

of food and locality. Chilean populations of *D. funebris* are good to investigate the role of *Drosophila* breeding sites in isolation between populations and our understanding of the speciation process in animal species.

Acknowledgments: Thanks are due to Enlace Proyecto ELN012/14, Universidad de Chile.

References: Brncic, D., and T. Dobzhansky 1957, Amer. Nat. 91: 127 – 128; Brncic, D., and P. Sánchez 1958, Amer. Nat. 92: 61 – 63; Carson, H.L., 1965, Chromosomal morphism in geographically widespread species of *Drosophila*. In: *The Genetics of Colonizing Species*. (Baker, H.G., and G.L. Stebbins, eds.), pp. 503 - 531, Academic Press, NY.



The development of hooks in larvae of the two isolates of *Drosophila gaucha*.

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Complex behaviors as food ingestion depend on movements of mouth anatomical components coordinated by the nervous system. In *Drosophila* larvae, the hook movement has a crucial role in ingestion of feeding items allowing food to get into the larval gut (Sewell, Burnet, and Connolly, 1974). Thus, investigations focused on the development of larval hooks are important to understand properly the larval age-related changes in feeding rate (Godoy-Herrera, Burnet, and Connolly, 2005). On the other hand, a comprehension of population variation in development patterns of *Drosophila* larva hooks may clarify the role of genetic and ecological factors in the origin and maintenance of morphological differences linked with food ingestion. We investigated the genetics of morphological changes of hooks through the whole of larval period in two isolates of *Drosophila gaucha* separated by over 1200 km. Specifically, we measured hook width through the whole of larval period of *D. gaucha*, a Neotropical, Latin American endemic species, belonging to the *mesophragmatica* group of species of *Drosophila* (Brncic and Koref-Santibañez, 1957). Populations of the species distribute from South Brazil (Campos de Jordan, CJ), through Uruguay and Argentina (Buenos Aires, BA). We conjectured that geographic variation in development patterns of hook morphology of *D. gaucha* larvae could be indicative of inter population genetic differences in larval feeding rates (Okada, 1963).

Climatic differences

The populations examined live in contrasting environments. The climate in Buenos Aires is temperate-humid (1147 mm of mean rain per year; annual mean temperature is 17.6°C; 25 m over the sea level). In Campos de Jordan (Brazil) the climate is tropical-height (1700 m over the sea level; 1566 mm of mean rain per year; annual mean temperature 13.6°C; Campos de Jordan is the only place in Brazil where snow falls in winter).

Crosses and collection of larvae

We established cultures of the BA and CJ isolates at 18°C. After 12 months, once the cultures were well established, we collected virgin individuals of the two sexes. Fifteen-day-old virgin males and females of the two populations were reciprocally crossed. Homogametic crosses within the strains were also made as controls for the inter-population crosses. We examined N = 50 larvae per each: (i) parental population, (ii) the two reciprocal F₁, (iii) the two out four F₂, and (iv) each one of the 4 out 8 backcrosses. That is, N = 50 per each of the 10 groups of genotypes. Groups of 30-40 inseminated females were allowed to oviposit for 3-4 h on plastic spoons filled with culture medium. Larvae eclosed around 48 h after the eggs are laid. Larvae were collected at successive 24 h intervals after emergence until 192 h of larval age.

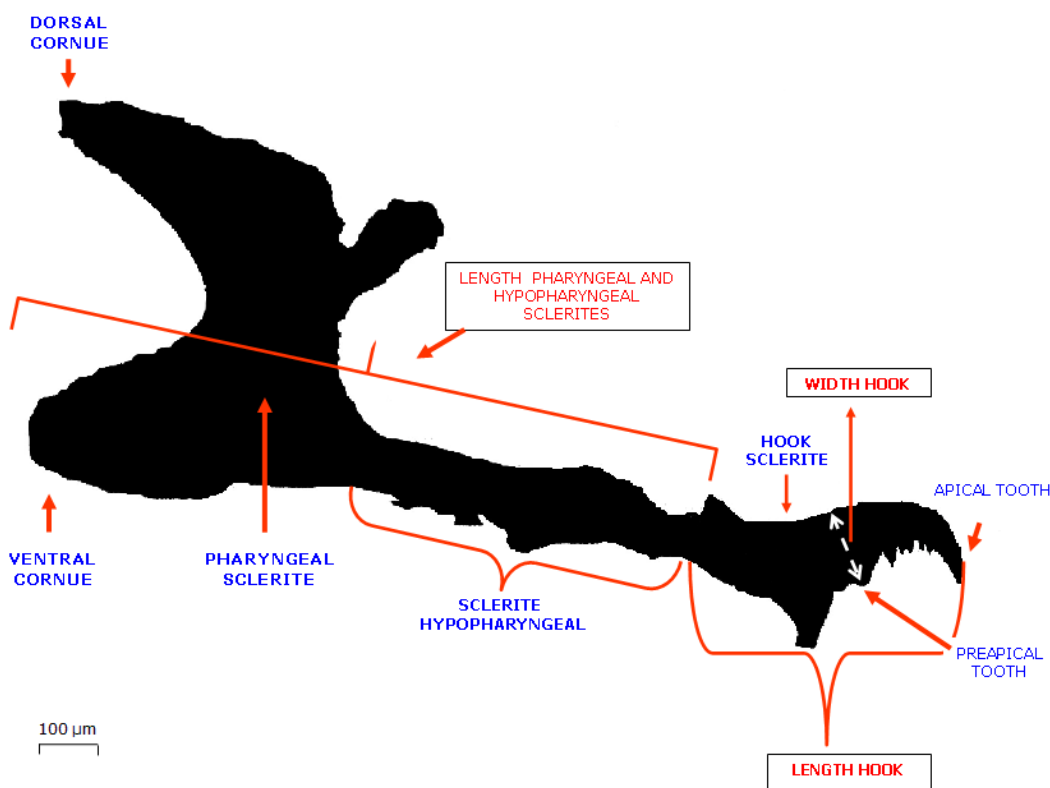


Figure 1. Hook of *Drosophila* larva. The traits measured are indicated by arrows. Hook width is indicated by white arrows.

Larval hooks

Larval hooks, $N = 50$ pairs of hooks per larval age and group of genotypes, were dissected at successive 24 h until 192 h of larval development. Figure 1 shows the hook traits measured. Here, we communicate the data on the development of larval hook width.

Figure 2 shows the changes in hook width between 24 to 192 h of larval development in the Buenos Aires and Campos de Jordan parental strains of *D. gaucha*, reciprocal F_1 , F_2 , and four backcrosses. In larvae of the BA and CJ parents and the two F_1 increase in hook width occurs at 72 and 144 h of larval age. By contrast, in the F_2 and backcross larvae the hook width increase occurs continuously from 24 until 192 h of larval age (Figure 2). These findings suggest that recombination of the BA and CJ genetic heritage substantially modified the development patterns of larval hooks in the F_2 and backcrosses generations.

We made an ANOVA to know whether the growing patterns of hooks in larvae of the ten groups of genotypes analyzed were statistically different. The F-values were: (i) between generations, $F_{3,312} = 3.08$, $P < 0.05$, (ii) between the BA and CJ parents, $F_{1,78} = 0.34$, $P > 0.05$, (iii) the F_1 versus the parents, $F_{2,234} = 0.07$, $P > 0.05$, (iv) the F_2 versus mean backcrosses, $F_{1,78} = 4.22$, $P < 0.05$, (v) between the four backcrosses, $F_{3,234} = 0.03$, $P > 0.05$. The results suggest genetic segregation for development of hook width in larvae of *D. gaucha*.

We carried on the analysis by making further scaling tests to estimate whether the data fitted to an additive-dominant model (Mather and Jinks, 1971). Means and variances of all generations were used. The joint scaling test was applied. It compares the observed generation means with expected values derived from the estimates of the $[m]$, $[d]$, and $[h]$ (details in Mather and Jinks, 1971). The comparison between observed and expected means assumes a χ^2 distribution with degrees of freedom three less than the number of family

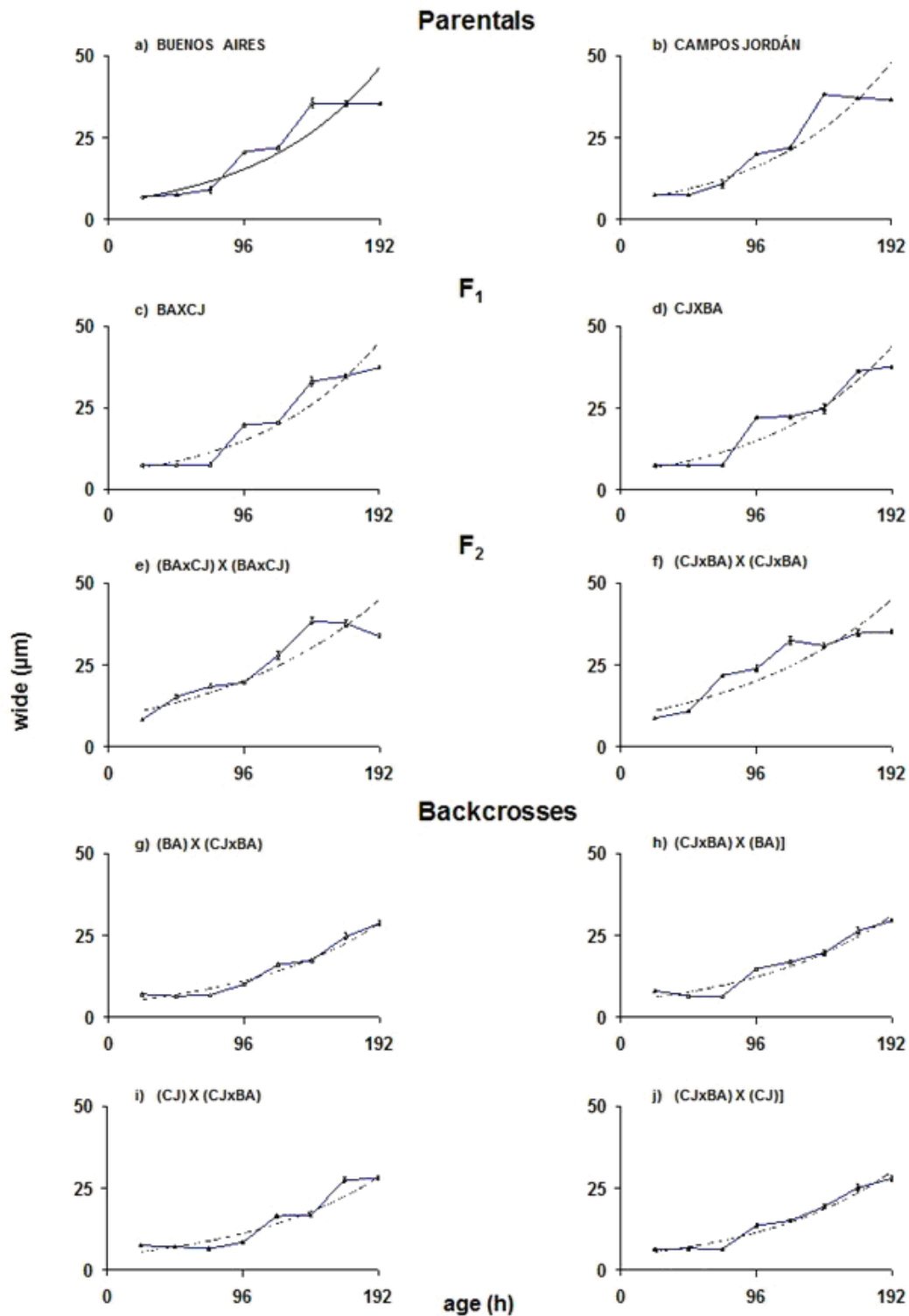


Figure 2. The development of hook width in larvae of *D. gaucha*. (i) parents: Buenos Aires (BA) and Campos de Jordan (CJ), (ii) the two reciprocal F₁, the two reciprocal F₂ and (iv) the four backcrosses. Other details in the text.

means available. We obtained a $\chi^2 = 2.37$, $df = 7$, NS , suggesting a genetic architecture for larval growing of hook width based principally on additive and dominant components.

The results support the conclusion that the Buenos Aires and Campos de Jordan populations of *D. gaucha* differ genetically in growing patterns of larval hooks. Thus, the F_2 and backcross larvae show hook width growing patterns different from those of the parental and F_1 larvae (Figure 2). These inter population differences emerge by introgression of genetic inheritance of the BA and CJ populations of *D. gaucha*. In fact, the two isolates show similar hook width growing patterns, but the F_2 and backcross larvae differ statistically with respect to the parents (Figure 2). We conclude that larvae of the two populations examined exhibit genetic differences for growing patterns of hook width. This is a morphological structure essential for ingestion of food.

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References: Brncic, D., and S. Koref-Santibañez 1957, *Evolution* 11: 300-311; Godoy-Herrera, R., B. Burnet, and K. Connolly 2004, *Heredity* 92: 14-19; Mather, K., and J. Jinks 1971, *Biometrical Genetics*. Chapman and Hall, London; Okada, T., 1963, *Evolution* 17: 84-98; Sewell, D., B. Burnet, and K. Connolly 1976, *Genet. Res.* 24: 163-173.



Length of feeding breaks in larvae of six species of the *mesophragmatica* group of *Drosophila*.

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The structure of feeding behavior of *Drosophila* larva – that is the arrangement and relations between the elements that participate in food ingestion – merits particular attention in view of the fact that the rate of feeding is linked with the larval growth and adult reproduction (Arizmendi *et al.*, 2008). Feeding of *Drosophila* larvae is conformed by periods of intense activity of the hooks in which food gets into the larval gut (feeding bout), interrupted by breaks that affect the rate at which food is ingested (feeding breaks; Green *et al.*, 1983). In the nature, it is also observed that a *Drosophila* larva feeds on decaying fruits by a continuous rhythm of raking movements of the hooks interrupted by frequent breaks (personal observations). We are interested in comparing the structure of larval feeding behavior of *Drosophila* in a phyletic group of species of *Drosophila*. To address this goal we examined duration of break length at 24 h of larval development, that is when feeding rate is low, and at 120 h of larval age when ingestion of food is maximized (Sewell, Burnet, and Connolly, 1975). We studied six species of the *mesophragmatica* group of *Drosophila*. The phylogenetic relationships between the species and the larval foraging behavior are known (Brncic and Koref-Santibañez, 1957; Del Pino and Godoy-Herrera, 1999; Godoy-Herrera, Burnet, and Connolly, 2005). These investigations may be of importance to understand the evolution of organization and functioning of the brain in a *Drosophila* larva.

The species studied were *Drosophila pavani*, *Drosophila gaucha*, *Drosophila brncici*, *Drosophila gasici*, *Drosophila mesophragmatica*, and *Drosophila viracochi*. With the exception of *D. gaucha* that lives in South Brazil, Uruguay, and Argentina, the other species can be collected in Andean habitats and *D. pavani* in Central Valley of Chile (Brncic and Koref-Santibañez, 1957). The flies were all reared in a constant environment under permanent light at 18°C in 300 cc glass bottles containing about 50 cc of Burdick's medium (1954). The six species have similar development times, to molt, wander and pupate (Koref-Santibañez, 1964; Del Pino and Godoy-Herrera, 1999).

Foraging behavior at 24 and 120 h of larval age was recorded individually under stereomicroscope, $N = 50$ larvae per species and age. Larvae tested were transferred to a Petri dish with agar covered by a film of fresh yeast paste. For each larva a new Petri dish was used. Observations were made at 22°C, 90% humidity