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ORIGINAL PAPER

The relative strength of different floral visitors driving floral evolution within a *Primula secundiflora* population

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Abstract

Floral visitor assemblages within plant populations are usually composed of different visitors, and the relative abundance of these visitors also varies. Therefore, identifying the relative strength of these floral visitors driving floral evolution within the population is an important step in predicting the evolutionary trajectory of floral traits. Using supplemental hand pollination and nectar-robbing exclusion treatments, we experimentally identified the relative strengths of legitimate pollinators (that visit flowers through the corolla tube entrance) and nectar robbers (that visit flowers by biting a hole in the corolla tube or using an existing hole) driving floral evolution within the *Primula secundiflora* population. We also estimated legitimate pollinator- and nectar robber-mediated selection separately for pin and thrum flowers. Both legitimate pollinators and nectar robbers mediated selection on pollination efficiency traits in *P. secundiflora* population. Legitimate pollinators mediated selection for wider corolla tubes, whereas nectar robbers mediated selection for longer corolla tubes. In addition, nectar robber-mediated selection on corolla tube length marginally varied between the pin and thrum flowers. Nectar robber mediated selection for longer corolla tube length in the pin flowers not in the thrum flowers. These results indicate that legitimate pollinators and nectar robbers within a population can drive differential evolutionary trajectories of floral traits.

Keywords Floral evolution · *Primula secundiflora* · Legitimate pollinator-mediated selection · Nectar robber-mediated selection · Female fitness

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Introduction

Identifying the causes of variation in floral evolution is key to understanding adaptive differentiation and speciation in local populations (MacColl 2011). In angiosperm plants, spatial and temporal variations in visitor assemblages will lead to differential evolutionary trajectories of floral traits, which is supported by increasing evidence in natural populations (Fenster et al. 2004; Harder and Johnson 2009; Brunet 2009; Gómez et al. 2009; Van der Niet et al. 2014; Chapurlat et al. 2015; Wu and Li 2017). However, visitor assemblages within a plant population are usually composed of different visitors, and the relative abundance of these visitors also varies (Zhao and Huang 2013; Wu and Li 2017). These floral visitors may cause divergent selective pressures, thus driving differential floral evolution. For example, the nectar guide size of *Mimulus luteus* increases with the increasing proportion of hummingbirds in the visitor assemblages, but the mean corolla size decreases with the increasing proportion of lepidopterans in the visitor assemblages (Medel et al. 2007). Therefore, directly identifying the relative strength of these floral visitors driving floral evolution within a population is an important step in predicting the evolutionary trajectory of floral traits.

In most *Primula* species (exceeding 90%), reproductive success relies on floral visitors (Mast and Conti 2006; Richards 2014). Variation in visitor composition may result in different selective pressures on floral traits (Ehrlén et al. 2002; Toräng et al. 2008; Vanhoenacker et al. 2010, 2013). In *Primula secundiflora* populations, the dominant visitor assemblages (bumblebees) are commonly composed of two main floral visitors (Zhu et al. 2015; Wu and Li 2017). One visitor commonly visits the flower through the corolla tube entrance, whereas the other visitor commonly visits the flower by biting a hole in the corolla tube or using a hole already made by other robbers (Fig. 1). According to the theory of Inouye (1980), we define the former as a legitimate pollinator and the latter as a nectar robber. In primrose populations, legitimate pollinators always increase female fitness for one morph and male fitness for the reciprocal morph because of asymmetrical disassortative mating (Charlesworth and Charlesworth 1979; Lloyd 1979; Lloyd and Webb 1992; Zhu et al. 2015). Legitimate pollinators may cause different reproductive success between the pin and thrum flowers in *P. secundiflora* and thus may generate different selective pressures on floral traits. Nectar robbing widely exists in tubular flowers, and most nectar robbing leads to fitness costs by negatively affecting pollinator visitation (Schlindwein et al. 2014; Maruyama et al. 2015; Geerts 2016). This will create conflicting selection on floral traits by legitimate pollinators and nectar robbers (Irwin et al. 2008; Irwin and Brody 2011; Wang et al. 2013; Maruyama et al. 2015). However, nectar robbers in *P. secundiflora* populations can increase fitness benefits. In particular, nectar robbers commonly improve pollen transfer from pin flowers to thrum flowers (Zhu et al. 2010, 2015). This may also cause different reproductive success between the pin and thrum flowers.

Indeed, morph-specific variation in reproductive success may lead to divergent evolutionary trajectories of floral traits (Nishihiro et al. 2002). For example, morph differences in the reproductive success of *Primula sieboldii* lead to selection for a higher stigma in the thrum flowers but not in the pin flowers (Nishihiro et al. 2002). Because of different pollination success between morphs, selection on corolla tube length is stronger in the thrum flowers than in the pin flowers in *P. vulgaris* and *P. veris* (Kálmán et al. 2007). In total, both legitimate pollinators and nectar robbers within a *P. secundiflora* population may cause variation in reproductive success between morphs and thus may generate differential selective pressures on floral traits. Studies that experimentally identify the relative strength

Fig. 1 Legitimate pollinator (**a**), nectar robber (**b**), and nectar-robbing hole (**c**) and its location on the corolla tube (**d**) in *Primula secundiflora*. The photo in d is from Zhu et al. (2010)

of these floral visitors driving floral evolution and how it varies between morphs are helpful for fully understanding the role of floral visitor-mediated selection on floral traits in heterostylous taxa.

In generalized pollination systems, there are two main approaches to separately identify the relative strength of legitimate pollinators and nectar robbers driving floral evolution. One is to compare the differences in selection between supplemental hand- and open-pollinated plants in populations. If a legitimate pollinator exerts selection on floral traits through fitness functions, then selection should be stronger in open-pollinated plants than in supplemental hand-pollinated plants (Sletvold and Ågren 2010; Bartkowska and Johnson 2012; Lavi and Sapir 2015). The second approach is to directly exclude the effects of nectar robbers on fitness functions (i.e., nectar robber exclusion treatment) and then compare the differences in selection between nectar robber exclusion-treated and non-nectar robber exclusion-treated plants in populations. If nectar robbers exert selection on floral traits through fitness functions, then the selection will differ between nectar robber exclusion-treated and non-nectar robber exclusion-treated plants (Brody 1997; Irwin and Brody

2011). In this study, we simultaneously used these two approaches to separately identify legitimate pollinator- and nectar robber-mediated selection on floral traits within the *P. secundiflora* population.

Primula secundiflora is a typical distylous, self- and intra-morph incompatible perennial herb. The reproductive success of this species relies on pollinators (Zhu et al. 2010; Wu and Li 2017). In the present study, we experimentally identified the relative strength of legitimate pollinator- and nectar robber-mediated selection on floral traits through female function. We also estimated legitimate pollinator- and nectar robber-mediated selection separately for the pin and thrum flowers. We quantified selection for five floral traits (flowering start, plant height, number of flowers, corolla tube length and corolla tube width), which are likely to influence the attractiveness for and pollination efficiency of floral visitors. We specifically want to know (1) whether legitimate pollinators and nectar robbers drive differential evolutionary trajectories of floral traits and (2) whether legitimate pollinator- and nectar robber-mediated selection on floral traits varies between the pin and thrum flowers.

Materials and methods

Study species and site

Primula secundiflora is a distylous [long-style and short-anther phenotype (pin); short-style and long-anther phenotype (thrum)], self- and intra-morph incompatible perennial herb that is widely distributed in the alpine regions of southwest China. This herb produces leaves in a basal rosette and typically has 3–43 flowers in a single umbel. The flowering period is from May to August, and the fruiting period is from August to September.

The studied primrose population was located in an open, wet grassland in Potatso National Park in Shangri-La, Southwest China (99°54′39.337″E, 27°48′48.66″N, 3424 m.a.s.l). Dominant floral visitors (bumblebees) to the population were composed of two main visitors: legitimate pollinators and nectar robbers. Legitimate pollinators, *Bombus convexus*, visited the flowers through the corolla tube entrance, whereas nectar robbers, *B. lucorum*, visited the flowers by biting a hole in the corolla tube or using an existing hole (Fig. 1). *Bombus richardsi* and *B. atrocinctus* always shift roles between legitimate pollinators and nectar robbers during visitation (Y. Wu, *personal observation*). When there is a hole in the corolla tube, *B. richardsi* and *B. atrocinctus* commonly use it to visit the flower; when there is not a hole in the corolla tube, these two bees commonly visit the flower through the corolla tube entrance. In addition, nectar robbers were composed of the primary nectar robbers and secondary nectar robbers in the primrose population (Y. Wu, *personal observation*). The flowers of *P. secundiflora* were always simultaneously visited by legitimate pollinators and nectar robbers. The intensity of nectar robbing was high in the population, with the percentage of flowers per plant robbed exceeding 80% (Y. Wu, *personal observation*).

Field experiments

To examine the independent effects of legitimate pollinators and nectar robbers on the phenotypic selection on floral traits, we separately manipulated the intensity of legitimate pollinators and nectar robbers in pollination treatment design (nectar robbing exclusion vs. nectar robbing exclusion + supplemental hand pollination, and open-pollinated control vs. nectar robbing exclusion, respectively).

In late May 2017, 480 individuals with flower buds were randomly selected and individually marked (240 pin individuals and 240 thrum individuals). We randomly assigned these individuals to each of the three pollination treatments (160 individuals for each treatment, including 80 pin individuals and 80 thrum individuals): open-pollinated control (C), nectar robbing exclusion + supplemental hand pollination (NREx + HP) and nectar robbing exclusion (NREx). For the NREx + HP treatment, all open flowers were first hand pollinated with cross-pollen (i.e., pin flowers were pollinated with pollen from the thrum flowers, and thrum flowers were pollinated with pollen from the pin flowers) from other non-treated individuals located at least 10 m from the target individual. Then, flowers were marked using transparent adhesive tape around the corolla tube (Fig. S1). This prevented nectar robbers from pricking holes in the corolla tube and removed the effect of nectar robbers. All flowers received supplemental hand-pollination at least once and NREx treatment through the flowering period. For the NREx treatment, all open flowers of each individual were marked using transparent adhesive tape around the corolla tube (Fig. S1). All flowers received NREx treatment through the flowering period. The transparent adhesive tape treatment did not influence the effect of legitimate pollinators (Table S1). In addition, nectar robbers did not try to breach the tape (Y. Wu, *personal observation*). The primrose population was visited three times per week through the flowering period. On each visit, we hand-pollinated new open flowers, placed transparent adhesive tape around the corolla tube for new open flowers and checked the previous transparent adhesive tape to ensure that it remained.

We recorded flowering start (Julian day, day of the year) for each individual when the first flower opened. At the onset of flowering, we measured the plant height of each individual in the experiment (distance from the ground to the topmost flower to the nearest 0.1 cm). For the first three open flowers of each individual, we measured corolla tube length (distance from the corolla tube entrance to the corolla tube bottom) and corolla tube width (width of the corolla tube entrance) to the nearest 0.01 mm with digital callipers. We recorded the number of flowers for each individual at the end of the flowering period.

To quantify female reproductive success, we recorded the number of fruits at maturation and collected all fruits from each individual to determine the number of seeds per fruit. For each individual, we estimated total seed production as a proxy of female fitness.

Statistical analysis

Two-way ANOVA was used to test the effects of morph (pin vs. thrum) and pollination treatments (C, NREx + HP and NREx) on floral traits (flowering start, plant height, number of flowers, corolla tube length and corolla tube width) and female reproductive success (fruit production, seeds per fruit and female fitness). To improve the normal distribution of the data, flowering start, plant height, number of flowers, corolla tube length, corolla tube width, fruit production, seeds per fruit and female fitness data were $\log_{10}$ transformed prior to analysis. The log-transformed data were only used for two-way ANOVA and not for other analyses in the present study.

Following the methods of Lande and Arnold (1983), we used multiple regression analysis to estimate net directional selection, legitimate pollinator-mediated selection and nectar robber-mediated selection. In the regression models, we separately used relative female fitness (individual female fitness/mean female fitness; using the original data and not the log-transformed data) as the response variable and the standardized five floral traits (with a mean of 0 and a variance of 1; using the original data and not the log-transformed data) as the explanatory variables. Due to the differences in corolla tube width and female fitness between the pin and thrum flowers, we estimated relative female fitness and standardized the floral traits separately for each morph and pollination treatment. In addition, we estimated selection gradients separately for each morph and pollination treatment. We quantified the selection gradients (β_i) using multiple linear regression models. We initially included cross-product terms (γ_{ij}, between floral traits) to quantify correlational selection. A few correlational selection gradients were statistically significant, and the variance inflation factors (VIFs) exceeded 10, which indicated substantial multicollinearity in these models (Quinn and Keough 2002). Consequently, we did not include the cross-product terms in the linear regression models. To test for multicollinearity in these linear regression models, we calculated the VIFs for the linear terms. All VIFs were < 2.1, indicating no multicollinearity problem (Quinn and Keough 2002).

To examine whether net directional selection varied between morphs, we used data from C plants in ANCOVA. In the model, we used relative female fitness as the response variable. We used the five standardized traits (flowering start, plant height, number of flowers, corolla tube length and corolla tube width), morph and trait × morph as the explanatory variables.

To test whether legitimate pollinator-mediated selection varied between morphs, we used data from both NREx + HP and NREx plants in ANCOVA. In the model, we used relative female fitness as the response variable. We used the five standardized traits, morph, pollination (NREx + HP vs. NREx), trait × morph, trait × pollination and trait × morph × pollination as the explanatory variables. Significant trait × morph × pollination indicated that legitimate pollinator-meditated selection varied between morphs. We further tested the effect of pollination treatments (NREx + HP vs. NREx) on linear selection gradients separately for each morph to determine whether there was significant legitimate pollinator-mediated selection.

To test whether nectar robber-mediated selection varied between morphs, we used data from both C and NREx plants in ANCOVA. In the model, we used relative female fitness as the response variable. We used the five standardized traits, morph, pollination (C vs. NREx), trait × morph, trait × pollination and trait × morph × pollination as the explanatory variables. A significant trait × morph × pollination term indicated that nectar robber-mediated selection varied between morphs. We further tested the effects of pollination treatments (C vs. NREx) on linear selection gradients separately for each morph to determine whether there was significant nectar robber-mediated selection.

To quantify legitimate pollinator-mediated selection, we subtracted the estimated selection gradients for each trait of NREx + HP plants ($\beta_{\text{NREx+HP}}$) from the estimate obtained for NREx plants (β_{NREx}) ($\Delta\beta_{\text{n-poll}} = \beta_{\text{NREx}} - \beta_{\text{NREx+HP}}$). To quantify nectar robber-mediated selection, we subtracted the estimated selection gradients for each trait of NREx plants (β_{NREx}) from the estimate obtained for C plants (β_{C}) ($\Delta\beta_{\text{n-robb}} = \beta_{\text{C}} - \beta_{\text{NREx}}$) (Irwin and Brody 2011; Chapurlat et al. 2015; Sletvold et al. 2015).

All analyses were performed with R 3.3.2. We used Excel (2007) and Photoshop CS4 to generate the graphs.

Results

Floral traits and female reproductive success

Corolla tube width varied between morphs ($F_{1,431} = 393.23$, $P < 0.001$) (Tables 1, S2). Corolla tube width was wider in the pin flowers than in the thrum flowers. The number of flowers, corolla tube length and corolla tube width did not vary among pollination treatments (Tables 1, S2). Flowering start and plant height varied among pollination treatments, but the variations in these two traits were weak (Table 1).

Pollination treatments significantly influenced female reproductive success in *P. secundiflora* (Tables 1, S2). The NREx + HP treatment increased female fitness for the pin and thrum flowers by 26.5% and 22.7%, respectively, compared to that of the C plants. The NREx treatment increased female fitness by 4.6% but reduced it by 16.1% for the pin and thrum flowers, respectively, compared to that of the C plants.

Phenotypic selection

Net directional selection on corolla tube length varied between morphs, as indicated by the significant morph × trait interaction obtained with ANCOVA ($F_{1,132} = 3.994$, $P = 0.048$). Longer corolla tube length was selected in the pin flowers; however, selection on this trait was not detected in the thrum flowers (Table S3; Fig. 2a, b). Increased flower production and wider corolla tube width were selected in both the pin and thrum flowers (Table S3; Fig. 2a, b).

Legitimate pollinator-mediated selection on floral traits did not vary between morphs ($P > 0.05$). Legitimate pollinator-mediated selection for taller plants was detected in the

Table 1 Floral traits and female reproductive success (mean ± SD) for the pin and thrum flowers of *P. secundiflora*

Traits	Pin n = 72/74/71	Thrum n = 72/73/70	Reproductive success	Pin n = 72/74/71	Thrum n = 72/73/70
Flowering start (Julian day)			*Fruit production*		
C	**165.3 ± 0.9**	**164.6 ± 0.9**	C	**8.5 ± 4.8**	**8.9 ± 4.6**
NREx + HP	**165.9 ± 1.3**	**165.5 ± 1.3**	NREx + HP	**10.9 ± 4.2**	**10.2 ± 5.1**
NREx	**165.4 ± 1.3**	**165.3 ± 1.3**	NREx	**9.5 ± 5.4**	**7.6 ± 5.0**
Plant height (cm)			*Seeds per fruit*		
C	**27.1 ± 6.7**	**27.0 ± 7.1**	C	**85.5 ± 24.1**	**79.3 ± 17.5**
NREx + HP	**27.1 ± 5.2**	**28.2 ± 5.3**	NREx + HP	**89.4 ± 20.1**	**87.0 ± 16.1**
NREx	**28.8 ± 6.2**	**28.7 ± 5.3**	NREx	**83.7 ± 16.9**	**70.9 ± 23.4**
Number of flowers			*Female fitness*		
C	13.8 ± 5.9	14.1 ± 7.6	C	**797.0 ± 539.7**	**748.7 ± 478.6**
NREx + HP	13.7 ± 4.6	13.6 ± 5.1	NREx + HP	**1008.6 ± 542.0**	**918.5 ± 535.7**
NREx	13.9 ± 5.6	13.9 ± 4.3	NREx	**833.7 ± 581.8**	**628.4 ± 527.0**
Corolla tube length (mm)					
C	10.07 ± 0.82	10.04 ± 0.75			
NREx + HP	9.90 ± 0.68	10.04 ± 0.59			
NREx	10.07 ± 0.66	10.00 ± 0.50			
Corolla tube width (mm)					
C	**3.50 ± 0.38**	**2.96 ± 0.29**			
NREx + HP	**3.55 ± 0.27**	**2.87 ± 0.31**			
NREx	**3.56 ± 0.43**	**2.95 ± 0.24**			

Sample sizes (n) are given in the following order: C: open-pollinated control, NREx + HP: nectar robbing exclusion + supplemental hand pollination, and NREx: nectar robbing exclusion. Significant differences ($P < 0.05$) in floral traits and female reproductive success between the morphs and/or among pollination treatments are indicated in bold

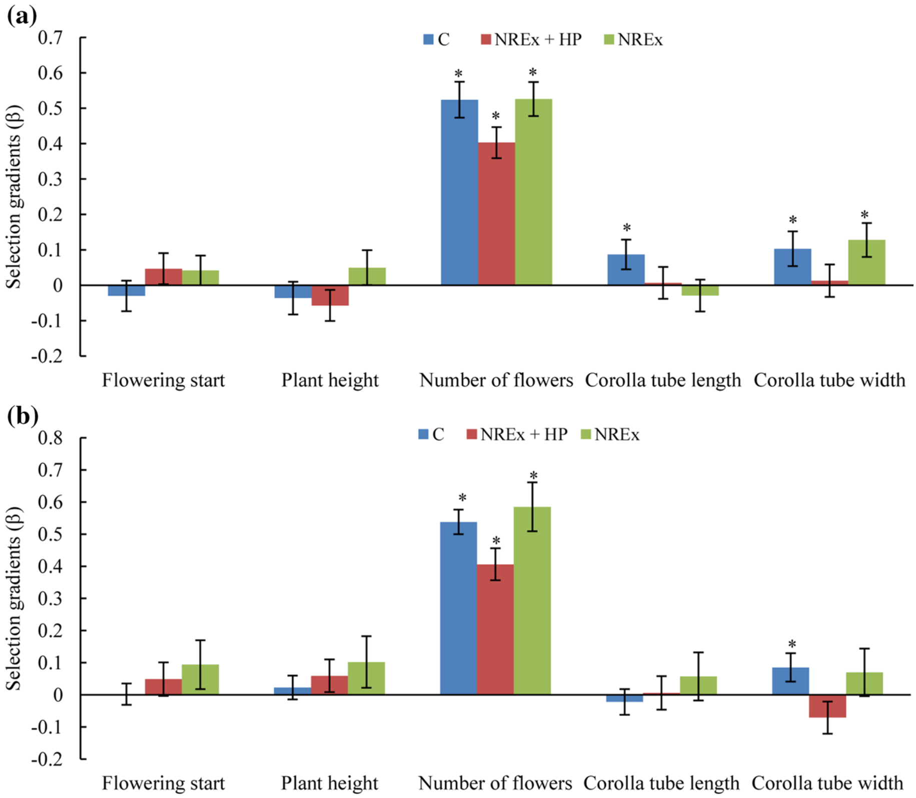

Fig. 2 Linear selection gradients ($\beta_i \pm SE$) on floral traits in the pin (**a**) and thrum (**b**) flowers in *P. secundiflora* population. Symbols above individual bars indicate the level of significance of the gradient. *$P < 0.05$

pin flowers (Table S3; Figs. 3a, S2a). Legitimate pollinator-mediated selection increased flower production in both the pin and thrum flowers but only partly [(0.123/0.524) * 100% = 23.5%; (0.179/0.538) * 100% = 33.3%] explained the net directional selection (Table S3; Figs. 3a, b, S2b, c). Legitimate pollinator-mediated selection for wider corolla tube width was marginally significant in the pin ($\Delta\beta_{n\text{-poll}} = 0.115$, $P = 0.087$) but not in thrum flowers ($\Delta\beta_{n\text{-poll}} = 0.141$, $P = 0.114$), and explained all of the selection on this trait (Table S3; Fig. 3a, b).

Nectar robber-mediated selection on corolla tube length marginally significantly varied between morphs ($F_{1,262} = 3.09$, $P = 0.080$). Nectar robber-mediated selection for longer corolla tube length was marginally significant in the pin flowers ($\Delta\beta_{n\text{-robb}} = 0.116$, $P = 0.067$) and explained all of the selection on this trait (Table S3; Figs. 3a, S2d). However, nectar robber-mediated selection on this trait was not detected in the thrum flowers.

Discussion

This study demonstrates that both legitimate pollinators and nectar robbers mediate selection on pollination efficiency traits in *P. secundiflora* population. Legitimate pollinators mediate selection for wider corolla tube widths, whereas nectar robbers mediate selection

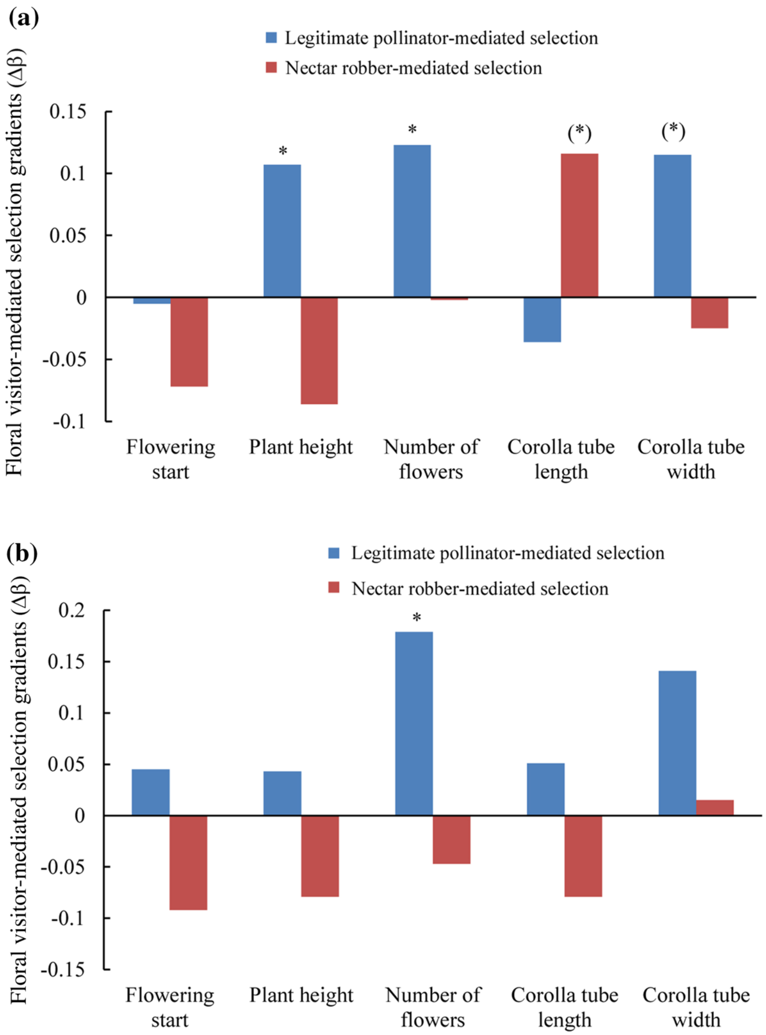

Fig. 3 Floral visitor-mediated selection on floral traits in the pin (**a**) and thrum (**b**) flowers in *P. secundiflora* population. Symbols above individual bars indicate the level of significance of the gradient (significant trait × pollination interaction). $*P<0.05$; $(*)P<0.1$

for longer corolla tubes. In addition, nectar robber-mediated selection on corolla tube length marginally varied between the morphs.

In many cases, pollinators and non-pollinators commonly use the same cues to distinguish individual plants and thus may always cause conflicting selection on the same floral traits (Strauss and Irwin 2004; Elzinga et al. 2007; Kessler and Halitschke 2009; Sletvold et al. 2015). Due to the trade-offs in attracting pollinators as well as seed predators, the calyx length of *Castilleja linariaefolia* experiences conflicting selective pressures by these two agents (Cariveau et al. 2004). In *Primula farinosa*, selection exerted by grazers favours the short-scaped morph, whereas pollinator-mediated selection favours the long-scaped morph (Ågren et al. 2013). However, our results indicated that legitimate pollinators and nectar robbers did not generate conflicting selection on floral traits. Indeed, these two floral visitors mediated selection on different target floral traits within the primrose population. Legitimate pollinators mediated selection for wider corolla tube widths, whereas nectar robbers mediated selection for longer corolla tube lengths. In tubular flowering plants, corolla tube width and corolla tube length always influence the morphological compatibility between flower and visitor, thus causing different reproductive success. As a result, these two traits always were exposed to selective pressures from visitors (Boberg and Ågren 2009; Huang et al. 2016). As far as we know, wider corolla tubes are expected to improve the mechanical fit between legitimate pollinators and flowers, thus increasing pollination efficiency. Long flower tubes have traditionally been viewed as a floral adaptation for pollination by long-tongued pollinators (Darwin 1859, 1877; Fenster 1991). However, nectar robbers commonly visit flowers by biting a hole in the corolla tube or using a hole already made by other insects. This seems to be a counter-selective force for increasing corolla tube length by nectar robbers (Lara and Ornelas 2001). Similar selective pressures are also indicated in *Salvia Mexicana*, *S. iodantha*, *Ipomoea hederifolia* and *Duranta erecta* (Lara and Ornelas 2001; Navarro and Medel 2009). In *D. erecta*, nectar robbers commonly generate selective pressure for longer flower tubes, and this pressure will balance specialized selection by legitimate pollinators (Navarro and Medel 2009). Natural robbery will reduce the attractiveness of flowers to legitimate pollinators, thus decreasing pollination success (Temeles and Pan 2002). However, a longer corolla tube may increase the visitation of nectar robbers (i.e., increasing accumulation of nectar volume) and thereby increase pollen transfer from the pin flowers to the thrum flowers in *P. secundiflora*.

Intriguingly, morph-specific variation in nectar robber-mediated selection on corolla tube length was observed. However, these findings deviate from the predictions in *P. vulgaris* and *P. veris* of stronger selection on corolla tube length in thrum flowers than in pin flowers (Kálmán et al. 2007). Our results revealed stronger selection for longer corolla tubes in the pin flowers than in the thrum flowers. Longer corolla tubes in the pin flowers may be beneficial to the accumulation of nectar volume and thus may increase the attractiveness of flowers to nectar robbers. Due to the positive effect on pollen transfer by nectar robbers, this selective pressure on corolla tube length may increase pollination success. In contrast, nectar robber-mediated selection for longer corolla tubes in the pin flowers increases the distance between stigma and anther and may affect the reciprocal herkogamy and morphological match between morphs (Kálmán et al. 2007; deVos et al. 2014), thus reducing pollination efficiency and reproductive success (Ferrero et al. 2011). Furthermore, it may be harmful to the maintenance of the heterostylous syndrome and may sometimes lead to the breakdown of heterostyly (Yuan et al. 2017). The benefits and costs of selection for longer corolla tubes imply that morph-specific variation in nectar robber-mediated selection on this trait may not persist in this primrose species.

In the present study, higher flower production was selected. As one of the most important attractive traits, higher flower production always creates a stronger floral display and advertisement and then obtains greater pollinator preference, thus increasing potential seed production (Grindeland et al. 2005; Sandring and Ågren 2009; Parachnowitsch and Kessler 2010). In addition, pollination treatments indicate that selection for higher flower production is attributed to legitimate pollinators and other agents (except for nectar robbers) in the experimental primrose population. This reflects shifts in the relative importance of legitimate pollinators and other agents (except for nectar robbers) on pollination success (Sletvold and Ågren 2010).

The importance of plant stature for pollination success should depend on vegetation height (Toräng et al. 2006), and field experiments suggest that pollinator-mediated selection on plant stature is stronger in tall plants than in short plants (Ehrlén et al. 2002; Ågren et al. 2006). Our results support this hypothesis. Intriguingly, legitimate pollinators mediate selection for taller plants with pin flowers, but the selective pressure is counteracted by other agents. At present, the limited experimental data partly suggest that legitimate pollinator-mediated selection on plant height may be limited by nectar robbers. However, the exact additive effects of legitimate pollinator- and nectar robber-mediated selection on this trait are unclear.

Estimates of selection on flowering phenology were not significant. In addition, the NREx + HP and NREx pollination treatments did not influence the selection on flowering phenology. This may suggest no direct links between flowering phenology and female reproductive success in the *P. secundiflora* population or that flowering phenology is not the target trait mediated by these two floral visitors. Furthermore, flowering phenology and other floral traits may be correlationally selected by pollinators because of an additive effect of these traits on pollination success (Chapurlat et al. 2015).

The present study has two limitations. One limitation of our estimates of the relative strength of legitimate pollinator- and nectar robber-mediated selection on floral traits is that we only consider the association between floral traits and female fitness. Variance in fitness may be higher for male function than for female function (Arnold and Wade 1984). Therefore, the use of only female function might underestimate the pollinator's effects on floral evolution. To comprehensively explore the pollinator-mediated selection on floral traits, analyses for both sex functions are needed (van Kleunen and Ritland 2004; Hodgins and Barrett 2008). The second limitation is that we only use a linear regression model to estimate directional selection on floral traits. Due to the specific and complex pollinator-plant interactions, floral traits may be under quadratic or correlational selection (Chapurlat et al. 2015). To address these issues, a sufficient sample size and multiple nonlinear regression analysis are needed in the future.

By using supplemental hand pollination and nectar-robbing exclusion treatments, we have directly shown the relative strength of different floral visitors driving floral evolution within the *P. secundiflora* population. The results of the present study indicate that legitimate pollinators and nectar robbers can mediate differential evolutionary trajectories of floral traits. This shows the potential force of different floral visitors driving floral evolution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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