

The current study confirmed the utility of the RAPD method, as supplementary too to other methods for obtained intraspecific and interspecific genetic markers, even without any previous DNA sequence information on the species studied.

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Breeding sites of Neotropical Drosophilidae (Diptera). II. Fallen fruits of *Citharexylum myrianthum* Cham. (Verbenaceae).

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One hundred fallen fruits of *Citharexylum myrianthum* Cham. (Verbenaceae) in several stages of rotting were collected by Dr. J.S. Morgante on the ground under one single tree growing on the border of a secondary Forest Reserve located at the Cidade Universitária "Armando de Salles Oliveira", within São Paulo City, state of São Paulo, Brazil. The reserve, which is composed of native and exotic trees (Rossi, 1994), represents a fragment of the Atlantic Forest. The fruits were all collected on 14 February 2000 and were kept in ¼ liter vials (20 fruits per vial) containing a layer of wet sand, under controlled temperature ( $23 \pm 1$  °C) and photoperiod (13 h : 11 h; L : D). From February 20 (first emergence) through March 21 (last emergence), the emerged imagoes were daily aspirated and they are listed in Table 1. The observations were made until April 7, when the remainings of the fruits were discarded.

A total of 416 adult insects (Diptera, Coleoptera and Hymenoptera) emerged, of which 82 % belonged to the family Drosophilidae (n = 341), and of these 56.9 % are *Zaprionus indianus* (n = 194) and the remaining 43.1 % belonged to the following, alphabetically ordered, 13 species of *Drosophila* (in parentheses the number of emerged flies): *D. arauna* (1), *D. capricorni* (2), *D. cuaso* (3), *D. kikkawai* (5), *D. griseolineata* (43), *D. maculifrons* (4), *D. nebulosa* (2), *D. paulistorum* (1), *D. parabocainensis* (1), *D. paraguayensis* (3), *D. polymorpha* (1), *D. trifilum* (1), and *D. willistoni* (80). It should be pointed out that two of the listed species (*D. kikkawai* and *Z. indianus*) are introduced to the neotropics and together they represented most (58.4 %) of the drosophilid flies that emerged from fruits of *Citharexylum myrianthum*.

*Zaprionus indianus* Gupta, 1970 is an invading species of Afrotropical origin, which is also widespread in the Australasian, Oriental and Palearctic regions, in addition to several Islands of the Atlantic and Indian Oceans. It is the most widespread fly (Tsacas, 1985; Chassagnard and Kraaijeveld, 1991; Chassagnard and Tsacas, 1993) of its genus (described by Coquillett in 1901),

Table 1. Drosophilids and other insects emerged in the laboratory from 100 fallen fruits of *Citharexylum myrianthum* Cham. (Verbenaceae) collected on February 14<sup>th</sup>, 2000 at the border of the forest reserve of the *Cidade Universitária "Armando de Salles Oliveira"*, São Paulo City, state of São Paulo, Brazil. In each cell, numbers on the left of the diagonal refer to males and those on the right, to females. The coleopterans were not identified regarding to sex.

| Insects                           | Emergence date: February 2000 |     |     |     |       |       |       |       |      |     |
|-----------------------------------|-------------------------------|-----|-----|-----|-------|-------|-------|-------|------|-----|
|                                   | 20                            | 21  | 22  | 23  | 24    | 25    | 26    | 27    | 28   | 29  |
|                                   | M/F                           | M/F | M/F | M/F | M/F   | M/F   | M/F   | M/F   | M/F  | M/F |
| <i>Drosophila arauna</i>          | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 1/0   | 0/0  | 0/0 |
| <i>Drosophila capricorni</i>      | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 1/0   | 1/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila cuaso</i>           | 0/0                           | 0/0 | 0/0 | 0/0 | 1/2   | 0/0   | 0/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila kikkawai</i>        | 0/0                           | 0/0 | 0/0 | 1/0 | 2/0   | 1/1   | 0/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila griseolineata</i>   | 0/0                           | 0/0 | 0/0 | 0/1 | 1/1   | 4/2   | 5/11  | 8/9   | 0/0  | 0/1 |
| <i>Drosophila maculifrons</i>     | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 2/1   | 0/0   | 0/0   | 0/1  | 0/0 |
| <i>Drosophila nebulosa</i>        | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 1/1   | 0/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila paulistorum</i>     | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 1/0   | 0/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila parabocainensis</i> | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 1/0   | 0/0  | 0/0 |
| <i>Drosophila paraguayensis</i>   | 0/0                           | 0/0 | 0/0 | 0/0 | 0/1   | 0/1   | 1/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila polymorpha</i>      | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/1   | 0/0   | 0/0   | 0/0  | 0/0 |
| <i>Drosophila trifilum</i>        | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 1/0   | 0/0  | 0/0 |
| <i>Drosophila willistoni</i>      | 1/0                           | 0/0 | 1/4 | 4/5 | 15/17 | 13/11 | 6/1   | 0/0   | 1/0  | 0/0 |
| <i>Zaprionus indianus</i>         | 0/0                           | 0/0 | 0/0 | 0/0 | 0/2   | 2/9   | 10/19 | 20/46 | 39/8 | 9/3 |
| <i>Anastrepha amita</i>           | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 0/0   | 0/0  | 0/0 |
| Chloropidae (Diptera)             | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 0/0   | 0/0  | 0/0 |
| Lonchaeidae (Diptera)             | 0/0                           | 0/0 | 0/0 | 0/0 | 0/0   | 0/0   | 0/0   | 0/0   | 0/0  | 0/0 |
| microhymenoptera                  | 0/0                           | 0/0 | 0/0 | 0/0 | 1/0   | 0/0   | 1/0   | 0/0   | 0/0  | 0/0 |
| Nitidulidae (Coleoptera)          | 0                             | 0   | 0   | 0   | 0     | 0     | 0     | 2     | 1    | 1   |
| TOTAL (Insecta)                   | 1                             | 0   | 5   | 11  | 43    | 52    | 55    | 88    | 50   | 14  |

  

| Insects                           | Emergence date: March 2000 |     |     |     |     |     |     |     |     |     |
|-----------------------------------|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                   | 1                          | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|                                   | M/F                        | M/F | M/F | M/F | M/F | M/F | M/F | M/F | M/F | M/F |
| <i>Drosophila arauna</i>          | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila capricorni</i>      | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila cuaso</i>           | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila kikkawai</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila griseolineata</i>   | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila maculifrons</i>     | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila nebulosa</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila paulistorum</i>     | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila parabocainensis</i> | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila paraguayensis</i>   | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila polymorpha</i>      | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila trifilum</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila willistoni</i>      | 0/0                        | 1/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Zaprionus indianus</i>         | 3/3                        | 5/1 | 4/4 | 4/1 | 0/2 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Anastrepha amita</i>           | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 1/0 | 3/3 | 6/2 | 1/1 | 1/1 |
| Chloropidae (Diptera)             | 0/0                        | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 | 0/1 | 0/0 | 0/0 | 0/1 |
| Lonchaeidae (Diptera)             | 0/0                        | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 0/1 | 0/0 | 0/0 |
| microhymenoptera                  | 0/0                        | 0/0 | 1/0 | 1/0 | 0/0 | 0/2 | 0/0 | 0/0 | 1/0 | 0/0 |
| Nitidulidae (Coleoptera)          | 5                          | 2   | 4   | 0   | 0   | 2   | 2   | 0   | 0   | 0   |
| TOTAL (Insecta)                   | 11                         | 9   | 13  | 6   | 4   | 5   | 9   | 9   | 3   | 3   |

Table 1. Continued

| Insects                           | Emergence date: March 2000 |     |     |     |     |     |     |     |     |     |
|-----------------------------------|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                   | 11                         | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 21  |
|                                   | M/F                        | M/F | M/F | M/F | M/F | M/F | M/F | M/F | M/F | M/F |
| <i>Drosophila arauna</i>          | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila capricorni</i>      | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila cuaso</i>           | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila kikkawai</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila griseolineata</i>   | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila maculifrons</i>     | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila nebulosa</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila paulistorum</i>     | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila parabocainensis</i> | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila paraguayensis</i>   | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila polymorpha</i>      | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila trifilum</i>        | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Drosophila willistonii</i>     | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Zaprionus indianus</i>         | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| <i>Anastrepha amita</i>           | 0/2                        | 1/1 | 1/0 | 1/1 | 3/4 | 1/0 | 0/1 | 2/0 | 1/1 | 0/0 |
| Chloropidae (Diptera)             | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| Lonchaeidae (Diptera)             | 0/0                        | 0/0 | 1/1 | 0/0 | 0/0 | 0/0 | 1/0 | 0/0 | 0/0 | 0/1 |
| microhymenoptera                  | 0/0                        | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/1 |
| Nitidulidae (Coleoptera)          | 0                          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| TOTAL (Insecta)                   | 2                          | 2   | 3   | 2   | 7   | 1   | 2   | 2   | 2   | 2   |

M = male; F = female

Note. The last emergence occurred on 21.III.2000 and no insect emerged on 20.III.2000.

which currently comprises 57 described species. Very recently (Vilela, 1999), it was also recorded for the first time from the American continent (Brazil), where it arrived most probably late 1998 or early 1999. Soon after it had been first recorded from Brazil (Santa Isabel, state of São Paulo) it was reported for the first time (Vilela *et al.*, 1999, 2000) as a pest that caused serious damage to commercial figs (*Ficus carica*) in the Valinhos area, a site also located in the state of São Paulo, Brazil.

As a generalist species (Chassagnard and Kraaijeveld, 1991) it is apparently becoming a semicosmopolitan species in the tropical zone of the world. In the process of monitoring its route in the Neotropical region, some native neotropical fruits, as well as exotic fruits, are being analyzed as potential breeding sites for the larvae of *Zaprionus indianus*. Here we report the results of the analysis we did regarding the fruits of *Citharexylum myrianthum*, a plant belonging to the Verbenaceae and endemic to eastern South America, from French Guiana to Brazil, where it ranges from the state of Amazonas to the state of Rio Grande do Sul (Pio Corrêa, 1984; Lorenzi, 1992). It is worthwhile to note that Lachaise and Tsacas (1984) reported 73 kinds of fruits belonging to 31 families of plants, both native and introduced to Africa, as breeding sites for the fruit breeder *Z. indianus*.

As there is no previously published paper regarding the use of fruits of *Citharexylum myrianthum* Cham. as breeding sites for Drosophilidae, it is impossible to assess if the introduction of *Zaprionus indianus* is causing, and if so at what extent, the exclusion of any native neotropical species of *Drosophila* from such a resource.

Other Diptera that emerged are ascribed to the families Chloropidae, Lonchaeidae and Tephritidae. No attempts were made to identify the flies of the first two families to genus or species level. Among the latter family only one species emerged. A total of 39 imagines (22 males and 17 females) of *Anastrepha amita* were reared from the fallen fruits, soon after the emergence of the drosophilids had finished (Table 1), and they represented 9.4 % of the emerged insects.

The genus *Anastrepha*, endemic of the American continent, comprises 18 groups of species (Aluja, 1994). *A. amita* belongs to the *fraterculus* group, which is widespread from USA (Texas and southern Florida) to northern Argentina. Their host plants are very diverse and at least seven out of the 27 species belonging to the *fraterculus* group are reported to have been reared from fruits of native host plants ascribed to three or more families (Norrbon *et al.*, 2000). Since some *Anastrepha* species attack fruits of commercial varieties, the fruit flies of this genus acquired the status of insect pests. According to host utilization, the *fraterculus* group includes generalist as well as specialist species. As far as it is known, *A. amita* Zucchi, 1979, described from two females collected in the state of Bahia (northeastern Brazil), is a specialist species (Zucchi, 2000) so far only reared from fruits of just one host plant (*Citharexylum myrianthum*). Apparently, the larvae of *A. amita* feed on the seed (D.S., unpublished data) instead of the fruit pulp as it has been reported for most species belonging to the *fraterculus* group. It is worthwhile to note that, probably associated with this fact, only one seed of the remainings of the 100 sampled fruits germinated in the wet sand inside the vials. *A. amita* has been recorded from the Caribbean Islands, northeastern, central and southeastern Brazil (Zucchi, 1979; McPherson *et al.*, 2000; Souza Filho *et al.*, 2000), and its distribution probably is dependent on the geographical range of its putatively unique host plant.

In the species description, Zucchi (1979) indicates that the mediotergite (*sensu* Norrbom *et al.*, 2000; referred to as metanotum and postscutellum by Zucchi) of *A. amita* presents a uniform yellowish color. In the sample of *A. amita* here reported, which was identified through the analysis of the aculeus tip, we have found a variation (polymorphism ?) in the mediotergite color pattern of both males and females. Among the 29 specimens analyzed (10 were not available) regarding to this character, 10 (7 males, 3 females) individuals present the uniform yellowish color (as stated in the original description) and 15 (7 males, 8 females) have two lateral dark brown markings, whereas in the 4 remaining specimens (2 males, 2 females) the markings, although present, are lighter. This variation seems also to be present in other species of the *fraterculus* group (Norrbon *et al.*, 2000).

As we have not individualized either the puparia of the Diptera or the pupae of the Coleoptera, it was not possible to associate undoubtedly the emerged parasitoids with their host species. However, microhymenopterans belonging to the families Braconidae and Figitidae (Eucoilinae) have been reported (Canal and Zucchi, 2000; Guimarães *et al.*, 2000) parasitizing immatures of *A. amita*. Two males (largest ones) out of the eight parasitoids (5 males, 3 females) emerged in the present sample were identified (using the key by Canal and Zucchi, 2000) as belonging to *Doryctobracon areolatus* (Braconidae), and they are likely to be emerged from the puparia of *Anastrepha amita*. The remaining six smaller microhymenopterans, which could be parasitoids of any of the emerged species of Diptera and/or Coleoptera or even hiperparasitoids, were tentatively identified as follows: *Asobara* sp. (Braconidae, 2 males emerged on 24 and 26.II.), Braconidae ? (one female, 06.III), *Leptopilina* sp. (Figitidae, Eucoilinae, one female, 21.III), *Aganaspis* aff. *pelleranoi* (Figitidae, Eucoilinae, one female, 06.III), Pteromalidae (one male, 03.III). A review of the wasps that parasite *Drosophila* species is to be found in Carton *et al.* 1986.

Additionally, 19 beetles belonging to the family Nitidulidae, undetermined regarding to sex, also emerged from the 100 sampled fruits.

Voucher specimens will be housed in the *Museu de Zoologia, Universidade de São Paulo*, São Paulo City, Brazil.

**Acknowledgments:** To Dr. João Stenghel Morgante (IBUSP) for kindly collecting and donating the fruits, and to Keiko Uramoto for identifying the species of *Anastrepha*. We are also indebted to FAPESP (98/10701-4), CAPES, CNPq and FINEP (66.93.0618.00) for financial support. **References:** Aluja, M., 1994, *Annu. Rev. Entomol.* 39: 155-178; Canal, N.A. and R.A. Zucchi 2000, *In: Malavasi, A. and R.A. Zucchi (eds), Moscas-das-frutas de importância econômica no Brasil, conhecimento básico e aplicado*, p. 119-126, Holos, Ribeirão Preto; Carton, Y., M. Bouletreau,

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*Notch* affects the development of *Drosophila* macrochaeta through lateral inhibition but that of wing veins through induction.

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We irradiated first instar larvae of *Drosophila melanogaster* with either the genotype  $w^a Ax^{59d} fl/M(1)o^{sp}$  or  $w^a Ax^{59d} ct/M(1)o^{sp}$  with 1000 R of X-rays in order to get fast-growing homozygous  $w^a Ax^{59d} fl w^a Ax^{59d} f$  or  $w^a Ax^{59d} ct/w^a Ax^{59d} ct$  clones on the *Minute* background. When investigating these clones, we found firstly that patches homozygous for the antimorphic gain-of-function allele,  $Ax^{59d}$ , of the *Notch* gene, and marked with *forked*, carried few, if any, sensory macrochaeta, but macrochaeta could develop along the clone borders. Secondly, we found that wing vein gaps homozygous for  $Ax^{59d}$ , marked with *cut*, were significantly longer ( $86 \pm 36$  hundredth of millimeter) than the gaps in the veins of the opposite wing ( $57 \pm 27$ ) ( $F = 9.533$ ;  $P = 0.003$ ). Further we observed that wing vein gaps extended slightly longer on the dorsal surface than on the ventral surface of the wing.

These results indicate that the effect of the *Notch* gene on the development of the sensory macrochaeta is based on lateral inhibition (which fact is already well-established), but the effect of *Notch* on the longitudinal growth of the wing vein is of inductive nature, which is a new observation.