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Mechanisms of flower coloring and eco-evolutionary implications of massive blooming events in the Atacama Desert

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The Atacama Desert, one of the driest places on earth, holds a rich biodiversity that becomes most appreciable in years when unusual rainfall accumulation triggers a phenomenon of explosive development of ephemeral herbaceous and woody desert species known as "desierto florido" or "blooming desert." Despite the scientific importance of this unique phenomenon only few studies have addressed the mechanisms of flower phenotypic divergence under the fluctuating environment provided by this recurrent event. We investigated the mechanisms of floral color diversity in *Cistanthe longiscapa* (Montiaceae), a dominant species across the ephemeral blooming landscape of Atacama Desert. Our analyses show that the variation in colors of *C. longiscapa* flowers result from petals containing betalain pigments with different absorption spectra. The different pigment composition of petals causes flower color differences in the visible and ultraviolet (UV) range of the spectrum. Through color vision models we show that *C. longiscapa* flowers are highly polymorphic in their color appearance for insect pollinators. Our results highlight the variable nature in flower color of *C. longiscapa* varieties blooming simultaneously in a geographical restricted area. Given the importance of color in attracting floral visitors, the observed color variability could contribute to increased cross pollination in extreme desert conditions, while accounting for complex and fluctuating histories of plant-pollinator interactions.

KEYWORDS

Atacama Desert, polymorphism, flower color, floral pigments, pollinators, color vision

Introduction

The Atacama Desert, one of the driest places on earth, stretches along the western border of South America from coastal regions between 18 and 30°S up to 5,000 m (Arroyo et al., 1988; Schulz et al., 2012). Arid (precipitation of ≤50 mm/y) and hyperarid climates (≤ 5 mm/y) can be recognized within this region, which in spite of its persistent aridity, undergoes pronounced rainfall fluctuations at interannual and interdecadal time scales. While interannual rainfall variability seems to be modulated to a large extent by El Niño-Southern Oscillation (ENSO), the pronounced low frequency changes during the past century, characterized by the occurrence of 'humid' (1920–1945; 1976–2002) and extremely dry (around 1910 and 1945–1975) periods, appears to be linked to large-scale climatic fluctuations in the Pacific region associated with the Interdecadal Pacific Oscillation (IPO) (Schulz et al., 2012). Rainfall fluctuations are directly associated with changes in the abundance of plants and animals, with intensive rainfall events inducing drastic changes in the desert landscape.

Despite representing one of the harshest environments of the planet, there is a rich biodiversity of plants and animals adapted to the unique extreme conditions of the Atacama Desert (Rundel et al., 1991). The colonization of the Atacama Desert by some plants and animals required a long lag time, occurring much later than the formations of its arid climates (Guerrero et al., 2013). In line with this, the evolution in some clades inhabiting this region seems to have occurred relatively recently (Guerrero et al., 2013). The highly diverse biota of the Atacama Desert becomes most appreciable in years when unusual accumulation of precipitation induces an explosive development of ephemeral herbaceous and woody desert species (Armesto et al., 1993; Vidiella et al., 1999; Jaksic, 2001). During these events, known as "desierto florido" or "blooming desert," different annual and geophyte species share dominance in a sequential appearance, triggering outbreaks of insects and vertebrates associated with these ecosystems (Vidiella et al., 1999; Chávez et al., 2019). Despite its scientific importance, little is known about the eco-evolutionary processes triggered by this unique phenomenon. For instance, plants native to this ecosystem have evolved traits associated not only with unpredictable dynamics of rainfall tolerance, but also traits to ensure pollination during short but massive blooming in the Desert (González and Pérez, 2010). Most of our current understanding of flower signals arises from non-arid biomes, while studies on the mechanisms underlying floral diversification and plant-pollinator interactions under such extreme environments are still scarce (Trunschke et al., 2021).

Variability in floral traits represents an essential condition to induce processes of floral isolation mediated by pollinators (Johnson, 2010). Pollinators rely on attributes such as color, shape and floral volatiles to detect and discriminate between flowers (Hirota et al., 2012; Reverté et al., 2016; Martínez-Harms et al., 2018). Variations in floral attributes can cause pollinators to behave selectively toward floral variants (Vickery, 1992; Salzmann and Schiestl, 2007; Dormont et al., 2014), inducing processes of floral isolation ("ethological isolation" *sensu* Grant, 1949) that can contribute to the formation and maintenance of reproductive barriers to hybridization. Variations in floral traits are common between species and can also occur naturally within species or as the result of hybridization events (Ippolito et al., 2004; Martínez-Harms et al., 2018, 2020). The eco-evolutionary mechanisms contributing to maintain intra-specific variations in floral attributes include processes such as selection by biotic and abiotic factors and non-adaptive processes such as equilibrium between gene flow and drift (reviewed in Narbona et al., 2018; Sapir et al., 2021). On the other hand, hybridization is considered a major source of genetic diversity, promoting phenotypic variability in plants (Goulet et al., 2017). Under such scenarios, pollinators can mediate processes of floral isolation that can lead to divergence in floral traits and the formation of new races or species. Understanding the mechanisms by which variations in floral characters arise and their consequences on floral visitors can provide valuable insights about the evolutionary processes derived from plant-pollinator interactions.

Previous studies have shown that differences in floral color can induce pollinators to behave selectively toward certain flowers (Vickery, 1992; Schemske and Bradshaw, 1999; Hirota et al., 2012). Flower color involves two optical phenomena: (*i*) the reflection and scattering of light by floral structures, and (*ii*) wavelength-selective absorption by floral pigments (Kay et al., 1981; Lee, 2007; Vignolini et al., 2012; Stavenga and van der Kooi, 2016; van der Kooi et al., 2017; Wilts et al., 2018). Although much remains to be studied about the optical properties of flowers, we know that due to the different refractive indices of the petals' cellular components, incident light is reflected and scattered (Vignolini et al., 2012; Stavenga and van der Kooi, 2016; van der Kooi et al., 2017; Wilts et al., 2018). Pigments, on the other hand, have been extensively characterized with respect to their spectral properties, biosynthesis and biological functions. Pigments accumulate and localize at different subcellular compartments of floral tissue, reducing light scattering and providing specific hue depending on their absorption spectrum (Exner and Exner, 1910; Kugler, 1963; Kay et al., 1981; Stavenga and van der Kooi, 2016). Therefore, variations in flower color can result from differences in the structure of flowers, differences in the floral content of pigments or a combined effect of the two phenomena.

Here we investigate the mechanisms of flower coloring of *Cistanthe longiscapa* (Barnéoud) Carolin ex M.A.Hershkovitz (Montiaceae), a dominant species across the ephemeral blooming landscape at the hyperarid Atacama Desert. A massive blooming of this species took place near the city of Caldera, Chile, during September-October 2021 (**Figures 1A,B**), that

consisted of two distinctive large patches dominated by purple and yellow flower color forms of *C. longiscapa* (**Figure 1C**). The flowering event called the attention of the general public all over the world and was widely reported in social media (France24, 2021; Reuters, 2021).

It is important to note that the yellow flower variant was initially described as *Calandrinia litoralis* Phil (Philippi, 1860) and was later synonymized with *C. longiscapa* based on herbarium material (Hershkovitz, 1991). In the areas where patches of yellow and purple flowers were close to each other reddish, pinkish and whitish flowers could be found in more abundance (**Figures 2A–H**, **4** and **Supplementary Figure 1**), floral variants that could have resulted from the cross pollination of the dominant varieties, could be found in more abundance than elsewhere (**Figures 2A–H**, **4** and **Supplementary Figure 1**). We extensively analyzed flower coloring with respect to: (*i*) their diversity; (*ii*) the mechanisms underlying color differences; and (*iii*) the implications of color diversity on the way flowers are perceived by pollinators. As a representative pollinator we consider the honeybee (*Apis mellifera*), an insect whose vision has been investigated in detail (Vorobyev and de Ibarra, 2012). Since the set of photoreceptors of most hymenopterans evaluated so far are similar to that of *Apis mellifera* (Peitsch et al., 1992), our analysis can be extended to other hymenopterans, which represents a guild of pollinators that have been observed visiting flowers of *C. longiscapa* (**Supplementary Figure 2**). We discuss our findings in the context of the ecological and evolutionary consequences of species inhabiting one of the driest places on earth.

Materials and methods

Plant material

Plant material was collected in the field and kept fresh until measurements and histological procedures were carried out. The flowers of *C. longiscapa* were studied at the peak of the flowering season in October 2021 in a location near the city of Caldera (−27.0667°S; −70.8178°W), where a massive flowering event of this species took place (**Figure 1**).

Photographic characterization of ultraviolet-patterns

A digital camera modified for increased ultraviolet (UV) sensitivity (EOS 10D, Canon USA Inc., Lake Success, NY, USA) equipped with a quartz lens (105 mm, UV-Nikkor, Nikon, Tokyo, Japan) was used to take images of flowers. For UV exposures, a narrow band-pass filter was used (Baader U-Venus-Filter, Baader Planetarium, Mammendorf, Germany), which

consists of a Schott UG11 substrate with dielectric coating that blocks wavelengths in the visible and infrared range while transmitting between 320 and 380 nm with a half-band-width of 60 nm. For exposures in the visible spectrum, we used a bandpass filter that transmits light between 400 and 700 nm.
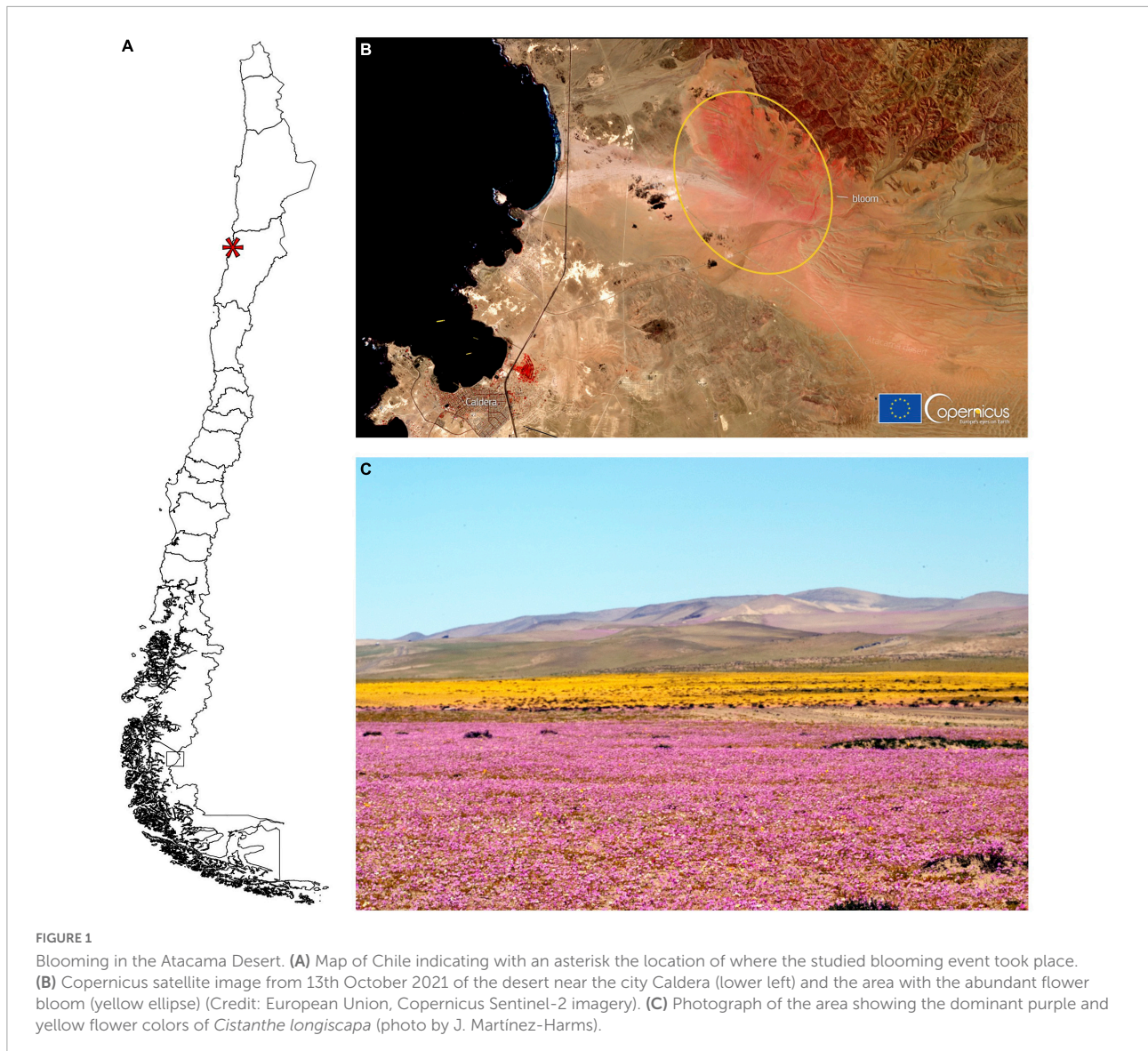
Anatomical study

To investigate petal structures, petal thickness and pigment distribution in flowers, cross sections of fresh plant material were examined. In order to provide mechanical support to floral components, petal pieces were embedded in a 10% agarose solution close to its solidification temperature (approximately 55°C). The agarose-embedded petal pieces were cut with a razor blade and the sections were observed and photographed under a microscope (Zeiss, Axioskop) (Stavenga and van der Kooi, 2016; van der Kooi and Stavenga, 2019).

Spectrophotometry

Reflectance and transmittance spectra of flowers were measured using an integrating sphere (Avantes Avasphere-50-REFL). For the reflectance measurements, petal pieces were illuminated from within the sphere by positioning them at the aperture of the integrating sphere and illuminating them with an optical fiber connected to a deuterium-halogen lamp (Avantes AvaLight-DH-S). The reflected light was collected by a second optical fiber connected to a spectrometer (Avantes Avaspec-ULS2048CL-EVO). Reflectance spectra of both the adaxial (R_a) and the abaxial (R_b) side were measured. For the transmittance measurements, petal pieces were illuminated from outside the sphere by an optical fiber connected to the deuterium-halogen lamp. Furthermore, in a survey of 110 flowers, reflectance spectra of petals were measured with a setup where an optical fiber connected to the deuterium-halogen lamp was used to illuminate patches of petals, while a second optic fiber connected to another spectrometer (Ocean Optics USD2000, Dunedin, FL, USA) collected the light reflected by the petal. The spectrometers were calibrated using a white reference standard (Avantes WS-2).

Kubelka-Munk analysis of the absorption and scattering characteristics of the flowers

The reflectance and transmittance of a flower petal depend on its structural components and their absorption and reflection/scattering characteristics. If the flower can be considered to consist of a stack of layers with specific absorption

FIGURE 1
Blooming in the Atacama Desert. **(A)** Map of Chile indicating with an asterisk the location of where the studied blooming event took place.
(B) Copernicus satellite image from 13th October 2021 of the desert near the city Caldera (lower left) and the area with the abundant flower
bloom (yellow ellipse) (Credit: European Union, Copernicus Sentinel-2 imagery). **(C)** Photograph of the area showing the dominant purple and
yellow flower colors of *Cistanthe longiscapa* (photo by J. Martínez-Harms).

and scattering parameters for each layer, the stack transfer
matrix is defined by (Stavenga and van der Kooi, 2016):

$$M_s = \frac{1}{T}\begin{bmatrix} T^2 - R_a R_b & R_b \\ -R_a & 1 \end{bmatrix} \tag{1}$$

with T the transmittance and R_a and R_b the adaxial and abaxial
reflectance, respectively. Here we treat the *Cistanthe* petals as a
single layer with equal reflectances for illumination from above
and below, $R_a = R_b = R$. The petal's scattering parameter S^* then
follows from

$$S^* = \{ln(1 - (p - q)\, R)/T\}/q \tag{2a}$$

with

$$p = (1 + R^2 - T^2)/(2R), \quad \text{and } q = \sqrt{p^2 - 1} \tag{2b}$$

and the absorption parameter then is

$$K^* = (p - 1)\, S^* \tag{2c}$$

The scattering and absorption coefficient, i.e., the scattering
and absorption per unit length of the petal medium, follow
with the petal thickness, d: $S = S^*/d$ and $K = K^*/d$
(Stavenga and van der Kooi, 2016).

Plotting loci of flowers in insect color space

Flower colors were plotted in a chromaticity diagram using
the receptor noise limited (RNL) color opponent model, which
is one of several diagrams used for displaying colors, most

FIGURE 2
Variously colored *Cistanthe longiscapa* flowers, petal anatomy and spectral characteristics. **(A, C, E, G)** RGB photographs. **(B, D, F, H)** UV photographs. **(I–L)** Transversal sections of the petals of the respective flowers above (scale bar, in **I**, 100 μm). **(M–P and Q–T)** Reflectance (R) and transmittance (T) spectra measured with an integrating sphere from distal and proximal petal areas, respectively; R_a – adaxial, R_b – abaxial. **(U–X)** Absorption (K^*) and scattering (S^*) parameters calculated with a Kubelka-Munk procedure; dist – distal, prox – proximal.

notably the Maxwell triangle (Neumeyer, 1980), the COC model (Backhaus, 1991), the color hexagon (Chittka, 1992) and several other models based on behavioral results of von Helversen (1972) (Brandt and Vorobyev, 1997; Vorobyev and Brandt, 1997; Garcia et al., 2017). According to the RNL model, colors can be depicted as points in a chromaticity diagram where the discriminability between colors is given by the Euclidean distance between points (**Figure 3D**). The greater the distance, the more reliable is the discrimination (Vorobyev and Osorio, 1998; Vorobyev et al., 2001a). This model has been used to describe color discrimination in the honeybee (Vorobyev and Osorio, 1998; Vorobyev et al., 2001a) and in a number of other animals (Goldsmith and Butler, 2003; Koshitaka et al., 2008; Lind et al., 2013). It is important to note that the RNL model predicts the lower limit for discrimination threshold that is set by the noise, which is achieved only after intensive training under controlled laboratory conditions. In more naturalistic conditions, color discrimination may significantly deviate from the predictions of the RNL model because the behavior of animals depend, among other factors, on their previous experience, physiological

state or the amount and type of reinforcement associated with stimuli (e.g., Giurfa, 2004; Avarguès-Weber et al., 2010; Reser et al., 2012; Avarguès-Weber and Giurfa, 2014; Martínez-Harms et al., 2014; Sommerlandt et al., 2016). Furthermore, the RNL model would often predict discrimination between the natural variability of the same flower type, indicating the need of behavioral validation of such predictions (Dyer et al., 2012; Garcia et al., 2018). For details of modeling see **Supplementary materials**. The analyses were performed using the PAVO package (Maia et al., 2019).

To describe the difference between flowers belonging to different groups, we used the Fisher Discriminant Analysis (Fisher, 1936). This method finds a direction in a multidimensional space that provides a best separation between the groups. The best separation is postulated to correspond to the maximum of the signal-to noise ratio, which is defined as the square root of the squared difference between the groups to their variance. This maximal signal-to noise ratio can be considered as a distance between clusters. Let μ_1 and μ_2 be the vector means of the two sets of multidimensional data, and Σ_1

and Σ_2 be the covariance matrices of these data, then the maximal separation is achieved along the direction $(\Sigma_1 + \Sigma_2)^{-1}(\mu_1 + \mu_2)$. The ratio of the square of the difference between the means to the variance along this direction is given by:

$$d = \sqrt{(\mu_1 + \mu_2)^T (\Sigma_1 + \Sigma_2)^{-1}(\mu_1 + \mu_2)} \qquad (3)$$

Note that the derivation of Eq. (3) is neither based on the assumption of normality of the distributions nor on the assumption of equality of variances of the groups (both these assumptions are used in a conventional linear discriminant analysis).

Modeling flowers as seen through the eyes of an insect pollinator

The honeybee has three spectral types of photoreceptors, maximally sensitive in the UV-, blue- and green-wavelength range, which are referred to as S, M, and L, for the short-, middle-, and long-wavelength-sensitive photoreceptor, respectively (Menzel and Backhaus, 1991). To create images of flowers as they are seen through the eyes of a honeybee, we applied the procedure described in Vorobyev et al. (2001b). Briefly, we made UV- and RGB-photographs of the flowers and also measured their reflectance spectra. We then calculated the quantum catch values for the L, M and S photoreceptors (LMS values) for the different flower regions assuming that the illumination was D65 daylight and substituted the RGB values in the images with the LMS values (Wyszecki and Stiles, 1982; see **Supplementary materials** for more details). The software for image processing written using Wolfram Mathematica 10, is available on request.

Results

Floral coloration

To characterize the differences in coloration between varieties of *Cistanthe longiscapa* flowers, we made RGB and UV photographs. **Figure 2** shows exemplary cases of flowers with yellow (**Figures 2A,B**), reddish (**Figures 2C,D**), purple (**Figures 2E,F**) and pink (**Figures 2G,H**) colored petals, revealing that flowers differ in their reflectance in the visible and UV range of the spectrum. Whereas the color in the (for humans) visible wavelength range was rather uniform (**Figures 2A,C,E,G**), the UV photographs show that some flowers have distinct differences between the distal and proximal areas, thus forming a UV reflection pattern usually referred to as a bullseye (**Figures 2B,D,H**).

Floral anatomy and pigment distribution

In order to visualize the petal structures and pigment distribution and to estimate petal thickness, cross sections of petals were carried out. This revealed that the thin petals of *C. longiscapa* are composed of a single cell layer and that pigments are distributed homogeneously in the petal cells (**Figures 2G–I**). The petal thickness was found to be $d = 80 \pm 20\,\mu\text{m}$.

Flower spectral characteristics

To characterize the differences between the distal and proximal petal areas, we measured reflectance and transmittance spectra of the set of flowers of **Figures 2A–H**. The reflectance spectra of the adaxial and abaxial sides appeared to be very similar for both the distal (**Figures 2M–P**) and proximal (**Figures 2Q–T**) petal areas. This allows the application of a single layer Kubelka-Munk type analysis, which yielded the petal's absorption and scattering parameters (**Figures 2U–X**).

The absorption in three wavelength ranges, i.e., the UV (<400 nm), the blue (400–500 nm) and the orange-red (500–650 nm) wavelength ranges, shows specific variations. The distal and proximal areas of the yellow flower of **Figures 2A,B** contain a pigment with peak absorption $\sim$465 nm. The proximal area contains additionally a UV-absorbing pigment. The pigmentation of the distal and proximal areas of the reddish flower of **Figures 2C,D** is very similar, but it contains additionally a pigment with peak absorption in the orange wavelength range ($\sim$570 nm). The purple flower of **Figures 2E,F** contains the same pigments in a much lower concentration, but the UV-absorbing pigment is dominant throughout the petal. The pink flower of **Figures 2G,H** instead has distally a large amount of the orange-absorbing pigment, which is less present proximally, but there the UV-absorbing pigment is dominant again.

Flower color diversity

Reflectance spectra measurements were further performed on flowers of 110 different plant individuals along a transect. In order to visualize the spectral differences between flowers, a principal component analysis (PCA) was performed, using the reflectance between 300 and 700 nm at intervals of 1 nm. The analysis revealed that two principal components (PCs) explained 84.5% of the variance in flower color. The analysis highlights the variability in the floral spectral reflectance of individual flowers blooming simultaneously in a geographically restricted area. PC1 accounted for 47% of the total variance and separated flowers with high reflectance between 400 and

600 nm from flowers with high reflectance in the UV range and above 600 nm (**Figure 3A**). PC2 accounted for 38% of the total variance and separated flowers with high reflectance in the UV and long wavelength range (above 500 nm) from flowers with high reflectance in the middle wavelength range (between 400 and 500 nm) (**Figure 3B**). Plotting PC1 versus PC2 scores for each flower results in a reflectance scatterplot that clusters flowers according to their spectral reflectance in different sections of the scatterplot in 4 clusters.

Flower color appearance for pollinators

Color loci of the 110 flowers mentioned above were plotted in the chromaticity diagram of the most common hymenopteran pollinator, the honeybee *Apis mellifera,* using the RNL model. Most hymenopterans investigated so far possess trichromatic color vision, with photoreceptors maximally sensitive in the UV, blue and green regions of the spectrum. Because trichromaticity represents the ancestral condition among arthropods (Briscoe and Chittka, 2001; van der Kooi et al., 2021), modeling a perceptual space using the spectral sensitivity of honeybees provides a good approximation about the way flowers are perceived by a wide variety of hymenopterans, pollinator guild that has been observed visiting flowers of *C. longiscapa* (**Supplementary Figure 2**). Our analysis revealed that the loci of *C. longiscapa* flowers distribute widely in the chromaticity diagram of the honeybee (**Figure 3D**). The flower colors belonging to human red, purple, white and yellow categories form clearly distinguishable clusters in the RNL diagram. The colors corresponding to pink and red human color categories slightly overlap in the RNL diagram. The separation between the clusters has been quantified using Fisher Discriminant Analysis and assessed as the ratio of the distance between the means to the standard deviation of the spread of the data along the line of the best separation between the clusters (Eq. 3). The results are presented in the **Table 1**. All separations, with the exception of that for the red and pink categories, significantly exceed 1, indicating that these clusters can be easily discriminated based on receptor signals of a honeybee, given that a suitable algorithm of discrimination between categories is used.
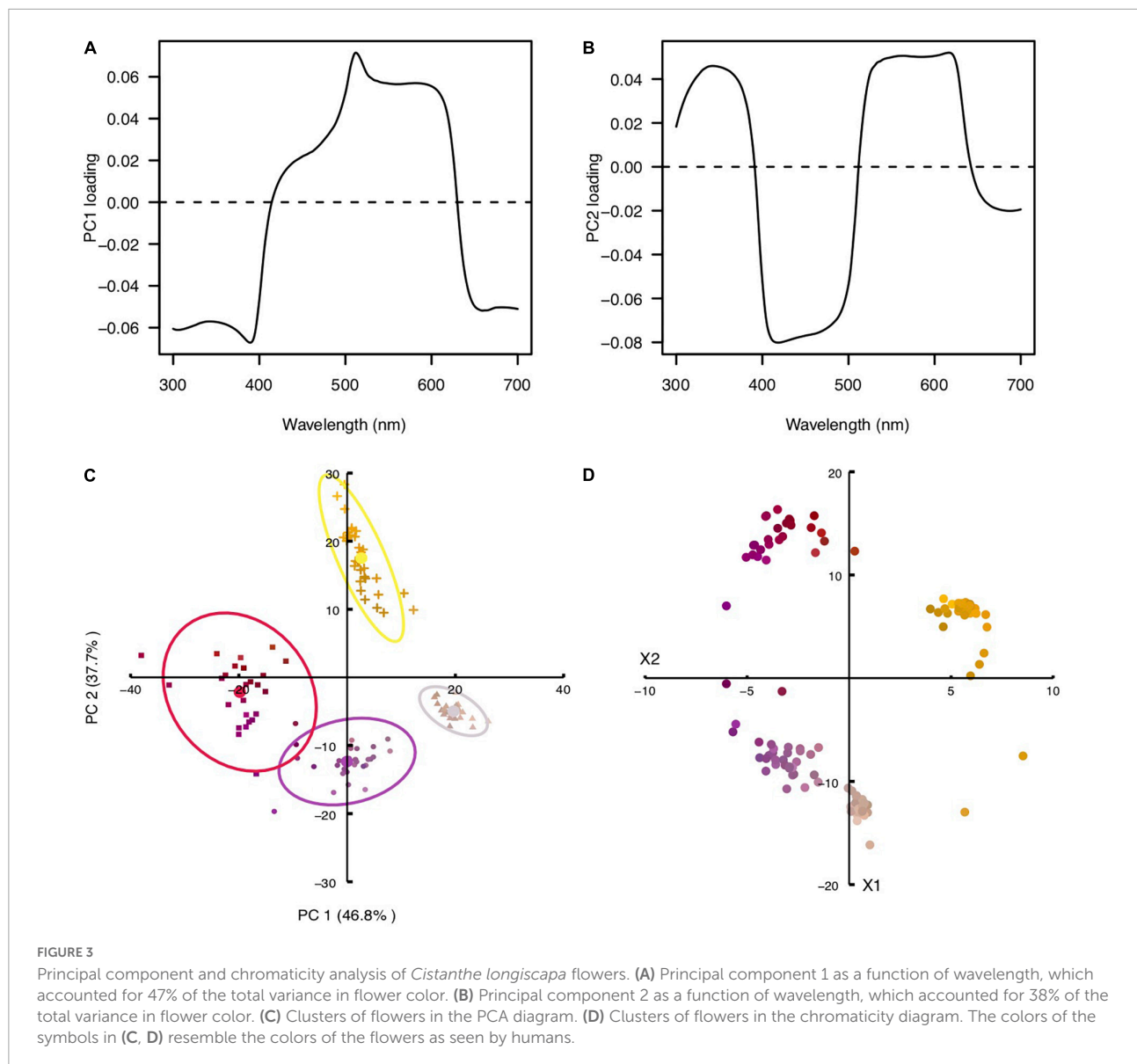
The fact that flower colors cluster in different sections of the chromaticity diagram indicates that for trichromatic hymenopterans, flowers from the different clusters appear different in color, thus falling in different color categories. For instance, flowers that appear yellow to humans, i.e., those with high reflectance above 500 nm, which vary in UV reflectance will have a different appearance for hymenopterans. Similarly, purplish flowers with low reflectance in the UV, appear different in color to those purplish flowers with appreciable UV reflectance. It is important to note that empirical evidence of color categories in bees is lacking (e.g.,

Benard and Giurfa, 2008). A matrix with the color perceptual distance between all flowers of *C. longiscapa* considered in this study is provided in the **Supplementary materials** (**Supplementary Figure 3**).

Since color vision of bees differ substantially from that of humans, the latter gives little insight into the appearance of flowers to bees (Vorobyev and de Ibarra, 2012). The reconstruction of flower images as they are seen through the eyes of bees illustrates the differences in appearance of flowers with similar spectral reflectance in the visible spectrum range but that differ in their UV-reflecting properties (**Figure 4**). This is the case when comparing yellow flowers with appreciable UV (**Figures 4A–C**) to yellow flowers having negligible levels of UV reflectance (**Figures 4D–F**). The same holds when comparing purple and pink flowers with different levels of UV reflectance (flowers of **Figures 4G–I** vs. flowers of **Figures 4J–L**). An interesting result is the similarity for honeybees of flowers that for humans appear quite distinguishable, as is the case for UV-reflecting pink flowers (**Figures 4J–L**) and the reddish flowers (**Figures 4M–O**). The color similarity of these flowers for bees is due to the lack of photoreceptors sensitive to red in the color vision system of honeybees, which in turn relies on UV photoreceptors. In case of flowers with low UV reflectance that appear white to humans, bees will not see these flowers as white given that they do not stimulate the three spectral types of bee photoreceptors (**Figure 4R**). Finally, flowers that appear uniform in color to humans with UV-bullseye patterns have a center-surround color pattern for bees (**Figures 4C,L,O**). Although the differences in flower appearance revealed by our results are not surprising considering the distinct UV sensitivity of insects, the analysis highlights that *C. longiscapa* flower color varieties occurring simultaneously in the Atacama Desert are highly variable in their color appearance for insect pollinators.

Discussion

Cistanthe longiscapa, due to its massive flowering events, represents a dominant feature across the ephemeral blooming landscape at the Atacama Desert. An interesting aspect of the 2021 blooming event was the co-occurrence of *C. longiscapa* plants displaying different flower colors. We investigated the mechanisms underlying those color differences and found that result from flowers having pigments with different absorption spectra (**Figures 2U–X**). The different pigment composition of petals causes flower color differences in the visible and UV range of the spectrum (**Figure 2**). The variability in *C. longiscapa* flower colors was visualized through a PCA analysis, which separates flowers in 4 clusters, based on their reflectance in different wavelength ranges (**Figures 3A–C**). Using color vision modeling we find that *C. longiscapa* flowers are highly polymorphic in their color appearance for insect pollinators and that their spectral reflectance differences in the visible and UV

FIGURE 3

Principal component and chromaticity analysis of *Cistanthe longiscapa* flowers. **(A)** Principal component 1 as a function of wavelength, which accounted for 47% of the total variance in flower color. **(B)** Principal component 2 as a function of wavelength, which accounted for 38% of the total variance in flower color. **(C)** Clusters of flowers in the PCA diagram. **(D)** Clusters of flowers in the chromaticity diagram. The colors of the symbols in **(C, D)** resemble the colors of the flowers as seen by humans.

range of the spectrum affect the way they are perceived by floral visitors (**Figures 3D**, **4**). Altogether, our results highlight the variable nature of floral color of *C. longiscapa* forms blooming simultaneously in a geographical restricted area. Given the importance of flower color for attracting floral visitors, the observed color variability could mediate the assurance of cross pollination under highly fluctuating pollinator environment conditions.

The *Cistanthe* are species belonging to Montiaceae that, as members of the Caryophyllales, owe their flower and fruit pigmentation to betalains (Chung et al., 2015) that accumulate in the vacuoles of the cells (Wink, 1997; Sadowska-Bartosz and Bartosz, 2021). The presence of betalains in flowers of *Cistanthe* is further supported by preliminary experiments on extracts from yellow, purple and pink flowers that reveal pigments with

different absorption spectra that are stable at pH from 3 to 7 (in preparation). The betalains are water-soluble, tyrosine-derived pigments, containing betalamic acid as their central chromophore (Stafford, 1994; Tanaka et al., 2008). In plants, betalains are taxonomically restricted to most families of the order Caryophyllales and their biosynthetic pathway is mutually exclusive with that of the more common anthocyanins (Stafford, 1994; Timoneda et al., 2019). Betalains comprise yellow-orange betaxanthins and red-violet betacyanins, with absorption peak wavelengths $\sim$470 nm and $\sim$536 nm, well corresponding to the pigments identified in the flowers of *C. longiscapa* (**Figures 2U–X**). Our results show that in the thin single cell layered petals of *C. longiscapa*, betalains co-occur with UV-absorbing pigments (**Figures 2U–X**), which most likely correspond to flavonoids (Narbona et al., 2021). On the other hand, the color of the
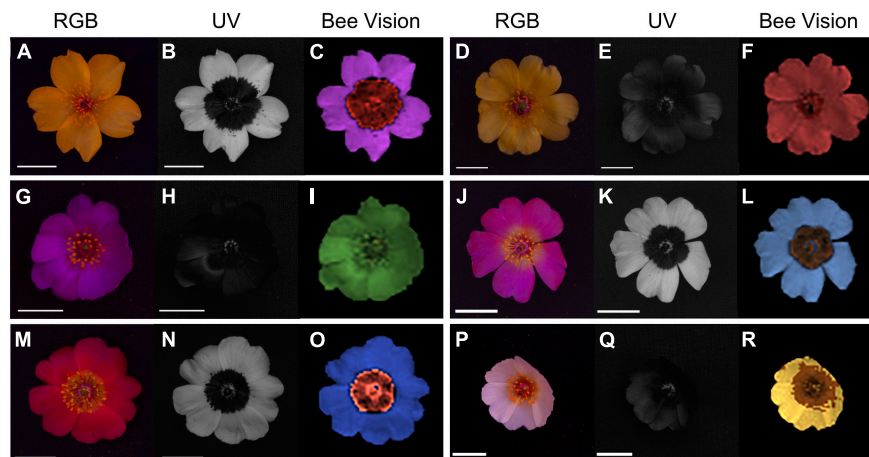
FIGURE 4
Color varieties of *Cistanthe longiscapa* flowers shown in RGB **(A,D,G,J,M,P)**, and UV photographs **(B,E,H,K,N,Q)** and images represented as they would be seen through the eyes of honeybees **(C,F,I,L,O,R)**.

reddish flowers seems to result from two types of betalains, with absorption peaks in the blue and orange wavelength range respectively, accumulating together in the vacuoles of the petal cells (**Figures 2J,V**). Betalains are of special interest regarding their evolutionary origin and homologous functional roles with anthocyanin pigments (Jain and Gould, 2015; Timoneda et al., 2019). While both classes of pigments have comparable spectral properties and have the same histological location, recent evidence indicates that they are effective as photoprotectants, in conferring tolerance to drought and salinity stress and as scavengers of reactive oxygen species in plants facing a variety of abiotic stressors (Jain and Gould, 2015).

Despite their functional similarities with anthocyanins, betalains also have distinctive properties. For instance, unlike anthocyanins, the color of betalains is stable between pH = 3-7 (Strack et al., 2003). This may provide advantages for species like *C. longiscapa*, which upon experiencing water stress switch the mode of carbon assimilation from C3 to Crassulacean acid metabolism (CAM), shifting from a relatively stable vacuolar pH to large diurnal pH fluctuations (Winter and Holtum, 2014; Jain and Gould, 2015; Ossa et al., 2022). Having stable pigments under such fluctuating conditions would ensure their functionality in a harsh, hyperarid environment such as the Atacama Desert (Jain and Gould, 2015). Despite the long interest in betalain pigments, they have been mostly studied in a few model species and much remains to be elucidated with respect to their diversity and biosynthetic and regulatory pathway. Evidence has accumulated about the variability of pigment content in flowers in response to abiotic stress along geoclimatic gradients (Koski and Ashman, 2016; Dudek et al., 2020). The variability in pigment composition revealed by our results points at *Cisthanthe* as suitable models to study betalain biosynthesis, as well as their adaptive role under extreme environments.

Plants native to the Atacama Desert have evolved traits associated not only with tolerance to abiotic stress, but also traits involved in ensuring reproduction during short but massive blooming in the Desert (González and Pérez, 2010). In angiosperms, female reproductive success is often constrained by the supply of pollen, a phenomenon known as pollen limitation, which has been associated with the stochastic nature of pollination (Burd, 1994; Larson and Barrett, 2000; Ashman et al., 2004). Pollen limitation is expected to be particularly strong in habitats such as the Atacama Desert, where unpredictable rainfall drives large interannual variations in plant cover and pollinator abundance (González and Pérez, 2010). Variable pollinator environments could also favor the evolution of strategies to cope with pollen limitation. For instance, the evolution of autonomous selfing (i.e., the ability of flowers to self-fertilize) could provide reproductive assurance in the absence of appropriate pollen vectors (Baker, 1955; Goodwillie et al., 2005). While empirical evidence for increased seed set due to autonomous selfing is available for a few annual species (Kalisz and Vogler, 2003; Pérez et al., 2006), its acquisition seems to be more difficult in perennial species, indicating that the adoption of selfing depends on the species' life form (Morgan et al., 1997). Alternatively, the evolution of floral attractive traits would also allow plants to cope with pollen limitation under fluctuating pollinator environments. A high investment in floral display or reward would increase the attraction of floral visitors and the probability of pollination (Billings and Mooney, 1968; Fabbro and Körner, 2004). An evaluation of the Atacama Desert flora showed that strong pollen limitation was not a general trend and that low pollen limitation levels in annual desert plants, such as *C. longiscapa*, result from the adoption of autonomous selfing as strategy to ensure pollination (González and Pérez, 2010). With respect

	Pink	Purple	Red	White	Yellow
Pink	0	5.9	1.7	18.4	8.6
Purple		0	6.0	3.2	11
Red			0	11.1	5.3
White				0	11.9
Yellow					0

to investment in reward, flowers of *C. longiscapa* do not provide nectar but provide copious amounts of pollen that might attract a variety of pollinators dependent on this type of resource. Although no association between flower size and pollen limitation was detected (González and Pérez, 2010), further studies are needed to evaluate the extent to which other attractive floral traits, such as color and volatiles, contribute to ensure reproduction in plants inhabiting the Atacama Desert.

The specialized floral morphologies observed in some species inhabiting the Atacama Desert suggest that pollinators have played an important role in the evolution of some desert plants. Mechanical and ethological isolation are the two proposed mechanisms by which pollinators would influence the evolution of flowering plants (Grant, 1949). Under processes of ethological isolation, pollinators' selective behaviors would induce the isolation of floral variants, contributing to the formation of reproductive barriers to hybridization, eventually leading to the development of new races or species (Grant, 1994). Two sympatric modes of origin of ethological isolation have been proposed: (*i*) hybrid speciation followed by the segregation of a new flower form that captures a new type of pollinator; (*ii*) the separation of a polymorphic variant for floral characters from other members of its population by flower-constant behavior and assortative mating (Grant, 1994). When seen through the eyes of honeybees, flowers of *C. longiscap*a can appear quite different from each other in color, providing a scenario that could potentially lead to processes of ethological isolation. Although the circumstances under which such polymorphism developed are unclear, the fact that *C. longiscapa* is a self-autonomous species suggests that pollinators may have promoted cross pollination between the two more common yellow and purple varieties, resulting in the more uncommon UV-reflecting pink and red forms. A higher incidence of autonomous selfing may have contributed to the persistence of those more uncommon forms throughout several generations (Buide et al., 2021). Several mechanisms have been proposed for the maintenance of high levels of variability in floral traits. For instance, negative frequency-dependent selection, although unlikely in pollen rewarding species, has been pointed out as a mechanism for maintaining color polymorphism in rewardless flower plants

(e.g., Gigord et al., 2001; Schiestl, 2005; Basist et al., 2021). Opposing selection regimes exerted simultaneously by mutualists and antagonists is also thought to contribute to the maintenance of flower color polymorphism (Irwin et al., 2003; Frey, 2004; de Jager and Ellis, 2014). An interesting example is *Claytonia virginica* (Montiaceae), whose color polymorphism is thought to be maintained through positive pollinator-mediated selection for redder flowers, whereas white flowers receive less damage from herbivores and pathogens (Frey, 2004). Spatial or temporal fluctuation in selection can also maintain phenotypic variation (Siepielski et al., 2009, 2013; Tang and Huang, 2010). A long-term study in the Mojave Desert inhabiting species, *Linanthus parryae*, demonstrated that flower color is subject to fluctuating selection exerted by temporal and spatial variation in spring precipitation (Schemske and Bierzychudek, 2001, 2007). We propose that understanding how biotic and abiotic factors affect flower evolution requires knowledge about the influence of those factors on specific traits along with historical insights about how those factors fluctuate in time and space. In the case of *C. longiscapa*, its outstanding flower color polymorphism may represent an ongoing process of divergence under a complex and fluctuating history of interactions with pollinators. Considering the historical nature of plant-pollinator interactions, the diversity of floral forms provided by *C. longiscapa* and other species across the Atacama Desert may also shed light about the sensory and cognitive capacities of the pollinators contributing to shape this unique flowering landscape.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JM-H, NP, and DS collected the data. JM-H, DS, and MV performed the analyses. JM-H drafted the manuscript. All authors revised subsequent versions and contributed to the design of the study.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.957318/full#supplementary-material

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