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Larval pupation behavior of the *mesophragmatica* species group of *Drosophila*.

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The *mesophragmatica* species group of *Drosophila* includes at least six sibling species (Brncic and Koref-Santibañez, 1957). Their phylogenetic relationships were established by Brncic *et al.* (1971) and Nair *et al.* (1971) by chromosomal and isoenzymatic studies. Del Pino and Godoy-Herrera (1999) found that the developmental patterns of larval foraging behavior in the group follow those phylogenetic relationships. Thus, in the *mesophragmatica* group, changes in ecologically relevant behaviors exhibited by the larvae during the feeding period paralleled genetic changes that occurred in evolutionary history of the group. Less known is whether changes in larval dispersal patterns involved in selection of pupation sites follow the established phylogenetic relationships among the species of the *mesophragmatica* group. This type of investigation may be of importance to understanding the evolution of behavioral patterns related with dispersal in heterogeneous environments, and it may reveal how closely related species have solved the problem of gaining access to and using resources like food and space.

The stocks employed in this work originated from flies collected by Brncic (1987): i) *D. gasici* (Cochabamba, Bolivia), ii) *D. brncici* (Bogotá, Colombia), iii) *D. viracochi* (Bogotá, Colombia), iv) *D. mesophragmatica* (La paz, Bolivia), v) *D. gaucha* (Buenos Aires, Argentina), and vi) *D. pavani* (La Florida, Santiago de Chile). They are kept by mass culture in the Faculty of Medicine, University of Chile. The flies were all reared in a constant environment at 18°C in half-pint bottles. In this work we used 100 eggs of each of the species above indicated which were distributed on separate cups of 2.0 × 2.5 cm filled with nutritive medium. The cups were deposited into transparent perspex boxes (see Godoy-Herrera *et al.*, 1989, for details). The bottoms of the boxes were filled with agar to a depth of 2 cm; then an area of agar of 9.0 × 4.5 cm was removed along one side to leave a dry surface. This was filled with yellow sand to a depth of 2 cm. Thus, the rearing cup was surrounded by agar and sand. The mature larvae of each of the species could choose to pupate either on agar, on the sand, or in the cup. The sand was humidified with 5 cc of distilled water. The number of pupae detected outside (the agar and sand) and inside the rearing cup was recorded prior to eclosion. We also recorded the number of pupae from each of the species found on and underneath agar and humid sand. Each experiment was replicated four times. The significance of the differences between number of pupae of *D. gasici*, *D. brncici*, *D. viracochi*, *D. mesophragmatica*, *D. gaucha*, and *D. pavani* found inside and outside the food cup was tested by using the G-test of Independence (Sokal and Rohlf, 1995). To test the significance of differences in the number of pupae found on and underneath the agar and humid sand, we applied a Chi-Square of independence R × C test. Before this we carried out unplanned tests of homogeneity for replicates made for each group in each experiment (the R × C test of independence, Sokal and Rohlf, 1995).

The statistical analysis showed that there were no significant differences within a species and among replicates in each experiment, and so we pooled the data as shown below. In Figure 1 is shown the preferences of *D. mesophragmatica*, *D. viracochi*, *D. gasici*, *D. brncici*, *D. gaucha*, and *D.*

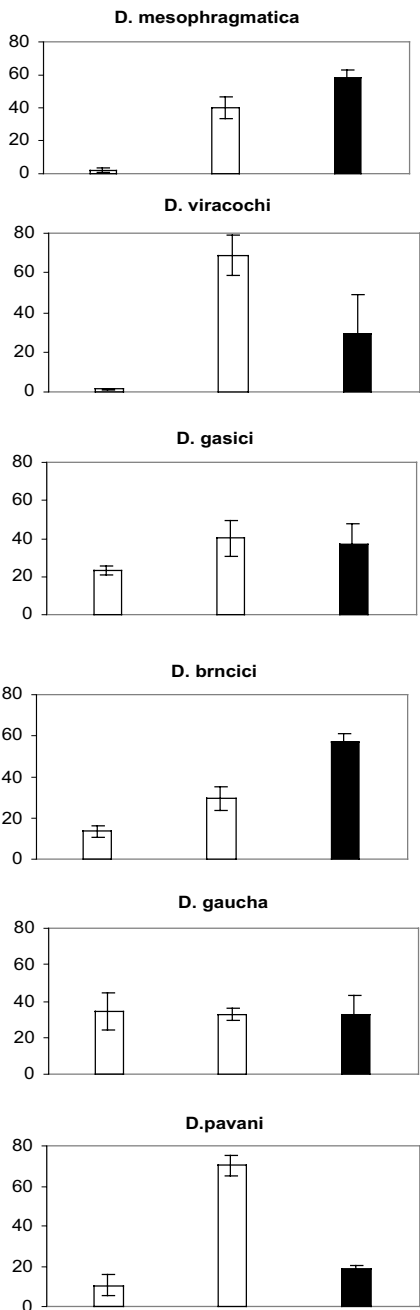


Figure 1. Percentage of pupae of *D. mesophragmatica*, *D. viracochi*, *D. gasici*, *D. brncici*, *D. gaucha*, and *D. pavani* on agar, sand, and in the cup. Each experiment was replicated four times (see details in the text).

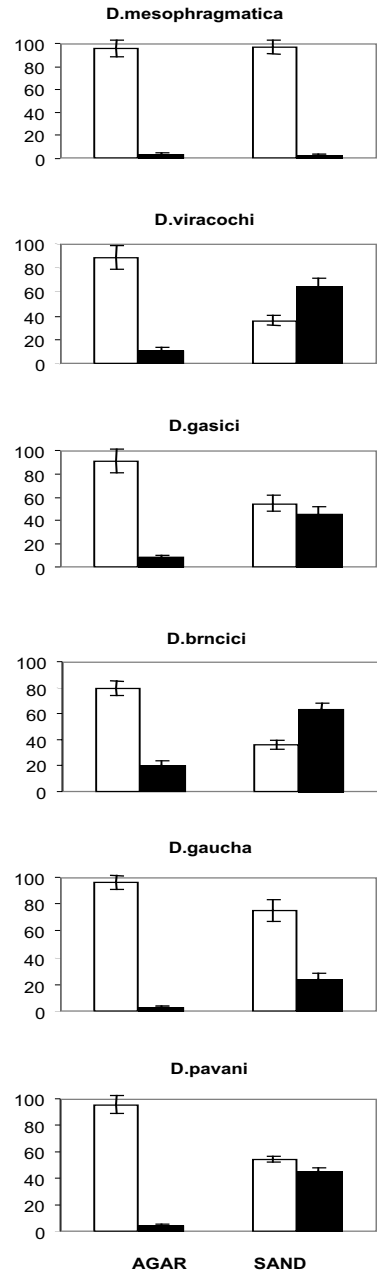


Figure 2. Percentage of pupae of *D. mesophragmatica*, *D. viracochi*, *D. gasici*, *D. brncici*, *D. gaucha*, and *D. pavani* found on and underneath agar and sand. Other details as in Figure 1.

pavani larvae to pupate on agar, moist sand, and the cup. *D. mesophragmatica* and *D. brncici* larvae prefer the sand, while those of *D. viracochi* and *D. pavani* pupate on agar. In contrast, *D. gasici* and *D. gaucha* larvae seem not to show preferences for the substrates to pupate, as shown by the percentages of pupae on the cup, agar and sand (Figure 1). The distributional pattern of pupae of the species is each statistically different with respect to the other ones (the G-test of independence). The Chi-Square values all exceed the critical value 5.99, $df = 2$, $p < 0.05$.

Figure 2 shows the percentage of pupae of the respective species found on and underneath agar and sand. A proportion of the larvae dug into the substrates, as shown by the pupae buried in humid sand and agar. However, *D. mesophragmatica* and *D. gaucha* larvae tend to pupate on the surface of the substrates and in the cup. The proportion of pupae buried in humid sand is higher than in agar for the all species tested, with the exception of *D. mesophragmatica* pupae which were found on the substrates (Figure 2). The distributional patterns of pupae found on and underneath the substrates differ among the species (Chi-square values exceed the critical value 7.81, $df = 3$, $p < 0.05$).

Larval dispersal patterns of *Drosophila* species previously observed to form puparia could be a part of adaptations of these preadults to distribute in heterogeneous environments. The results of this investigation suggest that in the same environments mature larvae of the *mesophragmatica* species group of *Drosophila* exhibit different dispersal patterns when searching for pupation sites. The larvae seem to differ in their responses to water content, compactness and texture of the substrates. Physical characteristics of the substrates also affect larval digging behavior, as shown by the proportion of pupae found underneath the agar and sand (Figure 2). Those characteristics could differ among the environments used by each of the species' lives in wild. Under these conditions, different selective pressures in combination with genetic drift could lead to divergence in larval pupation behavior between the species of the *mesophragmatica* group.

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