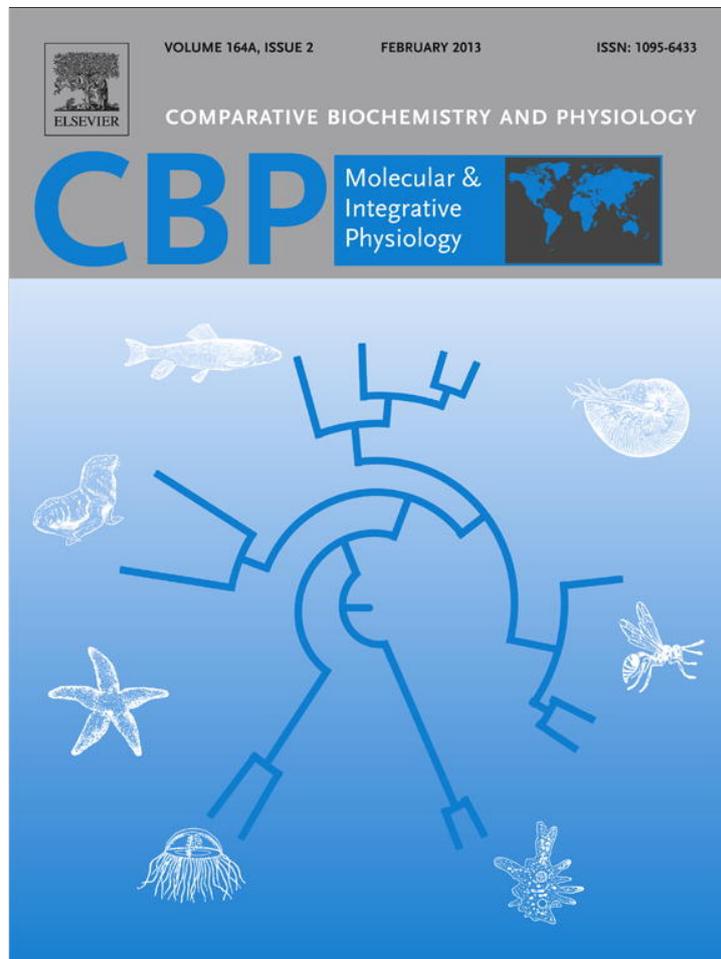


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## Meta-analysis of geographical clines in desiccation tolerance of Indian drosophilids

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## ABSTRACT

Tropical fruit flies (*Drosophilidae*) differ from temperate drosophilids in several ecophysiological traits, such as desiccation tolerance. Moreover, many species show significant differences in desiccation tolerance across geographical populations. Fruit flies from the tropical and subtropical Indian subcontinent show a clinal pattern for desiccation tolerance which is similar for more than a dozen species studied so far, suggesting adaptation to climatic differences. We performed a meta-analysis to investigate which particular climatic patterns modulate desiccation tolerance in natural populations of drosophilids. Latitude of the sampling site explained most of the variability. Seasonal thermal amplitude (fluctuations in temperature expressed as coefficient of variation) was the strongest climatic factor shaping desiccation tolerance of flies, while factors measuring humidity directly were not important. Implications for survival of flies after future climate change are suggested.

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## 1. Introduction

During their evolutionary history, widely distributed species have adapted to various biotic and abiotic conditions, and many display clear clines for various fitness-related traits across continents (Capy et al., 1993; Munjal et al., 1997; Starmer et al., 1997; James and Partridge, 1998; Karan et al., 1998a,b; Hoffmann et al., 2002; Ayrihac et al., 2004; Chown and Gaston, 2008; Rajpurohit et al., 2008c; Gaston et al., 2009). Naturally occurring latitudinal and altitudinal clines for such traits are promising targets to explore questions related to evolutionary physiology and climate change. It is generally considered that these clines reflect adaptive differentiation in response to different climates. Resistance of species to climatic extremes and reproductive output under different climatic conditions generally match expectations (Hoffmann, 2010). Precise predictions of these impacts must be based on an understanding of the mechanistic links between climate and organisms, and a consideration of evolutionary responses (Chown and Gaston, 2008; Gaston et al., 2009; Kearney et al., 2009; Hoffmann and Sgro, 2011).

*Drosophila* and *Sophophora* fruit flies (traditional genus *Drosophila* sensu lato, see Remsen and O'Grady (2002) for phylogenetic taxonomy and nomenclatorial changes) are classical models for population genetics and evolutionary ecology studies (Parsons, 1983). Understanding variability of environmental stress responses in different populations

and species in the genus *Drosophila* s.lat. can give important insights into understanding climatic adaptations, as well as help to understand factors determining distribution and abundance patterns on several continents differing in their abiotic and biotic conditions.

During the last two decades a considerable amount of work has been done with natural populations of south Asian drosophilids. These studies, covering approximately a dozen species, have focused on geographic variation in traits related to morphometrics, physiology and behavior. Substantial spatial heterogeneity has been detected in almost all traits across the Indian subcontinent, and this variation appears to be nonrandomly orientated with respect to latitude. The Indian subcontinent, where many drosophilids show clines for various fitness-related traits, is therefore an ideal place to identify patterns of clinal variation in stress-related traits under natural conditions. In this paper, we focus on desiccation tolerance in adults, a trait which has been investigated in a large number of species.

## 1.1. Desiccation tolerance and routes of water loss

Among fitness-related physiological traits, water balance is highly relevant for insects in natural habitats. Insects face a fundamental challenge to conserve body water as a result of their high surface area to body mass ratio. Desiccation tolerance is a complex physiological trait that may evolve through multiple mechanisms, each of them able to respond to selection (Graves et al., 1992; Hoffmann and Parsons, 1993; Gibbs et al., 1997; Chippindale et al., 1998). There are three main physiological ways by which insects can increase their tolerance to desiccation: by increasing their total body water content; tolerating

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**Table 1**

Multiple regression analysis of individual climatic factors with latitude, longitude and altitude combined in one model. Longitude was not significantly correlated with any of the climatic variables. Data originate from 22 weather stations across the Indian sub-continent with latitude ranging 8.28–32.4 °N and altitude 3–921 m. Abbreviations for climatic parameters are provided in the text. Ranges of measured values are indicated in parentheses. Multiple correlation coefficient (*r*), individual regression slopes (*b*) and significance (probability) of the relationship are figured. Statistically significant relationships based on the adjusted level  $p=0.00625$  are marked in bold font.

Climatic parameters	Latitude			Altitude		
	<i>r</i>	<i>b</i>	<i>p</i>	<i>b</i>	<i>p</i>	
$T_{max}$ (29–34.4 °C)	0.53	0.209	0.322	–0.519		0.021
$T_{min}$ (16.5–24.3 °C)	0.96	<b>–0.650</b>	$<10^{-6}$	<b>–0.568</b>		$<10^{-6}$
$T_{ave}$ (23.4–28.3 °C)	0.88	<b>–0.435</b>	<b>0.001</b>	<b>–0.677</b>		$<10^{-5}$
$T_{cv}$ (3.2–29.2%)	0.97	<b>0.961</b>	$<10^{-6}$	0.018		0.754
RH <sub>ave</sub> (46–79%)	0.86	<b>–0.689</b>	$2.10^{-5}$	–0.367		0.007
RH <sub>cv</sub> (4–43%)	0.78	<b>0.558</b>	<b>0.003</b>	0.351		0.047
RF <sub>ave</sub> (630–3228 mm)	0.65	–0.504	0.013	–0.264		0.166
WS (3.7–14 m/s)	0.31	–0.282	0.247	0.145		0.546

loss of a large proportion of water from the body; and reducing the rate of water loss (Chown and Nicolson, 2004). The rate of water loss through the cuticle and spiracles constitutes the most important process, with cuticular water loss generally accounting for >70% of overall water loss (Chown, 2002; Chown et al., 2006; Quinlan and Gibbs, 2006). Water conservation in drosophilids is achieved through behavioral and physiological mechanisms (Gibbs et al., 2003; Rajpurohit et al., 2008b). The contributions of other potential mechanisms to desiccation tolerance are less clear (Hoffmann and Harshman, 1999; Gibbs, 2002; Gibbs and Rajpurohit, 2010).

Whatever is the mechanism of desiccation resistance, it must be demonstrated in the evolutionary sense by either survival or death of the flies under stressful conditions. Thus, the measure widely used in literature (listed in Table 3) and analyzed in this article is survival time of 50% of individuals at low relative humidity and at a defined temperature (here 25 °C).

1.2. Desiccation tolerance in tropical drosophilids, the role of climatic variability

Interactions between temperature and humidity are complicated and significantly affect animal life under natural conditions. Temperature variation is more consistently associated with latitude than any other climatic variable, because latitude is the primary factor affecting the unequal solar heating of the Earth's atmosphere. Humidity patterns are more complicated. On a global scale, air becomes warmer and drier as it sinks at subtropical latitudes, creating a band of relatively dry surface conditions at approximately 30° north and south latitudes (Barry and Chorley, 2003; Quan et al., 2004). Interestingly, this pattern coincides closely with the upper latitudes of India. Locally, humidity is affected by geographical location (proximity to large bodies of water), elevation (water holding capacity of air decreases with increasing elevation), and rainfall (dependent on tree density and climatic patterns

such as monsoon and El Niño effects). Every geographical location is an admixture of various climatic parameters and imposes unique stresses on local drosophilids.

Physiological limits can influence the susceptibility of organisms to climate change and indicate potential vulnerability (Bernardo et al., 2007; Chown and Gaston, 2008; Williams et al., 2008; Gaston et al., 2009; Huey et al., 2009; Hoffmann and Sgro, 2011). *Drosophila* species exploit a wide range of habitats, including rain forests, tropics, deserts and mountains. It is generally argued that desiccation resistance is selected under hot and dry conditions, and data on Indian populations of several drosophilids support this contention (Parkash and Munjal, 1999). However, ectothermic insects also encounter desiccation stress at higher altitudes where water content of the air is reduced at low ambient temperature. Until recently there were no supporting data on whether these conditions also favor desiccation tolerance in drosophilids (Parkash et al., 2005; Rajpurohit et al., 2008c).

In the present study we integrate the results of numerous studies of clinal patterns (both latitudinal and altitudinal) for adult desiccation tolerance in drosophilids on the Indian subcontinent (Parkash et al., 1994; Karan and Parkash, 1998; Karan et al., 1998a; Parkash and Munjal, 1999; Parkash and Munjal, 2000; Parkash et al., 2005, 2008a, b; Rajpurohit et al., 2008b,c,d; Parkash et al., 2009a,c). A common explanation for latitudinal and altitudinal clines is that temperature is responsible, because the two geographical characteristics and temperature are strongly correlated. In the case of desiccation tolerance, however, geographical gradients in humidity may be more important.

Here we synthesize these earlier studies, focusing on the ecological basis of desiccation tolerance and its relationship to climate patterns (Parkash et al., 2010, 2011). We relied on a large climatic data set (>20 weather stations), including several weather parameters. Climatic data were obtained for the sites from where the original natural populations were sampled and used in a multivariate meta-analysis (more details in Materials and methods section). Long-term climate trends on the Indian subcontinent are described and their potential impact on the distribution and physiology of tropical and subtropical drosophilids is discussed.

2. Materials and methods

Climatic data were obtained for sites on the Indian subcontinent where natural drosophilid populations have been sampled by various scientific groups. Data for latitude, temperature maximum ( $T_{max}$ : annual average of daily maxima), temperature minimum ( $T_{min}$ : annual average of daily minima), average temperature ( $T_{ave}$ ), coefficient of variation of temperature ( $T_{cv}$ : standard deviation divided by average of 12 monthly averages of daily averages), relative humidity (RH<sub>ave</sub>), coefficient of variation of relative humidity (RH<sub>cv</sub>: the same as for  $T_{cv}$ ), average rainfall (RF<sub>ave</sub>), and wind speed (WS) were obtained from climatological tables (for the last 30 years) of the National Data Center, India Meteorological Department, Pune. Note that  $T_{cv}$  describes the seasonal variation, not circadian fluctuations or variation between years,

**Table 2**

Multiple regression analysis of  $T_{ave}$  (temperature average), RH<sub>ave</sub> (relative humidity average) and RF<sub>ave</sub> (rainfall average) in Monsoon season and rest of the year with latitude and altitude combined in one model. Data originate from same 22 weather stations (Table 1) across the Indian subcontinent with latitude ranging 8.28–32.4 °N and altitude 3–921 m. Multiple regression coefficient (*r*), individual regression slope (*b*) and significance (probability) of the relationship are figured. Statistically significant relationships based on the adjusted level  $p=0.006$  are marked in bold font. Monsoon: June to September.

Climate parameters	Season	Latitude			Altitude	
		<i>r</i>	<i>b</i>	<i>p</i>	<i>b</i>	<i>p</i>
$T_{ave}$	Monsoon	0.97	<b>0.17</b>	$<10^{-5}$	<b>–0.006</b>	$<10^{-6}$
	Rest of year	0.97	<b>–0.26</b>	$<10^{-5}$	<b>–0.005</b>	$<10^{-6}$
RH <sub>ave</sub>	Monsoon	0.66	<b>–0.53</b>	<b>0.005</b>	<b>0.006</b>	<b>0.002</b>
	Rest of year	0.54	–0.62	0.01	0.23	0.30
RF <sub>ave</sub>	Monsoon	0.44	0.26	0.27	0.25	0.29
	Rest of year	0.62	<b>–0.68</b>	<b>0.003</b>	0.55	0.01

**Table 3**

Geographical trends expressed as slope values (first line, *italics*) and probability (second line) of multiple regression of desiccation tolerance (survival time) with latitude and altitude in seven drosophilids. We analyzed all populations (numbers in parentheses) with available data about desiccation tolerance. Clines significant on the adjusted level of  $p = 0.007$  are highlighted in bold. The average desiccation tolerance (in h) calculated for each species by phylogenetically independent contrasts for the 21 °N is also given for the reference. Estimation did not converge for *Drosophila immigrans*.

Species	Latitude	Altitude	References	Desiccation tolerance (h) at 21 °N
<i>Sophophora melanogaster</i> Meigen, 1830 (12)	<b>0.64</b> <b>0.00038</b>	<b>0.47</b> <b>0.0028</b>	Shamina et al., 1993; Karan et al., 1998a, 1998b; Parkash and Munjal, 1999, 2000	22
<i>S. kikkawai</i> Burla, 1954 (10)	<b>0.87</b> <b>0.0016</b>	−0.09 0.61	Karan and Parkash, 1998; Parkash and Munjal, 1999	12
<i>S. ananassae</i> Doleschall, 1858 (13)	<b>0.90</b> <b>0.00012</b>	0.00 1.00	Karan et al., 1998a,b; Parkash and Munjal, 1999	8
<i>S. bipectinata</i> Duda, 1923 (6)	0.99 0.015	−0.132 0.55	Parkash et al., 1994; Parkash and Munjal, 1999	13
<i>Drosophila repleta</i> Wollaston, 1858 (5)	<b>0.99</b> <b>0.0042</b>	0.012 0.87	Parkash and Munjal, 1999	31
<i>D. immigrans</i> Sturtevant, 1921 (9)	−0.173 0.53	0.77 0.024	Parkash et al., 2008a,b	-
<i>Zaprionus indianus</i> Gupta, 1970 (13)	<b>0.92</b> <b>&lt;10<sup>−6</sup></b>	0.19 0.013	Karan et al., 1998a,b; Parkash and Munjal, 1999; Parkash et al., 2008a,b	31

such as caused by El Niño events. Climatic data from 22 weather stations were analyzed together with their geographical characteristics. Long-term temperature data (1901–2003) for the Indian subcontinent were obtained from the Indian Institute of Tropical Meteorology, Pune. The  $T_{ave}$  data for 102 years were calculated from the original database files (stored as  $T_{min}$  and  $T_{max}$ ).

Desiccation tolerance data on multiple populations of seven species of drosophilids were collected from published studies. We omitted a few other species from our analysis as these species either had restricted distribution or were food specialists. For example, *Drosophila nepalensis* was found only on certain flowering plants in the Himalayan foothills (S. Rajpurohit, unpublished data). All values used here were obtained by similar laboratory methods (see Karan et al., 1998a), i.e. we used data for the species where desiccation tolerance (survival time at low humidity) was measured at  $25 \pm 0.5$  °C temperatures only.

These values were used for simple and multiple regression models running in Statistica 9.1 (Statsoft Inc., Tulsa, Oklahoma USA) for each species separately (Tables 3–5). Stepwise regression with forward selection of geographical and climatic variables was used to identify the significant predictors of desiccation tolerance. Backward selection of the variables gave similar results. The models in this forward selection are penalized for complexity, it means the addition of another variable was merited given the loss of degrees of freedom caused by its inclusion. All climatic variables were tested both for entire year round and separately for the monsoon season and rest of year.

Standard probability values of 0.05 for decision of significance were lowered in the case of several independent parallel tests (6 or 7 species; Tables 3 and 4) by the Sidak correction (Abdi, 2007).

Phylogenetic information should be separated from the adaptive evolution within the geographic and climatic clines. To make all the species, which differ several times in their overall values of desiccation tolerance, comparable within a common regression analysis (Figs. 2–3) while putting aside the signal of phylogenetic relationship of species, we performed the following process with data collected from literature.

Our data first underwent a phylogenetic analysis assuming intra-specific variation in continuous traits (Ives et al., 2007; Revell and Reynolds, 2012) that reduced the multiple population data to a single specific fitted “mean.” The phylogenetic independent contrasts were calculated in R software package phytools (Revell and Reynolds, 2012), function `fitBayes(tr,des,ngen=10000,model=“BM”,method=“reduced”,control=list())`. The phylogenetic relationships between the six selected fruit fly species were combined from the Taxodros online database (Bächli, 2012) and phylogenies by Remsen and O’Grady (2002) and Yang et al. (2012) as follows: (((*Sophophora melanogaster*, *Sophophora kikkawai*), (*Sophophora ananassae*, *Sophophora bipectinata*)), *Zaprionus indianus*), *Drosophila repleta*). Real branch lengths were not available for all these species, so all edge lengths were set to the same value, producing a non-ultrametric tree. We found no violation of the independent contrasts analysis assumptions (see Garland et al., 1992) using the PDAP module (Midford et al., 2010) of Mesquite software (Maddison and Maddison, 2011).

For comparison, we calculated linear regressions of desiccation tolerance dependent on latitude for each species separately and then we calculated the expected value (geographic “mean”) for each species in the middle of subcontinental transect, i.e. at 21 degrees north (Table 3). The fitted mean values of desiccation tolerance calculated for each species by the phylogenetic independent contrasts method differed from the geographic means by 0 to 11%. We then calculated a

**Table 4**

Regression analysis of desiccation tolerance (survival time in hours) with individual climatic factors. Numbers of populations analyzed in each species are given in parentheses. Slope of regression (first line, *italics*) and significance ( $p$ , second line) are given, clines significant on the adjusted level of  $p = 0.0083$  are highlighted in bold font.

Species	$T_{max}$	$T_{min}$	$T_{ave}$	$T_{cv}$	RH	$RH_{cv}$	$RF_{ave}$	WS
<i>S. melanogaster</i> Meigen, 1830 (5)	−0.59 0.29	−0.48 0.42	−0.56 0.32	0.65 0.24	−0.25 0.68	0.014 0.98	0.56 0.33	−0.78 0.12
<i>S. kikkawai</i> Burla, 1954 (8)	−0.07 0.86	−0.38 0.35	−0.26 0.53	<b>0.85</b> <b>0.0076</b>	−0.03 0.95	−0.16 0.70	−0.03 0.95	−0.54 0.17
<i>S. ananassae</i> Doleschall, 1858 (8)	−0.49 0.21	−0.59 0.13	−0.62 0.10	<b>0.89</b> <b>0.0030</b>	−0.42 0.31	0.29 0.49	−0.08 0.86	−0.09 0.86
<i>S. bipectinata</i> Duda, 1923 (5)	0.61 0.28	−0.72 0.17	−0.26 0.67	<b>0.97</b> <b>0.0073</b>	−0.79 0.12	0.68 0.20	−0.77 0.13	0.32 0.61
<i>D. repleta</i> Wollaston, 1858 (4)	0.61 0.39	−0.42 0.58	0.19 0.81	0.98 0.023	−0.42 0.58	0.38 0.62	−0.46 0.54	−0.67 0.33
<i>Z. indianus</i> Gupta, 1970 (6)	0.33 0.53	−0.58 0.23	−0.08 0.88	<b>0.99</b> <b>0.00014</b>	−0.41 0.42	0.22 0.67	−0.42 0.40	−0.45 0.37

**Table 5**

Geographical and climatic clines expressed as slope values (first line, *italics*) and probability (second line) of multiple regression of desiccation tolerance (survival time in hours) with geographical and climatic variables in six drosophilids. Only those populations (numbers in parentheses) were analyzed where local climatic data were available. Forward stepwise selection was used to identify important predicting variables. Clines non-significant on 5% level are omitted and those significant on the adjusted level of  $p=0.0083$  are highlighted in bold font.  $T_{cv}$  = coefficient of variance of temperature;  $RH_{cv}$  = coefficient of variance of humidity; the other variables (originally three geographical and eight climatic variables, see Table 1) were not significantly correlated for any species analyzed.

Species	Latitude	Altitude	$T_{cv}$	$RH_{cv}$
<i>S. melanogaster</i> Meigen, 1830 (5)	4.18 0.020		–3.65 0.025	
<i>S. kikkawai</i> Burla, 1954 (8)	<b>1.01</b> <b>0.000007</b>			–0.42 <b>0.00048</b>
<i>S. ananassae</i> Doleschall, 1858 (8)			<b>1.01</b> <b>0.00010</b>	
<i>S. bipunctinata</i> Duda, 1923 (5)	<b>0.95</b> <b>0.0043</b>			
<i>D. repleta</i> Wollaston, 1858 (4)	<b>0.99</b> <b>0.0073</b>			
<i>Z. indianus</i> Gupta, 1970 (6)		0.26 0.015	<b>1.21</b> <b>0.00067</b>	0.12 0.014

relative desiccation tolerance (RDT) for each population as the ratio between survival time of that population and the fitted mean for that species. Values of RDT were then used for multiple regression with geographical and climatic factors.

Our comparative analysis inevitably includes a publication bias (see Hadfield and Nakagawa, 2009; Jennions et al., 2012) for widely distributed species that were sampled at many localities, but not the type of bias of including only studies with significant results, since the authors of original studies did not primarily search for clines.

### 3. Results

#### 3.1. Relationship between climatic and geographical variables

Within our transect of 22 climatic stations located near the sampling sites of drosophilids analyzed in this study, no climatic variable correlated significantly with longitude (data not shown). Three climatic variables (maximum temperature, average rainfall and wind speed) were not significantly correlated either with latitude or altitude within the entire year data. Two climatic variables were correlated with both latitude and altitude. These were minimum temperature and average temperature, which strongly decreased towards the north and in the mountains. Three climatic variables were significantly correlated only with latitude: relative humidity decreased, while coefficient of variation of temperature and coefficient of variation of relative humidity increased. In other words, temperature seasonally fluctuates much more in the north, and humidity is lower and fluctuates more in the north. When summarized, the northern locations are cold, dry and seasonally variable, while southern ones are warm, humid and moderate, with little seasonality (Fig. 1).

To investigate potential seasonal effects, we divided climatic data into two seasons, during the monsoon and the rest of the year. The monsoon season (June to September) is a major climatic phenomenon on the Indian subcontinent, with maximum rainfall occurring during this season throughout the region. Except for one station (Pondicherry; 27.83% reduction), an overall increase in rainfall in the monsoon season compared to the rest of the year was observed, ranging from 6 to 4425 per cent (data not shown). Average temperature was correlated with latitude during both seasons, but the direction of the relationship changed. Northern sites were warmer during the monsoon, but colder during the rest of the year. Relative humidity was significantly

(negatively) correlated with latitude only during the monsoon. Both  $T_{ave}$  and  $RH_{ave}$  significantly changed with altitude during the monsoon, but in opposite directions. Average rainfall decreased only with latitude during the rest of year (Table 2).

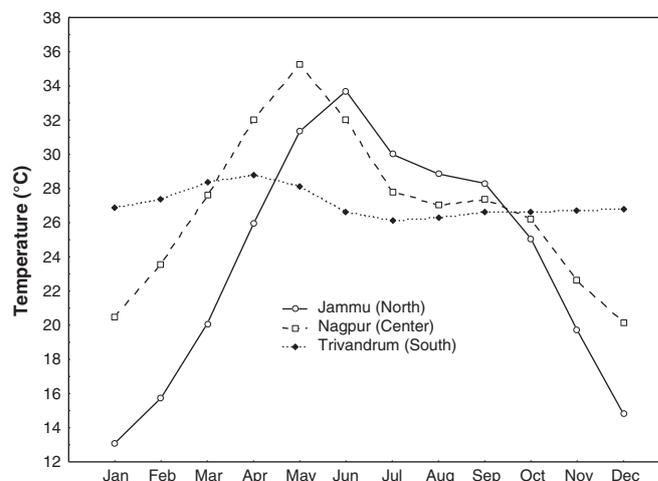
#### 3.2. Geographical and climatic clines of desiccation tolerance

Data on desiccation tolerance (survival time) were analyzed for seven drosophilids that were studied at five or more different locations. In five of these species, desiccation tolerance increased significantly with latitude (Table 3). Desiccation tolerance also increased with altitude in *S. melanogaster*, but not in other species (Table 3). Species differed approximately four-fold in desiccation tolerance; Table 3 lists desiccation tolerance for populations of each species collected at Nagpur (21.6 °N latitude, 310 m altitude).

Because local climate data were not available for all sites, fewer populations in each species could be analyzed for possible correlations of desiccation tolerance with climatic factors than in the previous geographical-only model. *Drosophila immigrans* was omitted from this analysis because weather station data were available for only four of its collection locations. When the regression was run with individual climatic factors on an annual basis, only temperature variability ( $T_{cv}$ ) was found to have a significant effect in four of six species (Table 4). Since there was an almost perfect correlation between  $T_{cv}$  and latitude, it was impossible to say which variable was driving the correlation with desiccation tolerance. When the seasons (monsoon and rest of year) were separated, latitude remained the only single significant predictor of desiccation tolerance.

To determine whether general environmental conditions related to geographical variables or specific climatic variables shaped the differences in desiccation tolerance between populations of several drosophilids, we analyzed the geographical and climatic variables using multiple regression. Among the six species analyzed, three showed strong positive correlations of desiccation tolerance with latitude (Table 5). In two other species, annual temperature fluctuations expressed as the coefficient of variation ( $T_{cv}$ ) was the factor most strongly correlated with desiccation tolerance. In *S. kikkawai*, humidity variability ( $RH_{cv}$ ) was also correlated, in addition to latitude. In *S. melanogaster*, no variables were significantly correlated with desiccation tolerance after correction for multiple comparisons.

To investigate overall patterns in desiccation tolerance, we merged data from these six species into a common model. Here it is important



**Fig 1.** Variation in average monthly temperature for three representative locations on the Indian subcontinent. Data were obtained from the National Data Center, India Meteorological Department, Pune. The altitudes (in meters a.s.l.) of three locations are as follows: Jammu 367; Nagpur 310; Trivandrum 64. There are several comparable locations (in altitude) in southern India with similar temperature profiles across the year as indicated in for Trivandrum.

to note that phylogenetic relationships between the species could mean that the data were not statistically independent. Because the species analyzed differed in their average survival time in desiccating conditions, we first calculated a relative desiccation tolerance (RDT) for each species. The phylogenetic independent contrast analyses with intraspecific variation slightly changed (below 5%) the RDT values. This characteristic increased strongly with both latitude (Fig. 2) and  $T_{cv}$  (Fig. 3) in simple regression models, but not with other climatic variables (Fig. 4). A model that used multiple regression of RDT dependent on both latitude (Lat) and temperature fluctuations ( $T_{cv}$ ) explained 70% of the variation ( $F_{2,33}=42$ ,  $p<0.0001$ ),  $RDT = 0.5 + 0.0096*Lat + 0.0156*T_{cv}$ .

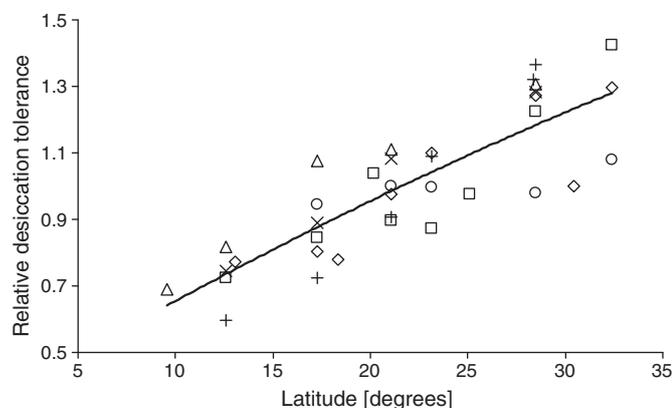
#### 4. Discussion

Seasonal temperature variability, expressed as the coefficient of variation ( $T_{cv}$ ) was the most important climatic factor explaining latitudinal variation in desiccation tolerance, in both simple and multiple regression models. However, latitude itself – a complex factor correlated with many climatic factors – was a surprisingly better predictor than individual climatic factors. This suggests that local adaptation is driven by multiple environmental variables whose individual effects may not be discernible.

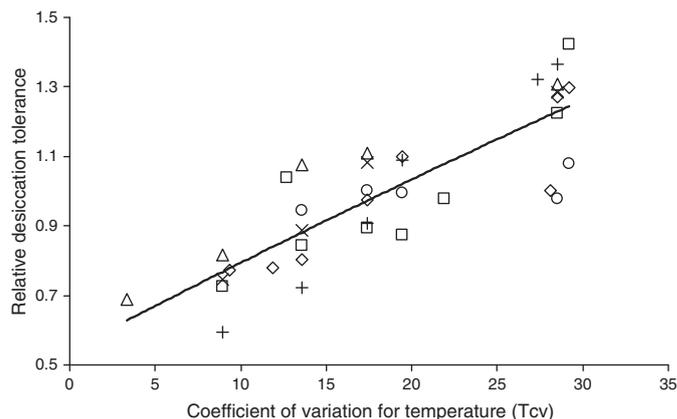
The patterns observed for desiccation tolerance in tropical and subtropical Indian drosophilids can be interpreted based on climatic adaptation, i.e. the flies experience gradients of climatic conditions (temperature and humidity) along the latitudinal range and have adapted to local conditions. The places where drosophilids were sampled had a relatively limited range of altitudes and corresponding climatic conditions, so we found no consistent altitudinal clines (Table 1). Circadian fluctuations in temperature and humidity would be higher and perhaps important in high altitudes, but not the seasonal variation analyzed in this study.

Clinal patterns that are thought to be associated with environmental variables could be driven by abiotic and/or biotic factors, which may include complex interactions with climate such as food availability/resources etc. The trait of interest, in this case desiccation resistance, may not even be the trait under selection, with clinal patterns driven by correlated selection on another trait. Similar patterns in multiple species may make this scenario less likely, but the fact that we find clinal patterns for desiccation resistance on the Indian subcontinent for *S. melanogaster* but not in Australia indicates that selection for desiccation resistance is driven by complex factors that may not be entirely related to climate (McKenzie and Parsons, 1974; Parsons, 1980; Davidson, 1990).

Recent studies (reviewed in Rajpurohit et al., 2008b; Parkash et al., 2008a) have found two key traits affecting desiccation tolerance in Indian drosophilids, body pigmentation and cuticular hydrocarbons



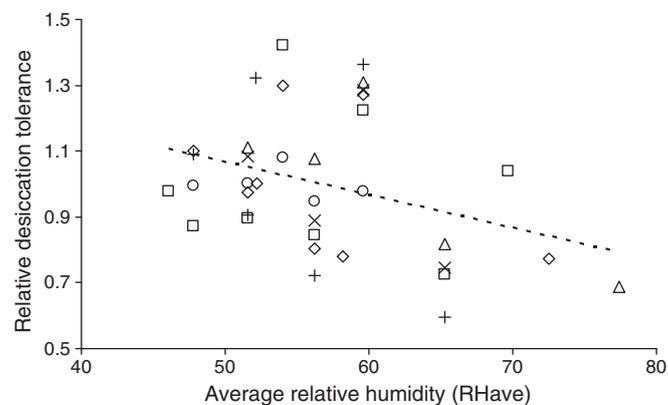
**Fig. 2.** Geographic cline of relative desiccation tolerance of six drosophilid flies. Values are relative to the mean survival time for all populations assayed in each species. Circle = *Sophophora melanogaster*, square = *S. kikkawai*, diamond = *S. ananassae*, triangle = *S. bipectinata*, x = *Drosophila repleta*, + = *Zaprionus indianus*.



**Fig. 3.** Climatic cline of relative desiccation tolerance of six drosophilid flies. Values are relative to the mean survival time for all populations assayed in each species. Circle = *Sophophora melanogaster*, square = *S. kikkawai*, diamond = *S. ananassae*, triangle = *S. bipectinata*, x = *Drosophila repleta*, + = *Zaprionus indianus*.

(CHC). The chemical nature of melanin molecules and CHC are strongly dependent on developmental temperature (Gibert et al., 2007; Gibbs and Rajpurohit, 2010). Thus, traits sensitive to temperature might play a major role in shaping clinal variation in desiccation tolerance in Indian drosophilids. Relatively few mechanistic studies of desiccation tolerance have been performed, but these have concluded that clinal patterns have diverse physiological bases (Parkash et al., 2011). We note that species differed in overall desiccation tolerance (Table 3). This may be caused by adaptation to different microclimates within a given region or may reflect phylogenetic differences (Gibbs and Matzkin, 2001).

On the Indian subcontinent, changes of humidity with latitude are significant and should be relevant to clines in desiccation tolerance. Thus, rainfall, average relative humidity and variability in relative humidity have been considered as the strongest selective agents (Parkash et al., 2011). However, total annual rainfall was not correlated with latitude and altitude within our data set (Table 1). Likewise we did not find any significant relationship of rainfall with desiccation tolerance (Table 4). Average relative humidity also did not explain variation in desiccation tolerance in any species.  $RH_{cv}$  (in addition to latitude) was significantly correlated with desiccation tolerance in the multiple regression model for only one species, *S. kikkawai*. Average humidity and rainfall were closely positively correlated during the year round and during the rest of year, and negatively correlated with latitude, but rainfall was positively correlated with latitude in the monsoon season. Effects of humidity and



**Fig. 4.** Relationship between relative desiccation tolerance of six drosophilid flies and year round average relative humidity. Values are relative to the mean survival time for all populations assayed within each species. Circle = *Sophophora melanogaster*, square = *S. kikkawai*, diamond = *S. ananassae*, triangle = *S. bipectinata*, x = *Drosophila repleta*, + = *Zaprionus indianus*. Trend line does not show a significant relationship.

rainfall on flies were weak (Table 4). They did not become significant explaining variables when the monsoon and rest of year were analyzed separately. Our somewhat surprising conclusion is that, despite the correlation of  $RH_{ave}$  and  $RH_{cv}$  with latitude, local humidity conditions do not seem to affect desiccation tolerance within species.

One possible explanation for this finding is seasonal differences in relative humidity. Rainfall on the Indian subcontinent is bimodal, with >90% of annual precipitation occurring during the monsoon in many areas (data not shown). Thus, selection for desiccation tolerance may be stronger during the rest of the year. However,  $RH_{ave}$  was not significantly correlated with latitude at this time, but was during the wet monsoon months.

Microclimate variation can also explain the lack of correlation between  $RH_{ave}$  and desiccation tolerance. Many of the collections used in our analysis were from fruit markets, so latitudinal differences and seasonal fluctuations experienced by these populations are probably smaller than macroclimatic variations. None of the species covered in this study are specialists, and all the species are easily available throughout the year from the reported sites (S. Rajpurohit, personal observations).

Five of seven species demonstrated positive significant slope values (clines) of desiccation tolerance against latitude (Table 3) which suggests common selection pressures are present. Specific reactions to climate variables also appeared in some species (Table 5). Thus, while there are parallel latitudinal trends, different local climatic conditions may have greater relative importance in different species. The slopes of the clines also appeared to differ among species. For example, relative desiccation tolerance of *S. melanogaster* increased with latitude less rapidly than relative desiccation tolerance of *Z. indianus* (Fig. 2). This may reflect greater gene flow or less climate-related adaptation to local conditions in *S. melanogaster*. Overall genetic variation may also be a factor; *Drosophila* climatic specialists from wet tropical rainforests of Australia lack genetic variance for desiccation resistance (Kellermann et al., 2009).

Overall, seasonal climate variability was more closely correlated with desiccation tolerance than mean conditions. Northern climatic conditions are cold, dry and variable while southern ones are warm and humid, with little seasonality. To survive these contrasting climatic conditions, northern populations have evolved multiple physiological mechanisms to conserve water, including increased melanization, greater amounts of cuticular lipids, and differences in energy storage patterns. Interestingly, though, different species

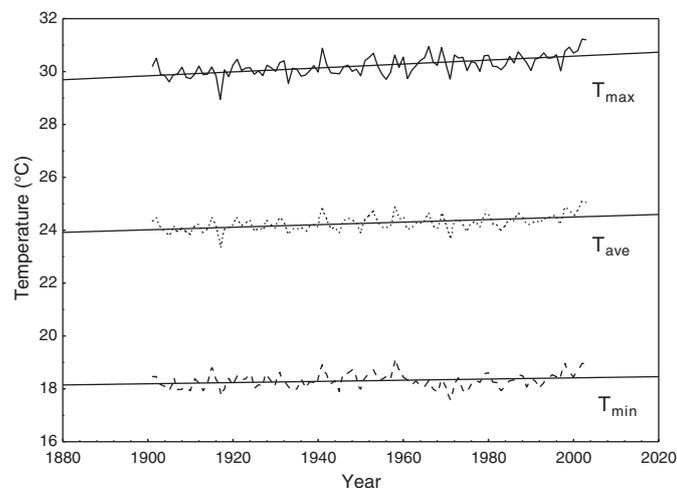
may use different mechanisms to achieve greater desiccation tolerance (Parkash et al., 2008a,b, 2009b, 2012).

Localities at higher altitude along the subcontinent are also characterized by much lower temperature and lower humidity (Mani 1968). Parkash et al. (2008a, 2009b) found that traits varying latitudinally also varied altitudinally along the Indian subcontinent. For all three physiological traits (melanization, desiccation tolerance and rate of water loss), they predicted trait values from multiple regression analysis as a simultaneous function of annual average temperature and relative humidity, and these predictions matched the observed values. This suggests temperature is a common selective force, with relative humidity also playing a supporting role in tropical mountains. However, these authors did not properly analyze the seasonal variation of these traits.

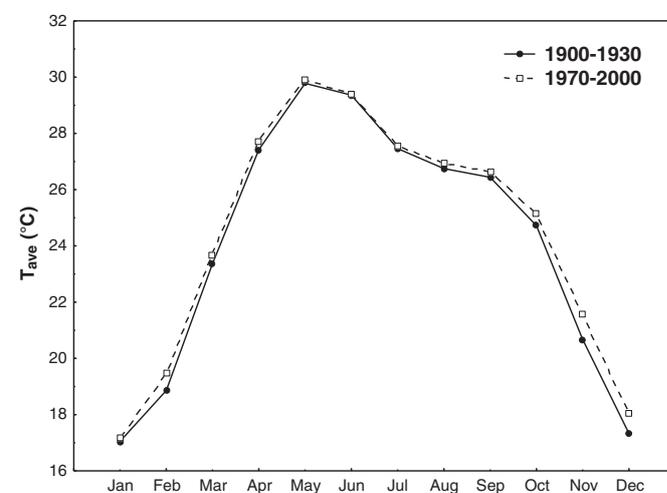
#### 4.1. Long-term temperature trends on the Indian subcontinent

In the last century, average, maximum and minimum temperatures have significantly increased on the Indian subcontinent (Fig. 5). Short generation time of drosophilids enables a fast evolutionary response to climatic changes, and thus these recent measurements (only 100 years) may be reflected in their actual physiological properties. Rajpurohit et al. (2008a,d) showed that  $T_{ave}$  increased between 1955 and 2003 in the western Himalayas. This increase in temperature has affected the distribution, species richness and diversity of insects in recent decades (Rajpurohit et al., 2008a,d). The regional climatic modeling system PRECIS (Providing Regional Climate for Impact Studies) developed by the Hadley Center suggested that there may be an approximate 3 °C rise in temperature as the 21st century progresses, with higher levels of warming in the northern parts of India (Kumar et al., 2006). Average temperatures in almost all months in India increased in the period 1970–2000 in comparison with 1900–1930 (significant at  $p < 0.001$ , based on  $t$ -tests for paired samples, see Fig. 6). These projected trends will impose greater effects on insects' physiology and distribution in general, which could ultimately affect the entire ecosystem at a broader level. The current distribution of several species of drosophilids along a latitudinal cline in India covers differences in average temperature of about 5 °C, comparable with the highest future temperature shifts predicted by global climatic models.

Several drosophilids demonstrate latitudinal clines in desiccation tolerance on the Indian subcontinent. Population variation is correlated with local climatic conditions, especially seasonal thermal amplitude ( $T_{cv}$ ). Physiological mechanisms for desiccation tolerance have been identified in many of these species. Indian drosophilids



**Fig. 5.** Trends of minimum, average and maximum temperatures observed in India from 1903–2003. Linear regression slopes for all three are statistically greater than zero ( $p < 0.05$ ). Data were obtained from the Indian Institute of Tropical Meteorology, Pune.



**Fig. 6.** Shift of monthly average temperatures observed between the period 1900–1930 and 1970–2000 on the Indian subcontinent. Data were obtained from the Indian Institute of Tropical Meteorology, Pune.

therefore may provide an excellent system for development and testing of species distribution models (Elith and Leathwick, 2009). It would also be informative to monitor changes in Indian drosophilids in the context of global climate change.

We suggest that southern Indian populations have the potential to adapt to the changing climate during the following decades. However, in those regions where climate variability will increase, fly populations may have to evolve greater desiccation tolerance or they may be replaced by populations that are already more desiccation tolerant. Populations from the north are likely to be selected for even greater desiccation tolerance, but current information does not allow us to predict whether they can do so. Future efforts should be made to monitor whether drosophilid populations persist or species' distributions shift, and ecological modeling of such patterns should be performed.

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