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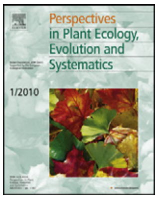

Research article

Pollination, biogeography and phylogeny of oceanic island bellflowers (Campanulaceae)

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ABSTRACT

We studied the pollination biology of nine island Campanulaceae species: *Azorina vidalii*, *Musschia aurea*, *M. wollastonii*, *Canarina canariensis*, *Campanula jacobaea*, *Nesocodon mauritianus*, and three species of *Heterochaenia*. In addition, we compared *C. canariensis* to its two African mainland relatives *C. eminii* and *C. abyssinica*. We asked to what extent related species converge in their floral biology and pollination in related habitats, i.e. oceanic islands. Study islands were the Azores, Madeira, Canary Islands, Cape Verde, Mauritius, and Réunion. Information about phylogenetic relationships of these species and their relatives were gathered from *atpB*, *matK*, *rbcL* and *trn*L-F regions, building the most complete phylogeny of Campanulaceae to date. Six of the island bellflower species were bird-pollinated and two (*A. vidalii* and *M. aurea*) were lizard-pollinated. Insects also visited some of the species, and at least *C. jacobaea* had both insect- and self-pollination. Several morphological traits were interpreted as adaptations to bird and lizard pollination, e.g. all had a robust flower morphology and, in addition, bird-pollinated species were scentless, whereas lizard-pollinated species had a weak scent. These examples of vertebrate pollination evolved independently on each island or archipelago. We discuss if these pollination systems have an island or mainland origin and when they may have evolved, and finally, we attempt to reconstruct the pollinator-interaction history of each species.

Introduction

Oceanic islands offer unique opportunities for studying the processes of species dispersal, establishment, and speciation, because their ecosystems often are biologically simple and geologically young and consequently, they may be relatively easy to comprehend (Kim et al., 2008). Long-distance dispersal events to oceanic islands usually only encompass a single species and not fragments of food webs (however, see Holt, 2010). Consequently, a species establishing on an oceanic island, experiences ecological release from its antagonists in its source continent (Janzen, 1985). On the island, it meets a community of native species to which it will be evolutionarily naïve and consequently, its potential interactions may be constrained (e.g. Aizen et al., 2008; Emerson and Gillespie, 2008). This different continental past or "ghost of linkage past" (inspired by Connell, 1980) has to be taken into account in order fully to understand the biology and, particularly, the biotic interaction context of an island species. However in spite of this different continental past, island lineages often converge in their evolution. Consequently, island selection regimes have to be very similar (e.g. Ricklefs and Schluter, 1993; Losos and Ricklefs, 2009). An example of island convergence is flightlessness in different bird lineages (Johnson and Stattersfield, 2008).

The well-studied bellflower family Campanulaceae has turned out to offer excellent opportunities in the study of this ecological-evolutionary compromise between the biotic past and presence of island species and their mainland ancestors (e.g. Olesen, 1985; Olesen and Valido, 2003a; Valido et al., 2004). We examined the extent of convergent or parallel evolution in pollination biology among related island bellflower species, all being limited in their distribution to tropical-subtropical oceanic islands. Recent studies have clarified the main phylogenetic relationships within the family (Eddie et al., 2003; Roquet et al., 2008, 2009; Haberle et al., 2009), and thus also its overall biogeographical history (Cellinese et al., 2009; Roquet et al., 2009), but these studies have also investigated the importance of pollinators to the evolution of the flower

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morphology within the family (Roquet et al., 2008). Here, we studied the pollination biology of nine island endemics of Campanulaceae and placed our results within a phylogenetic context encompassing the entire family. Specifically, we asked (1) to what extent related island bellflower species converge in their floral biology and pollination in related habitat, i.e. if specific island pollination morphs exist? And if so, (2) how can information about phylogeny and pollinator interactions, including both the past and the present pollinator fauna, explain the evolution of these island bellflowers?

Materials and methods

Study family

The Campanulaceae *sensu stricto* is a cosmopolitan (but mainly Eurasian) family with 600–950 species in 35–55 genera, sorted into three tribes: Campanuleae, Wahlenbergieae, and Platycodoneae (Eddie et al., 2003; Cosner et al., 2004; Roquet et al., 2008; Haberle et al., 2009). Platycodoneae and Wahlenbergieae *s. str.* clades are successive sisters to core Campanuleae (Fig. 1). Campanuleae *s. str.* mainly includes genera from the Northern Hemisphere, whereas Wahlenbergieae *s. str.* contains only Southern Hemisphere taxa (Cosner et al., 2004). The evolutionary history of Campanulaceae includes long-distance dispersal between continents and bursts of rapid diversification. The ancestor of the family may have originated in Asia, and later colonised Africa and the Mediterranean region (Haberle et al., 2009; Roquet et al., 2009). The initial diversification within the Platycodoneae is dated to 15–25 mya (million years ago), that of Wahlenbergieae *s. str.* to 15–20 mya, and that of the two main clades of Campanuleae to 10–15 mya (Roquet et al., 2009). Cooler and drier climate in Late Neogene and expansion of open steppe habitats in SW Asia and E Africa may have triggered these diversifications (Axelrod and Raven, 1972; Rohling et al., 1998; Fernandes et al., 2006). Tertiary climate change must also have affected the pollinators of the family because pollinators seem to have exerted a strong selective pressure on the floral traits, which are very diverse in the family (Roquet et al., 2008).

Within Campanuleae, open, rotate flowers are visited mainly by a diverse fauna of Syrphidae, Muscidae, small bees and *Xylocopa*, whereas more campanulate flowers are visited by a restricted fauna of *Bombus* species and other large bees (Skov, 1999; Roquet et al., 2008). However, the Campanulaceae also includes species pollinated by vertebrates, such as birds (Olesen, 1985) and lizards (Elvers, 1978; Hansen et al., 2006). Some taxa are selfers (e.g. *Campanula uniflora*, Ægisdóttir and Thórhallsdóttir, 2006; J.M. Olesen, pers. obs.; *C. dichotoma*, Nyman, 1992; *C. propinqua* and *C. fastigiata*, Roquet et al., 2008; *C. americana*, Galloway et al., 2003; the genus *Triodanis*, Trent, 1940; McVaugh, 1948; and island populations of the generally sexual *C. microdonta*, e.g. Inoue et al., 1996).

Insect-pollinated flowers are mainly blue-violet (can be also white or yellow), protandrous, scented, with small amounts of concentrated nectar, and have a "delicate" morphology. Among them, bee-pollinated flowers usually have a campanulate corolla, bee-fly- and butterfly-pollinated flowers are tubular, while flowers of selfers often are small and less conspicuous (e.g. Proctor et al., 1996). In contrast, bird-pollinated flowers are red, orange or yellow, scentless, with plenty of dilute nectar, and a robust morphology (e.g. Rodríguez-Gironés and Santamaria, 2004; Cronk and Ojeda, 2008; Dalsgaard et al., 2008; Rodríguez-Rodríguez and Valido, 2008). In a few cases, bird-pollination in Campanulaceae is expected to be relict. During glaciations and dry periods of the Neogene, mainland species may have partially disappeared, but survived in mesic refuges such as oceanic islands or mountaintops (Valido et al., 2004).

Study species and sites

Eleven Campanulaceae species were studied (Table 1). In addition, the floral biology of several greenhouse specimens of various origins was studied. Another bellflower described from Cape Verde Islands is *C. bravensis*, but the reported corolla colour and shape are not enough to discriminate it from *C. jacobaea*, which is included in our sample (Roquet et al., unpubl.). Recently, a third *Musschia* species, *M. isambertoi* M. Seq., R. Jardim, M. Silva & L. Carvalho, was described from the Desertas (Menezes de Sequeira et al., 2007). The differential characters of this species are: monocarpy (which is not the case for *M. aurea*), an unbranched inflorescence 1.5 m tall (0.4 m in *M. aurea*, 1–2 m in *M. wollastonii*), and anthers 2–3 times larger than those found in the other two *Musschia* species. However, sequences of *trnL-F* show no nucleotide difference between *M. isambertoi* and *M. aurea* (Roquet et al., unpubl.). Only one non-flowering individual/juvenile of *H. borbonica* was found at an altitude of 1730–1740 m on the trail from Hell-Bourg to Cap Anglais, Réunion. The plant was *c.* 0.4 m tall.

Flower morphology and pollinator observations

Flower measurements were made with a caliper. In early morning hours, volume of nectar per flower was measured as standing crop using micropipettes, and nectar sugar concentration was measured with a pocket refractometer and expressed as w/w% sucrose equivalents. Presence or absence of floral scent was scored from a sample of flowers stored for 24 h in a plastic bag. This crude method only allowed us to detect volatiles by the human olfactory sense. The site of secondary pollen presentation (typical to Campanulaceae; Erbar and Leins, 1989; Yeo, 1993) was identified and level of dichogamy, i.e. temporal order of maturation of anthers and stigma was scored by examining buds and flowers of different developmental stages. Compiled on field trips over 7 years, observations of pollinators were made in all populations. The amount of time spent per population varied from 12 (*C. eminii*) to 40 h (*N. mauritianus* and *C. canariensis*, population on the Anaga peninsula, Tenerife). Flower visitors are here called pollinators because contact with pollen brush and stigma in most cases was observed. All observations of pollinators are original unless otherwise stated. Insects were collected and identified later.

Phylogenetic inference and dating analyses

We retrieved sequences of four cpDNA regions (*atpB*, *matK*, *rbcL*, *trnL-F*) for all the Campanulaceae taxa available in Genbank (electronic supplement material ESM 1 – Tables 1 and 2). Sequences of *Abrophyllum ornans* (F. Muell.) Hook. f., *Cuttsia viburnea* F. Muell. and *Lobelia cardinalis* L. were added as outgroups. We sequenced the *trnL-F* region for 14 taxa in order to complete the sampling with the oceanic bellflowers studied here. The new sequences were added to Genbank (ESM 1 – Table 2).

Total DNA was extracted from herbarium material or silica gel-dried plant tissue using the "DNeasy Plant Mini Kit" (Qiagen Inc., Valencia, CA), according to the manufacturer's instructions.

PCR amplifications were performed with the thermocycler PTC-100TM Programmable Thermal Controller (MJ Research Inc.). The *trnL-F* region was amplified using the external primers "c" and "f" and internal primers "d" and "e" (Taberlet et al., 1991), amplifying the *trnL* (UAA) intron and the intergenic spacer between the *trnL* (UAA) 3′ exon and the *trnF* (GAA) 5′ exon. The PCR profile consisted of 1 min 35 s at 95 °C; 5 min at 80 °C, while DNA-polymerase (Ecotaq, Ecogen S. R. L.) was added; 34 cycles of 1 min denaturing at 93 °C, 1 min annealing at 50 °C, 2 min extension at 72 °C; and a final extension of 10 min at