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Are Signals of Local Environmental Adaptation Diluted by Laboratory Culture?

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ABSTRACT

Insects have the ability to readily adapt to changes in environmental conditions, however the strength of local environmental adaptation signals under divergent conditions and the occurrence of trait inertia after relaxation of selection, remains poorly understood, especially for traits of climate stress resistance (CSR) and their phenotypic plasticity. The strength of environmental adaptation signals depend on several selection pressures present in the local environment, while trait inertia often occurs when there is a weakening or removal of a source of selection. Here, using *Drosophila melanogaster*, we asked whether signals of adaptation in CSR traits (critical thermal limits, heat and chill survival and, desiccation and starvation resistance) persist after exposure to laboratory culture for different durations (two vs. ten generations) across four climatically distinct populations. We show that culture duration has large effects on CSR traits and can both amplify or dilute signals of local adaptation. Effects were however dependent upon interactions between the source population, acclimation (adult acclimation at either 18 °C, 23 °C or 28 °C) conditions and the sex of the flies. Trait plasticity is markedly affected by the interaction between the source population, the specific acclimation conditions employed, and the duration in the laboratory. Therefore, a complex matrix of dynamic CSR trait responses is shown in space and time. Given these strong interaction effects, 'snapshot' estimates of environmental adaptation can result in misleading conclusions about the fitness consequences of climate variability.

Introduction

The amount and nature of genetic variation can strongly influence traits and their adaptive capacity (Hoffmann and Willi, 2008; Edelaar and Bolnick, 2019), while the rate of evolution depends on the strength of selection acting upon the trait and the heritability of the trait (Hoffmann and Sgrò, 2011). Additionally, traits may also evolve due to genetic drift (random selection), but in such instances the direction usually varies at random (Willi et al., 2006; Santos et al., 2012) and, in turn, can influence multiple potential processes affecting individual or population level fitness (Edelaar and Bolnick, 2019).

There is a rich history of comparative studies of Drosophilidae (mainly in the genus *Drosophila*) as a model group to understand trait-environment relationships, geographic range structure, or evolutionary response to climate variability (e.g. Hoffmann et al., 2002; Kellerman et al., 2009; van Heerwaarden et al., 2009; Bush et al., 2016, 2016; Kellermann et al., 2018), and the potential cellular or genetic mechanisms underpinning environmental stress resistance (e.g. Sørensen and Loeschcke, 2001; Ransberry et al., 2011; Gerken et al., 2015). Although such studies using Drosophilidae as model taxa are prevalent, there are several potential shortcomings that remain contentious relating to the population genetic composition of the species under artificial holding conditions. Many comparative studies use Stock Center lines instead of, for example, newly established lines of wild flies. Alternatively, studies may use flies of mixed population, age or origin (e.g. some newly-established 'lines' are contrasted against others that have been in culture for many generations, e.g. Kellermann et al., 2012a, Kellermann et al., 2012b). This is perhaps less of an issue in studies that establish new lines from field collections and carefully control for potential genetic bottlenecks (e.g. Hoffmann et al., 2001a; Sgrò et al., 2010; Kellermann et al., 2017; but see discussion in Santos et al., 2012). Using species obtained from laboratory cultures for trait assessments and subsequent comparisons, instead of recently field-collected species or those reared for a standard amount of time in culture, has the potential drawback that the laboratory colony may not represent the field population in terms of trait diversity, phenotypic plasticity or genetic responses. Furthermore, it is often difficult to control for population bottlenecks, inbreeding effects, stochastic and/or founder population size effects and the potential interactions thereof (Santos et al., 2012; Ærsgaard et al., 2015; MacLean et al., 2018).

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Organisms typically respond to rearing conditions through laboratory acclimation (within generations) or adaptation (across generations) (reviewed in e.g. Hoffmann and Ross, 2018; MacLean et al., 2018). Most species examined to date show one or several traits that respond rapidly to a change in holding conditions, either within or between generations. Perhaps the most widely expected response is that laboratory adaptation results in decreased resistance to environmental stressors, indicating relaxation of stressors (e.g. Hoffmann et al., 2001b; Chown and Terblanche, 2007). Hoffmann and Ross (2018) found that upon introduction into the laboratory traits tended to change in the direction of increased fitness for Coleoptera, Diptera and Hymenoptera, however changes in Lepidoptera were often in the opposite direction. Studies investigating these effects show mixed results depending, at least partly, on the type of trait examined (e.g. temperature resistance vs. starvation resistance; MacLean et al., 2018). Regardless of any expected direction of effects, species kept in the laboratory for an extensive period are not likely subjected to the same stressors that maintain traits under field conditions through natural selection.

Adaptive capacity can be defined as the ability of organisms to respond to changes in environmental conditions, and any response depends on an oragnism's genetic and plastic makeup (Kellermann et al., 2009; Kellermann and Van Heerwaarden, 2019). In the broadest sense, phenotypic flexibility or plasticity indicates how much organisms can change between two environments. Plastic responses are thought to be important to climate change responses (Chevin et al., 2010) especially in variable environments (Rohr et al., 2018) although empirical evidence is mixed (e.g. Overgaard et al., 2011; Kellermann et al., 2020). How plasticity of stress resistance traits might vary under constant conditions remains unclear, despite that this could yield important insights into the nature of (mal)adaptive phenotypic plasticity, costs of plasticity, and its mechanistic underpinnings (Sgrò et al., 2016; Scheiner, 2018). Diverse *Drosophila* species show divergent responses to selection depending on the underlying adaptive capacity of the trait in question (Kellermann et al., 2009; Van Heerwaarden and Sgrò, 2014). It is therefore unlikely that the traits used in comparisons using lines of diverse origin or holding period have all responded equally to rearing under common conditions. There are two broad competing hypotheses for how phenotypic plasticity (stress resistance response after exposure to a thermal acclimation) is related to basal (innate) stress resistance (stress resistance response after exposure to a standard, benign, constant temperature) and therefore provide a general framework for theoretical expectations of how CSR traits may respond to laboratory rearing: first, that plasticity is traded-off against increased basal stress resistance ('trade-off' hypothesis), and second, that plasticity evolves independently of basal resistance ('constrained plasticity' hypothesis) (e.g. Stillman, 2003; Kellermann et al., 2018). To improve the robustness of data collected for various applications, field-collected species kept under laboratory conditions for a short period of time are argued to better represent the wild population's trait values (Najarro et al., 2015) but if this is indeed the case is a subject of renewed interest (Hoffmann and Ross, 2018; MacLean et al., 2018).

Here, we compared the same sets of CSR traits scored recently after establishment (i.e., at the 2nd generation (F_2)) or at several generations later (10th generation (F_{10})) in four *D. melanogaster* populations from climatically-distinct ecoregions, in order to estimate whether there is significant variation in CSR traits and their phenotypic plasticity. The second generation was chosen to represent the wild population but allowing sufficient time to increase the colony size and eliminate potential recent carry-over effects (e.g., poor parental nutrition) that might affect stress resistance, and the 10th generation to represent a standard laboratory period after which we expected the majority of any laboratory culture effects would have manifested and stabilized (Bertoli et al., 2010; Sambucetti et al., 2010; Santos et al., 2012). On the one hand, we expected a decrease in both basal resistance and their plastic responses due to laboratory culture, possibly driven by small founding population sizes or constant laboratory conditions, as previously shown for some stress resistance traits (Sgrò and Partridge, 2000; Hoffmann et al., 2001b). Moreover, in this case, we expected a decline in the plasticity of traits between the F_2 and F_{10} generation if plasticity is costly to maintain or coupled mechanistically to basal stress resistance. Alternatively, if plasticity and basal stress resistance are traded-off directly at the population level, basal stress resistance may decline in culture while plasticity could remain stable or even increase (or vice versa). If time spent in laboratory culture changes the basal stress resistance of the trait in question, this will mean that the species will no longer exhibit the resistance or adaptive capacity it showed in its natural environment and thus CSR estimations based on species kept in the laboratory for several generations might be of limited value depending on the specific research questions being asked. Additionally, should the basal resistance and adaptive capacity of *D. melanogaster* differ significantly depending on its source population, CSR trait estimates based on individuals from mixed origin might not represent the species as a whole. This will have implications on the way in which CSR traits are measured in future studies, particularly for estimating the adaptive capacity of a species in the face of climate change.

Materials and methods

Experimental lines and acclimation treatments

Hundreds of *Drosophila melanogaster* were collected from four diverse, climatically-distinct locations ((Citrusdal (32° 44.159′ S, 19° 02.436′ E), Durban (29°85.868′ S, 31°02.184′ E), Polokwane (22°97.613′ S, 30°44.647′ E) and Stellenbosch (33°93.210′ S, 18°86.015′ E)) across South Africa by placing traps consisting of buckets filled with mixed fruits (oranges, bananas, apples and lemons) in shaded locations in natural, semi-natural or disturbed habitats and home gardens. Mean, minimum and maximum temperature and relative humidity data for these locations are listed in Appendix Table S1 (Schulze, 2006). However, adaptation in thermotolerance could be found along microclimatic scales (see Duffy et al., 2015) and thus, further information on the microclimates of our study sites would have been useful but was outside the scope of our study. From each site, wild females were caught and placed individually in 325 mL plastic bottles containing a modified Bloomington standard cornmeal medium which consists of cornmeal, yeast, soy, dextrose and agar as well as nipagin and a phosphoric/ propionic acid mixture to counter microbial contamination (https://bdsc.indiana.edu/information/recipes/bloomfood.html). Flies were then placed at 23 °C (MRC LE-509, Holon, Israel) and 40%–60% relative humidity on a 12-hour day/night cycle.

Flies were randomly assigned to two different treatment groups where half of them were allowed to breed until the second generation (F_2) and half to breed until the tenth generation (F_{10}). Flies were tipped into new food at regular intervals to avoid overcrowding the bottles and to keep the number of flies per bottle constant at *c.* <50 flies per bottle. As soon as flies emerged at each generation, they were mixed between bottles. For both groups (F_2 and F_{10}) the flies were haphazardly split into three acclimation treatments within 24 h after eclosion. They were acclimated for 48 h at 18 °C, 23 °C and 28 °C respectively in climate chambers (MRC LE-509, Holon, Israel). These temperatures were chosen as recent studies have shown that relatively benign acclimation temperatures have an effect on CSR traits (Kellermann et al., 2017; MacLean et al., 2018). It was assumed that the effects induced in the 48 hours' acclimation period will last several days (for similar rationale see e.g., Loeschcke et al., 1997) since we were not interested in documenting any highly transient trait variation. However, Loeschcke et al. (1997) used much higher temperatures, but we wanted to ascertain whether more benign temperature treatments would still lead to significant differences. After the 48 h acclimation period, flies were placed back at 23 °C until they were between 5 and 7 days old (about 24 to 48 h), which is considered standard practice (e.g. Sgrò et al., 2010; Kellermann et al.,

2012a), after which CSR traits were scored. Adult flies used for CSR trait estimation were randomly chosen from the different bottles.

Climate stress resistance (CSR) traits

Temperature traits

Critical thermal maximum (CT_{MAX}) and Critical thermal minimum (CT_{MIN}) were determined by taking 15 male and 15 female, 5–7 day old flies from each acclimation treatment and placing them in 0.6 mL microcentrifuge tubes. The microcentrifuge tubes were placed on a foam "boat" inside a circulating programmable refrigeration bath (Huber CC-410wl, Huber, Offenburg, Germany) filled with water for CT_{MAX} and with ethanol for CT_{MIN} (to prevent the bath liquid freezing at low temperatures). Fine-gage (36-SWG) Type T thermocouples connected to a handheld two channel digital thermometer (Fluke 54 series II, Fluke Cooperation, China) were placed inside one of the tubes and on the "boat" surface. For both CT_{MAX} and CT_{MIN} flies were equilibrated at 23 °C for 15 min before ramping started. The water bath was then ramped up (CT_{MAX}) or down (CT_{MIN}) at 0.1 °C/min. The flies were checked intermittently for coordinated movement. CT_{MAX} and CT_{MIN} were scored as the temperature at which the fly lost all mobility (after all spasms have ceased and death ensues) (Lutterschmidt and Hutchison, 1997) and flies were gently poked with a piece of fishing line to ensure that CT_{MAX}/CT_{MIN} was reached. The ramping rate of 0.1 °C/min allows comparisons to be more readily made with some of the existing literature of lethal temperatures of Stock Center-derived *Drosophila* species (Overgaard et al., 2011; Kellermann et al., 2012b).

Heat and chill survival were determined by placing 15 male and 15 female flies (5–7 day old) from each acclimation treatment individually into microcentrifuge (0.6 mL) tubes and placing them on the same foam boat setup described for the CT_{MAX}/CT_{MIN} measurements. Flies were placed at 38 °C for 1 hour for the heat survival treatment and at 0 °C for 2 h for the cold survival treatment (following Bechsgaard et al., 2013). After the treatments, flies were placed at 23 °C for 24 h after which survival was scored.

Survival traits

For desiccation resistance, 5–7 day old flies (15 males and 15 females) from each acclimation treatment were placed individually into empty glass vials (12 mL) sealed with gauze and then transferred to an airtight desiccator 80–90% filled with silica gel (Merck, South Africa). The desiccator was placed in a dark incubator to suppress activity, at 23 °C and <10% relative humidity (modified from Kellermann et al., 2012a). A hygrochron iButton (DS1923 iButton, Maxim, Sunnyvale CA, USA) was placed inside the desiccator to confirm the temperature and relative humidity during the experiment. Survival was scored four to five times a day until the first fly died and was then scored hourly until all flies had died.

To determine starvation resistance, 15 male and 15 female 5–7 day old flies per acclimation were placed individually into glass vials containing 5 mL 0.5% agar solution (Matzkin et al., 2009). The vials were sealed with moist cotton wool, to maintain high levels of humidity (typically constant >95% humidity for several days) and placed at 23 °C. Mortality was scored at the same time each day until all flies had died.

As a control group, 15 male and 15 female (5–7 day old) flies were placed in glass vials containing standard cornmeal medium covered with gauze and placed in an incubator at 23 °C. Survival was scored every second day until the first death observed and then daily thereafter until all flies had died.

Statistical analyses

The effect of the acclimation regimes, source population and the number of generations spent in the laboratory on each of the separate traits of CSR of *D. melanogaster* were determined in R software v. 3.5.1 (R Development Core Team, 2018). Significance levels were set at $p<0.05$.

For the thermal limits data, a generalized linear model (GLM) with a gaussian distribution and an identity link function were run using the '*MASS*' (Venables and Ripley, 2002) package. CT_{MAX} or CT_{MIN} was used as dependent variables and the independent variables were population (Stellenbosch, Citrusdal, Durban or Polokwane), acclimation (18 °C, 23 °C, 28 °C), sex (male or female), line and the interactions thereof. An analysis of deviance table was then computed from the outputs of the GLM using a type 3 Anova function in the '*car*' package (Weisberg, 2019) to examine the main effects and interactions between the different variables.

For heat and cold survival, a GLM with a binomial distribution and a logit link function was run using the '*MASS*' (Venables and Ripley, 2002) package to assess the main effects and interactions of source population, generation, acclimation and sex on the percentage survival 24 h after exposure to a potentially lethal temperature. An analysis of deviance table was also computed for the heat and chill survival data using the anova.glm function in "*MASS*". All GLM models were checked for overdispersal by inspecting the residual deviance relative to the degrees of freedom (Crawley, 2012).

For the desiccation and starvation survival experiments, Kaplan-Meier survival curves were derived using the '*survival*' package in R (Therneau and Grambsch, 2000), to illustrate how proportion survival changes over time for the different acclimation regimes, generations, populations and line. The Cox-proportional hazards model, also in the '*survival*' package, was used to determine the statistical significance of survival time on the predictor variables of desiccation and starvation, determining the main effects and interactions of founder populations, treatments, acclimation and sex on survival time.

As multiple comparisons were done, p values were adjusted with the Benjamini-Hochberg (aka "*fdr*") method using the p.adjust function in the R "*base*" package. Additionally, we wanted to look at the interactions between the different independent variables and their effect on the dependent variables for all the traits. This was done by drawing interaction plots using the plot_model function in the "*sjPlot*" (Lüdecke, 2019a), "*sjmisc*" (Lüdecke, 2018) and "*ggplot2*" (Wickham, 2016) packages in R.

Results

Our results indicated significant interactions with a combination of founder population, time spent in culture, specific acclimation conditions and sex influencing the CSR traits. There was a strong interaction effect of population x generation x acclimation on the CSR traits of CT_{MAX} (χ^2_3=47.19, $p<0.0001$), CT_{MIN} (χ^2_3=15.17, $p<0.006$), cold survival (χ^2_3=36.23, $p<0.0001$) and desiccation resistance (χ^2_3=46.64, $p<0.0001$). Additionally, the interaction between founder population and duration in culture significantly influenced all CSR traits tested (Table 1).

Overall, there were large effects of culture duration on CSR traits, however the direction of this effect was trait, population, acclimation and sex dependent. For both basal and plastic resistance, CT_{MAX} tended to be higher (i.e., increased resistance) with longer time spent in culture (F_{10}) compared to the more recently collected individuals (F_2), whilst CT_{MIN} tended to decrease with time spent in culture (i.e., increased resistance) in all populations except for Durban (Fig. 1). Heat survival remained relatively stable with time in culture, whilst chill survival either increased or decreased with time spent in culture depending on source population, acclimation temperature and sex (Fig. 2). Desiccation resistance decreased with time spent in culture for all populations except Polokwane where there was a marginally significant increase. Lastly, starvation resistance decreased with duration in culture in the Citrusdal and Stellenbosch populations, but increased in the Polokwane and Durban populations (Fig. 3).

Table 1
Summary of the minimum adequate models for critical thermal maximum (CT_{MAX}) and critical thermal minimum (CT_{MIN}), heat survival, chill survival, desiccation resistance and starvation resistance for four populations of *D. melanogaster* (Citrusdal, Durban, Polokwane and Stellenbosch), indicating the chi-square value (χ^2), degrees of freedom (*d.f.*) and the p-value. Significance set to $p<0.05$, all values shown in bold are significant.

Stress Assay	Effect	χ^2	*d.f.*	p-value
CT_{MAX}	**Population**	**18.73**	**3**	**<0.002**
	Generation	**9.54**	**1**	**<0.005**
	Acclimation	**16.3**	**2**	**<0.0004**
	Sex	**11.34**	**1**	**<0.002**
	Population x Generation	**35.32**	**3**	**<0.0001**
	Population x Acclimation	**24.68**	**6**	**<0.0002**
	Generation x Acclimation	**9.87**	**2**	**<0.004**
	Population x Sex	**20.38**	**3**	**<0.0006**
	Generation x Sex	**6.61**	**1**	**<0.02**
	Acclimation x Sex	**15.01**	**1**	**<0.0005**
	Population x Generation x Acclimation	**47.19**	**6**	**<0.0001**
	Population x Generation x Sex	**10.60**	**3**	**<0.03**
	Generation x Acclimation x Sex	**15.81**	**1**	**<0.0004**
	Population x Generation x Acclimation x Sex	**17.50**	**3**	**<0.002**
CT_{MIN}	**Population**	**18.24**	**3**	**<0.003**
	Generation	**6.60**	**1**	**<0.02**
	Acclimation	0.44	2	0.54
	Sex	2.88	1	0.12
	Population x Generation	**13.71**	**3**	**<0.009**
	Population x Acclimation	**16.98**	**6**	**<0.004**
	Generation x Acclimation	**6.83**	**2**	**<0.02**
	Population x Sex	**9.90**	**3**	**<0.04**
	Generation x Sex	**6.65**	**1**	**<0.02**
	Acclimation x Sex	**1.32**	**1**	**0.28**
	Population x Generation x Acclimation	**15.17**	**6**	**<0.006**
	Population x Generation x Sex	**12.51**	**3**	**<0.02**
	Generation x Acclimation x Sex	**6.73**	**1**	**<0.02**
	Population x Generation x Acclimation x Sex	**14.90**	**3**	**<0.006**
Heat survival	**Population**	**8.17**	**3**	**<0.05**
	Generation	1.20	1	0.27
	Acclimation	1.02	2	0.31
	Sex	**26.78**	**1**	**<0.0001**
	Population x Generation	**11.55**	**3**	**<0.01**
	Population x Acclimation	**52.28**	**6**	**<0.0001**
	Generation x Acclimation	**9.76**	**2**	**<0.002**
	Population x Sex	**9.19**	**3**	**<0.03**
	Population x Generation x Acclimation	**16.19**	**6**	**<0.002**
	Population x Generation x Sex	**18.01**	**5**	**<0.0005**
	Population x Acclimation x Sex	**3.23**	**6**	**0.35**
	Population x Generation x Acclimation x Sex	**12.18**	**7**	**<0.007**
Cold survival	**Population**	**20.85**	**3**	**<0.0003**
	Generation	**10.61**	**1**	**<0.003**
	Acclimation	2.52	2	0.16
	Sex	**7.57**	**1**	**<0.006**
	Population x Generation	**50.03**	**3**	**<0.0001**
	Population x Acclimation	4.85	6	0.18
	Generation x Acclimation	**15.85**	**2**	**<0.0001**
	Population x Sex	**24.31**	**3**	**<0.0001**
	Population x Generation x Acclimation	**35.35**	**6**	**<0.0001**
	Population x Generation x Sex	**14.18**	**5**	**<0.003**
	Population x Acclimation x Sex	**46.30**	**6**	**<0.0001**
	Population x Generation x Acclimation x Sex	**15.87**	**7**	**<0.002**
Desiccation resistance	**Population**	**14.14**	**3**	**<0.008**
	Generation	**21.52**	**1**	**<0.0001**
	Acclimation	4.00	2	0.70
	Sex	1.16	1	0.32
	Population x Generation	**37.62**	**3**	**<0.0001**
	Population x Acclimation	**12.99**	**6**	**<0.02**
	Generation x Acclimation	**26.58**	**2**	**<0.0001**
	Generation x Sex	**6.53**	**2**	**<0.03**
	Population x Generation x Acclimation	**46.64**	**6**	**<0.0001**
	Population x Generation x Sex	**18.02**	**3**	**<0.002**
	Generation x Acclimation x Sex	**7.73**	**1**	**<0.02**
	Population x Generation x Acclimation x Sex	**24.14**	**3**	**<0.0001**
Starvation resistance	**Population**	**36.08**	**3**	**<0.0001**
	Generation	**12.10**	**1**	**<0.002**
	Acclimation	**18.67**	**2**	**<0.0002**
	Sex	0	1	0.99
	Population x Generation	**15.36**	**3**	**<0.0005**
	Population x Acclimation	**20.47**	**6**	**<0.0007**
	Generation x Acclimation	**7.56**	**2**	**<0.02**

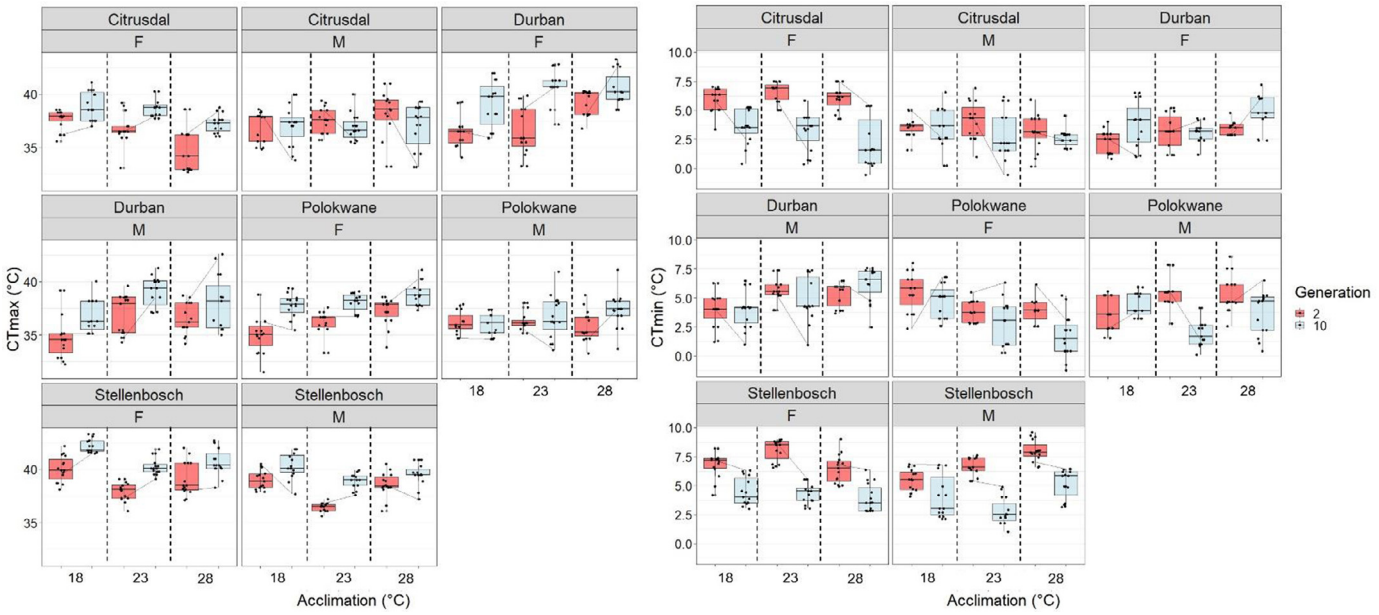

Fig. 1. The effect of source population (Citrusdal, Durban, Polokwane, or Stellenbosch), time spent in culture (F2 vs F10 generation), developmental acclimation (18 °C, 23 °C or 28 °C) and sex (male or female) on CT_{MAX} (°C) (left) and CT_{MIN} (°C) (right) estimates in *Drosophila melanogaster*. Boxplot indicating median CT_{MAX} (°C) and CT_{MIN} (°C), upper and lower quantiles and maximum and minimum values (whiskers), with raw data overlaid (black dots).

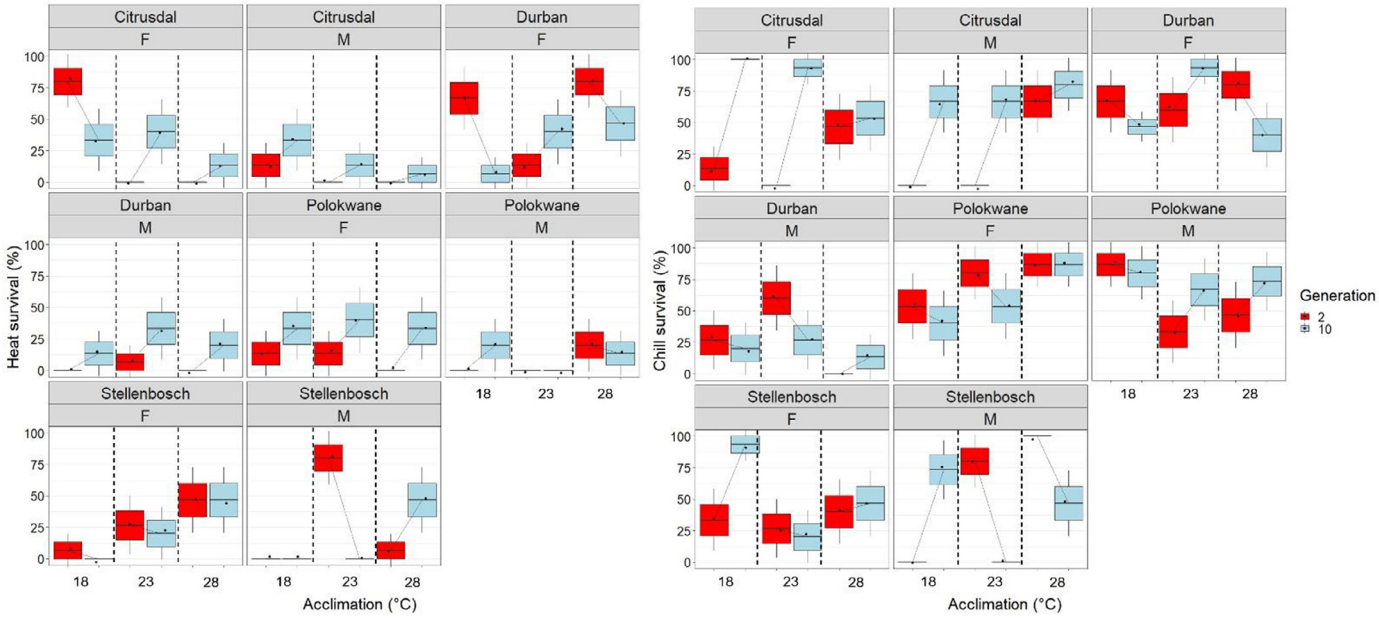

Fig. 2. The effect of source population (Citrusdal, Durban, Polokwane, or Stellenbosch), time spent in culture (F2 vs F10 generation), developmental acclimation (18 °C, 23 °C or 28 °C) and sex (male or female) on heat (left) and chill survival (right) (%) estimates in *Drosophila melanogaster*. Box and whiskers indicate mean, standard error and mean ± 1.96 SE.

There was also marked variation between the *D. melanogaster* populations for all the CSR traits measured: CT_{MAX} (χ^2_3=18.73, p<0.002), CT_{MIN} (χ^2_3=18.24, p<0.003), heat (χ^2_3=8.17, p<0.05) and chill survival (χ^2_3=20.85, p<0.0003), and the survival traits of desiccation (χ^2_3=14.14, p<0.008) and starvation resistance (χ^2_3=36.08, p<0.0001) (Table 1; Figs. 1, 2 and 3).

Lastly, acclimation treatments had strong effects on CT_{MAX} (χ^2_1=16.30, p<0.002) and starvation resistance (χ^2_1=18.67, p<0.0002) (Table 1). Both CT_{MAX} and starvation resistance responded differently to thermal acclimation across the four populations (Fig. 1 and 3). Additionally, sex differences in CSR trait values were found for CT_{MAX} (χ^2_1=11.34, p<0.002), heat survival (χ^2_1=26.78, p<0.0001) and cold survival (χ^2_1=7.57, p<0.006) (Table 1).

Discussion

Inertia in traits after relaxation of selection (e.g., upon introduction from a stressful to a more benign or optimal environment), or the strength of any local environmental adaptation signal under divergent conditions, remains poorly understood, especially for traits of climate stress resistance (CSR) and their phenotypic plasticity. Laboratory colonies of model taxa, such as *Drosophila*, are frequently used to answer

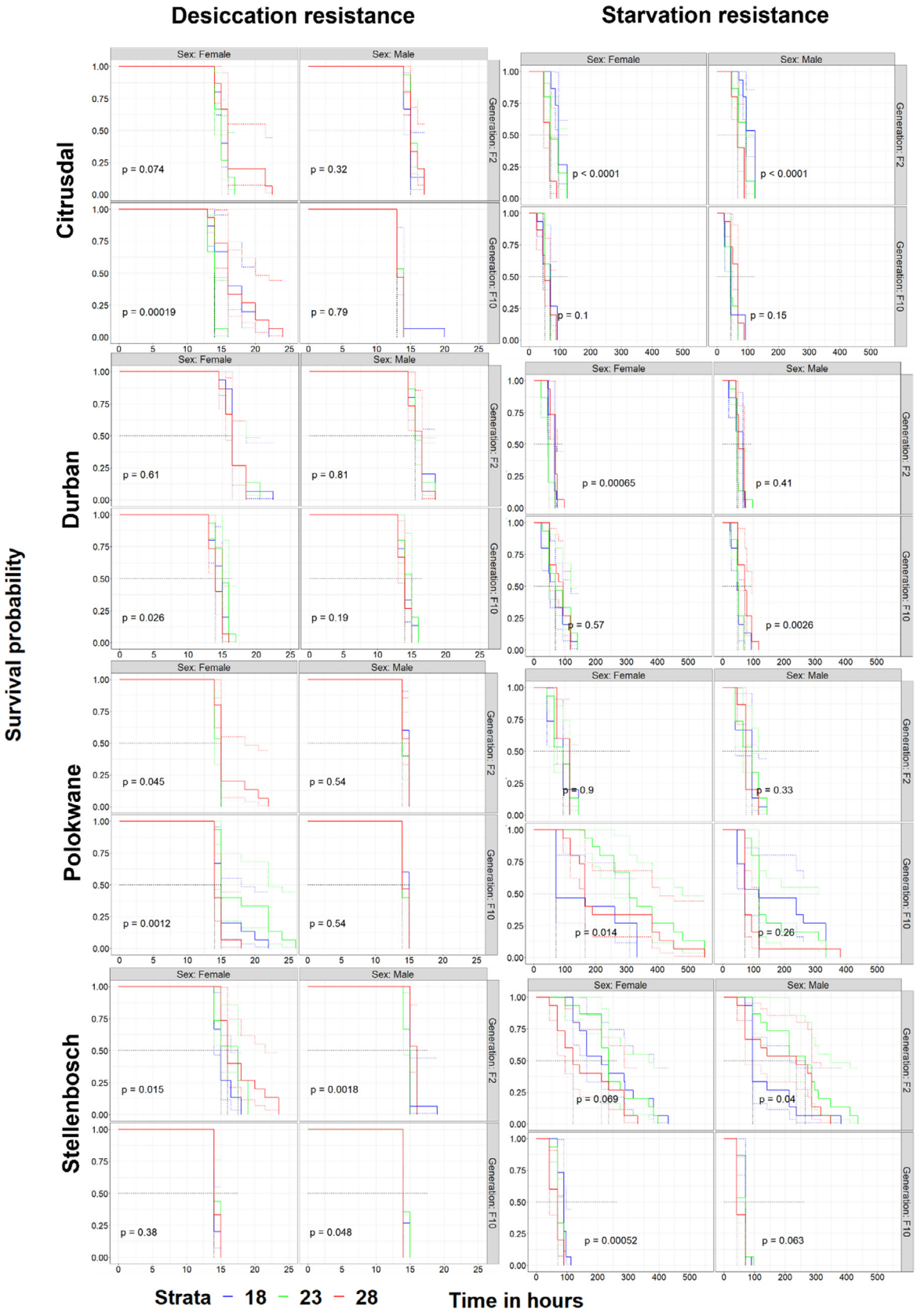

Fig. 3. The effect of source population (Citrusdal, Durban, Polokwane, or Stellenbosch) (represented by the rows), time spent in culture (F2 vs F10 generation), developmental acclimation (18 °C (blue), 23 °C (green) or 28 °C (red)) and sex (male or female) on desiccation (left column) and starvation (right column) resistance (time in hours) estimates in *Drosophila melanogaster*. Differences between acclimation treatments are indicated on each plot.

questions surrounding trait-environment relationships and the fitness consequences thereof, as well as evolutionary mechanisms governing geographic range limits, (e.g. Hoffmann et al., 2002; Bush et al., 2016; van Heerwaarden et al., 2016; Kellermann et al., 2018). Here, we asked whether the choice of founder population significantly influences CSR traits, and perhaps more importantly, the phenotypic plasticity of CSR traits, shortly after colony establishment (at F_2) or after ten generations (F_{10}) of standard culture conditions in four *D. melanogaster* populations from distant (>200kms), climatically distinct ecoregions in South Africa.

Our results show that CSR traits are influenced by a combination of source population, time spent in culture and specific acclimation temperature. This has broad implications for studies that use laboratory cultures to take a single 'snapshot' of stress resistance to infer patterns of local environmental adaptation or vulnerability to climate, in addition to a range of other critiques that can be raised when estimating species' vulnerability (Terblanche and Hoffmann, 2020; Clusella-Trullas et al., 2021). Many studies either empirically determine or compile estimates of CSR traits for Drosophilidae species derived from cultures of mixed laboratory pedigree to draw inferences about various evolutionary or ecological processes (e.g. Matzkin et al., 2009; Kellerman et al., 2012a;Kellermann et al., 2012b; Overgaard et al., 2014). Such trait comparisons are increasingly being used to model or infer biogeographic responses to climate change. Based on the estimates of CSR traits and the plasticity determined here, we argue that these might be problematic for drawing inferences about environmental niches, thermal specialization or trait-environment associations, if time in culture and any laboratory adaptation or genetic drift is unaccounted for, especially if attempting to decipher plasticity as a compensatory process (e.g. Rohr et al., 2018; Kellermann et al., 2020; Weaving et al., 2022).

As expected, each population varied in its CSR traits in a manner that could be interpreted as showing local environmental adaptation (Table 1), an interpretation that is in keeping with much of the foregoing literature (e.g., Hoffmann et al., 2002; Sgrò et al., 2010; Kellerman et al., 2012b; Erić et al., 2022). Flies from warmer or drier ecoregions generally withstand heat and/or desiccation better than flies from cooler sites and vice versa. However, our results indicated that source population has a pronounced influence on the direction, rates and extent of variation in response to the laboratory environment, and these responses were trait dependent. Moreover, the plasticity of these traits also appears to be varying in a manner that appears haphazard; we cannot find strong evidence to support the hypothesis of constrained plasticity or the alternative hypothesis of consistent trade-offs between basal and plastic stress resistance that might be expected if the one avenue of stress resistance evolves at the expense of the other. Again, this is in keeping with the literature on among-population comparisons of thermal plasticity (e.g. Sgrò et al., 2010) although we focus here on a broader suite of stress traits assayed at two distinct timepoints in culture.

It remains unclear what might be driving this complex inter-population CSR trait x time interaction effect, but it is likely the outcome of a combination of population genetic (dispersal and/or standing genetic variation) factors and local climate factors selecting for fitter genotypes via one or a few key individual or population-level processes (Edelaar and Bolnick, 2019). This may further be facilitated by variation in gut microbiota between the populations which may drive variation in environmental stress responses, and which may also vary during time in laboratory culture (Henry et al., 2018). Ørsted et al. (2018), for example, showed that differences in environmental conditions (developmental temperatures) directly influences the expression of genetic variation for cold tolerance in *D. melanogaster*. Thus, differences in local climate conditions may have resulted in differential gene expression and response to environmental stressors in our populations (and see Gerken et al., 2015 for discussion on cold stress resistance and plasticity). Population-specific adaptive solutions to similar environmental stress, i.e. pleiotropy, underlying CSR traits and their plasticity (Gerken et al., 2015) seems the most plausible explanation in our case, but requires further experimental work to directly assess the validity of this proposal and any such work will need to be interpreted within the context of these population's and their specific trait responses.

Our results also show substantial variation between the F_2 and F_{10} generations for the traits of CT_{MAX}, CT_{MIN}, cold survival, desiccation, and starvation resistance. However, the direction of the effects is mixed and interacts with the source population, specific acclimation temperature used and sex. An increase in stress resistance was found for CT_{MAX} and CT_{MIN} whilst the direction of change for chill survival was more erratic and haphazard, once again depending on the multiple other factors tested. The direction of change for desiccation and starvation resistance was also markedly population dependent (Fig. 3). Our results are therefore similar to those of Simões et al. (2008) who found that starvation resistance could increase or decrease depending on time of collection and source population. Hoffmann et al. (2001b), however found a decrease in resistance to desiccation and starvation with prolonged time in laboratory culture, although their cultures had been reared in the laboratory for far longer (~60 generations) than in our study. To our knowledge, there is sparse information on the effect of laboratory culture on temperature stress traits, except for Griffiths et al. (2005) who found no effect of laboratory culture on heat knockdown in *Drosophila birchii* reared for five to seven generations in the laboratory. Changes in CSR traits with increasing time in laboratory culture could also possibly be linked to morphological variation and is something that could be examined in future , although this was outside the scope of our study. Additionally, whether these intergenerational CSR trait changes increase or remain stable with an increase in the number of generations spent in laboratory culture is unknown and could also represent another avenue for future study.

Similar to many studies, we found generally strong acclimation temperature effects on CSR traits (e.g., for CT_{MAX} and starvation resistance, and CT_{MIN} (except in the case of Polokwane) (Table 1)). Typically, acclimation temperature interacted with several other variables in our minimal adequate models, such as founder population and number of generations spent in the laboratory, and thus influence CSR trait estimates. Importantly, our results indicate that the basal and plastic responses vary markedly depending on founder population and number of generations spent in laboratory. In other words, CSR traits vary in complex ways in space and time, perhaps more so than is typically appreciated or widely reported. However, populations did not respond similarly to these interaction effects with resistance increasing in some cases and decreasing in others (Appendix; Figure S1).

In conclusion, our results show clear evidence that CSR trait estimates are influenced by source population, time spent in culture and acclimation temperatures and, therefore, these and other possible influencing factors should be accounted for when performing comparative studies. Biased or skewed estimates of CSR traits are particularly problematic for risk or vulnerability assessments, and this will have far-reaching implications for measuring, inferring and thus mitigating, climate change impacts.

Data statement

All data and data analysis scripts are available in the Mendeley repository: link for reviewers: https://data.mendeley.com/datasets/b7y38jj8mr/2.

CRediT authorship contribution statement

Elizabeth J. Huisamen: Validation, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization, Funding acquisition. **Minette Karsten:** Supervision, Validation, Writing – review &

editing, Visualization, Funding acquisition. **John S. Terblanche:** Conceptualization, Supervision, Validation, Writing – review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

I have shared the link to my data in the main manuscript. It is published on the Mendeley data depository.

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Supplementary materials

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