Flower colour segregation and flower discrimination under the bee vision model in the polymorphic *Lysimachia arvensis*

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Running head: Flower colour heritability in *L. arvensis*

Abstract

Floral colour determines pollinator behaviour, strongly affecting plant-mating systems. *Lysimachia arvensis* has blue- and red-flowered plants and colour inheritance remains largely unknown. A control of floral colour based on one locus, with the red allele as dominant, has been proposed. This proposal cannot explain the sporadic appearance of other floral colours in wild populations. We studied floral colour segregation in *L. arvensis* and assessed the possibility that pollinators can visually distinguish colour morphs by using Chittka's hexagon model, sigmoidal model of bee discrimination and experimental studies on pollinator attendance for two years. Hand crossing between morphs originated a homogeneous F1 with salmon-coloured flowers. In the F2, blue, red, salmon morphs and other plants with intermediate colours appeared, suggesting that more than one single locus are involved in colour segregation. Results from the sigmoidal discrimination model suggest that blue, red and salmon flowers can be discriminated by pollinators. In fact, pollinators showed strong colour constancy and discriminated against the salmon morph. Our study shows that "Flower colour" is a natural marker to assess the rate of crossing between morphs. The extreme rarity of salmon flowers in wild populations and flower constancy of *L. arvensis* pollinators indicates assortative mating.

Key words: *Anagallis*, Chittka hexagon, floral evolution, flower colour discrimination, pollinator preference

INTRODUCTION

Angiosperms exhibit a markedly high diversity of flower colours, with sister species usually differing in intensity, hue or colour pattern of the corolla (e.g. Rausher 2008; Smith and Rausher 2011; Lagomarsino et al. 2017). This diversity implies that there have been numerous evolutionary transitions in the colour of flowers (Weis 1995; Rausher 2008). Flower colour is often correlated with other floral traits, resulting in the common recognition of "pollination syndromes" (Fenster et al. 2004). Flower colour has an enormous importance as a claim in the attraction of pollinators that may have preferences for some colours over others (Chittka and Menzel 1992), so that transitions to different colours may represent adaptation to different sets of pollinators (Faegri and van der Pijl 1966; Grant 1993; Fenster et al. 2004; Rausher 2008). In general, the colour of the flowers is due to the presence of pigments (Kay et al. 1981; Van der koi et al. 2016). There are four large groups of pigments: chlorophylls, carotenoids, betalains and flavonoids. Among these, anthocyanins, a group of flavonoids, are the most important floral pigments and are produced in a well-known and conserved biosynthetic pathway in angiosperms (Rausher et al. 1999).

Flower colour polymorphism is the presence of more than one colour morph, genetically determined, within the populations of a species (Huxley 1955). This phenomenon appears by a spontaneous mutation in the biosynthetic route of the pigments that give colour to the flowers. Once a coloured mutant appears in a population, this recent polymorphism can be lost or maintained depending on biotic or abiotic selective factors and gene drift (Narbona et al. 2018). Pollinator preferences play a fundamental role in the maintenance or loss of the flower colour polymorphism. Pollinators can show innate preferences for some colours over others (Shrestha et al. 2013, 2016; Van der Kooi et al. 2018) causing directional selection on a determinate colour morph and leading to the loss of polymorphism (Waser and Price 1981). However, balancing selection imposed by pollinators can result in the maintenance of

63 polymorphism. Thus, in the rewardless *Dactylorhiza sambucina* (L.) Soó, pollinators visit
64 different colour morphs in alternation as they switch to a different morph when they visit an
65 empty flower, thus maintaining colour polymorphism (Gigord et al. 2001). Similarly, if
66 species are visited by a wide variety of pollinators, they can show preferences for different
67 colour morphs thereby maintaining colour polymorphism (Schemske and Bradshaw 1999).
68 The behaviour of pollinators can induce changes in plant fertility, cross-pollination ratios, and
69 pollen flow among colour morphs (Malerba and Nattero 2012). These changes can lead to the
70 genetic differentiation of individuals with different flower colour, promoting ultimately the
71 speciation processes (Servedio et al. 2011). However, in order for pollinators to discriminate
72 between floral colours and act as selection agents, it is imperative they can differentiate them
73 visually. Therefore, a subjective evaluation of the floral colours according to the human
74 vision can lead to misleading interpretations in relation to the behaviour of pollinators, being
75 necessary an objective measurement of colours and their evaluation according to the visual
76 system of pollinators.
77 *Lysimachia arvensis* (L.) U. Manns & Anderb. is a tetraploid annual species, native to the
78 Mediterranean Basin and Europe that presents flower colour polymorphism. In natural
79 populations, there are plants with blue and red flowers, and these colours are due to the
80 presence of different types of anthocyanins. Malvidin is mainly responsible for the blue
81 colour and pelargonidin for the red colour (Wiering and de Vlaming in Harborne 1968;
82 Ishikura 1981). Selective abiotic factors influence a geographic distribution pattern of colour
83 morphs, with blue being much better represented in more xeric environments (Arista et al.
84 2013). In addition, in Mediterranean environments, pollinators show a higher preference for
85 the blue morph and the red morph has lower fitness; despite this, it remains in the populations
86 although in a low proportion (Ortiz et al. 2015).

The inheritance of flower colour in *L. arvensis* is unknown, and unravelling it could help to understand the maintenance of the red morph in Mediterranean populations, despite being subject to negative selection (Arista et al. 2013). In a simple scenario, if a recessive allele were responsible for the red colour, it would be protected in the heterozygotes that would show the blue dominant phenotype. However, in an oral communication in a Congress in 1910, Weiss explained that in experimental crosses between plants with flowers of different colour, the F1 obtained was all homogeneously red. Therefore, he concluded that the flower colour in *L. arvensis* depended on a single gene with two alleles, being the red allele dominant over the blue. The fact that flowers of intermediate colour do not usually appear in natural populations would support this dominance-recessive relationship between the two alleles. Later, Marsden-Jones & Weiss (1938) confirmed that result, although in some populations they found some plants of *L. arvensis* with flowers of salmon colour and others of pale blue colour. Salmon-flowered plants, although rare, had also been described previously by other authors who had suggested a hybrid origin between blue and red morphs since they only appeared when the two morphs, blue and red, coexist (Hoffmann 1879; Pax 1905). However, Marsden-Jones & Weiss (1938) found these plants in monomorphic red populations, and thus they attributed salmon plants to spontaneous mutations. In a recent sampling, over 19 mixed populations of *Lysimachia arvensis* in Western Europe, salmon-flowered plants appeared in two of them (Jiménez-López et al., unpub results). Their scarce representation in populations makes it difficult to know if they result from spontaneous mutations or by crossing between the red and the blue morphs. In the latter case, only a low frequency of crossing between morphs or a low success of the progeny of that crossing would explain the almost absence of salmon-flowered individuals in mixed natural populations.

The objectives of the present work are: (1) to establish if the salmon morph results from the crossing between the blue and the red morph in *Lysimachia arvensis*, (2) to know how the flower colour is inherited, (3) to characterize quantitatively the flower colours that can appear in this species by using the model of colour vision of Chittka (1992) and (4) to determine if they can be differentiated by bees in two ways, by calculating their discrimination probabilities from the sigmoidal-shaped model by Garcia et al. (2017), and by studying pollinator attendance in experimental stands during two reproductive cycles.

MATERIAL AND METHODS

Heritability of flower colour

To study the inheritance of colour in *Lysimachia arvensis*, hand pollinations were carried out in the greenhouse. Flower colour segregation was first quantified in offspring based on human vision. The plants used originally came from seeds obtained in natural populations of Hinojos (Spain), Tanger (Morocco), Tabarka (Tunisia) and Corsica (France). These plants were grown in a greenhouse, and by manual self-pollinations two successive generations were obtained to select pure colour lines. These pure lines, blue (B) and red (R), were used as parental (P) in this study. Crossings were carried out between parents of the same colour and different colour in order to obtain the F1. Crosses between parents of different colours were carried out in both directions, that is, the blue plants as pollen donors and the red plants as pollen receiver (RxB, n = 84 crosses) and the red plants as pollen donors and the blue as receiver (BxR, n = 88). The F1 seeds obtained were put to germinate in Petri dishes in germination chambers under 16h of light at 22°C and 8h of darkness at 15°C and seedlings were grown in the greenhouse. In this F1, different types of pollinations were made to obtain the F2. Some F1 plants were self-pollinated (N=149 pollinations), others were crossed with each other (N = 43

crosses), others were backcrossed with blue parental (N = 34 crosses), and others with red parental (N = 39 crosses). All seeds produced by this F1 were germinated and the resulting seedlings were grown in greenhouses until flowering (2907 plants).

Flower colour characterization

To characterize quantitatively floral colours of *L. arvensis* plants obtained from the crossing program previously described, the reflectance spectra of the petals of a subsample of plants were measured. The reflectance was measured in 88 parental plants (44 B and 44 R), 38 F1 plants (S thereafter; 15 from BxR and 23 from RxB) and 41 F2 plants obtained from self-pollination of the F1. Reflectance was also measured in 53 plants from the F1 backcrosses with both parents (19 from SxB, 5 from BxS, 14 from SxR and 15 from RxS). In each plant, the reflectance of the adaxial surface of a petal was measured, discarding the basal part corresponding to the centre of the flower (bull's-eye). To do that, a JAZ A1465 double-beam spectrophotometer from Ocean Optics, equipped with a UV-visible light source and capable of measuring reflectance between 190 and 890 nm was used. Reflectance spectra of the measured flowers are deposited at the open repository of the Universidad de Sevilla (https://idus.us.es/xmlui/).

Model of flower colour vision

To assess how petals are perceived by bees, the reflectance values between 300 and 700 nm obtained in each measurement were elaborated and represented in the colour hexagon model. This model was developed by Chittka (1992) integrating experimental data related to the reception of visual signals by bees and the translation of these signals in the bee brain. The colour hexagon is a two-dimensional representation in which each reflectance spectrum corresponds to a point defined by its Cartesian coordinates; a detailed description of how to

transfer the reflectance data to the colour hexagon can be seen in Chittka & Kevan (2005). This model allows quantifying the contrast of a flower with the general green background as the Euclidean distance between the point generated by the flower spectrum and the centre of the hexagon; in addition, it allows the categorization in a conventional manner of the colours perceived by bees placing them in six colour categories (Chittka 1992).

The Chittka model also allows quantifying the colour contrast of colour between two flowers perceived by the bees as the Euclidean distance in the hexagon between the points generated by their colour spectra, 0.1 being the threshold value for colour discrimination. However, recent behavioural studies modelled by particular bee species have reported that colour discrimination depends on context (Dyer and Chittka 2004; Dyer 2006) and follow sigmoidal-shaped functions (Garcia et al. 2017, 2018). To assess the capacity of bees for discrimination between both parental morphs (blue and red) and both F1-hybrid types (BxR and RxB), Euclidean distances were calculated for all possible pairs of flowers between twelve flowers of each of those four classes (blue, red, BxR and RxB). From those data, the discrimination capacity by bees for those pairs of flowers were calculated by using the 3-parameter logistic function described by Garcia et al. (2017). Given that the main pollinators of *L. arvensis* are Apoideae species and its flowers have blue anthocyanins, we selected the models for blue stimuli for both *Apis mellifera* L. and *Bombus terrestris* L. We used the median values of K, r and Mo parameters for those models from S-4 supplementary material from Garcia et al. (2017).

Pollinator preferences on colour morphs

To ascertain pollinator preferences on colour morphs, and so their discrimination capacity, we recorded pollinator visitation to parental and F1 hybrid plants during two reproductive seasons. We constructed artificial stands with a similar number of flowers of each of the three

colours that where intermingled. Each stand occupied an area of 0.5 m^2 and insect visitations were recorded by observing each stand for 10-min periods. All observations were made during sunny conditions between 9:00 and 15:00h to totalize 9 hours of censuses per year. In each census the number of flowers of each morph visited and the transitions between colour morphs made by pollinators were recorded. Differences in the number of visits per morph and census were analysed by means of a GLM model with Poisson distribution of errors and log link function with morph colour and year as main factors and considering their interaction. Differences in the frequency of transitions made by pollinators among colour morphs each year were analysed by pooling together data from all the censuses and using chi-square tests of frequencies.

RESULTS

Heritability of flower colour

All offspring obtained from crosses BxB and RxR was homogeneous and showed the same colour as the parents (N = 350 individuals observed in each case), which confirms the purity of the blue and red lines selected as parental. The crosses between plants of different colour, BxR and RxB, also originated a homogeneous offspring salmon in colour (N = 1199 individuals analysed, Fig. 1). In addition, these individuals presented a bull's-eye (ring of colour at the base of the petals) similar in size to that of the blue morph but larger than that of the red morph (Fig. 1). The self-pollination of the F1 originated 707 blue plants, 926 red and 452 salmon, but also appeared 51 individuals with intermediate colours between red and salmon (Fig. 1). The backcrosses of the F1 with each of the parents also gave rise to these four phenotypes, but in different proportions. When the backcross was performed with the blue parent, offspring showed mainly blue flowers (n = 407 plants), whereas when it was carried out with the red parent offspring was predominantly red (n = 496).

Flower colour characterization

The blue morph of *L. arvensis* reflected mainly in the ultraviolet, violet and blue, in the range between 330-450 nm (Fig. 2A). In contrast, the red morph reflected in the spectrum for yellow, orange and red (600-700 nm) with a reflectance peak in the ultraviolet (350 nm; Fig. 2A). In the hexagon model, the blue morph was found within the UV-Blue sector and the red in the UV sector (Fig. 3A). Both colour morphs were clearly separated from the center of the hexagon and from each other.

The F1 showed peaks of reflectance very similar to those of the red parent, although with higher reflectance in the blue-violet wavelength (Fig. 2B). The spectra of all F1 individuals were virtually identical regardless of the direction of the crossing (BxR or RxB). The F1 flowers were placed in the UV sector of the hexagon (Fig. 3B), very close and even overlapped with the UV-Blue sector. The spectra of the F2 obtained by self-pollination of the F1 with (N = 41) appeared separated into two large groups, one similar to the blue morphotype (N = 8) and another similar to that of the red or salmon flowers (N = 33, Fig. 2G). When the F2 was represented in the colour hexagon, eight plants coincided with the blue parent and the rest were placed in the area between red and the F1 salmon (Fig. 3E). Both groups were clearly differentiated from the center and from each other. The backcross between the F1 salmon and the blue parent resulted in two groups of plants according to their reflectance spectra (Fig. 2 C, D), one was in the UV-Blue sector of the hexagon and the other in the UV sector with a small part of the UV-Blue sector (Fig. 3C). These groups of plants were separated from each other and with the center of the hexagon. The reflectance spectra of the offspring from the backcross between the F1 salmon and the red morph, in either of the two senses, was the same as those of the red and salmon flowers (Fig. 2E, F). When the red morph acted as a pollen receiver a more heterogeneous range of spectra appeared in the F2 than when the red morph acted as pollen donor. All the offspring from the crosses were found

236 in the UV sector of the hexagon (Fig. 3D), although some of them were also located near the
237 UV-Blue sector.
238 Distances between each colour morph and the center of the hexagon were larger than 0.1; the
239 blue flowers showed the largest distances (median 0.37) and the salmon F1 the shortest
240 (median 0.21). The largest Euclidean distances were found between the blue flowers and both
241 the red and the salmon flowers (Supplementary materials, Appendix 1). Median distances
242 between red and salmon flowers were lower, about 0.1 (Fig. 5). The discrimination
243 probabilities among morphs calculated by using the *Apis mellifera* parameters were
244 practically 100% (Fig. 4; Supplementary materials, Appendix 2). The discrimination
245 probabilities calculated with *Bombus terrestris* parameters were similar and thus, results are
246 not shown in the main text (but see Supplementary materials, Appendix 3). According to
247 those models, pollinators would even discriminate intramorph flowers with a high probability
248 (Fig. 4).
249
250 *Pollinator preferences*
251 *Halictus* bees were the sole floral visitors of *L. arvensis* flowers and showed significant
252 differences in attendance to colour morphs (chi-square= 24.586, 2 df, $p<0.001$). Blue flowers
253 received the highest number of visits each year, followed by red flowers and finally by the
254 salmon flowers. Differences between years were also significant (chi-square=8.647, 2 df,
255 p=0.003) as pollinator visits were less abundant in 2017. However, the colour morph-by-year
256 interaction was not significant (chi-square=2.917, 2 df, p=0.233), indicating the same trend in
257 pollinator attendance to each morph each year (Fig. 5A). Pollinators showed a strong and
258 significant floral colour constancy in both years, as once they visited a colour morph, most
259 transitions were made towards the same colour morph (Fig. 5B). When pollinators moved
260 between different colour morphs, the most frequent transition was towards the blue, then

towards the red and finally towards the salmon flowers (Fig. 5B). Transitions from blue or red flowers to salmon flowers were the less preferred by pollinators in both years.

DISCUSSION

The results obtained in this work clearly indicate that the plants with salmon flowers results from the crossing between the pure red and blue morphotypes of *L. arvensis*. The F1 obtained was 100% homogeneous and showed an intermediate colouration between those of their two parents. This result indicates that there is no dominance-recessivity relationship between the colour alleles of *L. arvensis*, as was previously described (Marsden-Jones & Weiss 1938), a codominance situation being more likely. It is possible that in some lighting circumstances, these salmon plants could be categorized as "red" in human vision, possibly leading to Weiss (1910) and to Marsden-Jones & Weiss (1938) to assume that the red allele was dominant. In fact, the quantitative measures of colour placed these salmon plants very close to the red ones, although with a clearly different pattern.

The fact that F1 is homogeneous in colour suggests that the colour of the flowers in *L. arvensis* follows a characteristic segregation of a monogenic character, as described for floral colour in other species (Malerba and Nattero 2012). In fact, in the backcross between the blue morphotype and the F1, the proportion of individuals with blue flowers was 0.33. Likewise, in the F2 the proportion of individuals with blue flowers was 0.20, similar to that obtained in the cross between heterozygotes (0.25) of a monogenic character. However, the remaining individuals obtained in these crosses showed flowers which colours ranged from salmon to red, and they were clearly differentiated in human vision. This variation could indicate that there is more than one gene involved in the flower colour segregation in this species. However, *L. arvensis* is a tetraploid and the colour segregation obtained could be adjusted to the presence of two copies of the same gene (four alleles) in each individual. Thus, pure lines used as parental would have four alleles for the blue or red colour and the F1 would have two

alleles of each colour, giving rise to a phenotype with an intermediate colour between blue and red. The F2 obtained from self-pollination of the F1 would originate pure blue plants and pure red plants at a frequency of 1/16 each, salmon plants at a frequency of 6/16, and plants intermediate in colour at a frequency of 8/16. The proportions obtained experimentally do not match with these frequencies, being blue and red plants much more frequent than expected and intermediate plants much less frequent. In *Lysimachia monelli* (L.) U. Manns & Anderb., a sister species of *L. arvensis* with the same colour polymorphism, manual crosses between pure lines of blue and red flower plants give rise to an F1 similar to the red progenitor, but in F2 a third morphotype with pink flowers that differ subtly from red ones appears (Freyre and Griesbach 2004). The authors proposed a model of three genes to explain the inheritance of floral colour in this species. In our case, the results obtained are not in accord with the three gene model, but neither with any other segregation based on simple models of few genes, with or without epistatic interactions between them. Thus, the number of genes responsible for the flower colour in *L. arvensis* and the relationship between the genes remain unsolved.

Taking into account that anthocyanins are responsible for the colour of both the blue and the red flowers of *L. arvensis* (Harbone 1968), colour differences could be due to mutations of structural and/or regulatory genes of the biosynthetic pathway of these pigments. In flowers with anthocyanins, transitions from blue to red are relatively frequent (Rausher 2008) and are usually produced by the inactivation of one of two genes, F3'5'H or F3'H, of the anthocyanin pathway (Zufall and Rausher 2004; Rausher 2008). In previously studied cases, such as *Penstemon* (Wessinger and Rausher 2014), *Antirrhinum* (Ishiguro et al. 2012), *Phlox* (Hopkins and Rausher 2011), *Hibiscus* (Gettys 2012) or *Silene* (Casimiro-Soriguer et al. 2016), the F3'5'H coding genes and their regulators are responsible for the colour change. In these cases, a difference in the expression of a regulatory gene causes differences in the

311 concentration of the anthocyanins which results in variations in the flower colour intensity.
312 This kind of variation has appeared in the F2 offspring of *L. arvensis* and could indicate that
313 some regulatory gene in the anthocyanin pathway could be involved in the expression of the
314 floral colour, although this possibility would require a transcriptomic study of floral colour in
315 *L. arvensis.*
316 Regarding the perception of the colours of the *L. arvensis* flowers by pollinators, blue flowers
317 were placed at UV-blue sector, and red flowers at UV sector clearly separated from the blue
318 flowers, as already reported by Ortiz et al. (2015). Blue flowers were also separated from
319 salmon flowers but in contrast, red and salmon flowers appeared along a continuum in the
320 colour hexagon. Despite the close position of red and salmon flowers in the Chittka hexagon
321 model, discrimination probabilities calculated from sigmoidal functions clearly suggest a high
322 capacity of pollinators for discrimination between them. In fact, pollinator visits recorded
323 experimentally during two years support the idea of between-morph discrimination; *Halictus*
324 bees showed colour constancy behaviour when visiting *L. arvensis* flowers, which indicates
325 that it is capable of discrimination among the three flower colours. The greater bull's-eye of
326 salmon flowers could also contribute to its differentiation from the red morph.
327 When the three different flower colours were exposed to *Halictus* bees, they always showed
328 the highest preference for the blue and the lowest for the salmon flowers. The consistent
329 preference of pollinators for blue flowers found in this study had been already recorded in
330 mixed populations of red and blue morphs (Ortiz et al. 2015). Increased colour contrast with
331 the background has been shown to increase the probability of correct target flower
332 identification by several bee species, which is determinant for a quick detection of the flowers
333 (Chittka et al. 2001). The blue morph showed the highest distance to the center of the
334 hexagon and so the greatest contrast with the background. Therefore, pollinators would be
335 able to detect more easily the blue flowers than red or salmon flowers.

The high floral colour constancy showed by *L. arvensis* pollinators indicates that most pollen flow occurs within the same colour morph (assortative mating), although some intermorph flow occurs. The fact that in the mixed natural populations individuals with salmon flowers hardly appear indicates that the crossing between blue and red morphs rarely occurs and/or other reproductive barriers could be also preventing the recruitment of salmon plants in natural populations. Thus, the scarcity of hybrid plants in natural populations would indicate a considerable degree of reproductive isolation between blue and red morphs of *L. arvensis.* Our study shows that "Flower colour" could be used in *L. arvensis* as a natural marker to determine both the rate of crossing between morphs and that of salmon individuals with their parents.

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Captions of figure

Figure 1. Flowers of *Lysimachia arvensis*. A: blue (top left), red (top right) and salmon F1 (bottom) morphs. B: sample of individuals resulting from the self-pollination of F1 or from the backcrosses of F1 with its parents.

Figure 2. Reflectance spectra of the flower color of *Lysimachia arvensis*. A: blue and red morphs. B: F1 resulting from the cross between red and blue morphs (grey BxR, black RxB). C, D: offspring resulting from the backcross between the F1 and the blue morph acting as pollen receiver (C) or pollen donnor (D). E, F: offspring resulting from the backcross between the F1 and the red morph acting as pollen receiver (E) or pollen donnor (F). G: F2 offspring resulting from self-pollination of F1. Means and standard deviations are shown. In panels A, C, D, E, F and G, grey lines correspond to reddish flowers under human vision and black lines to bluish flowers under human vision.

Figure 3. Representation of the flower colour of *Lysimachia arvensis* in the hexagon model proposed by Chittka (1992) based on the perception of color by bees. A: Blue and red morphs. B: F1 resulting from the cross between blue and red morphs. C: backcross between F1 and the blue morph. D: backcross between the F1 and the red morph. E: F2 offspring resulting from self-pollination of F1.

Figure 4. Euclidean distances and discrimination probabilities between pairs of flowers of *L. arvensis*. Median and range values are shown. Euclidean distances were calculated according to hexagon model by Chittka (1992) and discrimination probabilities according to sigmoidal-shaped functions by Garcia et al. (2017) (see text for details).

Figure 5. Pollinator preferences and transition between flowers in experimental stands during two consecutive years. A: Mean number of pollinator visits per census at blue, red or salmon flowers. B: Transition between flower colours made for pollinators each year. Each pie shows the transitions from blue, red or salmon flowers to blue, red or salmon flowers each year. In each pie, different letters indicate significant differences.

Figure 1

Figure 2

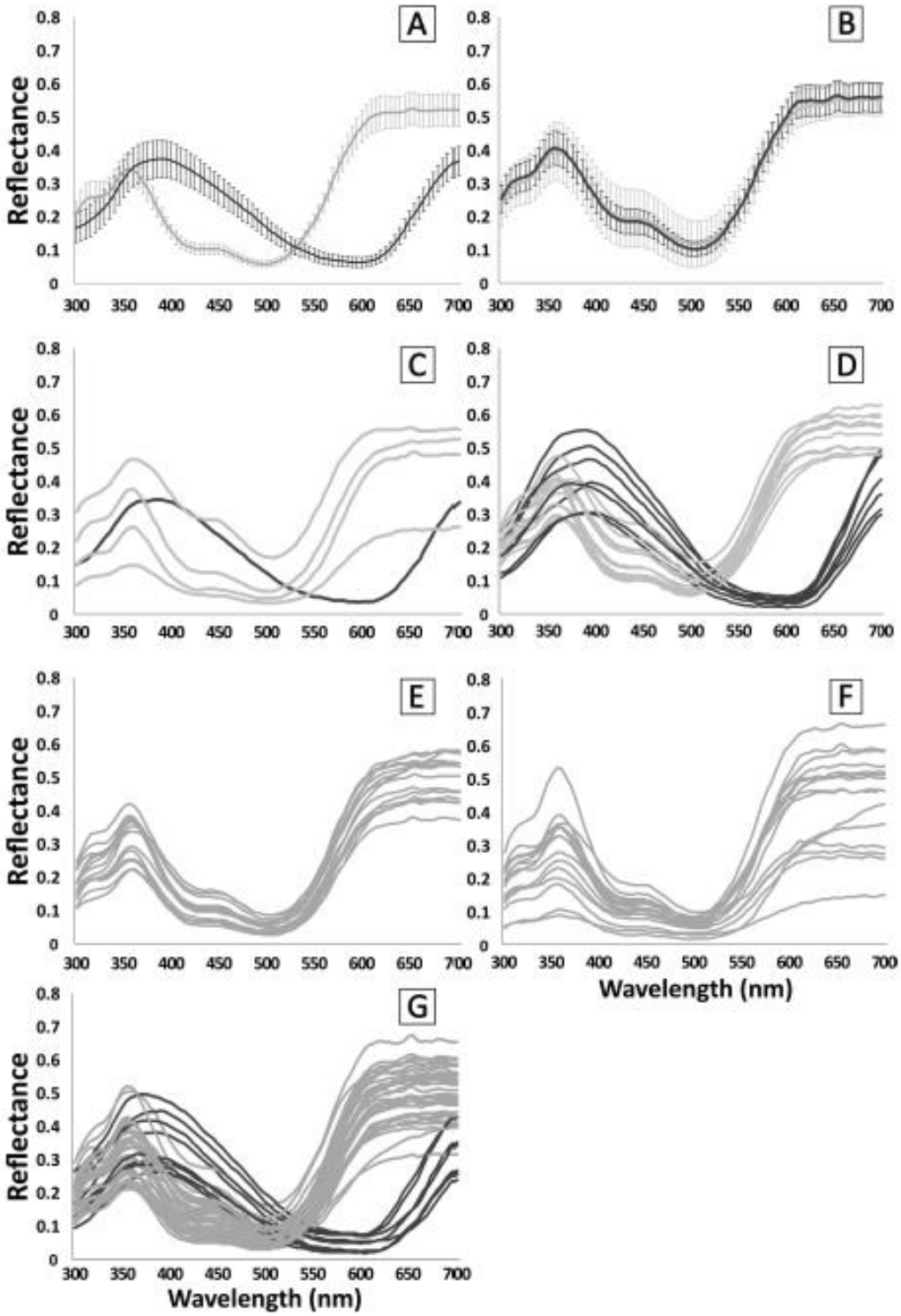

A
B
C
D
E
F
G
Reflectance
Wavelength (nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
300
350
400
450
500
550
600
650
700

Figure 3

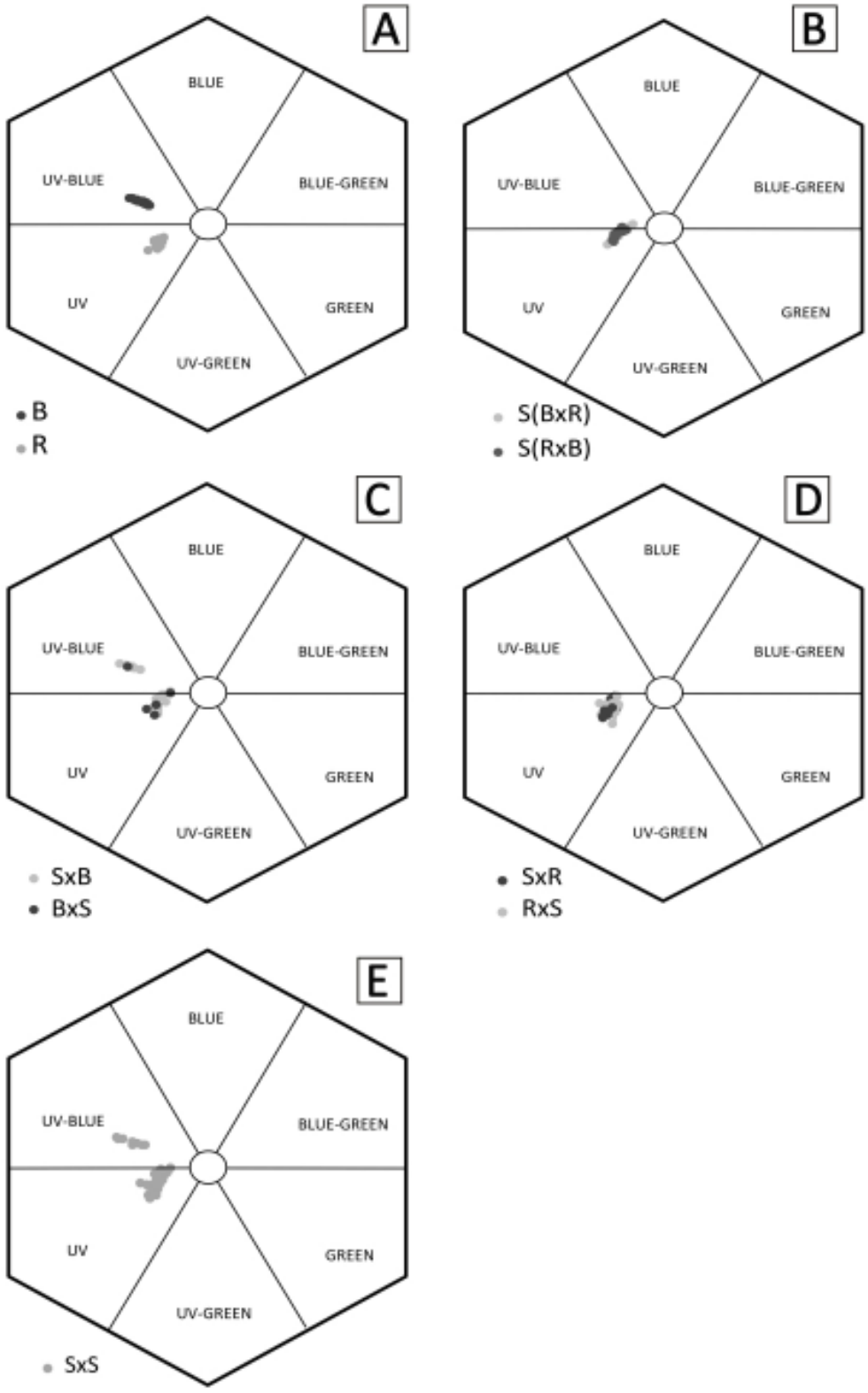

A
BLUE
UV-BLUE
BLUE-GREEN
UV
GREEN
UV-GREEN
B
R
B
BLUE
UV-BLUE
BLUE-GREEN
UV
GREEN
UV-GREEN
S(BxR)
S(RxB)
C
BLUE
UV-BLUE
BLUE-GREEN
UV
GREEN
UV-GREEN
SxB
BxS
D
BLUE
UV-BLUE
BLUE-GREEN
UV
GREEN
UV-GREEN
SxR
RxS
E
BLUE
UV-BLUE
BLUE-GREEN
UV
GREEN
UV-GREEN
SxS

Figure 4

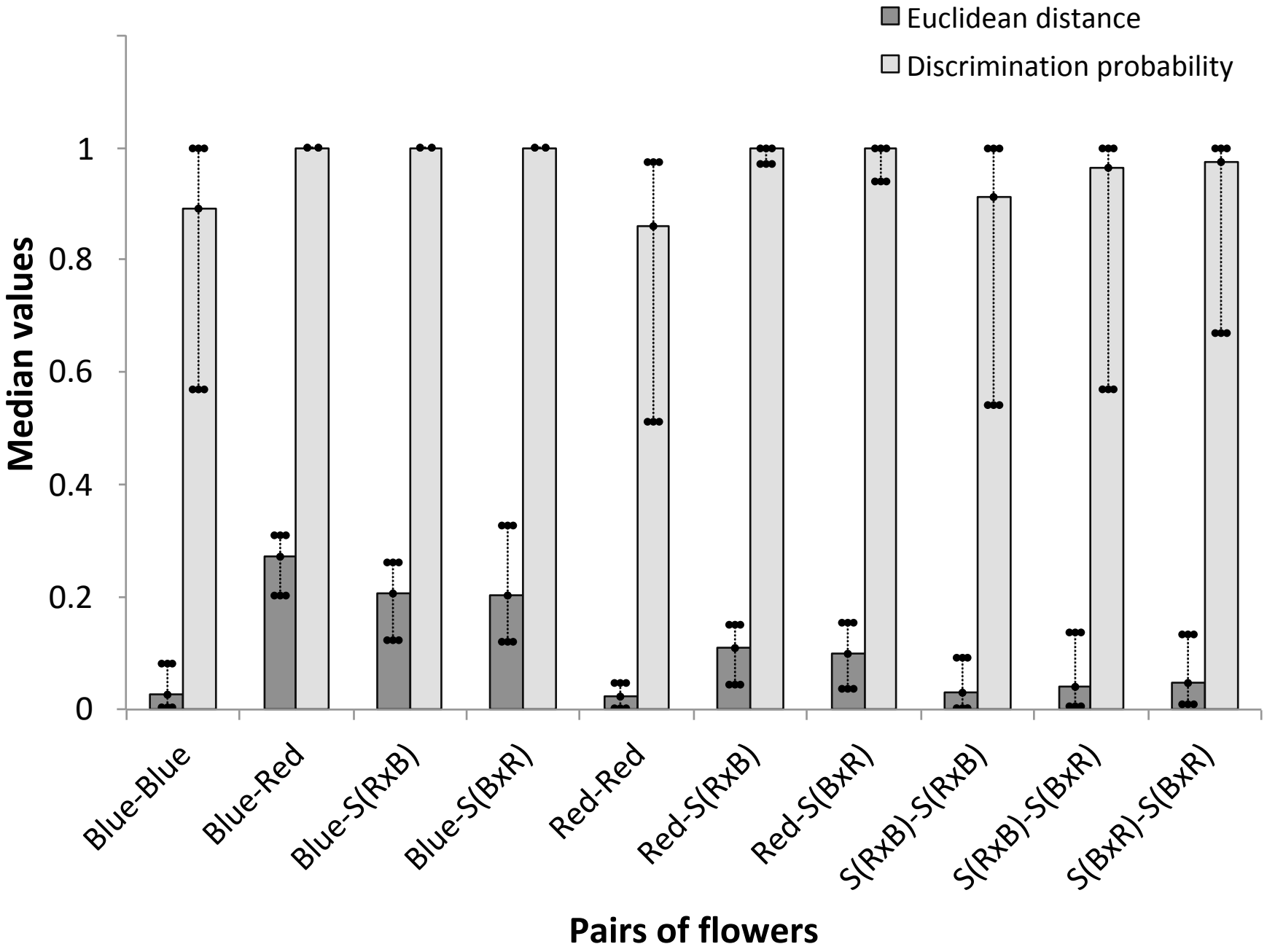
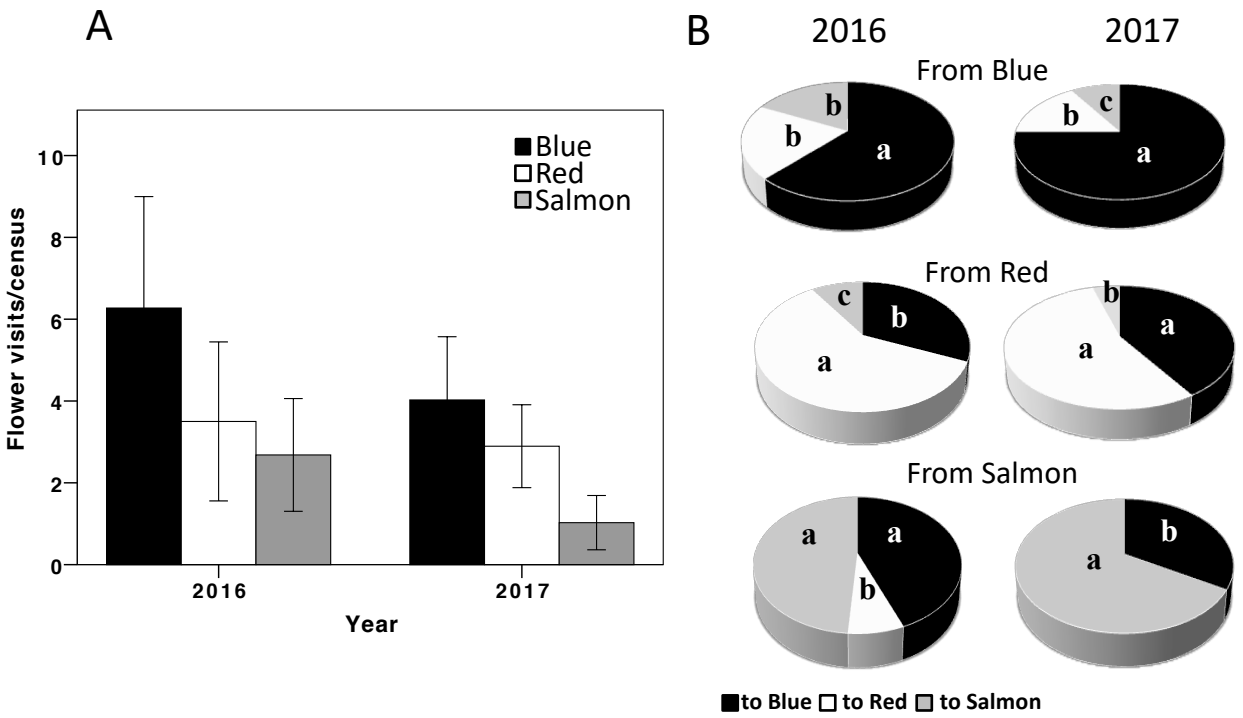

Euclidean distance
Discrimination probability
Median values
1
0.8
0.6
0.4
0.2
0
Blue-Blue
Blue-Red
Blue-S(RxB)
Blue-S(BxR)
Red-Red
Red-S(RxB)
Red-S(BxR)
S(RxB)-S(RxB)
S(RxB)-S(BxR)
S(BxR)-S(BxR)
Pairs of flowers

Figure 5


A
Blue
Red
Salmon
Flower visits/census
0
2
4
6
8
10
2016
2017
Year
B
2016
2017
From Blue
From Red
From Salmon
to Blue
to Red
to Salmon