





RESEARCH ARTICLE

Bird-flower colour on islands supports the bee-avoidance hypothesis

Cristina Rodríguez-Sambruno¹  | Eduardo Narbona¹  | José Carlos del Valle²  | Alfredo Valido³ 

¹Department of Molecular Biology and Biochemistry Engineering, Pablo de Olavide University, Seville, Spain

²Department of Plant Biology and Ecology, Sevilla University, Seville, Spain

³Island Ecology and Evolution Group, Institute of Natural Products and Agrobiology (IPNA-CSIC), San Cristobal de la Laguna, Spain

Correspondence

Alfredo Valido

Email: avalido@ipna.csic.es

Funding information

Fundación Cajacanarias/La Caixa, Grant/Award Number: 2022CLISA29; 'Investigo' (PRTR-NextGenerationEU); Spanish Ministry of Science and Innovation, Grant/Award Number: PID2020-116222GB-100

Handling Editor: Alison Brody

Abstract

1. Many insular plant species inhabiting different archipelagos worldwide present typical ornithophilous floral traits (e.g. copious nectar, red-orange colours), but most of them are visited by insectivorous/granivorous birds and lizards, which act as generalist pollinators. Oceanic islands promote these ecological interactions mainly due to the scarcity of arthropods. Our goal is to understand how these generalist interactions contribute to the shift of floral traits from entomophily (mainland) to ornithophily or saurophily (island), where specialist nectar-feeding birds have not inhabited.
2. We used the well-known pollination interactions occurring in the Canary Islands to evaluate two proposed ecological hypotheses, bee-avoidance or bird-attraction, explaining evolutionary transitions of floral traits. Specifically, we studied the flower colour conspicuousness of bird-pollinated Canarian species visited by birds and lizards with their closest relatives from the mainland mainly visited by bees. We analysed the chromatic contrast of flower colours using visual models of bees, birds and lizards and the achromatic contrast in visual models of bees. We also compared reflectance spectra marker points of flowers with available spectral discrimination sensitivities of bees and birds.
3. Using a phylogenetically corrected framework of independent plant lineages, our results revealed that bird-pollinated Canarian species showed lower chromatic contrast according to bees and lizard visual models than their mainland relatives, but similar chromatic contrast for bird vision. In addition, reflectance spectra marker points of the Canarian species were displaced to the longest wavelengths, far from the wavelengths of maximum discrimination of bees, but close to birds.
4. We conclude that the avoidance of bees would be a primary ecological strategy explaining the evolutionary transitions of flower colours from melittophily to ornithophily. The lower conspicuousness of bird-pollinated Canarian flowers in lizards is perhaps a side effect of the bee-avoidance strategy rather than an independent evolutionary strategy. Together, these findings provide insights into

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Functional Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

how vertebrate generalist pollinators can also lead to divergence of floral traits in insular habitats, but also in other arthropod-poor habitats.

KEYWORDS

bee avoidance, bird attraction, Canary Islands, chromatic contrast, flower colour evolution, ornithophily, plant–animal interactions, visual modelling

1 | INTRODUCTION

Most flowering plants are pollinated by animals, with bees (Apoidea) the main group of pollinators (Ollerton et al., 2011). However, thousands of plants worldwide depend on birds for pollination, that is ornithophily (Abrahamczyk, 2019; Cronk & Ojeda, 2008), including specialized nectarivores, such as hummingbirds and sunbirds, but also birds with a more generalist diet such as passerine birds feeding on insects, seeds or fleshy fruits (Abrahamczyk, 2019; Valido et al., 2004). The importance of lizards (Squamata: Sauria) as pollinators (i.e. saurophily) has also been recognized in many parts of the world, especially on islands (Correcher et al., 2023; Hervías-Parejo et al., 2020; Olesen & Valido, 2003).

In general, oceanic islands, as well as other ecosystems such as high mountains or deserts, have low arthropod abundance, limiting the availability of food for generalist-insectivorous vertebrates (i.e. birds and lizards; e.g. Janzen, 1973). This scarcity of food prompts these vertebrates to expand their trophic niche by feeding on nectar/pollen or fruits (Abrahamczyk, 2019; Correcher et al., 2023; Olesen & Valido, 2003), and acting such as mutualist agents. In this sense, the pollinator shifts from entomophily (i.e. insect-based pollination on the mainland) to ornithophily or saurophily (after its colonization to islands) could also promote evolutionary changes on floral traits exerted by these new pollinators (Cronk & Ojeda, 2008; Abrahamczyk, 2019; see also Shrestha et al., 2016 for evolution of floral traits in an island with a predominately or exclusively dipteran pollinator fauna). This can be especially advantageous for insular plants, whose gene pool is often limited (Frankham, 1997). For example, it is known that both specialist and generalist birds promote gene flow among plants through their pollination behaviours, facilitating the transfer of pollen over greater distances compared to bees (Bezemer et al., 2016; Gamba & Muchhala, 2022).

In the Canarian archipelago, at least six species of generalist passerine birds (Fringillidae, Paridae, and Sylviidae) and five species of lizards (Lacertidae and Gekkonidae) frequently visit flowers for nectar/pollen of at least 15 native plant species from seven families (Appendix S1: Table S1; Ojeda, 2013; Valido et al., 2004; Valido & Olesen, 2010). All these plant species are included in the so-called 'Macaronesian bird-flowered element' (Olesen, 1985) and can share similar floral characteristics such as red-orange flower colour, copious and hexose-rich nectar, and loss of conical cells on the epidermal surface (Dupont et al., 2004; Ojeda, 2013; Ojeda et al., 2016; Ollerton, Cranmer, et al., 2009; Valido et al., 2004). These sets of floral traits are termed 'pollination syndromes' and have evolved in

response to natural selection to attract effective pollinators or avoid the least effective ones (e.g. Ollerton, Alarcón, et al., 2009).

Here, we focus on floral colour because it plays a major role in attracting birds (and lizards) or repelling bees, that is functioning as an exclusion mechanism (Chittka & Menzel, 1992; Cronk & Ojeda, 2008), and flower colour perceived by a specific pollinator would depend on its visual system. For example, bees have trichromatic vision with peaks of sensitivity in UV, blue and green regions of the light spectrum (Peitsch et al., 1992; Appendix S1: Figure S1). Birds and lizards are tetrachromatic, with peak sensitivities in the UV, blue, green, and red (Hart et al., 2000; Martin et al., 2015; Appendix S1: Figure S1). Thus, flowers only presenting reflectance in the red region of the light spectrum are more inconspicuous to bees, because they have lower sensitivity in this region than birds and lizards (Chittka & Waser, 1997). This characteristic has been pointed out as a 'bee-avoidance' strategy (see e.g. de Camargo et al., 2019; Lunau et al., 2011), which has been shown to be the strongest force compared to a 'bird-attraction strategy', even though both strategies can act simultaneously (Castellanos et al., 2004). On the other hand, bees show innate preferences for blue colours, while birds like red flowers, but do not have innate preferences for any colour (e.g. Lunau et al., 2011).

Several plant traits such as floral colour, micromorphology of petals, chemical composition and volume of nectar have been studied in order to understand how a bird-pollination syndrome evolved in the Canarian plants (Dupont et al., 2004; Ojeda et al., 2013, 2016; Ollerton, Cranmer, et al., 2009). However, these studies did not incorporate flower colour and the visual system of pollinators in their mainland ancestors. Here, we analyse the floral colour spectral traits of all Canarian species included in the 'Macaronesian bird-flowered element' and their closest mainland relatives and compare their conspicuousness according to the vision models of their main pollinators, bees, birds, and lizards. Additionally, reflectance spectra marker points of flowers were compared with the available spectral discrimination sensitivities of bees and birds to estimate how well the flower colours matched the colour discrimination ability of pollinators, respectively (Chittka & Menzel, 1992; Shrestha et al., 2013a). Consequently, we hypothesized that bird-pollinated Canarian species should be less conspicuous to bees than their mainland relatives, whereas island and mainland species should be similarly conspicuous to birds. Our results verify this hypothesis and represent the first comprehensive analysis of flower colour evolutionary transitions that includes visual models of their specific pollinators in a phylogenetically corrected framework of independent plant lineages.

2 | MATERIALS AND METHODS

2.1 | Study species and sites

We selected Canary plant species belonging to the so-called 'Macaronesian bird-flowered' element defined as 'putatively pollinated' by birds and lizards (Olesen, 1985; $N=14$; see Appendix S1: Table S1). Here, we named them the 'Canarian bird-flowered element', including strict bird-pollinated species whose flowers are mainly visited by birds and lizards and presenting typical ornithophilous flower traits, and opportunistic ones whose flowers are highly visited by bees and other insects, as well as by birds and lizards (Appendix S1: Table S1). The flower traits of the last group are more diverse and cannot be unambiguously assigned to any specific pollination syndrome. For example, the flower of *Teucrium heterophyllum* is pink-red in colour (characteristic of ornithophily), but its nectar contains mainly sucrose (characteristic of melittophily or specialist birds pollinators but indigestible to opportunistic passerine birds; Dupont et al., 2004). Thus, opportunists are intermediate between insect- and bird-pollinated species.

For each Canary plant genus, for comparison we selected a close relative from the mainland according to recently published phylogenies (see Appendix S1: Figure S2). *Canarina canariensis* was an exception. Its closest relatives (*C. abyssinica* and *C. eminii*) are East African (Olesen et al., 2012), and we were unable to locate them in any botanical garden, and consequently, *C. canariensis* was removed from comparative analysis. To test the repeatability of our results, we also considered a 'alternative comparative group', consisting of the second closest relative species of the mainland (Appendix S1: Figure S2). The scale of inference and the scale at which the factor of interest is applied (Table 1). All research were realized under sampling permits of environmental agencies of Andalusian (RS-427/10) and Canary (2022/6131) governments, and also from the insular governments (cabildos) of Gran Canaria (FLA10-2022), Tenerife (AFF 33/22), La Gomera (864/2022), and La Palma (2022/3411).

2.2 | Reflectance measurements

The flower reflectance spectra were made with USB-2000 and Jaz A1465 Ocean Optics spectrophotometers (Duiven, The Netherlands) equipped with a top sensor system deuterium-halogen standardized light source, DT-MINI-GS-2 and DH-2000 lamps, respectively, and a coaxial fiber cable (QR-400-7-UV-VIS-BX; Ocean

Optics). Reflectance corresponds to the proportion of a standard white reference tile (WS-1-SS; Ocean Optics). For all measurements, we kept the distance between the petals and the measuring probe constant and with an angle of illumination and reflection fixed at 45°. Spectra data were processed with OceanView software (version 2.0.8; Ocean Optics) and calculated in 5-nm wide spectral intervals in the range of 300–700 nm. For this, three to 10 flowers (one flower per individual plant) were selected from each species to capture any possible intra-specific variability. Fresh flowers were placed in ziplock bag and transported inside a cooler with ice until later measurement in the lab (within the same day), with no apparent colour change or sample degradation (see del Valle et al., 2015 for a similar procedure). We obtained the reflectance of the main part of the flower, considered as such the part of the petal generating the highest advertising display for pollinators (sensu Dafni et al., 1997). For *Digitalis* we measured the external part of the upper lip as the main part, and for *Anagyris*, the wings were the main part (Figure 1). The Canary and mainland *Lotus* species differ in shape as the Canary ones present resupinate flowers; Canary *Lotus* species have well-developed wings, while the main pollinator attractor of mainland *Lotus* is the banner. The remaining species show actinomorphic flowers, thereby we used the inner, apical part of the petals (Figure 1). Reflectance spectra from *Teucrium montanum* were obtained from the Floral Reflectance Database (ID 1780; Arnold et al., 2010). Data for all species considered in this study are available in Rodríguez-Sambruno et al. (2023).

2.3 | Flower colour conspicuousness to pollinators: Chromatic and achromatic contrasts

First, we used the 'getspec' function of the 'pavo' R-package (Maia et al., 2019) to load the colour spectral data between 300 and 700 nm of each flower. To reduce noise, the data were smoothed with a span of 0.25 and negative values were corrected by setting the minimum value to zero and scaling other values accordingly ('prospec' function). We used the function 'aggplot' to aggregate the spectra of the flowers of the same species and then plot the average and SD values (represented by shaded areas). The lines were coloured according to human vision using the 'spec2rgb' function. Then, we represented the colour loci of each plant species by using available vision models of the honeybee (*Apis mellifera*, Apidae), bumblebee (*Bombus terrestris*, Apidae), the UV sensitive (UVS) Eurasian blue tit (*Cyanistes caeruleus*, Paridae) and the European wall lizard (*Podarcis muralis*, Lacertidae) as the closest relative to

TABLE 1 Replication statement of the statistical analysis in this study.

Hypotheses	Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Spectral signatures; Chromatic and achromatic contrasts; Marker points	Species	Species	14 Canary species, 7 mainland species (3–10 individuals each species)

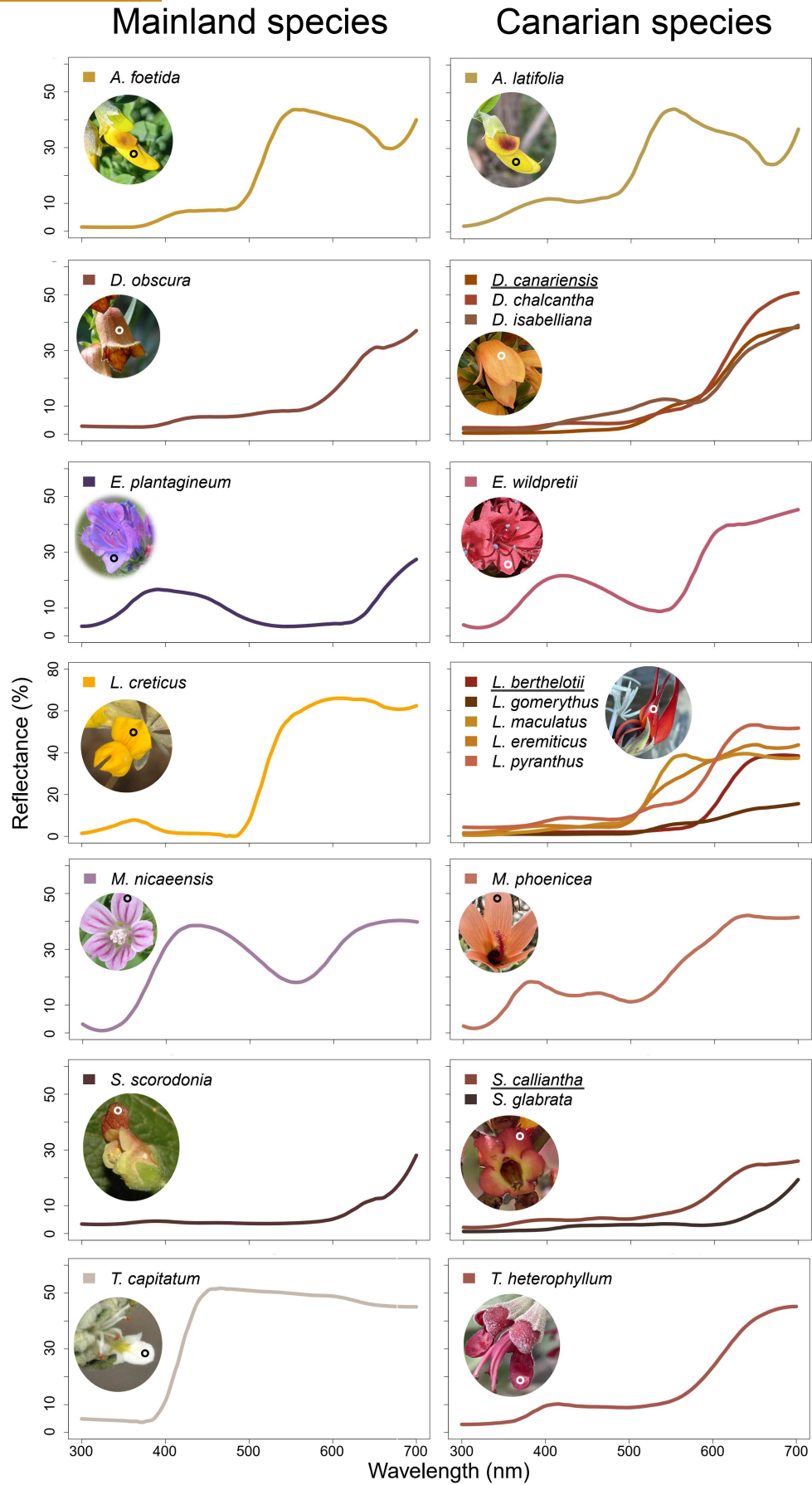


FIGURE 1 Comparative spectral signatures of mainland and Canarian species. The species in the photos are underlined in the legend and the colour of the lines represents the floral colour according to human vision. The measured locations are indicated with black or white circles in the photos. The variation within species in reflectance spectra is shown in Appendix S1: [Figure S3](#) for simplicity. All photos are property of the authors except *M. nicaeensis* (<https://flora-on.pt/#/h5flu>).

the Canarian endemic lizards (g. *Gallotia*, Lacertidae) with available visual system data. A detailed description of the visual systems of these species is shown in Appendix S1: [Methods](#), [Figure S1](#).

Quantum catches in each photoreceptor were calculated using the 'vismodel' function in 'pavo' and considering standard daylight (D65 irradiance) as illumination and von Kries colour correction (Arnold et al., 2010; Maia et al., 2019). Additionally, we used the green foliage provided by the 'pavo' package as background, which is the average spectrum of 230 green leaves provided by Chittka (1992). For the specific case of bees, we considered a hyperbolic transformed quantum catch. Then, we use the 'colspace' function ('pavo') to interpret the quantum catches in hexagonal and tetrahedral colour spaces for the vision systems of bees and lizards/birds, respectively. This function also calculates the chromatic and achromatic contrasts, that is the parameters used to estimate the conspicuousness of different pollinators. The chromatic contrast represents the contrast of colour between the flower and the background; it was calculated in the vision models as the Euclidean distance between the colour loci of flowers and the achromatic centre of the colour space (van der Kooij & Spaethe, 2022). Larger chromatic contrast values indicate more conspicuousness for the specific pollinators to distinguish the flower from the background. In the bee visual model, chromatic contrast values under 0.11 Euclidean distance units are considered to be unreliably discriminated for bumblebees (Dyer, 2006); being this threshold level conservative given that other hymenopterans may show higher colour discrimination ability (Garcia et al., 2017). The achromatic contrast (also known as green contrast) was calculated only in the bee vision model as the difference in excitation between colour loci and the background only in the green photoreceptor (more details in Appendix S1: [Methods](#)).

2.4 | Flower colour discrimination abilities of pollinators: Analysis of marker points

The visual system of pollinator shows optimal colour discrimination in certain regions of the light spectrum, which generally coincides with the region in which the sensitivity of two photoreceptors overlaps (Chittka & Menzel, 1992; Shrestha et al., 2013a). However, the reflectance spectra of flowers can show regions with sharp changes (i.e. steep slopes) that are termed marker points (Shrestha et al., 2013a). If reflectance spectra marker points coincide with the wavelengths for optimal discrimination ability of a pollinator group, it can be considered as a sign of adaptation of the floral colour to the animal's visual system (de Camargo et al., 2019; Dyer et al., 2012; Shrestha et al., 2013a, 2016). Thus, we calculated marker points for both Canarian and mainland plant species. For this, we use the

software 'Spectral-MP' (Dorin et al., 2020) and identify a marker point as a 10% change of amplitude in a 50nm range with a smoothing window of ± 10 data points and considering five data points to look ahead when performing slope change detection. For the species whose peak of reflectance does not exceed 10% (i.e. *Lotus gomerythus*, *Scrophularia glabrata*, and *S. lyrata*), we considered a marker point as a 5% change of amplitude in a 50nm range instead of 10%, because we considered it to be more proportional to the spectra of these species. In addition, we calculated the relative frequency of marker points in 10nm bins differentiating between Canarian and mainland species.

It is proposed that if floral colour has evolved to enhance discrimination by a specific pollinator, reflectance spectra marker points should coincide with the wavelengths of maximum discrimination of the pollinator (Shrestha et al., 2013a). In the case of bees, these maximum discrimination wavelengths are 400 and 500 nm (Chittka & Menzel, 1992; Shrestha et al., 2013a), whereas for UVS birds are 416, 489 and 557 nm (Shrestha et al., 2013b). The wavelengths of maximum discrimination for lizards have not yet been studied. To measure the matching between the reflectance spectra marker points and the pollinators' wavelengths of maximum discrimination, we used two metrics: the minimum absolute deviations (minADs) and the mean absolute deviation (MAD). The minADs correspond to the minimum distance between the marker points of a species and a specific wavelength of maximum discrimination of a specific pollinator, while the MADs takes into account all the wavelengths of maximum discrimination of a pollinator and the closest marker point to them (Shrestha et al., 2013a).

2.5 | Statistical analyses

To make statistically independent comparisons among species, we use phyANOVAs, ('phytools' R-package; Revell, 2012) with 10,000 simulations for each test. To do so, we first chose the accepted names of the plant species over the synonyms following the taxonomic name resolution service (TNRS; Boyle et al., 2013). Then we built the phylogeny of plant species using 'phylo.maker' ('V. PhyloMaker' R-package; Jin & Qian, 2019), which was derived from angiosperm megatrees as a phylogenetic backbone, and the function 'phylo.maker' to generate the phylogenetic tree (Appendix S1: [Figure S2](#)). *Anagyris latifolia*, *Lotus creticus*, and *L. gomerythus* were not included in the megaphylogeny, but we added them using recent published phylogenies (see Appendix S1: [Figure S2](#)). We use phyANOVAs to compare the groups -Canarian and mainland- for the achromatic contrast (only for bees vision) and the chromatic contrast for bees, birds, and lizards; MAD for bees and birds; and minAD for each wavelength of maximum discrimination by bees and birds.

All statistical analyses were performed in R version 4.2.1 (R Core Team, 2022).

3 | RESULTS

3.1 | Spectral signatures

Reflectance spectra captured a high variety of flower colours of plant species from the mainland. These species encompassed four distinctive groups: species with blue-pink colours with reflectance in the UV, blue and red regions (i.e. *E. plantagineum* and *M. nicaeensis*), species with yellowish flowers with an increasing slope at about 500–600 nm (i.e. *A. foetida*, *D. obscura*, and *L. creticus*), species with red flowers with reflectance curves with an increasing slope at about 650 nm (i.e. *S. scorodonia*), and species with white flowers with reflectance in the entire visible region of the spectrum (i.e. *T. capitatum*) (Appendix S1: Figure S3). A similar diversity was also found for the alternative set of mainland species (Appendix S1: Figure S4). In contrast, most of the reflectance spectra of bird-pollinated Canary species showed a trend to produce orange to red flowers with a general absence of reflectance in the UV, blue and green regions of the spectra and an increasing slope from 500 to 650 nm. Exceptions to this pattern were *A. latifolia*, *E. wildpretii* and *M. phoenicea* (Figure 1 and Appendix S1: Figure S3), which showed moderate reflectance in the UV and blue regions of their reflectance spectra.

3.2 | Chromatic and achromatic contrasts

In the honeybee colour space, the mainland species showed flower colours that occupied all colour categories except UV (Figure 2). On the contrary, bird-pollinated Canary species were closely distributed around the centre in the hexagonal colour space that mainly occupied the green sector of the hexagon. All mainland species, except *D. obscura*, showed chromatic contrasts higher than the threshold level of 0.11 Euclidean distance units from the centre of the hexagon, while only 40% of the bird-pollinated Canary species exceeded this level (Appendix S1: Figure S5). Thus, bird-pollinated Canary species showed significantly lower chromatic contrast values than mainland species for honeybee (mean \pm SD: 0.128 ± 0.1 and 0.225 ± 0.131 , respectively; phylANOVA: $F_{1,19} = 3.6$, $p = 0.0032$; Figure 3). Similar results were found using the visual model of the bumblebee (0.136 ± 0.104 and 0.271 ± 0.140 , respectively; phylANOVA $F_{1,19} = 6.3$, $p = 0.0004$; Appendix S1: Figure S6). The achromatic contrast showed a similar trend, with bird-pollinated Canary species showing significantly lower values than mainland species for honeybee (0.14 ± 0.084 and 0.208 ± 0.112 , respectively; phylANOVA $F_{1,19} = 2.41$, $p = 0.0109$; Figure 3). Similar results were found using the visual model of the bumblebee (0.141 ± 0.08 and 0.203 ± 0.116 , respectively; phylANOVA $F_{1,19} = 2.08$, $p = 0.0209$; Appendix S1: Figure S6).

In the bird colour space, mainland species showed flower colours with hues that were distributed towards the UV-blue-red vertices of the tetrahedron, while bird-pollinated Canary species tended to be closer to the red photoreceptor (Figure 2). However, the chromatic contrast of the Canary and mainland species was statistically similar (0.276 ± 0.115 and 0.242 ± 0.079 , respectively; phylANOVA $F_{1,19} = 0.47$, $p = 0.31$; Figure 3 and Appendix S1: Figure S5).

In the colour space of lizards, mainland species were also distributed towards the UV-blue-red vertices of the tetrahedron (Figure 2). Here, bird-pollinated Canary species were distributed around the centre with a weak tendency towards the red vertex. Surprisingly, the chromatic contrast was statistically lower for bird-pollinated Canary species compared to mainland species (0.149 ± 0.072 and 0.207 ± 0.086 , respectively; phylANOVA $F_{1,19} = 2.62$, $p = 0.0084$; Figure 3 and Appendix S1: Figure S5). Similar results were obtained with the alternative dataset for mainland species (Appendix S1: Table S2 and Figure S7).

3.3 | Marker points

The flowers of mainland species showed reflectance spectra with marker points distributed between 345 and 654 nm. Bird-pollinated Canary species showed marker points distributed in a similar range of wavelengths (352 to 659 nm), but more concentrated around 600 nm (Appendix S1: Figure S8). In the visual system of bees, bird-pollinated Canary species had higher MAD values than mainland ones, and this difference was only marginally significant (mean \pm SD: 89.3 ± 36.5 and 64.9 ± 55.2 , respectively; phylANOVA $F_{1,19} = 1.47$, $p = 0.056$; Table 2 and Appendix S1: Figure S9a). This trend is basically caused by the significantly lower values of minAD_{400} of mainland species compared to bird-pollinated Canary species, but the values of minAD_{500} were similar for both groups of species (Table 2 and Appendix S1: Figure S9a).

In the UVS bird visual system, mainland species had MAD values similar to insular ones (45.1 ± 33.1 and 47.2 ± 19.7 , respectively; phylANOVA $F_{1,19} = 0.03$, $p = 0.8002$; Table 2 and Appendix S1: Figure S9b). The values of minAD_{416} were significantly lower on the mainland than in bird-pollinated Canary species, but minAD_{557} showed the opposite pattern (Table 2 and Appendix S1: Figure S9b). When the alternative dataset from the mainland was used, we found a similar pattern, with significant differences for MAD_{bees} , minAD_{400} , minAD_{500} , minAD_{416} , and minAD_{489} (Appendix S1: Table S3 and Figure S10).

4 | DISCUSSION

We showed that species of the 'Canarian-ornithophilous element' were less conspicuous to bees and lizards than their closest relatives in the Mediterranean Basin. On the contrary, the island and mainland species were equally conspicuous for birds. These results are consistent with the 'bee-avoidance hypothesis' (i.e. red flowers

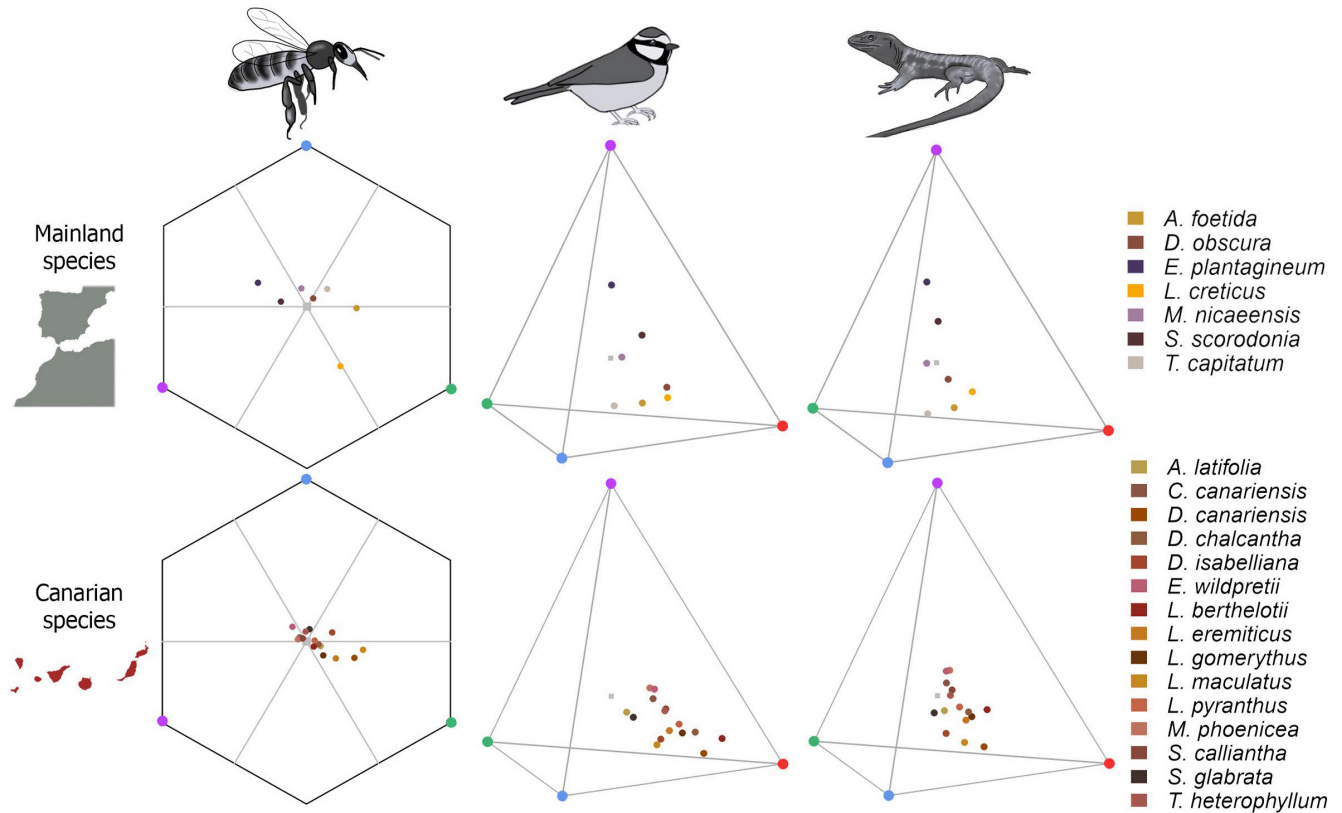


FIGURE 2 Colour loci of all species used in this study according to different colour vision models. Mainland species (main comparative group) are represented in the first row and Canarian species in the second row. Each column corresponds to a colour space model: (left) hexagonal colour space for the trichromatic vision of honeybees (*A. mellifera*, Apidae); (centre) tetrahedral colour space model for UVS birds (*C. caeruleus*, Paridae); and (right) tetrahedral colour space model for lizards (*P. muralis*, Lacertidae). The colour of the points represents the floral colour according to human vision. The coloured circles on the vertices represent the maximum signals in the blue, green, UV and red photoreceptors. Animal silhouettes (CRS).

avoid bees allowing them to be mainly visited by birds), which is also in agreement with other reports that study the evolution of flower colour in both specialist and generalist bird-pollinated species on a larger scale (Chen et al., 2020; de Camargo et al., 2019). The lower conspicuousness of bird-pollinated Canarian flowers to lizards was puzzling, but it is perhaps a side effect of the bee-avoidance strategy of Canarian plant species rather than independent evolutionary strategy due to the similarity between the sensitivities of their cones to bees (see below). The evolutionary transition to red/orange flowers reported here has occurred in several plant lineages in the Macaronesian archipelagos and even repeatedly in some genera (e.g. *Lotus*; Cronk & Ojeda, 2008; Ojeda et al., 2016).

4.1 | Changes in flower reflectance spectra between mainland and bird-pollinated Canarian floras

We detected a pattern of variation between insular and mainland species in flower colour spectra. In general, the bird-pollinated Canarian species were predominantly reflected in the red region with colour loci located near the centre in the colour hexagon of

bees, resulting in low conspicuousness (Chittka & Waser, 1997). Bees do detect red flowers, if they also reflect UV and/or blue light, as is the case for red flowers from the Mediterranean Basin (León-Osper & Narbona, 2022). However, none of the Canarian species belong to the strict bird-pollinated group (practically only effectively pollinated by birds and lizards; Appendix S1: Table S1) had reflectance in short or medium wavelengths, which suggests a 'private' communication channel with birds and lizards (Lunau et al., 2011). On the other hand, plant species belonging to the opportunistic bird-flower group (visited frequently by both vertebrates and bees; Appendix S1: Table S1) showed additional reflectance in UV and blue regions (e.g. *A. latifolia*, *M. phoenicea* and *E. wildpretii*). These results suggest that, in contrast to the strict bird-pollinated species, the opportunistic ones are in some way also conspicuous to bees.

4.2 | Flower colour conspicuousness to main groups of pollinators

The detected decrease of conspicuousness of bird-pollinated Canarian species to bees as compared to their mainland relatives suggests a lack of detectability at short and long distances,

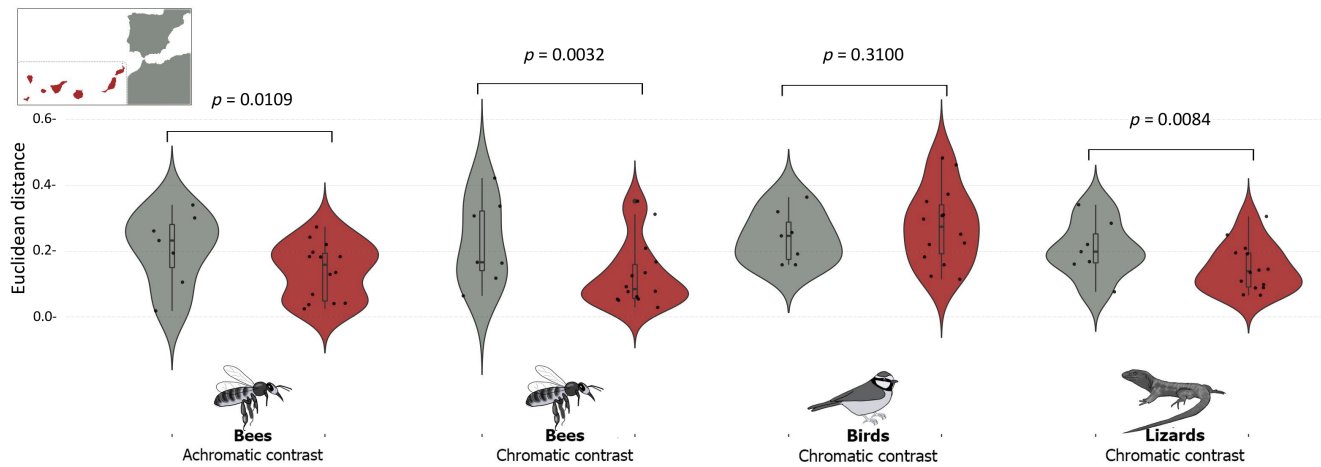


FIGURE 3 Violins with boxplots representing the distribution of chromatic contrast values (Euclidean distance units) obtained from the vision models of honeybees (*A. mellifera*, Apidae), UVS birds (*C. caeruleus*, Paridae), and lizards (*P. muralis*, Lacertidae) and achromatic contrast values (Euclidean distance units) obtained from the vision model of bees. Mainland species (main comparative group) are represented in grey violins, and bird-pollinated Canary species are represented in red violins. Slim points represent the values of the species, and thick points represent outliers. p -value resulting from phyANOVA tests that compare mainland with bird-pollinated Canary species is shown. Animal silhouettes (CRS).

	Mainland		Canary Islands		$F_{1,19}$	Phylogenetic p
	Mean	SD	Mean	SD		
<i>Bees vision</i>						
MAD	64.9	55.2	89.3	36.5	1.47	0.0555
minAD ₄₀₀	111.9	90.7	168.4	67.8	2.60	0.0090
minAD ₅₀₀	75.9	64.8	87.5	40.5	0.26	0.4682
<i>UVS birds vision</i>						
MAD	45.1	33.1	47.2	19.7	0.03	0.8002
minAD ₄₁₆	105.0	82.9	157.0	58.8	2.78	0.0073
minAD ₄₈₉	80.7	60.9	98.5	40.5	0.64	0.2237
minAD ₅₅₇	72.6	38.2	44.5	22.7	4.53	0.0011

Note: Smaller values of MAD and minAD indicate the proximity between the marker points of the flowers and the wavelengths of maximum colour discrimination for each pollinator group. This is interpreted as a better fit of the floral colour to the specific pollinator visual system (see Section 2).

suggested by chromatic and achromatic contrast, respectively (Chittka & Waser, 1997; Giurfa et al., 1997). These results also match previous findings noted by Ollerton, Cranmer, et al. (2009), who found low achromatic and chromatic contrast values in *C. canariensis*, *D. canariensis*, and *L. berthelotii* according to the bee vision model. Although arthropods are relatively scarcer on oceanic islands (Abrahamczyk, 2019; Janzen, 1973; Olesen & Valido, 2003), our results suggest that the flower colour of bird-pollinated Canary species seems to have evolved to avoid bees. This would be advantageous because bird pollination is found to increase the spatial scale of intraspecific gene flow in comparison with bees (Bezemer et al., 2016; Gamba & Muchhala, 2022). In fact, previous studies have found the same strategy acting on flower colour evolution of plant species worldwide that are visited by specialist nectar-feeding birds (e.g. Chen et al., 2020; de Camargo et al., 2019). For instance, loss of conical cells that facilitate bee landing or changes

in nectar chemical composition which may be hardly digestible for birds (Dupont et al., 2004; Ojeda et al., 2016).

In addition to the lower chromatic contrast of Canary species with respect to mainland ones in the bee vision model, we also found significantly lower values in the lizard vision model. This result was unexpected because the visual systems of birds and lizards include four photoreceptors (Hart et al., 2000; Martin et al., 2015). However, the difference in sensitivity in the long wavelength photoreceptor between the visual systems of UVS birds and lizards (peaks at 600 vs. 560 nm, respectively), and the overlap in the green region of two photoreceptors in the lizard visual system could explain these differences (Appendix S1: Figure S1). In fact, the sensitivity of the long-wavelength photoreceptor of lizards seems more similar to those of the bees than those of UVS birds. Because of this, we consider this result as a side effect of the bee-avoidance strategy, but further studies are needed.

TABLE 2 Average values of mean absolute deviation (MAD) and minimum absolute deviation (minAD) of spectral reflectance of marker point metrics for plant species from mainland and Canary Islands (values are related to wavelength of maximum discrimination of bees and birds), its standard deviations and results of phylogenetic ANOVA testing differences among group means using the main comparative group from mainland. Significant differences are indicated in boldface.

4.3 | Adjustment of bird-pollinated Canary flora to the colour discrimination abilities of pollinators

Analysis of marker points also supports the bee-avoidance hypothesis. Bird-pollinated Canary species have evolved reflectance spectra with marker points as far as possible from the wavelengths of maximum discrimination of bees (400 and 500 nm) but close of UVS birds (557 nm), as is found in other study systems (de Camargo et al., 2019; Shrestha et al., 2013b). The marker points of mainland species were closer to the wavelengths of maximum discrimination of bees, as is also known from other bee-pollinated plant species (León-Osper & Narbona, 2022; Shrestha et al., 2013a), while the marker points of bird-pollinated Canary species only presented cues of adaptation to the wavelengths of maximum discrimination of birds in minAD_{557} . The differences in minAD_{416} and minAD_{489} are similar to what we found for the vision of bees, probably due to the proximity of these two wavelengths of maximum discrimination for birds (416 and 489 nm) and for bees (400 and 500 nm). With this closeness between wavelengths of maximum discrimination of bees and birds, the only 'option' for bird-pollinated Canary species to adapt to the vision of birds, while remaining inconspicuous to bees is to develop marker points around 557 nm, which matches our findings. Therefore, our results also align with previous predictions by Shrestha et al. (2013b) on the Old-World flora pollinated by UVS birds. At this respect, they anticipated that bird-pollinated flowers in Africa and Asia would exhibit a shift in the wavelengths of marker points, moving closer to the UVS optimum of 557 nm.

5 | CONCLUSIONS

Using two complementary approaches, chromatic/achromatic contrasts and reflectance spectra marker points that correspond to the spectral discrimination of pollinators, our study suggests avoidance of bees as the main strategy explaining the evolutionary transitions of flower colours from melittophily to ornithophily in the 'Canary-ornithophilous element'. Although empirical data are still scarce, evidence shows that Canary-ornithophilous plants visited by birds have a higher fruit and seed set than those with vertebrates excluded (e.g. Rodríguez-Rodríguez & Valido, 2008, 2011). In this regard, it is known that birds also increase pollen and therefore gene flow within and among plant populations due to their higher mobility (Bezemer et al., 2016; Gamba & Muchhala, 2022). Thus, avoiding bees would positively affect the evolution and persistence of these insular plant populations. Our findings are relevant not only to understand the resulting diversification of floral traits in insular plant species (Shrestha et al., 2016) but especially to disentangle how generalist vertebrate pollinators can promote floral evolution in different mainland ecosystems characterized by poor diversity and abundance of arthropods as pollinators.

AUTHOR CONTRIBUTIONS

Alfredo Valido and Eduardo Narbona conceived and planned the study and carried out field work. Cristina Rodríguez-Sambruno,

José Carlos del Valle, Eduardo Narbona and Alfredo Valido analysed data and discussed results. Cristina Rodríguez-Sambruno, Eduardo Narbona and Alfredo Valido wrote a first draft of the manuscript. All authors contributed to the final draft.

ACKNOWLEDGEMENTS

We thank Juan Arroyo, Manuel Arechavaleta, Salvador Cruz, Félix Medina, Paulino Melchor and Beneharo Rodríguez for the localization of some plant populations, and Jens M Olesen and Jeff Ollerton for constructive comments on the manuscript. Besides national parks of Garajonay (La Gomera) and Taburiente (La Palma) provided facilities to access to some plant populations. A.V. is supported by Fundación Cajacanarias/La Caixa (2022CLISA29), and the program 'Investigo' (PRTR-NextGenerationEU). E.N. and J.C.d.V. are funded by Spanish Ministry of Science and Innovation (PID2020-116222GB-I00). We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.


DATA AVAILABILITY STATEMENT

Datasets for all studied species are available in Digital.CSIC repository at <https://doi.org/10.20350/digitalCSIC/15709> (Rodríguez-Sambruno et al., 2023). Besides, one sample of each species was deposited in the Floral Reflectance Database (<http://www.reflectance.co.uk/>).

STATEMENT ON INCLUSION

Our research combines the expertise of four authors, including scientists located in the islands and mainland where the study took place. All authors actively participated in the early stages of research and study design to ensure that the broad range of perspectives they bring were considered from the very beginning. We made a point of citing relevant literature published by scientists from the region whenever applicable.

ORCID

Cristina Rodríguez-Sambruno  <https://orcid.org/0000-0001-7415-3665>

Eduardo Narbona  <https://orcid.org/0000-0003-1790-6821>

José Carlos del Valle  <https://orcid.org/0000-0001-6023-6208>

Alfredo Valido  <https://orcid.org/0000-0001-8426-8634>

REFERENCES

- Abrahamczyk, S. (2019). Comparison of the ecology and evolution of plants with a generalist bird pollination system between continents and islands worldwide. *Biological Reviews*, 94, 1658–1671. <https://doi.org/10.1111/brv.12520>
- Arnold, S. E. J., Faruq, S., Savolainen, V., McOwan, P. W., & Chittka, L. (2010). FReD: The floral reflectance database—A web portal for analyses of flower colour. *PLoS One*, 5, e14287. <https://doi.org/10.1371/journal.pone.0014287>

- Bezemer, N., Krauss, S., Phillips, R., Roberts, D. G., & Hopper, S. D. (2016). Paternity analysis reveals wide pollen dispersal and high multiple paternity in a small isolated population of the bird-pollinated *Eucalyptus caesia* (Myrtaceae). *Heredity*, 117, 460–471. <https://doi.org/10.1038/hdy.2016.61>
- Boyle, B., Hopkins, N., Lu, Z., Raygoza Garay, J., Mozzherin, D., Rees, T., Matasci, N., Narro, M., Piel, W. H., Mckay, S., Lowry, S., Freeland, C., Peet, R., & Enquist, B. (2013). The taxonomic name resolution service: An online tool for automated standardization of plant names. *BMC Bioinformatics*, 14, 16. <https://doi.org/10.1186/1471-2105-14-16>
- Castellanos, M., Wilson, P., & Thomson, J. (2004). 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, 17, 876–885. <https://doi.org/10.1111/j.1420-9101.2004.00729.x>
- Chen, Z., Niu, Y., Liu, C. Q., & Sun, H. (2020). Red flowers differ in shades between pollination systems and across continents. *Annals of Botany*, 126, 837–848. <https://doi.org/10.1093/aob/mcaa103>
- Chittka, L. (1992). The colour hexagon: A chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A*, 170, 533–543. <https://doi.org/10.1007/BF00199331>
- Chittka, L., & Menzel, R. (1992). The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A*, 171, 171–181. <https://doi.org/10.1007/BF00188925>
- Chittka, L., & Waser, N. (1997). Why red flowers are not invisible to bees. *Israel Journal of Plant Sciences*, 45, 169–183. <https://doi.org/10.1080/07929978.1997.10676682>
- Correcher, E. J., Hervías-Parejo, S., Carnero, R. D. Y., Sauroy-Toucouère, S., & Traveset, A. (2023). Environmental and morphological drivers of mutualistic plant-lizard interactions: A global review. *Ecography*, 2023, e06425. <https://doi.org/10.1111/ecog.06425>
- Cronk, Q., & Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59, 715–727. <https://doi.org/10.1093/jxb/ern009>
- Dafni, A., Lehrer, M., & Kevan, P. G. (1997). Spatial flower parameters and insect spatial vision. *Biological Reviews*, 72, 239–282. <https://doi.org/10.1111/j.1469-185X.1997.tb00014.x>
- de Camargo, M. G. G., Lunau, K., Batalha, M. A., Brings, S., de Brito, V. L. G., & Morellato, L. P. C. (2019). How flower colour signals allure bees and hummingbirds: A community-level test of the bee avoidance hypothesis. *New Phytologist*, 222, 1112–1122. <https://doi.org/10.1111/nph.15594>
- del Valle, J. C., Buide, M. L., Casimiro-Soriguer, I., Whittall, J. B., & Narbona, E. (2015). On flavonoid accumulation in different plant parts: Variation patterns among individuals and populations in the shore campion (*Silene littorea*). *Frontiers in Plant Science*, 6, 939. <https://doi.org/10.3389/fpls.2015.00939>
- Dorin, A., Shrestha, M., Herrmann, M., Burd, M., & Dyer, A. G. (2020). Automated calculation of spectral-reflectance marker-points to enable analysis of plant colour-signalling to pollinators. *MethodsX*, 7, 100827. <https://doi.org/10.1016/j.mex.2020.100827>
- Dupont, Y. L., Hansen, D. M., Rasmussen, J. T., & Olesen, J. M. (2004). Evolutionary changes in nectar sugar composition associated with switches between bird and insect pollination: The Canarian bird-flower element revisited. *Functional Ecology*, 18, 670–676. <https://doi.org/10.1111/j.0269-8463.2004.00891.x>
- Dyer, A. G. (2006). Discrimination of flower colours in natural settings by the bumblebee species *Bombus terrestris* (Hymenoptera: Apidae). *Entomologia Generalis*, 28, 257–268. <https://doi.org/10.1127/entom.gen/28/2006/257>
- Dyer, A. G., Boyd-Gerny, S., McLoughlin, S., Rosa, M. G. P., Simonov, V., & Wong, B. B. M. (2012). Parallel evolution of angiosperm colour signals: Common evolutionary pressures linked to hymenopteran vision. *Proceedings of the Royal Society B: Biological Sciences*, 279, 3606–3615. <https://doi.org/10.1098/rspb.2012.0827>
- Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, 78, 311–327. <https://doi.org/10.1038/hdy.1997.46>
- Gamba, D., & Muchhala, N. (2022). Pollinator type strongly impacts gene flow within and among plant populations for six neotropical species. *Ecology*, 104, e3845. <https://doi.org/10.1002/ecy.3845>
- García, J. E., Spaethe, I., & Dyer, A. G. (2017). The path to colour discrimination is S-shaped: Behaviour determines the interpretation of colour models. *Journal of Comparative Physiology A*, 203, 983–997. <https://doi.org/10.1007/s00359-017-1208-2>
- Giurfa, M., Vorobyev, M., Brandt, R., Posner, B., & Menzel, R. (1997). Discrimination of coloured stimuli by honeybees: Alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A*, 180, 235–243. <https://doi.org/10.1007/s003590050044>
- Hart, N. S., Partridge, J. C., Cuthill, I. C., & Bennett, A. T. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: The blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative Physiology A*, 186, 375–387. <https://doi.org/10.1007/s003590050437>
- Hervías-Parejo, S., Nogales, M., Guzmán, B., Trigo, M. M., Olesen, J. M., Vargas, P., Heleno, R., & Traveset, A. (2020). Potential role of lava lizards as pollinators across the Galápagos Islands. *Integrative Zoology*, 15, 144–148. <https://doi.org/10.1111/1749-4877.12386>
- Janzen, D. H. (1973). Sweep samples of tropical foliage insects: Effect of seasons, vegetation types, elevation, time of day, and insularity. *Ecology*, 54, 687–708. <https://doi.org/10.2307/1935359>
- Jin, Y., & Qian, H. (2019). V.PhyloMaker: An R package that can generate very large phylogenies for vascular plants. *Ecography*, 42, 1353–1359. <https://doi.org/10.1111/ecog.04434>
- León-Osper, M., & Narbona, E. (2022). Unravelling the mystery of red flowers in the Mediterranean Basin: How to be conspicuous in a place dominated by hymenopteran pollinators. *Functional Ecology*, 36, 2774–2790. <https://doi.org/10.1111/1365-2435.14166>
- Lunau, K., Papiorek, S., Eltz, T., & Sazima, M. (2011). Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology*, 214, 1607–1612. <https://doi.org/10.1242/jeb.052688>
- Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2019). pavo 2: New tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution*, 10, 1097–1107. <https://doi.org/10.1111/2041-210X.13174>
- Martin, M., Le Galliard, J. F., Meylan, S., & Loew, E. R. (2015). The importance of ultraviolet and near-infrared sensitivity for visual discrimination in two species of lacertid lizards. *Journal of Experimental Biology*, 218, 458–465. <https://doi.org/10.1242/jeb.115923>
- Ojeda, D. I. (2013). The Macaronesian bird-flowered element as a model system to study the evolution of ornithophilous floral traits. *Vieraea*, 41, 73–89. <https://doi.org/10.31939/vieraea.2013.41.06>
- Ojeda, D. I., Santos-Guerra, A., Oliva-Tejera, F., Valido, A., Xue, X., Marrero, A., Caujapé-Castells, J., & Cronk, Q. C. B. (2013). Bird-pollinated Macaronesian Lotus (Leguminosae) evolved within a group of entomophilous ancestors with post-anthesis flower color change. *Perspectives in Plant Ecology, Evolution and Systematics*, 15, 193–204. <https://doi.org/10.1016/j.ppees.2013.05.002>
- Ojeda, D. I., Valido, A., Fernández de Castro, A. G., Ortega-Olivencia, A., Fuertes-Aguilar, J., Carvalho, J. A., & Santos-Guerra, A. (2016). Pollinator shifts drive petal epidermal evolution on the Macaronesian Islands bird-flowered species. *Biology Letters*, 12, 20160022. <https://doi.org/10.1098/rsbl.2016.0022>
- Olesen, J. M. (1985). The Macaronesian bird-flower element and its relation to bird and bee opportunists. *Botanical Journal of the Linnean Society*, 91, 395–414. <https://doi.org/10.1111/j.1095-8339.1985.tb01010.x>

- Olesen, J. M., Alarcón, M., Ehlers, B. K., Aldasoro, J. J., & Roquet, C. (2012). Pollination, biogeography and phylogeny of oceanic Island bellflowers (Campanulaceae). *Perspectives in Plant Ecology, Evolution and Systematics*, 14, 169–182. <https://doi.org/10.1016/j.ppees.2012.01.003>
- Olesen, J. M., & Valido, A. (2003). Lizards as pollinators and seed dispersers: An island phenomenon. *Trends in Ecology & Evolution*, 18, 177–181. [https://doi.org/10.1016/S0169-5347\(03\)00004-1](https://doi.org/10.1016/S0169-5347(03)00004-1)
- Ollerton, J., Alarcón, R., Waser, N. W., Price, M. V., Watts, S., Cranmer, L., Hingston, A., Peter, C. I., & Rotenberry, J. (2009). A global test of the pollination syndrome hypothesis. *Annals of Botany*, 103, 1471–1480. <https://doi.org/10.1093/aob/mcp031>
- Ollerton, J., Cranmer, L., Stelzer, R. J., Sullivan, S., & Chittka, L. (2009). Bird pollination of Canary Island endemic plants. *Die Naturwissenschaften*, 96, 221–232. <https://doi.org/10.1007/s00114-008-0467-8>
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Peitsch, D., Fietz, A., Hertel, H., de Souza, J., Ventura, D. F., & Menzel, R. (1992). The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A*, 170, 23–40. <https://doi.org/10.1007/BF00190398>
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Rodríguez-Rodríguez, M. C., & Valido, A. (2008). Opportunistic nectar-feeding birds are effective pollinators of bird-flowers from Canary Islands: Experimental evidence from *Isoplexis canariensis* (Scrophulariaceae). *American Journal of Botany*, 95, 1408–1415. <https://doi.org/10.3732/ajb.0800055>
- Rodríguez-Rodríguez, M. C., & Valido, A. (2011). Consequences of plant-pollinator and floral-herbivore interactions on the reproductive success of the Canary Islands endemic *Canarina canariensis* (Campanulaceae). *American Journal of Botany*, 98, 1465–1474. <https://doi.org/10.3732/ajb.1100146>
- Rodríguez-Sambruno, C., Narbona, E., del Valle, J. C., & Valido, A. (2023). Data from: Bird-flower colour on islands supports the bee-avoidance hypothesis. *Digital CSIC*. <https://doi.org/10.20350/digit.alCSIC/15709>
- Shrestha, M., Dyer, A. G., Boyd-Gerny, S., Wong, B. B. M., & Burd, M. (2013a). Evaluating the spectral discrimination capabilities of different pollinators and their effect on the evolution of flower colors. *Communicative & Integrative Biology*, 6, e24000. <https://doi.org/10.4161/cib.24000>
- Shrestha, M., Dyer, A. G., Boyd-Gerny, S., Wong, B. B. M., & Burd, M. (2013b). Shades of red: Bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist*, 198, 301–310. <https://doi.org/10.1111/nph.12135>
- Shrestha, M., Lunau, K., Dorin, A., Schulze, B., Bischoff, M., Burd, M., & Dyer, A. G. (2016). Floral colours in a world without birds and bees: The plants of Macquarie Island. *Plant Biology*, 18, 842–850. <https://doi.org/10.1111/plb.12456>
- Valido, A., Dupont, Y. L., & Olesen, J. M. (2004). Bird-flower interactions in the Macaronesian islands. *Journal of Biogeography*, 31, 1945–1953. <https://doi.org/10.1111/j.1365-2699.2004.01116.x>
- Valido, A., & Olesen, J. M. (2010). Pollination on islands: Examples from the Macaronesian archipelagos. In A. R. M. Serrano, P. A. V. Borges, M. Boeiro, & P. Oromí (Eds.), *Terrestrial arthropods of Macaronesia—Biodiversity, ecology and evolution* (pp. 249–283). Fundação para a Ciência e a Tecnologia.
- van der Kooij, C. J., & Spaethe, J. (2022). Caution with colour calculations: Spectral purity is a poor descriptor of flower colour visibility. *Annals of Botany*, 130, 1–9. <https://doi.org/10.1093/aob/mcac069>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Descriptive information about plant species included in this study showing their geographic distribution (MB, Mediterranean Basin; CI, Canary Islands), the bird-flowered element where Canarian species belong (O, opportunistic; S, strict), presence/absence of reflectance in the UV region (>10%), their main functional groups of pollinators (bees, birds, lizards) based on the bibliography review and our unpublished data (asterisks denote that pollinator information was obtained based on other species of the genus with similar flower morphology and available pollinator information), locality where the samples for colour were taken from and flower reflectance marker points. *Canarina canariensis* was not included in the comparative analysis, and the species in boldface constitute the alternative comparative group used from the mainland. See Material & Methods for details.

Table S2. Average values of chromatic contrast values (Euclidean distance units) obtained from each vision model and achromatic contrast values (Euclidean distance units) obtained from the vision model of honeybees and bumblebees, its standard deviations, and results of phylogenetic ANOVA testing differences among group means using the alternative comparative group from mainland. Significant differences are indicated in boldface.

Table S3. Average values of mean absolute deviation (MAD) and minimum absolute deviation (minAD) of spectral reflectance of marker point metrics for plant species from mainland and Canary Islands (values are related to wavelength of maximum discrimination of honeybees and birds), its standard deviations, and results of phylogenetic ANOVA testing differences among group means using the alternative comparative group from mainland. Significant differences are indicated in boldface.

Figure S1. Relative sensitivities of the photoreceptors of the studied pollinators: *Apis mellifera* (Apidae), *Bombus terrestris* subsp. *damalpinus* (Apidae), *Podarcis muralis* (Lacertidae) and *Cyanistes caeruleus* (Paridae). Modified from Chittka and Kevan (2005), Skorupski et al. (2007), Cronin et al. (2015) and Martin et al. (2015).

Figure S2. Chronogram of all the species included in this study. Mainland species are marked with grey branches and Canarian species with red branches. The species marked with asterisks constitute the alternative comparative group from the mainland following published phylogenies (Böhle et al., 1996; Fuertes-Aguilar et al., 2002; Bräuchler et al., 2004; Herl et al., 2008; Kelly & Culham, 2008; Mansion et al., 2009; Navarro-Pérez et al., 2013, 2015; Zhang et al., 2015; Salmaki et al., 2016; Villa-Machío, 2017; Jaén-Molina et al., 2021; Pérez-Vargas et al., 2021).

Figure S3. Spectral signatures of the main species included in this study. Lines represent the mean of all the individuals and the shades, the standard error. The colour of lines and shades represents the floral colour according to human vision. Asterisks on the spectral signatures of *Lotus berthelotii* and *Malva nicaeensis* represent their marker points.

Figure S4. Spectral signatures of the mainland species included in the alternative analysis. Lines represent the mean of all the individuals

and the shades, the standard error. The colour of lines and shades represents the floral colour according to human vision. Note that sample size was 1 for *Teucrium montanum*.

Figure S5. Individual values of chromatic contrast obtained from each vision model (honeybees, birds, lizards) and achromatic contrast obtained from the vision model of bees using the main species from mainland. Mainland species are represented in grey and Canarian species are represented in red. The dotted line represents the minimum contrast with the background that bees can discriminate reliably (Dyer et al., 2012). Animal silhouettes (CRS).

Figure S6. Violins with boxplots representing the distribution of chromatic contrast values (Euclidean distance units) obtained from the vision models of bumblebees (*Bombus terrestris* subsp. *damalpinus*, Apidae), UVS birds (*Cyanistes caeruleus*, Paridae), and lizards (*Podarcis muralis*, Lacertidae) and achromatic contrast values (Euclidean distance units) obtained from the vision model of bumblebees. Mainland species (main comparative group) are represented in grey violins and Canarian species are represented in red violins. Slim points represent the values of the species and thick points represent outliers. P-value resulting from phyANOVA tests that compare mainland with Canarian species is shown. Animal silhouettes (CRS).

Figure S7. Individual values of chromatic contrast obtained from each vision model (honeybees, birds, lizards) and achromatic contrast obtained from the vision model of honeybees using the alternative species from mainland. Mainland species are represented in grey and Canarian species are represented in red. The dotted line represents the minimum contrast with the background that bees can

discriminate reliably (Dyer et al., 2012). Animal silhouettes (CRS).

Figure S8. Distribution of reflectance spectra marker points of flowers of mainland (main comparative group) and bird-pollinated Canarian species, grey and red colours respectively. Marker points were binned each 10nm through 300–700nm. Dotted lines represent the wavelengths of maximum discrimination of bees (400 and 500nm; blue) and UVS birds (416, 489, and 557nm; orange). See Material & Methods for details.

Figure S9. Violins with boxplots representing the distribution of MAD and minAD for the discrimination optima of honeybees (a) and UVS birds (b) using the main species from mainland. Mainland species are represented in grey violins and Canarian species are represented in red violins. *p*-value resulting from phyANOVA tests that compare mainland with Canarian species is shown. Animal silhouettes (CRS).

Figure S10. Violins with boxplots representing the distribution of MAD and minAD for the discrimination optima of honeybees (a) and UVS birds (b) using the alternative species from mainland. Mainland species are represented in grey violins and Canarian species are represented in red violins. *p*-value resulting from phyANOVA tests that compare mainland with Canarian species is shown. Animal silhouettes (CRS).

How to cite this article: Rodríguez-Sambruno, C., Narbona, E., del Valle, J. C., & Valido, A. (2023). Bird-flower colour on islands supports the bee-avoidance hypothesis. *Functional Ecology*, 00, 1–12. <https://doi.org/10.1111/1365-2435.14493>