Adaptation for Flower Colour in Deceptive Orchids

Do genes controlling flower colour show a signal of adaptation in deceptive orchids in the genus *Disa*?

Introduction

It is estimated that about one third of orchid species have deceptive flowers (flowers that trick pollinators with promises of sex or food) (Johnson 2000). Four non-rewarding species in the genus *Disa* in South Africa have been shown to resemble sympatric rewarding flowers and to take advantage of the pollinators that visit those flowers (Johnson 1994, 2000, Anderson *et al.* 2005, Johnson and Morita 2006). These species are *Disa ferruginea*, *D. pulchra*, *D. nivea*, and *D. nervosa*, respectively. In each case, floral colour of the orchid closely matches that of the rewarding species. Though traditionally, deceptive orchids were considered to be either generalized food mimics (non-specific reward signal) or Batesian mimics (closely resembling one species), they are now considered to be able to persist between these two extremes (Johnson and Morita 2006). This might provide a good evolutionary argument for the existence of highly specialized mimics, since they could have arisen through a gradual process while remaining viable along the way. Are the four species mentioned above adapted to take advantage of their putative models? The resemblances in floral colour, floral morphology and flowering time suggest this is the case, but the hypothesis needs to be tested in a phylogenetic context (Johnson and Morita 2006). Even within a single species (*D. ferruginea*), there is the possibility of population adaptation: in one part of its range it mimics a red-flowered species and in another part of its range it mimics an orange-flowered species (Johnson 1994). This makes flower colour a worthwhile focus for finding evidence of adaptation. Within *Disa*, flower colours seem to be the result of a combination of anthocyanins and carotenoids and their relative amounts in specific perianth tissues (Tatsuzawa *et al.* 2010).

In addition to determining phylogenetic relationships within *Disa*, I propose examining the genomes of the four species mentioned above to determine if genes controlling flower colour show evidence of adaptation.

Experimental System

The orchid species mentioned are included below in Table 1 along with their putative models, flower colours, and pollinators. In order to provide a comparison for the adaptive signal as well as to determine phylogenetic relationships, genetic samples will need to be taken from other species of *Disa* as well. To compare flower colour in a phylogenetic context, colours of other species will have to be determined (or looked up).

Table 1. Some deceptive orchids of South Africa, the rewarding model species they resemble, and their primary pollinators. Data is from Johnson (1994, 2000), Anderson *et al*. (2005), and Johnson and Morita (2006).

Orchid	Model	Flower Colour	Pollinator
Disa ferruginea	*Tritoniopsis triticea*	red	*Meneris tulbaghia* (butterfly)
	Kniphofia uvaria	orange	
Disa pulchra	*Watsonia lepida*	pink	*Philoliche aethiopica* (fly)
Disa nivea	*Zaluzianskya microsiphon*	cream/ white	*Prosoeca ganglbaueri* (fly)
Disa nervosa	*Watsonia densiflora*	pink	*Philoliche aethiopica* (fly)

Experimental Design

Tissue samples from populations of each orchid along with samples from populations of other *Disa* species will be used for DNA extraction followed by PCR amplification and sequencing. Sequences of nuclear ribosomal internal transcribed spacer (ITS) regions will be used to construct a phylogenetic tree using parsimony and maximum likelihood methods.

Detecting a signal of adaptation will require sequencing genes controlling flower colour as well as other portions of the genome and comparing the variation in these regions across species and populations. Though the exact genes controlling flower colour may not be known presently, for the purposes of this proposal I will assume they have been identified or that genes similar to those controlling flower colour in other families have been experimentally shown to function similarly in *Disa* species. Whether the genes code for proteins involved in pigment biosynthesis or for factors controlling their cellular localization is assumed to already be determined. The analysis of the genes could include comparisons of variance as well as Tajima's D statistic and Fst measurements.

Possible Outcomes

Phylogenetic relationships are important for determining whether the trait in question (flower colour) is derived or plesiomorphic. For instance, the pink flower colours of *D. pulchra* and *D. nervosa* may have been independently derived for adaptation to their putative models, or they may be ancestral if the orchids are in the same clade or if pink flowers are found in most of the close relatives. Evidence that flower colour is derived supports the hypothesis that the trait is adaptive. Evidence that it is plesiomorphic makes adaptation less likely or at least more difficult to detect since if it occurred, it would have occurred much longer ago.

If the trait is under selection, we expect a decrease in genetic variation in the genes controlling the trait relative to neutral portions of the genome or relative to genes controlling the trait in species which are not under selection. The decreased variation could also be observed as low Fst values. Negative values of Tajima's D statistic would suggest that there has been recent directional selection for a given portion of the genome (Wright and Gaut 2005). Results which do not show reductions in variation or negative Tajima's D statistics could indicate that the genes are not under selection or that the choice of regions for comparison was poor. Those results may also occur if the time since the selective sweep is great enough to allow the genetic signal to decay.

Drawbacks

Whether a molecular signal is detected depends highly on the time since selection, since mutation and recombination can eliminate the signal (Wright and Gaut 2005). It seems possible that at least in some cases these orchid populations have maintained their flower colours for a long enough time to avoid detection of an adaptive signal. Despite this, the colour polymorphism in *D. ferruginea* suggests that divergence might be recent enough to detect a signal, since the populations are similar enough to be classified as the same species. The phylogeny produced as part of this experiment will help to clarify whether this is a reasonable conclusion.

References

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