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Pollination ecology and molecular systematics of Diuris (Orchidaceae)

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Abstract

The Australian terrestrial orchid genus *Diuris* is currently recognised to contain at least 61 species, with numerous new taxa expected to be recognised in the near future. Species are restricted to Australia, with the exception of *Diuris fryana*, which is endemic to Timor. Species of *Diuris* are well represented in the southern parts of western and eastern Australia, separated by the Nullarbor Plain, with a few species found in tropical Queensland. The eastern and western species mostly fall into morphologically distinct groups suggestive of distinct phylogenetic lineages.

Despite considerable variation between and even within species, *Diuris* species share certain important features. Most species occur in open forest and woodland and have flowers that bear a resemblance to Australian native 'egg and bacon' pea flowers of the tribes Bossiaeae and Mirbeliae, with which they are frequently sympatric. In some species, the resemblance is very close, in others it is more general. Most existing work on pollination in this taxon is of an anecdotal nature, with only one formal study prior to this project, of one species (*Diuris maculata* at Fern Tree Gully, near Melbourne, Victoria in 1986). The Beardsell *et al.* (1986) study proposed that this orchid was a non-rewarding floral mimic of pea flowers of the genera *Daviesia, Pultenaea* and *Dillwynia*. It was sympatric with the peas, with which it bore a visual resemblance, flowered at the same time and was visited by native bees, plus a wasp species that pollinated the pea flowers.

The overall purpose of my project was to advance knowledge of the pollination biology of *Diuris*. In particular, I planned to (i) test the effectiveness of AFLP markers for identifying the source of pollinium remnants collected from the bodies of putative

pollinators, (ii) conduct detailed pollination studies on two species in the Sydney region (*D. maculata* and *D. alba*) to test the generality of the conclusion of floral mimicry in *Diuris,* drawn from the 1986 study in Melbourne; (iii) survey pollinator interactions in a range of taxa (*D. aurea*, *D. punctata* and *D.* sp. aff. *punctata* (Mellong Swamp)), using pollination observations, imaging of ultraviolet visual cues, colorimetric analysis of putative model and mimic flowers, testing for nectar production and DNA-based identification of pollinaria removed from captured insects, to test for patterns in pollinator-plant associations, and (iv) place these observations in the context of a complete a phylogenetic analysis of the genus *Diuris.*

These main experimental findings are summarised as follows:

1. Amplified Fragment Length Polymorphism (AFLP) was shown not only to be capable of distinguishing many species, but also to possess high sensitivity. This latter feature had not been exploited previously. (See Chapter 2)
2. The pollination mechanism of *Diuris maculata* in this population was shown to be similar to the original Beardsell *et al.* (1986) study, but there were some significant differences – the timing of orchid flowering early in the flowering season of putative model pea species (cf. synchronised flowering in the Victorian population) and the role of male bees (cf. various male and female bees) in pollination, which may be quite common in *Diuris*. (See Chapter 3)
3. *Diuris alba* has flowers that are similar in form, but not colour, to other, putative pea-mimicking *Diuris* species. *Diuris alba* at Munmorah, New South Wales, was found to occur in a variety of

habitats including sites where pea flowers are absent or rare and the pollination success was found to be not dependent on pea flowers. This species was also found to produce a meagre nectar reward. (See Chapter 4)

4. Unpublished pollination data were obtained for a number of *Diuris* taxa (*Diuris aurea* (Castlereagh Nature Reserve), *D. punctata* (Castlereagh Nature Reserve), *D.* sp. aff. *punctata* (Mellong Swamp), *D. arenaria* (Tomaree National Park) and *Diuris sulphurea* form Stringy Bark Ridge, Pennant Hills. It was found that *D. aurea*, *D. punctata* and *D.* sp. aff. *punctata* showed pollination features consistent with Batesian-type floral mimicry of yellow 'egg and bacon' pea flowers, despite the latter two taxa having a white floral anthoxanthin base colour (with pink/purple suffusions). Additionally, preliminary data was obtained for *D. aurea* and *D.* sp. aff. *punctata* that showed higher pollination success for plants clustered some distance from yellow pea flowers than was obtained for plants scattered among yellow pea flowers. The taxon *D. arenaria* was shown to have higher reproductive success, when scattered amongst yellow 'egg and bacon' pea flowers than would be expected for a Batesian-type mimic, a result suggestive of a more generalised pollination strategy. Meagre nectar was found in one plant of *Diuris sulphurea* tested for nectar production, an interesting result that requires confirmation with further testing.

5. The molecular phylogenetic analysis of *Diuris* (Orchidaceae) based on AFLP and ITS (Internal Transcribed Spacers of Ribosomal DNA)

revealed three major clades and a basal species. *Diuris sulphurea* (subg. *Paradiuris*) is shown to a monotypic sister group to the rest of *Diuris*. (See Chapter 5)

The conclusions of the study can be summarised as follows:

1. Some species show close visual mimicry of specific model species (strict Batesian mimicry) e.g. *Diuris aequalis* is proposed to mimic *Gompholobium* spp.
2. Many species show a more generalised mimicry of peas (loose Batesian-type mimicry) e.g. *Diuris maculata* shows general similarity to 'egg and bacon' peas. Over its wide distribution (southern Victoria to north of Sydney, New South Wales) this orchid occurs at many sites and its pollination is likely to involve dozens of species of both peas and pollinators.
3. Some species show apparent dependence on mimicry while showing only aspects of similarity, with other different, perhaps flamboyant features suggestive of non-model mimicry e.g *Diuris* sp. aff. *punctata* (Mellong Swamp). This species shows pollination outcomes in the presence of the yellow pea *Dillwynia glaberrima* as expected for a yellow-flowered pea mimic. Its flowers show general pea-like form. However, the pink/purple floral colouration is quite different to the sympatric yellow peas. It is also noticeably fragrant. It is suggested that the flowers of this species are 'exciting' to a bee foraging on pea flowers because its flowers possess strong floral cues, which could be considered to transcend strict mimicry.

4. Generalised pollination e.g. *Diuris alba* from Munmorah, New South Wales. In this case flowers were shown to have pea-like floral form, but with different colour (white cf. yellow), fragrance and nectar. Plants were shown to have high pollination success both in the presence and absence of pea flowers. The pollination system was analysed using colorimetric analysis with a model of predicted colour-based foraging errors. This pollination system was proposed to have evolved from pea mimicry and not entirely disconnected from it. Aspects of Batesian-type mimicry, non-model mimicry, the 'magnet effect' and the presence of a meagre nectar reward may all contribute to high pollination success in varied environments.
5. *Diuris sulphurea* is the most widespread of all *Diuris* species in eastern Australia, forms a basal clade to the rest of the genus and may show ancestral pollination features. Such a widespread species is unlikely to mimic a single pea species and must show fairly generic pea mimicry. It has a colony-forming growth habit, produces nectar and has high reproductive success. An understanding of its pollination mechanism is likely to lead to insights of pollination evolution within the genus.

The phylogenetic data allow the following interpretations of current patterns of *Diuris* pollination:

1. The finding that *D. sulphurea* forms basal clade to all other *Diuris* suggests that this species may possess ancestral features, including pollination mechanism, which on the basis on preliminary evidence

would appear to be fairly generalised (guild) pea mimicry combined with nectar reward. Its pollination mechanism, combined with a colony-forming habit might be expected to promote significant self-pollination.

2. Knowledge of species groupings (clades) will aid in focusing pollination studies since clades can be expected to contain species sharing morphological and pollination features.

3. Yellow base colour can be inferred to be ancestral in *Diuris*, with pink/purple colouration being a synapomorphy in *Diuris* subg. *Diuris* sect. *Purpureo-albae* (plus some species within *Diuris* subg. *Xanthodiuris*, sect. *Pedunculatae*, e.g. *D. venosa*). Preliminary data suggest that despite the floral colour difference, species closely related to *D. punctata* appear to mimic yellow pea flowers and have a similar pollination mechanism, while another closely related species, *D. alba* has been shown to have generalised pollination. Therefore, a detailed understanding of pollination of the species *D. aurea*, *D. punctata*, and *D. alba* can be expected to provide considerable information about pollination within this large species group and also to provide important insights into the evolution of Batesian-type mimicry in this orchid group.

I propose the following hypothesis about the role of Batesian-type mimicy (which includes strict Batesian mimicry as commonly understood and looser forms, such as mimicry of a guild of Mullerian mimics) in the pollination systems of

Australian east coast *Diuris* species. Strict Batesian mimicry is highly specialised and inevitably leads to rarity, and there are consequently few examples. However, the looser type of Batesian mimicry (sometimes termed 'guild mimicry') exemplified by *Diuris maculata* permits the exploitation of many ecologically similar environments, in which basically similar, but distinct pea flower species may all serve as models for this orchid. Within *Diuris*, subg. *Diuris*, sect. *Purpureo-albae* there are numerous species with pink/purple, or white base colour. Many of these, paradoxically, appear to depend on association with yellow 'egg and bacon' peas for pollination. Phylogenetic evidence suggests that these species have undergone recent and rapid evolutionary radiation. This could be viewed as a shift toward even looser Batesian-type mimicry and could account for their evolutionary success. *Diuris alba* represents a group of species which have developed a sufficiently generalised pollination system to be independent of pea flowers, although high reproductive success in the presence of pea flowers would suggest that the link to pea mimicry is not completely broken. It thus may be reasonably termed a 'non-model' mimic and it likely benefits from the 'magnet effect' of being in the proximity of abundant rewarding species (which often happen to be pea flowers). As resemblance of this species to pea flowers is somewhat unclear, it may not be meaningful to view pea flowers as 'model' flowers, or indeed this orchid as a pea 'mimic'.

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Acknowledgements relating to each of the published research chapters can be found at the end of each of these chapters as they appeared in the published articles.

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