Differences in pollination success between local and foreign flower color phenotypes: a translocation experiment with *Gentiana lutea* (Gentianaceae)

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Background. The adaptive maintenance of flower color variation is frequently attributed to pollinators partly because they preferentially visit certain flower phenotypes. We tested whether *Gentiana lutea* − which shows a flower color variation (from orange to yellow) in the Cantabrian Mountains range (north of Spain) − is locally adapted to the pollinator community.

Methods. We transplanted orange-flowering individuals to a population with yellow-flowering individuals and vice versa, in order to assess whether there is a pollination advantage in the local morph by comparing its visitation rate with the foreign morph.

Results. Our reciprocal transplant experiment did not show clear local morph advantage in overall visitation rate: local orange flowers received more visits than foreign yellow flowers in the orange population, while both local and foreign flowers received the same visits in the yellow population; thus, there is no evidence of local adaptation in *Gentiana lutea* to the pollinator assemblage. However, some floral visitor groups (such as *Bombus pratorum*, *B. soroensis ancaricus* and *B. lapidarius decipiens*) consistently preferred the local morph to the foreign morph whereas others (such as *Bombus terrestris*) consistently preferred the foreign morph.

Discussion. We concluded that there is no evidence of local adaptation to the pollinator community in each of the two *G. lutea* populations studied. The consequences for local adaptation to pollinator on *G. lutea* flower color would depend on the variation along the Cantabrian Mountains range in morph frequency and pollinator community composition.

1   **Differences in pollination success between local and foreign flower color phenotypes: a**

2   **translocation experiment with *Gentiana lutea* (Gentianaceae)**

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26 **Abstract**

27 **Background.** The adaptive maintenance of flower color variation is frequently attributed to

28 pollinators partly because they preferentially visit certain flower phenotypes. We tested whether

29 *Gentiana lutea* – which shows a flower color variation (from orange to yellow) in the Cantabrian

30 Mountains range (north of Spain) – is locally adapted to the pollinator community.

31 **Methods.** We transplanted orange-flowering individuals to a population with yellow-flowering

32 individuals and vice versa, in order to assess whether there is a pollination advantage in the local

33 morph by comparing its visitation rate with the foreign morph.

34 **Results.** Our reciprocal transplant experiment did not show clear local morph advantage in

35 overall visitation rate: local orange flowers received more visits than foreign yellow flowers in

36 the orange population, while both local and foreign flowers received the same visits in the yellow

37 population; thus, there is no evidence of local adaptation in *Gentiana lutea* to the pollinator

38 assemblage. However, some floral visitor groups (such as *Bombus pratorum*, *B. soroensis*

39 *ancaricus* and *B. lapidarius decipiens*) consistently preferred the local morph to the foreign

40 morph whereas others (such as *Bombus terrestris*) consistently preferred the foreign morph.

41 **Discussion.** We concluded that there is no evidence of local adaptation to the pollinator

42 community in each of the two *G. lutea* populations studied. The consequences for local

43 adaptation to pollinator on *G. lutea* flower color would depend on the variation along the

44 Cantabrian Mountains range in morph frequency and pollinator community composition.

45

46   **Key words:** Flower color variation, Local adaptation, Pollinator preferences, Reciprocal

47   transplants, *Gentiana lutea*

48

49

50   **Introduction**

51   Floral evolution is primarily driven by pollinators (Bradshaw & Schemske, 2003; Whittall &

52   Hodges, 2007). The diversity in floral traits found in angiosperms, such as flower color, could be

53   related with transitions in these traits (Rausher, 2008), as a result of different selective pressures

54   exerted by pollinators with strong preferences for certain characteristics (Schemske & Bradshaw,

55   1999; Streisfeld & Kohn, 2007) or by changes in pollinator community composition (Ellis &

56   Johnson, 2009). Thus, floral diversification and speciation among closely related plant

57   populations may result from the isolation and/or local adaptation to the most efficient pollinators,

58   which increase the number of visits per plant depending on their flower preferences (Harder &

59   Johnson, 2009; and references therein).

60

61   The adaptive maintenance of flower color variation is frequently attributed to pollinators, in part

62   because they tend to promote assortative mating, i.e. preferentially visiting certain phenotypes

63   (Waser & Price, 1981; Stanton, 1987; Jones & Reithel, 2001). However, many other reasons

64   could explain the evolution of floral diversification, such as the potential uncoupling of the

65   evolution of floral traits from pollinator-mediated selection (Strauss & Whittall, 2006). For

66   example, pleiotropic effects on the biosynthetic pathways of floral pigmentation may drive the

67   evolution to alternative flower colors (Armbruster, 1993; Schemske & Bierzychudek, 2007;

68   Cooley, Carvallo & Willis, 2008).

69

70   Many studies support that animal pollinator preferences may cause selective pressures on flower

71   color, which may fluctuate depending on the structure and/or composition of the pollinator

72   community (Melendez-Ackerman & Campbell, 1998; Gómez & Zamora, 2000). In this context,

73   reciprocal transplant experiments between populations can be a powerful tool to assess how

74   changes in flower color preferences of the pollinator assemblage contribute to an adaptive

75   advantage of the local morph over the foreign morphs (Streisfeld & Kohn, 2007).

76

77   *Gentiana lutea* L. shows flower color variation along the Cantabrian Mountains (Northern

78   Spain). *Gentiana lutea* flowers are typically yellow (*G. lutea* var. *lutea*), though northwest

79   Iberian populations have orange-flowering individuals, which constitutes a different variety of

80   the species (*G. lutea* var. *aurantiaca;* Renobales, 2012). The pollinator assemblages are made up

81   mostly of bumblebee species (Veiga et al., 2015; Sobral et al., 2015). Previous studies conclude

82   that: (*i*) *Gentiana lutea* flower color variation does not result from adaptation to environmental

83   factors, such as elevation, temperature, radiation, and precipitation (Veiga et al., 2016); (*ii*)

84   *Gentiana lutea* is strongly dependent on pollinators for reproduction, and the plant has a positive

85   relationship between the number of pollinator visits and the number of seeds it produces (Losada

86   et al., 2015); (*iii*) there is a partial hybridization barrier among *G. lutea* color morphs (Losada et

87   al., 2015); (*iv*) *Gentiana lutea* flower color influences pollinator visits and its variation is related

88   to changes in pollinator community composition across populations, since part of this variation is

89   explained by different pollinator selective pressures exerted on flower color among *G. lutea*

90   populations (Sobral et al., 2015); and (*v*) the most abundant pollinators of our study species,

91   *Bombus terrestris* and *B. pratorum* (Veiga et al., 2015), possess photoreceptors with low

92 sensitivity to red colors; but other pollinators interacting with *G. lutea* plants, such as *Bombus*

93 *lapidarius,* has high sensitivity to red colors despite the lack of photoreceptors required (Peitsch

94 et al., 1992).

95

96 In order to determine whether *G. lutea* populations are locally adapted to the pollinator

97 community, we evaluated the effect of local and foreign flower color morphs on pollinator

98 visitation rate by means of reciprocal transplants in two *Gentiana* populations. More specifically,

99 we tested whether there is an adaptive advantage in the local flower color morph relative to the

100 foreign morph in pollinator visits by comparing the (*i*) overall pollinator assemblage, and (*ii*)

101 different visitor groups separately. We predicted that the local flower color morph would receive

102 more pollinator visits than the foreign morph, and that the two different color morphs would

103 receive visits by different pollinator groups in their local populations.

104

105 **Material and Methods**

106 **Plant species**

107 *Gentiana lutea* L. is an herbaceous perennial geophyte (Fig. 1). During summer, the rhizome

108 develops a flowering stalk of ca. 80 cm high, with a basal rosette formed by 4-8 oblong leaves.

109 Flowers grow in two opposed cymose groups (each one with 15 flowers) at different stalk levels,

110 above two elliptic leaves; and one flower crowns the top of the stalk. Flowers feature a rotate

111 corolla with 5-7 lobules, 5-7 free stamens, and a fixed ovary with two nectaries on the base. The

112 fruit is an ovoid capsule, holding elliptic and winged seeds of 3-4 mm (Renobales, 2012). This

113 species needs pollinators – mainly bumblebees – to produce seed (see Veiga et al., 2015; Sobral

114 et al., 2015).

115

116   *Gentiana lutea* flower color varies between orange and yellow (see Fig. 1) along the Cantabrian

117   Mountains (Northern Spain; Fig. 2). The western populations (west of Somiedo Natural Park)

118   have orange-flowering individuals, whereas the eastern populations (east of Puerto de Ventana)

119   have yellow-flowering individuals; and the intermediate populations show individuals with

120   flowers of a gradation between these two color morphs.

121

122   **Study site**

123   The study was conducted in July 2012 at the northwest of the Iberian Peninsula in two

124   populations, one located in Sierra de Ancares (orange-flowered population) and other in Puerto

125   del Pontón (yellow-flowered population), separated by approximately 160 km (Fig. 2).

126   Population size was estimated by the total number of *G. lutea* individuals (> 3000 in both

127   populations).

128

129   Flower color of 75 haphazardly selected flowers (3 flowers per plant, 25 plants) was measured

130   the previous year in both populations by means of a spectrometer (USB2000+; Ocean Optics,

131   Inc., Dunedin, FL) and petal color spectra were processed using the SpectraSuite® software

132   (Ocean Optics, Inc., Dunedin, FL, USA). The CIELab Colorimetric System (CIE, 2004) was

133   chosen to describe flower color variation in the visible range of the electromagnetic spectrum,

134   through three variables reduced by principal component analysis (for additional details, see

135   Veiga et al., 2015): *L* (brightness of color, from black to white), *a* (red color variation, from

136   green to red) and *b* (yellow color variation, from blue to yellow). Individuals from the Sierra de

137   Ancares population showed orange-colored flowers ($L = 16.42$; $a = 11.77$; $b = 23.61$), and from

138   the Pontón population, yellow ones ($L = 19.64$; $a = 3.84$; $b = 27.84$).

139

140   Although certain pollinator species may detect UV light, a previous study found no differences

141   among *G. lutea* populations and between individuals with different color morphs (orange or

142   yellow, discernible by human eye) in the UV light range (Veiga et al., 2015). Thus, we presume

143   that UV light does not drive local adaptation among populations, and consider flower color in

144   both, the UV and visible light ranges in this study.

145

146   **Experimental design**

147   In July 2012, a reciprocal transplant experiment was conducted to analyze local adaptation of

148   color morphs to pollinator assemblage of both populations (thanks to the field permission issued

149   by the Environmental Territorial Service institution from León, Regional Government of Castilla

150   and León, Territorial Delegation of Government of Spain - Identifier:

151   12_LE_325_RNA_PuebladeLilio_INV - Reference: 06.01.013.016/ROT/abp - File number:

152   AEN/LE/103/12). Given the difficulty of transplanting plants in pots due to their extensive

153   rhizomes, flowering stems used for the experiment were cut at the height of rhizome, placed in

154   plastic containers with water (0.5 L), sealed with adhesive tape, and buried at ground level to

155   prevent evaporation and ensure longer flower life. The effectiveness of this method was

156   previously determined by checking the duration of flowering and pollination in a control

157   population and verifying that the flowers were well-preserved in the first four days.

158

159   For the reciprocal transplant experiment, 15 flowering stems were cut in the orange population

160   (Ancares) and transferred to the yellow population (Pontón) immediately after collection, while

161   simultaneously, 15 yellow stems were cut in Pontón and transferred to the Ancares population

162   (foreign transplant treatment). Additionally, 15 plants of each population (orange in Ancares and

163   yellow in Pontón) were subjected to the same procedures (cut, transplanted, and buried) for the

164   local transplant treatment.

165

166   The same day, plant containers were placed in each population within 1 m^2 squares, set in

167   random layout by picking the grid point using a random number table. Additionally, 15 plants

168   were haphazardly marked to serve as a control in both populations. Thus, each population

169   contained 30 experimental plants and 15 control plants (45 plants per population, 90 plants

170   overall). Note that the stem height, the number of floral whorls per inflorescence, and the number

171   of flowers were measured in all plant individuals to correct for possible plant morphological

172   effects in the experimental results.

173

174   **Pollinator censuses**

175   We conducted 15 2-minute pollinator censuses on each of the 45 plants in both flower color

176   morph populations over 3 days. According to previous studies, the observation sampling effort

177   (30 minutes per plant, 1350 minutes in each population) is appropriate to obtain an adequate

178   representation of the pollinator spectra of this species and in this geographic area (see Materials

179   and Methods section in Veiga et al., 2015; Sobral et al., 2015). For each census, the species that

180   accessed the flowers and the number of flowers they visited were recorded.

181

182 Every day and before the census, the number of open flowers on each plant was counted to

183 correct for the effect of floral display in pollinator attraction. Plant censuses were randomly

184 conducted under sunny conditions between 0800 and 1800 hours. The number of visits per plant

185 (visitation rate per 30 min) was used as a measure of pollination success.

186

187 At the end of each census, a sample of pollinator taxa observed was collected and sent for further

188 identification by entomological specialists from the University of Oviedo (Spain). Correcting any

189 discrepancies between our visual identifications of bumblebee species and the lab

190 determinations, species were grouped for analyses into eight morphological categories based on

191 proboscis length (Obeso, 1992). The eight morphological groups were: group 1 (*Bombus*

192 *terrestris, B. lucorum*); group 2 (*B. hortorum, B. jonellus*); group 3 (*B. pratorum, B. soroensis*

193 *ancaricus, B. lapidarius decipiens*); group 4 (*B. wulflenii*); group 5 (*B. mesomelas*); group 6 (*B.*

194 *pascuorum*); group 7 (*B. (Psithyrus) rupestris*); group 8 (*B. hypnorum*).

195

196 **Statistical analyses**

197 Several generalized linear models (GLM) were performed, where the number of visits per plant

198 was the dependent variable. The locality (Ancares and Pontón), treatment (control, local

199 transplant, and foreign transplant), pollinator group (described earlier), and the interactions

200 among these factors were included as fixed effects. The models were performed for all pollinator

201 species pooled together and for each of the eight pollinator groups independently. Residuals of

202 the dependent variable were fitted in all models with a Poisson distribution and a logarithmic

203 link function.

204

205   Additionally, pairswise contrasts were performed to analyze the differences on the visitation rate

206   (number of visits per plant per 30 min) by the eight bumblebee groups between and within the

207   transplant treatments and control group averaged for both destination localities. To test for local

208   adaptation, the mean differences on visitation rate of group 1 and 3 bumblebees (the most

209   abundant groups) were compared between each pair of treatments applied for the translocation

210   experiment (foreign transplants, local transplants, controls), considering both localities (Ancares

211   and Pontón). All analyses were performed using SPSS Statistics 20 (IBM Corp., Somers, NY).

212

213   **Results**

214   **Pollinator censuses**

215   In total 951 insects (99% bumblebees belonging to the genus *Bombus,* and the remaining 1%

216   belongs to the genus *Apis* or the family Vespidae) visited the plants (538 in Ancares, and 413 in

217   Pontón), which represented 3268 total cumulative flower visits (see Supplementary file,

218   Supplemental [Data] S[1]). *Bombus terrestris* (group 1) and *Bombus pratorum* (group 3) were

219   the main flower visitor of *Gentiana lutea* plants in Pontón, with 447 and 372 total cumulative

220   visits respectively for each group (excluding control treatment, see Fig. 3). *Bombus soroensis*

221   *ancaricus + Bombus lapidarius decipiens* (group 3) and *Bombus (Psithyrus) rupestris* (group 7)

222   were the main flower visitors in Ancares (338 and 224 total cumulative visits respectively for

223   each group, only for transplant treatments), being the latest group absent in Pontón, and *Bombus*

224   *hypnorum* (group 8) was only present in this location (Fig. 3).

225

226   **Reciprocal transplants**

227   In Ancares, a population of orange morphs, more visits per 30 min were observed to orange- than

228   to yellow- flowering plants (32.5% versus 24.7% plants visited, respectively for each color

229   morph); whereas in Pontón, a population consisting of yellow flowers, there was no difference in

230   visitation rate between the two morphs (39.2% versus 41% plants visited, respectively for yellow

231   and orange morphs; see Supplemental [Data] S[2]). Number of visits per plant depended on the

232   floral visitor group ($P < 0.001$; Table 1). Additionally, the number of visits per plant by floral

233   visitor groups were affected differently per treatment: control, local transplant, and foreign

234   transplant (as suggested by the significant treatment*floral visitors interaction; $P < 0.05$; Table

235   1), and also by locality (as suggested by the significant treatment*floral visitors*locality

236   interaction; $P < 0.05$; Table 1).

237

238   Results of the GLM to analyze the locality and treatment effects on the number of visits per plant

239   for each pollinator group showed different flower color preferences, but only in the groups 1 (*B.*

240   *terrestris*) and 3 (*B. pratorum, B. soroensis ancaricus + B. lapidarius decipiens*; significant

241   treatment factor; group 1: $P < 0.05$; group 3: $P < 0.01$; Table 2). Group 1 and group 3 pollinators

242   visited control and local plants similarly in both color morph populations (Table 3; Fig. 4).

243

244   Group 1 visited foreign transplant individuals more often than local transplant individuals,

245   considering both locations (significant mean differences between foreign and local transplants; P

246   < 0.05; Table 3). Group 1 pollinators visited control and local plants similarly in both color

247   morph populations (Table 3; Fig. 4a). However, the absolute number of visits by these

248   pollinators was higher in Pontón (yellow morph population) than in Ancares (orange morph

249   population).

250

251 Group 3 visited local transplants more often than foreign transplants in both locations (no

252 significant treatment*locality interaction; Table 2). The number of visits by group 3

253 to control and foreign plants was equal considering both populations (Table 3; Fig. 4b). Once

254 again, the absolute number of visits by these pollinators was higher in Pontón (yellow-flowered

255 population) than in Ancares (orange-flowered population).

256

257 **Discussion**

258 Our results show that *Gentiana lutea* plants from both locations face different sets of flower

259 visitors with different color preferences − indicated by differences in visitation rate − suggesting

260 that these differences may play a role in floral color divergence patterns. We found that the

261 orange-flowering plants may be locally adapted to the pollinator assemblage since they received

262 more visits than the yellow-flowering transplants in Ancares, the population of orange morphs.

263 However, we found no evidence of local adaptation of yellow-flowering plants to the original

264 population since the number of bumblebee visits was similar to the orange-flowering transplants

265 in Pontón. This result differs from previous research, which suggested that flower color variation

266 among *G. lutea* populations is related to selective pressures exerted by different pollinator

267 assemblages (Sobral et al., 2015).

268

269 Several possible explanations may help to clarify our findings. The different responses obtained

270 in the Ancares and Pontón populations are likely due to variation in the pollinator assemblage

271 and/or floral visitor preferences between the two areas (as experimentally demonstrated, Jones &

272 Reithel, 2001). Ancares and Pontón have different pollinator assemblage and the species from

273   the Ancares population may prefer local orange-flowering plants, while the species from Pontón

274   population clearly show no preference for one of the two color morphs. Geographic variation in

275   floral visitor preferences can result from changes in pollinator composition, abundance, and

276   diversity (Price et al., 2005), even different plant populations that share the same pollinator

277   group receive different proportions of flower visits from each species or functional group

278   (Fenster et al., 2004; Tastard et al., 2014). Thus, pollinator-dependent foraging preferences may

279   cause shifts in the optimal floral phenotypes (see Gómez et al., 2009; and references therein).

280

281   Within the pool of *G. lutea* flower visitors in the Ancares population, the most abundant species

282   *(Bombus terrestris*) has low sensitivity for red color detection (Peitsch et al., 1992; Briscoe &

283   Chittka, 2001), which probably explains the reduction in this species visitation rate when

284   increasing orange-flowering individuals (Fig. 4a). We suspect that other pollinator species with

285   probably higher sensitivity to red color may increase fitness of the orange-flowering plants.

286   Therefore, the fact that they perceive red color does not imply that they necessarily would prefer

287   this color over others. In fact, *B. lapidarius,* present in other orange *G. lutea* populations

288   (personal observations), shows sensitivity for red colors; although it lacks red color receptors

289   (Kugler, 1943; Peitsch et al., 1992). If pollinator species with a higher sensitivity or innate

290   preferences for red colors are present, orange-flowered plants would increase their fitness

291   through the rising visitation rate by these pollinators. Therefore, variation of the pollinator

292   community composition might affect the selective pressures exerted on *G. lutea* flower color,

293   and ultimately affect flower color variation among populations via local adaptation.

294

295   A second explanation is that the preference of certain pollinator groups could be dependent on

296   the frequency of each morph in the population (i.e. the preferences for orange plants in Ancares

297   are diluted in a context of yellow plants in Pontón). Empirical studies that examine plant species

298   polymorphisms in flower color, morphology, or sex phenotype generally show negative

299   frequency dependence (see references in Harder & Johnson, 2009). However, in some cases this

300   selection occurred somewhat heterogeneously and studies have found that common (or highly

301   efficient) pollinators tend to exhibit positive frequency-dependent foraging, while less common

302   (or less efficient) species might exhibit negative frequency-dependence (Eckhart et al., 2006) or

303   positive frequency-dependence (Malerba & Nattero, 2012).

304

305   Our results show that differences in visitation rate between Ancares and Pontón populations

306   depend on floral visitors from Groups 1 and 3. Group 1 (*B. terrestris*) shows preferences for the

307   least abundant morph in the population (orange in Pontón; negative frecuency-dependence).

308   Conversely, Group 3 shows a preference for the most abundant morph (orange in Ancares;

309   positive frequency-dependence). Consequently, the preference of certain pollinator groups is

310   dependent on the frequency of each morph in a population (Smithson & Macnair, 1996). Theory

311   suggests that competition for floral resources might favor frequency-dependent foraging by some

312   pollinator species, possibly contributing to the maintenance of flower color variation by

313   frequency-dependent selection (Gigord, Macnair & Smithson, 2001; Eckhart et al., 2006).

314

315   Additionally, data from this work and previous studies show that the different pollinator

316   abundance, especially groups 1 and 3, varies between years in Ancares and Pontón populations,

317   but not selective pressures exerted on *G. lutea* flower color (see Sobral et al., 2015). However,

318   the pollinator assemblage composition can also vary substantially year-to-year (e.g. Herrera,

319   1988; Price et al., 2005). Bumblebee foraging may differ among years (Teräs, 1985), among sites

320   (Elam & Linhart, 1988; Jones & Reithel, 2001) and between queens and workers (Teräs, 1985;

321   Wesselingh & Arnold, 2000). The floral color choices of bumblebees appear not to be governed

322   by innate preference only, but also by environment conditions and colors of co-flowering plants

323   (Teräs, 1985). Consequently, preferences for different color morphs may change temporally

324   according to the community composition, generating a mosaic of selective pressures on floral

325   color. Therefore, further analyses considering data from multiple years are needed to clearly

326   support the hypothesis of flower color adaptation to local pollinator assemblages, even though

327   possible technical and logistical challenges that would lead to the long-term monitoring of the

328   transplant experiment.

329

330   In conclusion, the present study suggests no clear evidences of local adaptation to the pollinator

331   community in each of the two *G. lutea* populations studied, even though some floral visitor

332   groups (such as *Bombus pratorum*, *B. soroensis ancaricus* and *B. lapidarius decipiens*)

333   consistently preferred the local morph to the foreign morph whereas others (such as *Bombus*

334   *terrestris*) consistently preferred the foreign morph. Variation in pollinators foraging preference

335   and visitation rate could generate a mosaic of frequency-dependent selection in *G. lutea* along

336   the Cantabrian Mountains range. The consequences for local adaptation on *G. lutea* flower color

337   would thus depend on variation in morph frequency, pollinator community composition and their

338   effects on plant fitness.

339

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345

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446   **Table 1: Results of the generalized linear model to analyze the effect of color morphs of**

447   ***Gentiana lutea* on the visitation rate (number of visits per plant per 30 min) by eight**

448   **bumblebee groups in two areas of Northern Spain (the orange color morph was present at**

449   **the Ancares site, whereas the yellow morph was present at the Pontón site).**

450   We marked in bold the statistically significant factors ($P < 0.05$). Factors: locality (Ancares and

451   Pontón, orange and yellow morph population respectively); treatment (control, local transplants,

452   and foreign transplants); and floral visitor groups (group 1: *Bombus terrestris*, *B. lucorum*; group

453   2: *B. hortorum*, *B. jonellus*; group 3: *B. pratorum*, *B. soroensis ancaricus*, *B. lapidarius*

454   *decipiens*; group 4: *B. wulflenii*; group 5: *B. mesomelas*; group 6: *B. pascuorum*, group 7: *B.*

455   *(Psithyrus) rupestris*; group 8: *B. hypnorum*).

456

457

Factors	Wald Chi-Square	d.f.	*P*
Locality	0.702	1	0.402
Treatment	0.110	2	0.946
Treatment * Locality	4.336	2	0.114
Floral visitors	66.548	3	**< 0.001**
Treatment * Floral visitors	16.703	6	**0.010**
Treatment * Floral visitors * Locality	19.573	9	**0.021**

464 **Table 2: Results of the generalized linear model to analyze the effect of locality, treatment,**

465 **and the corresponding interaction between both, on the visitation rate (number of visits per**

466 **plant per 30 min) by two bumblebee groups (group 1 and 3) in two areas of Northern Spain**

467 **(Ancares and Pontón, the orange and yellow morph population respectively).**

468 We marked in bold the statistically significant factors (*P* < 0.05). Factors: locality (Ancares and

469 Pontón, orange and yellow morph population respectively); treatment (control, local transplants,

470 and foreign transplants); and floral visitor groups (group 1: *Bombus terrestris*, *B. lucorum*; group

471 3: *B. pratorum*, *B. soroensis ancaricus*, *B. lapidarius decipiens*). Note that we only included

472 results of the visitor groups that showed significant model effects (Group 1, Group 3).

473

Floral visitors	Factors	Wald Chi-Square	d.f.	*P*
	Locality	32.222	1	**< 0.001**
Group 1	**Treatment**	6.181	2	**0.045**
	Treatment * Locality	0.692	2	0.707
	Locality	1.867	1	0.172
Group 3	**Treatment**	16.331	2	**< 0.001**
	Treatment * Locality	0.114	2	0.945

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476 **Table 3: Results of pairwise contrasts to analyze the mean differences on the visitation rate**

477 **(number of visits per plant per 30 min) by bumblebee groups 1 and 3, averaged for two**

478 **areas of Northern Spain (Ancares and Pontón, the orange and yellow morph population**

479 **respectively) and per treatment (control, local transplant, foreign transplant).**

480 We marked in bold the statistically significant mean differences between the pair of treatments

481 ($P < 0.05$). Abbreviations: trans-local (local transplants), trans-foreign (foreign transplants) and

482 control (unmanipulated groups). Note that we only included results from pairwise contrasts that

483 showed significant mean differences between pair of treatments (i.e., pollinator groups 1 and 3).

484

Floral visitors	Pair of treatments		Mean-Difference	S.E.	d.f.	*P*
	Control	Trans-foreign	-5.090	2.801	1	0.069
	Control	Trans-local	1.390	2.331	1	0.551
Group 1	**Trans-foreign**	**Trans-local**	6.480	2.733	1	**0.018**
	Control	**Trans-foreign**	18.720	4.369	1	**< 0.001**
	Control	Trans-local	8.210	4.992	1	0.100
Group 3	**Trans-foreign**	**Trans-local**	-10.510	3.736	1	**0.005**

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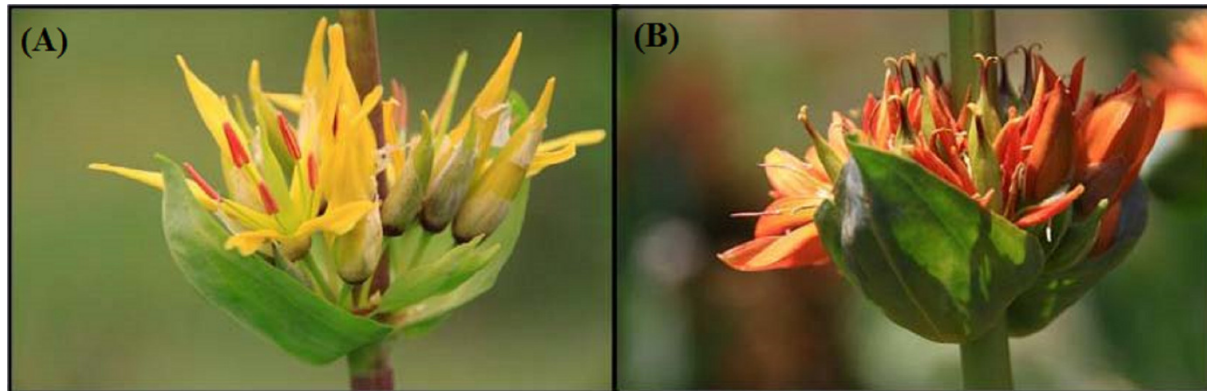
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495 **Figure 1: Color morphs of *Gentiana lutea* L. at northern Spain (A: var. *lutea*, the yellow**

496 **morph in Pontón location; and B: var. *aurantiaca*, the orange morph in Ancares location).**

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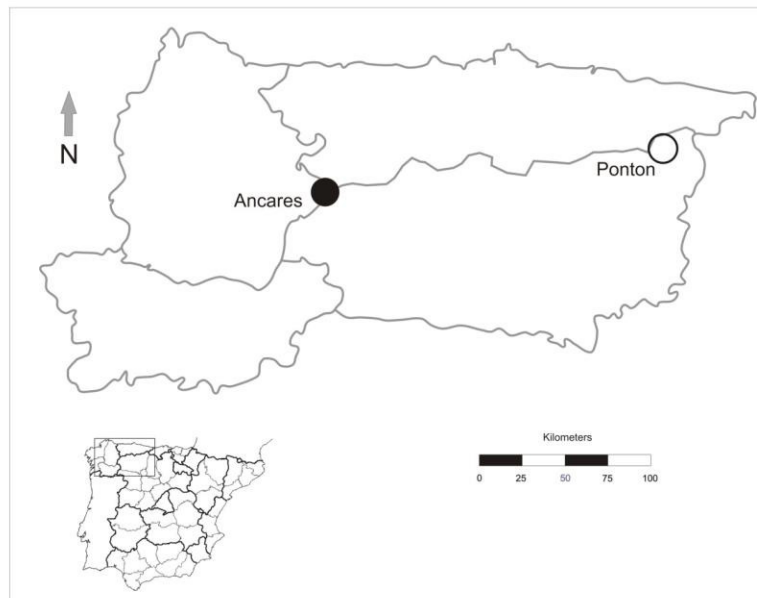
500   **Figure 2: Geographic location of *Gentiana lutea* populations at northern Spain in which**

501   **reciprocal transplants were conducted (black dot: Ancares, the orange morph population;**

502   **white dot: Pontón, the yellow morph population).**

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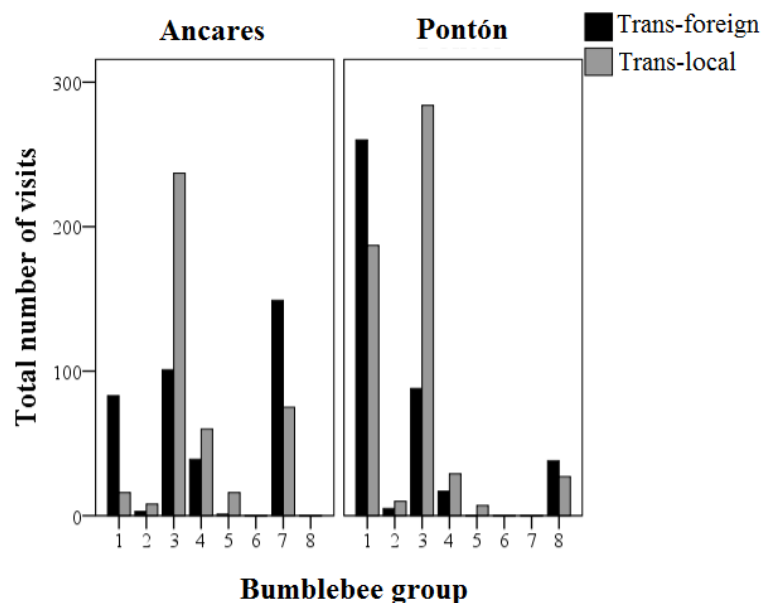
Figure 3: Total cumulative number of visits to the *Gentiana lutea* transplanted plants

(black-colored bars: foreign; grey-colored bars: local) by each group of bumblebees in the

two study sites at northern Spain (Ancares with the orange morph and Pontón with the

yellow morph).

Abbreviations: Trans-foreign (foreign transplant); Trans-local (local transplant). Floral visitor

groups were the following: group 1: *Bombus terrestris, B. lucorum*; group 2: *B. hortorum, B.*

jonellus; group 3: *B. pratorum, B. soroensis ancaricus, B. lapidarius decipiens*; group 4: *B.*

wulflenii; group 5: *B. mesomelas*; group 6: *B. pascuorum*, group 7: *B. (Psithyrus) rupestris*;

group 8: *B. hypnorum*.

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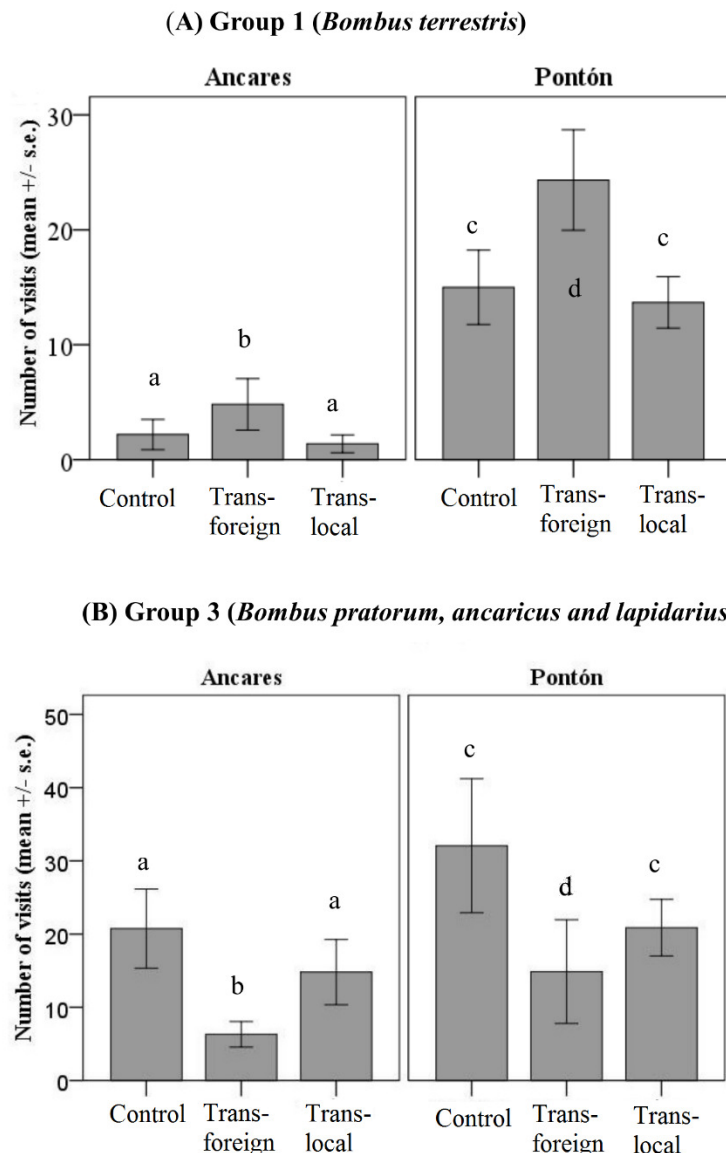
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516 **Figure 4: Number of floral visits per plant per 30 min (mean ± standard error) by two**

517 **bumblebee groups (A) group 1:** *Bombus terrestris*; **(B) group 3:** *B. pratorum, B. soroensis*

518 *ancaricus, B.lapidarius decipiens*; **depending on the locality at northern Spain (Ancares, the**

519 **orange morph population; and Pontón, the yellow morph population) and the experimental**

520 **treatment (control, local transplant, foreign transplant)**.

521   Abbreviations: Trans-foreign (foreign transplant); Trans-local (local transplant). Different letters

522   indicate statistical differences among treatments ($P < 0.01$).