

1 **Patterns of variation in distylous traits and reproductive consequences in *Erythroxylum***
2 **species and populations**

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12 **Variation in distylous traits of *Erythroxylum***

13

14 **PREMISE OF THE STUDY:** Distylous species possess two floral morphs with reciprocal
15 positioning of stigmas and anthers that is hypothesized to promote disassortative pollination.

16 Theoretical models predict equal morph frequencies, but many populations depart from the
17 expected 1:1 ratio, a pattern that often correlates with asymmetric mating between morphs
18 and/or presence of weak incompatibility system. Variation in reciprocity can also affect the
19 likelihood of disassortative pollination, and hence reproductive fitness.

20 **METHODS:** We described variation in incompatibility systems and morph ratio in four
21 *Erythroxylum* species to test if greater deviations from 1:1 ratios occur in populations of self-
22 compatible species. We used adaptive inaccuracy and described upper and lower organ
23 reciprocity in species and populations, and assessed the relationship of reciprocity to

24 population means and coefficients of variation for fruit set to test if reciprocity could predict
25 female reproductive success.

26 **KEY RESULTS:** Morphs occurred in 1:1 ratios in most populations of three *Erythroxylum*
27 species with distylous self-incompatibility. In self-compatible *E. campestre* populations
28 showed an excess of the long-styled morph, the short-styled morph, or were monomorphic for
29 the short-styled morph. We detected deviations from reciprocity, with total inaccuracy
30 ranging between 9.39% and 42.94%, and inaccuracy values were lowest in low organs.
31 Across populations, we found a positive relationship between inaccuracy and the coefficient
32 of variation of fruit set.

33 **CONCLUSIONS:** *Erythroxylum* species showed variation in the distylous syndrome, with
34 changes in the incompatibility system that corresponded with deviations from 1:1 morph
35 ratio, and variation in reciprocity that correlated with variation in female reproductive fitness.

36 **KEY WORDS:** breeding systems; Cerrado; disassortative mating; Erythroxylaceae;
37 heterostyly; isoplethy; monomorphism; morph bias; reciprocal herkogamy; sex
38 polymorphism.

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40 Heterostyly is a genetically controlled sex polymorphism described in 28 angiosperm
41 families, where plant populations possess two (distyly) or less commonly three (tristyly) floral
42 morphs (Barrett and Shore, 2008). In distylous species, the long-styled morph (L-morph)
43 presents the stigma above the anthers, while the stigma of the short-styled morph (S-morph)
44 appears below the anthers. Thus, floral morphs differ from one another in the positions in
45 which anthers and stigmas are reciprocally presented, an arrangement referred to as reciprocal
46 herkogamy (Barrett, 2002). Sex-organ reciprocity (L stigma-S anther and S stigma-L anther)
47 functions to promote cross-pollination between morphs (disassortative pollination) because

48 pollinators are likely to pick up pollen from the morphs on different parts of their bodies and
49 transfer pollen to stigmas at those same heights on flowers of the opposite morph (Darwin,
50 1877; Lloyd and Webb 1992a, b; Barrett, 2002). Together with reciprocal herkogamy,
51 heterostylous species commonly possess a heteromorphic self-incompatibility system
52 preventing self-fertilization and intra-morph cross-fertilization (Barrett and Richards, 1990),
53 and morph-specific ancillary characters (Ganders, 1979b; Dulberger, 1992). Hence, the
54 combination of herkogamy with reciprocally positioned high and low sexual organs should
55 reduce self-interference and pollen wastage on incompatible stigmas, and increase male
56 fitness (Barrett, 2002). At equilibrium, populations of distylous species are expected to show
57 a 1:1 ratio of style morphs (Pannell et al., 2005). Populations of many distylous species,
58 however, show L- or S-biased morph ratios or populations fixed for a morph. These
59 conditions are associated with the breakdown of the distylous incompatibility system and with
60 random stochastic events (Eckert and Barrett, 1992; Zhou et al., 2012, 2017). In addition, the
61 presence of intra-morph compatibility and changes in the rates of disassortative pollen
62 transfer can result in deviations of the morph ratio (Barrett and Hodgins, 2006; Brys et al.,
63 2008; Hodgins and Barrett, 2008; Pérez-Barrales and Arroyo, 2010; Consolaro et al., 2011;
64 Zhou et al., 2017).

65 An investigation of variability in the position of anthers and stigmas is an initial step
66 to interpret the ecological function and selection on the morphological features of the
67 distylous floral syndrome (Charlesworth and Charlesworth, 1979; Lloyd and Webb 1992a, b).
68 While reciprocity is based on the notion that reciprocal organs are placed at the same height,
69 heterostylous species display variation in high and low organ reciprocity, with anthers and
70 stigmas often deviating from the position of their “compatible” reciprocal organ (Pailler and
71 Thompson, 1997; Thompson and Dommée, 2000; Faivre and McDade, 2001; Pérez et al.,
72 2004; Ferrero et al., 2009, 2011a, b; Keller et al., 2012; Sá et al., 2016; Armbruster et al.,

2017). Deviations from perfect reciprocity (defined as exact similar position of reciprocal high and low reproductive organs) can lower the probability of disassortative pollination, reduce fruit and seed production, or even facilitate a breakdown of the floral polymorphism (Keller et al., 2014; Zhou et al., 2015; Jacquemyn et al., 2018; Wu et al., 2018; Brys and Jacquemyn, 2019; but see Simón-Porcar et al., 2015). Under the assumption that the function of heterostyly relies on the close matching between reciprocal organs (Darwin, 1877; Jacquemyn et al., 2018; Brys and Jacquemyn, 2019), a morphological analysis of reciprocity can help elucidate whether heterostylous species and populations are likely to have stable reproductive systems. Armbruster et al. (2017) developed a metric to measure reciprocity based on adaptive inaccuracy, with the aim of associating variation in reciprocity with reproductive fitness. For distylous species, inaccuracy is based on the concept that the adaptive optimum of an anther level is represented by the population mean of the reciprocal stigma and vice versa, so that if all anthers and stigmas of a population are at the same height, inaccuracy will be zero. Hence, inaccuracy is defined by the distance between the average organ height with regards the population mean of its reciprocal organ, and the population variance of each organ (Armbruster et al., 2009, 2017). Long and S individuals with stigma heights similar to their compatible anther should experience higher disassortative pollen transfer and hence higher seed or fruit production (Armbruster et al., 2017; Jacquemyn et al., 2018; Brys and Jacquemyn, 2019). Hence, populations of heterostylous species with lower inaccuracy in reciprocity should have higher female reproductive success, as well as low variability in female reproductive success. Therefore, a negative relationship between inaccuracy and female fitness is expected, and a positive relationship between inaccuracy and female fitness variation is predicted. To our knowledge, population level associations between reproductive success and inaccuracy remain to be tested.

97 Erythroxyloaceae is a heterostylous family with four tropical genera and ca. 260 species
98 of small trees and shrubs (White et al., 2019). The genus *Erythroxyllum* P. Browne is the
99 largest of the family (ca. 250 species; White et al., 2019) and endemic to the neotropical
100 region. The main center of diversity is Brazil, with 114 species of which 74 are endemic
101 (Plowman and Hensold, 2004; Loiola et al., 2007), representing a monophyletic group within
102 the genus (White et al., 2019). Although knowledge of the reproductive biology of species is
103 scarce, *Erythroxyllum* species show variation in breeding system, with heterostylous species
104 displaying variation in the strength of heteromorphic incompatibility system and population
105 morph ratio (Burck, 1895; Arroyo and Cabrera, 1978; Ganders, 1979a; Domínguez, 1990;
106 Berry et al., 1991; Domínguez et al., 1997; Barros, 1998; Pailler et al., 1998; Bianchi et al.,
107 2000). A feature of *Erythroxyllum* flowers that could affect reciprocity of anthers and stigmas
108 is the presence of two stamen whorls, which may or may not show similar length within the
109 flower.

110 In this study we investigated four species of *Erythroxyllum* in Brazil to (i) describe the
111 incompatibility system of each species; (ii) describe variation in morph ratio among species
112 and populations; (iii) quantify the variation in the position of anthers and stigmas to
113 investigate inaccuracy of high and low reciprocal organs; and (iv) study the natural fruit set of
114 the morphs in different populations. These data were used to test the following predictions:
115 first, because previous research on *Erythroxyllum* species indicated some degree of self-
116 compatibility (Arroyo and Cabrera, 1978; Ganders, 1979a; Berry et al., 1991; Barros, 1998),
117 we predicted greater deviations from 1:1 morph ratio in self- and intra-morph compatible
118 species than in species with heteromorphic incompatibility system. Second, because
119 deviations from perfect reciprocity appear associated with lower disassortative pollen
120 deposition, which in turn should reduce fruit production (Jacquemyn et al., 2018; Brys and
121 Jacquemyn, 2019), we predicted a negative relationship between inaccuracy and average fruit

122 set at the population level. Also, we predicted a positive relationship between inaccuracy and
123 the coefficient of variation of fruit set as increases in inaccuracy would be associated with
124 higher uncertainty in disassortative pollen deposition.

125 **MATERIAL AND METHODS**

126 **Species and study areas**

127 The study species, *E. campestre* A.St.-Hil., *E. deciduum* A.St.-Hil., *E. suberosum* A.St.-Hil.
128 and *E. tortuosum* Mart., are found in the Brazilian Cerrado. In these species flowers are small
129 (ranging from 2,8 mm length in *E. campestre* to 4,5 mm in *E. suberosum*) with creamy white
130 petals (Fig. 1), and visited by various species of bees and wasps, or less frequently, by
131 butterflies and flies (Barros, 1998; R. Matias, University of Brasília, personal observation). At
132 the base, petals possess a nectariferous ligule appendage forming a small tube. Flowers have
133 ten stamens with filaments fused at the base, forming a short staminal tube around the
134 superior ovary. Stamens are organized in two whorls with five stamens each, placed opposite
135 to the sepals and petals. In the study species, the two stamen whorls in L flowers appear at
136 different heights but occur at similar heights in S flowers (Fig. 2; other *Erythroxylum* species
137 show variation in the two stamen whorls of L and S flowers; Pailler et al., 1998). Flowers of
138 both morphs are tricarpellate with only one fertile locule containing a single fertile ovule
139 (Loiola et al., 2007; Silva et al., 2016). In the present study, eleven Cerrado areas from
140 Central Brazil were selected for the population survey. Details on region, area name and
141 coordinates are included in Appendix S1 (see the Supplementary Data with this article). In
142 total, 26 populations were surveyed. Species and population surveys were completed during
143 the main flowering season between August and November of 2015 and 2016.

144 **Incompatibility system**

145 Using hand-pollinations, the incompatibility system was assessed in one population per
146 species in 2016 (populations 3, 10, 16 and 24; located in the same area). Between 16-19
147 flowering individuals per species were tagged, all of which were exposed to the following
148 treatments: (1) facilitated self-pollination using pollen from the same flower, (2) intra-morph
149 and (3) inter-morph cross-pollination by applying pollen from different plants of the same and
150 different morph respectively, (4) autonomous self-pollination by bagging unopened flowers,
151 (5) agamospermy by emasculating flowers and bagging them to prevent insect visitation, and
152 (6) control treatment as open pollination under natural pollination conditions. To describe the
153 incompatibility system a total of 2-7 flowers were used per treatment and on each individual
154 such that all plants used in the experiment received all treatments. Flowers were bagged
155 before and after the manipulations with small tulle bags to prevent uncontrolled pollen arrival,
156 except for the control treatment. Fruit development was assessed after two weeks.

157 *Erythroxylum* flowers produce a single seed so that fruit set corresponds to seed set. Data
158 were analysed using generalized linear mixed-effects models (hereafter GLMMs) to
159 investigate the effect of treatment, morph and interaction term on fruit set (number of fruits
160 formed/number of flowers used in each treatment per individual). Individual plants were
161 included as a random factor. Fruit production was modelled with a binomial distribution using
162 the probit function. The analyses were conducted using the `glmer` function in the `lme4`
163 package in R (Bates et al., 2015). To obtain the significance of each factor, a type-II analysis
164 of variance was conducted using the `Anova` function in the `car` package (Fox, 2015). The
165 `lsmeans` function with a Tukey adjustment from the package `emmeans` (Lenth, 2016) was
166 used as a post hoc test to detect significant differences between treatments. All statistical
167 analyses in this study were performed in the R statistical environment (R Core Team, 2019).

168 **Population morph ratios**

169 Population morph ratios were assessed during surveys conducted in 2015. In each area, all
170 plants intercepted along a 1000-2000m. transect were classified as L- or S-morph. Because
171 the areas investigated included at least two *Erythroxylum* species (see Appendix S1),
172 flowering individuals were verified to identify the species and floral morph. *Erythroxylum*
173 individuals do not appear to propagate clonally and the sampling of genets was unambiguous.
174 In all populations, chi-square (χ^2) analysis with Yates correction was used to assess whether
175 population morph ratios departed from isoplethy (1:1), using $\alpha = 0.05$.

176 **Floral morphology and reciprocal herkogamy**

177 During the survey of population morph ratio, flowers were collected (n = 1-3 flowers per
178 individual plant; 18-49 individuals per population) and fixed in 70% ethanol for
179 measurements. Flowers were dissected, photographed and traits measured using ImageJ 1.45s
180 software (Rasband, 2011; <http://imagej.nih.gov/ij/>). The traits measured included stigma and
181 anther height, using as a reference point the top of the ovary (Fig. 2). Anther height of each
182 anther level was measured in the L-morph (Fig. 2). Differences between morphs and
183 populations in stigma and anther height per species were analysed with linear mixed-effects
184 models (hereafter LMMs) using the lmer function in the lme4 package (Bates et al., 2015).
185 Morph, population and interaction term were included in the models as fixed factors, and
186 individual plants as a random factor. Significance of each factor was tested with type-II
187 analysis of variance as described above. Residual analysis was used in order to ensure model
188 assumptions were met (homoscedasticity and normal distribution). Because the aim of the
189 analysis consists of detecting differences between morphs, we excluded monomorphic and
190 highly anisoplethic populations.

191 Population mean and variance in anther and stigma height were used to investigate
192 adaptive inaccuracy in reciprocity as described by Armbruster et al. (2017). In heterostylous
193 species, the phenotypic optimum of L stigmas corresponds to the population mean of S anther

194 height, and the phenotypic optimum of S anthers corresponds to the population mean of L
195 stigma height. Similarly, the phenotypic optimum of S stigmas corresponds to the population
196 mean of L anther height, and the phenotypic optimum of L anthers corresponds to the
197 population mean of S stigma height (Darwin, 1877; Barrett 2002; Armbruster et al., 2017).
198 Inaccuracy in reciprocity is then estimated as the contribution of bias (differences in mean
199 height of reciprocal organs) and imprecision (variance in organ height) to departures from
200 close matching between reciprocal organs (Armbruster et al., 2009, 2017). We used the
201 population mean and variance of each organ type to calculate inaccuracy values for high (L
202 stigmas [S] and S anthers [A]; Eqn 1) and low organs (L anthers [a] and S stigmas [s]; Eqn 2).
203 Because L flowers have two stamen whorls of different lengths (Fig. 2), the low organ level
204 inaccuracy was estimated in two ways. The height measures of the two anther levels (aA and
205 aa for upper and lower anther level, respectively) were analyzed together in the same equation
206 (Eqn 2) by using the population mean and variance of the L anthers without distinction of
207 anther position. In addition, the contribution of each anther level to inaccuracy in reciprocity
208 was estimated by using upper (Eqn 3; aA) and lower anthers (Eqn 4; aa) separately. We
209 generated these two inaccuracy estimates because it is unknown whether one anther level
210 contributes more to the pollination of S stigmas, or whether both contribute equally. Hence,
211 the comparison of inaccuracy values obtained without distinction between the upper and
212 lower anther (a), and those estimated with the upper (aA) and lower (aa) L anther can be
213 helpful to infer the potential role of the two anther levels in disassortative pollen transfer
214 (Thompson et al., 2012). In the equations presented below, letters with bars correspond to the
215 population mean of organs whereas V corresponds to the variance of the organs (Armbruster
216 et al., 2017).

$$217 \quad \text{Inaccuracy}_{\text{high organs}} = (\bar{A} - \bar{S})^2 + V_A + V_S \quad \text{Eqn 1}$$

$$218 \quad \text{Inaccuracy}_{\text{low organs with both L anthers}} = (\bar{a} - \bar{s})^2 + V_a + V_s \quad \text{Eqn 2}$$

219
$$\text{Inaccuracy}_{\text{low organs with upper L anthers}} = (\overline{aA} - \bar{s})^2 + V_{aA} + V_s \quad \text{Eqn 3}$$

220
$$\text{Inaccuracy}_{\text{low organs with lower L anthers}} = (\overline{aa} - \bar{s})^2 + V_{aa} + V_s \quad \text{Eqn 4}$$

221 The unit of inaccuracy is trait units squared, and the results of high (Eqn 1) and low
222 organ inaccuracies (Eqn 2) were summed to provide a total inaccuracy value per population.
223 Equations 3 and 4 represent inaccuracy estimates for low organs using the upper (aA) and
224 lower (aa) anther of L flowers. To compare levels of inaccuracy between populations and
225 species, values were standardized by the squared mean of all anther and stigma heights
226 recorded for each population and adjusted to a proportional scale (Armbruster et al., 2017).
227 Low values of inaccuracy correspond to high levels of reciprocity, e.g., close matching
228 between reciprocal organs with low dispersal around the optimal values.

229 **Population variation of natural fruit set and relationship with inaccuracy**

230 Natural fruit production was studied in 12 of the 26 populations (2-4 populations per species)
231 in 2015. Ten to 27 individual plants were randomly selected in each population, and 4-102
232 flowers on each plant were tagged and left exposed to pollinators. Fruit development was
233 inspected 3-4 weeks later. Differences among populations in fruit set (number of fruits
234 formed/number of flowers marked) were analysed with a GLMM using the glmer function
235 with a binomial distribution and probit function. This analysis included the terms population,
236 morph and the interaction effect; individual plant was included as random factor. Significance
237 of each factor was tested with type-II analysis of variance; when a factor was statistically
238 significant, post hoc tests were conducted as described above. Sequential Bonferroni
239 correction was used to account for multiple tests.

240 Using the standardized inaccuracy values, LMMs were used to evaluate the
241 relationship between high (Eqn1) and low (Eqn 2) organ inaccuracy with female reproductive
242 success. Three populations of the self-compatible species were excluded from the analyses

243 because fruit set can result from either self- or cross-pollination (see results below).
244 Specifically, two analyses were conducted using inaccuracy values as the independent
245 variable and the mean and the coefficient of variation of fruit set for each population and
246 morph as dependent variables. In these analyses, species and population nested within species
247 were included as random effects in the models. To obtain the statistical significance of the
248 models, we performed a type-II analysis of variance. Because the sample size was small (only
249 nine populations and a total sample size of 18, including two morphs per population), the term
250 morph was not included in the analyses (a model incorporating morph showed that this term
251 was not statistically significant in the analyses, results not shown).

252 **RESULTS**

253 **Incompatibility system**

254 In *E. campestre* autonomous self-pollination and all hand pollination treatments rendered
255 similar fruit set ($P > 0.875$ for all comparisons), while agamospermy and open pollination
256 treatments produced lower fruit set ($P < 0.006$ for all comparisons; Table 1 and Fig. 3). In the
257 rest of species, fruit set after the inter-morph cross-pollination treatment was much higher
258 than all the other treatments ($P < 0.006$ for all comparisons; Table 1 and Fig. 3), with the
259 exception of the open pollination treatment in *E. deciduum*, suggesting absence of pollen
260 limitation for this species. In all cases, there were no statistically significant differences
261 between morphs (Table 1). Taken together, these results indicate that *E. campestre* is a self-
262 compatible species, whereas *E. deciduum*, *E. suberosum* and *E. tortuosum* present the typical
263 heteromorphic self-incompatibility of distylous species.

264 **Population morph ratios**

265 Of the eight populations of *E. campestre*, three were isoplethic (had equal numbers of L and S
266 plants) and five showed variation in the morph ratio, with one population fixed for the S-

267 morph, two populations biased for the L-morph and two populations biased for the S-morph
268 (Table 2; Fig. 4). Populations of the remaining species displayed 1:1 morph ratio, except for
269 one L-biased population of *E. suberosum* and one L-biased population of *E. tortuosum* (Table
270 2; Fig. 4).

271 **Floral morphology and reciprocal herkogamy**

272 All species showed statistically significant differences in the position of anthers and stigmas
273 between morphs (Tables 3, 4), and all species other than *E. deciduum* showed differences
274 between populations. For stigma height, the interaction term morph * population was
275 statistically significant for all species except for *E. deciduum* (Table 4). The interaction term
276 for the upper anther of the L-morph was statistically significant and marginally significant for
277 *E. campestre* and *E. deciduum* respectively, while the interaction effect using the lower L
278 anther was significant in all species (Table 4). These results suggest that populations of
279 species harbor substantial variation in anther and stigma height.

280 Values of standardized total inaccuracy calculated without distinction between the L
281 anthers whorls ranged between 9.39% and 42.94% (Table 5). *Erythroxyllum suberosum* and *E.*
282 *tortuosum* displayed the lowest inaccuracy values per species, ranging between 9.39% and
283 20.01% in *E. suberosum* and between 13.93% and 21.64% in *E. tortuosum* (Table 5).
284 Inaccuracy values in *E. deciduum* were the largest of all species, ranging between 38.07% and
285 42.94%. *Erythroxyllum campestre* showed substantial variation in inaccuracy (Table 5), with
286 populations showing low values (e.g., 13.48% in population 8) and high values (e.g., 41.16%
287 in population 5), covering the range displayed by all species. Low organ inaccuracy estimated
288 with the upper and lower L anther considered separately revealed different patterns. In *E.*
289 *campestre*, *E. suberosum* and *E. tortuosum* inaccuracy was smaller when S stigmas were
290 analyzed against the upper anthers of L flower (s-aA), with values ranging from 0.70% to
291 9.08%. For these species, inaccuracy values estimated with the upper anther level ranged

292 between 4.01% and 22.51% (Table 5). In contrast, *E. deciduum* showed the opposite pattern,
293 with larger inaccuracy values for the upper anthers ranging between 53.28% and 71.52%, and
294 between 1.59% and 3.54% for the lower anthers. For *E. campestre*, *E. suberosum* and *E.*
295 *tortuosum* high organs displayed larger inaccuracy values than low organs, whereas low organ
296 inaccuracy in *E. deciduum* was larger than the high value. However, this appeared to result
297 from the large contribution to inaccuracy of the upper L anther (Table 5).

298 **Population variation of natural fruit set and relationship with inaccuracy**

299 Populations of *E. campestre* (the only self-compatible species) and *E. tortuosum* showed
300 similar patterns of fruit set, with no differences between morphs, populations or the
301 interaction effect (Table 1; Fig. 5). *Erythroxyllum deciduum* displayed substantial variation in
302 fruit set among populations and for the morph * population term, with similar fruit set
303 between morphs in populations 9 and 12, and larger fruit set for the L-morph in population 10
304 (Table 1; Fig. 5). Fruit set in *E. suberosum* differed among populations, but no differences
305 were detected between morphs (the interaction term was not statistically significant; Table 1;
306 Fig. 5).

307 The relationship between mean fruit set of L and S plants and organ inaccuracy (using
308 the low organ inaccuracy value without distinction between the upper and lower L anthers)
309 was not significant ($\chi^2 = 0.253$; $df = 1$; $P = 0.615$). In contrast, there was a positive and
310 significant relationship between the coefficient of variation of fruit set and measures of
311 inaccuracy ($\chi^2 = 7.961$; $df = 1$; $P = 0.005$; Fig. 6), suggesting that greater inaccuracy
312 reciprocity values correlate with greater variation in fruit set.

313 **DISCUSSION**

314 In the present study, we described the incompatibility system, variation in morph ratio and
315 upper and lower organ reciprocity in four distylous species of *Erythroxyllum*, and used those

316 data to investigate associations between inaccuracy in sex-organ reciprocity and the
317 population mean and coefficient of variation in fruit set among populations. We predicted a
318 negative relationship between inaccuracy and fruit production, because low inaccuracy values
319 correspond to high reciprocity, which in turn should increase disassortative pollen transfer
320 and female fitness (Jacquemyn et al., 2018; Brys and Jacquemyn, 2019). While we did not
321 detect a negative relationship of inaccuracy with mean fitness as measured by fruit
322 production, we found a positive relationship between inaccuracy in reciprocity and the
323 coefficient of variation of fruit set. Although this result was based on a limited number of
324 populations, it suggests that the morphological variation of anthers and stigmas, and the
325 magnitude of overlap between reciprocal organs affects the function of distyly in
326 *Erythroxyllum*.

327 We found that in most of the populations of the species with the typical heteromorphic
328 incompatibility system, namely *E. deciduum*, *E. suberosum* and *E. tortuosum*, L and S plants
329 occurred at equal frequency. In contrast, populations of *E. campestre* displayed variation in
330 morph ratio, including isoplethic, L-biased, S-biased and S-monomorphic populations. Unlike
331 other *Erythroxyllum* species (Ganders, 1979a; Domínguez, 1990; Pailler et al., 1998; Bianchi
332 et al., 2000; Silva et al., 2007), *E. campestre* is self-compatible and all crosses formed seeds.
333 The 1:1 morph ratio in populations of distylous species is often associated with the presence
334 of a heteromorphic incompatibility system (Pailler and Thompson, 1997; Pailler et al., 1998;
335 Sá et al., 2016). In self-compatible species or intra-morph compatible species, promotion of
336 legitimate pollination and disassortative mating is also an important mechanism for the
337 maintenance of the polymorphism, and changes in mating patterns can drive changes in
338 morph ratio, and the fixation of a morph (Barrett and Hodgins, 2006; Pérez-Barrales and
339 Arroyo, 2010, Simón-Porcar et al., 2014; Zhou et al., 2015; Ferrero et al., 2017).
340 Unfortunately, we were unable to obtain pollinator observations or estimate pollen transfer

341 rates to understand if departures of the 1:1 ratio correlated with rates of legitimate pollination
342 and the function of distyly (but see discussion below), particularly in the self-compatible
343 *Erythroxyllum* species. Variation in morph ratio could also be explained in the context of
344 stochastic population events, such as habitat fragmentation, reduction of population size or
345 founder events, which are often accompanied by changes in the incompatibility system and
346 selection of autonomous self-pollination (Eckert and Barrett, 1992; Brys et al., 2008; Costa et
347 al., 2016; Zhou et al., 2012, 2017). However, our population data per species is limited and
348 larger population surveys would be necessary to model the relationship between random
349 population events, breakdown of the incompatibility system and the loss of the polymorphism
350 in *Erythroxyllum*.

351 We used adaptive inaccuracy to quantify reciprocity. Adaptive inaccuracy uses the
352 mean and the variance to interpret the adaptive significance of the position of anthers and
353 stigmas in relation to pollen pick-up and delivery (Armbruster et al., 2009, 2017), which is
354 critical for the function of heterostyly (Armbruster et al., 2006). In our analysis, when
355 averaged across multiple populations, the values at species level were higher than those
356 obtained in species of *Primula* L. (Primulaceae; Armbruster et al., 2017) and *Pulmonaria* L.
357 (Boraginaceae; Jacquemyn et al., 2018), suggesting that reciprocity in distylous *Erythroxyllum*
358 species is lower and less accurate. Our results showed that high organs had larger inaccuracy
359 values than low organs. In *Primula* and *Pulmonaria*, high organs contributed more strongly to
360 inaccuracy, a pattern attributed to developmental variation, which is often greater in large
361 organs (Armbruster et al., 2017; Jacquemyn et al., 2018; Brys and Jacquemyn, 2019). In
362 flowers with long tubes and epipetalous stamens, like those in *Primula* and *Pulmonaria*,
363 anther height is usually influenced by floral development and the correlation between the
364 stamens and the floral tube, which might then affect the placement of anthers and stigmas,
365 and therefore reciprocity, with larger effects for high organs if S anthers express more

366 variability in their height (Faivre, 2000; Thompson and Dommée, 2000; Faivre and McDade,
367 2001; Pérez-Barrales et al., 2014; Santos-Gally et al., 2015). *Erythroxyllum* species present
368 open and relatively small corollas. Filaments are relatively free and only fused at the base of
369 the ovary and not to the corolla. While our results agreed with an apparent tendency of larger
370 inaccuracy values in high organs, as detected in both *Primula* and *Pulmonaria*, the
371 morphology of *Erythroxyllum* flowers probably imposes low developmental restrictions, and
372 allows expression of more variability in organ height position compared to epipetalous
373 flowers. This might explain the relatively larger inaccuracy values compared to those in
374 *Primula* and *Pulmonaria* (Armbruster et al., 2017; Jacquemyn et al., 2018; Brys and
375 Jacquemyn, 2019). Future morphological studies of floral development will be useful to gain
376 insights into the importance of floral development in the expression of reciprocity (Faivre,
377 2000; Faivre and McDade, 2001).

378 In our study, inaccuracy values of low organs were influenced by the presence of two
379 anther whorls in the L-morph. Of all species, *E. deciduum* showed the greatest differences
380 between the two inaccuracy values for low organs (s-aA and s-aa), and consequently great
381 imprecision between S stigmas and the upper anthers of L flower (s-aA). We believe that high
382 inaccuracy values can decrease the amount of disassortative pollination, but pollination
383 studies are necessary to test this hypothesis in *Erythroxyllum* species. In *Narcissus assoanus*
384 Dufour ex Schult. f., a style dimorphic species with intra-morph compatibility and with two
385 anther whorls in L and S flowers, female fertility of S flowers increased when pollen donors
386 were the low L anthers rather than the low S anthers, a fertility pattern explained by the closer
387 match of S stigmas with the low L anthers than the low anther level of S flowers (Thompson
388 et al., 2012).

389 The data presented here add additional evidence that heterostylous species often depart
390 from theoretical expectations of high reciprocity (Pailler and Thompson, 1997; Thompson and

391 Dommée, 2000; Faivre and McDade, 2001; Keller et al., 2012; Armbruster et al., 2017;
392 Jacquemyn et al., 2018). The ultimate goal of understanding the evolutionary significance of
393 heterostyly is to establish a relationship between variation in reciprocity, disassortative pollen
394 transfer and reproductive success. We found a positive and significant relationship between
395 inaccuracy and the coefficient of variation in fruit set. These findings agree with recent
396 experiments evaluating how inaccuracy in reciprocity influences disassortative pollination in
397 distylous *Pulmonaria* (Jacquemyn et al., 2018) and *Primula veris* L. (Brys and Jacquemyn,
398 2019). In these studies, disassortative pollen transfer increased in L and S flowers with greater
399 reciprocity (e.g. low inaccuracy). Consistent with pollen deposition patterns, seed set in *P.*
400 *veris* declined with increasing inaccuracy, indicating a strong link between disassortative
401 pollen deposition and seed set (Brys and Jacquemyn, 2019). It is therefore possible that larger
402 inaccuracy in *Erythroxylum* populations decreased legitimate pollen deposition, making
403 disassortative mating unpredictable, which in turn would result in greater fruit set variation.
404 This result shows that variation in reciprocity could potentially have far-reaching effects on
405 patterns of fruit production, and may even initiate or facilitate the breakdown of this floral
406 polymorphism, especially if these changes are accompanied by modifications in the
407 heteromorphic incompatibility system (Barrett and Shore, 2008).

408 We did not detect a relationship between inaccuracy and mean fruit set. *Erythroxylum*
409 species produce only one seed per flower, and in the presence of a heteromorphic
410 incompatibility system, a small amount of compatible pollen may be sufficient to fertilize a
411 single ovule. This contrasts with species with a larger ovule number, like *Primula veris*,
412 where disassortative pollen transfer correlated with seed set (Brys and Jacquemyn, 2019).
413 Alternatively, variation in fruit set could be explained in the context of pollen limitation
414 (Knight et al., 2005). For example, in the experiment to describe the incompatibility system,
415 fruit set after open pollination was smaller than the legitimate hand-pollination pollination

416 treatment (Fig. 3). The low fruit set could be explained under low visitation rate or inefficient
417 pollination service. Fruit production under natural open pollination was substantially different
418 among populations, which probably reflects geographic variation in the pollinator service.
419 Future research in *Erythroxylum* should incorporate pollinator observations to understand how
420 pollinator interactions contribute to disassortative pollen transfer. Ultimately, data on
421 pollinator behavior and efficiency can help to establish a clear relationship between
422 inaccuracy, pollen flow dynamics and the function of distyly in *Erythroxylum*.

423 In this study, we described the variation of the distylous syndrome in *Erythroxylum*
424 species and populations, and how this variation is partitioned between morphs. We conclude
425 that the most important features of typical distyly, namely the morphological syndrome and
426 heteromorphic incompatibility system, seem to be conserved in *E. deciduum*, *E. suberosum*
427 and *E. tortuosum*. The morphological syndrome is present in *E. campestre*, but this species is
428 self-compatible and populations displayed great variation in morph ratio, including S-
429 monomorphic populations. Variation in morph ratio and the presence of self-compatibility
430 may indicate failure in the function of the polymorphism. Monomorphism might be a derived
431 condition, as reported in other distylous species and populations (Mast et al., 2006; Zhou et
432 al., 2012; Ruiz-Martín et al., 2018). As different clades of *Erythroxylum* generally occur in
433 distinct geographic areas (White et al., 2019), the presence of monomorphism in species that
434 occur in the Brazilian Cerrado and Northern Venezuela (*E. undulatum*; Berry et al., 1991)
435 possibly supports the hypothesis of multiple independent origins of monomorphism from
436 distylous ancestors. Taken together, our findings suggest that *Erythroxylum* is a promising
437 genus to further investigate the selection, function and evolution of heterostyly.

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447 **AUTHOR CONTRIBUTIONS**

448 All authors have made substantial contributions to conception, design and interpretation of
449 data. R.M. collected and analyzed the data. R.M. and R.P.B. led the writing. H.C. helped with
450 discussion and text revision.

451 **DATA AVAILABILITY STATEMENT**

452 All data needed to evaluate the conclusions in the paper are present in the paper and/or the
453 Supplementary Materials. Additional data related to this paper may be requested from the
454 authors.

455 **LITERATURE CITED**

- 456 Armbruster, W.S., R. Pérez-Barrales, J. Arroyo, M.E. Edwards, and P. Vargas. 2006. Three-
457 dimensional reciprocity of floral morphs in wild flax (*Linum suffruticosum*): a new twist on
458 heterostyly. *New Phytologist* 171: 581–590.
- 459 Armbruster, W.S., T.F. Hansen, C. Pélabon, R. Pérez-Barrales, and J. Maad. 2009. The
460 adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of*
461 *Botany* 103: 1529–1545.
- 462 Armbruster, W.S., G.H. Bolstad, T.F. Hansen, B. Keller, E. Conti, and C. Pélabon. 2017. The
463 measure and mismeasure of reciprocity in heterostylous flowers. *New Phytologist*
464 215: 906–917.

- 465 Arroyo, M.T.K., and M. Cabrera. 1978. Preliminary self-incompatibility tests for some
466 tropical cloud forest species in Venezuela. *Incompatibility Newsletter* 8: 72–76.
- 467 Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3:
468 274–284.
- 469 Barrett, S.C.H., and H.J. Richards. 1990. Heterostyly in tropical plants. *Memoirs of the New*
470 *York Botanical Garden* 55: 35–61.
- 471 Barrett, S.C.H., and K.A. Hodgins. 2006. Floral design and the evolution of asymmetrical
472 mating. In L.D. Harder, and S.C.H. Barrett [eds.], *Ecology and evolution of flowers*, 239–
473 255. Oxford University Press, Oxford, UK.
- 474 Barrett, S.C.H., and J.S. Shore. 2008. New Insights on Heterostyly: Comparative Biology,
475 Ecology and Genetics. In V.E. Franklin-Tong [ed.], *Self-incompatibility in flowering*
476 *plants—evolution, diversity, and mechanisms*, 3–32. Springer-Verlag, Berlin, Germany.
- 477 Barros, M.G. 1998. Sistemas de polinização em espécies simpátricas de *Erythroxyllum* P. Br.
478 (*Erythroxyllaceae*) do Brasil. *Brazilian Journal of Botany* 21: 159–166.
- 479 Bates, D., M. Maechler, B. Bolcher, and S. Walker. 2015. Fitting linear mixed-effects models
480 using lme4. *Journal of Statistical Software* 67: 1–48.
- 481 Berry, P.E., H. Tobe, and J.A. Gómez. 1991. Agamospermy and the loss of distyly in
482 *Erythroxyllum undulatum* (*Erythroxyllaceae*) from northern Venezuela. *American Journal of*
483 *Botany* 78: 595–600.
- 484 Bianchi, M.B., P.E. Gibbs, D.E. Prado, and J.L. Vesprini. 2000. Studies on the breeding
485 systems of understorey species of a Chaco woodland in NE Argentina. *Flora* 195: 339–
486 348.

- 487 Brys, R., H. Jacquemyn, and T. Beeckman. 2008. Morph-ratio variation, population size and
488 female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). *Journal of*
489 *Evolutionary Biology* 21: 1281–1289.
- 490 Brys, R., and H. Jacquemyn. 2019. The impact of individual inaccuracy of reciprocal
491 herkogamy on legitimate pollen deposition and seed set in a distylous self-incompatible
492 herb. *Journal of Ecology* 00: 1–13.
- 493 Burck, W. 1895. Over de eigenaardige heterostylie der bloemen van *Erythroxyton*.
494 *Nederlandsch Kruidkundzg Archief* 6: 254–262.
- 495 Cesaro, A.C., and J.D. Thompson. 2004. Darwin’s cross-promotion hypothesis and the
496 evolution of stylar polymorphism. *Ecology Letters* 7: 1209–1215.
- 497 Charlesworth, B., and D. Charlesworth. 1979. The maintenance and breakdown of distyly.
498 *The American Naturalist* 114: 499–513.
- 499 Consolaro, H., S.C.S. Silva, and P.E. Oliveira. 2011. Breakdown of distyly and pin-
500 monomorphism in *Psychotria carthagenensis* Jacq. (Rubiaceae). *Plant Species Biology* 26:
501 24–32.
- 502 Costa, J., S. Castro, J. Loureiro, and S.C.H. Barrett. 2016. Variation in style morph
503 frequencies in tristylous *Lythrum salicaria* in the Iberian Peninsula: the role of
504 geographical and demographic factors. *Annals of Botany* 117: 331–340.
- 505 Darwin, C. 1877. *The Different Form of Flowers of the Same Species*. John Murray, London,
506 UK.
- 507 Domínguez, C.A. 1990. Consecuencias ecológicas y evolutivas del patrón de floración
508 sincrónico y masivo de *Erythroxyton havanense* Jacq. (Erythroxytonaceae). Ph D
509 dissertation, Universidad Nacional Autónoma de México, México.

510 Domínguez, C.A., G. Avila-Sakar, S. Vizquez-Santana, and J. Mairquez-Guzmain. 1997.
511 Morph-biased male sterility in the tropical distylous shrub *Erythroxyllum havanense*
512 (Erythroxyllaceae). *American Journal of Botany* 84: 626–632.

513 Dulberger, R. 1992. Floral polymorphisms and their functional significance in the
514 heterostylous syndrome. In S.C.H. Barrett [ed.], *Evolution and function of heterostyly*, 41–
515 84. Springer, Berlin, Germany.

516 Eckert, C.G., and S.C.H. Barrett. 1992. Stochastic loss of style morphs from populations of
517 tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* 46: 1014–
518 1029.

519 Faivre, A.E. 2000. Ontogenetic differences in heterostylous plants and implications for
520 development from a herkogamous ancestor. *Evolution* 54: 847-858.

521 Faivre, A.E., and L.A. McDade. 2001. Population-level variation in the expression of
522 heterostyly in three species of Rubiaceae: does reciprocal placement of anthers and stigmas
523 characterize heterostyly? *American Journal of Botany* 88: 841–853.

524 Ferrero, V., J. Arroyo, P. Vargas, J.D. Thompson, and L. Navarro. 2009. Evolutionary
525 transitions of style polymorphisms in *Lithodora* (Boraginaceae). *Perspectives in Plant*
526 *Ecology, Evolution and Systematics* 11: 111–125.

527 Ferrero, V., I. Chapela, J. Arroyo, and L. Navarro. 2011a. Reciprocal style polymorphisms are
528 not easily categorised: the case of heterostyly in *Lithodora* and *Glandora* (Boraginaceae).
529 *Plant Biology* 13: 7–18.

530 Ferrero, V., S. Castro, J.M. Sánchez, and L. Navarro. 2011b. Stigma-anther reciprocity,
531 pollinators, and pollen transfer efficiency in populations of heterostylous species of
532 *Lithodora* and *Glandora* (Boraginaceae). *Plant Systematics and Evolution* 291: 267–276.

- 533 Ferrero, V., S.C.H. Barrett, D. Rojas, J. Arroyo, and L. Navarro. 2017. Associations between
534 sex-organ deployment and morph bias in related heterostylous taxa with different stylar
535 polymorphisms. *American Journal of Botany* 104: 1–12.
- 536 Fox, J. 2015. Applied regression analysis and generalized linear models, 3rd ed. Sage,
537 London, UK.
- 538 Ganders, F.R. 1979a. Heterostyly in *Erythroxyllum coca* (Erythroxyllaceae). *Botanical Journal*
539 *of the Linnean Society* 78: 11–20.
- 540 Ganders, F.R. 1979b. The biology of heterostyly. *New Zealand Journal of Botany* 17: 607–
541 635.
- 542 Hodgins, K.A., and S.C.H. Barrett. 2008. Asymmetrical mating patterns and the evolution of
543 biased style-morph ratios in a tristylous daffodil. *Genetics Research* 90: 3–15.
- 544 Jacquemyn, H., M. Gielen, and R. Brys. 2018. Is sexual organ reciprocity related to legitimate
545 pollen deposition in distylous *Pulmonaria* (Boraginaceae)? *Oikos* 127: 1216–1224.
- 546 Keller, B., J.M. de Vos, and E. Conti. 2012. Decrease of sexual organ reciprocity between
547 heterostylous primrose species, with possible functional and evolutionary implications.
548 *Annals of Botany* 110: 1233–1244.
- 549 Keller, B., J.D. Thomson, and E. Conti. 2014. Heterostyly promotes disassortative pollination
550 and reduces sexual interference in Darwin’s primroses: evidence from experimental
551 studies. *Functional Ecology* 28: 1413–1425.
- 552 Knight, T.M., J.A. Steets, J.C. Vamosi, S.J. Mazer, M. Burd, D.R. Campbell, M.R. Dudash, et
553 al. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of*
554 *Ecology, Evolution, and Systematics* 36: 467–497.

- 555 Lenth, R.V. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical*
556 *Software* 69: 1–33.
- 557 Lloyd, D.G., and C.J. Webb. 1992a. The evolution of heterostyly. *In* S.C.H. Barrett [ed.],
558 Evolution and function of heterostyly, 151–178. Springer, Berlin, Germany.
- 559 Lloyd, D.G., and C.J. Webb. 1992b. The selection of heterostyly. *In* S.C.H. Barrett [ed.],
560 Evolution and function of heterostyly, 179–207. Springer, Berlin, Germany.
- 561 Loiola, M.I.B., M.F. Agra, G.S. Baracho, and R.T. Queiroz. 2007. Flora da Paraíba, Brasil:
562 Erythroxyloaceae Kunth. *Acta Botanica Brasilica* 21: 473–487.
- 563 Mast, A.R., S. Kelso, and E. Conti. 2006. Are any primroses (*Primula*) primitively
564 monomorphic? *New Phytologist* 171: 605–616.
- 565 Pailler, T., and J.D. Thompson. 1997. Distyly and variation in heteromorphic incompatibility
566 in *Gaertnera vaginata* (Rubiaceae) endemic to La Reunion Island. *American Journal of*
567 *Botany* 84: 315–327.
- 568 Pailler, T., L. Humeau, and J.D. Thompson. 1998. Distyly and heteromorphic incompatibility
569 in oceanic island species of *Erythroxyllum* (Erythroxyloaceae). *Plant Systematics and*
570 *Evolution* 213: 187–198.
- 571 Pannell, J.R., M.E. Dorken, and S.M. Eppley. 2005. ‘Haldane’s Sieve’ in a metapopulation:
572 sifting through plant reproductive polymorphisms. *Trends in Ecology & Evolution* 20:
573 374–379.
- 574 Pérez, R., P. Vargas, and J. Arroyo. 2004. Convergent evolution of flower polymorphism in
575 *Narcissus* (Amaryllidaceae). *New Phytologist* 161: 235–252.

576 Pérez-Barrales, R., and J. Arroyo. 2010. Pollinator shifts and the loss of style polymorphism
577 in *Narcissus papyraceus* (Amaryllidaceae). *Journal of Evolutionary Biology* 23: 1117–
578 1128.

579 Pérez-Barrales, R., V.I. Simón-Porcar, R. Santos-Gally, and J. Arroyo. 2014. Phenotypic
580 integration in style dimorphic daffodils (*Narcissus*, Amaryllidaceae) with different
581 pollinators. *Philosophical Transactions of the Royal Society B* 369: 20130258.

582 Plowman, T., and N. Hensold. 2004. Names, types and distribution of neotropical species of
583 *Erythroxyllum* (Erythroxyllaceae). *Brittonia* 56: 1–53.

584 R Core Team. 2019. R: A language and environment for statistical computing. R Foundation
585 for Statistical Computing, Vienna, Austria. Website <http://www.R-project.org>

586 Rasband, W.S. 2011. Image J, version 1.45s. National Institutes of Health, Bethesda,
587 Maryland, USA.

588 Ruiz-Martín, J., R. Santos-Gally, M. Escudero, J.J. Midgley, R. Pérez-Barrales, and J. Arroyo.
589 2018. Style polymorphism in *Linum* (Linaceae): a case of Mediterranean parallel
590 evolution? *Plant Biology* 20: 100–111.

591 Sá, T., M.T. Furtado, V. Ferrero, R. Pérez-Barrales, E.B. Rodrigues, I.G. Santos, and H.
592 Consolaro. 2016. Floral biology, reciprocal herkogamy and breeding system in four
593 *Psychotria* species (Rubiaceae) in Brazil. *Botanical Journal of the Linnean Society* 182:
594 689–707.

595 Santos-Gally, R., A. de Castro, R. Pérez-Barrales, and J. Arroyo. 2015. Styler polymorphism
596 on the edge: unusual flower traits in Moroccan *Narcissus broussonetii* (Amaryllidaceae).
597 *Botanical Journal of the Linnean Society* 177: 644–656.

598 Silva, L., C.L. Fernandes, E. Simão, and A.T. Nakamura. 2016. Development of anthers and
599 pollen in Brazilian heterostylic species of *Erythroxyllum* (Erythroxyllaceae): an ecological
600 approach. *Pakistan Journal of Botany* 48: 2047–2055.

601 Silva, F.J.T., M.R.M. Schwade, and A.C. Webber. 2007. Fenologia, biologia floral e
602 polinização de *Erythroxyllum cf macrophyllum* (Erythroxyllaceae), na Amazônia Central.
603 *Revista Brasileira de Biociências* 5: 186–188.

604 Simón-Porcar, V.I., R. Santos-Gally, and J. Arroyo. 2014. Long-tongued insects promote
605 disassortative pollen transfer in style-dimorphic *Narcissus papyraceus* (Amaryllidaceae).
606 *Journal of Ecology* 102: 116-125.

607 Simón-Porcar, V.I., T.R. Meagher, and J. Arroyo. 2015 Disassortative mating prevails in
608 style-dimorphic *Narcissus papyraceus* despite low reciprocity and compatibility of
609 morphs. *Evolution* 69: 2276–2288.

610 Thompson, J.D., and B. Dommée. 2000. Morph-specific patterns of variation in stigma height
611 in natural populations of distylous *Jasminum fruticans*. *New Phytologist* 148: 303–314.

612 Thompson, J.D., A.C. Cesaro, and J. Arroyo. 2012. Morph ratio variation and sex organ
613 reciprocity in style-dimorphic *Narcissus assoanus*. *International Journal of Plant Sciences*
614 173: 885–893.

615 White, D.M., M.B. Islam, and R.J. Mason-Gamer. 2019. Phylogenetic inference in section
616 *Archerythroxyllum* informs taxonomy, biogeography, and the domestication of coca
617 (*Erythroxyllum* species). *American Journal of Botany* 106(1): 154–165.

618 Wu, L.-Y., F.-F. Chang, S.-J. Liu, W.S. Armbruster, and S.-Q. Huang. 2018. Heterostyly
619 promotes compatible pollination in buckwheats: Comparations of intraflower, intraplant,
620 and interplant pollen flow in distylous and homostylous *Fagopyrum*. *American Journal of*
621 *Botany* 105: 108–116.

- 622 Zhou, W., S.C.H. Barrett, H. Wang, and D.-Z. Li. 2012. Loss of floral polymorphism in
623 heterostylous *Luculia pinceana* (Rubiaceae): a molecular phylogeographic perspective.
624 *Molecular Ecology* 21: 4631–4645.
- 625 Zhou, W., S.C.H. Barrett, H. Wang, and D.-Z. Li. 2015. Reciprocal herkogamy promotes
626 disassortative mating in a distylous species with intramorph compatibility. *New Phytologist*
627 206: 1503–1512.
- 628 Zhou, W., S.C.H. Barrett, H.-D. Li, Z.-K. Wu, X.-J. Wang, H. Wang, and D.-Z. Li. 2017.
629 Phylogeographic insights on the evolutionary breakdown of heterostyly. *New Phytologist*
630 214: 1368–1380.

631 SUPPORTING INFORMATION

632 Additional Supporting Information may be found online in the supporting information section
633 at the end of the article.

634 **APPENDIX S1.** Locations of the 26 populations studied. DF= Federal District; GO= Goiás;
635 MG= Minas Gerais.

636 TABLES

637 **TABLE 1.** Results of the analysis of variance for the comparisons of the fruit set to describe
638 the incompatibility system and natural fruit set of four *Erythroxylum* species.

Source of variation	<i>E. campestre</i>			<i>E. deciduum</i>			<i>E. suberosum</i>			<i>E. tortuosum</i>		
	x ²	df	P	x ²	df	P	x ²	df	P	x ²	df	P
Incompatibility system												
Morph	0.424	1	0.514	0.091	1	0.763	1.165	1	0.559	3.627	1	0.057
Treatment	46.440	5	<0.001	55.617	5	<0.001	45.984	5	<0.001	16.518	5	<0.01
Morph * Treatment	6.053	5	0.301	8.493	5	0.131	0.354	5	0.997	0.000	5	1.000
Natural fruit set												
Morph	0.837	1	0.360	0.274	1	0.601	0.000	1	0.996	0.481	1	0.488
Population	4.053	2	0.132	30.852	2	<0.001	23.464	3	<0.001	0.267	1	0.605
Morph * Population	0.177	2	0.915	7.901	2	0.019	2.869	3	0.412	0.000	1	0.978

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641 **TABLE 2.** Morph ratio and chi-square analyses to test deviations from isoplethy (1:1 ratio) in
 642 populations of four *Erythroxylum* species.

Species Population	Number of plants (S:L-morph)	df	χ^2	P	Characterization of populations
<i>E. campestre</i>					
1	40 (21:19)	1	0.03	0.874	Isoplethy
2	56 (17:39)	1	7.88	< 0.001	Anisoplethy (↑L)
3	39 (21:18)	1	0.10	0.749	Isoplethy
4	39 (32:7)	1	14.77	< 0.001	Anisoplethy (↑S)
5	37 (1:36)	1	31.24	< 0.001	Anisoplethy (↑L)
6	39 (19:20)	1	0.00	1.000	Isoplethy
7	43 (43:0)	1	41.02	< 0.001	Monomorphic
8	41 (39:2)	1	31.61	< 0.001	Anisoplethy (↑S)
<i>E. deciduum</i>					
9	41 (18:23)	1	0.39	0.532	Isoplethy
10	40 (18:22)	1	0.23	0.635	Isoplethy
11	39 (15:24)	1	1.64	0.200	Isoplethy
12	34 (13:21)	1	1.44	0.230	Isoplethy
<i>E. suberosum</i>					
13	32 (12:20)	1	1.53	0.216	Isoplethy
14	34 (14:20)	1	0.74	0.391	Isoplethy
15	48 (22:26)	1	0.19	0.665	Isoplethy
16	40 (26:14)	1	3.03	0.082	Isoplethy
17	42 (21:21)	1	0.00	1.000	Isoplethy
18	35 (16:19)	1	0.11	0.735	Isoplethy
19	30 (7:23)	1	7.50	0.006	Anisoplethy (↑L)
20	72 (40:32)	1	0.68	0.409	Isoplethy
<i>E. tortuosum</i>					
21	42 (27:15)	1	2.88	0.090	Isoplethy
22	49 (23:26)	1	0.08	0.775	Isoplethy
23	31 (9:22)	1	4.65	0.031	Anisoplethy (↑L)
24	39 (22:17)	1	0.41	0.522	Isoplethy
25	37 (22:15)	1	0.97	0.324	Isoplethy
26	42 (19:23)	1	0.21	0.643	Isoplethy

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651 **TABLE 3.** Flower morphometrics (in mm) of four *Erythroxyllum* species: sample size for the
652 two floral morphs, mean (\pm variance) of the height of each organ type, and mean (\pm variance)
653 of upper and lower anthers in the L-morph.

Species Population	<i>n</i> L- morph	<i>n</i> S- morph	L stigmas (S)	S anthers (A)	S stigmas (s)	L anthers (a)	Two anther level of the L-morph	
							Upper anthers (aA)	Lower anthers (aa)
<i>E. campestre</i>								
1	18	17	3.11 (0.52)	3.25 (0.54)	1.47 (0.20)	0.88 (0.11)	1.11 (0.10)	0.64 (0.02)
2	58	25	2.49 (0.22)	2.13 (0.10)	1.40 (0.03)	0.84 (0.08)	1.06 (0.04)	0.62 (0.02)
3	36	36	3.50 (0.34)	2.99 (0.09)	1.44 (0.02)	1.06 (0.12)	1.31 (0.08)	0.81 (0.03)
4	12	62	3.10 (0.14)	3.51 (0.13)	1.65 (0.05)	1.01 (0.12)	1.25 (0.07)	0.76 (0.04)
5	42	2	2.40 (0.57)	1.95 (0.01)	1.02 (0.04)	0.84 (0.15)	1.13 (0.09)	0.55 (0.05)
6	28	30	3.73 (0.51)	3.90 (0.16)	1.79 (0.05)	1.12 (0.25)	1.47 (0.18)	0.76 (0.07)
7	0	19	-	3.47 (0.45)	1.77 (0.10)	-	-	-
8	1	33	2.61	2.23 (0.12)	1.17 (0.02)	1.02 (0.11)	1.26	0.78
Average			2.99 (0.38)	2.93 (0.20)	1.46 (0.06)	0.97 (0.13)	1.23 (0.09)	0.70 (0.04)
<i>E. deciduum</i>								
9	40	30	3.58 (0.18)	4.21 (0.61)	1.51 (0.08)	2.61 (1.27)	3.64 (0.27)	1.58 (0.13)
10	44	39	3.77 (0.30)	3.85 (0.17)	1.49 (0.06)	2.84 (1.47)	3.87 (0.62)	1.80 (0.16)
11	39	30	3.73 (0.19)	4.22 (0.53)	1.43 (0.09)	2.57 (1.21)	3.61 (0.17)	1.52 (0.04)
12	33	30	3.54 (0.21)	3.80 (0.51)	1.49 (0.05)	2.62 (0.99)	3.52 (0.17)	1.72 (0.16)
Average			3.66 (0.22)	4.02 (0.46)	1.48 (0.07)	2.66 (1.24)	3.66 (0.31)	1.66 (0.12)
<i>E. suberosum</i>								
13	25	6	4.29 (0.31)	4.35 (0.11)	2.12 (0.03)	1.51 (0.35)	2.03 (0.12)	1.00 (0.06)
14	30	20	4.53 (0.29)	4.47 (0.37)	2.03 (0.04)	1.59 (0.66)	2.18 (0.46)	1.00 (0.16)
15	45	38	4.57 (0.58)	4.48 (0.26)	2.16 (0.07)	1.73 (0.54)	2.31 (0.32)	1.16 (0.09)
16	18	38	4.88 (0.56)	4.81 (0.51)	2.26 (0.10)	2.06 (0.54)	2.65 (0.21)	1.46 (0.15)
17	29	31	5.83 (0.53)	4.95 (0.52)	2.28 (0.06)	2.30 (0.70)	2.99 (0.32)	1.61 (0.12)
18	49	39	4.90 (0.19)	4.54 (0.22)	2.07 (0.05)	1.82 (0.38)	2.36 (0.11)	1.27 (0.06)
19	40	14	5.14 (0.27)	4.30 (0.81)	2.05 (0.05)	2.04 (0.45)	2.60 (0.18)	1.47 (0.07)
20	27	55	4.19 (0.41)	3.71 (0.29)	1.75 (0.06)	1.38 (0.34)	1.85 (0.15)	0.91 (0.08)
Average			4.79 (0.39)	4.45 (0.39)	2.09 (0.06)	1.80 (0.50)	2.37 (0.23)	1.24 (0.10)
<i>E. tortuosum</i>								
21	15	47	3.91 (0.32)	3.91 (0.27)	1.61 (0.06)	1.27 (0.44)	1.85 (0.16)	0.69 (0.03)
22	42	37	3.87 (0.34)	3.99 (0.36)	1.61 (0.04)	1.28 (0.40)	1.80 (0.21)	0.76 (0.03)
23	36	19	3.88 (0.30)	3.51 (0.10)	1.49 (0.05)	1.13 (0.31)	1.60 (0.13)	0.65 (0.03)
24	42	50	4.48 (0.27)	4.12 (0.33)	1.64 (0.05)	1.40 (0.34)	1.92 (0.09)	0.88 (0.04)
25	31	47	4.64 (0.22)	4.18 (0.44)	1.64 (0.10)	1.50 (0.60)	2.21 (0.13)	0.79 (0.05)
26	45	15	3.70 (0.31)	3.40 (0.42)	1.49 (0.05)	1.01 (0.15)	1.34 (0.07)	0.69 (0.02)
Average			4.08 (0.29)	3.85 (0.32)	1.58 (0.06)	1.27 (0.37)	1.79 (0.13)	0.74 (0.03)

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661 **TABLE 4.** Results of the analysis of variance for the comparisons between floral morphs and
662 populations of stigma height and anther height of four *Erythroxyllum* species. High anther
663 height corresponds to the comparison between anthers of the S-morph and upper anthers of
664 the L-morph. Low anther height corresponds to the comparison between anthers of the S-
665 morph and lower anthers of the L-morph.

Source of variation	<i>E. campestre</i>			<i>E. deciduum</i>			<i>E. suberosum</i>			<i>E. tortuosum</i>		
	x ²	df	P	x ²	df	P	x ²	df	P	x ²	df	P
Stigma height												
Morph	622.7	1	< 0.001	1083.9	1	< 0.001	1955.4	1	< 0.001	3146.1	1	< 0.001
Population	105.1	4	< 0.001	1.3	3	0.717	118.7	6	< 0.001	49.7	5	< 0.001
Morph * Population	35.4	4	< 0.001	1.2	3	0.762	46.9	6	< 0.001	42.5	5	< 0.001
High anther height												
Morph	1165.8	1	< 0.001	14.7	1	< 0.001	870.6	1	< 0.001	1877.0	1	< 0.001
Population	189.0	4	< 0.001	3.9	3	0.271	131.9	6	< 0.001	76.0	5	< 0.001
Morph * Population	89.5	4	< 0.001	7.8	3	0.051	6.0	6	0.424	7.0	5	0.221
Low anther height												
Morph	2482.4	1	< 0.001	907.7	1	< 0.001	2780.1	1	< 0.001	5606.1	1	< 0.001
Population	180.5	4	< 0.001	0.9	3	0.835	136.5	6	< 0.001	35.7	5	< 0.001
Morph * Population	154.0	4	< 0.001	17.3	3	< 0.001	14.7	6	0.022	27.2	5	< 0.001

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677 **TABLE 5.** Estimates of inaccuracy in reciprocity in species and populations of *Erythroxyllum*
678 (standardized inaccuracy in parentheses). The values of inaccuracy were standardized by the
679 squared mean of all anther and stigma height recorded for each population. Total inaccuracy
680 for each population (column 4) is the sum of the inaccuracy values of high and low organs
681 (columns 2 and 3). Columns 5 and 6 include inaccuracy (absolute value in units of mm² and
682 percentage standardized) of the low organs (S stigmas and L anthers) considering separately
683 the upper (aA) and lower (aa) anthers of the L-morph.

Species Population	Inaccuracy by organ type		Total inaccuracy	Inaccuracy of the low organs considering the different height of anthers in the L-morph	
	High (S-A)	Low (s-a)		(s-aA)	(s-aa)
<i>E. campestre</i>					
1	1.08 (22.81%)	0.66 (14.04%)	1.74 (36.85%)	0.43 (9.08%)	0.90 (19.01%)
2	0.44 (15.07%)	0.42 (14.35%)	0.87 (29.42%)	0.18 (6.18%)	0.66 (22.51%)
3	0.69 (13.64%)	0.28 (5.49%)	0.96 (19.13%)	0.12 (2.29%)	0.44 (8.68%)
4	0.44 (8.24%)	0.58 (10.93%)	1.03 (19.17%)	0.28 (5.23%)	0.89 (16.61%)
5	0.77 (32.00%)	0.22 (9.16%)	0.99 (41.16%)	0.14 (5.67%)	0.30 (12.62%)
6	0.70 (10.02%)	0.75 (10.86%)	1.45 (20.89%)	0.33 (4.82%)	1.17 (16.90%)
8	0.26 (8.55%)	0.15 (4.93%)	0.42 (13.48%)	0.02 (0.70%)	0.17 (5.52%)
Average	0.63 (15.76%)	0.44 (9.96%)	1.07 (25.73%)	0.21 (4.85%)	0.65 (14.55%)
<i>E. deciduum</i>					
9	1.20 (13.49%)	2.56 (28.88%)	3.76 (42.37%)	4.89 (55.12%)	0.21 (2.40%)
10	0.47 (5.32%)	3.35 (37.62%)	3.82 (42.94%)	6.37 (71.52%)	0.32 (3.54%)
11	0.96 (10.74%)	2.58 (28.94%)	3.54 (39.67%)	4.99 (56.00%)	0.14 (1.59%)
12	0.79 (9.70%)	2.32 (28.36%)	3.12 (38.07%)	4.36 (53.28%)	0.26 (3.20%)
Average	0.86 (9.81%)	2.70 (30.95%)	3.56 (40.76%)	5.15 (58.98%)	0.23 (2.68%)
<i>E. suberosum</i>					
13	0.42 (4.48%)	0.76 (8.05%)	1.18 (12.53%)	0.16 (1.74%)	1.34 (14.28%)
14	0.67 (6.71%)	0.89 (8.93%)	1.56 (15.64%)	0.52 (5.23%)	1.25 (12.60%)
15	0.85 (8.08%)	0.80 (7.61%)	1.64 (15.69%)	0.42 (4.00%)	1.17 (11.20%)
16	1.07 (8.70%)	0.68 (5.55%)	1.75 (14.25%)	0.47 (3.80%)	0.89 (7.22%)
17	1.81 (12.30%)	0.76 (5.15%)	2.57 (17.46%)	0.88 (5.96%)	0.63 (4.28%)
18	0.54 (4.89%)	0.50 (4.50%)	1.04 (9.39%)	0.24 (2.20%)	0.75 (6.75%)
19	1.78 (15.62%)	0.50 (4.39%)	2.29 (20.01%)	0.54 (4.74%)	0.46 (4.01%)
20	0.93 (12.23%)	0.53 (7.02%)	1.46 (19.25%)	0.22 (2.84%)	0.85 (11.15%)
Average	1.01 (9.13%)	0.68 (6.40%)	1.69 (15.53%)	0.43 (3.81%)	0.92 (8.94%)
<i>E. tortuosum</i>					
21	0.59 (8.20%)	0.61 (8.53%)	1.20 (16.73%)	0.28 (3.87%)	0.93 (12.97%)
22	0.71 (9.80%)	0.55 (7.65%)	1.26 (17.45%)	0.29 (4.04%)	0.81 (11.21%)
23	0.54 (8.68%)	0.50 (7.94%)	1.04 (16.61%)	0.20 (3.13%)	0.79 (12.68%)
24	0.73 (8.61%)	0.45 (5.32%)	1.18 (13.93%)	0.23 (2.67%)	0.67 (7.91%)
25	0.86 (9.64%)	0.72 (8.04%)	1.58 (17.68%)	0.55 (6.11%)	0.88 (9.81%)
26	0.82 (14.24%)	0.43 (7.40%)	1.25 (21.64%)	0.14 (2.50%)	0.71 (12.28%)
Average	0.71 (9.86%)	0.54 (7.48%)	1.25 (17.34%)	0.28 (3.72%)	0.80 (11.14%)

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686 **FIGURE LEGENDS**

687 **FIGURE 1.** Flowers of *Erythroxyllum* species studied in the Cerrado of Central Brazil,
688 showing L- and S-morph of *E. campestre* (A, B), *E. deciduum* (C, D), *E. suberosum* (E, F)
689 and *E. tortuosum* (G, H).

690 **FIGURE 2.** Schematic representation of the relative position of stigmas and anthers in the S
691 and L-morph of *Erythroxyllum* species studied and the flower traits measured. Letters in
692 parentheses correspond to the initials used for each type of organ in the analysis of
693 reciprocity. For clarity, only four of 10 stamens per flower are drawn (two per whorl).

694 **FIGURE 3.** Mean \pm standard error of fruit set after hand-pollination treatments in individuals
695 of four *Erythroxyllum* species. Pollination treatments were: agamospermy (Agam.),
696 autonomous self-pollination (Self-aut.), facilitated self-pollination (Self-fac.), intra-morph
697 cross-pollination (Intra) and inter-morph cross-pollination (Inter). Results from flowers
698 exposed to natural pollinations are also shown (control). As there were no statistical
699 differences between morphs within each species (results not shown), the results for both
700 morphs are plotted together. Treatments with different letters were significantly different from
701 each other at the 0.05 level in post-hoc tests.

702 **FIGURE 4.** Geographic distribution and variation in morph ratio in 26 populations of four
703 *Erythroxyllum* species studied in Cerrado areas of Central Brazil. Black and grey segments
704 indicate the proportions of the L- and S-morph, respectively. Numbers correspond to the code
705 of each population. Details on sample size per population are provided in Table 2.

706 **FIGURE 5.** Population mean \pm standard error of natural fruit set in S (black bars) and L
707 plants (grey bars) in four *Erythroxyllum* species. Location of sampled populations are shown
708 in Figure 4. Populations with different letters were significantly different from each other at
709 the 0.05 level in post-hoc tests. Asterisks indicate statistically significant differences between

710 morph within populations or in overall, considering the 0.05 level in post-hoc tests. Sequential
711 Bonferroni correction was used to account for multiple tests. Except for *E. campestre*, natural
712 fruit set of these populations (mean and coefficient of variation) was used to investigate
713 associations with high and low inaccuracy (see Material and Methods for details).

714 **FIGURE 6.** Relationship between inaccuracy and the coefficient of variation in fruit set for S
715 (grey points) and L plants (black points) in nine populations of three different *Erythroxylum*
716 species. Dashed line shows predicted relationship and the shaded area indicates the 95%
717 confidence interval.