related to the effect of fragmentation on the *Drosophila* communities in the subtropical rain forests of South America.

References: Klaczko, L.B., 2006, Genetica 126: 43-55; Lacy, R.C., 1982, Evolution 36: 1265-1275; Lacy, R.C., 1983, Genetics 104: 81-94; Mateus, R.P., and F.M. Sene 2003, Biochem. Genet. 41: 219-233; Pavan, C., 1959, Bol. FFCLUSP 11: 1-81; Sene, F.M., F.C. Val, C.R. Vilela, and M.A.Q.R. Pereira 1980, Pap. Av. Zool. 33: 315-326; Vilela, C.R., 1992, Rev. Bras. Entomol. 36: 197-221; Vilela, C.R., and G. Bächli 2000, Bull. Soc. Entomol. Suisse 73: 49-65.

Methodology tune up for the assessment of desiccation resistance in natural populations of *Drosophila buzzatii*.

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Introduction

Arid environments impose strong selective pressures on organisms. Particularly, insects and other terrestrial arthropods are susceptible to water loss due to their small size, especially in desert environments (Gibbs *et al*., 1997). Comparative interspecific studies have showed that in the genus *Drosophila*, species from arid environments exhibit physiological characteristics that allow the maintenance of water balance (Marron *et al*., 2003). Intraspecific studies have focused mainly on *D. melanogaster*. These studies do not agree on the mechanisms involved in the ability to cope with desiccation conditions. In order to investigate how hydric stress explains ecological responses and distribution patterns, it appears appropriate to assess the adjustment to dry conditions and its intraspecific variation in desert dwelling species.

Certainly, populations can differ in a trait at a geographic scale, which could also be related to ecological diversity and/or mechanistic variations at lower organization levels. In this sense, a comparative study must focus on the genetic basis of the genotype-environment interaction (Mackay and Anholt, 2007), and how it provides support for a species dealing with challenging and heterogeneous conditions. Such a study requires a prior assessment of experimental conditions in pilot studies, in order to adjust the technique to the selected model species.

The objective of this work was to tune up a method for measuring desiccation resistance in *D. buzzatti*, a cactophilic and widely distributed species in southern South America, and finally to set an experimental design that allows us to detect within and among population variation and phenotypic plasticity by means of the response of flies to different desiccation treatments. The specific questions addressed are: i) how intense should a desiccation treatment be to exert an effect on survival?; ii) how subtle should desiccation be to reveal a differential effect among populations differently adjusted to dry conditions?; iii) which is the most accurate and representative response variable to measure the effect of desiccation treatments among individuals?; iv) does variation in the response to desiccation vary between males and females?; v) is there standing genetic variation (differences among isofemale lines) in the response to desiccation?; vi) does desiccation resistance vary among populations?

Materials and Methods

With this purpose, we carried out two experiments comparing isofemale lines derived from collections in two localities that differ dramatically in rainfall regimes: Montecarlo (Misiones, 26º 33´ 43.2" S, 54º 40´15.4" W, 2302 mm annual rainfall) and Ingeniero Juárez (Formosa, 23º 53´ 58.6" S, 61º 51´3.7" W, 740 mm annual rainfall).

All flies employed in the experiments were raised under optimal density conditions. At four days post-eclosion, flies were lightly anesthetized with CO_2. Ten flies were placed in individual 30 ml vials containing an amount of silica gel desiccant in the bottom of the vials that varied among treatments. A polyethylene sponge was used to keep flies in the upper part of the vial and prevent them from getting into direct contact with the desiccant. Finally, all vials were sealed with Parafilm. Flies were maintained in a chamber with photoperiod 12 l:12 d and 24Cº, and checked at hourly intervals for death, as indicated by failure to right themselves or to move their legs when vials were tapped or inverted (Gibbs *et al*., 1997). Mortality was scored at intervals of 2 h until all flies had died. For each vial we computed the mean survival time and the LT50 score, defined as the time taken for half the flies to die.

In the first experiment, four desiccation treatments differing in the amount of desiccant were tested: 1 g, 2 g, 3 g, and 4 g, as well as a control treatment (0 g). Three replicates for each combination of population, sex, and treatment were set up, making a total of 60 vials (30 vials per population).

Given that this experiment provided a good but scarce diversity of responses, we increased the number of replicates and decreased the difference among treatments in the amount of desiccant.

In the second experiment two additional isofemale lines were chosen from a pool of lines derived from the same populations described above and the treatments tested were: control (0 g), 0.5 g, 1 g, and 3 g of silica gel. We also increased the number of replicates to four. In this experiment the total amount of vials was 64, 16 per sex and population.

Results

Data sets were analyzed with factorial ANOVAs with population, treatment, and sex as categorical variables. Separate ANOVAs were carried out using LT50 and mean survival time of each vial as dependent variables.

In the first experiment, mean LT50 was not significantly different between populations (Montecarlo mean = 22.3 and Juárez mean = 20.9). However, differences between sexes were significant ($F_{1, 37} = 5.09$, $p = 0.029$), with females (mean = 22.63) having a greater desiccation resistance than males (mean = 20.48). The effect of treatment was also statistically significant ($F_{4, 37} = 19.64$, $p = 0.001$). Tukey´s tests showed that the control LT50 was significantly higher than all desiccation treatments ($p < 0.05$), while differences among treatments were not significant. The interaction sex × population was also significant ($F_{1, 37} = 7.18$, $p = 0.011$). In Figure 1a,b it is clear that Montecarlo females have a greater LT50 than both males of the same locality and males and females from Juárez.

Finally, the population × treatment interaction, though non-significant, was close to significance for LT50 ($F_{4, 37} = 2.35$, $p = 0.071$), suggesting that treatments did not affect in exactly the same fashion the flies of both populations. However, the population × treatment × sex interaction was significant ($F_{4, 37} = 3.05$, $p = 0.02$) and the results are illustrated in Figure 1a,b.

Likewise, a similar analysis was performed using mean survival time as the dependent variable. In this case, there were significant effects for all categorical variables and the interactions.

Mean desiccation resistance (mean = 19.8 h) was greater in Montecarlo than in Juárez (18.43 h, $F_{1, 37}$ = 7.009, p = 0.012), and females (mean = 20.19 h) were more resistant than males (mean = 17.96 h, $F_{1, 37}$ = 14.33, p = 0.001). We also detected a significant treatment effect ($F_{4, 37}$ = 27.62, p = 0.001), as for LT50. Mean survival differed significantly between the control and desiccation treatments; however, differences among treatments were not significant. The population × sex ($F_{1, 37}$ = 9.53, p = 0.004) and the population × treatment ($F_{4, 37}$ = 9.66, p = 0.001) interactions were highly significant. The former revealed the same pattern as the LT50, while the second showed each population responded differentially to desiccation treatments. Population × treatment × sex interaction in the ANOVA for mean survival showed a similar pattern to LT50 ($F_{4, 37}$ = 3.59, p = 0.014).

These results suggest fly survival in *D. buzzatii* is affected by desiccating conditions, and that desiccation resistance varies among populations.

The second experiment was designed to identify desiccating conditions that allow getting a deeper insight into the pattern of responses. An additional aim of the second experiment was to incorporate a different set of isofemale lines as a preliminary appraisal of intrapopulation variation.

Mean LT50 was significantly different between populations (Montecarlo mean = 13.3 and Juárez mean = 20.6, $F_{1, 46}$ = 326.38, p = 0.001) and between sexes ($F_{1, 46}$ = 23.45, p = 0.00001), with males showing greater values (mean = 15.9) than females (mean = 17.9). The effect of treatment was also significant ($F_{3, 46}$ = 18.32, p = 0.001). Tukey´s tests showed that LT50 in control vials was significantly higher than in all desiccation treatments ($p < 0.05$) and also detected statistical differences between 0.5 g and 3 g treatments. The interaction sex × population was significant ($F_{1, 46}$ = 13.055, p = 0.0007), Juárez males showed a higher mean LT50 than females of the same population, while there were no differences between sexes in Montecarlo. Finally, the interaction population × treatment was also significant ($F_{3, 46}$ = 22.636, p = 0.0001), survival in Montecarlo flies did not vary among treatments. In Figure 1c,d we present a summary of the patterns of variation ($F_{3, 46}$ = 1.31, p = 0.28).

Afterwards, the same analysis was performed using mean survival time as the dependent variable. In this case, there were significant effects for all categorical variables. Mean survival in Montecarlo (12.4 h) was significantly lower than in Juárez ($F_{1, 46}$ = 436.89, p = 0.001), and female mean survival (15.03 h) was lower than in males (17.16 h, $F_{1, 46}$ = 36.3, p = 0.001). Regarding the significant treatment effect ($F_{3, 46}$ = 32.02, p = 0.001), *post hoc* Tukey´s tests showed that differences between the control and all desiccant treatments were significant, while differences among treatments were not. The population × sex interaction was significant ($F_{1, 46}$ = 19.69, p = 0.001), with the same pattern exhibited by the LT50. Finally, the interaction population × treatment was also significant for mean survival ($F_{3, 46}$ = 34.88, p = 0.001), showing as with LT50, that mean survival time differed between control and desiccation treatments in both populations. Population × treatment × sex interaction for mean survival showed a pattern similar to LT50 ($F_{3, 46}$ = 1.20, p = 0.32).

Discussion

In comparing results from both experiments, we can conclude that there is an important degree of within-population variation, even with respect to the responses of males and females and among treatments. For instance, differences in survival time between sexes were of opposite sign in the two sets of experiments, and also between populations, Montecarlo flies showed greater desiccation resistance than Juárez flies in the first experiment, and the reverse was true in the second one.

On the other hand, the responses to different desiccation treatments varied across experiments. It should be noticed that for the second experiment we employed a smooth gradient of desiccation

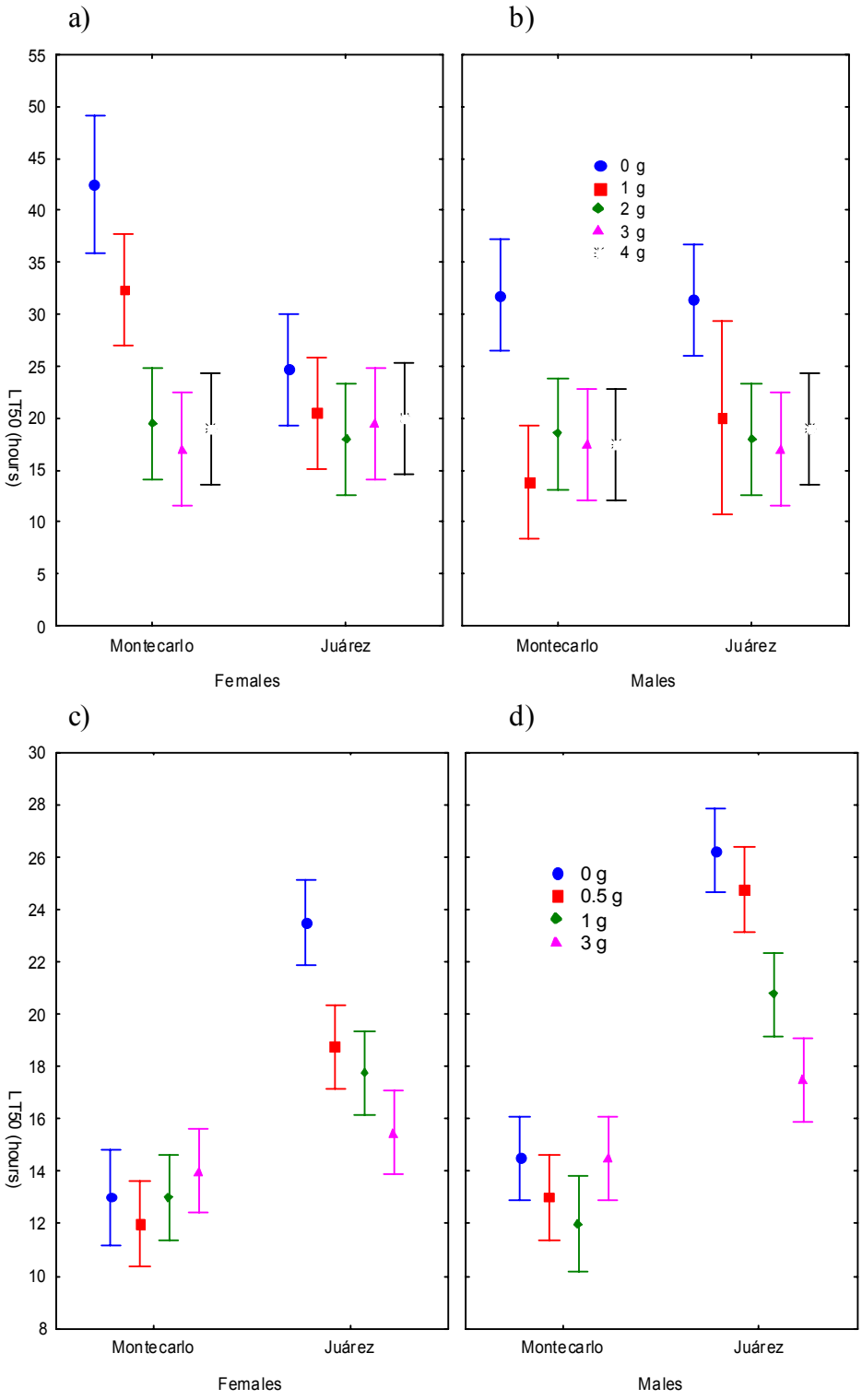

Figure 1. Interaction population × sex × treatment from ANOVAs on the first experiment: a) females, b) males; and second experiment: c) females, d) males. Each point represents the mean LT50 for different treatments, and vertical bars denote 0.95 confidence intervals.

conditions, allowing us to record variations in the responses among individuals under different intensities of desiccation. Thus, a finer gradient of desiccation stress would be an appropriate tool for assessing possible levels of adjustment in natural populations. Indeed, it would better resemble the variation of natural environmental conditions, as well as allow recording the possible plastic responses of flies.

Another objective of the experiments reported herein was to identify the most accurate variable that measures desiccation resistance. In this sense, we found that LT50 and mean survival time provided very similar information, although the former requires less effort and gives less weight to the effect of individual survival on the final measurement. Furthermore, LT50 is the most typical response variable used in desiccation studies, so we follow these criteria and choose it for further experiments.

Finally, our initial questions have been answered, and the information obtained leads us to define an experimental design that includes three desiccation treatments: control (0g), 0.5 g, and 3 g, since these treatments affected differently survival and their effect varied among populations. Both sexes provided complementary information; therefore, it is recommendable to assess desiccation resistance in females and males separately. Our study also revealed among-lines variation. Therefore, we plan to carry out a more comprehensive study assessing 10 isofemale lines per population and 5 replicates per line.

In conclusion, the results reported herein have been useful to define an experimental design to investigate desiccation resistance in natural populations of *D. buzzatii* for which there is no previous knowledge of traits related to resistance to arid conditions.

References: Gibbs, A.G., A.K. Chippindale, and M.R. Rose 1997, The Journal of Experimental Biology 200: 1821-1832; Mackay, T.F.C., and R.R.H. Anholt 2007, Trends in Genetics 23: 311-314; Marron, M.T, T.A. Markow, T.A. Kain, and A.G. Gibbs 2003, Journal of Insect Physiology 49: 261-270.

Insights about the phylogenetic relationship between *Zaprionus* and *Drosophila* from molecular and cytogenetic data of *α-esterase7* gene of *Zaprionus indianus.*

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Introduction

Zaprionus indianus is a species that occurs throughout Africa, India, and Saudi Arabia, as well as in islands of the Indian Ocean (Comores, Madagascar, Seychelles, Mauritius, and Réunion) and the Atlantic Ocean (Canary and Saint Helena) (Chassagnard and Kraaijeveld, 1991; Chassagnard and Tsacas, 1993). In the end of the 20th and the beginning of the 21st centuries, it spreads to South America (*e.g.*, Vilela, 1999; Castro and Valente, 2001; Gõni *et al.*, 2001; Vilela *et al.*, 2001; Tidon *et al.*, 2003) and then to Central and North America (van der Linde *et al.*, 2006).

Zaprionus indianus belongs to the subgenera *Zaprionus*, which contains 44 species from the Afrotropical region (Chassagnard and Tsacas, 1993; Chassagnard, 1996), genus *Zaprionus*