

2010) collected from a natural population. Eighteen out of the 20 tested loci had good amplification quality using a pool of individuals of a *D. griseolineata* strain, according to Laborda *et al.* (2009a). However, in the individual DNA samples from freshly collected specimens of this work, only seven (35%) showed amplification. Among these seven loci, two (Dmed^{UNICAMP}_ssr079 and Dmed^{UNICAMP}_ssr118) showed weaker amplification, even when annealing temperature higher (55°C) and lower (53°C) than that applied to obtain the fragment with the expected size were tested. Moreover, these loci were not amplified even using the touchdown PCR condition. The optimal annealing temperature for each primer that showed positive amplification ranged from 50°C to 56°C (Table 1).

The Dmed^{UNICAMP}_ssr107 locus, which showed no amplification for *Drosophila griseolineata* in the work of Laborda *et al.* (2009a), presented positive result in the tests performed in the present work. The proportion of *D. mediopunctata* loci that showed amplification in *D. griseolineata* (35%, Table 1) was lower than that found by Tractz *et al.* (2012) in *D. maculifrons* (50%), despite both belonging to the same group of species. The rate of amplification in *D. griseolineata* was higher when compared with *D. ornatifrons* (28%, Tractz *et al.*, 2012), of the *guarani* group. However, among the loci that showed positive amplification, only three (Dmed^{UNICAMP}_ssr087, Dmed^{UNICAMP}_ssr096, Dmed^{UNICAMP}_ssr118) coincided among *D. griseolineata* and the other two species. On the other hand, five coincident loci were obtained between *D. maculifrons* and *D. ornatifrons* (the same three above, plus Dmed^{UNICAMP}_ssr034 and Dmed^{UNICAMP}_ssr057).

These data indicated that the transferability of the loci described for *Drosophila mediopunctata* to species that belong to closely related groups is reduced in individual samples, which are more adequate for populational analyses. Moreover, despite the higher amplification rate in the *guaramunu* group to be in agreement with the close phylogenetic relationship of this group with the *tripunctata* group, closer than the relationship of the *guarani* group of *Drosophila ornatifrons* with the *tripunctata* group (Kastritsis, 1969; Kastritsis *et al.*, 1970; Hatadani *et al.*, 2009; Robe *et al.*, 2010), the higher number of in common amplified loci between *D. maculifrons* and *D. ornatifrons* than between both species of the *guaramunu* group reinforce the data of Laborda *et al.* (2009a), who postulated that there is no correlation between phylogeny and the results of interspecific amplification.

The microsatellite loci of *Drosophila mediopunctata* that showed good quality amplification in *D. griseolineata* indicated them to be adequate genetic markers to be applied in population studies using this species.

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Interplay between *Drosophila suzukii* and native *Drosophila* species in the Mediterranean area.

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Drosophila suzukii, an endemic to South East Asia, has become an invasive pest starting by the Mediterranean area and in less than 10 years has colonized almost all of Western Europe (Cini *et al.*, 2012). In Catalonia, the species was first detected in 2008 and near Barcelona in 2009 (Calabria *et al.*, 2012). In fly collections performed near Barcelona at the Font Gropa site—in the northern foothills of the Tibidabo hill at 400 m above sea level—in autumn 2012 and 2013, the species abundance was reported to be 8 to 10% of drosophilid flies (Canals *et al.*, 2013; Pineda *et al.*, 2014), suggesting that it was well established in the Barcelona area.

Here we report the presence of *D. suzukii* in two drosophilid collections performed in autumn 2011 (November 2 and 7) and in autumn 2014 (November 14) at the *Observatori Fabra* fields. This site that is described in Orengo and Prevosti (1996) is located, similarly to Font Gropa near Barcelona, but in this case in the southern foothills of the Tibidabo hill at 413 m above sea level. Flies were captured in the afternoon until dusk, netting over fermenting banana baits placed at regular intervals in the Fabra fields.

Drosophilids had been previously collected multiple times at the Fabra fields in the period extending from 1970 to 2007 (Orengo and Prevosti, 1996, and unpublished results). Records from those collections showed that the most common species in that location were *D. subobscura* and *D. simulans*. Those records also showed that both species experienced a population explosion in autumn, with *D. simulans* reaching its maximum abundance first and *D. subobscura* doing it subsequently (de Frutos and Prevosti, 1984; Orengo, 1994).

Table 1. Number (and %) of flies captured in the Fabra fields in autumn 2011 and 2014.

November 2, 2011				
Species	♀	♂	Total	
<i>D. subobscura</i> ¹	11	9	20	(13.99)
<i>D. simulans</i> ²	4	4	8	(5.59)
<i>D. buzzatii</i>	4	1	5	(3.50)
<i>D. suzukii</i>	51	58	109	(76.22)
other	1	0	1	(0.70)
Total	71	72	143	(100)
November 7, 2011				
Species	♀	♂	Total	
<i>D. subobscura</i> ¹	21	158	179	(10.09)
<i>D. simulans</i> ²	493	648	1141	(64.32)
<i>D. buzzatii</i>	11	12	23	(1.30)
<i>D. suzukii</i>	219	208	427	(24.07)
other	1	3	4	(0.23)
Total	745	1029	1774	(100)
November 14, 2014				
Species	♀	♂	Total	
<i>D. subobscura</i> ¹	198	15	213	(77.45)
<i>D. simulans</i> ²	16	13	29	(10.55)
<i>D. buzzatii</i>	13	11	24	(8.73)
<i>D. suzukii</i>	5	1	6	(2.18)
other	3	0	3	(1.09)
Total	235	40	275	(100)

¹ some *D. ambigua* flies could have been included as *D. subobscura*.

² some *D. melanogaster* flies could have been included as *D. simulans*.

period would be successively *D. suzukii*, *D. simulans*, and *D. subobscura*. This observation for the latter two species conforms to those recorded from previous collections in the same area and time period in which the first maximum species abundance in early autumn was that of *D. simulans* followed by that of *D. subobscura* (Orengo, 1994).

When we newly collected drosophilids at the Fabra fields in 2011—*i.e.*, only two years after Calabria *et al.* (2012) reported the presence at low frequency of *D. suzukii* near Barcelona—we found that this species was one of the two most abundant species in this location (Table 1). However, the *Drosophila* species abundance varied greatly between the two samples collected that autumn. Indeed, most flies collected on November 2 were *D. suzukii* individuals (76.22%), being *D. subobscura* the second most abundant species (Table 1). In the much larger sample collected that same year on November 7, the most abundant species was *D. simulans* (64.32%), followed by *D. suzukii* (24.07%) and by *D. subobscura* (10.09%; Table 1). The species abundances also varied greatly between the two samples collected in 2011 and that collected on November 14, 2014. In the latter sample, *D. subobscura* was the prevalent species, and *D. suzukii* was among the least frequent species. Since according to the Observatori Fabra records, climatic conditions at that site along November were very similar in 2011 and 2014, the collection data here reported would indicate that, in autumn, the relatively most abundant species along this

Our drosophilid collection data at Observatori Fabra would support previous observations from collections at Font Gropa indicating that *D. suzukii* is well established in the Barcelona area, even though its frequency was much lower in our last collection in autumn 2014. Its establishment in this area, as well as throughout Europe and North America, raises an important concern given the negative economical consequences of its females ovipositing in commercial fresh fruits and the subsequent damage to the corresponding crops. Moreover, our data would indicate that native species might be affected by the presence of this exotic species that would postpone their autumn population maxima, when they might be subjected to different environmental conditions.

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On the geographic distribution of the *Drosophila willistoni* group (Diptera, Drosophilidae) – updated geographic distribution of the Neotropical *willistoni* subgroup.

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Introduction

Drosophila willistoni species group comprises 24 Neotropical species, divided into three subgroups – *alagitans*, *bocainensis*, and *willistoni* (Bächli, 2015). The *willistoni* subgroup is composed of six sibling species: *D. willistoni*, *D. equinoxialis*, *D. tropicalis*, *D. insularis*, *D. pavlovskiana*, and *D. paulistorum*. The latter is actually a species complex. *Drosophila willistoni* was described as *Drosophila pallida* (Williston, 1896). Since this nomenclature was already used, Sturtevant (1916) changed it to *D. willistoni*. Dobzhansky and Pavan (1943) found two *willistoni*-like species, one more common and slightly smaller than the other. They believed that the more common species was *D. willistoni* and nominated the larger and less frequent species as *D. paulista*. Later, the authors perceived that, in fact, *D. paulista* was a synonym of the *D. willistoni* and, since then, the smaller species was nominated *D. paulistorum* (Dobzhansky and Pavan in Burla *et al.* 1949).

A few years later, three new siblings were described: *D. equinoxialis* (Dobzhansky, 1946), *D. tropicalis* (Burla and Cunha, 1949 in Burla *et al.*, 1949) and *D. insularis* (Dobzhansky, 1957 in Dobzhansky *et al.*, 1957). Also, Townsend (1954) found that *D. tropicalis* comprises two subspecies, *tropicalis* and *cubana*. *Drosophila tropicalis tropicalis* presents a southern distribution and the northernmost register is in Rio Branco, Brazil; while *D. tropicalis cubana* is a northern form and the southernmost register is in Jamaica (Townsend, 1954).

In 1959, Dobzhansky and Spassky discovered that *D. paulistorum* was not a unique species, but a cluster of six incipient species – Amazonian, Andean-Brazilian, Centroamerican, Guianan, Orinocan and