

## What's the buzz about *Ophrys fusca* and *O. dyris*? Tittle-tattle between two bee orchids in central Portugal

Joana A. ABREU<sup>1,2,3,\*</sup>, J.A. HAWKINS<sup>3</sup>, J. PELLICER<sup>4</sup>, Mike F. FAY<sup>4,5</sup>,  
H.C. COTRIM<sup>1</sup>

<sup>1</sup> Centre for Ecology, Evolution and Environmental Changes (CE3C), Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal

<sup>2</sup> Botanic Garden, National Museum of Natural History and Science, University of Lisbon, 1250-102 Lisbon, Portugal

<sup>3</sup> School of Biological Sciences, University of Reading, Whiteknights, Reading, Berkshire RG6 6BX, United Kingdom

<sup>4</sup> Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, United Kingdom

<sup>5</sup> School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia

\* joanaabreu@gmail.com

*Ophrys* is amongst the best known orchid genera, and is an established system for the study of pollinator-mediated floral evolution. Two species, *Ophrys fusca*. and *Ophrys dyris* (= *O. omegaifera* subsp. *dyris*) belonging to *Ophrys* section *Pseudophrys* are the focus of this study. Cases of introgression have been reported between these species, which have similar morphological characters and can be easily misidentified in the field. In order to better characterize the populations of these two taxa and its dynamics in central-Portugal, we integrated cytological and morphological and genetic diversity data between *O. fusca* and *O. dyris*, here focusing on the results regarding genome size, cytotype diversity and gene flow. Flow cytometry methods were used to assess genome size, and subsequently determine the ploidy level of 67 specimens, including the species and putative hybrids. Cytotypes were also confirmed based on chromosome counts from the roots of specimens of each species. Constancy of nuclear DNA content ( $1C = 11.19$  pg) and ploidy level ( $2n = 4x = 72, 74$ ) was documented among all the individuals analysed. Bayesian cluster analysis of 13 microsatellite loci and 167 individuals confirmed introgression and hybridization. Nevertheless, in this area of central Portugal, species seem to remain genetically circumscribed, as the number of genetic groups identified is two. Current results support the view of the Iberian Peninsula as a hotspot of polyploidisation in section *Pseudophrys*.