

1 **High incidence of pollen theft in natural populations of a buzz-**
2 **pollinated plant**

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13 **ABSTRACT**

14 More than 20,000 angiosperm species possess non-dehiscent anthers that open through small pores
15 at the anther's tip. These flowers are visited by bees that use vibrations to remove pollen, a
16 phenomenon known as buzz-pollination. However, some floral visitors fail to transfer pollen
17 efficiently, either through a mismatch of flower and insect size, or because they are unable to
18 buzz-pollinate. These visitors collect pollen, but provide little or no pollination, behaving as pollen
19 thieves. Although pollen theft is widespread in plants, few studies have quantified the incidence of
20 pollen thieves for buzz-pollinated plants. We use observations of natural populations and floral
21 manipulations of *Solanum rostratum* (Solanaceae) to investigate the incidence of pollen theft, find
22 morphological and behavioural differences between pollinators and thieves, measure the
23 pollination efficiency of visitors, and characterise the reproductive ecology of this herb. We found
24 that most visitors act as thieves, with <20% of all bees contacting the stigma. Insect visitors that
25 regularly failed to contact the stigma (illegitimate visitors), included buzzing and non-buzzing
26 bees, were significantly smaller, visited fewer flowers per bout and stayed longer in each flower
27 than (legitimate) visitors that regularly contact the stigma. Few flowers visited solely by
28 illegitimate visitors set fruit. Our results show that *S. rostratum* requires insect visitation to set
29 seed and natural populations experience moderate pollen limitation. We conclude that insect size,
30 relative to the flower, is the main determinant of whether a visitor acts as a pollinator or a pollen
31 thief in *S. rostratum*.

32

33 **Key words:** buzz-pollination, pollen larceny, pollen limitation, pollen theft, pollination efficiency,
34 *Solanum rostratum*.

35 INTRODUCTION

36 Floral larceny is a widespread phenomenon whereby visitors collect resources, such as pollen or
37 nectar, but provide little or no pollination (Inouye 1980; Irwin et al. 2010). This phenomenon is
38 well documented in plants visited by nectar-collecting foragers (e.g., Faegri and Van der Pijl 1966,
39 Irwin et al. 2010), but the prevalence and fitness consequences of floral larceny by pollen-
40 consuming visitors has received less attention (e.g., Renner 1983, Hargreaves et al. 2009).
41 Although floral larceny generally results in fitness costs for plants, the magnitude of its effects
42 depend on the type and frequency of visitors, as well as on the reproductive biology of the plant
43 itself (Irwin et al. 2001, Hargreaves et al. 2009). Therefore, to understand the ecological and
44 evolutionary consequences of pollen larceny, the incidence and characteristics of illegitimate
45 pollen consumption in natural plant populations must be assessed.

46 Floral larceny can occur with or without damage to floral structures. Inouye (1980) divided
47 larceny into theft and robbing, depending on whether “force” is used to access the floral reward
48 (pollen or nectar). For example, access to nectar by piercing a whole at the base of the corolla
49 tube, bypassing the sexual organs, is an example of robbing, whereas a visitor that is simply too
50 small to contact the sexual organs while collecting the resource is a case of floral theft. Moreover,
51 Hargreaves et al. (2009) defined pollen thieves as visitors that while removing pollen from
52 flowers, have a negligible contribution to cross-pollination. They subdivided pollen thieves into
53 habitual, those that never deposit pollen deposition onto stigmas, and conditional, which may
54 transfer pollen to stigmas, but represent the minimum in the continuum of pollination efficiency.
55 More generally, robbers can be defined as those that cause floral damage (e.g., piercing the corolla
56 tube, chewing through the anther wall), whereas thieves remove the reward but cause no unusual
57 physical damage to the flower (Inouye 1980, Irwin et al. 2010, Hargreaves et al. 2009).

58 Pollen larceny can affect plant fitness directly by reducing the pollen available for
59 fertilizing ovules, or indirectly by reducing the attractiveness of robbed flowers, and thus affecting
60 visitation by pollinators (Hargreaves et al. 2009). Pollen larceny might be particularly costly for
61 plants that use pollen as the main or only reward to attract pollinators (i.e., nectarless or pollen-
62 only flowers; Endress 1996), because unlike nectar, pollen cannot be replenished after it is
63 removed (Hargreaves et al. 2010). Moreover, pollen consumption either by the visitor or its
64 progeny (e.g., bees collect pollen to feed their larvae; Thorp 2000) diminishes the pollen available
65 to fertilize other flowers (but see Harder and Wilson 1997). To date, few studies have quantified
66 the incidence of pollen larceny in natural populations of pollen-only species.

67 Pollen-only flowers often possess anthers that release pollen through small pores or slits
68 (i.e., poricidal anthers; Buchmann 1983, Vallejo-Marín et al. 2010). These plants are visited
69 primarily by bees (Apoidea), although they may also occasionally receive visits by flies (Diptera),
70 beetles (Coleoptera), and butterflies (Lepidoptera; e.g., Larson and Barrett 1999). Bee visitors to
71 nectarless flowers with poricidal anthers are extremely diverse in terms of taxonomic affiliation,
72 behaviour, and morphological characteristics such as body size (Bernhardt 1995, Larson and
73 Barrett 1999, Duncan et al. 2004, Gao et al. 2006, Kawai and Kudo 2009, Liu and Pemberton
74 2009). For example, species with poricidal anthers in Melastomataceae and *Solanum* (Solanaceae)
75 are visited by bees that range in size from diminutive halictids (Halictidae) and stingless bees
76 (Meliponini) to large carpenter bees (Apidae: *Xylocopa* spp.) and bumblebees (Apidae: *Bombus*
77 spp.; Renner 1989, Larson and Barrett 1999, Anderson and Symon 1988, Raw 2000, Liu and
78 Pemberton 2009). Bees visiting plants with poricidal anthers use contrasting methods of pollen
79 collection. Typically, bees use vibrations (i.e., buzzing) to rapidly remove large quantities of
80 pollen (Buchmann and Hurley 1978, Buchmann 1983). Such buzz-pollination is strongly

81 associated with nectarless flowers with poricidal anthers, and occurs in thousands of plant species,
82 including crops such as tomatoes and potatoes (Vallejo-Marín et al. 2010, De Luca and Vallejo-
83 Marín 2013). However, some non-buzzing bees can also access pollen from these flowers, for
84 example by chewing the anther walls (e.g., *Trigona spp.*, Renner 1983), or simply by gleaning
85 pollen previously extracted by buzzing visitors (e.g., *Apis spp.*; Buchmann 1983). The different
86 morphologies and behaviours of pollen-collecting bees may cause variation in their ability to
87 transfer pollen from anthers to stigmas (effectiveness *sensu* Ne'eman et al. 2009), and therefore in
88 their efficiency to promote seed siring and production, thus affecting whether a floral visitor
89 behaves as a pollinator or as a pollen larcenist.

90 Body size is an important characteristic that determines the visitor's effectiveness to
91 contact the floral sexual organs during visitation, which has implications for visitor efficiency to
92 promote seed siring and production (Whalen 1979, Bernhardt 1995, Vivarelli et al. 2011). For
93 example, in invasive populations of Turkey berry (*Solanum torvum*, Solanaceae), in Florida, visits
94 by medium and large bees (*Euglossa viridissima* and *Xylocopa micans*; 4.81 ± 0.13 - 7.10 ± 0.24
95 mm thorax width; mean \pm S.E.) yield higher fruit set than visits by smaller halictids (2.5 ± 0.11
96 mm, Liu and Pemberton 2009). The low pollination efficiency of halictid bees seems to result
97 from mismatch in the size of the visitor relative to the flower, with bees that are relatively small
98 failing to contact the stigma. Similarly, visitors that destroy anthers while collecting pollen (e.g.,
99 *Trigona spp.*) will limit opportunities for subsequent visitation, and potentially affect pollen
100 dispersal (Renner 1983). Nevertheless, few studies have systematically documented the
101 morphological and behavioural characteristics that distinguish pollinators (legitimate visitors)
102 from pollen larcenists (illegitimate visitors) in buzz-pollinated plants.

103 Here we investigate the incidence and characteristics of pollinators and pollen larcenists in
104 natural populations of a buzz-pollinated herb, *Solanum rostratum* (Solanaceae). We characterize
105 the visitor assemblages in three *S. rostratum* populations in Central Mexico, and correlate the
106 morphology and behaviour of visitors with the likelihood that they contact the sexual organs
107 (effectiveness) while foraging for pollen. We then use experimental manipulations to estimate fruit
108 set (efficiency) following individual visits by putative pollinators and pollen larcenists. Finally, we
109 characterize the reproductive biology of *S. rostratum* across six populations to determine the
110 extent to which pollinator availability limits reproduction in this self-compatible, but outcrossing
111 species, and to examine the reproductive consequences of variation in the frequency of pollen
112 larceny among populations.

113 **METHODS**

114 **Study species**

115 *Solanum rostratum* Dunal (Solanaceae) is a self-compatible, annual herb distributed from central
116 Mexico to the Great Plains in the USA, which grows in dry and disturbed habitats (Whalen 1979,
117 Nee 1993). It is also adventitious or invasive in Canada, Asia, Europe, and Australia (Whalen
118 1979, Zhao et al. 2013). The bright yellow flowers of *S. rostratum* are nectarless and have five
119 poricidal anthers arranged in a cone at the centre of the flower. Like other species in *Solanum*
120 section *Androceras*, *S. rostratum* is heterantherous, presenting two morphologically and
121 functionally distinct sets of anthers in each flower (Whalen 1979, Vallejo-Marín et al. 2014). Four
122 centrally located yellow-coloured anthers provide pollen for visiting insects and are known as
123 feeding anthers (Bowers 1975, Vallejo-Marín et al. 2009). A single, usually darker, larger anther
124 located to either the right- or left side of the floral axis, produces 50-66% of the total number of

125 pollen grains per flower and contributes disproportionately to pollen reaching the stigmas of other
126 flowers, and is known as the pollinating anther (Vallejo-Marín et al. 2009, 2014). Visiting insects
127 usually ignore this pollinating anther (Bowers 1975, Vallejo-Marín et al. 2009). Flowers of *S.*
128 *rostratum* are enantiostylous, i.e., with mirror-image floral morphs that present the style and
129 pollinating anther opposite to each other, deflected either right or left side of the floral axis, with
130 the two floral morphs alternating along the inflorescence (Todd 1882, Jesson and Barrett 2002).

131 *Solanum rostratum* is buzz-pollinated by a wide diversity of bees (García-Peña 1976,
132 Harris and Kuchs 1902, Linsley and Cazier 1963, Bowers 1975, Jesson and Barrett 2005). During
133 buzz-pollination, a bee grasps the base of, usually, the feeding anthers with its mandibles and curls
134 its body around the anther cone. The bee then produces a series of high-frequency vibrations that
135 are transmitted to all anthers and cause pollen grains to expel forcibly from a pair of apical pores
136 from both feeding and pollinating anthers (De Luca et al. 2013). When flowers are visited by
137 pollinators of the appropriate size (medium to large bees), the feeding anthers deposit pollen on the
138 ventral side of the bee's thorax and abdomen, while the pollinating anther deposits pollen on the
139 lateral or dorsal surface of the abdomen (Vallejo-Marín et al. 2009). The placement of pollen from
140 the pollinating anther corresponds to the site of contact of the stigma in a flower of the opposite
141 morph (Jesson and Barrett 2005). The combination of the stereotyped behaviour of buzz-
142 pollinators (De Luca and Vallejo-Marín 2013), and the complex reproductive morphology of *S.*
143 *rostratum* results in a precise interaction between flower and pollinator that promotes both pollen
144 deposition and receipt (Armbruster et al. 2009) and pollen transfer between flowers.

145

146 **Study site**

147 We studied six populations of *S. rostratum* in Mexico (Table 1), which is the centre of the
148 distribution of this species, and also has an extremely diverse bee fauna, with an estimated 1,800-
149 2,000 species (Vergara and Ayala 2002). Populations were at least 43 km apart with a linear
150 distance of 470 km between the northernmost and southernmost ones. Plants vouchers specimens
151 were deposited at the National Herbarium of Mexico (MEXU). Population DHG (Dolores
152 Hidalgo, Guanajuato) occupied roadside near the town of Dolores Hidalgo, and population SLG
153 (San Luis, Guanajuato) occupied a fallow field near a rural town, both sites being characterised by
154 intense anthropogenic activity. Population TEM (Teotihuacán, Estado de México) also occurred in
155 a fallow field in an area of increasing anthropogenic activity surrounding a major archaeological
156 site. To conduct detailed pollinator observations (between September 2009 and 2011) we focused
157 on three populations, which were chosen because they best represented different environmental
158 conditions that *S. rostratum* usually inhabits: (1) CU (Ciudad Universitaria, Distrito Federal), in
159 the Pedregal de San Ángel nature reserve within Mexico City, which is a 237 ha urban nature
160 reserve characterized by xeric shrubland dominated by *Pittocaulon praecox* (= *Senecio praecox*;
161 Lot and Camarena 2009); (2) TP (Tehuacán, Puebla), located in an abandoned field in the
162 Tehuacán-Cuicatlán Valley, near San Juan Raya, in an area of xeric vegetation and extremely high
163 biodiversity (Casas et al. 2001); and (3) LP (Libres, Puebla), located approximately 120 km north
164 of TP, in an area with semiarid climate and subject to intensive agriculture and rapidly
165 urbanization (INEGI 2003).

166 **Pollination ecology**

167 We recorded floral visitors to *S. rostratum* during 30-min periods scattered throughout the day.
168 The time of observation in each population was adjusted to preliminary observations according to

169 the earliest and latest visit by floral visitors of *S. rostratum*. For populations LP and TP we
170 recorded at five times between 09:30 and 16:00 during seven days in 2010 (9-15 October), and
171 five days in 2011 (21 July-5 August). In population CU, visitation started earlier so we conducted
172 eight observations periods per day during seven days in 2011 (11 August-9 September) between
173 07:30-18:30. Observations were made in 5×5 m quadrats within the *S. rostratum* populations. In
174 summary, we conducted 115 h of pollinator observations in these three focal populations (35h,
175 37h, and 43h in populations TP, LP, and CU, respectively).

176 For each visitor species, we captured and measured at least ten individuals (one to four for
177 rare species) at the end of visitation bouts. All insects captured were cooled on ice, identified,
178 measured and then released. The following measurements were taken with digital callipers: 1)
179 body length (from the top of the head (vertex) to the tip of the abdomen, 2) thorax length; 3)
180 widest thorax width, 4) abdomen length, and 5) abdomen width. Four individuals of each species
181 were collected as voucher specimens for identifications, and deposited at the Universidad de las
182 Américas, Puebla (UDLA-P), Mexico. We analysed the morphological measurements of visitors
183 using principal component analysis (PCA, *princomp* function in *R* ver. 3.1.2; R Core Development
184 Team 2014) based on the correlation matrix, with data centred to zero, because PCA summarized
185 the variation of visitor morphology and the first principal component effectively estimates visitor size.
186 The diversity of floral visitors in each population was calculated with the Shannon-Wiener
187 diversity index.

188 During each visit, we recorded whether the visiting insect produced an audible buzz and
189 whether it contacted the feeding anthers, pollinating anthers, style/stigma, or multiple structures in
190 a single visit. In addition, we recorded the number of flowers visited per visitation bout and the
191 time spent in each flower. For bees, we removed the pollen load from the scopae and/or the rest of

192 the body and placed it in a microcentrifuge tube in 70% ethanol to determine an insect's fidelity to
193 foraging on *S. rostratum*. We mounted the pollen samples in fuchsine-glycerine jelly (Kearns and
194 Inouye 1993), and calculated the proportion of pollen grains from *S. rostratum* vs. other species
195 using a light microscope (Dialux 20EB, Leitz), with a minimum of 100 grains observed per
196 sample.

197 We classified each floral visit during which visitors contacted the anthers and/or stigma as
198 legitimate or illegitimate, depending on whether the visitor contacted the stigma. We used the
199 species' average proportion of legitimate visits over all floral visits (legitimate + illegitimate
200 visits) to classify them as legitimate ($\geq 50\%$ legitimate visits) or illegitimate ($\leq 20\%$ legitimate
201 visits) visitors. Illegitimate visitors collected pollen from flowers, but did not contact the stigma,
202 and can thus be considered as potential pollen larcenists. Difference in body size (mean of the first
203 principal component scores per species per population) between legitimate and illegitimate visitors
204 was compared using a one tailed *t*-test with unequal variances.

205 We analysed the effect of visitor type (legitimate or illegitimate) on pollinator visitation
206 using generalised linear mixed effects models (*glmer* function in the *lme4* package in *R*; Bates et
207 al. 2014). Bout length (number of flowers visited per individual visitor in a bout - 1) was analysed
208 using a negative binomial error distribution, and visit duration (average time spent per flower for
209 an individual visitor within a bout) was analysed using a gamma error distribution with a log-link.
210 Both analyses included population and bee species as random effects.

211 **Pollination efficiency experiment**

212 We estimated the efficiency of legitimate and illegitimate visitors in triggering fruit set, as a
213 measure of female reproductive success, in a pollination experiment in population CU. We

214 randomly selected *S. rostratum* inflorescences, removed all open flowers, and enclosed the
215 remaining floral buds using fine mesh. Experimental flowers were then exposed to visitors during
216 30-min periods from 10:00 to 13:30 when most visitor activity occurred. A single insect was
217 allowed to visit each flower, after which the flower was labelled and bagged again in fine mesh.
218 Unvisited flowers at the end of the observation period were bagged and used as controls. This
219 experiment was conducted during several days between 22 August and 9 September 2011. Six
220 weeks later, we collected all fruits produced by the labelled flowers. Differences in fruit set
221 between legitimate and illegitimate visitors were analysed using a generalised linear model with a
222 binomial error distribution (*glm* package in *R*), and statistical significance of visitor type was
223 evaluated using a likelihood ratio test.

224 **Reproductive system of *S. rostratum***

225 We used experimental manipulations to characterize the reproductive system in all populations
226 between 2009 and 2011. In each population, we assigned individual flowers to the following four
227 treatments according to Eckert et al. (2010). 1) Emasculation, for which we removed the anthers
228 before anthesis and used fine mesh bags to exclude pollinators, assessed whether *S. rostratum* can
229 set seed without pollination (e.g., through apomixis or agamospermy). 2) Pollinator exclusion, for
230 which the anthers were left intact, but the flowers were covered with fine mesh before anthesis,
231 assessed the ability of plants to self-fertilize in the absence of pollinators (autonomous selfing). 3)
232 Pollen supplementation, in which open-pollinated flowers were supplemented with additional
233 pollen extracted from the flowers of at least five individuals. Pollen was extracted from donor
234 flowers using an electric toothbrush, collected in a 1.5ml microcentrifuge vial and applied using a
235 toothpick to the stigma of the recipient flower. 4) Open pollination, involved unmanipulated

236 flowers exposed to natural pollination. Each treatment was represented by at least two flowers on
 237 each of 15 individuals per population ($2 \times 15 \times 6 = 180$ flowers per treatment). Fruit development
 238 was estimated two weeks after applying the treatments by determining whether the flower had
 239 dropped (unsuccessful fertilization) or a fruit had begun to form (successful fertilization). If a fruit
 240 was forming, it was bagged to prevent seed loss after maturation and seeds were collected and
 241 counted approximately six weeks later.

242 To determine whether natural populations were pollen limited, we used the pollen
 243 limitation index proposed by Larson and Barrett (2000): $L = 1 - (Op/Ps)$, where Op is the fruit or
 244 seed set in the open pollination treatment, and Ps is the fruit or seed set in the pollen
 245 supplementation treatment. We calculated the value of the index for each individual plant for fruit
 246 set (L_{FS}), seed set (L_{SS}) and pre-dispersal fitness (L_{Wpre}). We excluded individuals that
 247 lacked one of the experimental treatments. We calculated the index for pre-dispersal fitness as
 248 $L_{Wpre} = 1 - (Wpre_{Op}/Wpre_{Ps})$, where $Wpre_{Op}$ is the product of fruit set and the mean
 249 number of seeds per plant in the open pollination treatment, and $Wpre_{Ps}$ is the equivalent
 250 calculated for the pollen supplementation treatment. We subsequently calculated the mean pollen
 251 limitation index for each population. The pollen limitation index ranges from -1 to $+1$, with
 252 positive values indicating pollen limitation. We calculated 95% confidence intervals of the pollen
 253 limitation indices by bootstrapping with 1000 permutations (Gomez et al. 2010) using the *boot*
 254 package in *R* (Canty and Ripley 2014). We also analysed the effect of treatment (open pollination
 255 or pollen supplementation) on fruit set using generalised linear mixed models with binomial
 256 distribution (logit link), and on seed production using a Poisson distribution (log link). In both
 257 models, we used treatment type as a fixed effect, and both population-year and individual as
 258 random effects (*glmer* package in *R*).

259 **RESULTS**

260 The main visitors to *S. rostratum* were bees (Hymenoptera: Apoidea), and we rarely observed
261 visitors from other orders such as Diptera (in CU, TP and LP), Hemiptera, Coleoptera and
262 Lepidoptera (in TP only). In the case of Coleoptera, individuals were often observed eating the
263 corolla and/or the anthers, whereas Lepidoptera and Hemiptera did not seem to collect any reward
264 from the flowers. Diptera occasionally seemed to gather a very small amount of pollen from the
265 anther pores, but were not observed to contact the stigma during any visit. We therefore focus on
266 bees, as they were the most abundant, and the only visitors capable of collecting significant pollen
267 from *S. rostratum*, and of contacting the sexual organs during visits.

268 The diversity of bee visitors varied widely among the studied populations of *S. rostratum*.
269 TP had the highest diversity of floral visitors (Shannon-Weiner index = 1.85), followed by CU
270 (Shannon-Weiner index = 1.57), and LP had the lowest diversity index (Shannon-Weiner index =
271 0.66). Species of *Lasioglossum*, *Xylocopa* and *Apis mellifera* were common at all sites sampled
272 (Table 2). Visitor abundance differed among populations: at CU *A. mellifera* and *Exomalopsis*
273 *mellipes* represented more than half of the visitors; at LP *A. mellifera* was the most abundant
274 visitor (84% of the total); and at TP the most abundant visitors were *Augochlorella neglectula*,
275 *Exomalopsis pueblana*, *Augochlora* sp. and *Pseudaugochlora graminea* (Table 2). The most
276 abundant species also conducted most visits in their corresponding populations. However, at CU
277 rare species (e.g., *Thygater analis* and *Xylocopa* sp.) conducted as many visits as common species
278 (Table 2). Rare species (e.g., *T. analis*, *Xylocopa* sp., *Centris zacateca* and *C. mexicana*) visited
279 more flowers per bout (i.e., bout length) than other more common species at these three
280 populations (Table 2).

281 The duration of visits differed among species. Some species (*Centris spp.*, *T. analis* and
282 *Xylocopa sp.*) spent just a few seconds (1–2 sec) per visit compared with other species that spent
283 more time (4–52 sec) per visit (Table 2). Almost all *Centris*, *Thygater* and *Xylocopa* buzzed when
284 extracting pollen from flowers of *S. rostratum*. For other genera, such as *Lasioglossum*, only some
285 individuals buzzed when visiting. In contrast, *A. mellifera*, and *Augochlora sp.* did not buzz while
286 collecting pollen (Table 2).

287 **Effectiveness of legitimate and illegitimate visitors**

288 Visitors in the genera *Centris*, *Thygater*, and *Xylocopa* were classified as legitimate because
289 during most visits they contacted both the stigma and anthers of *S. rostratum* flowers (Table 3;
290 Fig. 1). The remaining 10 bee taxa were considered to be illegitimate visitors because they usually
291 did not contact the stigma (Table 3; Fig. 1). Both legitimate and illegitimate visitors had a high
292 percentage of *S. rostratum* pollen in the scopae or on their bodies (>73%; Table 2). Most of the
293 illegitimate visitors collected pollen primarily from the feeding anthers (55% of visitors) or from
294 both types of anthers (40%), rather than the pollinating anthers (5%; Table 3).

295 Population CU had the highest proportion of legitimate visitors (20% of visitors), and
296 accounted for 47% of all visits in this population (Fig. 2). By comparison, populations LP and TP
297 had a smaller proportion of legitimate visitors (3% and 2%, respectively), accounting for only
298 1.5% and 7% of the total of visits, respectively. *Apis mellifera* was the most abundant visitor in
299 population LP and performed almost all recorded visits (97%). In contrast, although *A. mellifera*
300 was abundant at site CU, it accounted for only 32% of floral visits. Similarly, *A. mellifera* was also
301 abundant at site TP, but it was rarely observed visiting *S. rostratum* flowers. Other illegitimate

302 visitors such as *Augochlora sp.*, *Exomalopsis pueblana* and *Pseudaugochlora graminea* conducted
 303 93% of the visits recorded in this population (Fig. 2).

304 The analysis of the morphological characteristics of floral visitors indicated clear
 305 differences between legitimate and illegitimate visitors. The first principal component explained
 306 91% of the variance in morphological characteristics and all eigenvectors were negative (Online
 307 Resource 1). Based on first principal component as a measure of overall visitor size, legitimate
 308 visitors were statistically larger than illegitimate visitors ($t = 4.88$, d.f. = 11.49, $P < 0.001$), which
 309 allowed legitimate visitors to contact the stigma while collecting pollen from the anthers (Table 3).
 310 In addition, the behaviour of the two types of visitors differed significantly. Legitimate visitors
 311 spent less time per flower (average visit duration 1.27 vs. 14.32 s for legitimate and illegitimate
 312 visitors, respectively; Fig. 3; Table 4), and visited more flowers per bout than illegitimate visitors
 313 (25.82 vs. 7.47 flowers on average for legitimate and illegitimate visitors, respectively; Fig. 3;
 314 Table 4). Legitimate visitors buzzed anthers during most pollination bouts, whereas illegitimate
 315 visitors included both buzzing (e.g., *Exomalopsis spp.*, *Lasioglossum spp.*, *Augochlorella*
 316 *neglectula*) and non-buzzing (e.g., *Apis mellifera*, *Augochlora sp.*) bees.

317 **Pollination efficiency**

318 A single visit by legitimate visitors (e.g., *Thygater analis* and *Xylocopa sp.*) triggered fruit
 319 production in 33% visits ($n = 12$), whereas no visits by illegitimate buzzing visitors (*E. mellipes*,
 320 *L. jubatum* and *L. Dialictus sp.*) triggered fruit set ($n = 12$). Interestingly, single visits by *A.*
 321 *mellifera*, a non-buzzing visitor, caused 18% fruit set ($n = 28$ flowers). However, the probability of
 322 setting a fruit after a single visit to a flower did not differ significantly between legitimate and
 323 illegitimate visitors (likelihood ratio test $\chi^2 = 2.498$, df = 1; $P = 0.114$). Unvisited flowers did not

324 set any fruits ($n = 10$), demonstrating that fruit production in *S. rostratum* requires pollinator
325 visitation.

326 **Reproductive biology of *S. rostratum***

327 *Solanum rostratum* strongly depended on pollinators to reproduce, and natural populations show
328 moderate pollen limitation. In the three studied populations, no fruits developed from flowers in
329 either the pollinator exclusion ($n = 153$ flowers in CU in 2009 and 2011) or emasculation ($n = 61$
330 flowers in LP in 2011, and TP in 2010) treatments. Moreover, five of six studied populations were
331 pollen-limited in both fruit production ($L_{FS} = 0.23 \pm 0.06$, CI = 0.1620–0.3815), and seed
332 number ($L_{SS} = 0.24 \pm 0.06$, CI = 0.2052–0.4458; Table 5). Similarly, the second estimate of
333 pollen limitation at the predispersal stage differed significantly from zero across all populations
334 ($L_{Wpre} = 0.33 \pm 0.07$, CI = 0.2334–0.5273; Table 5). Supplemented flowers produce
335 significantly more fruits (average fruit set 0.489 vs. 0.680 for open pollination and pollen
336 supplementation, respectively; Table 4), and slightly more seeds (53.54 vs. 57.28 for the open
337 pollination and pollen supplementation treatments, respectively; Table 4) than the open pollination
338 treatment. Our estimates of pollen limitation approximate to the effect of pollen supplementation
339 on seed set, because resource redistribution between developing fruits, and pollen-quality effects
340 (Ashman et al. 2004) could cause overestimates of pollen limitation. However, our results and
341 recent evidence of pollen limitation in invasive populations of *S. rostratum* in China (Zhang and
342 Lou 2015) suggest that reproduction in natural populations of this plant is often pollen limited.

343 **DISCUSSION**

344 Pollen larcenists commonly visit natural populations of a buzz-pollinated plant, *Solanum*
 345 *rostratum*. Both observation of stigmas contact during visitation and estimates of visitor'
 346 efficiency in producing fruits show that morphology and behaviour influence whether a floral
 347 visitor is likely to act as a pollinator or as a pollen thief. However, floral visitors should be
 348 characterized cautiously as pollinators or larcenists, as other factors such as the characteristics of
 349 the flower, and the composition of the pollinator fauna in each population, can influence the
 350 relative contribution of visitors to seed set. Even inefficient visitors can contribute to seed set
 351 when other pollinators are absent or rare. Nevertheless, the introduction of non-native species
 352 incapable of efficient pollen removal and transfer (e.g., *A. mellifera*), particularly in environments
 353 subject to intensive human-modification, is likely to alter selection on floral traits of buzz-
 354 pollinated species by increasing the frequency and magnitude of pollen larceny.

355 **Reproductive consequences of pollen theft**

356 For plants that offer pollen as the main or only reward, frequent pollen larceny can be
 357 reproductively costly (Hargreaves et al. 2009). Our study reveals that natural populations of buzz-
 358 pollinated *S. rostratum* suffer a high incidence of visits by pollen larcenists. In fact, between 55%
 359 and 95% of all visitors to *S. rostratum* act illegitimately, removing pollen but not consistently
 360 contacting the stigma. Furthermore, single visits by illegitimate visitors did not trigger fruit
 361 development. The exception was non-buzzing *Apis mellifera*, which occasionally contacted the
 362 stigma (18% of visits, Table 3), stimulating fruit set after single visits (18% fruit set). Thus, even
 363 inefficient *A. mellifera* visit may contribute to reproductive success in the absence of other more
 364 efficient pollinators.

365 We did not assess the effect of pollen larceny on male reproductive success. However,
366 pollen consumption linked with unreliable or null contact with the stigmas of other flowers
367 probably also imposes a severe male cost. For example, Lau and Galloway (2004) showed that the
368 presence of pollen-collecting halictids bees in natural populations of nectar-producing *Campanula*
369 *americana* reduces siring success. Similarly, Hargreaves et al. (2010) showed that adding pollen
370 thieves (*A. mellifera*) to experimental populations of the bird-pollinated, nectar-producing *Aloe*
371 *maculata* reduced total seed production. To our knowledge no studies have attempted to estimate
372 fitness costs of pollen theft in natural populations of buzz-pollinated species, and future work in
373 this area is critically needed.

374 **What determines whether a bee acts as a legitimate or illegitimate visitor?**

375 The efficiency of floral visitors in pollen transfer and triggering seed set varies along a continuum,
376 and therefore categorical classifications need to be considered into this context (Hargreaves et al.
377 2009). Moreover, pollinator efficiency may vary within individuals as pollen foraging has a
378 learned component (Raine and Chittka 2007), and floral larceny can be social transmitted within
379 (Leadbeater and Chittka 2008) and between species (Goulson et al. 2013). Nevertheless, our study
380 shows that, in *S. rostratum*, a major determinant of whether a bee contacts the stigma during floral
381 visitation and thus triggers seed set is based on morphological and specific behavioural
382 characteristics (i.e. the ability to buzz-pollinate). Legitimate visitors of *S. rostratum* were relatively
383 large-bodied bees, capable of buzz-pollination. They conducted short, numerous visits during a
384 pollination bout. In contrast, illegitimate pollinators were smaller bees, with or without the ability
385 to buzz-pollinate, that stayed longer in each flower and visited fewer flowers per bout. The
386 difference in body size between legitimate and illegitimate visitors should affect the likelihood that

387 they contact the sexual organs during visitation. A visitor is more likely to contact both the
388 pollinating anther and stigma if its size exceeds the separation between them (see Fig. 1, also see
389 Whalen 1979; Liu and Pemberton 2009). Given differences in flower size among plants, the same
390 visitor could act as a pollen thief in one species (larger flowers) but as a legitimate visitor in
391 another (smaller flowers; Hargreaves et al. 2009). Similarly, to the extent that the separation
392 between anther and stigma varies among populations of a single species, the same species of bee
393 may act as a legitimate or illegitimate visitor in different populations. The existence of closely
394 related species and populations in *Solanum* section *Androceras* with larger or smaller flowers
395 (Vallejo-Marín et al. 2014) provides an ideal study system to assess the extent to which the
396 correspondence between flower and visitor size influences whether a visitor becomes a pollinator
397 or a pollen thief.

398 **Pollination services by non-native bees**

399 The role of introduced *A. mellifera* as a pollinator of native plants has been a topic of debate (Aebi
400 et al. 2012; Ollerton 2012). In some cases, *A. mellifera* reduces fruit and seed set because of its
401 limited ability to transfer pollen relative to native bees (Gross and Mackay 1998; Hargreaves et al.
402 2009). In contrast, under certain conditions, such as fragmentation of habitat or low abundance of
403 native pollinators, *A. mellifera* can increase fruit and seed set in wild plants if its high foraging
404 activity compensates for its inefficiency at depositing pollen compared to native pollinators
405 (Cayuela et al. 2011; Sun et al. 2013). However, observations in agricultural crops suggest that
406 honeybees do not substitute the contribution of wild pollinators to fruit set and do not maximize
407 the fruit production in crops around the world (Garibaldi et al. 2013).

408 In our study, introduced honeybees, *Apis mellifera*, acted as conditional thieves, offering
409 some low efficiency pollination. In one study populations (LP), the vast majority of visitors and

410 visits involved honeybees (Fig. 2). This population is located in an area subject of considerable
411 human influence and habitat degradation, and it is very likely that the abundance of honeybees
412 reduces visitation by native pollinators. Whether honeybees are replacing native legitimate or
413 illegitimate visitors is currently uncertain. However, because honeybees do not buzz-pollinate (De
414 Luca and Vallejo-Marín 2013), the possible shift in pollinator community composition brought by
415 introduced species could alter the reproductive environment experienced by natural populations of
416 *S. rostratum* and other buzz-pollinated species (Dupont et al. 2004). To the extent that honeybees
417 replace larger, buzz-pollinating bees (e.g., *Thygater spp.* and *Xylocopa spp.*) in environments
418 heavily impacted by human activities, selection on traits that permit easy pollen removal (e.g.,
419 larger opening of anther pores), or that increase the probability of fruits being produced by mid-
420 sized bees (e.g., a closer distance between a flower's sexual organs) may be favoured in *S.*
421 *rostratum*. Additional work is necessary to understand the impact of non-native pollinators on the
422 reproduction and evolution of buzz-pollinated plants.

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Figure legends

Fig.1 Examples of legitimate (left panel: *Thygater analis*) and illegitimate visitors (right panel: *Augochloropsis metallica*) of *S. rostratum* in Ciudad Universitaria, Mexico City (CU) and San Juan Raya, Tehuacán, Puebla (TP), respectively. Photographs by LSM and CSM.

Fig.2 Proportions of legitimate and illegitimate visitors (n = 425, 116, and 376 visitors in populations CU, LP and TP) and the proportions of floral visits (n = 4903, 1890 and 1947 visits, respectively) observed at three sites: (a) Ciudad Universitaria, Mexico City (CU), (b) Libres, Puebla (LP), and (c) Tehuacán, Puebla (TP).

Fig.3 Box plots of the number of flowers visited in a bout (bout length, shown in \log_{10} scale; left panel), average visit duration per flower during a visitation bout (in seconds, shown in \log_{10} scale; middle panel), and thorax width (mm; right panel) between illegitimate (I) and legitimate (L) floral visitors to three Mexican populations of *S. rostratum*.

Online Resources

Online Resource 1 Principal Component Analysis (PCA) of the morphological characteristics of floral visitors of *Solanum rostratum* in natural populations in Mexico. Eigenvectors and percent variance explained by each of the first two principal components (PC1 and PC2).

Table 1 Characteristics of six populations of *Solanum rostratum* studied in central Mexico. Populations used to characterise floral visitors are identified with asterisks. Population CU was surveyed in 2009 and 2011.

Pop. Code	Population	Latitude (N)	Longitude (W)	Elevation (m)	Population size	Period of study
CU*	Ciudad Universitaria, Reserva del Pedregal, Distrito Federal.	19.32°	99.19°	2311	250	Sep-Dec 2009
					150	Aug-Sep 2011
DHG	Dolores Hidalgo, Guanajuato.	21.17°	100.90°	1891	50	Sep 2011
LP*	Libres, Puebla.	19.47°	97.67°	2373	1500	Jun-Aug 2011
SLG	San Luis la Paz, Guanajuato.	21.31°	100.51°	2002	50	Sep 2011
TEM	Teotihuacán, Estado de México.	19.68°	98.84°	2284	150	Sep 2011
TP*	San Juan Raya, Tehuacán, Puebla.	18.33°	97.57°	1670	500	Oct-Nov 2010

Table 2 Identity and characteristics of floral visitors of *Solanum rostratum* in three populations in central Mexico. **Number individuals** = number of insects visiting the flowers during the observation period. A single insect was followed since it entered the flower patch until it left (a visitation bout). **Number of floral visits** = total number of flowers visited per species; a visit was recorded if the insect landed on the flower regardless of the amount of time it spent there. **% Total visits** = percentage of visits relative to the total visits recorded in each population. **Bout length** = mean number of flowers visited \pm standard error, per species; mean was calculated as individuals per species, where we considered each visitor one visitation bout. **Visit duration in seconds** = mean length of visit \pm standard error, per species; the time that visitors spend in each visit from when they land on a flower until they leave it. **% Individuals buzzing** = percentage of individuals that produced vibrations while stationary in the flower, per species. **% *S. rostratum* pollen** = mean percentage of pollen grains from *S. rostratum* \pm standard error, per species; samples were collected from the pollen carried by the insect at the end of the visitation bout. **Thorax width** = mean widest thorax width of visitors in mm \pm standard error, per species; this was measured in a subsample of visitors per population.

Visitor	Number individuals	Number of floral visits	% Total visits	Bout length ± S.E.	Visit duration (sec) ± S.E.	% individuals buzzing	% <i>S. rostratum</i> pollen ± S.E.	Thorax width (mm) ± S.E.
Ciudad Universitaria, Distrito Federal (CU)								
<i>Apis mellifera</i>	139	1553	31.7	11.2 ± 1.1	13.3 ± 1.2	0	98.5 ± 0.5	3.9±0.1
<i>Exomalopsis mellipes</i>	133	813	16.6	6.1 ± 0.5	16.4 ± 0.7	71	93.2 ± 2.1	3.1±0.1
<i>Lasioglossum (Dialictus) sp.</i>	29	79	1.6	2.7 ± 0.4	22.2 ± 5	11	NA	1.5±0.2
<i>Lasioglossum jubatum</i>	37	139	2.8	3.8 ± 0.6	14.7 ± 1.5	19	89.2 ± 4.9	2.6±0.3
<i>Thygater analis</i>	62	1622	33.1	26.2 ± 2.8	1.2 ± 0.05	98	83.3 ± 11.1	5.1±0.2
<i>Xylocopa sp.</i>	25	697	14.2	27.9 ± 3.4	1.3 ± 0.03	100	92.5 ± 1.5	7.4±0.9
Total	425 individuals	4903 visits	100%	425 visitors	4903 visits	403 visitors	26 visitors	37 visitors
Libres, Puebla (LP)								
<i>Apis mellifera</i>	97	1810	95.8	18.7 ± 2.3	8 ± 0.2	0	98.7 ± 0.7	3.2±0.1
<i>Augochlora sp.</i>	3	13	0.7	4.3 ± 2.0	4.1 ± 0.9	0	73.0	1.8
<i>Lasioglossum (Lasioglossum) sp.</i>	8	19	1.0	2.4 ± 0.9	25.2 ± 7.3	37	100.0	3.4
Syrphid fly	4	20	1.0	5.0 ± 2.5	52.4 ± 10.3	0	NA	1.7±0.04
<i>Xylocopa sp.</i>	4	28	1.5	7.0 ± 4.3	1.1 ± 0.14	100	NA	NA
Total	116 individuals	1890 visits	100%	116 visitors	1890 visits	84 visitors	11 visitors	20 visitors
Tehuacán, Puebla (TP)								
<i>Augochloropsis metallica</i>	16	132	6.8	8.2 ± 2.0	10.6 ± 0.8	100	NA	2.9 ± 0.1
<i>Augochlorella neglectula</i>	87	342	17.6	3.9 ± 0.4	18.2 ± 1	99	78.9 ± 15.7	1.8±0.3

<i>Apis mellifera</i>	2	3	0.1	1.5 ± 0.5	2 ± 0	0	NA	NA
<i>Augochlora</i> <i>sp./Pseudoagochlora</i> <i>graminea</i>	76	558	28.7	7.3 ± 0.8	12 ± 0.5	98	93.3 ± 3.0	2.7±0.1
<i>Centris mexicana/ C.</i> <i>zacateca</i>	2	43	2.2	21.5 ± 8.5	2.1 ± 0.04	100	97.0	5.3±0.2
<i>Exomalopsis</i> <i>mellipes</i>	26	128	6.6	4.9 ± 0.8	12.2 ± 1	100	88.4 ± 6.3	3.7±0.2
<i>Exomalopsis</i> <i>pueblana</i>	102	459	23.6	4.5 ± 0.4	13.2 ± 0.5	100	93.0 ± 2.8	3.3±0.1
<i>Lasioglossum</i> <i>(Dialictus) sp.</i>	33	90	4.6	2.7 ± 0.4	22.2 ± 2.7	54	NA	1.0±0.2
<i>Lasioglossum</i> <i>(Lasioglossum) sp.</i>	28	102	5.2	3.6 ± 0.6	13.9 ± 1.2	96	91.0 ± 8.0	2.3±0.3
<i>Xylocopa cyanea</i>	4	90	4.6	22.5 ± 19.9	1.5 ± 0.06	100	NA	10.10
Total	376	1947 visits	100%	376	1947 visits	325 visitors	24 visitors	61 visitors
	individuals			visitors				

*In population TP we pooled observations of visits by *Augochlora sp.* with *P. graminea* because these species were difficult to distinguish in the field.

Table 3 Classification of legitimate and illegitimate visitors in three populations (CU, LP and TP) in central Mexico. Proportions of legitimate visits during which a visitor contacted only the stigma, both types of anthers and the stigma (FA, PA, ST), or one anther type (feeding or pollinating anther) and the stigma (FA/PA, ST) during all visits, including legitimate and illegitimate visits [if the visitor contacted only the feeding anthers (FA), the pollinating anther (PA) or both anthers types (FA, PA)].

Bee species	Sexual organ contacted during visit						Total visits contacting stigma and anthers	Total visits observed	Proportion of legitimate visits
	Anthers and stigma		Only anthers		Only stigma				
	FA/PA, ST	FA,PA, ST	FA	PA	FA, PA				
Legitimate visitors									
<i>Xylocopa cyanea</i>	0	3	0	0	0	0	3	3	1
<i>Xylocopa sp.</i>	0	26	0	0	0	0	26	26	1
<i>Thygater analis</i>	0	49	1	3	4	0	49	57	0.86
<i>Centris mexicana/</i>	0	1	0	0	1	0	1	2	0.5
<i>Centris zacateca</i>									
Sub-Total	0	79	1	3	5	0	79	88	0.9
Illegitimate visitors									
<i>Apis mellifera</i>	5	29	108	2	53	1	35	198	0.18
<i>Exomalopsis mellipes</i>	0	17	23	15	95	0	17	150	0.11
<i>Lasioglossum (Dialictus)sp.</i>	1	3	30	5	19	0	4	58	0.07
<i>Lasioglossum jubatum</i>	0	1	25	4	6	0	1	36	0.03
<i>Augochlorella neglectula</i>	1	0	57	3	22	0	1	83	0.01
<i>Augochlora sp.</i>	0	0	3	0	0	0	0	3	0
<i>Augochloropsis</i>	0	0	12	0	2	0	0	14	0

<i>metallica</i>									
<i>Exomalopsis pueblana</i>	0	0	33	5	56	0	0	94	0
<i>Lasioglossum</i>	0	0	26	0	7	0	0	33	0
<i>(Lasioglossum)sp.</i>									
<i>Pseudaugochlora</i>	0	0	58	0	6	0	0	64	0
<i>graminea*</i>									
Sub-Total	7	50	375	34	266	1	58	733	0.08
Grand Total	7	129	376	37	271	1	137	821	0.17

*In population TP we pooled observations of visits by *Augochlora sp.* with *P. graminea* because these species were difficult to

distinguish in the field.

Table 4 Effect of visitor type (legitimate vs. illegitimate) on (A) pollinator visitation, (B) pollen limitation. Pollinator visitation was analysed using a generalised linear mixed effects model with a gamma error for visit duration, and with a negative binomial for bout length -1. In the pollen visitation analysis, species and population were included as random effects. Fruit set was analysed with a generalised linear mixed effects model with binomial error, and seed set with a Poisson error. Pollen limitation analyses included population-year and plant as random effects. Only coefficients for fixed effects are shown. S.E. = standard error of the coefficient.

Response Variable	Explanatory variable	Coefficient Estimate (S.E.)	Test statistic	P-value
A) POLLINATOR VISITATION				
Visit duration	<i>Visitor type</i>			
	Illegitimate	2.613 (0.130)	$t = -14.41$	<0.001
	Legitimate	0.212 (0.166)		
Bout length	Illegitimate	1.471 (0.196)	$t = 5.163$	<0.001
	Legitimate	3.240 (0.342)		
A) POLLEN LIMITATION				
Fruit set	<i>Treatment</i>			
	Open pollination	-0.067 (0.238)	$z = 4.57$	<0.001
	Pollen supplementation	1.006 (0.235)		
Seed set	Open pollination	3.919 (0.048)	$z = 4.92$	<0.001
	Pollen supplementation	4.024 (0.213)		

Table 5 Fruit set—the percentage of flowers maturing into fruits—, and seed set—mean \pm standard error number of seeds — in six populations of *Solanum rostratum* in central Mexico. Flowers were exposed to natural pollination conditions either with (pollen supplementation treatment) or without (open pollination) addition of supplemental outcross-pollen. The mean value of the pollen limitation index (L) \pm S.E. for fruit set (L_{FS}), seed set (L_{SS}) and predispersal fitness (L_{Wpre}) are reported. Values of pollen limitation in bold are statistically significant, based on non-overlap of 95% CI (generated by bootstrapping with 1000 permutations). The values in parentheses are the number of flowers per treatment for the fruit set and the number of fruits counted in each population for the seed set.

	Population													
	CU			DHG		LP		SLG		TEM		TP		
	Fruit set	Fruit set	Seed set	Fruit set	Seed set	Fruit set	Seed set	Fruit set	Seed set	Fruit set	Seed set	Fruit set	Seed set	
	2009	2011	2011	2011		2011		2011		2011		2010		
Pollen supplementation	54.8	67.9	65.1 ± 3.5	70.4	55.6 ± 3.9	92.9	57.6 ± 3.1	50.6	65.4 ± 6.2	72.7	50.1 ± 3	67.9	58.2 ± 6.7	
	(42)	(28)	(17)	(27)	(15)	(28)	(24)	(28)	(8)	(44)	(27)	(28)	(16)	
Open pollination	42.2	50	63.5 ± 3.8	51.2	62.7 ± 3.1	62.1	46.1 ± 3.9	41.4	57.4 ± 6.2	47.4	39.1 ± 4.1	56.7	52.6 ± 4.8	
	(71)	(30)	(15)	(43)	(18)	(29)	(17)	(29)	(8)	(38)	(14)	(30)	(15)	
L_{FS}/L_{SS}	0.19 ± 0.17	0.29 ± 0.13	0.34 ± 0.12	0.19 ± 0.17	-0.41 ± 0.13	0.33 ± 0.12	0.39 ± 0.12	0.17 ± 0.19	0.43 ± 0.17	0.34 ± 0.12	0.48 ± 0.12	0.11 ± 0.14	0.15 ± 0.18	

L_Wpre	0.45 ± 0.13	0.07 ± 0.22	0.43 ± 0.13	0.31 ± 0.25	0.51 ± 0.13	0.19 ± 0.19
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