

RESEARCH ARTICLE

Effects of saturation deficit on desiccation resistance and water balance in seasonal populations of the tropical drosophilid *Zaprionus indianus*

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ABSTRACT

Seasonally varying populations of ectothermic insect taxa from a given locality are expected to cope with simultaneous changes in temperature and humidity through phenotypic plasticity. Accordingly, we investigated the effect of saturation deficit on resistance to desiccation in wild-caught flies from four seasons (spring, summer, rainy and autumn) and corresponding flies reared in the laboratory under season-specific simulated temperature and humidity growth conditions. Flies raised under summer conditions showed approximately three times higher desiccation resistance and increased levels of cuticular lipids compared with flies raised in rainy season conditions. In contrast, intermediate trends were observed for water balance-related traits in flies reared under spring or autumn conditions but trait values overlapped across these two seasons. Furthermore, a threefold difference in saturation deficit (an index of evaporative water loss due to a combined thermal and humidity effect) between summer (27.5 mB) and rainy (8.5 mB) seasons was associated with twofold differences in the rate of water loss. Higher dehydration stress due to a high saturation deficit in summer is compensated by storage of higher levels of energy metabolite (trehalose) and cuticular lipids, and these traits correlated positively with desiccation resistance. In *Z. indianus*, the observed changes in desiccation-related traits due to plastic effects of simulated growth conditions correspond to similar changes exhibited by seasonal wild-caught flies. Our results show that developmental plastic effects under ecologically relevant thermal and humidity conditions can explain seasonal adaptations for water balance-related traits in *Z. indianus* and are likely to be associated with its invasive potential.

KEY WORDS: *Zaprionus indianus*, Desiccation resistance, Phenotypic plasticity, Energy metabolites, Seasonal variation, Saturation deficit, Water balance

INTRODUCTION

Drosophila species have adapted to spatially and temporally varying habitats that differ in environmental variables such as thermal and humidity conditions (Gibbs and Matzkin, 2001; Gibbs, 2002; Ashburner et al., 2003). Under conditions of low humidity, various *Drosophila* species have evolved mechanisms to cope with desiccation stress by lowering their body water loss and have successfully colonized arid habitats, including deserts and/or high

altitudes in tropical and temperate regions (Hoffmann and Harshman, 1999; Gibbs, 2002; Hoffmann and Weeks, 2007; Parkash et al., 2008). Enhanced desiccation resistance of cactophilic *Drosophila* was described on the basis of highly desiccating conditions in deserts and it was inferred that desiccation stress has been an important selective factor in the evolution of xeric species compared with their mesic relatives (Gibbs and Matzkin, 2001; Gibbs, 2002). Furthermore, in *Drosophila melanogaster*, individuals acclimated to low humidity [40% relative humidity (RH)] survived significantly longer than their counter-replicates acclimated at 75% RH (Aggarwal et al., 2013). In *Drosophila simulans*, an enhanced level of desiccation has been reported after exposure to high temperature and low humidity (Bubliy et al., 2013). These studies have shown the effects of plastic changes on desiccation resistance in *D. melanogaster* and *D. simulans*. Thus, abiotic environments exert selection pressure on the water balance of drosophilids.

Seasonality is a dominant feature in determining distribution and affecting water balance physiology of small insects (Hadley, 1994; Chown and Nicolson, 2004; Parkash et al., 2009a,b). Seasonal variations have been reported for desiccation resistance and other stress-related traits in *D. melanogaster* (Mckenzie and Parsons, 1974; Parkash et al., 2009a). In *Drosophila serrata*, there is a genetically based increase in cold resistance after winter (Jenkins and Hoffmann, 1999), whereas in *Drosophila jambulina*, melanization and desiccation levels increase in response to the dry season (Parkash et al., 2009b). Likewise, *D. melanogaster* populations from Australia showed higher desiccation resistance in summer season when grown under season-specific simulated conditions in the laboratory (Hoffmann et al., 2005). Previous studies have shown that the magnitude of geographical variation in stress-related traits in *D. melanogaster* varies significantly between populations from different continents. For example, Indian populations of *D. melanogaster* exhibit steeper clines for desiccation resistance compared with conspecific populations from the Australian continent (Parkash and Munjal, 1999; Hoffmann and Harshman, 1999; Hoffmann and Weeks, 2007). This is due to the fact that significant seasonal variation occurs on the Indian subcontinent. Thus, differences in the extent of the seasonally varying environment (thermal as well as humidity conditions) are likely to cause different levels of selection pressure on stress-related traits in populations of various drosophilids from different continents.

Plastic responses due to simulated seasonally varying thermal conditions have been studied in natural populations of *D. melanogaster* from Australia (Hoffmann and Harshman, 1999; Hoffmann and Weeks, 2007). However, in these studies, the possible interactions between abiotic factors, temperature and relative humidity with desiccation resistance in natural environments have not been taken into consideration. In subtropical localities of north

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India, seasons differ significantly in temperature as well as humidity: the hot, dry summer is followed by a warm, humid rainy season, whereas autumn and spring seasons are moderately wet and warm (Parkash et al., 2009a,b; Aggarwal et al., 2013; www.tropmet.res.in; www.accuweather.com). Thus, stenothermal drosophilids are expected to evolve for desiccation resistance largely under the hot and dry summer season compared with other seasons. Since seasonal variations include changes in both temperature and humidity, a joint index called the saturation deficit should be considered to assess relationship between climatic conditions and water conservation in *Drosophila* species. *Zaprionus indianus* is a warm-adapted tropical species of African origin and has recently invaded tropical and subtropical regions of other continents, such as Brazil, and southern and central America (Tidon et al., 2003; Commar et al., 2012). Based on its invasive capacities in different parts of the world, this species has attained a sub-cosmopolitan status (Tidon et al., 2003; Commar et al., 2012). In Brazil, a higher frequency of *Z. indianus* flies was recorded in spring and summer (Tidon et al., 2003; Commar et al., 2012). Thus, it is likely that seasonally varying humidity and thermal conditions might affect distribution as well as resistance level to different climatic stressors in wild populations of *Z. indianus* from different continents.

Furthermore, natural populations of diverse *Drosophila* species are exposed to seasonal changes in relative humidity and temperature across the world, which may make coping with drought a crucial aspect of their ecology (Hadley, 1994; Chown and Nicolson, 2004; Parkash et al., 2009a,b). Previous studies on diverse insect taxa have mainly focused on inter-specific and intra-specific differences in desiccation resistance and its associated mechanisms (Parkash and Munjal, 1999; Gibbs and Matzkin, 2001; Parkash et al., 2008). However, assessment of plastic changes due to a combination of low and high temperatures and humidities under simulated seasonal conditions has received less attention. Season-specific thermal and humidity conditions can provide a way to understand the mechanistic basis of seasonally induced plastic changes for maintenance of homeostatic conditions of water balance-related traits. Moreover, seasonal differences in relative humidity and temperature represented in terms of a saturation deficit have the potential to impact water balance-related traits.

For seasonally varying populations of *Drosophila* species, mechanisms that increase resistance to climatic stresses need to be identified along with environmental cues/variables that change resistance patterns. This can help to identify associations of stress-related traits with specific environmental variables. Most studies on desiccation resistance in diverse insect taxa, as well as on a large number of *Drosophila* species, have employed assay conditions of <5% RH by using chemical desiccants (Hoffmann and Weeks, 2007; Parkash et al., 2009a,b). Few studies have assessed the effects of low humidity (~40% RH) on laboratory-reared populations of some *Drosophila* species (Chown and Nicolson, 2004; Aggarwal et al., 2013; Bublik et al., 2013). A better understanding of desiccation resistance at the interspecific level requires analysis of wild flies and their comparison with isofemale (IF) lines grown under season-specific simulated thermal and humidity conditions. Despite the fact that seasonality is a prominent feature for the Indian subcontinent, previous studies did not compare changes in desiccation or other abiotic stresses in the field, as well as laboratory populations of diverse *Drosophila* species.

In north Indian localities, there are five different seasons (spring, summer, rainy, autumn and winter) that ectothermic insects encounter in nature. In the current study, the tropical drosophilid *Zaprionus indianus*, which is active over four seasons (spring,

summer, rainy and autumn) was investigated. In the north Indian Rohtak region, relative humidity and temperature vary significantly across the seasons. We tested whether the desiccation resistance of laboratory-reared flies under seasonally varying simulated abiotic conditions could match that of wild flies directly captured during different seasons. The wild-collected and laboratory-reared flies were assessed for water balance-related traits and energy metabolites. We investigated the rate of utilization of different energy metabolites and seasonal differences in the energy budget of the flies. We also examined the association between the season-specific saturation deficit and the rate of body water loss in a seasonally varying wild population and those reared under simulated conditions in the laboratory.

MATERIALS AND METHODS

Fly collection and culture

Wild *Zaprionus indianus* Gupta 1970 ($N=350$ – 400 flies from each season) flies were collected by bait trap as well as net sweeping methods in four different seasons (spring, summer, rainy and autumn) from the Rohtak locality (latitude $28^{\circ}08'N$; altitude 220 m). For each season, ambient temperature and humidity were recorded every week (Table 1). We also calculated the saturation deficit (combined measure of temperature and humidity) for each season. Significant differences in mean average seasonal temperature (T_{avg}) and relative humidity (RH) were observed across seasons. For example, T_{avg} was higher for summer ($31.2^{\circ}C$) and rainy season ($30.2^{\circ}C$) and lower in spring ($20.6^{\circ}C$) and autumn ($23.2^{\circ}C$). But, for relative humidity, there were smaller differences in autumn and spring (spring, 49.3% ; autumn, 53.6%), these differences were higher between summer (39.6%) and rainy (79.5%) seasons (Table 1). For each season, wild-caught flies were directly analyzed for desiccation-related traits. Wild-caught females were used to initiate 20 IFs from each season, which were reared under simulated season-specific thermal and humidity conditions and were maintained on standard cornmeal-yeast-agar medium. For each season, cultures were maintained close to their ambient RH (summer, 40% ; rainy, 80% ; spring, 50% ; autumn, 54%) as well as ambient T_{avg} (summer and rainy, $28^{\circ}C$; spring, $21^{\circ}C$; autumn, $23^{\circ}C$). Laboratory analyses were made on G_1 flies after rearing under simulated conditions corresponding to four different seasons. For all cultures, larval density was kept low by limiting the egg laying period to 8 h, which resulted in 40–60 eggs per vial (37×100 mm size). Virgins were collected from newly eclosed flies within 1.5 h intervals using mild solvent ether anesthesia from each IF line and flies were sexed simultaneously based upon their genitalia and each sex kept in separate vials. All assays were performed on 8-day-old virgin flies from 20 IF lines with 10 replicates. Experiments were performed on the same temperature and relative humidity at which flies were grown from each season.

Table 1. Climatic variables for spring, summer, rainy and autumn seasons in Rohtak locality, north India

	Spring (March– April)	Summer (May–June)	Rainy (July– August)	Autumn (September– October)
T_{avg} ($^{\circ}C$)	20.6	31.2	30.2	23.2
RH (%)	49.3	39.6	79.5	53.6
Saturation deficit (mB)	12.21	27.48	8.48	13.61

Values are based on season-specific ambient temperature and humidity levels; saturation deficit was calculated from both these variables.

Desiccation resistance and assessment of cuticular lipid mass

Desiccation resistance and cuticular lipid mass were assessed in wild-caught flies ($N=60$) as well as IF lines ($N=20$ IF lines \times 10 replicates) reared under season-specific temperature and humidity conditions from each season as described in Parkash et al. (2008). For desiccation survival curve analysis, data were pooled from 20 IF lines.

Estimation of energy metabolites and body mass

Trehalose, glycogen, body lipid and protein content, and wet and dry body mass were estimated in wild-caught flies ($N=60$) and IF lines ($N=20$ IF lines \times 10 replicates) reared under season-specific temperature and humidity conditions for each season by following our previous methods (Kalra and Parkash, 2014).

Basic measures of water loss

Total body water content and dehydration tolerance (%) was estimated in IF lines ($n=20$ IF lines \times 10 replicates) reared under season-specific temperature and humidity conditions from each season following standardized methods (Telonis-Scott et al., 2006; Kalra and Parkash, 2014). Dehydration tolerance was estimated as the percentage of total body water lost to death due to desiccation and was calculated by the formula (wet body mass–body mass at death)/(wet body mass–dry body mass) \times 100. Total body water content was estimated as the difference between mass before and after drying at 60°C. The rate of water loss and water loss after hexane treatment was determined in wild-caught flies ($N=60$) and IF lines ($n=20$ IF lines \times 10 replicates) reared under season-specific temperature and humidity conditions from each season, following Wharton (1985) and Kalra and Parkash (2014).

Assessment of extractable hemolymph content

Hemolymph content and tissue water was estimated in IF lines ($n=20$ IF lines \times 10 replicates) as in Folk et al. (2001) and Kalra and Parkash (2014).

Saturation deficit

We calculated the saturation deficit of each season. Saturation deficit is a measure of the drying power of air and is calculated as: $SD=SVP-APV$ where SD is the saturation deficit (mB), SVP is the saturation vapor pressure (mB) and APV is the actual vapor pressure (in mB). The SVP is a function of temperature (in °C) and the actual vapor pressure is a function of relative humidity (Kleynhans and Terblanche, 2011).

Utilization of energy metabolites and hemolymph depletion

Energy metabolites (trehalose, glycogen, total body lipids or proteins) were measured in IF lines ($n=20$ IF lines \times 10 replicates) before and after utilization under desiccation stress in each season. Flies were subjected to different durations of desiccation stress (at 6 h intervals). Rate of utilization of each metabolite was calculated as a regression slope value as a function of desiccation stress duration (Kalra and Parkash, 2014). Rate of energy production and total energy budget was calculated using standard conversion factors following Schmidt-Nielsen (1990).

Statistical analysis

Population means (20 IF lines \times 10 replicates) are given \pm s.e.m. Changes in trait values across seasons were compared with ANCOVA (dry body mass as covariate) followed by Tukey's *post hoc* tests. Pearson correlations were calculated from 20 IF lines for each season. Rate of water loss between control and hexane-treated flies were compared using the Student's *t*-test. Statistical calculations and illustrations were made using Statistica v.5.0 (StatSoft) software.

RESULTS

Relative abundance of wild-caught flies of *Zaprionus indianus* from four different seasons (spring, summer, rainy, autumn) in a north Indian locality (Rohtak) is shown in Fig. 1A. The highest relative abundance of *Z. indianus* was observed in summer (42%) followed by the rainy season (32.5%). Spring (7.5%) and autumn (9.6%) showed

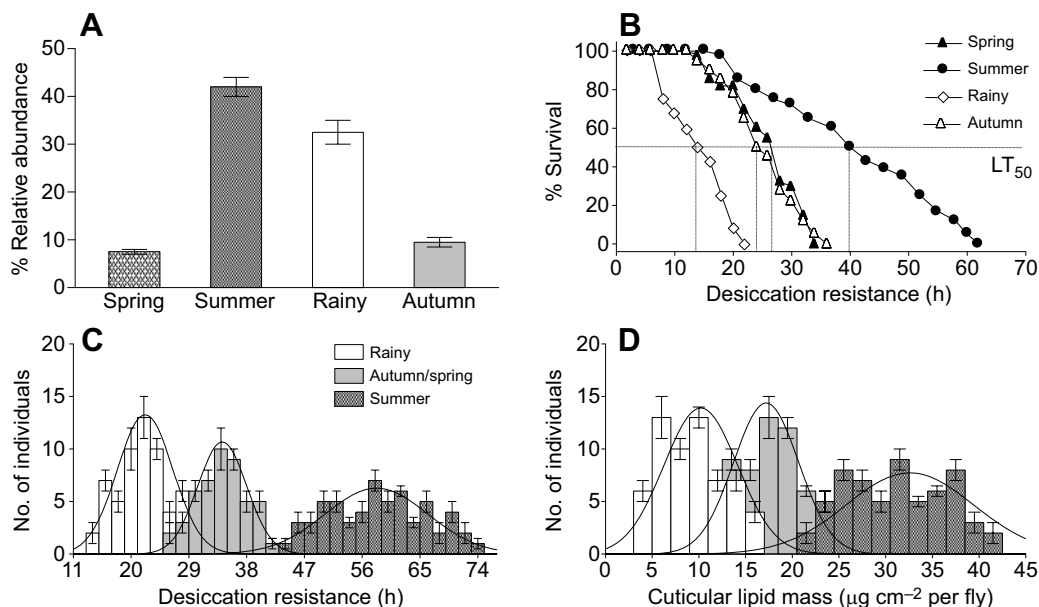


Fig. 1. Relative abundance and desiccation resistance of wild-caught *Zaprionus indianus* collected during four different seasons (spring, summer, rainy and autumn). Relative abundance (A), between-season variability in desiccation survival (B), desiccation resistance (C) and cuticular lipid mass (D) in wild-caught flies ($N=60$). Note that for autumn and spring seasons, values of desiccation resistance and cuticular lipid mass overlap.

Table 2. Desiccation resistance and lipid, carbohydrate and protein measurements in wild-caught *Z. indianus* (N=60 for each trait) over four seasons

	Spring	Summer	Rainy	Autumn
Desiccation resistance (h)	34.35±1.95 ^a	53.23±1.95 ^b	19.12±1.75 ^c	37.50±0.91 ^{a,d}
Cuticular lipid ($\mu\text{g cm}^{-2}$)	17.12±0.35 ^a	29.05±0.62 ^b	9.05±0.05 ^c	20.15±0.24 ^{a,d}
Trehalose ($\mu\text{g per fly}$)	76.58±1.76 ^a	92.16±1.63 ^b	62.52±1.25 ^c	80.15±1.06 ^{a,d}
Glycogen ($\mu\text{g per fly}$)	72.56±1.85 ^a	78.51±0.69 ^b	68.25±0.65 ^c	73.51±0.72 ^{a,d}
Lipid ($\mu\text{g per fly}$)	118.71±2.56 ^a	94.12±1.05 ^b	157.21±0.75 ^c	116.59±0.98 ^{a,d}
Protein ($\mu\text{g per fly}$)	63.15±0.85 ^a	66.88±0.91 ^b	61.05±0.25 ^c	63.01±0.78 ^{a,d}

Data are means±s.e.m. Different superscript letters denote significant differences (ANCOVA followed by Tukey's *post hoc* test, $P<0.05$).

lower relative abundance. Summer and the rainy season showed higher average temperature ($\sim 30^\circ\text{C}$); however, RH was twofold higher in the rainy season than summer season (Table 1). Smaller differences were observed in RH and T_{avg} in spring and autumn (Table 1). These two seasons were moderately wet and moderately warm. Saturation deficit was highest in summer (27.5 mB) and lowest in the rainy season (8.48 mB). Saturation deficit values were intermediate for autumn and spring seasons (Table 1).

Comparison of plastic effects for desiccation-related traits

Seasonal variation for desiccation resistance, energy metabolites and cuticular lipid mass for wild-caught *Z. indianus* from four seasons is given in Table 2. Desiccation resistance of summer flies was significantly higher than rainy season flies (ANCOVA with body mass as covariate, followed by Tukey's *post hoc* tests, $P<0.001$; Table 2). Desiccation survival curves illustrate the divergence of desiccation resistance in four different seasons in wild-caught flies (Fig. 1B). Spring and autumn season flies showed intermediate desiccation resistance compared with rainy and

summer seasons flies (Fig. 1C). Genetic divergence for desiccation resistance and cuticular lipid mass in wild-caught flies from four seasons is illustrated in Fig. 1C,D. There was higher variability for these traits in summer compared with the rainy season (Fig. 1C,D). These differences in desiccation resistance match the trehalose content – trehalose content was higher in summer than in the rainy season ($P<0.001$). However, glycogen and protein levels were slightly higher in summer ($P<0.05$) compared with levels in the rainy season. Lipid contents were higher in the rainy season flies than in summer flies. Furthermore, there were slight but non-significant differences in desiccation resistance and energy metabolites in autumn and spring flies. Cuticular lipid mass also showed plastic responses due to humidity and temperature variability (summer>spring~autumn>rainy; ANCOVA with body mass as covariate, followed by Tukey's *post hoc* tests, $P<0.001$; Table 2). We found similarity between trait values in seasonal populations reared in the laboratory when compared with wild-caught flies by ANCOVA (Table 3). Summer and rainy season flies showed statistical differences in desiccation resistance, cuticular

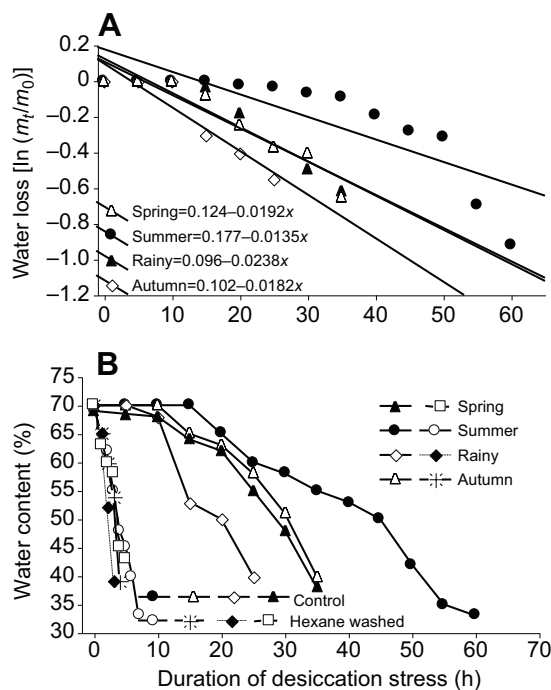


Fig. 2. Body water loss as a function of different durations of desiccation stress in *Z. indianus*. (A) Water loss was calculated according to Wharton's method (Wharton, 1985) for wild-caught flies ($N=60$) in four different seasons where m_t is the water lost at time t and m_0 is the initial water content. Slope values are shown for LT_{100} . (B) Loss of body water content in control flies and in flies treated with organic solvent (hexane).

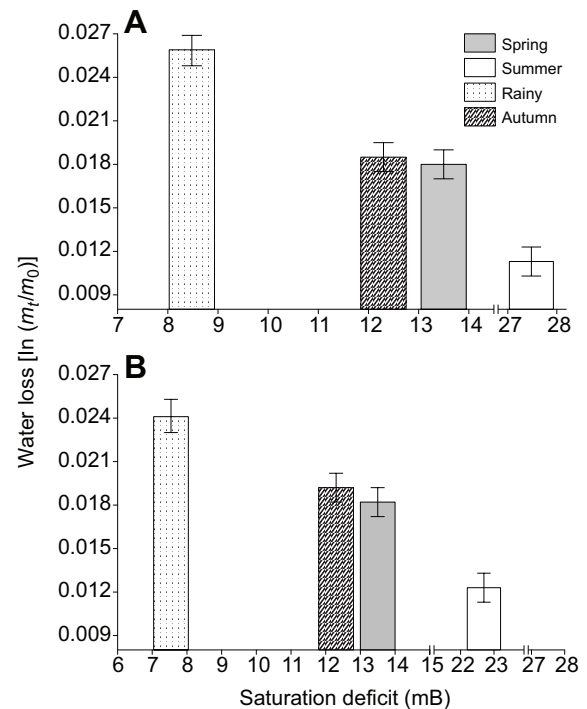


Fig. 3. Rate of water loss across a range of saturation deficits in *Z. indianus* either caught in the wild or raised in season-specific temperature and relative humidity conditions. Values were calculated according to Wharton's method (Wharton, 1985) for wild-caught (A) and laboratory-reared (B) *Zaprius indianus*. Data are means±s.e.m. of $N=60$ flies for each season.

Table 3. ANCOVA for desiccation-related traits of seasonally varying wild-caught versus laboratory-reared individuals (under simulated conditions) of *Z. indianus*

Traits		d.f.	Spring	Summer	Rainy	Autumn
Desiccation resistance (h)	MS	1	4.1 ^{n.s.}	1394.75*	364.18*	133.2 ^{n.s.}
	<i>F</i>	258	2.05	253.59	193.01	28.64
	Error	259	2.12	5.5	1.89	4.65
Cuticular lipid ($\mu\text{g cm}^{-2}$)	MS	1	5.21 ^{n.s.}	998.23*	365.21*	102.32 ^{n.s.}
	<i>F</i>	258	2.63	151.23	121.33	22.09
	Error	259	1.98	6.6	3.01	4.63
Trehalose ($\mu\text{g per fly}$)	MS	1	3.21 ^{n.s.}	652.21**	152.32*	98.54 ^{n.s.}
	<i>F</i>	258	2.11	85.25	74.25	37.9
	Error	259	1.52	7.65	2.05	2.62

** $P < 0.01$; * $P < 0.05$; n.s., not significant.

lipids and trehalose content but such differences were lacking in autumn and spring season flies (Table 3).

Comparison of rate of water loss and effect of organic solvent on rate of water loss

We used independent sets of experiments for different season flies to determine changes in the rate of body water loss in control versus flies exposed to different durations (spring, autumn: 0–25 h; summer: 0–60 h; rainy: 0–35 h) of desiccation stress (Fig. 2A). Wild-caught flies from the dry, hot summer season showed lower water loss than wet rainy season flies (Fig. 2A). Moreover, slope values of water loss increased significantly between LT_{50-100} (half lethal time to full lethal time) for all the seasons (spring: LT_{100} , 0.0192 \pm 0.008; LT_{50-100} , 0.0275 \pm 0.006; $P < 0.001$; summer: LT_{100} , 0.0135 \pm 0.005;

LT_{50-100} , 0.0198 \pm 0.004; $P < 0.001$; rainy: LT_{100} , 0.0238 \pm 0.007; LT_{50-100} , 0.0358 \pm 0.009; $P < 0.001$; autumn: LT_{100} , 0.0182 \pm 0.003; LT_{50-100} , 0.0281 \pm 0.004; $P < 0.001$). Wild-caught flies from all the seasons were treated with hexane and time series changes in percentage body water content are shown in Fig. 2B. Wild-caught flies grown under season-specific simulated growth conditions showed the same trends as wild-caught flies. There was a ~8–15% increase in rate of water loss after hexane treatment in flies from all the seasons (spring: control, 0.0182 \pm 0.001; hexane treatment, 0.154 \pm 0.003; $t = 56.01$, $P < 0.001$; summer: control, 0.0123 \pm 0.002; hexane treatment, 0.238 \pm 0.003; $t = 59.07$, $P < 0.001$; rainy season: control, 0.024 \pm 0.004; hexane treatment, 0.145 \pm 0.002; $t = 25.78$, $P < 0.001$; autumn: control, 0.0185 \pm 0.002; hexane treatment, 0.243 \pm 0.003; $t = 59.86$, $P < 0.001$).

Effect of saturation deficit on rate of water loss

Comparison of the saturation deficit in wild-caught and flies grown under season-specific simulated growth conditions is shown in Fig. 3A,B. Summer season flies have a higher saturation deficit and showed significantly lower water loss than rainy season flies. Values of water loss were intermediate for autumn and spring season flies. Thus, there was trade-off in saturation deficit and rate of water loss among seasonal *Z. indianus* (Fig. 3A,B). Despite similar ambient temperature, but 35% lower relative humidity, summer flies exhibited a significantly lower rate of water loss compared with rainy season flies (Fig. 4B). Similar trends were observed for desiccation resistance (Fig. 4A).

Differences in basic measures of water balance and dehydration tolerance

Data for wild-caught flies grown under season-specific simulated growth conditions are shown in Table 4. A comparative analysis of body mass showed slight differences in measures of wet and dry body mass ($P < 0.01$; Table 4). Rainy season flies showed higher body mass than summer season flies. Likewise, total water content, hemolymph content and hemolymph water content was also higher in rainy season flies than in summer flies. Hemolymph content was ~1.2-fold and hemolymph water content ~1.3-fold higher in rainy season flies. However, there was no difference in tissue water content between flies of different seasons. Interestingly, despite the smaller size of summer season flies, they have higher dehydration tolerance than rainy season flies (Table 4). Furthermore, there was a greater loss of hemolymph water under desiccation stress in summer flies than in rainy season flies, although no significant differences were observed in tissue water loss among the seasons. Thus, differences in dehydration tolerance are due to hemolymph water loss (Table 4).

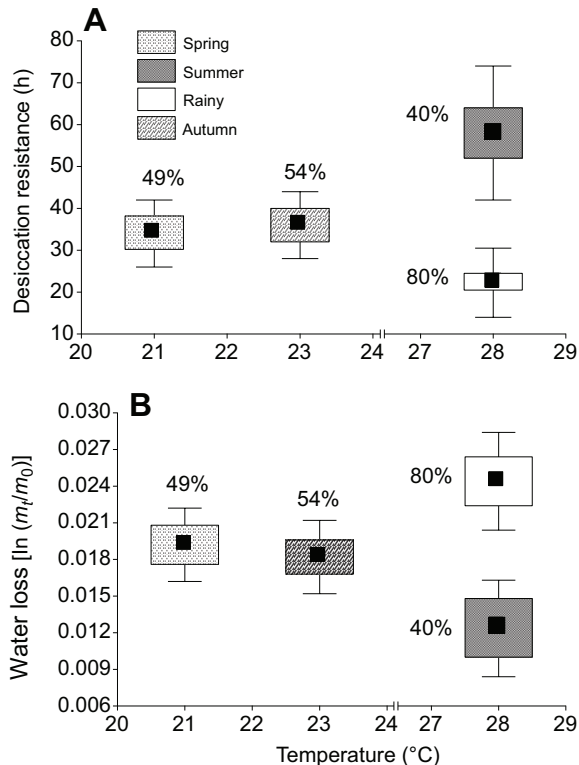


Fig. 4. Desiccation resistance and rate of water loss rate as a function of temperature for wild-caught *Z. indianus*. Desiccation resistance (A) and rate of water loss (B) is shown as a function of seasonal temperature for $N = 60$ flies for each season. Boxes represent s.e.m.; whiskers, s.d.; black squares, mean. Mean value is given alongside bars.

Table 4. Mass and different measures of water balance and dehydration tolerance in laboratory-reared *Z. indianus* under simulated season-specific conditions

	Spring (21°C, 50% RH)	Summer (28°C, 40% RH)	Rainy (28°C, 80% RH)	Autumn (23°C, 54% RH)
Body mass (mg)	2.086±0.018 ^a	1.921±0.014 ^b	2.166±0.028 ^c	2.081±0.011 ^{a,d}
Dry mass (mg)	0.625±0.009 ^a	0.571±0.008 ^b	0.649±0.005 ^c	0.624±0.008 ^{a,d}
Basic measures of hemolymph and tissue water				
Total water content (mg per fly)	1.461±0.007 ^a	1.351±0.002 ^b	1.517±0.002 ^c	1.457±0.006 ^{a,d}
Hemolymph water content (mg per fly)	0.693±0.007 ^a	0.588±0.008 ^b	0.751±0.005 ^c	0.692±0.007 ^{a,d}
Tissue water content (mg per fly)	0.768±0.006 ^a	0.763±0.003 ^a	0.767±0.008 ^a	0.765±0.006 ^a
Measures of dehydration tolerance				
Dehydration tolerance (%)	50.20±1.16 ^a	60.30±2.06 ^b	42.56±1.23 ^c	49.80±1.89 ^{a,d}
Water loss under desiccation stress (mg per fly)	0.745±0.006 ^a	0.814±0.007 ^b	0.645±0.004 ^c	0.742±0.005 ^{a,d}
Hemolymph water loss (mg per fly)	0.446±0.002 ^a	0.513±0.004 ^b	0.342±0.002 ^c	0.443±0.005 ^{a,d}
Tissue water loss (mg per fly)	0.299±0.002 ^a	0.301±0.008 ^a	0.303±0.005 ^a	0.298±0.002 ^a

Data are means±s.e.m.; *N*=20 IF lines×10 replicates. Different superscript letters denote significant differences (ANOVA followed by Tukey's *post hoc* tests, *P*<0.05).

Comparison of rate of metabolite utilization under desiccation stress

Utilization of different energy metabolites (carbohydrates or lipids or proteins) as a function of different durations of desiccation stress (6 h interval) was examined in flies grown under season-specific simulated growth conditions and slope values were compared (Table 5). The levels of trehalose decreased significantly with an increase in the duration of desiccation stress (Table 5). However, the rate of utilization of trehalose was the same for all of the seasons. Furthermore, the net energy budget gained from trehalose and energy budget used under desiccation stress was highest in summer season flies and lowest in rainy season flies and there was a lack of utilization of glycogen, lipids and protein under desiccation stress. Thus, under desiccation stress in *Z. indianus*, the energy budget is exclusively utilized from trehalose rather than any other energy metabolites (Table 5).

Trait correlations

Correlation values of wild-caught flies grown under season-specific simulated growth conditions are shown in Table 6. Correlation (based on 20 IF lines) of desiccation resistance with trehalose content and cuticular lipid mass for all the four seasons was positive and significant (*r*>0.80, *P*<0.001). For glycogen,

lipids and protein there was no correlation with desiccation resistance (Table 6).

DISCUSSION

Adaptation to seasonally varying warmer and drier environments requires water balance homeostasis for survival of *Drosophila* species under natural conditions (Tauber et al., 1998; Danks, 2007; Parkash et al., 2009a,b). In insects, desiccation resistance has been related to: (1) higher bulk water, (2) reduced rate of water loss, and (3) tolerating a larger proportion of overall water loss from the body (Hadley, 1994; Gibbs, 2002; Chown and Nicolson, 2004; Parkash et al., 2008; Kalra and Parkash, 2014; Kalra et al., 2014). Laboratory selection experiments have explored the genetic basis of desiccation resistance and water balance traits in *D. melanogaster* (Gibbs et al., 1997; Telonis-Scott et al., 2006). Nevertheless, the role of abiotic environmental conditions in determining physiological limits of desiccation resistance has been given less attention. There have been very few attempts to compare wild-caught flies of *Drosophila* with flies grown under season-specific thermal and humidity conditions. In the current study, *Z. indianus* were compared from four contrasting seasons that differ in relative humidity and temperature. After spring (mildly dry and warm), summer flies gained resistance to desiccation (~1.7-fold). However, in the

Table 5. Rate of metabolite utilization, net energy budget from carbohydrate, lipids and proteins under desiccation and energy budget used in desiccation for laboratory-reared *Z. indianus* under simulated season-specific conditions

	Spring	Summer	Rainy	Autumn
Rate of utilization (μg h ⁻¹)				
Trehalose	-0.825±0.071***	-0.821±0.091***	-0.826±0.007***	-0.828±0.008***
Glycogen	-0.00549±0.032 ^{n.s.}	-0.00556±0.040 ^{n.s.}	-0.00542±0.014 ^{n.s.}	-0.00551±0.011 ^{n.s.}
Lipid	-0.00442±0.025 ^{n.s.}	-0.00431±0.032 ^{n.s.}	-0.00440±0.012 ^{n.s.}	-0.00421±0.018 ^{n.s.}
Protein	-0.0212±0.032 ^{n.s.}	-0.0216±0.024 ^{n.s.}	-0.0215±0.021 ^{n.s.}	-0.0218±0.019 ^{n.s.}
Net energy budget (J mg ⁻¹)				
Trehalose	1.491±0.009	1.673±0.008	1.153±0.005	1.481±0.009
Glycogen	1.271±0.005	1.384±0.009	1.198±0.004	1.269±0.009
Lipid	5.581±0.015	3.724±0.012	6.214±0.016	5.606±0.014
Protein	1.161±0.011	1.236±0.014	1.133±0.013	1.174±0.012
Energy budget used in desiccation (J mg ⁻¹)				
Trehalose	0.634±0.004	0.704±0.007	0.442±0.003	0.633±0.001
Glycogen	0.0158±0.006	0.0167±0.004	0.0102±0.002	0.0109±0.003
Lipid	0.0353±0.005	0.0361±0.004	0.0359±0.002	0.0352±0.002
Protein	0.00089±0.00006	0.00078±0.00002	0.00087±0.00006	0.00077±0.00002

Data are means±s.e.m.; *n*=20 IF lines×10 replicates. Conversion factors: 17.6 J mg⁻¹ for carbohydrates, 39.3 J mg⁻¹ for lipids, 17.8 J mg⁻¹ for proteins. Slope value represents rate of metabolite utilization as a function of time (negative sign indicates that the metabolite level decreased with time under stress). n.s., not significant; ****P*<0.001.

Table 6. Correlation between desiccation resistance, cuticular lipids mass and energy metabolites in *Z. indianus* reared under simulated season-specific growth conditions

	Spring	Summer	Rainy	Autumn
Cuticular lipids mass ($\mu\text{g cm}^{-2}$)	0.81 \pm 0.05***	0.82 \pm 0.09***	0.81 \pm 0.03***	0.79 \pm 0.05***
Trehalose ($\mu\text{g per fly}$)	0.83 \pm 0.08***	0.89 \pm 0.06***	0.80 \pm 0.05***	0.82 \pm 0.03***
Glycogen ($\mu\text{g per fly}$)	0.13 \pm 0.18 ^{n.s.}	0.19 \pm 0.26 ^{n.s.}	0.08 \pm 0.15 ^{n.s.}	0.23 \pm 0.12 ^{n.s.}
Lipid ($\mu\text{g per fly}$)	0.03 \pm 0.12 ^{n.s.}	0.12 \pm 0.13 ^{n.s.}	0.07 \pm 0.12 ^{n.s.}	0.08 \pm 0.18 ^{n.s.}
Protein ($\mu\text{g per fly}$)	0.06 \pm 0.08 ^{n.s.}	0.09 \pm 0.17 ^{n.s.}	0.18 \pm 0.13 ^{n.s.}	0.10 \pm 0.09 ^{n.s.}

Values are mean \pm s.e.m.; $n=20$ IF lines \times 10 replicates; n.s., not significant; *** $P<0.001$.

subsequent rainy season, desiccation resistance decreased (\sim 2.6-fold) compared with that in summer flies. Autumn and spring flies showed slight differences in desiccation resistance (\sim 2 h; Table 2; Fig. 1). The summer population of *Z. indianus* showed a higher storage of energy metabolite (trehalose) and a threefold increase in the cuticular lipid mass (Table 2), and these traits correlated positively with desiccation resistance (Table 6). Furthermore, we found a trade-off for rate of water loss with saturation deficit across seasons: summer flies showed the lowest water loss with a higher saturation deficit compared with levels in the rainy season (Fig. 3A, B). Thus, increased dehydration stress due to a high saturation deficit in summer is compensated by plastic changes in cuticular lipids as well as trehalose, which are both related to water balance. Moreover, we observed modest statistical differences between wild and laboratory-reared flies for water balance-related traits in seasonal populations: seasonal changes in desiccation-related traits in wild-caught flies and those grown under simulated conditions are due to plastic changes (Table 3). Therefore, seasonal plasticity for desiccation resistance is associated with rearing under season-specific simulated temperature and humidity.

Effect of saturation deficit on desiccation resistance

Seasonality is a dominant feature of north Indian localities i.e. summers are very dry and hot whereas the rainy season is hot and very humid (Parkash et al., 2009a,b). However, temperature and humidity are mild in autumn and spring (Table 1). Therefore, seasonally varying populations of different drosophilids are likely to evolve strategies for maintaining an adequate body water balance under varying climatic conditions (Gibbs, 2002; Chown and Nicolson, 2004; Parkash et al., 2009a,b). In previous work on ectothermic drosophilids, climate change has been studied in terms of temperature only (Hadley, 1994; Chown and Nicolson, 2004). Nevertheless, the relative humidity level is also likely to affect the desiccation resistance of insects and very few studies have actually analyzed the effect of changes in humidity level on desiccation resistance (Kleynhans and Terblanche, 2011; Aggarwal et al., 2013; Bublly et al., 2013). In north India, the rainy season follows summer and there are significant seasonal differences in the saturation deficit of summer (27.5 mB) versus the rainy (8.5 mB) season. It is known that the saturation deficit (drying power of the air) enhances under high temperatures to create a low humidity environment. We observed that for the low humidity summer season (RH, 39.6%), the saturation deficit value increased substantially (more than three times; 27.5 mB in summers; 8.5 mB for rainy season) compared with levels for the highly humid (RH, 79.5%) rainy season, despite

the fact that both seasons have similar thermal conditions. Furthermore, the saturation deficit was intermediate for autumn and spring. Thus, our study illustrates the advantage of saturation deficit over RH as a formal index of desiccation stress when seasonally varying humidity and temperatures are compared. In the rainy season, atmospheric air is saturated with water and *Drosophila* species are less likely to experience desiccation stress than summer season flies. Thus, selection pressure for desiccation is higher in the summer than in the rainy season and flies are expected to evolve more desiccation resistance in summer season. Owing to the higher saturation deficit in summer, the probability of water loss is more, but this is compensated by the higher level of cuticular lipids in summer. In this study, we found that summer flies have higher desiccation resistance, with the lowest rate of water loss under conditions of the highest saturation deficit (Fig. 3A,B).

Some previous studies have shown a modest increase in desiccation resistance due to acclimation of adults of sibling species of *Drosophila* (Hoffmann, 1991; Bublly et al., 2013). For example, one study has reported enhanced desiccation resistance under quite low humidity acclimation of adult flies of *D. melanogaster* (Hoffmann, 1991), whereas another study on *D. simulans* has reported an enhanced level of desiccation after exposure to high temperature and lower humidity (Bublly et al., 2013). Moreover, our results have indicated higher desiccation resistance of summer season flies under a higher saturation deficit – i.e. under conditions of low humidity and high temperature. Furthermore, in the rainy season, where temperature and relative humidity were both highest, there was an enhanced rate of water loss (lowest saturation deficit) compared with levels in the other seasons (Fig. 4B). Thus, based upon our results and results obtained in previous studies, we argue that lower relative humidity rather than temperature should be used as a selection factor for flies to evolve their desiccation resistance.

Relationship between water-balance mechanisms and desiccation resistance

For laboratory populations of *Z. indianus* reared under varying temperatures and humidities, we partitioned different measures of water budget that supported the greater desiccation resistance of *Z. indianus* in summer (\sim 36 h higher) compared with in the rainy season. First, summer flies store more bulk water and because of this, have a \sim 6.5 h longer desiccation resistance than rainy season flies (bulk water difference between two seasons/water loss in summer season; 0.166/0.0241=6.5 h). Second, water loss (WL) differences between two seasons were compared (WL in summer=0.0123 mg h⁻¹; WL in rainy season=0.0241 mg h⁻¹; ratio=0.51), which can account for 21.5 h of increased desiccation resistance in summer than in the rainy season (desiccation resistance in rainy season/reduced water loss; 22.52/0.51=44.15; 44.15–22.52=21.5 h). Furthermore, as *Z. indianus* lost more water content before dying (dehydration tolerance) in summer than in the rainy season, this difference in dehydration tolerance can contribute \sim 7 h of increased desiccation potential in summer (between season difference in total water lost under desiccation/water loss per hour in summer season: 0.169/0.0123=13.56 h; 13.56–6.5 h=7.06 h). We found that *Z. indianus* exhibited \sim 36 h more desiccation survival in summer than in the rainy season. These calculations have suggested an increased desiccation resistance in summer compared with the rainy season as a result of differences in (1) bulk water content (\sim 18%, 6.5 h); (2) water loss (\sim 60%, 21.5 h); and (3) dehydration tolerance (\sim 20%, 7 h). Based upon these calculations, increased desiccation resistance in autumn compared

with the rainy season was contributed by (1) bulk water content (~20%, 2.3 h); (2) water loss (~60%, 6.6 h); and (3) dehydration tolerance (~20%, 2.2 h). Thus, *Z. indianus* maintains water balance by utilizing multiple mechanisms of water conservation by significantly reducing the rate of water loss in particular compared with the other two mechanisms and this might be due to seasonal changes in the amount of cuticular lipids in this species.

Role of cuticular lipids in dry and wet conditions

In insects, the exoskeleton is an important interface between the animal physiology and their environment. Insects in xeric environments generally have a thick cuticle to inhibit water loss as compared with insects in mesic environments (Hadley, 1994; Chown and Nicolson, 2004). Furthermore, scorpions (*Centruroides sculputratus*) collected in the summer have lower water loss rates than those collected in winter (Toolson and Hadley, 1979). In another study, seasonal changes in cuticular lipids have been reported in the beetle *Eleodes armata* (Hadley, 1977). Changes in the composition of cuticular lipids have been associated with improved desiccation resistance when adult flies of *D. melanogaster* were acclimated to desiccation stress (Blomquist and Bagnères, 2010; Stinziano et al., 2015). In the current study, we observed seasonal plasticity for cuticular lipid mass and found significant changes in the rate of water loss across seasons (Table 2; Fig. 2A). We observed approximately threefold higher cuticular lipid mass in summer flies compared with rainy season flies. Although seasonal changes include alterations in temperature as well as relative humidity, previous studies did not consider their combined effects on the quantity of cuticular lipids in different *Drosophila* species. In this study, we observed a significant increase in the quantity of surface lipids in flies collected in summer. Interestingly, summer and rainy season flies have similar ambient temperatures but differ mainly in relative humidity and therefore the tropical drosophilid *Z. indianus* might be affected significantly by the seasonal variation in the saturation deficit.

Seasonal variation in relative abundance

Zaprionus indianus is widespread in tropical and subtropical regions of the world (Commar et al., 2012). Owing to its rapid invasion from its native African origins to South America and oriental regions, it has attained a semi-cosmopolitan status (Tidon et al., 2003; Commar et al., 2012). The invasive capacity of *Z. indianus* on different continents has been argued on the basis of the niche shift hypothesis in contrast to niche conservatism (Commar et al., 2012). Thus, we may expect that changes in plastic responses for morphological and physiological traits of *Z. indianus* might contribute to the invasive capacity of this tropical drosophilid. There are few studies on the relative abundance of *Z. indianus* on the African as well as Indian subcontinent, whereas several recent studies have reported changes in its population dynamics in Brazil (Tidon et al., 2003; Silva et al., 2005; Commar et al., 2012). In the present work, we found an association between seasonal changes in the relative abundance of *Z. indianus* and desiccation-related traits in wild flies. In the current study, a higher relative abundance of *Z. indianus* was found in the hot summer season but this species has also adapted to the highly humid rainy season. High relative abundance in summer is favored by higher desiccation ability (higher amount of cuticular lipids and higher dehydration tolerance) to cope with desiccation stress. In contrast, in the rainy season, flies experience a lower saturation deficit (8.5 mB). Our current as well as previous collections from the laboratory over the years (our unpublished data) have shown a decline in the relative abundance of

Z. indianus in autumn and spring and a complete lack of distribution of *Z. indianus* in the winter season. *Z. indianus* is a predominantly tropical species that is sensitive to cold and optimal temperature for successful development of flies is 20–30°C. Thus, this species has shown plastic adaptation for both warmer and drier climatic conditions (summer) and also for warm and wet conditions (rainy season), which might have led to its tropical distribution worldwide.

Conclusions

For ectothermic insects, seasonal changes in temperature and humidity impact water conservation mechanisms. Despite numerous studies on desiccation resistance in laboratory populations of various *Drosophila* species reared under standard culture conditions (25°C and 60% RH), there have been few attempts to investigate seasonal adaptations in wild-caught flies as well as in flies reared in the laboratory under season-specific simulated growth conditions (Gibbs et al., 1997; Chown and Nicolson, 2004; Telonis-Scott et al., 2006). In the present work, seasonal variation in desiccation-related traits (cuticular lipids mass, dehydration tolerance, rate of water loss and accumulated levels of trehalose) due to plastic effects of seasonal simulated growth conditions correspond to similar changes observed in seasonally varying wild-caught *Z. indianus*. Thus, developmental acclimation or a plastic effect due to ecologically relevant season-specific growth conditions can explain changes related to desiccation resistance and water balance-related traits assessed directly in seasonal wild-caught flies. Furthermore, we found evidence in support of a seasonally varying saturation deficit, which affects evaporative body water loss in wild *Z. indianus* across four different seasons (spring, summer, rainy and autumn). For *Z. indianus*, seasonal changes in the relative abundance are likely to be associated with season-specific differences in desiccation resistance to cope with the saturation deficit. It may be argued that plasticity changes for climatic stress-related traits could be responsible for the greater invasive potential of *Z. indianus* and for it attaining its sub-cosmopolitan status. However, further studies are needed on the thermal adaptations of this species in the context of climatic warming and the associated changes in the saturation deficit.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

B.K. was involved in conceptualization, experimentation, interpretation and analysis of results. B.K. and R.P. both wrote and revised the text.

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