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Genetic differentiation between three populations of *Drosophila pavani* that live in different breeding sites in nature.

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Natural selection and genetic drift acting on populations of one species which exploit distinct resources originate genetic differences which may lead to reproductive isolation between them (Ridley, 1996). This is the first step in formation of new species (Coyne and Orr, 1998). *Drosophila pavani* is endemic to Chile, even if it has recently also been collected in Ecuador (Vela and Rafael, 2001). In Chile, *D. pavani* has been found to be an unified species, that is not divided into subspecies (Brncic, 1970). On the other hand, very little is known about the ecology of *D. pavani*. However, we have found that *D. pavani* can use as breeding sites a diversity of substrates which differ in several ecological features. Thus, Manriquez and Benado (1994) reported that the cactus *Echinopsis chilensis*, which may produce substances as alcaloids and triterpens (see Barker, 1990), is an endemic breeding site for *D. pavani*. Adults of the species also may emerge from overipe apples fallen on the ground. In this type of fruits, concentration of ethanol and acetic acid is high (Parsons, 1983). Thus, *D. pavani* represents a favorable material for study of reproductive isolation between populations which exploit different ecological resources. The purpose of this work is to investigate genetic differentiation between three geographically different populations of *D. pavani* with particular reference to intercrosses between them and molecular genetic markers. One population was formed with ancestors which had emerged from overipe tissue of *E. chilensis* growing in Til-Til, a dry place at 50 Km Northwest from Santiago (the Til-Til strain) and the other two strains were originated

Table 1. The F1 offspring obtained to reciprocally cross the Chillán, La Florida and Til-Til strains of *D. pavani*. The F2 and backcrosses are also shown. Ch = the Chillán strain; LF = the La Florida strain; T = the Til-Til strain.

Type of cross	Offspring		Male / female
	male	female	ratio
within strains			
♀ Ch x ♂ Ch	250	268	0.93
♀ LF x ♂ LF	386	379	1.02
♀ T x ♂ T	134	133	1.00
Between strains (F1 generation)			
♀ T x ♂ Ch	-	-	-
♀ Ch x ♂ T	178	229	0.78
♀ T x ♂ LF	-	-	-
♀ LF x ♂ T	140	147	0.95
♀ Ch x ♂ LF	347	348	1.00
♀ LF x ♂ Ch	262	342	0.77
F2 generation			
♀ (Ch x T) x ♂ (Ch x T)	262	279	0.94
♀ (LF x T) x ♂ (LF x T)	274	268	1.02
♀ (Ch x LF) x ♂ (Ch x LF)	304	279	1.02
♀ (LF x Ch) x ♂ (LF x Ch)	287	293	0.98
Backcrosses			
♀ Ch x ♂ (Ch x T)	-	-	-
♀ (Ch x T) x ♂ (Ch)	134	123	1.09
♀ T x ♂ (Ch x T)	306	289	1.06
♀ (Ch x T) x ♂ T	253	229	1.10

from ancestors which emerged from overipe apples collected in Chillán, 420 Km South away from Santiago (the Chillán strain), and in La Florida located in Santiago itself (the La Florida strain).

The stocks are kept by mass culture in the Human Genetic Program, Faculty of Medicine, University of Chile. They were all reared in a constant environment under constant light at 18°C.

Crosses: Twenty-day-old virgin males and females of the Til-Til, Chillán and La Florida strains were reciprocally crossed. The F2 and backcross generations were also obtained (Table 1). Homogametic matings within each strain were also performed to serve as control for the other crosses (see Table 1). After that, females of each cross were allowed to lay eggs for 4-5 days in half-pint bottles containing nutritive medium (Burdick, 1954). Previously, 3-4 drops of live yeast cream were deposited onto the surface of the medium. After 18-20 days the number of males and females emerged from each type of cross was recorded.

DNA Genetics Markers: Six larval genotypes of the Chillán strain and two other groups of seven of the Til-Til and La Florida strains were, respectively, used in this work. Before all DNA extractions, the specimens were individually cleaned and washed in sterile destilled water. Each larva was smashed in 200 µl of Chelex 5%. RAPD analysis was carried out using 8-mer oligonucleotide primers from Operon Technologies Inc: OP-G11, OP-M13, OP-A20, OP-G18 and OP-P04. The conditions of the PCR reactions were the same described by Iturra *et al*. (1998). After each PCR reaction, aliquots of amplified DNA from each of the individuals tested were electrophoresed on agarose gel containing 0.5% TBE. The individual samples obtained with each one of the primers were run together with 1 µl of DNA molecular weight marker to determine the approximate length of the PCR products. After that, we used the pattern bands of each of the larval genotypes to build individual matrices scoring presence (1) or absence (0) of each band. Genetic distances between individuals were estimated by using the Apostol (1994) and Sokal and Sneath (1963) methods included in the RAPD-Distance software. The agreement between both methods was estimated by the Mantel test. The corresponding dendrograms (see one of them in Figure 2) were also yielded by using the NTSYS-pc software (Rohlf, 1994).

Table 1 shows that Til-Til females crossed with males of the Chillán and La Florida strains do not produce offspring. However, the corresponding reciprocal crosses (Til-Til males × Chillán females, and Til-Til males × La Florida females) originate a substantial number of F1 adult flies of both sexes (Table 1). In contrast, the two reciprocal crosses between the Chillán and La Florida strains produce abundant F1 males and females. It is also interesting to note that all F1 flies produce abundant F2 fertile adult flies (Table 1). However, the F1 males, obtained to cross the Chillán and Til-Til strains, backcrossed with Chillán females do not produce offspring (Table 1). In contrast, the F1 females crossed with Chillán males originate adult flies of both sexes. Finally, the reciprocal backcrosses between the F1 and the

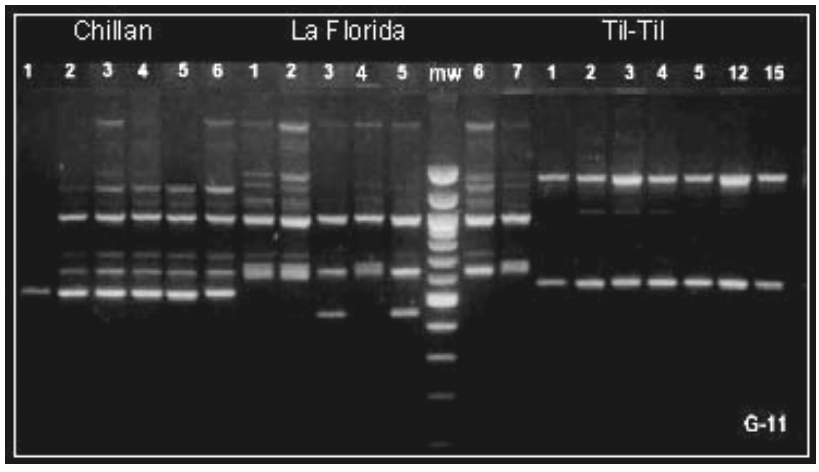

Figure 1. Gel electrophoresis of RAPD products by using G-11 primer. Figure shows the migration patterns of DNA bands of six genotypes of the Chillán strain, and samples of seven genotypes obtained, respectively, from the La Florida and Til-Til strains. (mw: molecular weight marker).

Til-Til strain produce abundant number of males and females (Table 1).

Figure 1 shows the migration patterns of DNA bands of the Chillán, La Florida, and Til-Til strains with the G-11 primer. With the exception of the A-20, M-20 and G-04 primers, the Chillán and La Florida strains show a great similarity of DNA banding patterns which clearly differ respect to those of the Til-Til strain.

Dendrogram (see Figure 2) obtained by using the UPGMA method (Rohlf, 1994) is in good agreement with Figure 1, that is the Chillán and La Florida populations yield very close clusters clearly different to the cluster of the Til-Til population (Figure 2). The mean of genetic distance between individuals within each of the samples is: i) the Chillán strain, 0.13 ± 0.07, ii) the La Florida strain, 0.20 ± 0.01, and iii) the Til-Til strain, 0.09 ± 0.01. These findings suggest that the sample of Til-Til flies is genetically more homogeneous that those of the Chillán and La Florida strains.

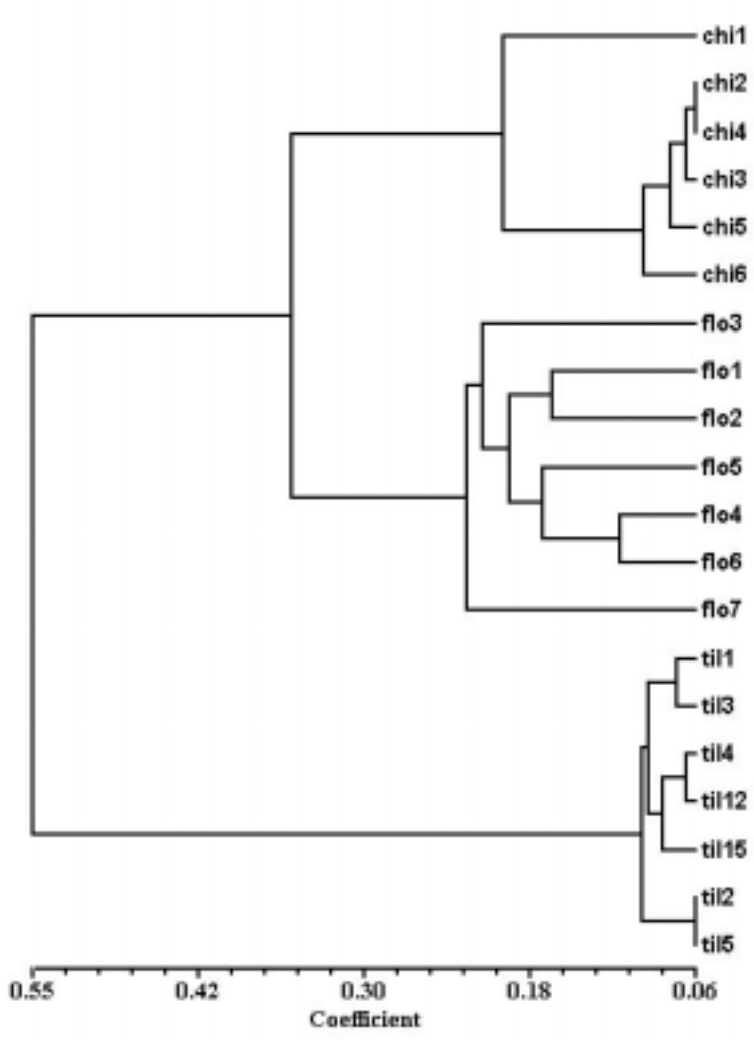

Figure 2. Dendrogram showing the relationships between samples of individuals of the Chillán, La Florida and Til-Til strain (see text). The number of larval genotypes tested were, respectively: i) the Chillán (chi) strain, 6; the La Florida (flo) strain, 7, and iii) the Til-Til (til) strain, 7.

The results of this work show that flies of the Til-Til population of *D. pavani* are in part genetically isolated from the Chillán and La Florida populations. These last two populations are separated by 420 Km, but the results (Table 1) suggest that there is not reproductive isolation between them. On the other hand, the La Florida and Chillán strains were formed with ancestors which had emerged from rotten apples. In contrast, the Til-Til strain was originated with flies emerged from overipe tissue of columnar cactus *E. chilensis*. We would like to suggest that the observed reproductive isolation may be a consequence of genetic differentiation between the examined *D. pavani* populations due to the process of adaptation to very different breeding sites. However, we have not investigated whether the observed restrictions in genetic flow are due to premating isolation or postzygotic reproductive isolation.

It is interesting to note that the results yielded with the genetic markers are in good agreement with the hypothesis of adaptation of the Til-Til population to a breeding site substantially different to those of the Chillán and La Florida strains. That is, the band patterns and cladogram (Figures 1 and 2) indicate that the Til-Til population is genetically very different to the Chillán and La Florida strains. Taken together, our findings suggest that the populations of *D. pavani* here studied have accumulated genetic differences expressed in partial reproductive isolation between the Til-Til and the Chillán and La Florida strains. The isolation could have built because the populations have climbed separate adaptative peaks that correspond to distinct ecological niches, as suggested by

the substrates used as breeding sites. We especulate that the Til-Til × Chillán, and Til-Til × La Florida hybrids could show intermediate phenotypes between the parental strains and thus they may removed by selection. We are planning to investigate this further.

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