

Biotic stress is driving divergence and sequence evolution in *Dactylorhiza fuchsii* and *D. incarnata*, a pair of species with distinct ecological preferences

Mark W. CHASE^{1,*}, Ovidiu PAUN², Mikael HEDRÉN³

¹ Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, TW9 3DS, United Kingdom

² Department of Systematic and Evolutionary Botany, University of Vienna, Vienna, Austria

³ Department of Biology, University of Lund, Sweden

* m.chase@kew.org

Although Orchidaceae are the largest family in the angiosperms, little is known about the genetic basis of this significant biological variation. We have taken advantage of the methods used to sequence genomes to investigate the genetic divergence between two food-deceptive European terrestrial orchids, *Dactylorhiza incarnata* and *D. fuchsii*, which we integrate with their distinct ecologies, which we also document. Clear lineage-specific adaptive features are identified, in particular elements of biotic defense (relating to attacks by viruses and bacteria), in agreement with the Red Queen hypothesis (which proposes that organisms must constantly adapt, evolve, and proliferate not merely to gain reproductive advantage, but also simply to survive while pitted against ever-evolving opposing organisms in an ever-changing environment). We show the two *Dactylorhiza* species inhabit distinct niches; they differ significantly with regard to soil acidity and tree cover, but also with respect to temperature evenness over the year and precipitation of the driest month. If maintained over generations, such deviating ecological preferences likely have triggered distinct selection that in combination to specific demographic histories have moulded different genomic landscapes in these two species. The current prevalence of *D. incarnata* within small, localized populations over a highly fragmented distribution is corroborated here with considerable levels of inbreeding in this taxon. In contrast, *D. fuchsii* currently grows in larger, more diffuse populations and exhibits higher levels of heterozygosity and greater genetic diversity. When the two species are grown in a common garden intermediate between the two, the major features under selection relate to adaptation/acclimation to abiotic conditions (primarily water relations). These genomic techniques are not very expensive and offer for the first time the possibility to investigate which factors in the life history of orchids are driving their evolution, making it possible for scientists to begin the process of developing a model of how orchid diversity has evolved.