

# Seasonal and Spatial Distribution of Two Sibling Species, *Drosophila melanogaster* and *Drosophila simulans* (Diptera: Drosophilidae) in Belgrade, Serbia

Sofija B. Pavković-Lučić, Vladimir D. Kekić

University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, 11000 Belgrade, Serbia;  
E-mails: sofija@bio.bg.ac.rs, kekić@eunet.rs

**Abstract:** In this paper we examined seasonal and spatial distribution of two sibling fruit fly species, *Drosophila melanogaster* and *D. simulans* in two adjacent habitats, *domestic* and *semidomestic*. Those species showed fine differences in temporal and spatial distribution. *D. melanogaster* was absolutely dominant in domestic habitat (balcony of the building). In park collections (*semidomestic* habitat), *D. simulans* became numerous in autumn collections, while *D. melanogaster* was recorded in significant proportion during summer months, July and August. In relation to the total catch of two species during “*Drosophila* season”, *D. melanogaster* was found in more than 99% of captured specimens in the *melanogaster/simulans* balcony collections ( $Z = 72.72$ ;  $P < 0.01$ ), while *D. simulans* was recorded in more than 70% of individuals collected in the park ( $Z = -60.10$ ;  $P < 0.01$ ). Differences in the seasonal and microspatial distribution of the two species as well as in their behaviour, reflect differences in their preferences for ecological factors in the niches, which they occupy.

**Keywords:** *Drosophila melanogaster*, *D. simulans*, seasonal distribution, spatial distribution, ecology, behaviour

## Introduction

The fauna of the family Drosophilidae (Diptera: Insecta) of the Central and Western Balkans has been continuously investigated for more than 40 years. It comprises about 60 species, the largest number of which (37) belongs to the most famous genus *Drosophila* (KEKIĆ *et al.* 1999a, KEKIĆ 2002, KEKIĆ 2009). The number of the Drosophilidae species in different habitats significantly varied accordingly to the anthropogenic influences: 48 species have been recorded up to now in *wild* habitats, 40 species in *semidomestic* habitats, and 12 in *domestic* habitats. The faunistic and ecological investigations have been most numerous in Serbia, where Vojvodina and the regions along the Danube River have been the most explored ones (KEKIĆ *et al.* 1999, KEKIĆ 2009).

During the faunistic and ecological research of the fauna of Drosophilidae in Belgrade (Serbia) special attention was paid to the seasonal and spa-

tial distribution of two sibling fruit fly species, *Drosophila melanogaster* and *D. simulans*. Both species are cosmopolitan and exhibit domestic status (PATTERSON, STONE 1952). They probably evolved in Africa (CAPY *et al.* 2004), but are also able to proliferate under temperate climates (DAVID *et al.* 2004). While *D. melanogaster* world colonisation was associated with early human migration, it is assumed that the worldwide expansion of *D. simulans* was much more recent (CAPY *et al.* 2004). Furthermore, *D. melanogaster* is considered as probably the most differentiated into geographic subpopulations, while *D. simulans* is absent in many geographic regions with suitable climates (see for review DAVID *et al.* 2007). On considering the general aspects of their development and ecology in nature, both species may have ten or more generations *per* year and, in terms of feeding/oviposition preferences, they are

generalists that oviposit in a wide variety of substrates (MARKOW, O'GRADY 2008).

*D. melanogaster* and *D. simulans* are morphologically very similar. Males are easily distinguished using their genitalia, while, for discriminating females, a comparison of check width and eye height is used (MCNAMEE, DYTAM 1993). In spite of the morphological similarity, these species do not hybridise in nature, owing to, among other things, some very important differences in mating behaviour, such as differences in olfactory profiles (FERVEUR, COBB 2010) and acoustic signals produced by males during courtship (MOULIN *et al.* 2004). Those behavioural barriers established through differences in sensory domains certainly contribute to prezygotic reproductive isolation in nature. However, under laboratory conditions, asymmetrical hybridizations have been observed, *i.e.* *D. melanogaster* females mate relatively easily with *D. simulans* males, while the reciprocal cross is rare (MOULIN *et al.* 2004). In such crosses, many genes interact to produce hybrid inviability or sterility (see for review MALLET 2006).

In this paper, we present results of investigation of a few aspects of the ecology of *D. melanogaster* and *D. simulans*, *i.e.* those considering seasonal and spatial distribution in two very close, but ecologically distinct microhabitats in Belgrade, Serbia.

## Material and Methods

### Systematics

*Drosophila melanogaster* Meigen, 1830 and *D. simulans* Sturtevant, 1919 belong to subgenus *Sophophora* Sturtevant, 1939 and to *Drosophila melanogaster* species group.

### Collecting of the flies

Following the methodology in our previous research (KEKIĆ 1999a, KEKIĆ 2002, KEKIĆ 2009), the flies were collected in two ecologically distinct habitats: a balcony at the apartment of one of the authors (on the sixth floor of the building) and a park in front of that apartment building. Both localities are very close to each other, being situated in an urban part of New Belgrade, Serbia. The balcony represented a “domestic” habitat (together with, for example, house interiors, market places, wine cellars, etc.), while the city park belonged to the “semidomestic” habitats (for categorisation of *Drosophila* habitats see KEKIĆ 2002).

A mixture of seasonal fruits with small amount of sugar and baker's yeast (to accelerate fermentation) was used in attracting the flies. In both habitats,

the flies were captured with the same baits. The substrate was prepared one day before the use and later placed in small portions on plastic plates (c. 300 g *per* plate) and distributed over the habitats. The flies attracted by fruit baits were captured using entomological net.

The flies were collected from March to October 2001, twice a day (at the time of their maximum activity, in the morning and evening), during 3 days within a single month.

### Identification

After capturing the flies they were kept in 70% alcohol until the identification that was performed according to the *Drosophilidae* keys to genera and species (BÄCHLI, BURLA 1985, BÄCHLI *et al.* 2004). All the collected specimens were identified, counted and kept in separate vials according to the habitat and species. The flies were then deposited in the Institute of Zoology, Faculty of Biology, University of Belgrade.

### Statistics

The differences in the number of the individuals from the two investigated species, which were captured at the two localities, were tested using a Z-test.

## Results and Discussion

The presence/absence of different *Drosophila* species in collections may vary seasonally and spatially as a result of the degree of their tolerance to abiotic (temperature, light, relative humidity) and biotic factors (competitive interactions, predation, etc.) In this paper we pay attention to the seasonal and spatial distribution of two sibling species, *D. melanogaster* and *D. simulans*. In addition, all the captured drosophilids were identified. Twenty-three species were found in the park collections. They belonged to 3 genera: *Chymomyza* Czerny, 1903 (1 species), *Drosophila* Fallén, 1823 (20 species) and *Scaptomyza* Hardy, 1849 (2 species) (Table 1). Only 7 species were recorded in the balcony collections, all of which belonging to genus *Drosophila* Fallén, 1823 (Table 1).

Since *D. melanogaster* and *D. simulans*, the species under our investigation, can often be found in the same area and even emerging from the same larval food resources (CAPY *et al.* 2004), the question was: how they share a relatively limited resources of the habitat, and how they coexist? Theoretically, the species in competition can coexist in a stable equilibrium if they use at least partially different resources or if resources are used with different efficiency. One possibility (insufficiently explored in

*Drosophila*), which makes the competition in the habitat less pronounced, is related to the impact of some regular seasonal changes in the composition of the habitat resources. It is important to establish the potential impact of different environmental factors on the efficiency with which the observed species uses available resources.

**Table 1.** List of the Drosophilidae species collected in two habitats (a balcony and a park) from March to October 2001. Abbreviations: “+” – present in samples, “-“ – absent in samples

Genus and Species	Park	Balcony
<b>Chymomyza Czerny, 1903</b>	+	-
<i>C. amoena</i> Loew, 1862	+	-
<b>Drosophila Fallén, 1823</b>	+	+
<i>D. ambigua</i> Pomini, 1940	+	-
<i>D. bifasciata</i> Pomini, 1940	+	-
<i>D. busckii</i> Coquillett, 1901	+	+
<i>D. cameraria</i> Haliday, 1833	+	-
<i>D. funebris</i> (Fabricius, 1787)	+	+
<i>D. hydei</i> Sturtevant, 1921	+	+
<i>D. immigrans</i> Sturtevant, 1921	+	+
<i>D. kuntzei</i> Duda, 1924	+	-
<i>D. littoralis</i> Meigen, 1830	+	-
<i>D. melanogaster</i> Meigen, 1830	+	+
<i>D. obscura</i> Fallén, 1823	+	-
<i>D. phalerata</i> Meigen, 1830	+	-
<i>D. repleta</i> Wollaston, 1858	+	+
<i>D. rufifrons</i> Loew, 1873	+	-
<i>D. simulans</i> Sturtevant, 1919	+	+
<i>D. subobscura</i> Collin, 1936	+	-
<i>D. testacea</i> von Roser, 1840	+	-
<i>D. transversa</i> Fallén, 1823	+	-
<i>D. tristis</i> Fallén, 1823	+	-
<i>D. unimaculata</i> Strobl, 1893	+	-
<b>Scaptomyza Hardy, 1849</b>	+	-
<i>S. graminum</i> (Fallén, 1823)	+	-
<i>S. pallida</i> (Zetterstedt, 1847)	+	-

Whether *D. melanogaster* and *D. simulans* have very similar requirements in terms of feeding and egg laying, they were rarely seen in the same place at the same time and in equal numbers (KEKIĆ *pers. obs.*). Usually, they alternate each other in space and time. The observed changes in the composition of these species in two different sinantropic habitats (the park and the balcony) under our research clearly illustrate this fact.

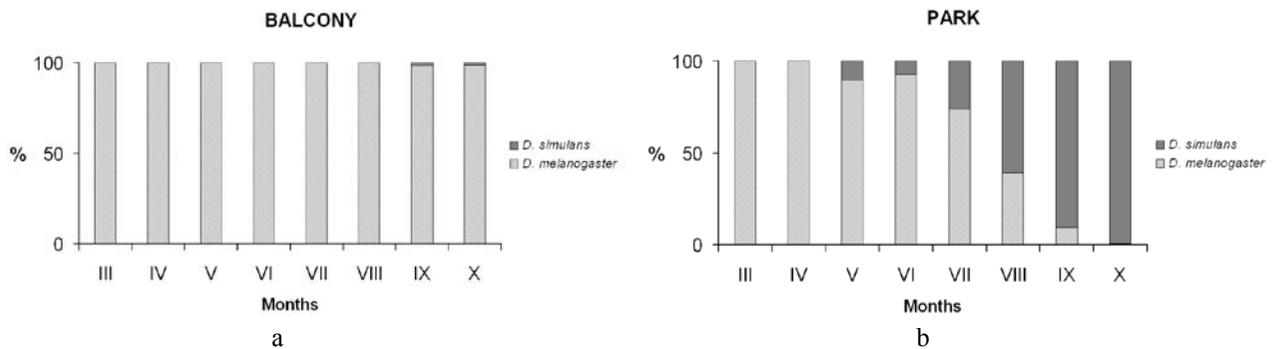
The seasonal and spatial distribution of *D. melanogaster* and *D. simulans* in the two habitats during “*Drosophila* season” (from March to October) are presented in Table 2 and Fig. 1 (a and b).

*D. melanogaster*, a species strongly associated with humans, was absolutely dominant in the “balcony collections” (Fig. 1a, Table 2). This result supports our previous research, where *D. melanogaster* was the dominant species in some domestic habitats, reaching 96% of the inspected flies (KEKIĆ 2002). Contrary to *D. melanogaster*, *D. simulans* was extremely rarely found on the balcony. It was not recorded in the balcony collections from March to August and was significantly less abundant than *D. melanogaster* in September ( $Z = 23.67$ ,  $P < 0.01$ ) and October ( $Z = 39.86$ ,  $P < 0.01$ ). This finding is consistent with those of David and colleagues, who pointed that *D. simulans* never enter human buildings, whereas *D. melanogaster* frequently does so (DAVID, TSAKAS 1983, DAVID *et al.* 2004). The reasons for this behavioural difference might include “light intensity, a direct perception of what is a building, and also alcoholic vapors when *D. melanogaster* concentrates in wine cellars” (MCKENZIE, PARSONS 1972).

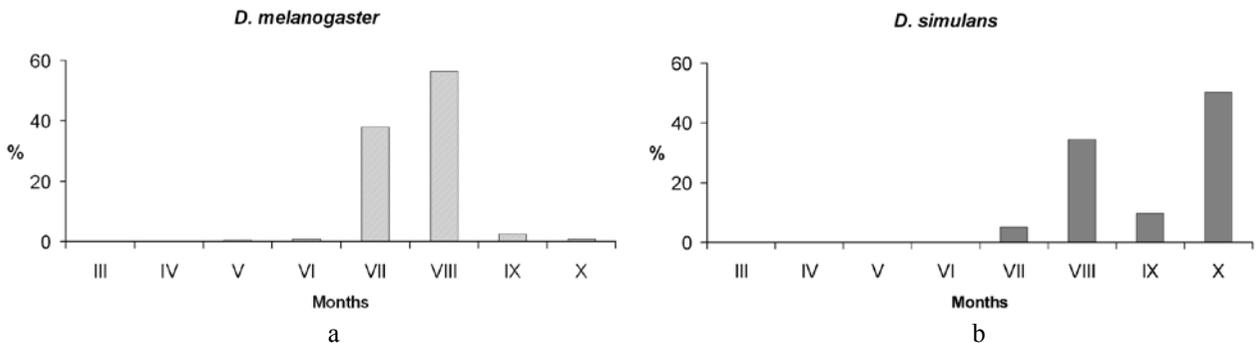
In the park collections, a noticeable shift of the two species is observed seasonally (Fig. 1 b, Table 2). *D. simulans* was absent in the collections sampled in the park during March and April. In the May, June and July collections *D. melanogaster* was significantly more numerous than *D. simulans* ( $Z = 5.060$ ,  $P < 0.01$ ;  $Z = 6.41$ ,  $P < 0.01$ ;  $Z = 25.80$ ,  $P < 0.01$ , respectively). In the August, September and October

**Table 2.** Number of individuals of *D. melanogaster* and *D. simulans* captured in two habitats (a balcony and a park)

Habitat/ Species	Month	March	April	May	June	July	August	September	October	Total
Balcony										
<i>D. melanogaster</i>		7	15	99	161	288	215	146	409	1340
<i>D. simulans</i>		0	0	0	0	0	0	2	4	6
Park										
<i>D. melanogaster</i>		2	1	9	13	525	779	36	14	1379
<i>D. simulans</i>		0	0	1	1	182	1199	339	1750	3472



**Fig 1.** The percentages of individuals of *D. melanogaster* and *D. simulans* captured in two habitats (a – balcony, b – park)



**Fig. 2.** a) Percentages of *D. melanogaster* flies captured from March to October in the park collections in relation to the total catch. b) Percentages of *D. simulans* flies captured from March to October in the park collections in relation to the total catch

collections *D. simulans* significantly outnumbered *D. melanogaster* ( $Z = -18.89$ ,  $P < 0.01$ ;  $Z = -31.29$ ,  $P < 0.01$ ;  $Z = -82.67$ ;  $P < 0.01$ , respectively).

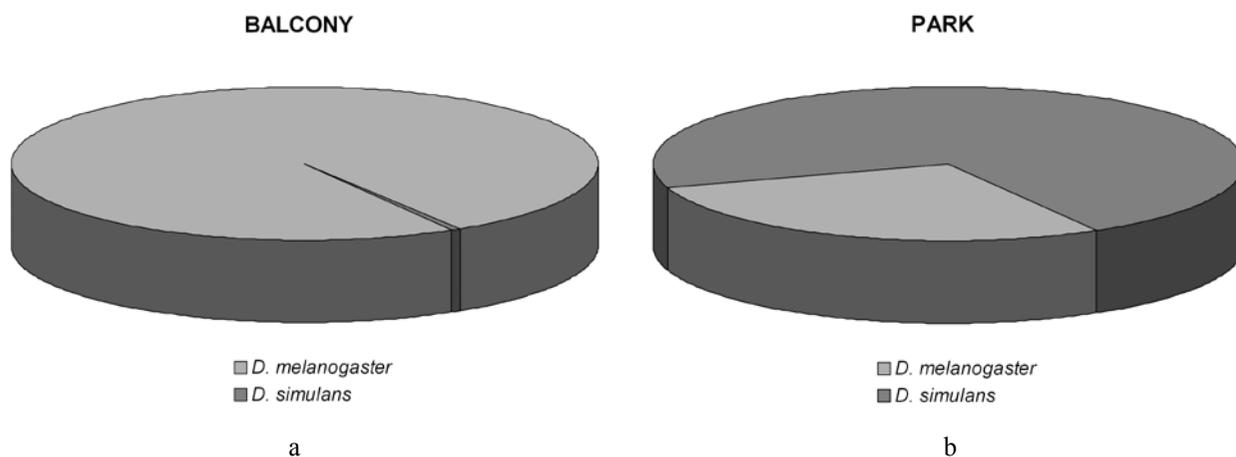
Fig. 2 presents the seasonal distribution of the two species in the park collections in relation to the total catch of a given species. In relation to the total *D. melanogaster* catch, the mentioned species was abundant during the summer months (July and August) (Fig. 2a, Table 2), while *D. simulans* became more numerous in the autumn and in October/November it reached its peak (Fig. 2b, Table 2). The abundance of individuals of those species in our samples over temporal gradient implies their different preference for environmental factors, such as temperature, light intensity, relative humidity, and amount of precipitation.

The results about the seasonal distribution of the two species are in agreement with some similar, previously reported investigations. For example, in Switzerland, the majority of *D. melanogaster* specimens were captured in June and July (BÄCHLI *pers. comm.*). In France, *D. melanogaster* also outnumbered *D. simulans* in summer, while the proportion of *D. simulans* increased with the decrease in temperature and could make more than 80% of the total catch in autumn (GRAVOT 2000).

Fig. 3 (a and b) was obtained from sampling both species at both localities from March to October. Out

of 1346 *D. melanogaster* – *D. simulans* flies sampled in the balcony collections, 1340 (99.55%) belonged to *D. melanogaster*, whereas only 6 individuals (0.45%) belonged to *D. simulans* (Fig. 3a). That difference is highly significant ( $Z = 72.72$ ;  $P < 0.01$ ). Out of 4851 flies caught in the park, 3472 (71.57%) belonged to *D. simulans* and 1379 (28.43%) to *D. melanogaster* (Fig. 3b), with that difference being also statistically significant ( $Z = -60.10$ ;  $P < 0.01$ ). The sampling of the flies throughout the season clearly demonstrated that *D. melanogaster* prevailed on the balcony, while *D. simulans* was dominant in the park collections.

In *Drosophila*, “species distribution reflects the distribution of their particular resources as well as their particular combinations of ecophysiological tolerances” (MARKOW, O’GRADY 2008). The divergence in the ecological niches used by two species is obviously influenced by different environmental factors. According to DAVID *et al.* (2004), two of those factors are the most important: the temperature and alcoholic resources. Data obtained from ecophysiological approaches usually describe *D. simulans* as more cold-adapted than its sibling. *D. melanogaster* is more tolerant to high temperature: it breeds at 31°C, while, at that temperature *D. simulans* exhibits a very low viability (DAVID *et al.* 2004). In competition experiments *D. melanogaster* eliminates *D.*



**Fig. 3.** Percentages of *D. melanogaster* and *D. simulans* in the balcony (a) and park collections (b) in relation to total *D. melanogaster* – *D. simulans* catch

*simulans* at 25°C, while, at temperatures lower than 20°C, *D. simulans* is a better competitor (TANTAWY, SOLIMAN 1967). Furthermore, contrary to *D. simulans*, *D. melanogaster* is polymorphic at the alcohol dehydrogenase (*Adh*) locus (DAVID, BOCQUET 1975): it has much higher ethanol tolerance and is able to use artificial fermenting resources (DAVID *et al.* 2004). This fact may, at least partially, help in explaining its permanent presence in human buildings, wine cellars, greengroceries, markets, etc. Finally, the two species under our investigation differ in preference for light intensities (DAVID *et al.* 2004), which also may contribute to difference in their distribution in time and/or space.

Fruit flies can travel a few hundred meters *per* day in search for food, oviposition site, shelter or

mate (TAYLOR *et al.* 1984). According to our results, the two sibling species do not share habitat in the same way and significantly differ in preferences for some important environmental factors. In this respect, the use of some similar habitat resources (*e.g.* food) is enabled in different times and at different places. Those ecologically and behaviourally important differences between *D. melanogaster* and *D. simulans* certainly contribute, along with the previously mentioned differences in sensory domains, to their isolation in nature.

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## References

- BÄCHLI G., H. BURLA 1985. Diptera: Drosophilidae. Insecta Helvetica, Zürich. 115 p.
- BÄCHLI G., C. R. VILELA, S. A. ESCHER and A. SAURA 2004. The Drosophilidae (Diptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica, 39. Brill, Leiden and Boston. 362 p.
- CAPY P., P. GIBERT and I. BOUSSY (eds.). 2004. Preface. – In: *Drosophila melanogaster; Drosophila simulans: so similar, so different*. Contemporary Issues in Genetic and Evolution. Kluwer Academic Publishers, The Netherlands, 1-3.
- DAVID J., C. BOCQUET 1975. Similarities and differences in latitudinal adaptation of two *Drosophila* sibling species. – *Nature*, **257**: 588-590.
- DAVID J. R., L. TSAKAS 1983. L'évolution des Drosophilides: l'œil du paléontologiste et l'œil du biologiste généticien. – *Colloques Internationaux du Centre National de la Recherche Scientifique*, **330**: 249-257.
- DAVID J. R., R. ALLEMAND, P. CAPY, M. CHAKIR, P. GIBERT, G. PETAVY and B. MORETEAU 2004. Comparative life-histories and ecophysiology of *Drosophila melanogaster* and *D. simulans*. – In: CAPY P., P. GIBERT, I. BOUSSY (Eds.): *Drosophila melanogaster, Drosophila simulans: so similar, so different*. Kluwer Academic Publishers, Netherlands, 151-164.
- DAVID J. R., F. LEMEUNIER, L. TSACAS and A. YASSIN 2007. The historical discovery of the nine species in the *Drosophila melanogaster* species subgroup. – *Genetics*, **177**: 1969-1973.
- FERVEUR J.-F., M. COBB 2010. Behavioral and evolutionary roles of cuticular hydrocarbons in Diptera. – In: BLOMQUIST G. J., A.-G. BAGNÈRES (Eds.): *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press, 325-343.
- GRAVOT E. 2000. Interactions entre la pourriture acide de la vigne et les populations de drosophiles dans la région Bordelaise. Thèse de Doctorat, Université Paris VI, 100 p.
- KEKIĆ V. 2002. The Drosophilidae (Drosophilidae, Diptera) of Yugoslavia. – In: ČURČIĆ B. P. M., M. ANDELKOVIĆ (Eds.): *Genetics, Ecology, Evolution*. Monographs, Volume VI, Institute of Zoology, Faculty of Biology, University of Belgrade: 109-120.
- KEKIĆ V. 2009. The Drosophilidae (Diptera) of Fruška Gora Mountain. – In: ŠIMIĆ S. (Ed.): *Invertebrates (Invertebrata)*

- of the Fruška Gora Mountain. Matica Srpska, Department of Natural Sciences, 63-84.
- KEKIĆ V., S. PAVKOVIĆ-LUČIĆ, J. MILOŠEVIĆ, N. POPOVIĆ and N. J. MILOŠEVIĆ 1999. Studies of Drosophilidae (Diptera) in Yugoslavia. XII. Collections from Belgrade and Sremska Kamenica at Danube coast. – In: GIOKAS S., A. LEGAKIS, R. POLYMERI, S. SFENTHOURAKIS, M. THESSALOU-LEGAKI AND A. ZENETOS (Eds.): Contributions to the Zoogeography and Ecology of the Eastern Mediterranean region. The Hellenic Zoological Society, **1** (suppl.): 1-7.
- KEKIĆ V., G. BÄCHLI and S. PAVKOVIĆ-LUČIĆ 1999a. Drosophilidae fauna (Diptera) of former Yugoslavia. – In: GIOKAS S., A. LEGAKIS, R. POLYMERI, S. SFENTHOURAKIS, M. THESSALOU-LEGAKI AND A. ZENETOS (Eds.): Contributions to the Zoogeography and Ecology of the Eastern Mediterranean region. The Hellenic Zoological Society, **1** (suppl.): 9-15.
- MALLET J. 2006. What does *Drosophila* genetics tell us about speciation? – *Trends in Ecology and Evolution*, **21**: 386-393.
- MARKOW T. A., P. O'GRADY 2008. Reproductive ecology of *Drosophila*. – *Functional Ecology*, **22**: 747-759.
- MCKENZIE J. A., P. A. PARSONS 1972. Alcohol tolerance: an ecological parameter in the relative success of *Drosophila melanogaster* and *Drosophila simulans*. – *Oecologia* (Berlin), **10**: 373-388.
- MCMAMEE S., C. DYTAM 1993. Morphometric discrimination of the sibling species *Drosophila melanogaster* (Meigen) and *D. simulans* (Sturtevant) (Diptera: Drosophilidae). – *Systematic Entomology*, **18**: 231-236.
- MOULIN B., T. AUBIN and J.-M. JALLON 2004. Why there is a one-way crossability between *Drosophila melanogaster* and *D. simulans*. – *Genetica*, **120**: 285-292.
- PATTERSON J. T., W. S. STONE 1952. Evolution in the genus *Drosophila*. MacMillan, New York. 610p.
- TANTAWY A. O., M. H. SOLIMAN 1967. Studies on natural populations of *Drosophila*. 6. Competition between *Drosophila melanogaster* and *D. simulans*. – *Evolution*, **21**: 34-40.
- TAYLOR C., J. R. POWELL, V. KEKIĆ, M. ANDELKOVIĆ and H. BURLA 1984. Dispersal rates of species of the *Drosophila obscura* group: implications for population structure. – *Evolution*, **38**: 1397-1401.

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