

## The genus *Neottia*, an early step in shift from autotrophy to mycoheterotrophy in orchids?

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Many plant families evolved mycoheterotrophy as a way of gaining nutrients, but the evolution of such nutritional shift has been little studied. We investigated mycorrhizal associations and a nutritional mode of two orchid species, a common ecological generalist *Neottia ovata* and *N. cordata*, a species of peatbog spruce forests. The species are closely related to a non-green *N. nidus-avis*, associated with ectomycorrhizal Sebaciales, clade A. We investigated the course of germination at 18 sites in the Czech Republic, analyzed fungal spectra of both seedlings and 39 *N. ovata* and 21 *N. cordata* adults growing in 32 European sites in different habitats and measured the natural content of stable isotopes. Seeds of both species suffered high mortality during two years of *in situ* germination. Whereas *N. ovata* produced few protocorms at both

*N. ovata* and *N. cordata* localities, *N. cordata* never reached a protocorm stage. We found Sebaciales, clade B, in both seedlings and adults, independent of habitat type and site geography. The spectra of accompanying fungal species differed according to habitat: the meadow individuals associated frequently with saprophytic Tulasnellaceae, whereas the forest individuals associated more often with ectomycorrhizal fungi. Natural content of stable <sup>13</sup>C and <sup>15</sup>N isotopes in forest specimens, however, did not indicate significant nutrient gain by mycoheterotrophy. *Neottia* species form mycorrhiza with Sebaciales, clade B, in various habitats. This association together with the co-occurrence of ectomycorrhizal fungi at forest sites could serve as a predisposition for the shift to full mycoheterotrophy in *Neottia*.

## Endophytic fungi associated with natural populations of *Vanilla* species in south-west Colombia

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The genus *Vanilla* (Orchidaceae) is of economic interest as the source of the natural vanilla fragrance, derived principally from cultivated plants of the species *V. planifolia*. The clade of fragrant species (*V.* subgenus *Xanata*, section *Xanata*) has a natural distribution in the Neotropics. Work in this research group has identified populations of ten *Vanilla* species in the region. Orchid species, including those of *Vanilla*, have an intimate relationship with mycorrhizal fungi to provide nutrients during seedling establishment and often in mature plants. Colombia has over 3,300 orchid species, but

little is known regarding the diversity of orchid mycorrhizal fungi essential for the establishment and survival of these species. We aim to characterize the diversity of fungi associated with the root system of adult plants of *Vanilla* species native to the diverse ecosystems of south-west Colombia, which include both humid tropical rainforest and sub-xerophytic habitats. Three methodologies are being implemented: 1) isolation of fungi from root portions colonized with mycorrhizal fungi; 2) isolation from single pelotons; and 3) identification of fungi present through direct PCR amplification using