

Research



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The most effective pollinator principle applies to new invasive pollinators

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G. L. Stebbins' most effective pollinator principle states that when pollinators are not limiting, plants are expected to specialize and adapt to the most abundant and effective pollinator species available. In this study, we quantify the effectiveness of bees, hummingbirds and hawkmoths in a Chilean population of *Erythranthe lutea* (Phrymaceae), and examine whether flower traits are subject to pollinator-mediated selection by the most effective pollinator species during two consecutive years. Unlike most species in the pollinator community, the visitation rate of the recently arrived *Bombus terrestris* did not change substantially between years, which together with its high and stable pollen delivery to flower stigmas made this species the most important in the pollinator assemblage, followed by the solitary bee *Centris nigerrima*. Flower traits were under significant selection in the direction expected for short-tongue bees, suggesting that *E. lutea* is in the initial steps of adaptation to the highly effective exotic bumblebee. Our results illustrate the applicability of Stebbins' principle for new invasive pollinators, and stress their importance in driving flower adaptation of native plant species, a critical issue in the face of biotic exchange and homogenization.

1. Introduction

From the early outstanding predictions including the hawkmoth with the extraordinary tongue capable of sipping nectar from the 30 cm long Malagasy star orchid tube [1], the metrical match between flower traits and suitable body parts of pollinator species has been considered as one of the most remarkable examples of fine-tuned adaptations moulded by natural selection (e.g. [2–4]). This high level of specialization is expected to evolve in plants subject to selection by abundant and effective pollinators, that is, by those pollinator species that contribute most to the mean plant population fitness. This hypothesis, known as the 'most effective pollinator principle' after Stebbins [5], highlights the two key components of pollinator activity that determine high levels of flower adaptation to pollinators—the frequency and effectiveness of flower visits. When these criteria are not satisfied, plants are expected to evolve generalist strategies, and lack of specialization to the most effective pollinator is expected. Most studies have hitherto examined plant adaptation and specialization in native pollination systems, where pollinators and plants have a long history of interaction [6–9]. However, the arrival and establishment of exotic pollinator species in local ecosystems may quickly shift plant traits toward new optimal states, provided exotics become more abundant and effective than native pollinators.

In this study, we examine Stebbins' principle in an Andean plant–pollinator community, where bees, hummingbirds and hawkmoths converge in the use of the Andean monkeyflower, *Erythranthe lutea* (Phrymaceae). In doing so, we recorded their visitation rate and the number of pollen grains deposited on virgin stigmas to estimate their effectiveness. As floral traits involved in the attraction and mechanical fit with effective pollinators are expected to be under pollinator-mediated selection, we estimated selection coefficients on flower tube

length and corolla size, two floral traits often under pollinator-mediated selection [8–10].

2. Material and methods

(a) Natural history and study site

This study was performed in Juncal (32°51' S, 70°08' W, 2398 m elevation, Chile), during two consecutive summer seasons (2016 and 2017). *Erythranthe lutea* is a perennial self-compatible herb inhabiting streams and wetlands from sea level to 3650 m between 29° and 45° S. Even though *E. lutea* is self-compatible, pollen vectors are needed to ensure successful fertilization [10].

(b) Pollinator assemblages, pollen limitation and pollen deposition effectiveness

We recorded the mean visitation rate (V_R) per pollinator species in both years. The pollen deposition effectiveness per single visit (D_v), that is, the number of pollen grains delivered by flower visitors onto virgin stigmas, was recorded by removing and storing the stigmas just visited by a pollinator in vials containing 70% ethanol. Once in the laboratory, after fixation and staining, the number of pollen grains was recorded under a binocular microscope. The pollen deposition effectiveness per unit time (D_t) for every pollinator species was calculated by multiplying V_R , D_v and r , r being the proportion of flowers having receptive stigmas. All the field measurements were performed in the morning in a patch approximately 50 m². To maximize the number of virgin stigmas, all the flowers in anthesis were removed from the patch the night before measurements, leaving only flower buds to the next morning. In consequence, we observed first-day opened flowers and the fraction of receptive flowers at the time of observation (r) was always 1 [11]. For the purposes of this study, the index D_t is appropriate as it encapsulates in one single measure the two most important factors involved in Stebbins' principle, the frequency of visits and pollinator effectiveness.

The most effective pollinator principle states that flower evolution towards the most efficient pollinator may occur when pollen is not limited in the environment. We evaluated this criterion by estimating the pollen limitation index (L): $L = 1 - (C/S)$, where C and S are the number of seeds produced by naturally pollinated flowers, and by flowers manually supplemented with an excess of pollen, respectively. Values near to zero indicate absence of pollen limitation in the environment. Detailed information on procedures can be found in the electronic supplementary material.

(c) Phenotypic selection

We estimated the selection coefficients of putatively functional flower traits by randomly choosing 100 plants per year and recording the mean tube length (mm) and mean corolla size (mm²) from three flowers per plant (electronic supplementary material, figure S1). At the same time when flower traits were measured, we collected three capsules from the same plant and their seed number was recorded in the laboratory to have a fitness estimate through the female sex function. The strength of pollinator-mediated selection acting upon floral traits was estimated from linear selection gradients using the multiple regression approach of Lande & Arnold [12] (see electronic supplementary material for more information).

3. Results

(a) Pollinator assemblages and visitation rate

The composition of pollinator assemblages was relatively similar between years (62.5% similarity). However, when

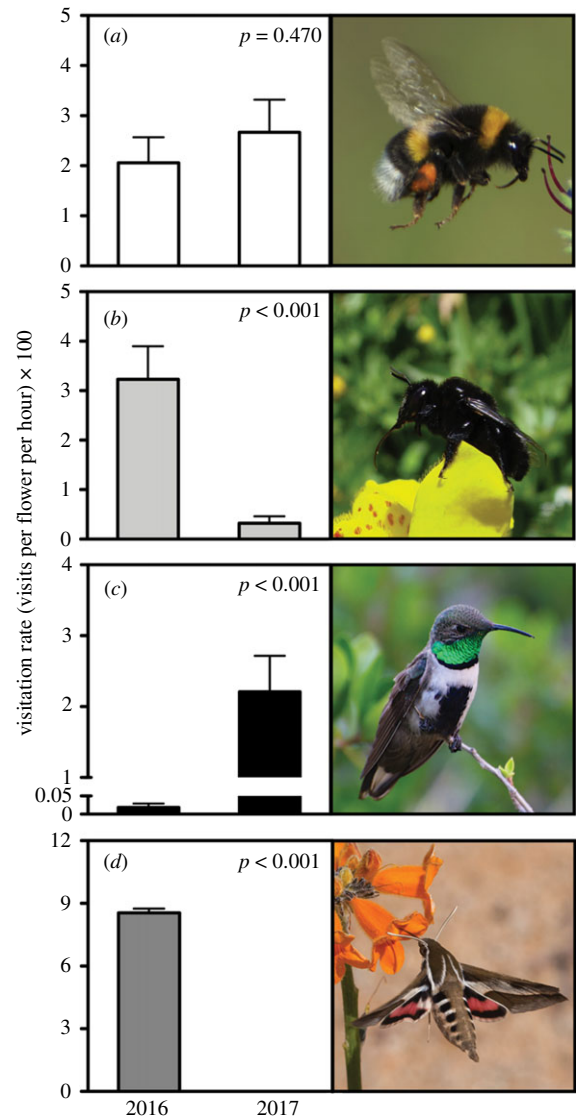


Figure 1. Mean visitation rate (s.e.) of (a) *Bombus terrestris*, (b) *Centris nigerrima*, (c) *Oreotrochilus leucopleurus* and (d) *Hyles annei* on the Andean monkeyflower, *Erythranthe lutea*, during the flowering seasons of 2016 (104 h of observation) and 2017 (138 h of observation). Credit for photograph of *H. annei*: J. P. de la Harpe.

visitation rate was included in the analysis, similarity decreased to 25%, CI: 2–52% (see electronic supplementary material). This decrease was determined to a large extent by the change in visitation rate of the solitary bee *Centris nigerrima*, the hummingbird *Oreotrochilus leucopleurus* and the hawkmoth *Hyles annei* (figure 1 and table 1). Besides native pollinators, the exotic bumblebee *Bombus terrestris* has been present in the site at least since 2009, showing an increasing abundance over the years (figure 2a), as is usually observed for invasive species in the first stages of the establishment process [14,15]. This bumblebee presented a more stable pattern, making 2.06 ± 5.46 and 2.67 ± 7.31 (mean \pm s.e.) visits per flower per hour in 2016 and 2017, respectively (figure 1a and table 1).

(b) Pollen limitation, pollination effectiveness and natural selection

The pollen limitation index was low in the two years (2016: $L = 0.28$; 2017: $L = 0.34$) (electronic supplementary material, table S1), indicating that pollinator availability was not

Table 1. Mean visitation rate \pm s.e. (V_v), pollen deposition effectiveness per single visit (D_v) and per unit time (D_t) of every flower visitor recorded on *E. lutea*. The nomenclature for pollen deposition effectiveness follows [11]. Numbers in parentheses in D_v -values show the number of stigmas analysed. Dashes in D_v columns indicate lack of stigmas for pollen counting, in most cases owing to low pollinator abundance. Percentages in parentheses in D_t -values represent the contribution of each pollinator species to the total on a yearly basis. Asterisks in D_t columns indicate that estimates were calculated using the D_v -value recorded in the alternative year.

species	visitation rate (V_v , visits per flower per hour) $\times 100$			pollen deposition effectiveness			mean
	2016	2017	2016	2017	2016	2017	
	per single visit (D_v)			per unit time ($= D_v \times V_v \times r$)			
Hymenoptera							
<i>Bombus dahlbomii</i>	0.18 \pm 1.05	0	345.8 \pm 404.7 (8)	—	0.64 (4.2%)	—	0.64 (4.3%)
<i>Bombus terrestris</i>	2.06 \pm 5.46	2.67 \pm 7.31	158.1 \pm 260.2 (126)	191.3 \pm 292.7 (39)	3.26 (21.4%)	5.10 (37.0%)	4.18 (27.9%)
<i>Centris chilensis</i>	0.85 \pm 3.12	0.42 \pm 1.67	103.2 \pm 207.1 (32)	315.6 \pm 658.2 (13)	0.88 (5.8%)	1.32 (9.6%)	1.1 (7.4%)
<i>Centris nigerrima</i>	3.23 \pm 7.16	0.32 \pm 1.54	201.1 \pm 334.8 (101)	—	6.49 (42.6%)	0.64* (4.6%)	3.57 (23.9%)
<i>Corynura chloris</i>	0	0.01 \pm 0.07	—	—	—	—	—
<i>Hypodynerus</i> sp.	0.004 \pm 0.04	0.02 \pm 0.10	—	—	—	—	—
<i>Megachile saulcyi</i>	1.33 \pm 3.61	0.03 \pm 0.13	112.4 \pm 246.6 (52)	—	1.50 (9.8%)	0.03* (0.2%)	0.77 (5.2%)
<i>Megachile semirufa</i>	0.06 \pm 0.53	0.04 \pm 0.25	—	37.0 \pm 25.0 (3)	2.22* (14.6%)	1.48 (10.7%)	1.85 (12.4%)
<i>Svastrides melanura</i>	0.01 \pm 0.11	0	38.4 \pm 33.4 (7)	—	0.01 (0.1%)	—	0.01 (0.1%)
Lepidoptera							
<i>Hyles annei</i>	8.55 \pm 22.12	0	1.3 \pm 6.2 (44)	—	0.11 (0.7%)	—	0.11 (0.7%)
<i>Pseudolucia</i> sp.	0	0.004 \pm 0.03	—	—	—	—	—
<i>Tatochila</i> sp.	0.02 \pm 0.17	0.03 \pm 0.17	—	—	—	—	—
<i>Vanessa carye</i>	0.01 \pm 0.09	0.01 \pm 0.04	0 (1)	0 (1)	0	0	0
Diptera							
<i>Scaeva melanostoma</i>	0.01 \pm 0.05	0.01 \pm 0.12	—	—	—	—	—
<i>Bombyliidae</i>	0.02 \pm 0.23	0	4 (1)	—	0.08 (0.5%)	—	0.08 (0.5%)
Apodiformes							
<i>Oreotrochilus leucopleurus</i>	0.02 \pm 0.11	2.21 \pm 6.03	—	236.5 \pm 433.1 (48)	0.05* (0.3%)	5.23 (37.9%)	2.64 (17.6%)

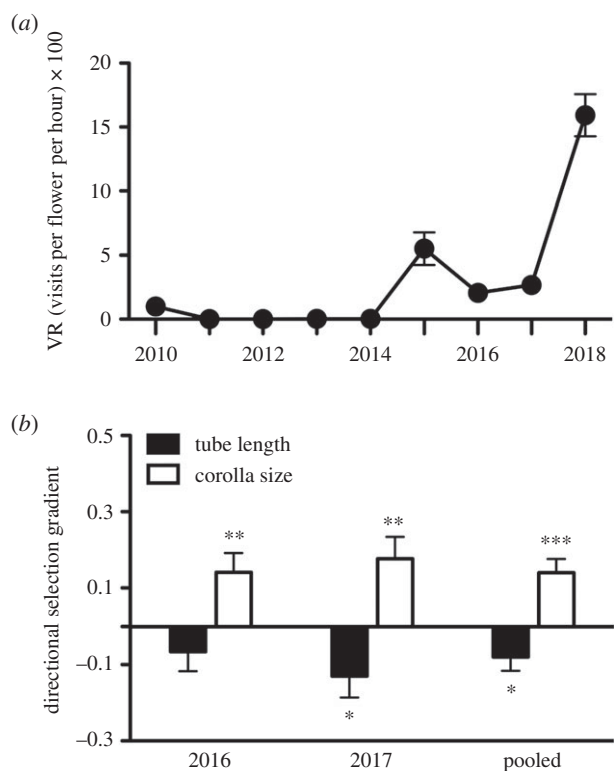


Figure 2. (a) Pattern of temporal change in the mean (s.e.) visitation rate of *Bombus terrestris* during 9 consecutive years. Data from 2010 to 2012 were obtained from [13], and those from 2013 to 2018 are unpublished from a long-term research in the study site. (b) Directional selection β -coefficients (s.e.) for tube length and corolla size in 2016, 2017 and in the 2-year pooled analysis. * $p < 0.05$, ** $p < 0.01$.

limiting in the study site, which satisfies an important criterion of Stebbins' principle.

The number of pollen grains delivered onto stigmas per pollinator species (D_v) and the resulting pollen deposition effectiveness (D_t) are reported in table 1. In 2016, the species with the highest D_t values were the solitary bee *C. nigerrima* and the exotic *B. terrestris*. In 2017, the most effective pollinators were the hummingbird *O. leucopleurus* and *B. terrestris*. Regarding mean effectiveness during the two years, *B. terrestris* was the most effective one, followed by *C. nigerrima* and *O. leucopleurus*. Likewise, the invasive bumblebee was the less variable between years (56%), followed by *C. nigerrima* (914%) and *O. leucopleurus* (19 360%), indicating that *B. terrestris* had not only the highest mean effectiveness but also the most stable one.

The two flower traits of *E. lutea* here examined were more variable between than within plants (electronic supplementary material, table S2), and represented important targets of pollinator-mediated selection (figure 2b). Pollinators were more likely to facilitate the reproduction of plants with short flower tubes in 2017 and in the 2-year pooled data ($\beta_{2017} = -0.13$, $\beta_{\text{pooled}} = -0.08$). Likewise, pollinator-mediated selection consistently favoured flowers with large corollas ($\beta_{2017} = 0.14$, $\beta_{2018} = 0.18$, $\beta_{\text{pooled}} = 0.14$).

4. Discussion

Our aim was to examine the extent to which flower traits of *E. lutea* were under selection by the most efficient pollinator species as predicted by Stebbins' principle. Combining correlational and experimental approaches, we showed that the bee *C. nigerrima*, the hawkmoth *H. annei* and the hummingbird *O. leucopleurus*

provided variable pollination service to *E. lutea*, in part because of their intermittent visitation rate to flowers and low effectiveness in pollen deposition. In spite of being established only in the last few years in the study site, *B. terrestris* was the most important pollinator for *E. lutea*. This observation is consistent with the selection to reduce flower tube length in the plant population. While flowers of *E. lutea* have a tube length of 35.8 mm on the average ($N = 100$ flowers), the shorter tongue length of *B. terrestris* (mean = 6.3 mm, range = 5.6–7.0 mm) relative to the second most important pollinator, the solitary bee *C. nigerrima* (mean = 9.0 mm, range = 8.2–9.8 mm), may favour short-tubed flowers as expected for bee-pollinated species [7,16]. Likewise, large corollas had a reproductive advantage over small-sized corollas. Previous evidence in this system indicates that bees visit corollas 1.25-fold larger than the hummingbird [10], which is consistent with the idea that *E. lutea* is in the process of adapting its flower phenotype to the effective bee pollinators. The question whether variation in the pollinator community across years reinforces the importance of *B. terrestris* in moulding the floral phenotype of *E. lutea* stresses the need for long-term studies of phenotypic selection in this system.

One important assumption of Stebbins' principle is that pollinator effectiveness is tightly coupled to the strength of pollinator-mediated selection. This assumption has been critically examined in optimality models that restrict the generality of Stebbins' model to cases where the marginal fitness gain of specialization exceeds the marginal fitness loss of adapting to the many less efficient pollinators [17]. The relatively depauperate Chilean pollinator assemblages (4.25 pollinators per plant species on the average, [18]) offer little gain from evolving generalized flower phenotypes, and, therefore, high marginal fitness loss can be expected relative to specialization on the most effective pollinator.

Although the arrival and establishment of exotic pollinators in new environments is often associated with ecological risks and unknown detrimental consequences for local communities [19], to our knowledge their importance in driving flower adaptation of native plants has been poorly addressed in the literature [20]. This is unfortunate, as we are just beginning to understand the ecological and evolutionary consequences of invasive species in the face of biotic exchange and homogenization [14,21]. It is likely that plant phenotypic and genetic adjustments caused by recently arrived pollinators are more common than previously thought. For instance, it is now well established that evolution can occur rapidly in response to invasive species [22–24], which implies that native plant responses to highly effective exotic pollinators may occur on a time scale of a few years after their arrival and establishment in new habitats. All these facts together indicate that invasive species may, under some circumstances, become quickly the most important ones in local communities, influencing not only ecological but also evolutionary processes. Results from this study suggest that novel functional roles adopted by invasive pollinators in new habitats may be more complex than previously thought, and need to be addressed before implementation of conservation programmes.

Ethics. This research was in accordance with the Ethics and Biosecurity protocols of the University of Chile (1809-FCS-UCH).

Data accessibility. Data are available as electronic supplementary material and in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.m21q0th>) [25].

Authors' contributions. R.M. planned the study and wrote the paper. All authors participated in data acquisition and contributed to the manuscript, approved the final version, and are accountable for the work.

Competing interests. We declare we have no competing interests.

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