

Annual climatic effects on the autumnal drosophilid fauna composition at the Font Gropa site (Tibidabo, Barcelona)

Maria GALLUDO^{1,4}, Jordi CANALS^{1,4}, Laura PINEDA-CIRERA^{1,4}, Carla ESTEVE^{1,4}, Maria ROSSELLÓ^{1,4}, Raquel MADRENAS^{1,4}, Joan BALANYÀ^{1,2}, Conxita ARENAS³ and Francesc MESTRES^{1,*}

1. Dept. Genetics, Microbiology and Statistics. Section Biomedical genetics, Evolution and Development. Universitat de Barcelona. Barcelona (Spain).

2. IRBio – Biodiversity research Institute. Universitat de Barcelona. Barcelona (Spain).

3. Dept. Genetics, Microbiology and Statistics. Section Statistics. Universitat de Barcelona. Barcelona (Spain).

4. All these authors contributed equally to this research.

* Corresponding author, F. Mestres, E-mail: fmestres@ub.edu

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Abstract. The abundance of different drosophilid species was studied in a series of six consecutive years during autumn at the Font Gropa site (Tibidabo, Barcelona). Furthermore, the relation between these abundances and the following climatological variables (Tmean: mean temperature, Tmax: maximum temperature, Tmin: minimum temperature, Hm: mean humidity and Rf: rainfall) were studied. The most common species observed at this location were *D. subobscura*, *D. melanogaster*/*D. simulans* and the invasive species *D. sukukii*. Other species were trapped depending on the year (*D. immigrans*, *D. buzzatii*, *D. cameraria*, *D. phalerata* and *D. hydei*), and the values of several diversity indexes were computed for each annual collections. In general, in those years where *D. melanogaster*/*D. simulans* flies were abundant, *D. subobscura* and *D. sukukii* were in low frequencies. From the analyses of the climatic variables and the three most abundant species, it seemed that *D. subobscura* would need environmental conditions characterized by a low Tmin, not a very high Tmax (but with a large difference between both), a certain degree of humidity and scarce rainfall. *D. sukukii* presented a similar pattern, but not so accused, whereas *D. melanogaster* / *D. simulans* group would need high Tmin, low Tmax (with a small difference between Tmin and Tmax values) and low levels of humidity and rainfall. Finally, in these autumnal samples our results would indicate a certain association between the abundances of *D. subobscura* and *D. sukukii*, although their breeding sites are clearly different.

Key words: Drosophilidae, diversity, climate, adaptation, invasive species.

Introduction

Scientifically, global warming has been recognized as a real fact by almost all researchers who investigate this topic (Cook et al. 2016). In these studies, *Drosophila* genus is considered an excellent biological system to monitor climatic change. In different *Drosophila* species, many genetic markers have been used for this purpose, being the chromosomal inversions one of the most outstanding (Orengo & Prevosti 1996, Rodriguez-Trelles & Rodriguez 1998, Solé et al. 2002, Levitan 2003, Balanyà et al. 2004, 2006, 2009, Levitan & Etges 2005, Umina et al. 2005, Zivanovic and Mestres 2010, 2011, Zivanovic et al. 2012, 2014, 2015, Arenas et al. 2018). Recently, we have studied the effect of different climatic variables on *D. subobscura* inversion polymorphism (Galludo et al. 2018). We trapped *D. subobscura* at the Font Gropa site (Tibidabo hill, Barcelona), in the same season (early autumn) during five consecutive years (2011 to 2015).

As a product of collecting *D. subobscura*, other *Drosophila* species were collected and identified (Canals et al. 2013, Pineda et al. 2014, Esteve & Mestres 2015, Rosselló et al. 2016, Madrenas et al. 2017). It is well known that organisms are directly affected by climatic factors (and other environmental conditions) and need to adapt to them and also to the changes that consequently are generated in the ecosystems (Parmesan & Yohe 2003, Pearson & Dawson 2003, Atkins & Travis 2010, Thomas 2010, Gilman et al. 2010, Hoffmann & Sgrò, 2011). The species of *Drosophila* are not an exception and many studies have been carried out (Atkinson and Shorrocks 1977, Avelar et al. 1987, Holt 1990, Krimbas 1993, Powell 1997, Tidon 2006). For all these reasons, the main aim of this research was to analyze the effect of several climatic variables on the diversity and abundance of distinct *Drosophila*

species at the Font Gropa site. A secondary objective was to monitor the abundance of the invasive pest *D. sukukii*, which was first detected in Europe in 2008 (Calabria et al. 2012) and spread very fast along the continent (Cini et al. 2012, Asplen et al. 2015, Arnó et al. 2016, Lavrinienko et al. 2017).

Material and Methods

Fly sampling

Drosophilids were collected during early autumn in six consecutive years (2011 to 2016) at the Font Gropa site, which is located on the foothills of the Tibidabo Mountain (at 400 m above sea level) in the city limits of Barcelona. The vegetation is typically Mediterranean, mainly composed of pines (*Pinus pinea*) and ilexes (*Quercus ilex*) with brushwood (*Arbutus*, *Ruscus*, *Erica*, *Hedera*, *Rubus*, *Smilax*, *Laurus* and others). Along a trail, 12 fermenting banana baits were laid on the ground 5 to 10 m apart from each other. The total length of the trapping trail was approximately 100 m. The baits were located at the base of trees, in a humid environment with no direct insolation. Flies were netted in intervals of 15-30 minutes from afternoon (4 p.m.) to dusk (7:30 p.m.), using always the same procedure. The trapped drosophilids were kept in vials with standard culture medium at 17 °C and were identified in the laboratory the next day. As *D. melanogaster* and *D. simulans* are considered sibling species and, because their females are morphologically nearly identical, they have been grouped together. Males could be identified, and in all cases *D. simulans* was strongly dominant. Additionally, H' (Shannon diversity index) and J (Shannon uniformity index) were computed for each sample. These indices are often used because they present appropriate properties for the ecology of *Drosophila*, although they have a bias when the number of species is small and/or certain species are over-dominant (Shannon and Weaver 1949, Argemí et al 1999, Avondet et al. 2003).

Climatic data and statistical analyses

We selected the following climatic variables: mean temperature (Tmean), maximum temperature (Tmax), minimum temperature

(Tmin), mean humidity (Hm) and rainfall (Rf). They were studied for the average of July, August and September, in order to detect a possible effect of summer conditions on the autumnal *Drosophila* collections. Values of these climatic parameters were obtained from the Observatori Fabra, located only 2 km from the trapping site. All meteorological information was downloaded from the website: <http://www.fabra.cat/>

To analyze the similarities between years with regard to climatic variables (Tmean, Tmax, Tmin, Hm and Rf), the UPGMA clustering method using the Euclidean distance was used. As a measure of the quality of the obtained dendrogram, the cophenetic correlation coefficient was considered. Both the resemblance between years (based on species composition) and between species (in samples from different years) were evaluated using the Morisita-Horn index (Horn 1966). Using this index, an UPGMA dendrogram of the samples was obtained and the cophenetic correlation was also computed. Furthermore, we analyzed the differences between these groups of years or species with a permutational multivariate ANOVA (PERMANOVA) analysis followed by the post-hoc Tukey method, and a Multi-dimensional Scaling (MDS) graph was performed. Finally, to analyze the climate influence on species abundance a Redundant Analysis (RDA) was computed and the effects of the climate variables on the *Drosophila* species, as well as on the community in different years, were also tested using General Linear Models (GLM). Tmean was removed from this kind of analysis (data not shown) because it provided little information, as it was a daily mean, which was then averaged for all days of the month and finally averaged again for the three summer months (July, August and September). All analyses were carried out using the R package (CRAN, <http://CRAN.R-project.org>).

Results

The number of drosophilids per species and years, with the corresponding estimates of H' and J indices are presented in Table 1. From a qualitative point of view, *D. subobscura* was the most abundant species in 2011 and 2013, whereas *D. melanogaster/D. simulans* was in 2012, 2015 and 2016. In 2014, the abundance of these species was rather similar: 30.53% and 38.94% for *D. subobscura* and *D. melanogaster/D. simulans* group, respectively. Interestingly, *D. suzukii* presented large variations, from 40.84% (2011) to 1.27% (2016). However, in three collection years this species showed similar values:

9.22% (2012), 8.02% (2013) and 8.97% (2015). The remaining five species reported (*D. immigrans*, *D. buzzatii*, *D. cameraria*, *D. phalerata* and *D. hydei*) were rather sporadic and trapped in low frequencies. The composition of species generated that the higher and lower estimates of H' and J were those from 2014 and 2016, respectively. The UPGMA dendrogram showing the similarity between years in species composition using the Morisita-Horn index is shown in Fig. 1. It is possible to observe two main groups of years: one comprising 2012, 2015 and 2016, and the other containing 2011, 2013 and 2014. This classification corresponds to the years with low and high values for the diversity indices, respectively. If the composition is analyzed in detail, the first group was characterized by a large abundance of *D. melanogaster/D. simulans* (over 80%) and small frequencies of *D. subobscura* (from 6.86% to 12.07%) and *D. suzukii* (1.27% to 9.22%). The second group had in common the number individuals of *D. subobscura* and *D. suzukii* together was higher than that of the *D. melanogaster/D. simulans*. The cophenetic correlation was rather high (0.77), indicating that the dendrogram reproduced properly the distribution of the years. However, the PERM-

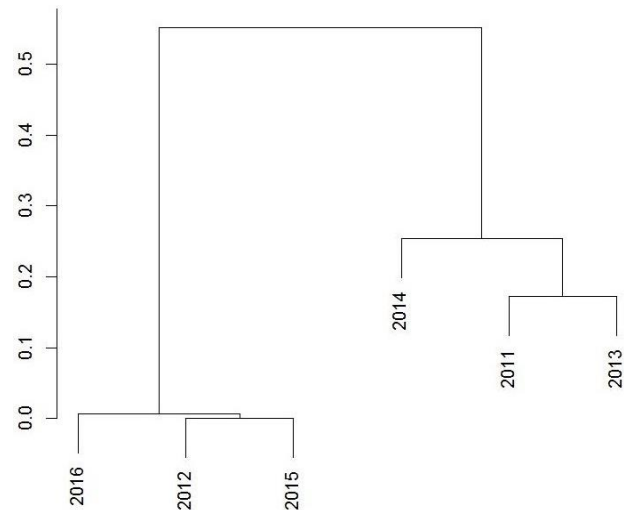


Figure 1. Dendrogram showing the similarity between years in species composition using the Morisita-Horn index.

Table 1. Number of individuals (n) and percentage (%) per species and year collected at the Font Gropa site (Barcelona, Spain). For each sample, the values of S (number of species), H' (Shannon diversity index) and J (Shannon uniformity index) indices are also presented.

SPECIES	YEAR											
	2011		2012		2013		2014		2015		2016	
	n	%	n	%	n	%	n	%	n	%	n	%
<i>D. subobscura</i>	484	57.63	35	6.86	298	62.87	87	30.53	262	7.69	66	12.07
<i>D. melanogaster/D. simulans</i>	13	1.53	414	81.17	120	25.31	111	38.94	2805	82.31	471	86.10
<i>D. suzukii</i>	343	40.84	47	9.22	38	8.02	58	20.35	306	8.97	7	1.27
<i>D. immigrans</i>	0	0	13	2.55	12	2.53	19	6.67	0	0	0	0
<i>D. buzzatii</i>	0	0	0	0	0	0	0	0	1	0.03	0	0
<i>D. cameraria</i>	0	0	0	0	0	0	1	0.35	33	0.97	0	0
<i>D. phalerata</i>	0	0	1	0.20	6	1.27	9	3.16	1	0.03	2	0.37
<i>D. hydei</i>	0	0	0	0	0	0	0	0	0	0	1	0.18
TOTAL	840	100	510	100	474	100	285	100	3408	100	547	100
S	4		5		5		6		6		5	
H'	0.763		0.678		0.990		1.363		0.624		0.474	
J	0.550		0.421		0.615		0.761		0.348		0.294	

References: 2011 (present research), 2012 (Canals et al. 2013), 2013 (Pineda et al. 2014), 2014 (Esteve and Mestres 2015), 2015 (Rosselló et al. 2016) and 2016 (Madrenas et al. 2017).

ANOVA analysis on these groups did not found significant differences ($p=0.4731$). Moreover, it is possible to construct an UPGMA tree for the different species over years measuring their similarity based also in the Morisita-Horn index (Fig. 2). Four groups can be observed from left to right: the first constituted only by *D. hydei* (a single individual was found in the 2016 sample); the second containing *D. immigrans* and *D. phalerata* (species that were collected together in 2012, 2013 and 2014); the third with *D. buzzatii* (only one individual detected in the 2015 sample), *D. cameraria* (a single individual trapped in 2014, but 33 in 2015) and *D. melanogaster/D. simulans* (usually abundant when the frequencies of *D. subobscura* and *D. suzukii* were small); and finally the last group with *D. subobscura* and *D. suzukii* which tended to be together in many collections. In this study, the cophenetic correlation was high (0.95), showing that the graphical reconstruction fits well with the reported data. The PERMANOVA analysis (see Fig. 3) on these groups indicated significant differences between the species groups ($p=0.0339$) and the post-hoc Tukey multiple comparisons only showed significant differences between the group formed by *D. subobscura* and *D. suzukii* and the group formed by *D. melanogaster/D. simulans*, *D. buzzatii* and *D. cameraria* ($p=0.0443$).

Using the climatic variables Tmean, Tmax, Tmin, Hm and Rf, an UPGMA tree was constructed to analyze the resemblances among years (Fig. 4). In this case, the cophenetic correlation did not present a high value (0.61). It is worth comparing the distributions of years according to species (Fig. 1) with that corresponding to climatic variables (Fig. 4). A clear resemblance seems to have appeared with years 2012 and 2015, and a slight one for 2016. We hypothesize that it could have been due to the dominant presence of the *D. melanogaster/D. simulans*. Therefore, it could be deduced that there is a certain relation between species and climate. To study the climate influence on species abundance, the Redundant Analysis (RDA) was computed using the three most abundant species (*D. subobscura*, *D. melanogaster/D. simulans* and *D. suzukii*) (Fig. 5), and GLM models were performed with the climate variables as regressors. It was possible to observe that *D. subobscura* and *D. suzukii* followed a similar pattern with regard to the climatic factors studied, whereas the *D. melanogaster/D. simulans* group was very different. In this analysis, Tmin was the most important climatic variable, followed by Tmax and later, but with a similar magnitude, Rf. Focusing on the species, *D. subobscura* would need environmental conditions characterized by a low Tmin although it is not significant ($p=0.399$), not a very high Tmax ($p=3.97e-12$), a certain degree of humidity ($p<2e-16$) and scarce rainfall ($p<2e-16$). *D. suzukii* would also require low Tmin ($p<2e-16$), but not as low as *D. subobscura*, it would need not a very high Tmax ($p<2e-16$) and humidity ($p=0.0168$), but a little less than *D. subobscura*, and almost absence of rainfall ($p<2e-16$). Therefore, both species present certain similarities with regard to the climatic variables studied. Finally, *D. melanogaster/D. simulans* would be characterized by a high Tmin ($p<2e-16$), low Tmax ($p<2e-16$) (with a small difference between the Tmin and Tmax values), and low levels of humidity ($p=0.0168$) and rainfall ($p<2e-16$). If the analysis was repeated, but adding the species that presented non-negligible frequencies (*D. immigrans*, *D. cameraria*, *D. phalerata*), the climatic variables do not seem to pro-

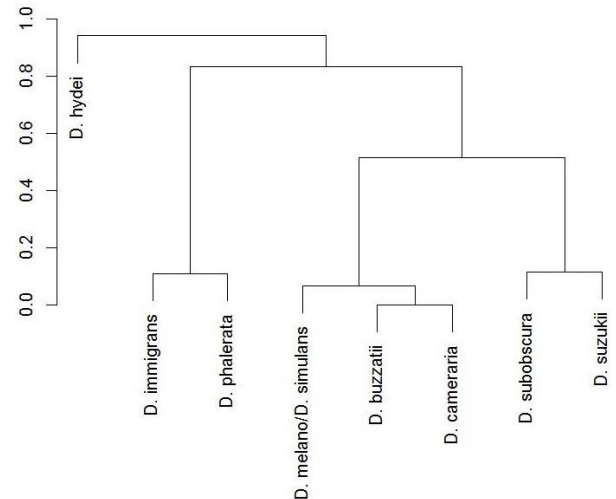


Figure 2. Dendrogram presenting the similarity among species over years using the Morisita-Horn index.

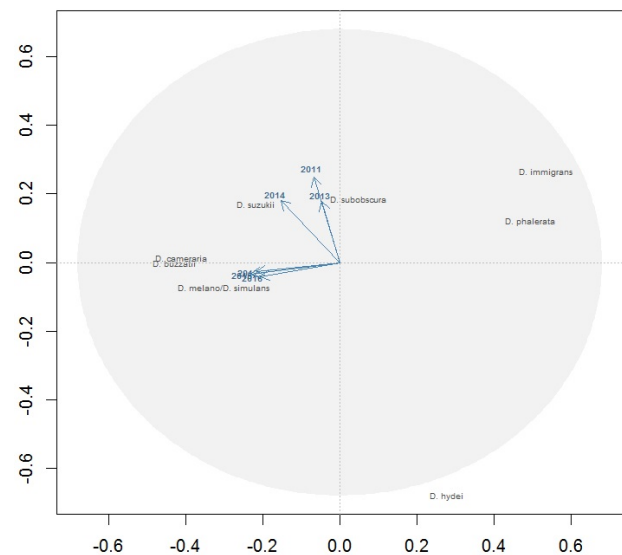


Figure 3. Graphical representation of the MDS for the abundance of all species along the years. Goodness of fit = 85%.

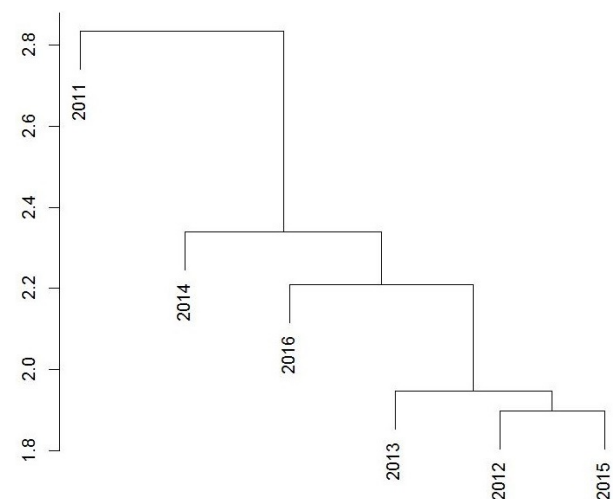


Figure 4. Dendrogram showing the resemblances among years using the following climatic variables: mean temperature (Tmean), maximum temperature (Tmax), minimum temperature (Tmin), humidity (Hm) and rainfall (Rf).

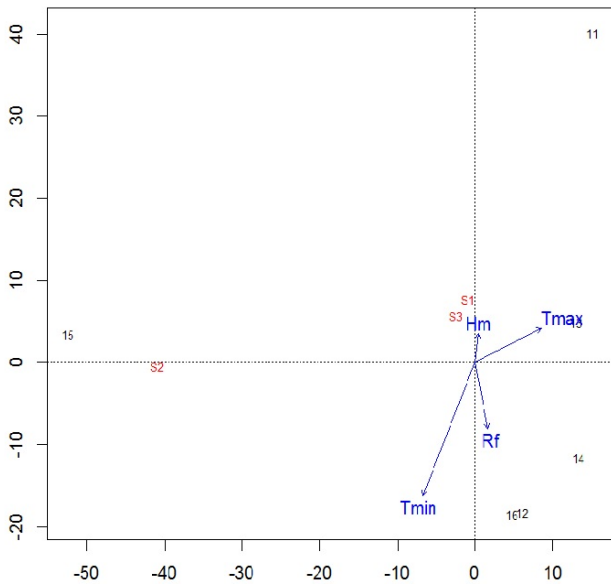


Figure 5. Graphical representation of the RDA for the three most abundant species. S1, S2 and S3 stand for *D. subobscura*, *D. melanogaster/D. simulans* and *D. suzukii*, respectively. The climatic variables used are: maximum temperature (Tmax), minimum temperature (Tmin), humidity (Hm) and rainfall (Rf). Individual numbers indicate the studied years (11 = 2011, 12 = 2012, etc.).

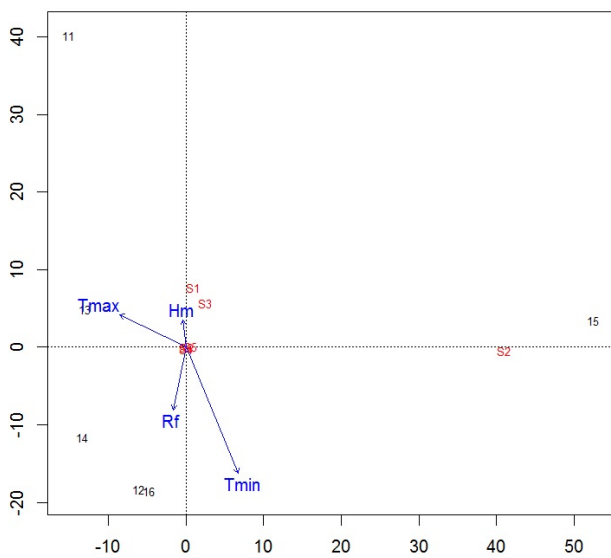


Figure 6. Graphical representation of the RDA, but adding three more species (S4: *D. immigrans*, S5: *D. cameraria* and S6: *D. phalerata*). All other symbols are the same as those in figure 4.

duce substantial effects on them (Fig. 6). This result could be attributed to the fact that these species were not collected in several years and, in several samples where they were present, the number of individuals was scarce (Table 1). Finally, as the difference between Tmin and Tmax seemed relevant for the most abundant species (*D. subobscura*, *D. melanogaster/D. simulans* and *D. suzukii*), a new RDA was computed using this parameter (Fig. 7). Analyzing the results, *D. subobscura* benefited by a rather larger difference between both measures of temperature ($p=0.0002$). The same effect, but not as accused, was observed for *D. suzukii* ($p<2e-16$). Conversely, *D. melanogaster/D. simulans* would require a small difference between Tmin and Tmax ($p<2e-16$). When we ana-

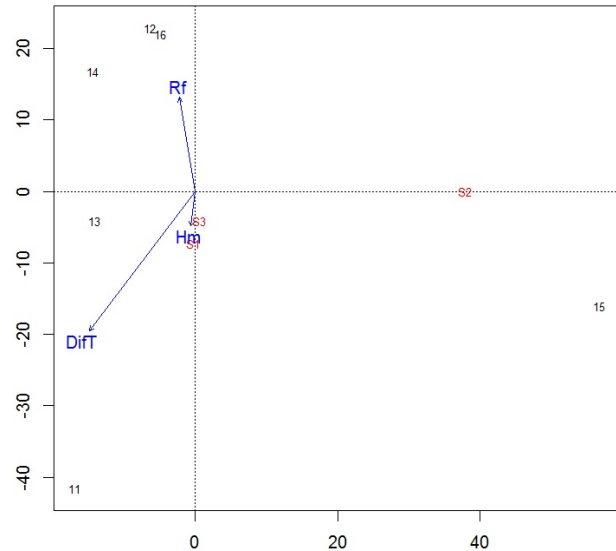


Figure 7. Graphical representation of the RDA, but substituting the variables Tmax and Tmin by their difference (DifT). All other symbols are the same as those in figure 4.

lyzed the association between climatic variables and the community in different years, the community would need environmental conditions characterized by a high Tmin ($p<2e-16$), low Tmax ($p<2e-16$) with a small difference between the Tmin and Tmax values ($p<2e-16$), a certain degree of humidity ($p<2e-16$) and scarce rainfall ($p<2e-16$).

Discussion

Many species of *Drosophila* genus are considered model organisms in diverse fields of research. For instance, one of them is the study of their interactions with other species and environmental conditions (Ecology), and also in which ways they change over time (Evolution). Although extensive knowledge on these topics is currently available, more information is still needed and the present research is a further contribution. The taxonomy and systematics of the genus have been extensively studied (Markow & O'Grady 2006), and in the nineties it was estimated that 3,341 species belonged to the Drosophilidae (Powell 1997), whereas the *Drosophila* genus contains more than 1,600 (O'Grady & DeSalle 2018). In general, drosophilids are considered organisms specialized to breed in decomposing plants and fungi. Although their origin was likely in the tropics, at present they are distributed throughout our planet in different habitats. It is worth pointing out that a reduced group has adapted to become human commensals and considered "domestic". The great evolutionist Dobzhansky (1965) considered the following species in this "domestic" group: *D. melanogaster*, *D. simulans*, *D. immigrans*, *D. hydei*, *D. ananassae*, *D. montium*, *D. funebris*, *D. virilis*, *D. repleta* and *D. busckii*. The first four species of this list were collected in our research. Although we are not able to morphologically differentiate *D. melanogaster* females from those belonging to *D. simulans*, from the trapped males the second species would seem dramatically more abundant (in all years together: 25 male *D. melanogaster* versus 1308 *D. simulans*). This observation would confirm that in Mediterranean temperate climate *D. melanogaster* is

being displaced by *D. simulans* in the open field (Argemí et al. 1999, David et al. 2004), although in late summer and early autumn a peak of *D. simulans* expansion was described (Argemí et al. 1999, 2003). With regard to *D. subobscura*, it could also be considered as 'domestic' because it is a generalist species that lives in woods and parks close to human activity (Krimbas 1993). Furthermore, a peak of species expansion has been reported in autumn (Krimbas 1993, Argemí et al. 1999, Araúz et al. 2009). For these reasons, it was one of the common species collected in our study. Therefore, trapping 'domestic' *Drosophila* species was expected, being the Font Gropa a collecting site in an edge of a woodland adjacent to an area inhabited by humans. To find *D. cameraria* and *D. phalerata* was also a common result, because they are competing species that breed in fungi. We observed many mushrooms along the trapping trail in different years, *D. phalerata* being more abundant than *D. cameraria*, with the exception of 2015 (Table 1). Shorrocks and Charlesworth (1980) reported this interspecific competition (*D. phalerata* being also more common than *D. cameraria*) in England during August, and probably the general climatic conditions of this month would be similar to those found in Font Gropa site during autumn. In a monthly survey considering two periods widely separated in time, *D. phalerata* was generally more abundant than *D. cameraria* in Bordils (Girona, 70 km NE of Barcelona, Spain) (Argemí et al. 1999). However, to find one specimen of *D. buzzatii* was actually unexpected, because it breeds in cacti of the *Opuntia* genus (Fontdevila et al. 1981) and this kind of plant was absent in the trapping place. Finally, the invasive species *D. suzukii* was rather abundant, with the exception of 2016 (only 1.27% of individuals sampled). From the beginning of its European invasion, the species spread very quickly and most of their populations seem stable along the years. In the Font Gropa during autumn, the presence of red fruits (for instance *Rubus*, *Arbutus*, *Ruscus* or *Smilax*), which are the preferred breeding site of *D. suzukii*, would favor the permanence of a stable population.

The analysis of the relation between the climatic variables studied and the most common species at the collecting site (*D. melanogaster/D. simulans*, *D. subobscura* and *D. suzukii*) yielded valuable results. In general, temperature (in its different aspects), humidity and rainfall are considered as relevant factors in *Drosophila* species distribution and abundance (Hoffmann & Parsons 1991, Krimbas 1993, Powell 1997, van Heerwaarden & Hoffmann 2007, Reusch & Wood 2007). From a climatic point of view, the years 2012 and 2015 are very similar (Fig. 4), and their proportion of the three major species is almost identical (Table 1). An outstanding result from our study was that *D. subobscura* and *D. suzukii* appeared closely associated in two distinct analyses, the cluster (Fig. 2) and the RDA (figs. 5, 6 and 7). The latter would indicate that at Font Gropa during autumn both species require similar conditions for the climatic variables studied. The general pattern for both species would be a low T_{min} , not a very high T_{max} , a certain degree of humidity and scarce rainfall. However, *D. subobscura* would be more favored than *D. suzukii* by a rather large difference between T_{min} and T_{max} . Nevertheless, the breeding site is different, because *D. subobscura* is considered rather generalist, but *D. suzukii* is a pest species parasitizing mainly red fruits as cherries, straw-

berries and grapes, although it can use other fruits as plums, blackberries, blueberries or peaches (Lee et al. 2011, Walsh et al. 2011, Cini et al. 2012, Farnsworth et al. 2017). This association between both species was not recently detected in a May 2018 collection by Lagares and Mestres (2018), because *D. subobscura* was in a high proportion (95.36%) whereas *D. suzukii* was scarce (0.66%). Finally, valuable information was provided when the difference between T_{max} and T_{min} was studied (Fig. 7). It would seem that *D. subobscura* was adapted to a large range between both temperatures (T_{min} and T_{max}), *D. suzukii* presented a similar pattern although with a lower range, and the group *D. melanogaster/D. simulans* needed a small difference. These observations for *D. subobscura* and *D. melanogaster/D. simulans* agree with the pattern of diurnal activity in the Mediterranean location of Bordils (Girona, NE of Spain) detected by Argemí et al. (2000). Therefore, the adaptation of these species to a global warming scenario would likely be different. Finally, from our autumnal study, it was outstanding to discover that, even though *D. subobscura* and *D. suzukii* used distinct breeding sites, their behavior in front of the climatic variables analyzed was actually similar and there was an association of their abundance in those years where *D. melanogaster/D. simulans* group is not the dominant species.

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