Wing centroid size	♀	18°C (42)	30.73	T	—	Temperature \times sex ^{***}	
		15/21°C (47)	-0.52		0.17		-3.11
		12/24°C (25)	-1.27		0.20		-6.42
	♂	18°C (34)	+4.27	0.18	—	Temperature ^{***}	
		15/21°C (35)	+4.68	0.18	+23.55		
		12/24°C (36)	+2.66	0.18	+25.99		
Wing loading	♀	18°C (42)	10.24	T	—	Temperature ^{NS}	
		15/21°C (47)	+0.06		0.062		+0.91
		12/24°C (25)	-0.02		0.074		-0.31
	♂	18°C (33)	-0.94	0.068	—	Sex ^{***}	
		15/21°C (35)	-0.91	0.067	-13.90		
		12/24°C (35)	-0.81	0.067	-12.19		
Wing aspect	♀	18°C (42)	1.312	T	—	Temperature ^{**}	
		15/21°C (45)	+0.024		0.010		2.44
		12/24°C (25)	+0.026		0.011		2.24
	♂	18°C (33)	+0.011	0.011	—	Sex ^{NS}	
		15/21°C (35)	+0.032	0.010	1.03		
		12/24°C (35)	+0.021	0.010	3.08		
Longevity (d)	♀	18°C (45)	27.19	T	—	Temperature ^{NS}	
		15/21°C (48)	+1.93		2.15		+0.94
		12/24°C (30)	+2.49		2.33		+1.07
	♂	18°C (40)	-0.29	2.15	—	Sex ^{NS}	
		15/21°C (41)	+1.00	2.13	-13.50		
		12/24°C (40)	-0.23	2.15	-12.19		

Restricted max likelihood model estimates, SE, test statistics, and statistical significance are given. The factors included in the best model are highlighted in bold and symbols indicate analysis of deviance *P* values.

^a Mean and standard errors presented in logits. The means are converted into proportions by $P = 1/(1 + 1/e^x)$.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, nonsignificant.

found ($\chi^2_2 = 6.13$; $P = 0.047$) because of a larger difference in growth rates of males between treatments.

Body size, as represented by length of hind tibia and wing centroid size, was similar in the low temperature fluctuation and constant 18°C treatments but body size was significantly smaller in the high fluctuation treatment (tibia: $\chi^2_2 = 124.98$, wing: $\chi^2_2 = 150.96$; $P < 0.001$) (Table 1; Fig. 4). Male body size was significantly larger than female body size (tibia: $\chi^2_1 = 3799.83$, wing: $\chi^2_1 = 1773.82$; $P < 0.001$). Further, a significant interaction was found between sexes and temperatures: adult body size was becoming more

similar at the high fluctuation treatment (tibia: $\chi^2_2 = 20.86$, wing: $\chi^2_2 = 24.84$; $P < 0.001$). Comparing the three constant temperatures, the flies conformed to the temperature-size-rule (Blanckenhorn 1997a; Shi et al. 2012, 2013) (larger body size at cooler temperatures; tibia: $\chi^2_2 = 833.49$, wing: $\chi^2_2 = 810.34$; $P < 0.001$). Wing loading (wing centroid size/hind tibia length) did not significantly vary with fluctuating temperature regime but was significantly greater in males ($\chi^2_1 = 513.00$; $P < 0.001$) (Table 1; Fig. 4). Wing aspect (wing length/width), showed significantly higher values (i.e., narrower wings) at fluctuating temperatures compared with the constant (18°C) temperature

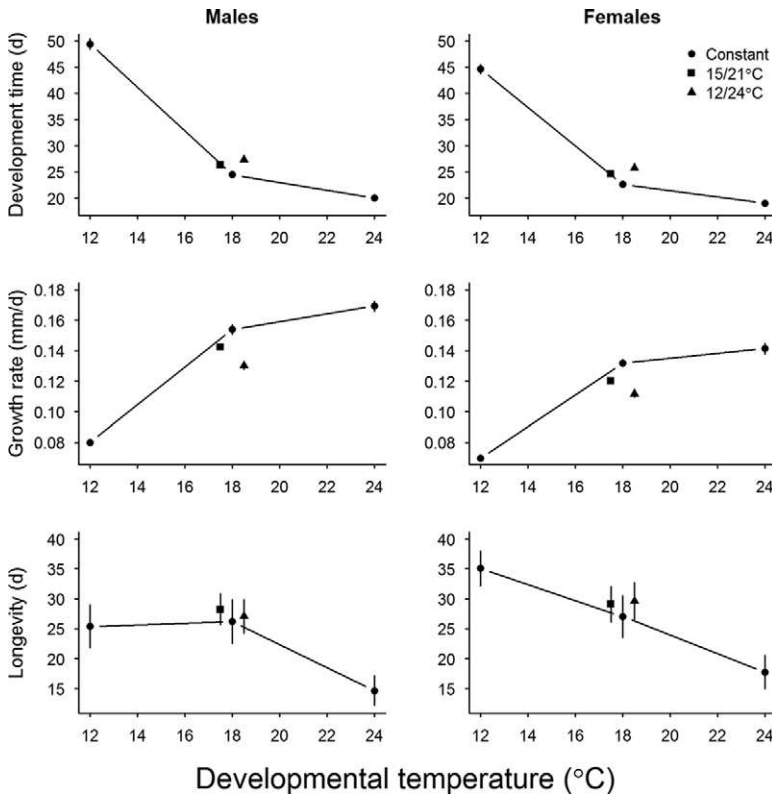


Fig. 3. Reaction norms (raw data) for life-history traits of the yellow dung fly *Scatophaga stercoraria* reared and two fluctuating treatments (15/21 and 12/24°C; 12/12 h cycle) with a mean of 18°C. Development time, growth rate and longevity with 95% CIs (not shown when smaller than the symbols).

($\chi^2_2 = 10.54$; $P = 0.005$) (Table 1; Fig. 4). Comparing wing aspect among the three constant temperatures there was a significant interaction between temperature and sex ($\chi^2_2 = 9.57$; $P = 0.008$) with males having relatively wider wings at the extreme temperatures (Supp. Table 1 [online only]; Fig. 4).

Discussion

The expression of standard life-history traits (body size, development time, growth rate, and mortality) of the yellow dung fly has been well investigated across a wide thermal range using constant temperatures (Blanckenhorn 1997b, Blanckenhorn et al. 2010). The effects of fluctuating temperatures have not been studied in much detail despite the potentially high thermal variability associated with its juvenile habitat, except for at low temperature (Blanckenhorn 1997a). Further, we analyzed wing size and shape for the first time. We expected that high temperature fluctuation (12/24°C) would depress the mean because the (concave) high temperature effect would dominate the response, resulting in retarded development, smaller body size, and/or higher mortality than the corresponding mean temperature (18°C) or low fluctuation (15/21°C) treatments. Alternatively, especially small temperature fluctuations may even be generally ben-

eficial since dung flies are likely adapted to substantial temperature heterogeneity experienced in their natural habitat (Ward and Simmons 1990). Performance at low temperature fluctuations (15/21°C) mostly did not significantly differ from that at constant 18°C, never being significantly better for any of the fitness-related traits, but occasionally worse (longer development time and slower growth rate). This smaller or absent effect was expected because at this temperature range the thermal performance curve for development rate of yellow dung flies is quasi linear (Blanckenhorn 1999). The larger influence of high temperature fluctuations (12/24°C) on performance was also mostly in accordance with expectations from Jensen’s inequality alone (Ruel and Ayres 1999) given strongly nonlinear concave functions at high temperatures. Performance changes in response to environmental variation caused by nonlinearity of reaction norms have to be considered nonadaptive because they can solely derive from mathematical properties that are difficult to interpret functionally. Positive effects of environmental variation on performance, however, likely indicate that organisms have adapted to such natural fluctuations, even though the concrete mechanism remains unclear.

Given that the fluctuating treatments and the 18°C constant treatment shared a common mean, the re-

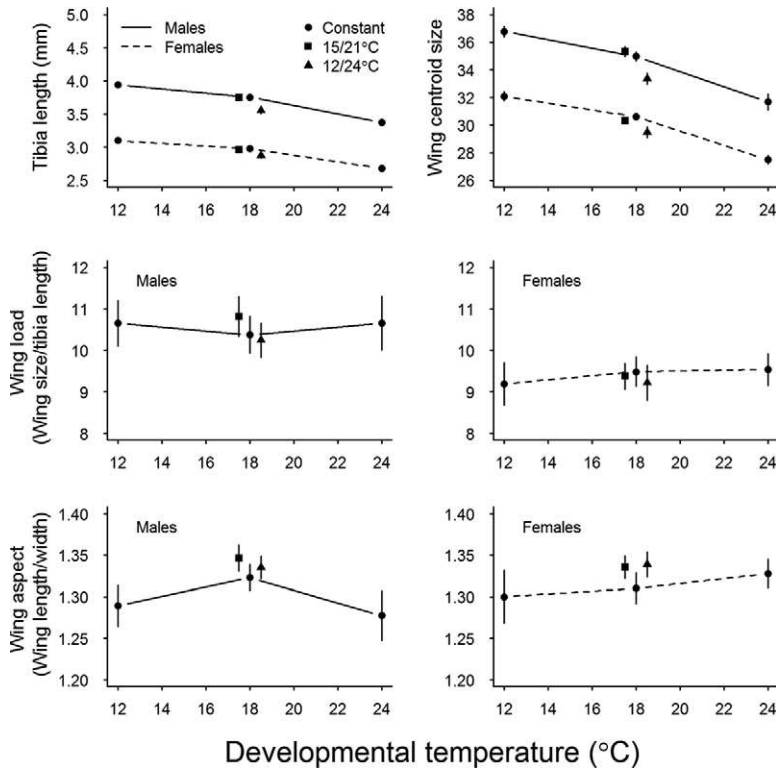


Fig. 4. Reaction norms (raw data) for morphological traits of the yellow dung fly *Scatophaga stercoraria* reared at constant mean temperature treatments (12, 18, and 24°C) and two fluctuating treatments (15/21 and 12/24°C; 12/12 h cycle) with a mean of 18°C. Hind tibia length, wing centroid size, and wing loading and wing aspect with 95% CIs (not shown when smaller than the symbols).

sulting lower growth rate and body size and longer development time in the fluctuating treatments probably reflect constraints on development rather than adaptive phenotypic plasticity because both fast development and/or large body size, all else being equal, typically confer higher fitness (Partridge et al. 1987, Blanckenhorn 2000, Kingsolver and Pfennig 2004, Dmitriew 2011). Body size is regulated by a large set of genes (Carreira et al. 2009), and the underlying genetic architecture has been shown to change with temperature (van Heerwaarden and Sgro 2011), which could render this trait relatively sensitive to fluctuations. It is well-known that temperature drives selection on body size and plastic responses as seen in this study and numerous others (Bochdanovits and de Jong 2003). We cannot exclude that smaller body size at maturity may be advantageous if activity is primarily confined to the high end of the temperature scale. Nevertheless, the summer decline of yellow dung fly populations in Central European habitats strongly suggests that the flies remain inactive during these periods or at least do not engage much in reproductive activities during hot spells (Blanckenhorn et al. 2001, Blanckenhorn et al. 2010).

Longevity when fed only on sugar water (yellow dung flies are predatory) likely indicates body condition because resources accumulated during develop-

ment prolong life on this suboptimal diet (Reim et al. 2006). Here females lived longer than males only at the low temperature (12°C; Fig. 3). The response to developmental temperature fluctuations, however, did not differ between the sexes even though the shapes of the reaction norms of the constant temperature treatments were quite different (curvilinear and linear, respectively). The large lifespan difference found (only) at low developmental temperatures could potentially be explained by sex specific sensitivity and/or age-dependence in reacting to lacking protein sources, which are required for both sexes for the production of sperm and eggs (Blanckenhorn et al. 2010). Males lived longer than females in a study where prey was provided in addition to sugar (Blanckenhorn 1997b).

The effect of fluctuating developmental temperature on wing loading and wing shape has rarely been addressed and no studies had investigated wing shape in yellow dung flies to date. There is some evidence from *Drosophila* studies that wings become wider with more area at low developmental temperature, in line with an adaptive explanation that reduced wing loading or greater wing aspect may improve flight in the cold (Frazier et al. 2008, Loh et al. 2008). Our data did not support this hypothesis for the yellow dung fly. Either there was no effect of rearing temperature

(wing loading and female wing aspect; Fig. 4), or values were lower at both hot and cold (constant) temperatures (male wing aspect). There was a sex difference in wing loading, which was consistently higher in males. Hence, males have a larger wing area relative to hind tibia length. This could be because of selection, as males sometimes have to fly off carrying their female with them in case of take-over attempts or disturbance. However, allometric differences of the abdomen, which will be enlarged in gravid females, would predict higher wing loading of females, opposite to what we found. Temperature fluctuation produced greater wing aspect than at the constant temperature with the same mean. This was especially intriguing in the males because it directly opposes the pattern of the extreme constant temperatures despite the fact that the high fluctuating temperature regime cycled between these two temperatures. The *Drosophila* wing has proven readily amenable to independent evolutionary shape changes in selection experiments in even very small wing compartments (Weber 1992). Accommodation or compensatory growth in adjacent wing areas convene global homeostasis (García-Bellido 2009), so it is possible that temperature fluctuation may interfere with such a mechanism if different genes controlling growth in different regions also exhibit different temperature specific expression (Debat et al. 2009). All in all this suggests that wing morphometric analysis may constitute a sensitive measure of stress resulting from temperature heterogeneity in this economically important species (Hoffmann et al. 2005).

The investigated traits did not always follow the expected patterns derived from the reaction norms. Similar responses in longevity of the sexes were observed despite clearly different reaction norms (Fig. 3), as well as smaller body size (tibia length) and greater wing aspect under high temperature fluctuations, despite largely linear reaction norms (Fig. 4). This indicates that not all trait responses can be inferred from the above-mentioned mathematical properties associated with nonlinear reaction norms. An important aspect here is probably the capacity (or lack hereof) of an organism to acclimate to a change in temperature and the associated time lag to reach optimal performance at a given temperature (Gabriel 2005, Kristensen et al. 2008). Heat shock proteins are, for instance, often induced at stressful temperatures at faster rates than they disappear upon reversal to a more benign temperature (Sørensen et al. 2003). Folguera et al. (2011) found higher expression levels of heat shock proteins with higher temperature fluctuation in the woodlouse *Porcellio laevis* Latreille, and showed that this pattern was correlated with less efficient physiological processes in agreement with our findings.

The increase in mean temperatures associated with global warming has been argued to benefit ectotherms in temperate zones because they will move toward their physiological optima (Deutsch et al. 2008). However, if temperature variance accompanies the mean temperature increase, the positive effects may par-

tially be outweighed by the negative effects mediated by the variance, such as the size reduction or prolonged development found in this and other studies (Petavy et al. 2001a, Kjærsgaard et al. 2012). The implication is that laboratory experiments may overestimate performance under natural conditions, especially when temperature variation is not taken into account, which is problematic when assessing conservation status of a species and modeling future scenarios. However, our data also show that the use of constant stressful temperatures (here 12 and 24°C) probably lead to overestimation of the detrimental effects on development and longevity because temperatures will usually return to more benign states on a daily basis. The extreme constant temperature treatments typically resulted in the most extreme phenotypic responses (Supp. Table 1 [online only]).

Besides the importance of climate change, most organisms experience considerable temperature heterogeneity in their lifetime (Cossins and Bowler 1987, Kingsolver 2000, Gibbs et al. 2003). Therefore, it is somewhat peculiar that the great majority of laboratory experiments have been conducted only at constant rather than more natural variable temperatures. This is likely because of a combination of the corresponding ease of experimenting and interpreting the outcomes. Some ecological aspects may, however, be left out or even misinterpreted because a change in temperature regime away from the prevailing conditions experienced in the natural environment can alter existing associations between species or phenotypes (Brakefield and Mazzotta 1995, Blanckenhorn 1997a, Brakefield and Kesbeke 1997, Kingsolver et al. 2004, Ragland and Kingsolver 2008, Fischer et al. 2011).

Most of the results of this study contrast a recent study by Fischer et al. (2011) who found mainly positive effects of temperature fluctuation in the butterfly *Lycaena tityrus* (Poda). Several factors could explain these differences. Even if the temperature regimes are comparable in terms of means and amplitudes among the two studies, the butterfly study used a multistep temperature function making temperature changes more gradual than what we used here. As a result, acclimation to the extreme temperatures may be more efficient and exposure times shorter. The different larval habitats likely influence the species in different ways. Dung patches gradually change in nutritional value and eventually dry out as they decompose. Food quality may be more constant for the butterfly larvae, which may however be more exposed sudden shifts in weather conditions. Such differences highlight the need for detailed studies of temperature fluctuations across taxonomic groups. When fluctuating temperature has been used in experiments, this usually involved only one variable treatment that was compared with constant temperature treatments. However, a number of recent studies have been conducted using two or more fluctuating temperature regimes (Petavy et al. 2001a,b; Schaefer and Ryan 2006; Ragland and Kingsolver 2007; Folguera et al. 2008, 2009, 2011; Ragland and Kingsolver 2008; Wu et al. 2009; Merakova and Gvozdk 2009; Bozinovic et al. 2011). Hence, there

is an increasing awareness of the potential importance of developmental temperature fluctuation.

In conclusion, it seems yellow dung flies canalize development in the face of temperature fluctuation, but to different degrees that are trait dependent. Beneficial effects associated with moderate but not extreme temperature fluctuations during development, both of which occur frequently in nature, are largely absent in contrast to what some studies find (Fischer et al. 2011). Increasing environmental variation associated with climate warming could therefore affect yellow dung fly populations negatively and thereby the decomposition of the dung of livestock. Effects of constant extreme temperatures here were mostly greater than those of corresponding fluctuating regimes; therefore, we advocate more frequent use of ecologically relevant fluctuating temperature regimes in conjunction with constant regimes in the yellow dung fly and other species.

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