Molecular Phylogenetics and Evolution 62 (2012) 306–318

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

The origin of bird pollination in Macaronesian *Lotus* (Loteae, Leguminosae)

Isidro Ojeda [a,*], Arnoldo Santos-Guerra [b], Ruth Jaén-Molina [c], Felicia Oliva-Tejera [c], Juli Caujapé-Castells [c], Quentin Cronk [a]

[a] *The Biodiversity Research Centre, Botanical Garden and Centre for Plant Research, Department of Botany, University of British Columbia, 6804 SW Marine Drive, Vancouver, Canada V6T 1Z4*
[b] *Jardin de Aclimatación de la Orotava, Unidad de Botánica-ICIA, Puerto de la Cruz, Canary Islands, Tenerife, Spain*
[c] *Jardin Botánico Canario "Viera y Clavijo" – Unidad Asociada CSIC, Las Palmas, Gran Canaria, Spain*

ARTICLE INFO

Article history:
Received 16 March 2011
Revised 27 September 2011
Accepted 1 October 2011
Available online 12 October 2011

Keywords:
Bird pollination
Species paraphyly
Rhyncholotus
Pedrosia
Macaronesia
Canary Islands
Molecular phylogeny
Dating
CYCLOIDEA

ABSTRACT

The four red-flowered, apparently bird-pollinated *Lotus* species from the Canary Islands have previously been classified in their own genus, *Rhyncholotus*. Currently, they are considered as a separate section within genus *Lotus*, distinct from other herbaceous Canarian congeners which are yellow-flowered and bee-pollinated. A combined analysis of four nuclear regions (including ITS and three homologues of *CYCLOIDEA*) and three plastid regions (*CYB6*, *matK* and *trnH-psbA*) nests the four bird-pollinated species within a single extant species of bee-pollinated *Lotus* (*L. sessilifolius*), in a very extreme example of species paraphyly. Therefore, our data compellingly support the hypothesis that the Macaronesian *Lotus* species with a bird pollination syndrome are recently derived from entomophilous ancestors. Calibration of the phylogenetic trees using geological age estimates of the most recent islands (La Palma and El Hierro) together with oldest ages of Fuerteventura indicates that bird pollination evolved ca. 1.7 Ma in the Canarian *Lotus*. These four bird-pollinated species share a most recent common ancestor (MRCA) with *L. sessilifolius* that dates to about 2.2 Ma. Our analyses further suggest that the evolution of the bird pollination syndrome was likely triggered by the availability of new niches in La Palma and Tenerife as a result of recent volcanic activity.

1. Introduction

The phytogeographic region of Macaronesia consists of five volcanic archipelagos (Azores, Madeira, the Salvage Islands, the Canary Islands and Cape Verde) as well as a region of the African mainland (southern Morocco and the former Spanish West Africa) known as the "Macaronesian enclave" (Fig. 1) (Peltier, 1973; Sunding, 1979). The Macaronesian flora has a high degree of endemism, 20% overall (Humphries, 1979) and 40% in the Canary Islands alone (Santos-Guerra, 1999). It also has a broad range of altitudinal zones (0–3700 m), island ages (0.8–21 Ma) and distances from main continental areas of Europe or Africa, with the islands ranging from 95 to 1600 km (Carracedo, 1994; Carracedo et al., 2002; Humphries, 1979).

Bird pollination is reported in this region for at least 11 endemic plant species from six genera: *Canarina* and *Musschia* (Campanulaceae), *Isoplexis* (Scrophulariaceae), *Echium* (Boraginaceae), *Lotus* (Leguminosae) and *Navaea* (Malvaceae). These species possess several features associated with opportunistic nectar-feeding birds, including red–orange flowers, abundant dilute nectar, diurnal anthesis, extended flower life span, and loss of scent and landing platform (Dupont et al., 2004; Olesen, 1985, 1988; Olesen and Valido, 2003a; Ollerton et al., 2009; Rodríguez-Rodríguez and Valido, 2008; Valido et al., 2004).

Recent studies have suggested at least five passerine birds, the Canarian chiffchaff (*Phylloscopus canariensis*), the blue tit (*Parus teneriffae*), the Sardinian warbler (*Sylvia melanocephala*), the canary bird (*Serinus canarius*) and chaffinch (*Fringilla coelebs*), as the main pollinators of these species (Ollerton et al., 2009; Rodríguez-Rodríguez and Valido, 2008; Sletzer, 2005; Valido et al., 2002). Despite the relatively large number of lineages with a bird pollination syndrome in this region, to date there are only four studies that unequivocally demonstrate the role of these opportunistic nectar feeders as effective pollinators, specifically in *Isoplexis*, *Canarina* and *Navaea* (González and Fuertes, 2011; Ollerton et al., 2009; Rodríguez-Rodríguez and Valido, 2008, 2011). In the remaining groups only nectar foraging observations have been reported.

Several hypotheses have been suggested to explain the origin and maintenance of this pollination syndrome in Macaronesia: (1) the "de novo specialist" hypothesis states that presumably extinct specialist nectarivorous birds on the islands exerted

---

[*] Corresponding author. Address: The Biodiversity Research Centre and Department of Botany University of British Columbia, Room 3529, 6270 University Blvd, Vancouver, Canada V6T 1Z4. Fax: +1 604 822 2016.

E-mail address: isidro@interchange.ubc.ca (I. Ojeda).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.10.001

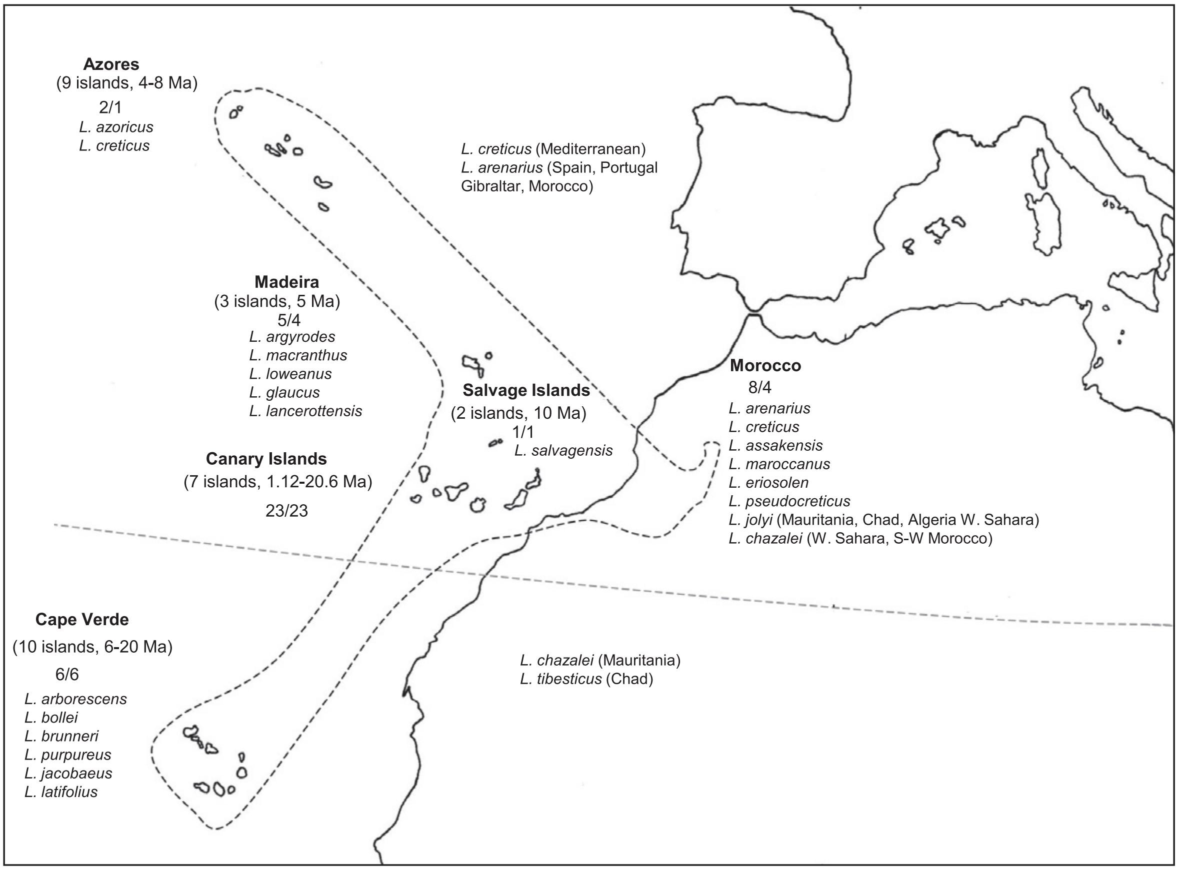

Fig. 1. Geographical distribution of *Lotus* section *Pedrosia* and rhyncholotus in the five Atlantic volcanic archipelagos of the Macaronesian region (Madeira, Azores, the Salvage islands, the Canary Islands and Cape Verde islands), Europe and Africa. In each archipelago, we indicate the no. of species/no. of endemic species. The age of each Canarian island after emergence was taken from Carracedo et al. (2002). The phytogeographic region of Macaronesia is indicated by dashed lines, including a portion of the Africa mainland denoted as the "Macaronesian enclave" (Peltier 1973; Sunding 1979; Kim et al. 2008).

selective pressures on the flowers, followed by their further maintenance by opportunistic birds after the extinction of the specialist birds (Olesen, 1985; Vogel et al., 1984); (2) the "relict" hypothesis suggests that the selection and evolution took place in mainland areas before the plant taxa colonized the islands, once in the islands the specialist bird was replaced by non specialist nectarivorous passerines (Valido et al., 2004); (3) the "de novo opportunistic" hypothesis suggests that the floral traits observed in these species evolved in the islands under the selective pressure of current opportunistic birds (Dupont et al., 2004; Valido et al., 2004).

To date, evidence from *Navaea* (González and Fuertes, 2011), *Canarina* (Fuertes-Aguilar et al., 2002) and *Isoplexis* (Bräuchler et al., 2004; Rodríguez-Rodríguez and Valido, 2008) suggests that these floral features are plesiomorphic and may be relictual. Bird-pollinated species in these genera do not have derived positions within their respective phylogenies. It is therefore likely that the evolution of these flower features occurred on the mainland before the colonization of Macaronesia. However, in the remaining cases (*Lotus*, *Muschia* and *Echium*) this is still unresolved (Valido et al., 2004) and it is unknown when these flower features evolved within these groups. Bird-pollinated *Echium* (Böhle et al., 1996; Dupont and Skov, 2004; Valido et al., 2002) and *Lotus* (Allan et al., 2004) have derived positions within entomophilous clades and this may indicate a recent origin in the Canary Islands. Due to incomplete sampling and the low resolution of the internal transcribed spacer (ITS) used in previous studies in *Lotus* (Allan et al., 2004; Degtjareva et al., 2006), it has not yet been possible to determine unequivocally the phylogenetic origin of bird pollination or the most closely related entomophilous species.

1.1. Bird-pollinated Macaronesian Lotus

Bird pollination occurs in four species of Macaronesian *Lotus* (Olesen, 1985; Ollerton et al., 2009; Valido et al., 2004). These are normally placed in their own section *Rhyncholotus*, but we will refer to them as the "rhyncholotus group" as our current analysis does not support their status as a section (see Section 4). These taxa have several floral traits associated with bird pollination, including: large flowers, great quantities of dilute nectar (mainly composed of hexose sugars), red–orange flower color, long-lived flowers, and upward flower orientation (Dupont et al., 2004; Olesen, 1985; Ollerton et al., 2009; Valido et al., 2004). These species are apparently pollinated by opportunistic nectar-feeders, and two species (the Canarian chiffchaff and the blue tit) have been reported foraging on cultivated individuals of at least two of these species (Ollerton et al., 2009; Sletzer, 2005).

The bee-pollinated species of section *Pedrosia*, together with those of the bird-pollinated rhyncholotus group, represent an example of an island radiation within Macaronesia. Currently, *Pedrosia* and *Rhyncholotus* are recognized as a two separate sections within *Lotus* (Degtjareva et al., 2006; Sandral et al., 2006; Valido et al., 2004), the former with about 36 species and the latter with four species (Sandral et al., 2006). Previous studies have addressed the morphology of Macaronesian *Lotus* (Degtjareva et al., 2006; Sandral et al., 2006). The main diagnostic feature common to both sections (*Pedrosia* s.l.) is the presence of a tooth (also referred to as a 'forked style') on the ventral side of the style (Kramina and Sokoloff, 1999). *Rhyncholotus* has been separated from *Pedrosia* only on the basis of differences in the floral traits