indicates that the level of heterozygosity of all these three enzyme loci did not fluctuate to a large extent between places TSR to AKL.

The high frequency of heterozygosity for esterase coding genes indicates heterozygous superiority in *D. ananassae*. Due to the presence of heterozygotes, there is balancing selection in the population, *i.e.*, the different forms of genotypes have their representation. The extent of heterozygosity is known to vary from population to population. Enzymes that are polymorphic may also be showing variation in the frequency of heterozygotes. Those being more adaptive in heterozygous condition would have a higher frequency of heterozygotes than other polymorphic enzymes. Thus, esterases in *D. ananassae* seem to confer hetrozygous superiority for all the loci concerned, although population-wise differences do exist among them.

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Foreign invasive pests *Drosophila suzukii* (Matsamura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) threaten fruit production in northwestern Argentina.

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Abstract

The sub-tropical region of northwestern Argentina (Tucuman province) shelters a major soft fruit production and exporting industry. *Drosophila suzukii* (spotted-wing *Drosophila*; SWD) is a major global pest of soft fruits because females can lay eggs under the epidermis of healthy, ripening fruit. Recently, Argentina was invaded by the SWD, which has quickly spread to all cardinal points, showing a great ability of adaptation to different climates and fruit crops. We report for the first time the presence of two invasive drosophilid species, SWD and *Zaprionus indianus* (African fig fly), in the sub-tropical rainforest of the Yungas (Köppen-Geiger climate classification CWa), adjacent to a high-value fruit production region, in the province of Tucumán (northwestern Argentina). Both species were recovered from wild guava fruit (*Psidium guajava*). The SWD was found in healthy, ripe fruit attached to the trees (65%) and in damaged fruit collected from the ground (35%), while *Z. indianus* was only recovered from damaged fruit collected from the ground (100%). *Zaprionus indianus*, SWD, and other drosophilids accounted for 86.6%, 7.1%, and 6.3%, respectively, of the total of drosophilids found. The presence of both invasive insects in the region, especially SWD, is a threat for the local berry industry. Since SWD can complete its life cycle in guavas, these fruits would allow the sustainability of SWD populations during the seasons in which commercial berry crops are not in production. Berry growers and plant protection agencies should promptly take measures to limit these pests' dispersion to commercial fruit fields. Keywords: Spotted-wing *Drosophila*; African fig fly; *Psidium guajava*

Introduction

Argentina currently exports over 1.9 million tonnes of fruits and vegetables each year, generating revenues of around 1.7 billion dollars. This condition makes Argentina one of the largest produce exporting countries of the southern hemisphere (Fundación Exportar, 2014), being citrus, berries, pome fruits, and stone fruits the most exported. The subtropical region of northwestern Argentina (Tucuman province), where the rainforest is part of the landscape, is a major soft fruit producer and exporter (Funes *et al*., 2017).

The spotted wing *Drosophila* (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a highly polyphagous invasive pest from South East Asia (Walsh *et al*., 2011), detected for the first time in Europe (Cini *et al*., 2012) and North America in 2008 (Hauser, 2011), and in South America (Brazil) in 2013 (Deprá *et al*., 2014). From then on, this species has colonized Europe and America affecting a wide range of host plants.

The SWD is considered an important global pest of soft fruits, because females are capable of laying eggs under the epidermis of healthy, ripening fruit, using their powerful, sclerosed and serrated ovipositor. In the last 3 years, Argentina has been literally invaded by the SWD, which has quickly spread to all cardinal points and different fruit crops (Cichón *et al.,* 2015; Santadino *et al*., 2015; Lue *et al*., 2017); however, there are no reports on the presence of SWD in sub-tropical regions of northwestern Argentina.

Zaprionus indianus Gupta (Diptera: Drosophilidae) or African fig fly is native to sub-Saharan Africa and, like SWD, has also rapidly spread to tropical and subtropical regions (Silva Commar *et al*., 2012). This drosophilid infests mainly damaged fruit of about 80 species from over 31 plant families (van der Linde *et al.,* 2006; Yassin and David, 2010). However, valuable crops, such as *Ficus carica* L. (fig) and *Dimocarpus longan* Lour. (longan) have been severely affected by this fly from Brazil to the USA (van der Linde *et al*., 2006; Vilela and Goñi, 2015). In Argentina, *Z. indianus* was first reported in 2006 from decaying fruits of a wide range of native and cultivated host plants surveyed in northeastern Argentina, including the oriental semiarid lands of Tucumán (Lavagnino *et al*., 2008), but this finding has received little attention.

Wild and cultivated guava (*Psidium* spp.) species are reported as *Z. indianus* and *D. suzukii* hosts in Brazil (Vilela and Goñi, 2015), México (Lasa *et al*., 2016), and the USA (van der Linde *et al*., 2006). Even though in Argentina guavas are not grown with economic purposes, guava trees are very common in the backyards of rural homes for family consumption (Ovruski *et al*., 2005). Additionally, many local or native people harvest guavas from the subtropical rainforests for the production of juices and jams, which are traded in informal fairs nearby (Telam, 2017). In Tucumán, feral guava (*Psidium guajava* L.) fruit is found in the foothills of the mountain rainforest, known as the Yungas (Grau and Aragón, 2000). The Yungas border the humid piedmont, which hosts most of the soft fruit orchards of the region (Funes *et al*., 2017). Since there is no information about both invasive drosophilid species in the subtropical region of northwestern Argentina, and considering the potential economic losses that these pests could cause to the fruit industry, the objectives of this study were to determine the species composition of drosophilids infesting "feral" guavas in the Yungas, and their relative abundance and prevalence.

Material and Methods

During a routine tephritid fruit fly monitoring in guava fruit, a large number of unusual drosophilid specimens were observed in the collected samples. The studied area is in Horco Molle (26°45′00"S, 65°20′00"W, 500–600 m elevation; Tucumán province, Argentina), within the "Sierra de San Javier" park, in the southernmost end of the sub-tropical Yungas forest. The site is characterized by disturbed secondary vegetation (exotic and native plant species combined) surrounded mainly by large citrus orchards (Ovruski *et al*., 2005) and soft fruit crops. Horco Molle´s climate is classified as "humid warm-temperate" with a rainywarm season from October through April, and a dry-cold season from May through September. Mean annual rainfall ranges from 1300 to 1600 mm, with an average annual temperature of 18°C.

From a group of 30 wild guava trees (*Psidium guajava* L., Myrtaceae) selected in the sampling site, six trees were randomly chosen for the study. Knowing that in this location ripe guava fruit are more abundant in late summer/early autumn (Ovruski *et al*., 2005), all fruit samples were collected in March, 2016.

Five undamaged early maturing fruit (partially yellow guava, with mottled green spots and soft texture) were harvested from the selected trees, and five damaged, ripe fruit were collected from the ground below each tree canopy. In both cases, fruit were chosen randomly. Each fruit sample was placed individually into a cloth bag (20 cm diameter and 30 cm depth) and transported in a plastic tray to the lab (Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales, LIEMEN, Tucumán, Argentina).

Guava fruits were rinsed with a 30% sodium benzoate solution, and weighed. Each fruit sample was placed in a plastic tray (48 × 28 × 15 cm) with a slotted bottom, which was placed over another plastic tray of the same size but without perforations. A 5-cm sand layer was used as pupation substrate in the second tray. Both trays were tightly covered with organdy cloth. The double tray method was used to prevent the contact between fruit and sand, in order to minimize fungal growth and bacterial contamination. Samples were kept in a dark room with no climate control, with temperatures ranging from 22° to 27°C. Sand was sifted once a week to recover drosophilid pupae for a 1-month period, after which all fruit were dissected to search for remaining drosophilid larvae or pupae inside each fruit.

Drosophilid pupae were transferred to glass cups (21 cm diameter, 9 cm depth) filled with sterilized moist vermiculite. Cups were covered with a piece of organdy cloth and held until adult emergence. Adult drosophilid specimens were identified to species using taxonomic keys (Markow and O'Grady, 2006). Species identification was based on external morphology and on the terminalia of both sexes. Voucher specimens were placed in the entomological collection of Fundación Miguel Lillo (FML) in Tucumán, Argentina.

Results and Discussion

Total sampled fruit weight was 3.25 kg, with an individual mean fruit weight of 54.2 ± 9.9 g (SD). This quantity of fruit yielded 387 drosophilid puparia, from which a total of 239 resulted in emerged adults (Table 1), that were identified as *Z. indianus* (207 individuals; 86.6%), SWD (17 individuals; 7.1%), and *Drosophila* spp. (15 individuals; 6.3%; probably *D. melanogaster* and *D. simulans* among others).

Approximately, 65% SWD adults were recovered from guavas collected from the tree canopy, while the remaining 35% were recovered from fruit collected from the ground. Regarding to *Z. indianus* and *Drosophila* spp., 100% of the adults were recovered only from fruit lifted from the ground (Table 1).

SWD is the first drosophilid species found in the subtropical region of northwestern Argentina with capability of laying eggs below the epidermis of healthy, ripe fruit, and of developing in the fruit. In Argentina, this frugivorous fruit fly has recently been recorded in very contrasting environments at different latitudes, fruit species, and climates (Cichón *et al*., 2015; Santandino *et al*., 2015; Lue *et al*., 2017) (Table 2). In fact, the ability of *D. suzukii* to adapt to different environments and hosts has enabled this species to establish in tropical and subtropical regions in both hemispheres (dos Santos *et al*., 2017).

In the present study, *D. suzukii* was the only drosophilid species recovered from undamaged guava fruit harvested from the plant, which is consistent with the literature. SWD has been reported in several countries infesting a great variety of fresh commercial fruits, such as blueberry, blackberry, raspberry, strawberry, cherry, plum, peach, pear, grape, fig, kiwi, and guava (Van Timmeren and Isaacs, 2014; Wang *et al*., 2016; Lasa *et al*., 2017), as well a wide range of non-crop fruits, including guavas (Arnó *et al*., 2016; Kenis *et al*., 2016).

On the other hand, *Z. indianus* was the dominant drosophilid species found in damaged, fallen fruit. As reported previously, *Z. indianus* had been recovered from cultivated peaches (*Prunus persica* (L.) Stokes) in Vipos (Lavagnino *et al*., 2008), located in the semi-arid region of the Tapia-Trancas basin (Tucumán, Argentina). This site is located in the northeastern part of the Tucumán province, where climate is warm semi-arid, with precipitations around 450 mm and permanent water deficit (Zuccardi and Fadda, 1985). Nevertheless, we found *Z. indianus* in a completely different environment. Our sampling site is located ≈60 km south of Vipos, in a very contrasting environment: the Yungas rainforest (humid and perhumid piedmont region), with annual rains ≈1000 mm and positive water balance (Zuccardi and Fadda, 1985). Köppen-Geiger climate classification for Vipos is BSh while for Horco Molle it is CWa, which reflects the plasticity of *Z. indianus,* an issue previously discussed by other researchers (da Mata *et al*., 2010; Calabria *et al*., 2010).

Table 1. Total and relative abundance and sex ratio of Drosophilidae species, recovered from guava fruits collected from the tree canopy and from the ground in Horco Molle, Tucumán, Argentina.

Sampled Tree	Fruit origin	Nº of Fruit	Nº of puparia	Drosophilidae species					
				Z. indianus		*D. suzukii*		*Drosophila spp.*	
				Nº of adults	Sex ratio (%) [a]	Nº of adults	Sex ratio (%)	Nº of adults	Sex ratio (%)
1	Canopy	5	9	0	-	4	75.0	0	-
	Ground	5	85	50	58.0	0	-	7	
2	Canopy	5	1	0	-	1	100	0	-
	Ground	5	66	34	44.1	2	50.0	3	
3	Canopy	5	11	0	-	4	50.0	0	-
	Ground	5	41	25	44.0	1	0	0	-
4	Canopy	5	3	0	-	1	0	0	-
	Ground	5	82	40	40.0	2	50.0	3	
5	Canopy	5	2	0	-	1	100	0	-
	Ground	5	59	34	32.4	1	100	0	-
6	Canopy	5	15	0	-	0	-	0	-
	Ground	5	13	24	54.2	0	-	2	

[a] Sex ratio = proportion of females on the total number of emerged adults.

Table 2. Name, geographical location, climate type and description, annual precipitation and annual average temperature of sites where the SWD was reported in Argentina, including the present study.

Province	Location name	Location latitude	Associated fruit species	Climate type (Köppen-Geiger)[4]	Climate description	Annual rain (mm)	Annual average temperature (ºC)
Río Negro[1]	Choele Choel	39°17′09″S, 65°39′15″W	Raspberry	BSk	local steppe	268	15.4
Buenos Aires[2]	Lobos	35°11′11″S, 59°05′46″W	Blueberry	Cfa	warm and temperate	1008	16.1
La Rioja[3]	Anillaco	28°48’23’’S, 66°56’27’’W	Pear	BWh	desert	330	20.0
Tucumán	Horco Molle	26°45′00”S, 65°20′00”W	Guava	Cwa	warm and temperate	949	19.4

[1]Cichón *et al*., 2015; [2]Santandino *et al*., 2015; [3]Lue *et al*., 2017; Kottek et al., 2006.

Our findings are very similar to those reported by Fartyal *et al*. (2014), who found both *D. suzukii* and *Z. indianus* affecting sweet orange (*Citrus sinensis* L.) and guavas in subtropical environments of India. They observed that *D. suzukii* was the only drosophilid found in healthy fruit attached to the plant, and that *Z. indianus* only emerged from damaged fruit bearing in the tree or collected from the ground.

The sex ratio, defined as the proportion of adult females on the total number of adults (Table 1), varied from 32.4% to 58.0% for *Z. indianus* in fruit lifted from the ground, which is consistent with previous reports in guava (56%; Lasa *et al*., 2017). Regarding to SWD, the small number of specimens found was not enough to make any discussion about sex ratio. In more integral studies, SWD sex ratios were 58% in guavas collected from the tree and 66% in damaged guavas lifted from the ground (Lasa *et al*., 2017).

An important issue to address in future research is the interaction between both drosophilid species, as shown in other studies. Strawberry fruit injured by SWD adults facilitated the infestation by *Z. indianus*, showing the opportunistic ability of African fruit fly adults to infest damaged fruit (Bernardi *et al*., 2017).

In hosts like guavas, SWD can complete its life cycle in 15 d under lab conditions, indicating that guavas allow the sustainability of SWD populations during the seasons in which commercial berry crops are not in production (Rebollar-Alviter *et al*., 2015). As pointed out before, in the sub-tropical region of northwestern Argentina, guavas share the same geographical space with commercial berry orchards. Therefore, our results should be taken as a warning signal for growers and government plant protection agencies.

Our findings reveal the need of increasing the studies about the drosophilid community in this region, including studies on population dynamics, interactions between species, potential natural enemies, geographical distribution, host range within non-crop plants, and potential dispersion of both pest species to neighboring orchards.

Given that SWD is considered a key pest of several fruit crops worldwide (Arnó *et al*., 2016; Bolda *et al*., 2010; Wang *et al*., 2016), its presence in the subtropical region of northwestern Argentina is a threat for the local fruit industry and for native non-crop fruit species. Prompt measures should be taken in order to limit this pest dispersion to commercial orchards and to natural plant sanctuaries.

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Annual record for chromosomal polymorphism in a natural population of *Drosophila pseudoobscura* from Morelos, Mexico.

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Abstract

A seasonal survey of *Drosophila pseudoobscura* was performed in a natural population from Tres Marías, Morelos, Mexico, in order to determine fluctuations in the relative frequencies of inversions in the third chromosome of this species. This study corresponds to an analysis of 1126 third chromosomes, among which we were able to detect 10 different gene arrangements. Two of them, CU and TL, represent the dominant couple and the pair EP/SC as well six minor and sporadic gene arrangements complete the genetic structure of the population. The chromosomal constitution of the population is similar to other nearby populations reported with respect to the number of inversions and dominant pairs. Changes in frequency are related to climatic fluctuations in the locality and ascribed to the adaptability of the chromosomes to climatic conditions as have been observed in many populations. Key words: *Drosophila,* inversions frequencies, temporal changes

Introduction

Based on works of Painter (1934) and Bridges (1935), salivary glands of *Drosophila melanogaster* promoted a continuous series of studies concerning a chromosomal pattern in several species of the genus *Drosophila.* In the western hemisphere such species as *D. pseudoobscura, D. persimilis, D. robusta, D.willistoni*, and *D. nebulosa,* among others, have been extensively studied cytologically. All of them possessed an enormous diversity of chromosomal polymorphism in their genome (Krimbas and Powell, 2000).

We refer now to *D. pseudoobscura, a* species that inhabits temperate climes and with a geographic distribution from southwestern Canada, western region of USA, whole Mexico and Guatemala, and a small colony in Colombia. It inhabits mainly coniferous forests and it has even been found in other habitats with different vegetation. It lives in areas with altitudes above sea level between 1800 and 3000 meters and is easily cultured in the laboratory.

The chromosomal polymorphism in *D. pseudoobscura* is present mainly in its third chromosome with at least 40 different gene arrangements or inversions described among other authors by Dobzhansky and Epling (1944), Olvera *et al.* (1979), and Powell (1992). Concerning its chromosomal constitution a population could be uniform, present only one kind of inversion; even so, the most common is the presence of up to 6-7