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SCIENTIFIC NOTE

# First Record of *Drosophila buzzatii* (Patterson & Wheeler) (Diptera: Drosophilidae) Emerging from a Non-Cactus Host

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**Abstract**
*Drosophila buzzatii* (Patterson & Wheeler), a typical cactophilic species of the repleta group, is registered for the first time emerging from Melon (*Cucumis melo*) in western Argentina. The analysis of inversion polymorphism and genetic diversity of mitochondrial cytochrome oxidase subunit I gene (mtCOI) provided additional evidence that corroborated the presence of a high proportion of *D. buzzatii* among the flies emerged from melon. This finding set the scenario for a broader range of possible hosts and host-related distribution and dispersion for this widespread species.

*Drosophila buzzatii* (Patterson & Wheeler) belongs to the repleta group, one of the largest and complex species groups in the genus *Drosophila* (Wasserman 1982, Vilela 1983). *Drosophila buzzatii* is widely distributed in South America (Manfrin & Sene 2006, Soto *et al* 2010), but it has also recently passively colonized the Old World and Australia (Fontdevila 1989, Barker 2013). Emergence records reveal that the main hosts are rotting cladodes of prickly pears (genus *Opuntia*), although it also breeds on other cactus genera of the family Cactaceae such as *Cereus*, *Trichocereus*, and *Pilosocereus* (Hasson *et al* 1992, Fanara *et al* 1999, Manfrin & Sene 2006, Oliveira *et al* 2012). We report the first record of *D. buzzatii* emerging from the commercial fruit *Cucumis melo* ("Honeydew melon," afterwards Melon) in a natural population. This observation constitutes a novelty for an otherwise ecological specialist of the repleta group.

The population sampled is located near the town of Lavalle, Province of Mendoza, Argentina (32°43′00″S, 68°35′00″W; 647 m asl). Fly collection was carried out in two areas that are 2.1 km apart. The first one is a 1.5 km$^2$ plantation of *Prunus domestica* (Rosaceae, plum) and the other is a 1.2 km$^2$ plantation of *Vitis vinifera* (Vitaceae, grape) with a cultivated patch of 0.3 km$^2$ of *Cucumis melo* (melon). We did not detect any cactus species within and between the sites of collection as well as 5 km around the collecting area. Adult flies were collected by net sweeping on yeasted banana baits. Adult flies were sorted by sex, and females were placed in individual vials (isofemale lines) containing a laboratory culture medium (David 1962). We also collected rotten fruits in both sampling areas. Rotten fruits were isolated in 1.5 L glass containers and taken to the lab. During the following 2 weeks after field collection, we recovered all emerging flies every day, and the flies emerging from the same host (Plum. Grapes, or Melon) were kept together for 8 days in bottles with laboratory culture medium. This procedure ensured that flies of all species reached sexual maturity and that females were inseminated by males developed in the same host. Subsequently, flies were sorted by sex, and females were used to establish a set of isofemale lines from females emerged from fruits. Males (collected and emerged) and one

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Table 1 Number of adult flies by sex of *Drosophila melanogaster*, *D. simulans*, and *D. buzzatii* collected with banana baits in both two samples areas (plum and grapes/melon) and emerged flies from rotting pockets of plum, grapes, and melon in Lavalle, Province of Mendoza, Argentina.

| | Collected | | | | Emerged | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plum | | Grapes/Melon | | Plum | | Grapes | | Melon | |
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| *D. melanogaster* | 91 | 222 | 114 | 227 | 82 | 99 | 74 | 61 | 37 | 50 |
| *D. simulans* | 32 | 125 | 35 | 60 | 43 | 49 | 28 | 37 | 6 | 5 |
| *D. buzzatii* | 2 | 4 | 20 | 55 | 0 | 0 | 0 | 0 | 35 | 36 |

male progeny from the isofemale lines (collected and emerged) were classified to species by inspection of the genitalia. Nearly 1000 adult flies were collected using banana baits, and more than 600 flies emerged from the fruits (Table 1). Considering both samples, collected and emerged, the 64.9, 25.8, and 9.3% were identified as *Drosophila melanogaster* (Meigen), *Drosophila simulans* (Sturtevant), and *D. buzzatii*, respectively. Species proportions were compared between collected and emerged from fruits by means of contingency tests. The result of this analysis indicate that the species proportions were homogeneous across samples ($\chi^2_2 = 4.12$, $p = 0.13$) suggesting that the frequency of emerged flies can explain the composition of adult flies sampled. However, we observed a bias in the emergence record among the Drosophila species when the resource was considered (Fig 1). Certainly, *D. buzzatii* only emerged from Melon, an observation that constitutes the first record that this species can utilize non-cactus resources. To corroborate these results, one progeny larva of each *D. buzzatii* isofemale line that were generated from melon emergences was analyzed cytologically using standard techniques (Fontdevila *et al* 1981). The results revealed that polytene chromosome karyotypes of all isofemale lines correspond to *D. buzzatii*. Furthermore, the population is polymorphic for three more common second chromosome arrangements: *standard* (38%), *j* (54.3%), and $jz^3$ (7.7%); whereas the rest of the chromosomes were monomorphic. Interestingly, the inversion frequencies observed are similar to other populations of the same biogeographical area (Hasson *et al* 1995, Soto *et al* 2010), suggesting that the use of Melon as resource do not impose a differential selective regime on the inversion polymorphism. In this sense, the nutritional profiles of Grapes, Plum, and Melon (Agriculture Research Service 2015) are comparable to *Opuntia sulphurea* (Carreira *et al* 2014). Therefore, the absence of emerged *D. buzzatii* flies from grapes and plum may be attributed to the small sizes of these fruits that cannot allow the completion of larval development, rather than putative nutritional constraints imposed by these fruits.

Finally, we evaluated mitochondrial cytochrome oxidase subunit I gene (mtCOI) molecular diversity of *D. buzzatii*. Briefly, we followed the manufacturer's protocol for the PUREGENE DNA Purification Kit (QIAGEN Inc., Valencia, CA) to extract total DNA from four females (whole flies) of *D. buzzatii* collected in Grapes/Melon area. A 572-bp fragment of the mtCOI gene was amplified from all the

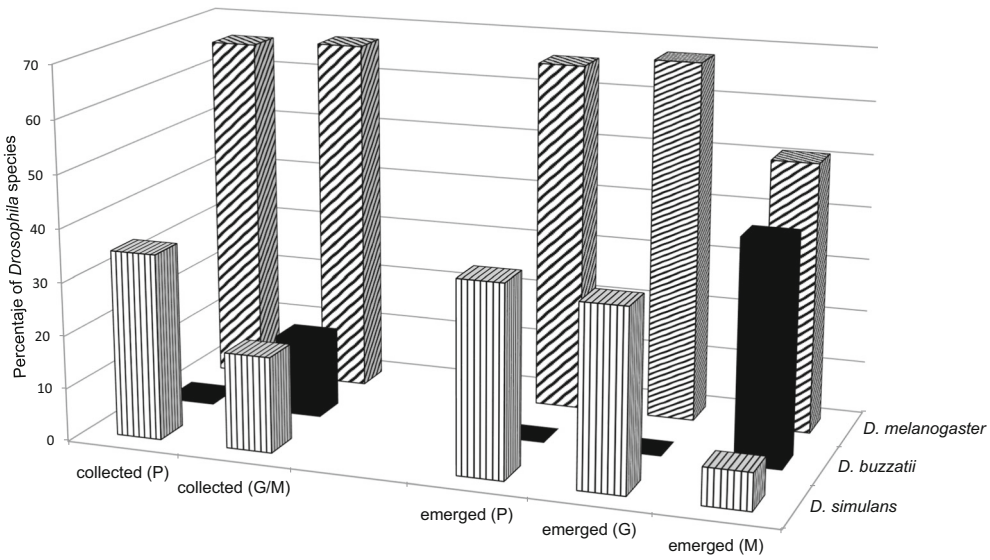


Fig 1 Percentage of *Drosophila melanogaster*, *D. simulans*, and *D. buzzatii* collected in plum (P) and grapes/melon (G/M) sample areas and emerged from plum (P), grapes (G), and melon (M) resources.

individuals analyzed using primers (de Brito *et al* 2002) 1406f (5′-CAATTTATCGCCTAACTTCAGCC-3′) and 2191r (5′-CCCGGTAAAATTAAAATATAAACTTC-3′). PCR products were purified using Accuprep PCR Purification Kit (Bioneer Corp, Alameda, CA). The DNA template reaction for sequencing was prepared according to the BigDye Terminator Cycle Sequencing Ready Reaction kit manual (Applied Biosystems, Foster City, CA) using the same primers as those in the amplification step. The sequences obtained were aligned using ClustalW 1.8 (Thompson *et al* 1994), and edited in BioEdit (Hall 1999). Genetic diversity was calculated using DnaSP 4.20 (Librado & Rozas 2009). The analysis of the results revealed the presence of 3 haplotypes (h), 22 polymorphic sites (S), and a haplotypic diversity (Hd) of 0.833 (±0.222). The nucleotide diversity per site ($\pi$) was 0.02023 (±0.0107) while the average number of nucleotide differences (k) and Watterson estimator ($\Theta_W$) were 11.17 and 12, respectively. These estimates are not different from other populations in Argentina according to a Molecular Analysis of Variance ($\Phi ST = 0.02$, $P = 0.13$) (Lipko, unpublished results).

In summary, our results indicate that *D. buzzatii* breeds on *Cucumis melo* (melon), a host that can play a major role in the expansion from the center of origin of *D. buzzatii*, located in the central region of Argentina (Fontdevila 1989), to new areas as a consequence of the commercial trade involving this natural host.

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