

Bird pollination of Canary Island endemic plants

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ABSTRACT

The Canary Islands are home to a guild of endemic, threatened bird pollinated plants. Previous work has suggested that these plants evolved floral traits as adaptations to pollination by flower specialist sunbirds, but subsequently they appear to have co-opted passerine birds as sub-optimal pollinators. To test this idea we carried out a quantitative study of the pollination biology of three of the bird pollinated plants, *Canarina canariensis* (Campanulaceae), *Isoplexis canariensis* (Veronicaceae) and *Lotus berthelotii* (Fabaceae), on the island of Tenerife. Using colour vision models, we predicted the detectability of flowers to bird and bee pollinators. We measured pollinator visitation rates, nectar standing crops, as well as seed set and pollen removal and deposition. These data showed that the plants are effectively pollinated by non-flower specialist passerine birds that only occasionally visit flowers. The large nectar standing crops and extended flower longevities (>10days) of *Canarina* and *Isoplexis* suggests that they have evolved bird pollination system that effectively exploits these low frequency non-specialist pollen vectors and is in no way suboptimal. Seed set in two of the three species was high, and was significantly reduced or zero in flowers where pollinator access was restricted. In *L. berthelotii*, however, no fruit set was observed, probably because the plants were self incompatible horticultural clones of a single genet. We also show that, while all three species are easily detectable for birds, the orange *Canarina* and the red *Lotus* (but less so the yellow-orange *Isoplexis*) should be difficult to detect for insect pollinators without specialised red receptors, such as bumblebees. Contrary to expectations if we accept that the flowers are primarily adapted to sunbird pollination, the chiffchaff (*Phylloscopus canariensis*) was an effective pollinator of these species.

INTRODUCTION

The endemic flora of the Canary Islands, situated off the west coast of North Africa, has fascinated biologists for centuries and in more recent times has provided some excellent examples of island radiations of genera (e.g. Lems 1960; Silvertown 2004; Carine et al. 2004). These islands are diverse in their habitats and the plant communities which they sustain, and in places harbour relics of an archaic, pre-ice age flora. The evergreen laurel forest, which in the Tertiary period covered much of Southern Europe and North Africa, survives only here and on a few other Atlantic islands. Within these habitats there exists a guild of endemic plant species with flowers unlike those anywhere in Europe or the Mediterranean basin, and which superficially appear to be adapted in morphology to bird pollination. Where did these flowers come from, how did their unusual appearance evolve, and which animals pollinate them?

The pollination biology of Canarian plants has generally not been well studied (Garcia 2000), but these bird guild flowers have received considerable attention because, although they show the hallmarks of belonging to a bird pollination syndrome, there are no specialist flower visiting birds on the islands. This discrepancy was first noted by Vogel (1954) who hypothesised that the original pollinators of these plants were sunbirds (Nectariniidae) which had become extinct. Vogel et al. (1984) conjectured that the flowers are remainders of a flora that was Trans-Saharan in the Tertiary, and that the flowers were pollinated by birds specialised on nectar feeding. The assumption is that these pollinators vanished in the Pliocene, and so did their flowers – everywhere except in the Canaries, where the flowers survive to this day. Vogel et al. (1984) further suggested that some Palearctic bird species which colonised the islands during the Pleistocene discovered the “orphaned” bird flowers of the islands as welcome carbohydrate resources, and that they are adequately pollinated by these passerine species. Field work by various researchers since then has tended to confirm that the flowers of these plants are visited by passerine birds not specialised for flower visitation (e.g. Olesen 1985, Valido et al. 2002, 2004; Dupont and Olesen 2004), but there has been relatively little attempt to quantify the pollination services of these birds and to assess their effectiveness as pollinators (but see Rodríguez and Valido 2008). Such an assessment is necessary in order to determine whether the “typical” bird flower characteristics of these species have indeed been the result of selective pressures enforced by the passerine bird flower visitors (Dupont and Olesen 2004). Alternatively, are the traits largely the result of earlier evolution for sunbird pollination, and non-flower specialist passerines were co-opted as secondary pollinators following dispersal of these lineages to the sunbird-free Canary Islands, or the extinction of sunbirds from these islands?

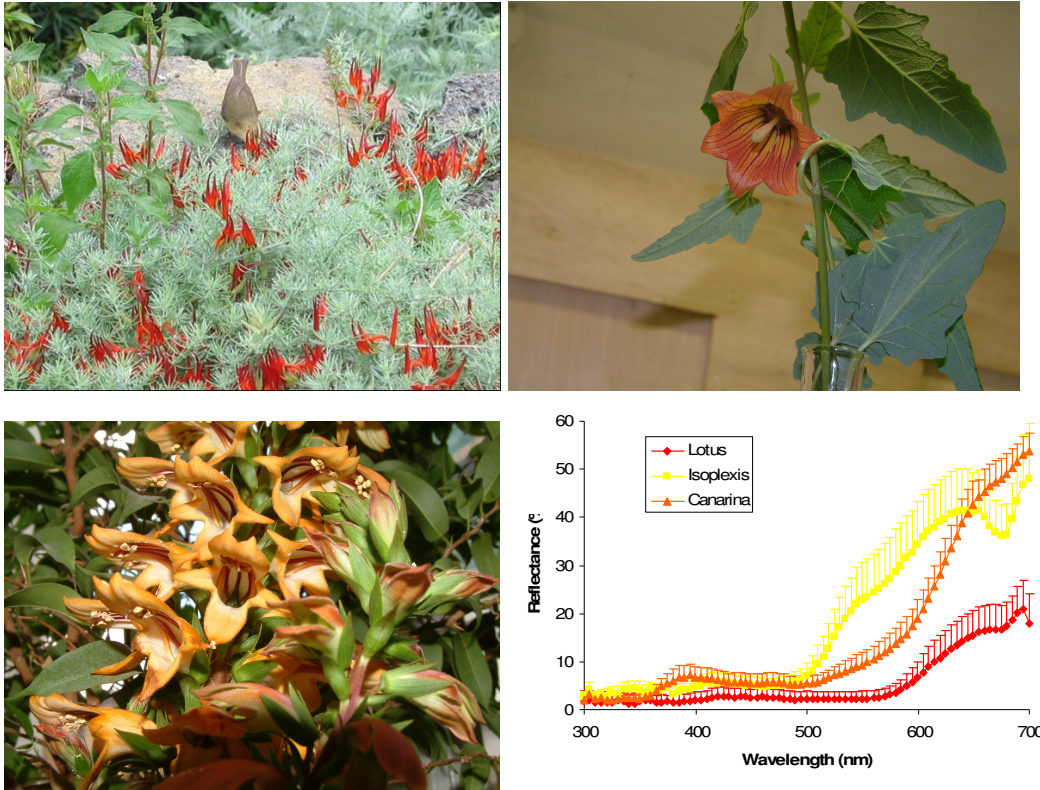


Fig. 1. The flower species studied and their spectral reflectance. Top left: *Phylloscopus canariensis* visiting the flowers of *Lotus berthelotii*; top right: a flower of *Canarina canariensis* in a laboratory; bottom left: an inflorescence of *Isoplexis canariensis*. Bottom right: average reflectance spectra (+SD) of *Lotus berthelotii*, *Canarina canariensis* and *Isoplexis canariensis*. Measurements were performed using an AvaSpec-2048 spectrophotometer. Five flowers were measured per species, with 20 scans averaged per flower, and an integration time of 100 ms. Lower error bars have been removed to aid interpretation.

Studying the reproductive biology of these plant species is also important because some of them are rare and threatened by extinction, whilst others may well already be extinct (Vogel et al. 1984; Olesen 1985; Bramwell and Bramwell 2001). In this study we focussed on two of the more common bird pollinated species on the island of Tenerife: *Canarina canariensis* (Campanulaceae) and *Isoplexis canariensis* (Veronicaceae ex Scrophulariaceae). In addition we were able to collect some data on *Lotus berthelotii* (Fabaceae) in cultivation on the island. All of the putatively bird pollinated *Lotus* species are extremely rare or possibly extinct in the wild (Bramwell and Bramwell 2001). This is echoed by the review of Valido et al. (2004) which could provide virtually no observations of flower visitors: although not ideal, observations made in cultivation may be the only feasible possibility for these species.

We conducted field work on the island of Tenerife to answer the following questions:

1. What are the main flower visitors of these species? Are they largely birds, or are insects also involved?
2. What are the nectar characteristics of these flowers, in terms of volume, sugar concentration and diurnal production rates?

3. How do the floral colours appear to insects and birds, are they detectable to these visitors, and is there evidence that the flowers' colour signals are specifically designed to address only the visual system of one pollinator type?

We also collect preliminary data on how effective are these flower visitors at removing and depositing pollen, and discuss the possibility that the flowers might be specifically adapted to bird visitors that are not, in turn, specialised flower visitors.

MATERIALS AND METHODS

Field work on *Canarina canariensis* and *Isoplexis canariensis* was undertaken from the beginning of March until mid May 2005 at a range of sites in Las Montañas de Anaga, Tenerife (28° 32' – 33'N, 16° 12' - 16'W). The altitude was 618-750m a.s.l. A list of all sites, with GPS coordinates, is available from JO on request. Field observations on *Lotus berthelotii* were performed in the small botanical garden at El Parque del Drago, Icod de Los Vinos, Northern Tenerife. Subsequent laboratory work took place in LC's lab at Queen Mary College, London and JO's lab at the University of Northampton. Field and laboratory techniques followed standard pollination biology protocols as described by Kearns and Inouye (1993) and Dafni et al. (2005).

Species descriptions

The following is based on our own observations and information in Bramwell and Bramwell (2001), except where otherwise noted:

Canarina canariensis (Campanulaceae) is a scrambling perennial herb, common in the shaded understorey of the laurel forest zone of Tenerife. It also occurs on some of the other Canary Islands. The orange-reddish bell shaped flowers are large (3 to 6cm in height) and pendulous, produced singly from leaf axils. The fruit is fleshy and edible. The genus *Canarina* contains only three species, the other two of which are native to tropical East Africa (Mabberley 1990)

Isoplexis canariensis (Veronicaceae ex Scrophulariaceae) is an upright small shrub, locally frequent in the laurel forest zone of Tenerife, but rare (and possibly extinct) on some of the other islands. Dense spikes of orange, zygomorphic flowers up to 1.5m in height are produced from leaf rosettes. The fruit is a dry capsule. Recent molecular phylogenetic analysis indicates that the genus *Isoplexis* is nested within *Digitalis* and should be considered taxonomically distinct only at sectional level (Bräuchler et al. 2004).

Lotus berthelotii (Fabaceae) is a scrambling to pendulous, slightly woody perennial herb, originally found on forest cliffs at only a few sites in Tenerife. It is now almost extinct in the wild, but is common in cultivation. The scarlet, zygomorphic flowers have a long, slender keel and wing petals held erect, and are profusely produced. The fruit is a dry legume. *L. berthelotii* belongs to a large, probably monophyletic clade of *Lotus* species that is endemic to the Canary Islands (Allan et al. 2004).

To avoid confusion caused by the identical specific epithets, we have chosen to refer to *I. canariensis* as *Isoplexis* and *C. canariensis* as *Canarina*. Likewise *L. berthelotii* is referred to simply as *Lotus*.

Analysis of flower colour

The spectral reflectance function (SRF) of flower petals was measured with a spectrophotometer (AvaSpec-2048, Avantes, Netherlands) relative to a white reflection standard, using a Deuterium-Halogen light source (DH 2000; Ocean Optics

(Dunedin, FL, USA). We were interested in assessing the extent to which the flower colours stimulated the various colour receptor types of bees and birds (Kevan et al. 2001). These can be assessed if the reflectance function of the object, the spectral sensitivity function of the receptor in question, and the illumination power spectrum are known. As an illumination spectrum, we used daylight norm function D65 (Wyszecki and Stiles 1982).

The flowers appear to be visited primarily by two passerine birds *Phylloscopus canariensis* (the Canary chiffchaff) and *Parus caeruleus* (the blue tit; see results below). Blue tits have four types of cones, whose sensitivity is determined by the opsin visual pigment as well as oil droplets and ocular media that filter the incoming light; the receptors are maximally sensitive in the UV ($\lambda_{\max}=374\text{nm}$), the blue ($\lambda_{\max}=455\text{nm}$) the green ($\lambda_{\max}=539$) and the red ($\lambda_{\max}=607$) (Hart et al. 2000; Hart 2001; Hart and Vorobyev 2005). Data for the spectral sensitivity of the chiffchaff are not available; however, all passeriform birds studied so far possess a tetrachromatic set of cones, with relatively little interspecific variation in the tuning of photopigments (Bowmaker et al. 1997; Hart 2001). Among 12 different passerines studied, for example, the wavelengths of maximum absorbance ranged from only 355–380nm for the UV pigment, 440–454nm for the short-wave pigment, 497–504nm for the medium-wave pigment, and 557–567 for the long-wave pigment (Hart 2001). The blue tit thus serves as a typical example for a passerine bird, and is used here to substitute for the chiffchaff as well.

For bees, we were specifically interested in how the flowers might appear to the local bumblebee subspecies *Bombus terrestris canariensis*. To this end, we modelled the responses generated by the flower signals in the bees' UV ($\lambda_{\max}=348\text{nm}$), blue ($\lambda_{\max}=436\text{nm}$) and green receptors ($\lambda_{\max}=538\text{nm}$), using these bees' continental conspecifics *Bombus terrestris dalmatinus* (Skorupski et al. 2007). The relative amount of light absorbed by each photoreceptor class is given by P :

$$P = R \int_{300}^{650} S_i(\lambda)I(\lambda)D(\lambda)d\lambda \quad (1)$$

and the variable R is the coefficient of adaptation to a green foliage background (I_B).

$$R = 1 / \int_{300}^{650} S(\lambda)I_B(\lambda)D(\lambda)d\lambda \quad (2)$$

The transduction of photoreceptor absorption (P) into receptor excitations (E) is given by:

$$E = P/(P+1) \quad (3)$$

For details see Gumbert et al. (1999) and Chittka (1997). For bees specifically, we were interested in the question of whether the bird flowers might be cryptic for bees, as an adaptation to exclude them as visitors. For this purpose, colour loci of the stimuli were calculated in the hexagon colour space, where coding is performed by two unspecified colour opponent mechanisms and colour distance is calculated as the Euclidean distance between stimuli loci in colour space (Chittka 1997). We also evaluated green contrast (i.e. the difference in green receptor signal generated by the

background and respective flower type), since this contrast has previously been shown to aid long range detection of flowers (Giurfa et al. 1996; Chittka and Raine 2006). As follows from formulae 1-3 above, the green receptor signal for the background (green foliage) is 0.5, where the receptor's entire (normalised) response range is from zero to unity; hence green contrast can range from 0 (no contrast; if the receptor signal for a given target is 0.5) to 0.5 (maximum contrast; the receptor signal is either zero, or maximum).

For passerine birds, we chose to model flower colours in a 3-dimensional colour opponent space. This is because n-1 colour opponent dimensions are necessary for coding the information from n colour receptors (Chittka 1996). We chose the following three colour opponent mechanisms to correspond to the axes of the colour space:

1. $x = E_U - 1/3 * (E_B + E_G + E_R)$
2. $y = E_B - 1/3 * (E_U + E_G + E_R)$
3. $z = E_G - 1/3 * (E_U + E_B + E_R)$

Note that equal excitation of the receptors yields zero excitation in the opponent processes, i.e. a spectrally neutral stimulus. The existence of these opponent mechanisms remains to be determined, but note that the precise nature of the colour opponent dimensions is largely arbitrary for colour coding, so long as the mechanisms are approximately orthogonal (Chittka 1996).

Visitors to the flowers

Observations of flower visitors were made in several populations throughout March and April, typically at a distance of not less than 10m, using binoculars. We observed visitors during daylight hours, from dawn to early evening. Observations of *Canarina* were made at ten populations over three months, comprising a total of 2312 flowers for 130.6 hours. Due to the scrambling habit of *Canarina* it was not possible to determine the number of individual plants, but it was not less than 500. *Isoplexis* was observed at four populations, containing a total of 791 inflorescences on at least 100 plants, for 39.1 hours. For *Lotus*, we observed approximately 3090 flowers for 19.9 hours at two sites within a botanical garden, recording 88 flower visits. Once again, the sprawling habit of the plant makes it impossible to determine the exact number of individuals, and in this case we suspect that the whole population was clonal (see below).

The variation in observation effort per species reflects the relative rarity and accessibility of the first two species (*Isoplexis* tends to occur infrequently in small patches on inaccessible rock outcrops, compared to the much more common and accessible *Canarina*) and that observations of *Lotus* were limited to a single, cultivated population at Dragon Tree Park during the second half of April only.

Floral biology, nectar production, and pollen removal and receipt

Floral longevities were assessed by tagging flowers in late bud stage and observing them from anthesis to flower wilting. Nectar standing crop of flowers was measured by randomly sampling previously un-sampled flowers throughout the day and the pattern of nectar production during the day was assessed by repeat-sampling the same, unbagged flowers every two hours from approximately 0800 onwards. In both cases we used either 0.5, 1, 3, 10, 20, 25 or 200 μ L glass microcapillary tubes

(Carl Roth GmbH, Karlsruhe, Germany). Nectar sugar concentration was measured using hand held sugar refractometers (Bellingham and Stanley).

Flowers of all species were initially observed to determine recognisable stages of development. Subsequently, flowers were categorised as follows: *Canarina* – early anthesis, mid anthesis (male stage) and late anthesis (female stage); *Isoplexis* – pre anthesis and post anthesis; *Lotus* – early anthesis and late anthesis.

Flowers from these stages were collected, stamens and pistils separated, and stored in 70% ethanol in individual Eppendorf tubes prior to analysis in the UK. In the laboratory, stigma pollen loads were counted under a dissecting binocular microscope, and the number of pollen grains remaining in anthers was determined using a Coulter Particle Counter.

Fruit production and breeding systems

Assessments were made of proportional fruit production in the populations and patches of all species by counting developing fruit and unfertilized flowers. The capacity for plants to set seed autogamously was investigated by bagging flowers of all species prior to anthesis and observing fruit production. All statistical analyses were performed using SPSS 8.0 for Windows (1997, SPSS Inc. Chicago, IL, USA). Averages are presented as mean \pm one standard deviation.

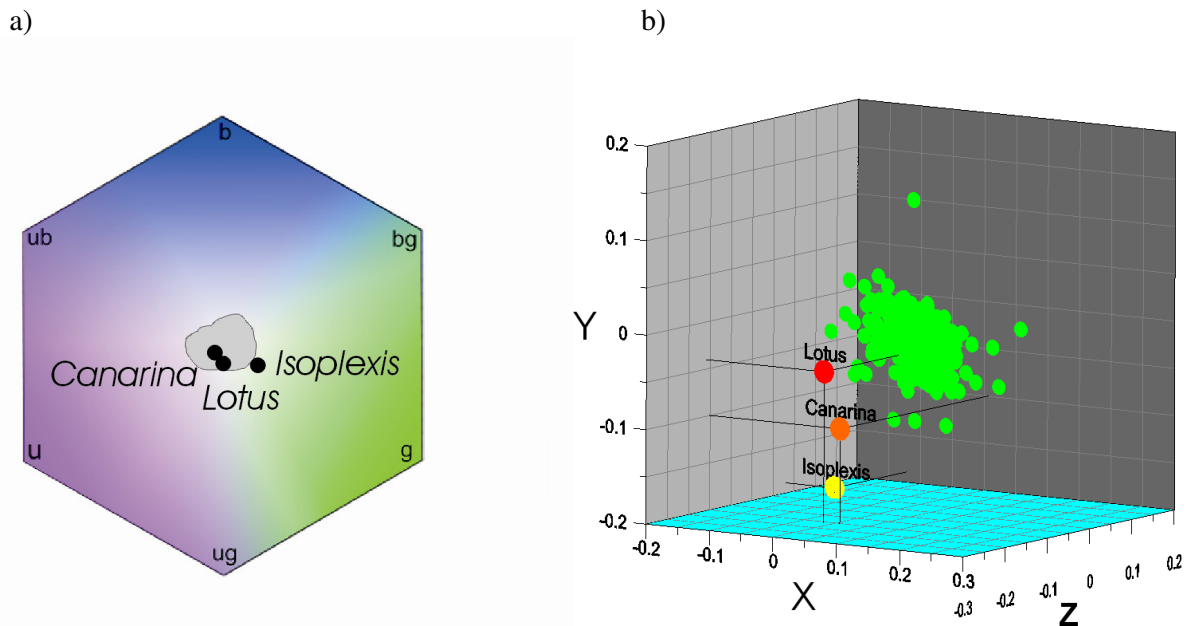


Fig. 2 – Colour loci of *Lotus berthelotii*, *Canarina canariensis* and *Isoplexis canariensis* in the colour space of trichromatic bees (a) and in the three-dimensional colour space of birds (b), shown with the corresponding areas of colour space occupied by green leaves from 230 plant species (Chittka 1996). a) In the colour hexagon space of bees, two of three of the plant species fall within the area covered by green foliage (shown in grey) and are therefore predicted to be relatively hard to detect. b) Conversely, in the 3 dimensional colour space for passerine birds, all three flowers fall clearly outside the volume of colour space that is occupied by green foliage.

RESULTS

Analysis of flower colour

The spectral reflectance functions of the three flower species under investigation (*Lotus berthelotii*, *Canarina canariensis* and *Isoplexis canariensis*) are displayed in Fig.1. None of the species reflect much UV light; the yellow-orange *Isoplexis* reflects a considerable amount of green light in addition to its strong reflectance in the green-red range. The orange *Canarina* and red *Lotus* reflect light predominantly above 600nm. For bees, both of these species are predicted to be poorly detectable by colour, since they fall into the same area of colour space as that occupied by green foliage (Fig.2a). *Isoplexis* stimulates a bee's green receptors somewhat more strongly, and therefore stands out from a green foliage background, although not conspicuously so. By definition (see methods) the centre of the colour hexagon corresponds to the average reflectance of green foliage – thus colour contrast to the centre of colour space (maximum possible = 1) provides a measure of conspicuousness of flowers against green foliage. Most bee-pollinated flowers have contrast values between 0.2 and 0.4 (Chittka 1997); the value is 0.14 for *Isoplexis* (providing moderate detectability) but only 0.06 for *Lotus* and 0.02 for *Canarina*, indicating very poor detectability for bees.

	BEE			BIRD			
	UV	BL	GR	UV	BL	GR	RE
<i>Isoplexis</i>	0.44	0.41	0.57	0.45	0.40	0.57	0.75
<i>Lotus</i>	0.25	0.24	0.18	0.25	0.23	0.14	0.42
<i>Canarina</i>	0.43	0.43	0.41	0.49	0.41	0.37	0.67

Table 1 Relative voltage signals in bumble bee (*Bombus terrestris*) and bird (blue tit *Parus caeruleus*) photoreceptors. These signals can range from 0 (no excitation at baseline voltage) to one (maximum excitation).

Of particular interest in assessing the detectability of a target for bees is *green contrast*, i.e. the difference in green receptor signal that is generated between a target and its backdrop (Giurfa et al. 1996). Green contrast is used particularly from longer distances or when flowers are small (Giurfa et al. 1996; Chittka and Raine 2006). Flowers that produce high green contrast and colour contrast are particularly well detectable (Giurfa et al. 1996). Positive green contrast (the target stimulates the receptor more than the background) appears to yield somewhat greater detectability than negative contrast (Giurfa et al. 1996). The maximum theoretical green contrast is 0.5 (see methods).

Green contrast is low (and negative: -0.09; see Table 1) for *Canarina*, so that this species combines low colour contrast with low green contrast, and is thus predicted to be poorly detectable for bees. *Lotus*, while having a low colour contrast (see above) has a relatively large (but negative: -0.32; Table 1) green contrast, is therefore predicted to be somewhat better detectable than *Canarina*. *Isoplexis* combines moderate colour contrast with low green contrast (0.07; Table 1) and is therefore not entirely cryptic, but also not well detectable for bees.

Conversely, in the colour space of passerine birds, the flowers of all three species clearly contrasted with green foliage (Fig.2b) and are therefore predicted to be easily detectable for passerines. Receptor excitation values (table 1) for the 3 flower species differ from 0.5 (the value for the average leaf) strongly in at least 3 out of 4 photoreceptors, indicating that these flowers will contrast strongly with green foliage backdrop.

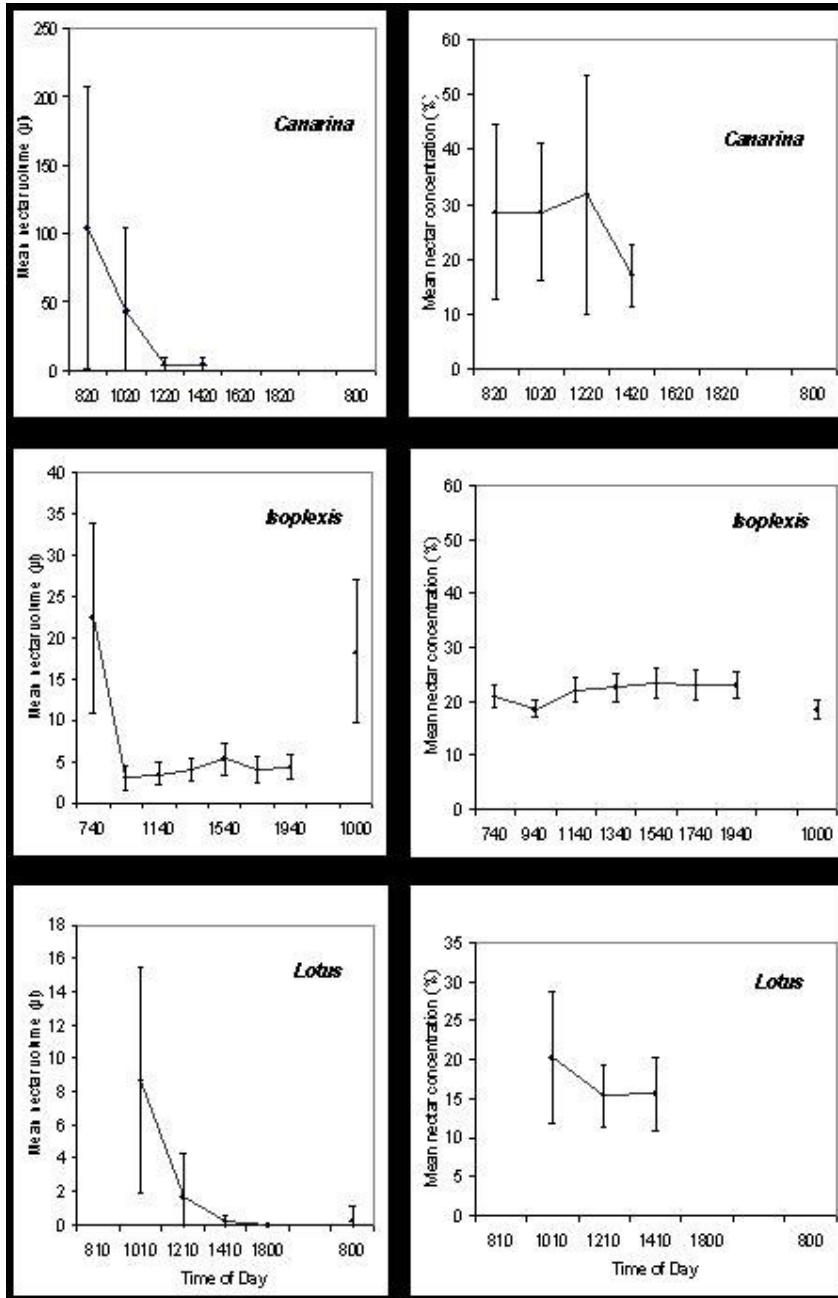


Fig. 3. Nectar characteristics of *Lotus berthelotii*, *Canarina canariensis* and *Isoplexis canariensis*. Nectar standing crop and sugar concentration (+SD) are shown as a function of time of day from repeat-sampled, unbagged flowers. Sample sizes are: *Canarina* n= 5 flowers; *Isoplexis* n=9 flowers; *Lotus* n=8 flowers. Repeat sampling of flowers of other individuals show similar patterns but are not presented due to mismatch in sampling times.

Floral biology and nectar production

Individual flower longevity was relatively long for both *Canarina* (18.1 ± 4.4 days, $n=15$ flowers on 15 plants) and *Isoplexis* (13.7 ± 2.7 days, $n=82$ flowers on 13 plants). Floral longevity could not be calculated for *Lotus*, but Olesen (1985) records that they remain viable for at least 10 days if unpollinated.

The three species produced moderate to very large quantities of low to medium concentrated nectar with significant standing crops and hourly production rates. For flowers randomly sampled throughout the day, *Canarina* mean nectar standing crop volume was $109.8 \pm 75.6\mu\text{l}$ ($n=58$ flowers from at least 10 plants across 3 sites) whilst the mean sugar concentration was $16.4 \pm 6.5\%$ ($n=53$ flowers); *Isoplexis* mean nectar standing crop volume was $24.8 \pm 18.5\mu\text{l}$ ($n=78$ flowers on 22 plants from 13 sites) and the mean sugar concentration was $29.5 \pm 11.9\%$ ($n=78$ flowers); finally, *Lotus* mean nectar standing crop volume was $13.5 \pm 8.2\mu\text{l}$ ($n=33$ flowers from 2 separate patches) and the sugar concentration was $32.0 \pm 14.0\%$ ($n=33$ flowers).

Standing crop of nectar was typically high in all three species in the morning (Fig. 3), above the volumes found in flowers commonly visited by bees, which are typically below one microlitre (Heinrich 1979). Repeated sampling of these flowers drained them of nectar which was only slowly replenished during that same day, although in *Isoplexis* nectar volume had recovered to the level of the beginning of the previous day by 10:00 (Fig. 3). Nectar concentration remained relatively constant near 20% during the day in *Isoplexis* and *Lotus* (Fig. 3). It should be noted that such low nectar concentrations are relatively unattractive for bees (Heinrich 1979). In *Canarina*, nectar production started at slightly higher levels (near 30%) in the morning, but fell to near 20% later in the day (Fig. 3).

For the three species, we calculated mean nectar production rates per hour (NPR^{hr}) of $7.1 \pm 24.9\mu\text{l}$ for *Canarina*, $2.0 \pm 0.7\mu\text{l}$ for *Isoplexis* and $0.1 \pm 1.7\mu\text{l}$ for *Lotus*. Therefore, whilst the three species had similar patterns of nectar presentation, with peak standing crop volumes in the morning, the hourly rate of production of *Canarina* was over 3.5 times as much as for *Isoplexis*, which in turn was 20 times as great as for *Lotus*. To humans, the viscous nectar of *Isoplexis* tastes bitter, and has a tongue-numbing effect, indicating the presence of secondary compounds, possibly alkaloids.

Visitors to the flowers

Flower visitors to these species were relatively infrequent in our study, but for all three taxa by far the commonest visitor was a passerine bird, *Phylloscopus canariensis*, the Canary chiffchaff. Honeybees (*Apis mellifera*) from nearby hives were occasionally observed to visit *Canarina* flowers and collect pollen, and a second passerine species *Parus caeruleus*, the blue tit, visited *Isoplexis* flowers on three occasions, and *Lotus* on one occasion. The lizard *Gallotia galloti* was twice seen feeding from *Lotus* flowers. The following calculations are all based on visitation rates from the two species of birds, though it appears that the main flower visitor to these three species is *Phylloscopus canariensis*.

In *Canarina*, a total of 35 visits were recorded, the visitation rate ranging from 0.00 to 0.02 visits per inflorescence per minute, with a mean visitation rate of 0.003 (± 0.02) birds per flower per minute, or approximately 1 visit every 5.5 hours. In *Isoplexis*, seventy six visits were recorded, with a visitation rate ranging from 0.00 to 0.01 visits per inflorescence per minute (mean = 0.001 ± 0.0021), which is equivalent

to approximately 1 visit every 16 hours. For *Lotus*, the visitation rate ranged from 0.0000 to 0.0002 visits per inflorescence per minute (mean = 0.0001 ± 0.0001), which is approximately 1 visit every 7 days.

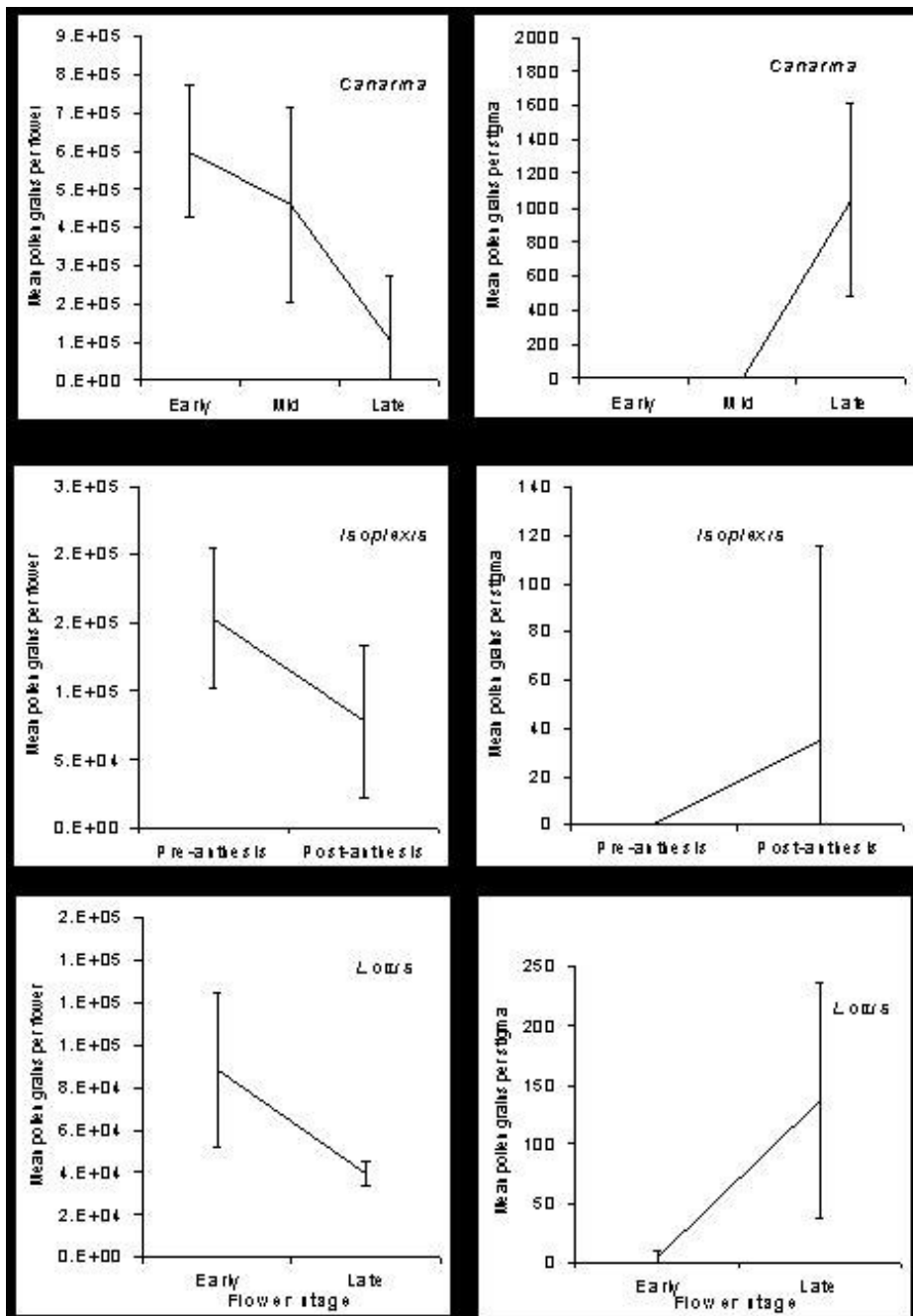


Fig. 4 – Pollen receipt on stigmas and pollen dispersal of the three species at different stages of flower anthesis. Samples sizes are: *Canarina* – early anthesis (n=9 flowers), mid anthesis (n=29) and late anthesis (n=48); *Isoplexis* – pre anthesis (N=18) and post anthesis (n=20); *Lotus* – early anthesis (n= 29) and late anthesis (n = 29).

Canarina therefore had the highest average rate of flower visitation, some 3 times greater than the visitation rate to *Isoplexis*, which correlates with the much higher rate of nectar production in *Canarina*. However, the very different inflorescence morphologies of these two species, and the relative inaccessibility of *Isoplexis* populations, meant that we could only compare flower visits for *Canarina* with inflorescence visits for *Isoplexis*; the *per flower* rate of visitation for *Isoplexis* will be much lower than this. *Lotus* had the lowest rate of visitation, which correlates with this species having the lowest rate of nectar production. The public park setting for these observations was clearly unnatural and may not reflect the natural rate of visitation: although the birds appeared habituated to the presence of people, the density of flowers was unnaturally high, which would negatively affect the per flower visit rate.

The visit times of birds to individual flowers were similar for all three species, and typically only one to a few seconds per flower. However the different growth forms and inflorescence morphologies of the three species meant that bird behaviour was different. On the scrambling, lax *Canarina*, birds would perch in nearby vegetation, or very occasionally hang upside down from the flower, to access nectar, visiting only a single flower before departing. On *Isoplexis*, the birds would perch on the upright flower spikes and move between them, feeding from two or three flowers per spike, and spending up to two minutes feeding from them. For *Lotus*, which is a low, spreading species, birds would move through a patch, feeding rapidly from numerous flowers, again staying up to two minutes in the patch.

Pollen removal and receipt

All three species produced a large amount of pollen per flower, typically of the order of high tens of thousands to mid hundreds of thousands of grains per flower (Fig. 4). In open pollinated flowers, pollen remaining per flower declined by 50% to 90% as the flowers aged and were visited by birds (Fig. 4). This pollen removal was mirrored by an increase in stigma pollen receipt in all three taxa, averaging thousands of grains per stigma in *Canarina*, tens to hundreds of grains in *Isoplexis*, and hundreds in *Lotus* (Fig. 4). Ovaries of *Canarina* typically contain more than 1000 ovules and those of *Isoplexis* several hundred ovules. In *Lotus* ovule numbers were lower, and we were therefore able to obtain exact counts, which ranged from 12 to 46 (mean= 28.2; SD= 11.5; n=13). Assuming that most of the pollen is received from other individuals (which is likely given the behaviour of the pollinators in only visiting a few flowers per plant – see above), these species are therefore unlikely to be pollen limited as adequate pollen to fertilise all ovules is usually found on the stigmas of late stage flowers (though see comments below regarding *Lotus* fruit set).

Fruit production and breeding systems

The fruit set of open pollinated flowers of *Canarina* was scored at eight different sites and overall mean fruit set was $62 \pm 14\%$ (n = 506 flowers, range 40-128 flowers per site; range of fruit set across the sites 44% - 83%). In the flower bagging experiments, bagged flowers (n = 16) had zero fruit set and did not set seed; 50% of the unbagged flowers (n = 16) at the same site set fruit. Seed set from open-pollinated flowers of *Canarina* is usually very high (mean = 1164 ± 496 seeds per flower, n = 5 fruit). These data indicate that *Canarina* cannot autogamously self pollinate and requires pollinators for fruit and seed set.

In contrast, *Isoplexis* can autogamously self pollinate, but open pollination yielded far greater seed-set. Unbagged inflorescences had a mean seed-set of $146.2 \pm$

137 seeds per flower (median = 84.0; n=169 fruits) whereas bagged inflorescences had a mean seed-set of 13.6 ± 25.7 per flower (median = 0.00; n=39 fruits; Mann-Whitney U = 469; $p < 0.0001$).

Despite an extensive search of the *Lotus* in cultivation at the botanic garden, no fruits were found in either patch at this site. We suspect that the origins of these plantings are from vegetative propagation of only a single clone, as the species is likely to be self-incompatible (Olesen, 1985).

DISCUSSION

The main pollinators of *Canarina canariensis*, *Isoplexis canariensis* and *Lotus berthelotii* in our study were passerine birds, and in particular the Canarian chiffchaff *Phylloscopus canariensis*. Insects were infrequent visitors, mainly opportunistic taxa such as honeybees. Oddly, native bees were not observed to visit these flowers, despite being abundant at our field sites. *Bombus terrestris canariensis*, which is the only species of bumblebee in the Canary Islands, has a peculiar preference for red flowers when tested in the laboratory (Chittka et al. 2004). Such a red preference was also described in the North American species *Bombus occidentalis* (Briscoe and Chittka 2001), a species which heavily exploits red hummingbird flowers for nectar (Irwin and Brody 1999). We therefore might have expected *Bombus terrestris canariensis* to visit the red reflecting flowers of *Canarina* and *Lotus*, but none were ever seen at the any of the three focal plant species (Stelzer et al. 2007).

Flower visitation rates to all species were low, ranging from on average 1 visit every 5.5 hours for *Canarina*, to 1 visit every 16 hours for *Isoplexis*, and to 1 visit every 7 days for *Lotus*. The latter result is probably lower than would naturally occur because of the artificially high density of flowers found when this species is grown as a horticultural bedding plant.

The flowers of all three species secreted large to very large volumes of moderately concentrated nectar, which accumulates in the flowers, producing a high standing crop of nectar, as appears typical of flowers pollinated by generalist birds, but not sunbirds (Johnson and Nicolson 2007).

Despite the low visitation rate of the pollinators, flowers of these plants accumulate high stigmatic pollen loads, and disperse the majority of their pollen (Fig. 4). Consequently, natural rates of fruit set for *Canarina* and *Isoplexis* are high. The critical factor here is that *Canarina*, *Isoplexis* and *Lotus* have very long lasting flowers, and at the average rate of visitation cited above (and assuming all visits are during daylight, circa 12 hours per day), a flower of *Canarina* could be expected to be visited approximately 40 times during its life span, a flower of *Isoplexis* 10 times, and a *Lotus* flower approximately once (though see comments above). We do not know how much pollen the birds carry, but the estimated lifetime visits per flower, and our data on fruit set, shows that the flowers of these species are being adequately serviced by their pollinators. Passerine birds such as the chiffchaff are not usually specialised flower visitors, neither in feeding habits nor in terms of morphology, and are typically insectivorous. However the chiffchaff is an opportunistic feeder that has often been recorded visiting flowers for nectar and has been found with pollen adhering to its bill and head (Clement 1995). In addition we observed chiffchaffs taking nectar from a range of other plants in Tenerife and in England.

The flowers of *Isoplexis*, *Canarina*, *Lotus*, and probably and some of the other Canarian bird guild species, appear to be specifically adapted to pollination by passerine birds which feed on a broad range of foods as well as nectar and are not frequent flower visitors. Adaptations of these flowers to low frequency pollination

are the long-lasting flowers, the large volume of nectar which is persistent in the flower ensuring that there is always a significant and predictable reward available. At the same time, the nectar might be unappealing to bees because of the relatively low sugar concentration, as well as bitter substances (e.g. in *Isoplexis*). Dupont et al. (2004) have found that all the flowers under investigation here are hexose dominated, and contain only traces of sucrose (which can be digested by specialised nectar feeders such as bees and hummingbirds, but not apparently by passerines not specialised on exploiting flowers (Dupont et al. 2004). Thus the sugar contents might indicate that the flowers have specifically evolved traits to employ non-specialised birds as pollen vectors (Dupont et al. 2004). Other adaptations such as the generally orange-reddish flower colour and the lack of obvious scent are typical of some other bird pollinated species and may be viewed as convergent evolution in relation to avian pollination (Proctor et al. 1996).

Specialisation on passerines that are in turn not specialised for flower feeding may explain why repeated nectar removal from *Canarina* flowers eventually resulted in flowers ceasing nectar production (Fig. 3) and, in some cases, losing turgor and collapsing (unpublished data). The flowers of *Canarina canariensis* are large and robust (as is common for bird pollinated flowers) and would normally be expected to withstand nectar removal which was comparable to quite a modest rate of pollinator visitation (one visitor removing all nectar every two hours). However, this is a much higher rate of nectar removal than is experienced by *C. canariensis* under normal circumstances, and, although the data are preliminary and circumstantial, we interpret this as evidence that these flowers evolved under a regime of infrequent (though still reliable) visitation that is still maintained today, rather than a more frequent regime of visits by sunbirds or other specialised flower feeders. Far from being an example of a relict pollination system, bird pollination in *Canarina* and the other species might provide an example of efficient adaptation in relation to generalist passerine birds which, although they visit flowers only occasionally, do so regularly enough to be recruited as pollen vectors. We note that “specialist” flower-feeding birds such as sunbirds and even hummingbirds usually include other items such as insects in their diet. In this respect the pollinating passerines on the Canary Islands are at one end of a spectrum of bird dietary generalisation-specialisation in relation to nectar feeding.

The pollination niche which *Canarina*, *Isoplexis* and *Lotus* are exploiting is characterised by utilising pollen vectors which are infrequent, though consistent, flower visitors. Why has such an unusual bird pollination system evolved in the Macaronesian islands? Dupont et al. (2004) suggest that this may be because low abundance of other pollinators has allowed passerine birds to broaden their feeding niches to include nectar sources, and that the plants have evolved accordingly. However despite their low species richness, populations of native bees are abundant on the islands and are widely utilised as pollinators by other plant species (Garcia 2000; Dupont et al. 2003; Dupont and Skov 2004; Ollerton et al. 2007) and often forage on flowers in close proximity to *Canarina* and *Isoplexis* (Stelzer et al. 2007). It is, however, possible that the flowers might indeed have originally evolved for pollination by specialised birds as suggested by Vogel et al. (1984); Valido et al. (2004) and that, following extinction of these specialised avian pollinators, the switch to insect pollination might have evolved crossing an adaptive valley, while utilising generalised bird pollinators might have required fewer evolutionary modifications.

Passerine pollination has also been recently documented in a mainland European plant species, *Anagyris foetida* (Fabaceae) by Ortega-Olivencia et al. (2005). The flowers of this species are also quite long lived: up to 14 days if they

remain unpollinated, and up to 10 days if they are pollinated (Valtueña et al. 2007). Functionally specialised pollination by generalist passerines, characterised by extended floral longevities, may therefore have evolved a number of times in other plant species, but we have failed to recognise it because it does not fit exactly with our expectations of the ecology of a bird pollinated plant, and in particular the low visitation rate by what is viewed as a sub-optimum pollinator. The lifespans of individual flowers have been shown to be an adaptation that balances the maintenance costs of the flowers against the rate of fitness gain in terms of stigmatic pollen receipt and pollen dispersal from anthers (Ashman and Schoen 1994). The ESS model of Ashman and Schoen (1996) predicts that flowers should be long lived when both daily floral maintenance costs and pollinator visitation rates are low. Hourly rates of nectar secretion (a significant component of flower maintenance) are relatively modest in these species. In addition, the flowers of *Canarina*, *Isoplexis*, *Anagyris* (and to a lesser extent *Lotus*) are quite waxy, and *Canarina* and *Isoplexis* grow mainly in the cool semi-shade of the laurel forests, all of which suggests that daily water expenditure per flower is relatively low. The flower longevities of these species places them in the top 10% of measured floral lifespans, on a par with tropical orchid species, and within the predicted range of the negative correlation between flower visitation rate and floral longevity (see Figures 5.1 and 5.4 respectively in Ashman and Schoen 1996). This is compelling evidence that the extended flower lifespans that we have documented are adaptations to low frequency pollination by chiffchaffs and others passerines.

Hummingbird pollinated flowers in Puerto Rico typically possess flowers that last only one day, whilst in southern Australia, mean floral longevity of bird pollinated species is 12 days on average (reviewed by Primack 1985). In South Africa, flowers pollinated by generalist birds only rarely last three weeks (S.D. Johnson, pers. comm.) as they do in our study. This shows that “bird pollination” is a variable pollination system, and flowers which are primarily hummingbird, sunbird or honeycreeper pollinated show subtly different blends of flower and nectar characteristics which can often be related to the behavioural, morphological and physiological characteristics of different bird groups (Johnson and Nicolson 2007; Nicolson and Fleming 2003). For example, hummingbirds often hover to access flowers, whilst sunbirds rarely do; sunbird pollinated plants therefore often have stout inflorescence stems that the birds use as perches. Generalist passerine pollination should therefore be seen within the context of a range of flower phenotypes (including nectar production and longevity) which reflect the visiting birds and their behavioural particularities. For these Canary Island endemic plants, and *Anagyris* in Europe, a low rate of pollen removal and receipt functions well as a pollination system.

A classical notion in evolutionary biology is that ecological specialisation is a hazardous dead-end street. In this view, if the ecological conditions change, specialists cannot cope and are therefore faced with extinction. This might have been the case if the specialised avian pollinators of these Canary Islands flowers had become extinct long ago. However, the rarity (and possible current extinction) of some of the Canary Island bird flowers seems to be related to habitat destruction as land is taken for agriculture and tourism (reviewed by Francisco-Ortega 2000; Bramwell and Bramwell 2001) rather than loss of specialised pollinators.

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