Watteyn et al. 2021

1 **Trick or Treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae)**

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ABSTRACT

Natural pollination of species belonging to the pantropical orchid genus *Vanilla* remains poorly understood. Based on sporadic records, euglossine bees have been observed visiting flowers of Neotropical *Vanilla* species. Our research aimed at better understanding the pollinator attraction mechanism of the Neotropical species *Vanilla pompona*, a crop wild relative with valuable traits for vanilla crop improvement programs. Using video footage, we identified floral visitors and examined their behavior. The flowers of *V. pompona* attracted *Eulaema cingulata* males, which distinctively displayed two behaviors: floral scent collection and nectar search; with the latter leading to pollen removal. Morphological measurements of floral and visitor traits showed that other *Eulaema* species may also act as potential pollinators. Additionally, we recorded natural fruit set in three populations and over a period of two years, tested for nectar presence and analyzed floral fragrances through gas chromatography - mass spectrometry. We observed a low natural fruit set (2.42%) and did not detect nectar. Twenty floral volatile compounds were identified, with the dominant compound trans-carvone oxide previously found to attract *Eulaema cingulata* males. We hypothesize a dual attraction of *Eulaema cingulata* males to *V. pompona* flowers, based on floral fragrance reward as the primary long-distance attraction, and food deception for successful pollen removal. Further research confirming this hypothesis is recommended to develop appropriate conservation policies for *Vanilla* crop wild relatives, which are the primary reserves of this crop's genetic variation.

KEYWORDS

46   1. INTRODUCTION

47   Plant-pollinator interactions are typically mutualistic, with a reward offered by the plant in

48   exchange for a pollination service (Wright & Schiestl, 2009). Within the orchid family

49   (Orchidaceae), approximately two-thirds of all species reward their pollinators with nectar, floral

50   fragrances, oils or food hairs (van der Pijl & Dodson, 1966; Nilsson, 1992; Williams, 1982;

51   Shrestha et al., 2020), while the remaining are believed to be deceptive (Dafni, 1984; Ackerman,

52   1986). Among the floral fragrance-rewarding species, odor-seeking male orchid bees (Apidae:

53   Euglossini) have been reported to be responsible for the pollination of hundreds of Neotropical

54   orchid species (Dodson et al., 1969; Williams & Whitten, 1983). These males gather volatile

55   compounds from floral or other sources to be used as courtship attractants, a phenomenon known

56   as the "Perfume Flower Pollination Syndrome" (PFPS) (Dodson, 1975; Kimsey, 1984; Schemske

57   & Lande, 1984; Eltz et al., 2003, 2005; Cappellari Rabeling & Harter-Marques, 2010).

58   The pantropical orchid genus *Vanilla* Plum. ex Mill. (1754) consists of 118 species (Karremans et

59   al., 2020). Only those native to the Neotropics and belonging to the section *Xanata* produce

60   aromatic pods or beans, such as *Vanilla planifolia* Andrews and its crop wild relatives (CWRs).

61   Commercial vanilla plants are almost exclusively hand-pollinated, leading to satisfactory bean

62   yields (Havkin-Frenkel & Belanger, 2018; Hu et al., 2019). Vector-mediated natural pollination,

63   however, generally results in cross-pollination and increases genetic variability. The latter

64   augments survival probabilities in changing environments and contributes to the development of

65   potentially interesting crop traits related to, amongst others, pest resistance and bean quality.

66   Studies on natural vanilla pollination are scarce but crucial to safeguard the long-term survival of

67   this crop and its CWRs, which are the primary reserve of a crop's genetic variation and a

68   prerequisite for crop improvement programs (Castañeda-Álvarez et al., 2016; Flanagan &

69   Mosquera-Espinosa, 2016).

70   The intrageneric diversity in floral morphology suggests the evolution of different pollination

71   mechanisms (Cameron & Soto Arenas, 2003; Soto Arenas & Dressler, 2010). Even though

72   spontaneous autogamy has been reported (Bateman et al., 2004; Van Dam et al., 2010; Pansarin,

73   2016), most *Vanilla* species are suspected to depend on animal vectors for pollination (Pansarin et

74   al., 2014; Gigant et al., 2016; Chaipanich et al., 2020). Members of the Euglossini tribe are

75   suggested as pollinators of the Neotropical species (Ackerman, 1983a; Lubinsky et al., 2006;

76   Householder et al., 2010; Soto Arenas & Dressler, 2010; Pansarin & Pansarin, 2014; Anjos et al.,

77   2017), but the specific mechanisms are still poorly understood. Our study aimed at better

78   understanding the pollinator attraction mechanism and reproductive biology of the Neotropical

79   species *Vanilla pompona* Schiede (*Vanilla* sect. *Xanata*), by a) identifying its floral visitors and

80   comparing their behaviors, b) confirming its pollinator species and quantifying natural fruit set, c)

81   identifying floral rewards, and d) predicting an optimal morphological fit between flower and

82   pollinator to determine other potential pollinator species.

83   **2. MATERIALS AND METHODS**

84   **2.1. Study area and species**

85   The study took place in Costa Rica and Peru, in respectively the Osa Peninsula and the Madre de

86   Dios region (Appendix A - Figure S1a-b). Both regions are recognized biological hotspots

87   (Kohlmann et al., 2010) with a tropical wet climate (Atrium Biodiversity Information System,

88   2011; Holdridge, 1967; Kappelle, 2003) and host several *Vanilla* species (Householder et al., 2010;

89   Janovec et al., 2013; Karremans et al., 2020; Watteyn et al., 2020).

90 *Vanilla pompona*, listed by the IUCN as Endangered (Herrera-Cabrera, 2020), has a distribution

91 from Mexico to Brazil and Peru (Soto Arenas & Dressler, 2010). Flowers consist of three sepals,

92 two lateral petals, and one large trumpet-shaped petal known as the lip or labellum. The latter is

93 partially fused to the column, which bears a single ventral stigma, separated by the rostellum from

94 the anther holding two pollinia-like pollen masses, that are deposited as smears. Each flower lasts

95 for one day and starts to wither after midday.

96 **2.3. Floral visitors and their behavior**

97 Floral visitors were observed in the three Costa Rican and Peruvian study sites (hereafter *V.*

98 *pompona* populations) during the flowering period of January-February 2019 (120 h of

99 observation) and September 2018 (64 h of observation), respectively. We checked for newly-

100 opened flowers and observed them from 0600 h to 1400 h, corresponding to the time of anthesis

101 until the flowers start to wither. Cameras (CANON SX60 HS, EOS M) were located at a minimum

102 distance of three meters from the flower to avoid disturbance. Videos were recorded upon visitor

103 approaches (Costa Rica) or with tele macro mode activated (Peru). We screened the video footage

104 to identify floral visitors, record their visiting time, and examine their behavioral patterns.

105 Subsequently, we assigned them to five behavioral categories, following the behavioral hierarchy

106 of bee attraction: (a) approaching, (b) landing on tepals or labellum, (c) landing on labellum, (d)

107 entering labellum, and (e) successfully removing pollen. To model the effect of floral visitor genus

108 and time of the day on this ordinal bee behavior classification, we used a series of mixed logistic

109 regression models, and sequentially compared each pair of consecutive behavior categories

110 (Appendix B) (Zuur et al., 2009).

111 **2.4. Pollinator identification and pollination success**

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112   The floral visitors that removed pollen were identified at species level and determined by sex.

113   Additionally, bees carrying pollen were caught during the flowering period in Costa Rica and Peru,

114   using baits shown to be attractive compounds: eugenol, 1,8-cineole, and methyl salicylate

115   (Dressler, 1981; Ackerman, 1989; Roubik & Hanson, 2004). The identity of the pollen was

116   confirmed by morphological comparison with *V. pompona* pollen using high microscope

117   magnification. Bees were identified using the key in Roubik and Hanson (2004). To test for

118   autogamy, ten inflorescences from two Peruvian populations were bagged the day before anthesis

119   with an insect proof nylon bag using a ground-based tool (Scaccabarozzi et al., 2020). Fruit set

120   within the Costa Rican populations was quantified during two flowering periods (2019 and 2020).

121   We counted the number of inflorescences per population, flowers per inflorescence, and developed

122   fruits per inflorescence. The calculated fruit set was then compared among the three populations

123   using the non-parametric Kruskal-Wallis test and Dunn's post-hoc tests with multiple comparison

124   correction (Benjamini-Hochberg adjustment to control the False Discovery Rate) and the two

125   flowering periods using the non-parametric Mann-Whitney test.

126   **2.5. Floral rewards**

127   To determine if pollination is based on a nectar reward, we bagged flowers the day before anthesis

128   (Corbet, 2003) and visually inspected 20 flowers from the Costa Rican populations for secretory

129   glands under a stereoscopic microscope. Attempts to collect nectar using microcapillary tubes (2

130   µL) were undertaken in 10 of these flowers. Since no secreted fluids were observed or collected,

131   we applied the rinsing method for small nectar volumes (Morrant et al., 2009; Power et al., 2017)

132   in eight additional flowers. The rinsed water was analyzed using High-Performance Liquid

133   Chromatography (HPLC) (Appendix C).

134   Floral fragrances of 12 flowers were collected by static headspace sampling (Tholl et al., 2006)

135   during the 2020 flowering period in two Costa Rican populations. The sampling was conducted

136   between 7:00 am and 11:00 am, corresponding to the activity peak of potential pollinators (Dodson

137   et al., 1969; Ackerman, 1983b; Armbruster et al., 2000; Gostinski et al., 2016), thus expected floral

138   scent productivity peaks (Whitten, 1985; Hills & Williams, 1990). Control samples were collected

139   simultaneously from bags filled with ambient air. The scent traps were analyzed by gas

140   chromatography-mass spectrometry (GC-MS). We used the same methodology for data analysis

141   and processing, and compound identification and attractiveness as described in Hetherington-Rauth

142   & Ramirez (2016) (Appendix C). The compound peak areas of the final set of floral volatiles were

143   converted to relative frequencies and averaged per compound among the samples to reveal

144   potential patterns within the floral bouquet.

145   **2.6. Flower and floral visitor morphology**

146   We measured and compared pollinator dimensions with floral traits to assess the pollinator

147   morphological-fit to flower structure. Bee traps were located in the vicinity of the Peruvian and

148   Costa Rican populations. The same methodology was used as described in Section 2.4. The bees'

149   total length, mesosoma height and diameter, and extended tongue length were measured using a

150   caliper. Next, 32 *V. pompona* flowers were measured across the Costa Rican populations. Measured

151   traits were: height (H1), width (W) and length (L1) of the labellar tube, height of the labellar tube

152   below the anther (H2), thickness of the column at height of the anther (T_Co), height of the labellar

153   callus (T_Ca), height of the labellar keels (T_La), and length of the pedicel (L2) (Figure 1a).

154   Further derived variables were estimated to assess the size of the labellar tube: "An", labellar tube

155   entrance measured at the apex of the anther (H2 - T_La) and "Cn", labellar tube measured at the

156   height of the callus, below the rostellar flap (H2 - T_Ca).

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157 **3. RESULTS**

158 **3.1. Floral visitors and their behavior**

159 Of the 123 recorded floral visitors, 82.1% were Euglossini, belonging to the genera *Eulaema* (n =

160 73) and *Euglossa* (n = 28), and 17.9% were Trigonini of the genus *Trigona* (n = 22). The behavioral

161 comparison analysis showed that most bees approaching the flowers subsequently landed on them

162 (Appendix B - Table S1). Closer towards the end of the morning, the chance that a bee lands on a

163 flower decreases significantly (Appendix B - Figure S2). Approaching *Euglossa* or *Eulaema* are

164 predicted to land more on the tepals than on the labellum, where they show a probing behavior of

165 brushing the flower structure with their foretarsi (Appendix B - Table S2). The chance that an

166 approaching *Trigona* lands on the labellum edge or enters the labellar tube is higher compared to

167 *Euglossa* or *Eulaema*, and all *Trigona* display a probing behavior of touching the flower surface

168 with their proboscis or antennae. *Eulaema* or *Euglossa* that enter the labellum, visit the flower for

169 less than five seconds and remain inside for about two seconds. As they exit, an extended tongue

170 was observed. These rapid visits suggest unsuccessful attempts of nectar foraging. Despite the

171 observation of three different bee genera in both Costa Rica and Peru, only individuals belonging

172 to the genus *Eulaema* (n = 5) successfully removed pollen when entering the labellum (Appendix

173 B - Figure S3). Overall, landing bees display two main reward-approach behaviors on different

174 flower parts: probing (Video S1) or attempts for nectar search (Video S2).

175 **3.2. Pollinator identification and pollination success**

176 The five bees observed removing the pollen of *V. pompona* flowers were identified as male

177 *Eulaema cingulata*. Furthermore, using chemical baits, we caught five male *Eulaema cingulata*

178 that carried *V. pompona* pollen masses attached to their scutellum (Figure 1b). Fruit set at

179 inflorescence level was significantly different among the populations ($H(2) = 7.72$, $P = 0.02$), with

180 Piro-Filanance (4.75% $\pm$ 9.71%) having a higher fruit set compared to Miramar (1.26% $\pm$ 4.78%;

181 $P = 0.01$) and Piro-Rio (1.24% $\pm$ 4.57%; $P = 0.02$). No difference was found between the two years

182 ($H(1) = 0.42$, $P = 0.52$). The autogamy test resulted in zero fruit development.

3.3. Floral rewards

184 No nectaries were observed after visually inspecting 20 *V. pompona* flowers and no nectar could

185 be collected via microcapillary tubes. The rinsing method followed by HPLC analysis detected

186 total sugars (glucose, fructose, sucrose) in trace (< 0.03 mg) amounts (four samples) or non-

187 quantifiable amounts (five samples) (Appendix C - Figure S4). We detected a total of 20 floral

188 fragrance compounds emitted by *V. pompona* flowers (Table 1). One of them could not be

189 identified. The most representative compounds of the floral bouquet were trans-carvone oxide

190 ($35.70 \pm 14.85\%$), limonene ($17.45 \pm 6.00\%$), limonene oxide, trans ($16.72 \pm 6.87\%$) and limonene

191 oxide, cis ($8.97 \pm 6.80\%$), followed by terpinolene ($6.94 \pm 3.60\%$), carvone ($4.42 \pm 1.50\%$),

192 myrcene ($3.34 \pm 2.36\%$), 1-R-alpha-pinene ($2.61 \pm 2.30\%$) and (-)-beta-pinene ($1.33 \pm 0.93\%$)

193 (Appendix C - Figure S5). The other compounds were present in low amounts (mean relative peak

194 area < 1%).

195 **Table 1.** Identified floral volatile compounds, Kovats retention indices, retention times (min), and

196 mean relative peak areas (%) calculated from the total peak area in the GC-MC chromatogram

197 from *Vanilla pompona* flowers (*N* =12). *SD* = standard deviation.

Compound	Kovats retention index[‡]	Retention time (min)[‡]	Relative peak area (%)[†] (mean ± *SD*)
alpha-pinene oxide	1077.59	7.69	0.04 ± 0.04
1-R-alpha-pinene	934.44	5.18	2.61 ± 2.30
(-)-beta-pinene	977.62	5.95	1.33 ± 0.93

cis-carveol	1222.48	10.03	0.15 ± 0.07
carvone	1248.58	10.42	4.42 ± 1.50
trans-dihydrocarvone	1207.43	9.81	0.32 ± 0.10
trans-carvone oxide	1277.30	10.84	35.70 ± 14.85
cis-carvone oxide	1283.64	10.94	0.70 ± 0.32
limonene	1031.50	6.89	17.45 ± 6.00
limonene oxide	1116.07	8.35	0.22 ± 0.09
limonene oxide, cis	1140.32	8.74	8.97 ± 6.80
limonene oxide, trans	1147.06	8.85	16.72 ± 6.87
S-(-)-limonene	1327.05	11.55	0.35 ± 0.16
linalool	1097.99	8.06	0.05 ± 0.11
myrcene	990.73	6.35	3.34 ± 2.36
cis-beta-ocimene	1048.99	7.20	0.35 ± 0.13
cis-beta-terpineol	1068.62	7.54	0.03 ± 0.03
terpinolene	1093.05	7.97	6.94 ± 3.60
L-4-terpineol	1190.86	9.55	0.15 ± 0.07
unknown-1	1199.53	9.69	0.16 ± 0.07

198 ‡ Retention time (minutes) and Kovats retention indices for each floral compound; † Mean relative peak area and standard deviation of floral

199 compounds, calculated by dividing the corresponding floral compound peak area by the total peak area of all floral compounds per sample, and

200 averaging the samples. The sum of the peak areas of all identified compounds was 1.29×10^9 mV/sec.

201 **3.4. Flower and floral visitor morphology**

202 A total of 83 *Eulaema* (3 species), 690 *Euglossa* (20 species), and 32 *V. pompona* flowers were

203 measured. The trait "An" (8.40 ± 0.35 mm) and "Cn" (6.95 ± 0.36 mm) showed the best fit with

204 the height of the pollinator species *Eulaema cingulata* (7.73 ± 0.31 mm) (Figure 1c). In fact,

205 *Eulaema cingulata* fits exactly between these two traits. The *Euglossa* species (3.95 ± 0.58 mm)

206 were too small to fall into the An-Cn gap, whereas the other *Eulaema* species, *Eulaema*

207 *bombiformis* (9.39 ± 0.39 mm) and *Eulaema meriana* (8.98 ± 0.44 mm), were too large.
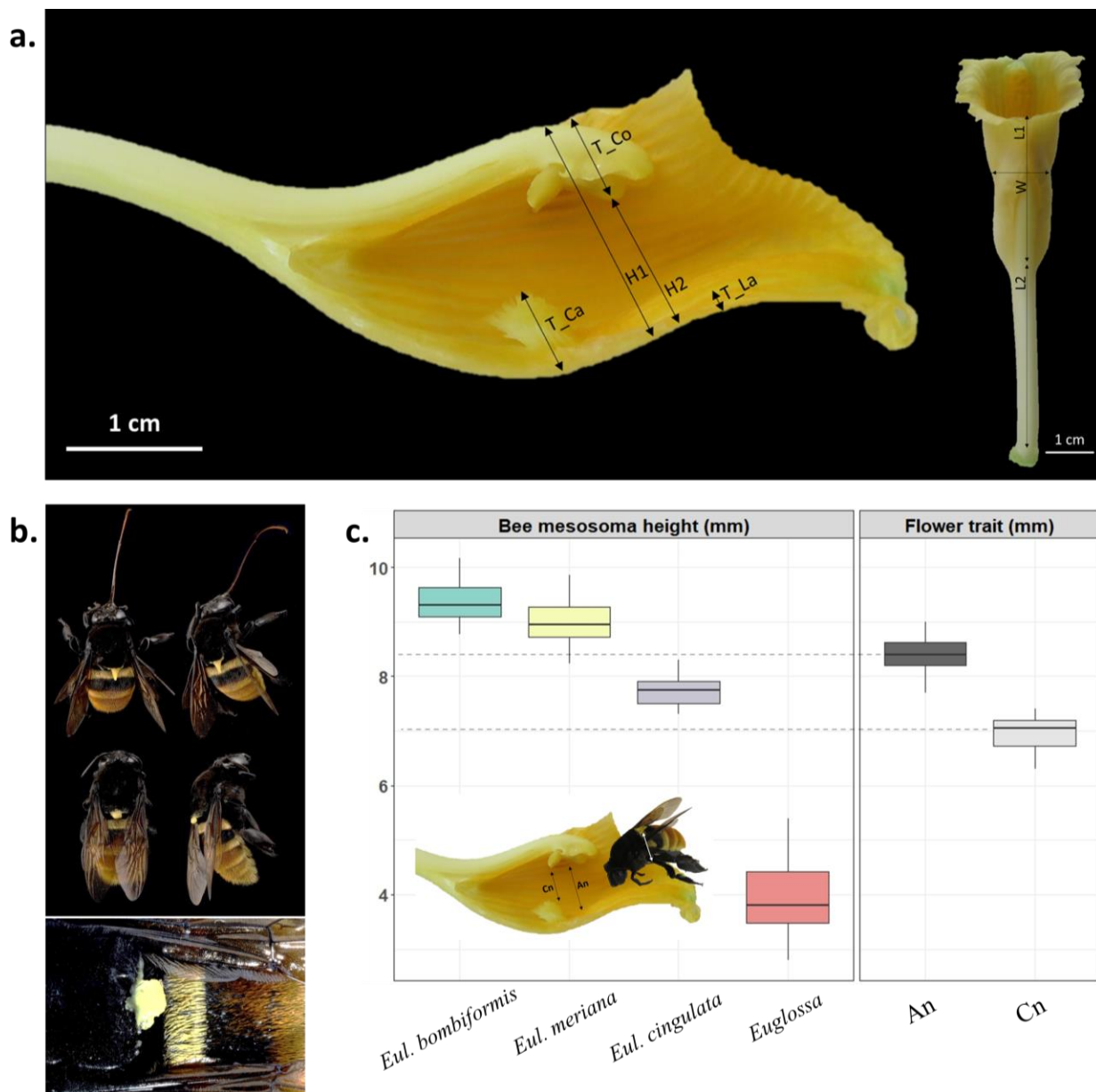
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Watteyn et al. 2021

Figure 1. (a) Measured *V. pompona* floral traits: height (H1), width (W) and length (L1) of the labellar tube, height of the labellar tube below the anther (H2), thickness of the column at the height of the anther (T_Co), height of the labellar callus (T_Ca), height of the labellar keels (T_La), and length of the pedicel (L2). **(b)** Caught male *Eulaema cingulata* bees with attached pollen of *V. pompona* and extended tongue (top), and attached *V. pompona* pollen in close-up (bottom). **(c)** Comparison of the mesosoma heights (mm) of the bee species *Eulaema bombiformis*, *Eulaema*

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215 *meriana*, *Eulaema cingulata* and *Euglossa* spp. with the flower morphological traits "An" (mm)

216 and "Cn" (mm) of *V. pompona*. Photographs by AK and CW (vouchers at Jardín Botánico

217 Lankester and Museo de Entomología Klaus Raven Büller).

218 **4. DISCUSSION**

219 *Vanilla pompona* flowers are visited by bees of the genera *Eulaema*, *Euglossa* and *Trigona*. As

220 confirmed by both the autogamy test and video footage, sexual reproduction is vector-mediated.

221 Only male *Eulaema cingulata* were observed removing pollen and caught with *V. pompona* pollen

222 masses. They are therefore suspected to act as effective pollinators in both Costa Rica and Peru.

223 Yet, it is not possible to exclude females nor other species as potential pollinators.

224 Bee behavioral patterns were assessed and compared. Towards the end of the morning, a decrease

225 in number of landing bees suggests that flowers become less attractive due to senescence close to

226 midday or after previous floral visits, as reported earlier for several plant species (van Doorn,

227 1997). *Trigona* landed almost exclusively on the labellum and exhibited a probing behavior

228 indicative of foraging (Armbruster, 1984; Slaa et al., 1998; Nieh, 2004; Lichtenberg et al., 2010;

229 McCabe & Farina, 2010; Grajales-Conesa et al., 2012). *Euglossa* and *Eulaema* were observed more

230 frequently on the tepals, repeatedly brushing with their foretarsi, then hovering around while

231 transferring compounds to their hind tibia, indicating fragrance collection (Dressler, 1968; Whitten

232 et al., 1989; Eltz et al., 2003). Some *Euglossa* and *Eulaema* did not collect fragrances but entered

233 directly into the labellum. These visits were brief, after which they quickly exited the flower

234 showing an extended tongue, suggesting nectar search. Only *Eulaema cingulata* successfully

235 removed the pollen after searching for nectar in the labellum.

236 **4.1. The dual pollinator attraction hypothesis**

237   The floral volatile compound composition was quantified for the first time in *V. pompona*. Trans-
238   carvone oxide, limonene and limonene oxide together represented 78.84% of the floral perfume.
239   Previous field bioassays have demonstrated that the compound trans-carvone oxide attracts
240   *Eulaema cingulata* and other *Eulaema* species, that collect and store it in their hind legs (Whitten
241   et al., 1986, Etlz et al., 2006; Milet-Pinheiro & Gerlach, 2017). Flowers of *Catasetum* species,
242   known to be pollinated through PFPS by different *Eulaema* species, also contain trans-carvone
243   oxide (Whitten et al., 1986; Gerlach & Schill, 1991; Milet-Pinheiro & Gerlach; 2017). Hence, this
244   compound most likely evolved in perfume-rewarding plant species as an attractant for *Eulaema*
245   pollinator species (Whitten et al., 1986; Brandt et al., 2019). The specific role of the other identified
246   compounds and their interaction with the *Eulaema* attractant trans-carvone oxide could be explored
247   further since studies have demonstrated that appropriate compound blends filter out certain bee
248   pollinator species (Williams & Dodson, 1972; Whitten et al., 1986).

249   For pollination to occur, bees need to actively crawl into the labellum. Curiously, none of the
250   observed male *Eulaema cingulata* exhibiting fragrance collection behavior on the tepals
251   subsequently moved into the labellum. We distinctly observed male *Eulaema cingulata* entering
252   the labellum and exiting the flowers with extended tongues, indicative of nectar search. The
253   identification of sugar traces suggests that *V. pompona* flowers deceive their pollinators, which is
254   conform with our observations of scarce and speedy visits in the labellum and a low natural fruit
255   set (2.42%) (Scopece et al., 2010; Scaccabarozzi et al., 2020). To tease apart whether the deception
256   is based on resembling a general flower image or a specific model species (Johnson and Schiestl,
257   2016; Caballero-Villalobos et al., 2017), advertising signals shared by *V. pompona* and co-
258   occurring *Eulaema cingulata*-pollinated species could be explored. Sugar traces most likely
259   encourage the bees to crawl inside the labellum, but there exists quite some debate whether to

260   decide if this is a food deceptive or semi-rewarding strategy. In case of a semi-rewarding strategy,

261   however, we would expect revisits since traces do not satisfy the bees' high energy demands. In

262   accordance with Shrestha et al. (2020), we suggest that sugar traces are deceitful, but the nature of

263   the deceit is more complex compared to complete nectar absence and definitely requires a more in-

264   depth comprehension.

265   Combining our behavioral and phytochemical data, we hypothesize a dual pollinator attraction in

266   *V. pompona* flowers, employing floral fragrance rewards as initial attraction and food deception to

267   induce pollen removal and deposition. The volatile compounds, especially trans-carvone oxide,

268   possibly provoke the primary long-distance attraction of male *Eulaema cingulata* to a flowering

269   population. *V. pompona* flowers only last for one day and start to wither after midday. This

270   restricted time lapse may have led to the development of floral fragrance rewards to boost pollinator

271   attraction. Yet, the attempt for nectar search is essential for pollen removal and seems to occur via

272   food deception as the short-distance attraction, taking place when these male bees, now present in

273   the surroundings of the flowering *V. pompona*, are foraging for food resources. Studies involving

274   flower manipulation experiments (i.e. removing fragrant compounds) or bee tagging could confirm

275   our proposed hypothesis and examine if this mechanism has been evolved across other Euglossini-

276   pollinated *Vanilla* species.

277   **4.2. Morphological goodness-of-fit for pollen removal by *Eulaema***

278   We assessed the "goodness-of-fit" between flower and bee to ensure pollen removal and showed

279   that the body height of the pollinator *Eulaema cingulata* perfectly fits in between the flower traits

280   "An" and "Cn". Given that vanilla flowers have a single aperture for entrance and exit, the callus

281   serves as a positioning structure, bringing the bee close to the rostellum. This results in the latter

282   being moved backwards, displacing the anther and releasing the pollen on the bee's scutellum. It

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283 explains why the height of the labellar tube at the callus is slightly smaller than the bee height and

284 demonstrates the key role of the labellar tube and the callus, both highly conserved features in

285 *Vanilla* sect. *Xanata*. Since the chemical baits did probably not attract all available *Eulaema* species

286 and the floral and bee morphological traits show intraspecific variation, more bee species could fall

287 within the "An-Cn gap". This is beneficial from a plant's perspective since dependence on a single

288 pollinator species might hazard a plant's population survival, especially when subjected to

289 environmental pressures (González-Varo et al., 2013; Traveset et al., 2017).

5. CONCLUSION

291 We hypothesize that *V. pompona* pollination by male *Eulaema cingulata* is based on floral

292 fragrance attraction towards a flowering population and food deception to induce pollen removal

293 and deposition. This proposed dual pollinator attraction mechanism can be explained by the need

294 to (a) attract bees from long distances to flowering populations, and (b) lure bees into the labellum

295 for successful pollination. Our study offers novel insights into plant-pollinator interactions within

296 the *Vanilla* genus and could be corroborated by further research on the attraction of both sexes to

297 *V. pompona* flowers, the presence of sugar traces as a semi-deceptive mechanism, the pollinator

298 species diversity across *V. pompona*'s distribution within the Neotropics, and the effects of

299 environmental changes on these interactions. Pollination studies are essential to define proper

300 conservation policies, thereby preserving the natural habitat of both vanilla and its pollinators and

301 assuring their long-term survival and agricultural application.

305 interpreted the collected data. CW, DS and APK wrote the first draft of the paper. All other authors

306 reviewed and approved the final paper. We would like to give a special thanks to José Pérez

307 Fuentes, Jessica Fonseca Prendas, Marvin Lopez Morales, Andrea Aromatisi, Manuel Huinga

308 Escalante, Luis Llerena and Osa Conservation for their help during the fieldwork, Mauricio

309 Fernández to determine the sex of the *Eulaema* specimens, the Ramirez Lab to help us with

310 analysing floral volatiles, the Insectopia lab and Clorinda Vergara Cobian for their help with bee

311 identification, and the environmental associations Arbio Peru, Simbio Onlus, Camino Verde.

FUNDING

CONFLICT OF INTEREST

318 The corresponding author confirms on behalf of all authors that there have been no involvements

319 that might raise the question of bias in the work or conclusions reported or in the conclusions,

320 implications, or opinions stated.

DATA AVAILABILITY STATEMENT

322 Upon acceptance, the data that supports the findings of this study will be made openly available in

323 the Dryad Digital Repository.

SUPPORTING INFORMATION

325 Additional Supporting Information may be found online in the Supporting Information section.

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SUPPORTING INFORMATION

Appendix A – Study area and study sites in Costa Rica and Peru

Figure S1. Map showing the study sites in (a) Osa Peninsula, Costa Rica (Miramar 8°26'20" N, 83°21'54" W, Piro 8°24'5" N, 83°20'36" W), and (b) Madre de Dios region, Peru (Baltimore 13°23'38" S, 69°44'59" W, Kapievi 13°1'17 S, 69°20'4" W, Belo Horizonte 12°28'38" S, 69°2'59" W).

Appendix B – Mixed logistic regression models for ordinal bee behavior classification

Table S1. Output of Bayesian multilevel behavioral comparison models to sequentially compare behavioral categories between floral visitor genera and time of the day. The intercept term is the genus *Euglossa* of the categorical variable bee genus. LOOIC = leave-one-out cross-validation, WAIC = Widely Applicable Information Criteria, *SE* = standard error, CI = credibility interval.

Table S2. Percentage of landing bees per genus displaying a defined reward approach type on different parts of *Vanilla pompona* flowers (T = tepals; LE = labellum edge; LT = labellum tube), whereby "probing" refers to touching the flower surface with the proboscis and antennae and / or brushing with foretarsi, and "nectar search" refers to entering straight into the labellum chamber, and showing an extended tongue when entering or departing the flower. *N* = total number of landing bees per genus. *SD* = standard deviation.

Table S3. Mean fruit set (%) of *Vanilla pompona* populations at the three study sites in Costa Rica, measured during the flowering period of 2019 and 2020. *SD* = standard deviation.

Figure S2. Response curve of the behavior landing, shown over time and per bee genus; demonstrating the chance over time of the day that a bee individual belonging to a certain bee genus will land on a *Vanilla pompona* flower.

532   **Figure S3.** Percentage of floral visitors per bee genus that (1) approaches, i.e. shows interest but

533   not necessarily lands, (2) lands, (3) probes, (4) enters into the labellum, (5) successfully removes

534   the pollen of *V. pompona* flowers.

535   **Appendix C – Floral rewards**

536   **Figure S4.** HPLC-chromatogram of a *V. pompona* sample extracted from the labellum via three

537   successive rinses (0.5 mL each) of de-ionized UHPLC gradient water. The green line represents a

538   standard mixture of three sugars (sucrose, glucose, fructose). The blue line shows the sugar content

539   of the sample. The limits of detection (LOD) was determined by the signal to noise (S/N) ratio

540   method and estimated as the minimum concentration providing a S/N ratio of 3:1.

541   **Figure S5.** Floral volatile compound composition of *Vanilla pompona* flowers, calculated by

542   averaging the relative frequency of each individual compound among the twelve samples.

543   **Supporting Videos**

544   **Video S1.** *Eulaema* and *Euglossa* demonstrating a probing behavior on *Vanilla pompona* flowers,

545   i.e. collecting fragrances from the tepals (sepals and lateral petals).

546   **Video S2.** *Eulaema cingulata* entering the labellum of a *Vanilla pompona* flower as an attempt for

547   nectar search, demonstrated by the extended tongue when entering and exiting the floral tube, and

548   successfully removing the pollen in case of a morphological fit between bee and flower.