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Predictability rather than amplitude of temperature fluctuations determines stress resistance in a natural population of *Drosophila simulans*

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Abstract

The adaptability of organisms to novel environmental conditions depends on the amount of genetic variance present in the population as well as on the ability of individuals to adjust their phenotype through phenotypic plasticity. Here, we investigated the phenotypic plasticity induced by a single generation's exposure to three different temperature regimes with respect to several life-history and stress-resistance traits in a natural population of *Drosophila simulans*. We studied a constant as well as a predictably and an unpredictably fluctuating temperature regime. We found high levels of phenotypic plasticity among all temperature regimes, suggesting a strong influence of both temperature fluctuations and their predictability. Increased heat tolerance was observed for flies developed in both types of fluctuating thermal environments compared with flies developed in a constant environment. We suggest that this was due to beneficial hardening when developing in either fluctuating temperature environment. To our surprise, flies that developed in constant and predictably changing environments were similar to each other in most traits when compared to flies from the unpredictably fluctuating environment. The unpredictably changing thermal environment imposed the most stressful condition, resulting in the lowest performance for stress-related traits, even though the absolute temperature changes never exceeded that of the predictably fluctuating environment. The overall decreased stress resistance of flies in the unpredictably fluctuating environment may be the consequence of maladaptive phenotypic plasticity in this setting, indicating that the adaptive value of plasticity depends on the predictability of the environment.

Introduction

Natural populations are exposed to environmental changes on yearly, seasonal and daily scales. These changes affect fitness-related traits such as stress resistance, survival and reproductive output (Fisher, 1930; Endler, 1986). A response of an organism exposed to novel environments is required to maintain high fitness, and this response depends on a combination of the amount of adaptive genetic variation (Wagner *et al.*, 1997; Flatt, 2005) and on the ability to adjust the phenotype through phenotypic plasticity (Pal, 1998; Lande, 2009; Chevin & Lande, 2010; Pelabon *et al.*, 2010). Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes in response to changes in internal or external environmental conditions (Bradshaw, 1984; Scheiner, 1998).

The relation between natural selection and phenotypic plasticity is not yet completely understood (Via, 1992; Scheiner, 1993; Czesak *et al.*, 2006). On the one hand, a trait with a high level of phenotypic plasticity could respond to environmental changes without relying on genetic variation. As a consequence, high plasticity might therefore relax selection pressure and limit the evolutionary response (West-Eberhard, 2003).

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On the other hand, phenotypic plasticity can facilitate evolutionary responses by creating different phenotypes during a short period (Ghalambor *et al.*, 2007), as suggested by the rapid increase of phenotypic variation due to phenotypic plasticity after experiencing new environmental conditions (Pal, 1998; Lande, 2009). If no costs were involved in a plastic response, organisms in nature would be expected to show infinite phenotypic plasticity, as having a plastic genotype always offers greater ability to adapt and thereby providing higher fitness compared with a nonplastic genotype (Pigliucci, 1996; DeWitt *et al.*, 1998). Costs of plastic responses are expected to be associated with the continuous monitoring of the environmental conditions, the acquisition and processing of this information, and the activation of all mechanisms necessary to develop the phenotype that best aligns with the new environmental conditions (DeWitt *et al.*, 1998).

The fitness of an insect exposed to daily environmental fluctuations is expected to be especially dependent on the ability to adjust its phenotype through phenotypic plasticity. The adaptive value of plasticity for a given trait depends on several factors such as the rate of environmental change (Utz *et al.*, 2014), the reversibility of the plastic response (Utz *et al.*, 2014), the amplitude (Terblanche *et al.*, 2010) and the predictability of temperature variations (Arnoldini *et al.*, 2012; Hallsson & Bjorklund, 2012; Ketola *et al.*, 2013). Both theoretical (Reed *et al.*, 2010) and empirical (Hallsson & Bjorklund, 2012) studies suggest that phenotypic plasticity might increase after selection in a predictably changing temperature compared with a constant temperature regime, reiterating that phenotypic plasticity might be an efficient mechanism to cope with predictable environmental changes. In constant environments, the maintenance of the plasticity machinery might be an unnecessary waste of resources and decreased levels of phenotypic plasticity compared with organisms selected in fluctuating environments are expected. Bet-hedging strategies and risk-spreading adaptations are two indirect ways of organisms to cope with unpredictable environmental changes (Frank & Slatkin, 1990; Biron *et al.*, 1998; Hopper & Rosenheim, 2003), because there is not a single phenotype with an optimal performance in all potentially occurring environments. Bet-hedging strategy is defined as a 'strategy that reduces the temporal variance in fitness at the expense of a lowered arithmetic means fitness' (Ripa *et al.*, 2010). Thus, in unpredictable environments, a large plastic response might lead to decreased fitness (maladaptive phenotypic plasticity), if the expression of a specific phenotype was based on inconsistent environmental cues (Lande & Arnold, 1983; Gomulkiewicz & Kirkpatrick, 1992). Thus, the selective past of a population is expected to continue affecting the fitness of organisms when they are exposed to novel environmental conditions (e.g. Hallsson & Bjorklund, 2012).

In this study, we investigated the effect of a single generation of maintenance at constant, predictably or unpredictably fluctuating temperature on the expression of plasticity in life-history- and stress-resistance-related traits of a population of *Drosophila simulans*. The mass population has been collected recently in an environment characterized by high amplitude and predictability of temperature changes (Bahrndorff *et al.*, 2009). Small differences were expected between flies developed under predictably and unpredictably fluctuating temperature regimes, as the last two temperature regimes were very similar to each other (see Material and Methods section). The consequences of fluctuating compared with constant temperatures with the same mean on individual traits was not easily predicted due to the asymmetrical effect on trait performance curves around the mean as described by Jensen's inequality. Thus, even if the mean temperatures were the same in all temperature regimes, larger differences were expected when comparing flies from the two fluctuating regimes with the constant environment (Fischer *et al.*, 2011; Bahar *et al.*, 2012). The metabolic gain at higher temperatures could both be either beneficial or costly and likely trait specific depending on the activated mechanisms. Fluctuating temperature regimes were expected to lead to a better performance of flies in thermal stress resistance due to beneficial acclimation during the development under these regimes (Hoffmann *et al.*, 2003; Sejerkilde *et al.*, 2003; Chown & Terblanche, 2007).

Material and methods

Origin of population

A population of *D. simulans* was collected in '*località la Casella*' (760 m asl.), close to Bologna, Italy (44.271647 N, 11.04686 E), in the first half of August 2012. '*località la Casella*' was chosen as the site of collection as the daily and seasonal temperature fluctuations in this part of Italy are highly predictable (Bahrndorff *et al.*, 2009). Moreover, the same population has been used in several studies providing useful information regarding plastic responses and genetic variance for several life-history traits (Trotta *et al.*, 2007, 2010). Nine traps were placed with a mixture of banana and peach pulp not closer than ten metres from each other around wild apple trees. The traps were transferred to the laboratory where the eggs hatched and juveniles developed. All emerged flies were sexed, and approximately 450 mated females were placed individually in vials with 7 mL standard oatmeal-sugar-yeast-agar medium. Five males and five females from the offspring of each of the original 450 females were picked randomly to create the mass population (4500 individuals), randomly divided among 18 bottles with 60 mL standard food in a cli-

mate chamber at 23 °C constant and a 16:8 h light: dark (L:D) cycle for two generations before setting up isofemale lines and starting the experiment. Flies contained in bottles were mixed prior to the initiation of every new generation.

Isofemale lines

To investigate the levels of phenotypic plasticity of a genotype in different environmental conditions, we established 25 isofemale lines randomly from the mass population after two generations in the laboratory at constant 23 °C and 16:8 L:D. Each of the females was allowed to lay eggs for 2 days, from 4 to 6 days of age in 7-mL vials with standard medium. The progeny of each isofemale line (F1) were collected in a 60-mL plastic bottle over 48 h. F1 flies were aged for 4 days before egg laying to ensure the presence of mated females. Density and developmental time were controlled by transferring batches of 40 eggs into 7-mL vials. Vials with F2 eggs were randomly split among the three temperature treatments (see below). 48 h after eclosion of F2 flies, sexes were separated under light CO_2 anaesthesia. Twenty females for each F2 isofemale line were placed in 7-mL vials with fresh food for another 48 h before assessing traits, to avoid post-anaesthesia effects (Nicolas & Sillans, 1989). Different isofemale lines were used for each trait investigated.

Environments

The offspring of each of 25 isofemale lines were tested in three different environmental conditions, constant (C), predictably fluctuating (PF) and unpredictably fluctuating (UF) temperatures (Fig. 1). The average temperature of 23 °C was identical in all environments, as well as the light cycle of 16:8 (L:D). The light intensity changed during the day following a bell-shaped curve, to imitate the sun light cycle. At 8:00 a.m. in the morning, when the day began, the light switched on and increased intensity gradually reaching the daily peak (approximately 6000 lumen) at 4:00 p.m. Past this point, the light intensity gradually decreased until it turned off at 00:00 a.m. Daily variations of temperature were different in the three environments (Fig. 1). Constant environment (C) had constant 23 °C throughout the day; predictably fluctuating environment (PF) repeated a constant daily fluctuation following a two-step (day and night, respectively) sinus function, where the day began at 8:00 a.m. with a temperature of 23 °C, followed by an increase in temperature until 28 °C at 4:00 p.m. At 00:00 a.m., the temperature was 23 °C again and continued to decrease quickly until it reached the minimum temperature of 13 °C at 4:00 a.m. From 4:00 a.m. to 8:00 a.m., the temperature returned to the starting point of 23 °C. The third treatment, the unpredictably fluctuating environment (UF), followed

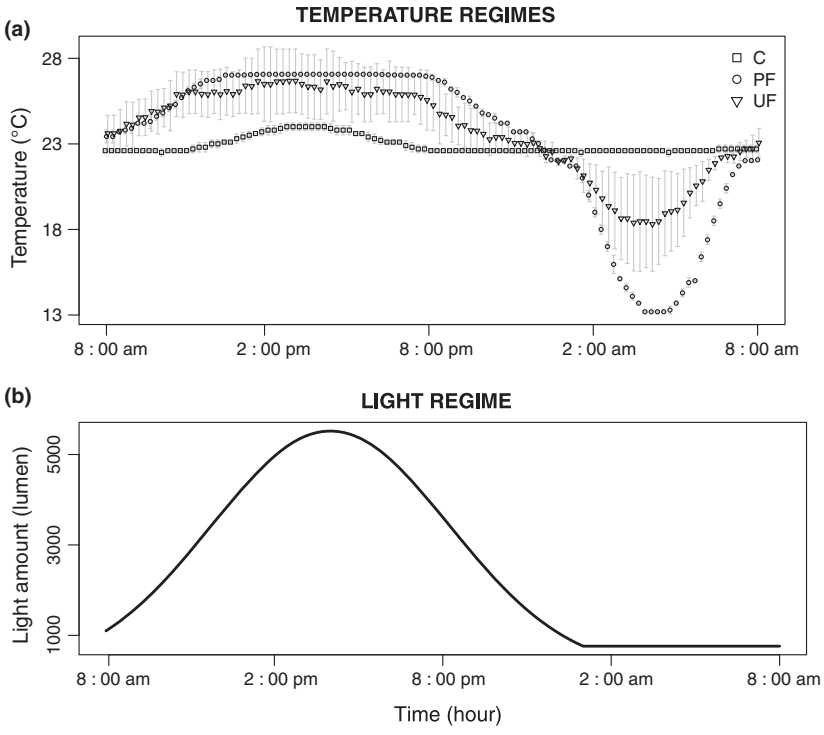

Fig. 1 (a) The registered temperatures for each environment from a 2-week period during the test. Squares represent the constant temperature treatment (C), circles correspond to the predictably fluctuating temperature treatment (PF), and triangles stand for the unpredictably fluctuating temperature treatment (UF). Note that the standard error for the unpredictably fluctuating treatment has a greater variance, indicating that the temperature in this regime varies among days without following a predictable pattern. (b) Light intensity over 24 h.

a similar sinus function where the maximum and minimum temperatures, at 4:00 p.m. and 4:00 a.m., respectively, were randomly determined, but never exceeding the extremes of 28 °C and 13 °C, respectively. The temperature at 8:00 a.m. and at 00:00 a.m. was 23 °C degrees, equal to the predictably fluctuating and constant treatments.

Phenotypic assessments

At the day of the test, flies from all three temperature regimes were moved to a common 23 °C climate room at 8:00 a.m., when the temperature in all three regimes was 23 °C.

For all traits investigated (except for egg-to-adult viability and developmental time, see below), ten females from each isofemale line were assayed.

Productivity
Productivity was assessed as number of eclosed flies generated by a single female. A 3-day-old female was kept with two males in a 4-mL vial with normal food for 3 days.

Egg-to-adult viability and developmental time
The number and time until eclosion of adult flies was assessed out of 40 eggs. Developmental time was measured as the time interval between egg laying by parental flies and progeny emerging of about 120 eggs for each isofemale line. To increase the precision of estimating developmental time, parental flies laid eggs for a short period of 8 h. The egg-to-adult viability was measured as the success of 120 eggs for each isofemale line. The egg-to-adult viability and developmental time were scored in three vials for each isofemale line every 8 h.

Time to heat knock-down
Heat resistance was assessed by placing individual females in 5-mL glass vials (diameter = 15 mm, height = 45 mm). The vials were positioned in a rack and submerged into a water bath set to 38 °C and with an effective water temperature of 37.5 °C. Time to knock-down was scored as time flies could tolerate high temperature before falling into a coma where they could no longer move any body part.

Chill coma recovery time
For cold resistance, individual females were placed in 5-mL glass vials and submerged in a cold water bath (real and set temperature of the water bath were 0 °C and −1 °C, respectively) for 3 h before being moved to the laboratory at room temperature. Chill coma recovery time was scored as time until the flies spontaneously woke up from the coma and stood up on all six legs.

Desiccation
To score desiccation tolerance individual females were placed into 5-mL vials sealed with gauze to ensure that the air could circulate. Vials were placed into an airtight tank with silica gel to keep the relative humidity close to 0%. Desiccation tolerance was scored as time to death.

Starvation
Starvation tolerance was measured as time flies could tolerate food deprivation. Individual females were placed into a vial with 2 mL of water and agar solution. Flies were checked every 8 h until all flies had died.

Body size
About 10 females were used to estimate body size from each isofemale line; right wings of each fly were dissected and mounted on glass slides in lactic acid/ethanol (6:5). From each wing, 11 landmark coordinates were recorded using ImageJ 1.46r software (National Institute of Health, USA) and the body size of an individual wing was estimated by the centroid size of its landmarks configuration (Slice, 1996).

Statistical analyses

The phenotypic plasticity was tested by a full factorial two-way analysis of variance (ANOVA) with isofemale line and treatment as fixed effects. All assumptions were met before running the ANOVA. The effect of treatment and the effect of isofemale line were tested for all traits except egg-to-adult viability, for which the experimental design only allowed to test the effect of treatment. When the analyses showed differences between treatments, Tukey's *post hoc* comparisons were run to specify which treatment was different from the others. The interaction terms between line and treatment of the fully crossed design were used to estimate the level of genetic variance for plasticity.

Results

Our main contrast of experimental treatments showed a significant effect of constant (C), predictably fluctuating (PF) and unpredictably fluctuating (UF) environments for all life-history and stress-resistance traits (Table 1, Fig. 2), suggesting the expression of ample plasticity in the base population in response to varying temperature regimes. The interaction between isofemale line and treatment, interpreted as genetic variance for plasticity, was significant for time to heat knock-down, desiccation and starvation resistance, body size and developmental time but not for productivity and chill coma recovery time (Table 1, Fig. S1).

In general, no simple overall pattern was identified, suggesting that each trait responded in a trait-specific

Table 1 Results for the different life-history and stress-resistance traits from full factorial model ANOVA and likelihood ratio chi-square (LR).

	Treatment		Line		Line × treatment	
	F	*P*	*F*	*P*	*F*	*P*
TDK	$38.4_{(2,530)}$	< 2.6E-16	$8.1_{(19,530)}$	< 2.6E-16	$2.5_{(38,530)}$	4.2E-06
PR	$3.6_{(2,482)}$	0.02	$3.2_{(21,482)}$	2.50E-05	$1.2_{(39,482)}$	0.21
CCRT	$19.5_{(2,509)}$	3.7E-05	$2.9_{(19,509)}$	6.9E-09	$1.1_{(38,509)}$	0.34
DES	$17.7_{(2,596)}$	3.3E-08	$3.5_{(24,596)}$	4.2E-08	$2.5_{(48,596)}$	1.8E-07
DT	$970_{(2,2529)}$	< 2.6E-16	$18.3_{(29,2529)}$	< 2.6E-16	$9.1_{(58,2529)}$	< 2.6E-16
ST	$11.5_{(2,700)}$	1.2E-05	$6.2_{(25,700)}$	< 2.6E-16	$1.8_{(50,700)}$	4.3E-04
BS	$78.8_{(2,624)}$	< 2.6E-16	$8.8_{(23,624)}$	< 2.6E-16	$1.4_{(46,624)}$	0.04
	LR	*P*	LR	*P*	LR	*P*
VIA	$54.2_{(2)}$	< 1.7E-14	$277.5_{(29)}$	< 2.6E-16	$240.4_{(57)}$	< 2.6E-16

TKD, time to heat knock-down; PR, productivity; CCRT, chill coma recovery time; DES, desiccation; DT, developmental time; ST, starvation; BS, body size; VIA, egg-to-adult viability.
Degrees of freedom are given within parentheses. The table shows the F-ratio (F) with associated probability (*P*) for all traits except VIA, where the likelihood ratio chi-square test (LR) with associated probability (*P*) is given.

manner (Fig. 2). The results of pairwise comparisons between regimes are reported in Table 2. Flies in the C regime had higher productivity compared with flies in PF and UF regimes, which showed similar values; the highest viability was recorded for flies reared in the UF regime compared with flies developed in the other two regimes. The fastest developmental time was observed in flies in the UF regime, followed by the flies in the C regime, and the longest developmental time was shown in flies developed in PF. The highest heat resistance was found in flies developed in PF and UF. Flies had a longer chill coma recovery time in UF compared with the C and PF regimes. Desiccation tolerance was highest in flies in the C regime, flies kept in PF showed an intermediate desiccation tolerance, and the lowest tolerance was observed in flies kept in the UF regime. Flies in C and PF had a similar starvation tolerance and higher compared with flies in UF. Flies in the C regime had the largest body size, whereas flies in PF showed the smallest body size with an intermediate body size shown by flies in UF (Table 2, Fig. 2).

Discussion

This study investigated how life-history and stress-resistance traits of a population of *D. simulans* were affected by a single generation of development under constant (C), predictably (PF) or unpredictably (UF) fluctuating temperature regimes. In agreement with other studies with various insect species (Colinet *et al.*, 2007; Hance *et al.*, 2007; Ragland & Kingsolver, 2008; Terblanche *et al.*, 2010), and with our expectation based on Jensen's inequality theory, we found differences in phenotypic performance between flies developed in different temperature regimes for all traits investigated. Our results show a general better performance of flies developed in a constant thermal environment (except for productivity), and interestingly, flies developed in the PF regime had a more similar performance to flies developed in the C rather than flies developed in the UF regime, with no differences in three of eight traits investigated. Thus, the expectation of differences mainly occurring between flies developed under either fluctuating regimes when compared to flies developed under a constant temperature regime was not confirmed.

The characteristics of temperature variation during acclimation are of great importance for the modulation of physiological and life-history traits via plastic responses (Niehaus *et al.*, 2012). For example, in *Drosophila*, constant and daily fluctuating temperatures with the same mean were shown to lead to different developmental time in survival and reproduction (Petavy *et al.*, 2001), as well as to introduce differential gene expression in *Aphidius colemani* (Colinet *et al.*, 2007). In *Ceratitis capitata*, low temperature variability (± 1 °C) resulted in an increased fitness and stress resistance, whereas higher levels of temperature variability (± 5 °C) led to trait-specific responses, with a not always increased performance (Terblanche *et al.*, 2010). Thus, Although Jensen's inequality predicts that the responses of organisms to fluctuating regimes are not symmetrical, with a stronger effect due to increased compared with decreased temperatures (Ruel & Ayres, 1999), the results of this and previous studies suggest that the exact effect of fluctuations is dependent on the experimental set-up and that it is trait specific.

Importantly, even if the amplitude of temperature variation was high in the fluctuating regimes (15 °C in the PF and randomly from 0 to 15 °C in the UF regime), the temperatures never reached stressful conditions for *D. simulans* (Fig. 1). This leads to the

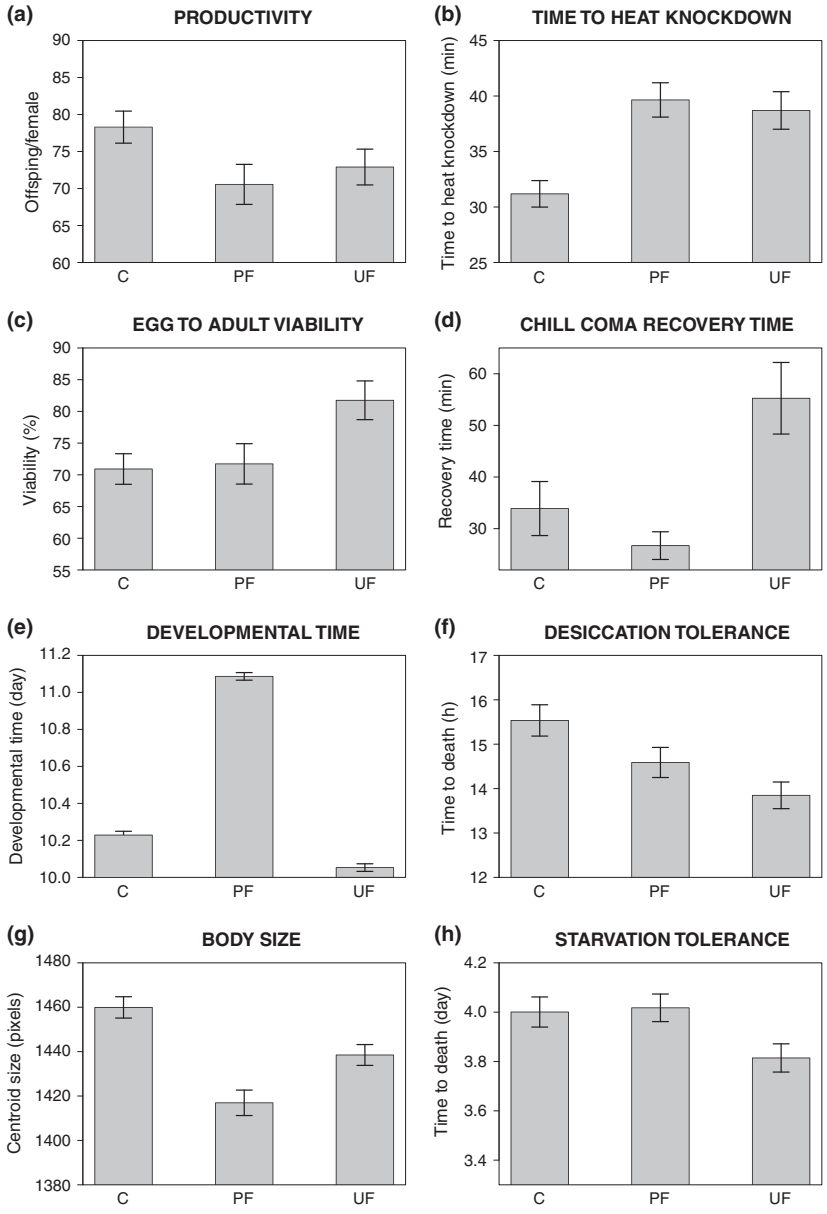

Fig. 2 Mean stress-resistance- and life-history-related traits ± standard error for each temperature regime given as the mean of around 20 assayed isofemale lines for each environment: constant temperature regime (C), predictably fluctuating temperature regime (PF) and unpredictably fluctuating temperature regime (UF). The first column refers to life-history-related traits (a = productivity; c = egg to adult viability; e = developmental time and g = body size), and the second column refers to stress-resistance traits (b = time to heat knockdown; d = chill coma recovery time; f = desiccation tolerance and h = starvation tolerance). The trait value for each isofemale line was based on the average of approximately ten individual females, except for developmental time and egg-to-adult viability, which due to the nature of these traits were based on the mean of three vials. The same letter close to the error bar indicates no significant difference.

conclusion that the induced effects of fluctuating temperature regimes were not the result of experiencing daily stressful temperatures (Deutsch *et al.*, 2008; Zeh *et al.*, 2014), but purely the effect of daily temperature variation. The UF temperatures were never exceeding the absolute high and low temperatures of PF and, on average, the amplitude of temperature variation was smaller in UF than PF. Thus, considering the limitations imposed to the model in order to mimic a natural environment, the level of unpredictability in UF could be considered to be low (Fig. 1).

We found that the UF regime affected mainly the stress-resistance rather than the life-history traits of flies. Regarding life-history traits, the only trait where UF flies showed a decreased performance was productivity, which was lower compared with flies in the C but similar to the flies in the PF regime. Instead, flies in the UF regime showed the lowest stress resistance in three of four stress-related traits.

The decreased stress tolerance of flies developed in UF leads to the conclusion that an unpredictable change of temperature has a stronger effect on flies' performance than the fluctuation of temperature itself, at least if the amplitude of variation does not lead to extreme temperatures (Deutsch *et al.*, 2008; Terblanche *et al.*, 2010; Zeh *et al.*, 2014). This low performance in

Table 2 Tukey test on the comparisons between temperature regimes: constant environment (C), predictably fluctuating environment (PF) and unpredictably fluctuating environment (UF) for each trait.

	C vs. $PF_{(1,378)}$		C vs. $UF_{(1,354)}$		PF vs. $UF_{(1,352)}$	
Traits	*F*	*P*	*F*	*P*	*F*	*P*
PR	4.8	0.02	3.2	0.04	0.2	0.76
DT	75.2	1.0E-12	5.3	0.01	152.3	1.0E-18
TKD	19.3	2.7E-05	13.4	2.3E-04	0.2	0.75
CCRT	0.1	0.88	16.2	7.0E-05	14.2	1.6E-04
DES	3.7	0.03	13.6	2.20E-04	3.1	0.04
ST	0.1	0.83	3.8	0.03	5.2	0.01
BS	33.2	1.60E-07	10.9	7.80E-04	7.8	3.60E-03
VIA	$0.4_{(1,57)}$	0.84	$7.8_{(1,58)}$	7.20E-03	$5.1_{(1,57)}$	0.03

TKD, time to heat knock-down; PR, productivity; CCRT, chill coma recovery time; DES, desiccation; DT, developmental time; ST, starvation; BS, body size; VIA, egg-to-adult viability; *F*, F-ratio; *P*, probability associated with F-ratio.
Degrees of freedom are given within parentheses.

the UF regime seems to be the result of the inability of flies to anticipate the degree of temperature changes in an environment that does not give any clue about how it is going to change exactly, even though the environment can be anticipated to change according to the daily rhythm dictated by the model (some increase during day and some decrease during night). A continuous monitoring and sampling of the environmental conditions leading to the required fine tuning of the physiology occurring at benign temperatures as used here and as experienced normally in the field (Overgaard & Sørensen, 2008) thus seems to be more costly than the actual physiological adjustments themselves and might even lead to the occurrence of a maladaptive plasticity response in an environment that changes with low predictability (Hallsson & Bjorklund, 2012). The experimental population was collected in an environment characterized by high predictability and large amplitude of temperature fluctuations (Bahrndorff *et al.*, 2009) resembling to some degree our fluctuating temperature regimes. However, we found that flies selected in a natural environment characterized by high predictability of temperature fluctuations showed a general better performance in life-history and stress-resistance traits when developed in a constant environment compared with flies developed in both fluctuating temperatures environments. We had no clear expectation about how a natural population might be affected by developing in a constant temperature regime. A constant temperature potentially is a stressful condition as the natural relation between the photoperiod and the thermoperiod, where an average increased temperature is expected with increasing light intensity, is disjoined. For example, it was shown to have a negative effect on cold resistance in aphids (Colinet *et al.*, 2007). However, in flies that developed in the C regime, we found a generally better performance compared with flies developed in PF, suggesting that any deleterious impact of stress or costs of the C regime were minor. Most likely, no changes of temperature during the day allowed flies to avoid unnecessary costs related with a plastic response machinery (DeWitt, 1998) and they were free to use the energy saved to cope better with a stressful situation. A bigger body size and the greatest productivity in flies in C compared with flies from PF and UF regimes appear to confirm this.

A significant 'line by treatment' interaction was found in six of the investigated traits (Fig. S1) (heat, desiccation and starvation tolerance, body size, viability and developmental time), indicating genetic variance for plasticity in these traits. In other words, different isofemale lines responded differently to different temperature regimes. In contrast, no 'line by treatment' interaction for productivity and chill coma recovery time was found, which suggests genetic canalization for plasticity under different environmental conditions for these traits (Wagner *et al.*, 1997; Flatt, 2005). Several studies have shown that productivity is not affected by environmental transitions (Stearns *et al.*, 1995; Wagner *et al.*, 1997) due to an environmental canalization of productivity. Our results show significant plasticity for productivity, but that this plasticity was canalized as different lines showed a similar adjustment of productivity in the different temperature regimes. We did not find genetic variance for plasticity in chill coma recovery time. The recovery time after a cold shock is related with the ability of organisms to regain ion and water homeostasis (Hosler *et al.*, 2000; MacMillan & Sinclair, 2011). The mechanisms determining the plastic response in recovery of homeostasis might be genetically constrained between lines, resulting in all isofemale lines having chill coma recovery time affected in a similar way by different temperature regimes.

With regard to heat tolerance, we hypothesized and we found an increased thermal stress resistance in flies from the PF and UF compared with the C regime. Temperatures experienced during development are expected to influence thermal stress resistance of

organisms, and flies exposed to warmer temperatures have been shown to have higher heat resistance (beneficial acclimation) (Huey *et al.*, 1991; Loeschcke & Hoffmann, 2007). However, *D. simulans* do not show a geographic cline for time to knock-down along the Australian east coast (Arthur *et al.*, 2008), contrary to other species of *Drosophila* like *D. melanogaster* (Hoffmann & Weeks, 2007). The absence of a geographic cline for heat resistance might indicate that the tolerance of *D. simulans* to heat stress originates from phenotypic plasticity or genetic variation for plasticity rather than genetic differentiation for this trait (van Heerwaarden & Sgro, 2011). The increased heat tolerance found in flies developed in both fluctuating regimes compared with flies developed in a constant temperature regime was not in agreement with what was found by Overgaard *et al.* (2011) where no differences were found between flies kept in a constant fluctuating temperature regarding heat tolerance. The increased heat tolerance in both fluctuating temperature regimes could be explained by considering the daily fluctuation of temperature to be sufficient to activate such an adaptive plastic (hardening) response. The population collected for the present study usually experienced great temperature variation in nature in its past, and due to the predictability of temperature fluctuations, flies were expected to be able to anticipate temperature changes with behavioural or physiological responses. The underlying mechanism of this beneficial effect of fluctuating temperatures needs further investigation.

In conclusion, the main differences in life-history and stress-resistance traits in response to different thermal regimes were found in the unpredictable regime mainly regarding stress resistance when compared to the other two, and not, as expected, in the constant regime when compared to the two fluctuating temperature regimes. The general poorer performance of UP flies indicates that unpredictability of temperature changes is more stressful than the amplitude of daily temperature variation in a natural population of *D. simulans*. The results also indicate the dual nature of the adaptability of a plastic genotype in predictable and unpredictable regimes: on one hand, phenotypic plasticity is likely to be a good adaptive response to predictable fluctuations of temperature, whereas on the other hand, adjusting the phenotype based on a plastic mechanism comes at a price in many traits when the environment changes with low predictability, even if this occurs within the permissive thermal limits. In addition, the selective past of a population also plays an important role for life-history and stress-resistance traits, even though in this case, originating from a natural environment where the temperatures change with great levels of predictably did not yield any apparent benefits of coping with a fluctuating environment. The results of this study have important ecological implications, considering that a decreased predictability has been shown to be one of the clearest consequences of the globally changing climate.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Mean stress resistance and life history related traits ± standard error for each isofemale line in each temperature regime given as the mean of around 10 females for each line: constant temperature regime (C), predictably fluctuating temperature regime (PF) and unpredictably fluctuating temperature regime (UF).

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