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ORIGINAL ARTICLE

Altitudinal variation in body size and resistance to stress in *Drosophila melanogaster* and *D. simulans* (Diptera: Drosophilidae) in southern Turkey

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Abstract. Tolerance of cold and heat and body size are traits that are important in thermal selection. Latitudinal and altitudinal transects include environments that gradually change in temperature. However, while there are studies on the effects on body size and cold tolerance in natural populations sampled mostly along latitudinal transects, there are few such studies along altitudinal transects. Resistance to starvation and desiccation, which are also thought to be affected by temperature, are the focus of studies on clines. In this study, we measured the variation in tolerance of cold (chill coma recovery time), body size, resistance to starvation and desiccation in isofemale lines of *Drosophila melanogaster* and *D. simulans* that originated from four locations (50 m – 1500 m) along an altitudinal transect in the southern part of Turkey. This revealed clines in the tolerance of cold and body size along the altitudinal transect with some degree of difference between these two species, whereas there were no clines in resistance to starvation and desiccation. This study revealed some differences and similarities between the sibling species *D. melanogaster* and *D. simulans*.

INTRODUCTION

Distribution of species and their phenotypic variation in many quantitative traits are shaped by different environmental factors (temperature, air pressure, humidity etc.). Spatially or temporally changing environmental conditions act as selective agents driving the evolution of traits. Organisms cope with these varying selective pressures by local adaptation to different environmental conditions, which eventually generates clines in traits (Endler, 1977). Temperature is one of the most important environmental factors affecting various traits, especially in ectotherms (Cossins & Bowler, 1987) and thus thermal limits are reported for many species (Stillman & Somero, 2000; Duarte et al., 2012; van Heerwaarden et al., 2012).

The reproducibility of the latitudinal clines for many traits, including body size (Calboli et al., 2003) and cold tolerance, suggests that natural selection is more important than demographic or other stochastic processes. Similarly, the environmental variation along altitudinal transects is very similar to that along latitudinal transects. However, latitudinal and altitudinal transects show a remarkable difference in terms of geographic scales; the difference between the lowest and highest latitude can cover thousands of kilometres, whereas it can be only a few kilometres in altitudinal transects (Bubliy & Loeschcke, 2005). Even so, they show similar environmental patterns especially in climatic factors like temperature. In this way, altitudes are thought to mirror latitudes (Klepsatel et al., 2014) and thus similar clinal patterns are expected along altitudinal transects. Besides these differences and similarities, altitudinal transects occur over relatively short geographical scales, which may allow a relatively high gene flow between locations compared to latitudes and provide an opportunity to test the possibility of local adaptation in spite of gene flow (Keller et al., 2013).

Drosophila melanogaster is a cosmopolitan species, which originated from sub-Saharan Africa and colonized Eurasia, America and Australia (David & Capy, 1988) and is the main organism used for investigating the adaptive basis of clines (Fabian et al., 2015). *Drosophila*, especially *Drosophila melanogaster*, are well studied in terms of latitudinal variation in traits including body size, abdominal pigmentation, ovariole number, resistance to starvation and desiccation, tolerance of heat and cold etc. (Capy et al., 1993; Karan & Parkash, 1998; Ricardo et al., 1998; Land et al., 1999; Rouault et al., 2004; Goenaga et al., 2013) and clines in genetic signatures from SNP level to genome-wide scale (Fabian et al., 2015). And these latitu-

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dinal clines in *D. melanogaster* are accepted as adaptations to the different environments along transects (Mitrovski & Hoffmann, 2001; Verrelli & Eanes, 2001; Bettencourt et al., 2002; Sezgin et al., 2004; Schmidt & Paaby, 2008). Body size and cold tolerance are very well studied and positively correlated with increase in latitude in *D. melanogaster* (Ricardo et al., 1998; Land et al., 1999; Ayrinhac et al., 2004; Hoffmann et al., 2005b). Unlike the above, the reported clines in resistance to starvation and desiccation differ greatly in different geographical regions, particularly along latitudinal transects (Robinson et al., 2000; Hoffmann et al., 2001, 2005b; Goenaga et al., 2013). On the other hand, compared to the preponderance of latitudinal studies, there is less information on variation in clines along altitudinal transects and the results differ greatly (Sørensen et al., 2005; Parkash et al., 2012; Keller et al., 2013).

D. simulans is a sibling of *D. melanogaster* and although they differ greatly in various traits they have much in common (Capy & Gibert, 2004). *D. simulans* also originated in Africa and similarly spread to most temperate areas except West Africa and Asia in which it is almost totally absent (Lachaise & Silvain, 2004). There are many comparative studies on many traits along latitudinal transects of these two sibling species, including fertility, alcohol tolerance, resistance to starvation and desiccation and tolerance of cold, which indicate that *D. melanogaster* is more resistant to cold, starvation and desiccation than *D. simulans* (Tantawy & Mallah, 1961; McKenzie & Parsons, 1972; Parsons, 1977; Dickinson et al., 1984; Capy et al., 1993; Hoffmann & Parsons, 1993; Karan & Parkash, 1998; Gibert et al., 2004). However, comparative studies of altitudinal clines are scarce for these siblings.

Most studies on clines in different ectoderms including *Drosophila* were carried out to improve our understanding of local adaptation to environments (Kennington & Hoffmann, 2010) and most of these studies were based on latitudinal rather than altitudinal transects. Thus, in this study, we aimed to determine the altitudinal clines in body size, cold tolerance, resistance to starvation and desiccation in natural populations of the sibling species *D. melanogaster* and *D. simulans*. For this purpose, we sampled flies at four locations along an altitudinal transect (0–1500 m) in the South of Turkey and recorded their body sizes, chill coma recovery times (CCRT) and resistances to starvation and desiccation.

MATERIAL AND METHODS

Field work and establishment of isofemale lines

Flies were collected along an altitudinal transect from sea level (Adana province) to 1500 m (Tufanbeyli) in the Mediterranean part of Turkey in September of 2011 (Table 1). Mean annual temperature and mean annual precipitation for the locations were obtained from Worldclim Version1 (Hijmans et al., 2005) and are presented in Table 1. Flies were collected from banana baits (average of 20 baits per location were placed in a diameter of 2–3 km, the minimum distance between any two adjacent locations being about 40 km) and female flies caught were used to establish isofemale lines. All females were individually placed in a single vial immediately after collection. All lines were maintained at 25°C and 65% RH (relative humidity) in a fly chamber with a 16L : 8D cycle at Hacettepe University until all experiments were completed. Species identification for all lines was carried out in the F1 generation by examining male genitalia and then every line was defined as either *D. melanogaster* or *D. simulans*.

Experiments

Body sizes were recorded for wild-caught flies (from which isofemale lines were generated) kept at –20°C in sample tubes until their wings were removed. Only left wings were used for the wing centroid size measurements (as a proxy for body size). Each wing was mounted on a glass microscope slide and fixed using Entellan (Merck). Photographs of the wings were taken using a Leica MZ16A microscope at a fixed magnification. Wing measurements were determined using the tps programs (tpsUtil, tpsDig and tpsRelw) of F. James Rohlf (http://life.bio.sunysb.edu/morph/). Centroid sizes were obtained using tpsRelw after 11 landmarks were digitized on the images using tpsDig2.

Chill-coma recovery assays were carried out at the same time for both species reared in the same environmental conditions. Each group (population) consisted of 6 to 7 isofemale lines. 3–5 day old virgin female flies were transferred to empty tubes, which were placed in melting ice at 0°C for 16 h (David et al., 1998). After the cold shock, each group (isofemale lines) of flies was placed in a petri dish in the laboratory at 23–25°C and the recovery times (the time elapsed for a fly to stand up after the cold shock) were recorded for 3 h.

In the resistance to starvation assay, each experimental group (population) consisted of 3 to 6 isofemale lines and 3–5 day old virgin female flies from all groups were put into vials containing 5 ml of 0.5% agar ensuring that the flies had access only to water. Each vial was observed every four hours and the number of dead flies recorded, until all the flies had died (Matzkin et al., 2009).

For the resistance to desiccation, each group (population) consisted of 3 to 6 isofemale lines. 3–5 day old virgin female flies were put in vials with 3 g of silica gel in the bottom. The number of dead flies in each vial was recorded every hour until all flies had died (Parkash et al., 2005).

Statistical analysis

A three-way mixed effect ANOVA (SAS PROC MIXED v. 9.4, SAS Inc., Cary, NC) was used to analyse the variation in chill-coma recovery time and resistance to starvation and desiccation. The model for between species components was y = u + Species + Location + Location (Line) + Species * Location + E, where u is overall mean, Species is the fixed effect and Line is the random effect nested in Location, E is the error. The model was modified

Table 1. Geographic and environmental information for the locations sampled.

Location	N	E	Altitude (m)	Annual mean temperature (°C)	Annual mean precipitation (mm)
Adana	37.03028	35.32139	50	19.3	685
Horzum	37.62556	35.83556	700	13.7	710
Saimbeyli	37.98611	36.09139	1050	11.7	592
Tufanbeyl	38.25806	36.21972	1500	8.4	521

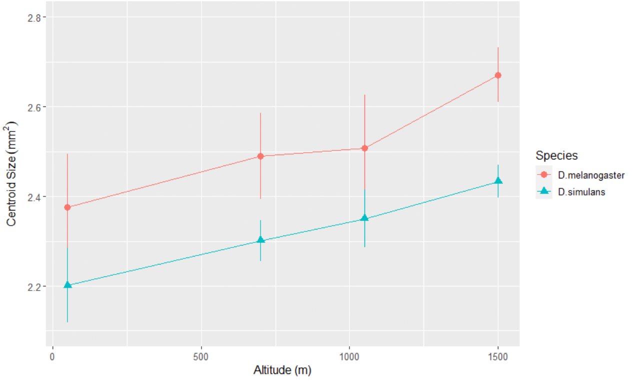

Fig. 1. The trends in mean body size of *D. melanogaster* and *D. simulans* collected at different altitude (points indicate mean and bars indicate 95% CI).

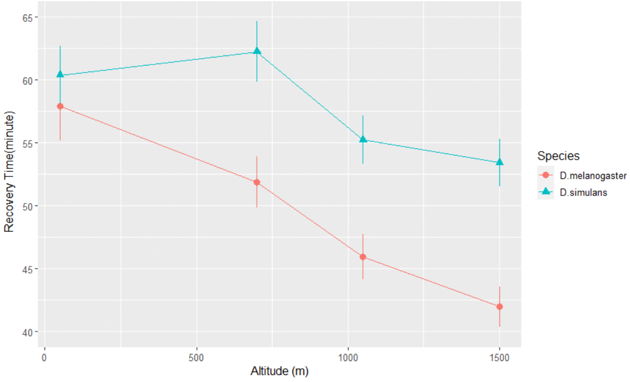

Fig. 2. The trends in mean chill coma recovery time of isofemale lines of *D. melanogaster* and *D. simulans* that originated from different altitudes (points indicate mean and bars indicate 95% CI).

to y = u + Location + Location (Line) + E, when the traits were analysed at the within-species level, Line being the random effect nested in the Location as before. A two-way ANOVA and one-way ANOVA were used to analyse the variation in body size between-species and within-species levels, respectively.

All statistical analysis and estimates of variance components were carried using PROC MIXED and VARCOMP modules implemented in SAS v. 9.4 and plots were drawn using the ggplot2 package (Wickham, 2009) in R Core Team, 2021.

RESULTS

We determined the body size, chill coma recovery, resistance to starvation and desiccation in the laboratory of 3rd generation isofemale lines of *D. melanogaster* and *D. simulans* that were reared from females collected along an altitudinal transect.

Body size

Individual body sizes were measured in terms of the wing centroid sizes of females collected in the field (as a proxy for body size). Table 2 shows mean body sizes for both species. In accordance with previous reports, the body size of wild caught female flies of *D. melanogaster* was bigger than that of *D. simulans* at all locations where they occurred sympatrically and body size increased significantly from lowland to highland locations for both sibling species, which implies a Bergman type cline in body size (Fig. 1). The increase in body size between the lowest and the highest locations was 11.2% for *D. melanogaster* and 9.5% for *D. simulans*. However, intraspecific comparisons between locations (data not shown) revealed few differences: For *D. melanogaster*, the location was highly significant for body size (Table 3), but this significance was due to the result for the highest location Tufanbeyli, at which mean body size differed markedly from that recorded at the other locations (Table 2). On the other hand, although body size increased with altitude, pairwise comparisons of the difference between the three locations (Adana-Horzum-Saimbeyli) were not statistically significant for *D. melanogaster* (data not shown). For *D. simulans*, the location was also highly significant and showed a pattern more or less similar that of *D. melanogaster*, with the Tufanbeyli location very different from the three other locations as in *D. melanogaster*. In addition, the lowest location Adana differed from Saimbeyli and the difference between Adana and Horzum was marginally insignificant (p = 0.052, data not shown).

Chill-coma recovery

There was a similar cline in chill coma recovery time for both species, but in the reverse direction to body size (Fig.

Table 2. Mean values of traits for both species and populations, with the number of flies and isofemale lines (in parentheses) used in the analysis.

	Body size (wing centroid size)		Chill coma recovery (minute)		Starvation resistance (h)		Desiccation resistance (h)	
	N	CS ± SE	N	Recovery time ± SE	N	Hours to death ± SE	N	Hours to death ± SE
D. melanogaster	4	2.54 ± 0.04	4	49.17 ± 1.27	4	70.29 ± 1.14	4	20.80 ± 0.31
Adana	9	2.37 ± 0.05	158 (6)*	57.91 ± 1.39	99 (5)	66.36 ± 1.1	114 (5)	20.29 ± 0.29
Horzum	13	2.49 ± 0.04	184 (7)	51.88 ± 2.01	131 (6)	71.94 ± 1.26	75 (4)	19.76 ± 0.37
Saimbeyli	9	2.51 ± 0.05	180 (7)	45.96 ± 0.90	156 (6)	74.51 ± 1.17	160 (6)	21.69 ± 0.26
Tufanbeyli	17	2.67± 0.03	181 (7)	41.98 ± 0.80	121 (5)	66.3 ± 1.0	103 (4)	20.76 ± 0.37
D. simulans	4	2.35 ± 0.02	4	57.72 ± 1.07	4	72.43 ± 1.36	4	16.23 ± 0.29
Adana	24	2.20 ± 0.04	139 (6)	60.39 ± 1.15	87 (4)	70.74 ± 1.12	65 (3)	17.09 ± 0.30
Horzum	56	2.30 ± 0.02	137 (7)	62.26 ± 1.23	126 (6)	72.10 ± 1.21	104 (5)	14.92 ± 0.26
Saimbeyli	47	2.35 ± 0.03	144 (6)	55.24 ± 0.98	101 (5)	73.64 ± 1.19	88 (4)	16.82 ± 0.30
Tufanbeyli	81	2.43 ± 0.01	148 (7)	53.43 ± 0.96	40 (3)	74.05 ± 2.74	54 (3)	16.74 ± 0.33

CS – centroid size; SE – standart error of the mean * numbers in the parentheses indicates the number of isofemale lines used.

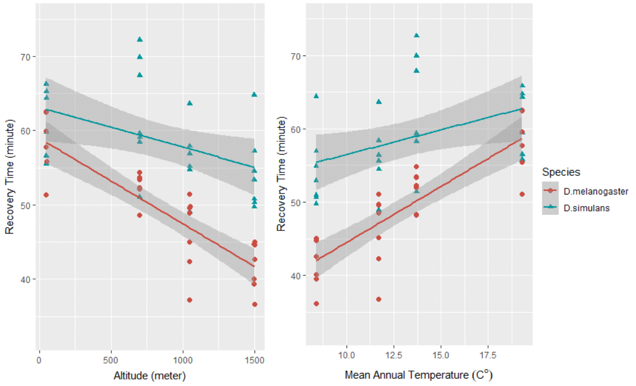

Fig. 3. Altitudinal clines in chill coma recovery times for both species. That for *D. melanogaster* is clearer (for altitude adjusted R-squared: 0.71, p < 0.001; for temperature adjusted R-squared: 0.69, p < 0.001) than that for *D. simulans* (for altitude adjusted R-squared: 0.17, p = 0.02; for temperature adjusted R-squared: 0.14, p = 0.03). Right graph relative to mean annual temperature and left graph to altitude (shaded areas denote 95% confidence intervals).

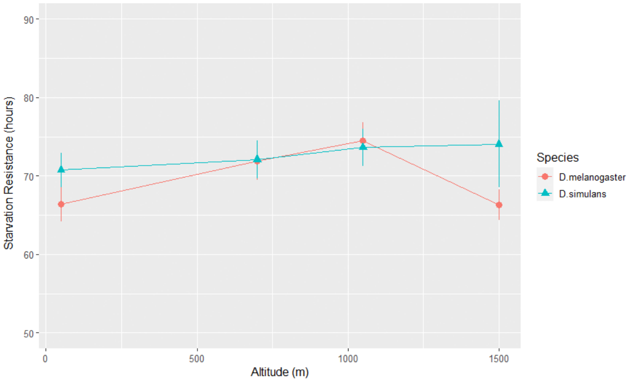

Fig. 4. Trends in the resistance to starvation (mean time to death) for isofemale lines of *D. melanogaster* and *D. simulans* that originated from different altitudes (points indicate means and bars indicate 95% CI).

2). Although the cline in chill coma recovery time in both species was similar, *D. melanogaster* at all locations recovered faster (~ 15%) than *D. simulans*. Interspecies ANOVA showed that the between species difference was highly significant (p < 0.001) (Table 3) and species effect accounted for 15.4% of total variation (9.9% for location effect and 6.3% for line effect). Even though the pattern of the clines for both species were similar, there were differences between species when each was analysed separately: For *D. melanogaster*, the ANOVA clearly indicates that recovery time differed significantly between locations (Table 3) and decreased gradually from lowland to highland locations (Figs 2, 3). In addition, location (site sampled) accounted for 20.04% of total variation whereas line accounted for only 3.6% of the total variation for *D. melanogaster* and the difference in the recovery time between the lowest and highest location is 27.5%. However, in *D. simulans* the effect of location differed between Adana-Horzum (50 m – 700 m) and Saimbeyli-Tufanbeyli (1050 m – 1500 m), but decreased linearly from lowland to highland locations in *D. melanogaster* (Fig. 3). Unlike *D. melanogaster*, line accounted for 13.5% of total variation while location accounted for only 6.2% for *D. simulans* and the difference in recovery time between the lowest and highest location was 11.5%, which is quite low in comparison with *D. melanogaster*.

Resistance to starvation

There was no statistical difference between the two species with regard to resistance to starvation (Table 3). *D. simulans* seems to be slightly, but not significantly, more resistant to starvation than *D. melanogaster* based on the means for all locations. The interspecies Anova, revealed no significant differences at species and location levels, with the only significant difference being between lines (i.e. within locations), which also accounted for a considerable amount of the total variation (15.4%). Intraspecies ANOVA revealed a similar pattern in which location is not significant, but lines were for both *D. melanogaster* and *D. simulans* and accounted for 16.7% and 11.2% of total variation, respectively. Overall, the altitudinal patterns in the resistance to starvation for both species are similar (Fig. 4). Unlike body size and chill coma recovery, resistance to starvation does not change with altitude.

Table 3. Intraspecies and interspecies ANOVA results with variance component values for all the traits investigated.

Level	Source	Body size			Chill coma recovery				Starvation resistance				Desiccation resistance			
		DF	Mean Square	F Value	DF	Mean Square	F Value	% Var. Comp.	DF	Mean Square	F Value	% Var. Comp.	DF	Mean Square	F Value	% Var. Comp.
	Species	1	1.3144	**41.878*****	1	24762.00	**49.46*****	**15.35**	1	976.81	1.46^{NS}	0.09	1	3002.96	**49.04*****	**47.61**
	Location	3	0.5836	**18.594*****	3	8276.12	**16.53*****	**9.90**	3	1110.57	1.63^{NS}	1.81	3	121.92	1.98^{NS}	2.25
Species	Species*Location	3	0.0129	0.412^{NS}	3	1271.11	2.54^{NS}	2.02	3	615.91	0.91^{NS}	0.00	3	20.52	0.33^{NS}	0.00
	Line(Location)	–	–	–	45	520.33	**3.17*****	**6.28**	32	735.35	**4.97*****	**15.36**	26	66.02	**8.97*****	12.87
	Error	248	0.0314		1218	163.97		66.44	821	148.00		82.73	729	7.36		37.27
D. melanogaster	Location	3	0.1904	9.281***	3	8312.45	**21.1*****	**20.04**	3	1953.47	2.37^{NS}	4.90	3	87.64	1.14^{NS}	1.21
	Line(Location)	–	–	–	23	396.98	**2.23*****	**3.62**	18	847.44	**5.77*****	**16.70**	15	80.53	**9.35*****	**25.94**
	Error	44	0.0205		676	178.13		76.33	485	146.79		78.40	433	8.61		72.84
D. simulans	Location	3	0.4061	**12.04*****	3	2026.31	**3.29***	**6.19**	3	131.80	0.24^{NS}	0.00	3	68.41	1.54^{NS}	4.40
	Line(Location)	–	–	–	22	649.29	**4.44*****	**13.47**	14	591.24	**3.95*****	**11.16**	11	46.23	**8.37*****	**27.62**
	Error	204	0.0337		542	146.31		80.33	336	149.74		88.84	296	5.52		67.98

*p < 0.05, **p < 0.01,***p < 0.001, NS – not significant.

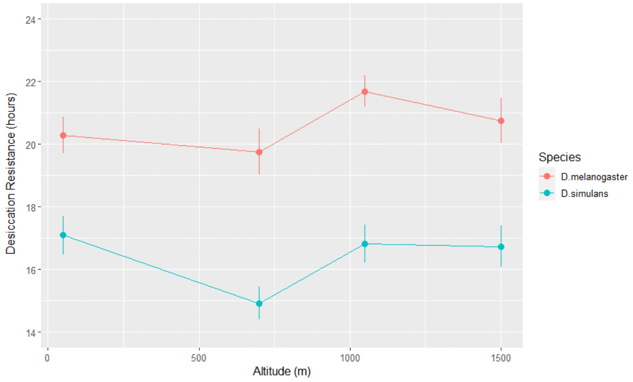

Fig. 5. Trends in the resistance to desiccation (mean time to death) for isofemale lines of *D. melanogaster* and *D. simulans* that originated from different altitudes (points indicate means and bars indicate 95% CI).

Resistance to desiccation

Although both species are strikingly similar, *D. melanogaster* was much more resistant to desiccation than *D. simulans* (Fig. 5, Table 2) and the interspecies ANOVA revealed that this difference is highly significant (Table 3). However, this similarity does not imply that the clines are similar: species accounts for nearly half of the variation (47.6%) whereas line (within location) accounts for only 12.9%. Again, at the intraspecies level, only line is significant and this effect explains 25.9% and 27.6% of the total variation for *D. melanogaster* and *D. simulans*, respectively. In summary, there seems to be no relationship between resistance to desiccation and altitude in either species.

DISCUSSION

In this study, clines in body size and resistance to stress along an altitudinal gradient for two closely related species, *D. melanogaster* and *D. simulans*, were determined. Significant altitudinal clines in body size and recovery from chill coma were recorded for intra- and interspecies differences and there are very strong similarities between the species. On the other hand, there were no clines in both species for resistance to desiccation and starvation.

Although there are many studies on clines in species of *Drosophila*, most of them are latitudinal rather than altitudinal clines (Hodkinson, 2005). Therefore, clines in body size are very well documented in terms of latitude for different continents and species of *Drosophila*, but not for altitudinal gradients (Pitchers et al., 2013; Klepsatel et al., 2014). From an altitudinal perspective, our results indicated that body size increase from lowland to highland in *D. melanogaster* and *D. simulans.* This finding is comparable to the results of previous studies on latitudinal and altitudinal clines in body size (Capy et al., 1993; James et al., 1997; Land et al., 1999; Loeschcke et al., 2000; Dahlgaard et al., 2001; Sambucetti et al., 2006; Kennington & Hoffmann, 2010; Pitchers et al., 2013; Klepsatel et al., 2014; Fabian et al., 2015). By using the body (wing) sizes of wild-caught female flies, our study differs from the majority of other studies in which laboratory reared strains were used for measuring body size. In a similar study along a latitudinal transect, body size of wild-caught *Drosophila melanogaster* also increased with latitude and were on average smaller than the laboratory strains (James et al., 1997). However, there is also evidence that there are no clines in body size of many wild-caught insects along altitudinal gradients (Dillon et al., 2006) possibly because body size is a plastic trait that is influenced by many factors (larval density, nutrient abundance, developmental temperature etc.) other than ambient temperature (Klepsatel et al., 2014). But this conclusion is based on studies on different insect orders and may not be the case for *Drosophila*. However, according to a previous study (Bubliy & Loeschcke, 2005) no clines in body size of sympatrically occurring *D. buzzatii* and *D. simulans* occur along an altitudinal transect. However, the maximum altitude (886 m) in that study was nearly half of the maximum altitude (1500) in our study and therefore the thermal gradient was insufficient and the locations were closer (max 5.5 km) than in our study, which may have allowed a relatively high gene flow, especially for a species like *D. simulans*. Therefore, these conflicting results may be because of a small difference in temperature along a short transect, or the effect of a higher gene flow disrupting local adaptation. Based on our results, there were clines in the body sizes of wild-caught flies along an altitudinal transect and this is also supported by the preliminary study (previous year in the same season) along the same transect (data not given). This indicates that the cline in body size is not incidental but subject to thermal selection, at least along the transect we used.

An altitudinal cline was also evident for recovery from chill coma in both of the species studied. This is in accordance with the majority of previous studies on clines in cold tolerance in different species of *Drosophila* (Hallas et al., 2002; Hoffmann et al., 2002, 2005a; Ayrinhac et al., 2004; Collinge et al., 2006; Arthur et al., 2008; Bridle et al., 2009; Parkash et al., 2010; Sisodia & Singh, 2010). Of those studies, the results of only three were equivocal in terms of latitudinal or altitudinal clines in cold tolerance for three species of *Drosophila*; *D. melanogaster*, *D. birchii* and *D. buzzatii* (Hoffmann et al., 2001; Griffiths et al., 2005; Sørensen et al., 2005). *D. buzzatii*, however, is (Sørensen et al., 2005) a more warm-adapted species than *D. melanogaster* and adaptation to cold may not be of major importance for this species. *D. birchii* is restricted to rainforest habitats in Australia and New Guinea (Griffiths et al., 2005) and may be subject to more important selective pressures than temperature. Thus, most of these studies indicate that clines in cold tolerance vary and are subject to thermal selection along both latitudinal and altitudinal transects in widely distant geographic regions.

Unfortunately, there is not a similar study on the tolerance of cold for these sibling species along the same altitudinal transect. So, we cannot compare the cold tolerance profiles of these two species with that of other species determined in a similar way. Even though both species' CCRT's decreased with increase in altitude there were differences between the two species. First, for chill coma recovery time, *D. melanogaster* was superior to *D. simulans* when the same location means are compared. *D. melanogaster* recovered faster and thus was more cold tolerant than its sibling species. Despite clines in cold tolerance being recorded for both species, that for *D. melanogaster* was more linear than that for *D. simulans*. *D. simulans* CCRT's can be grouped into two phenotypic classes, one for low and one for high locations. However, *D. melanogaster* CCRT's decreased linearly from lowest to the highest altitude. We think this might be related to differences in the incidence of dispersal (gene flow) between species and the absence of cosmopolitan inversions in *D. simulans* that may respond to altitudinal changes in ecological parameters (Weeks et al., 2002; Anderson et al., 2003). It is well documented that *D. simulans* has lower Fst values than *D. melanogaster*, which indicates a higher gene flow in the former species (Machado et al., 2016; Sedghifar et al., 2016). This putative gene flow effect may diminish the strength of selection and interrupt local differentiation in *D. simulans*, especially when the distance between regions is not far enough to allow local differentiation. This may account for the nonlinearity of the cline in cold tolerance in *D. simulans* recorded in this study. The expectation for *D. melanogaster* is that there is gene flow between locations, although to a lesser extent. However, unlike its sibling species, *D. melanogaster* populations carry cosmopolitan inversions, which might constitute a barrier against high levels of gene flow and allow local differentiation with respect to temperature resulting in a stronger local adaptation, which seems to be the case in our study.

In our study, no altitudinal clines in resistance to starvation or desiccation were recorded for either species. There were, however, significant differences in the resistance to desiccation recorded for the two species, in which *D. melanogaster* was on the average (for all locations) 20% more resistant. This between species difference accounts for nearly half of the total variance (47.6%). Unlike resistance to desiccation, there was no difference in the resistance to starvation between species. Moreover both species exhibit population specific patterns for these traits in which only effects of line were significant, implying that the phenotypic differences were mainly due to particular genotypes (lines) in the populations and the environmental factors expected to affect these traits may not be strong or constant enough across locations. The existence of clines in resistance to desiccation and starvation among *Drosophila* populations has long been a controversial issue in ecological evolutionary studies (Goenaga et al., 2013). Interestingly, the studies that record clines for resistance to desiccation and starvation in *Drosophila* are for India and South America, in which resistance to desiccation was positively correlated and resistance to starvation negatively correlated with increase in latitude or altitude (Karan & Parkash, 1998; Parkash et al., 2005, 2008, 2012; Goenaga et al., 2013). There are few studies, however, that report no clines in these traits in the same geographic regions (Robinson et al., 2000; Goenaga et al., 2010). On the contrary, most studies carried out in Australia indicate no clines for resistance to desiccation (Hoffmann et al., 2001, 2005a; Hallas et al., 2002; Arthur et al., 2008) and only one reports a cline in resistance to desiccation along a latitudinal transect for the rainforest species *D. birchii* (Griffiths et al., 2005), for which there is also a report of cline for resistance to starvation. However, two studies report the existence of latitudinal clines for resistance to starvation (Hoffmann et al., 2001; Arthur et al., 2008). All these results imply that resistance to desiccation is more evident in particular regions in India and South America, where habitats are very different from those in the rest of the world. And these very different environments may not be exactly mirrored in other regions with similar latitudinal or altitudinal transects. On the other hand, environmental factors affecting resistance to starvation seem to be more common and could occur similarly in different regions resulting in similar clines.

In conclusion, to our knowledge, this is the first study on the variation in body size, cold tolerance, resistance to desiccation and starvation along an altitudinal transect for the two cosmopolitan sibling species, *D. melanogaster* and *D. simulans*. Altitudinal clines were recorded for both species for body size and recovery from chill coma but not for resistance to desiccation and starvation. Even though both species have similar phenotypic clines, they differed in their tolerance of cold; for example, in *D. simulans* it was nonlinear and unlike that for the chill coma recovery time of *D. melanogaster*. We suggest that the underlying reason for this difference should be further investigated at both the genetic, ecological and demographic levels with the aid of modern genomic tools.

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CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

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