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RESEARCH PAPER

Nectar robbers influence the trait–fitness relationship of *Primula secundiflora*

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Direct and indirect effects; inter-morph variation; nectar robbers; *Primula secundiflora*; trait–fitness relationship.

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ABSTRACT

- The trait–fitness relationship influences the strength and direction of floral evolution. To fully understand and predict the evolutionary trajectories of floral traits, it is critical to disentangle the direct and indirect effects of floral traits on plant fitness in natural populations.
- We experimentally quantified phenotypic selection on floral traits through female fitness and estimated the casual effects of nectar robbing with different nectar robbing intensities on trait–fitness relationships in both the L- (long-style and short-anther phenotype) and S-morph (short-style and long-anther phenotype) flowers among *Primula secundiflora* populations.
- A larger number of flowers and wider corolla tubes had both direct and indirect positive effects on female fitness in the *P. secundiflora* populations. The indirect effects of these two traits on female fitness were mediated by nectar robbers. The indirect effect of the number of flowers on female fitness increased with increasing nectar robbing intensity. In most populations, the direct and/or indirect effects of floral traits on female fitness were stronger in the S-morph flowers than in the L-morph flowers. In addition, nectar robbers had a direct positive effect on female fitness, but this effect varied between the L- and S-morph flowers.
- These results show the potential role of nectar robbers in influencing the trait–fitness relationships in this primrose species.

INTRODUCTION

In angiosperms, the trait–fitness relationship influences the strength and direction of floral evolution (Harder & Johnson 2009; Van der Niet *et al.* 2014). The traits are expected to have direct and indirect effects on plant fitness in natural populations, and the indirect effects are commonly mediated by biotic factors, with the exception of pollinators (Scheiner *et al.* 2000; Valdés & Ehrlén 2017). For example, increased flower production will increase the attractiveness to pollinators, thus directly increasing plant fitness (Sandring & Ågren 2009; Cuartas-Domínguez & Medel 2010; Parachnowitsch & Kessler 2010). However, larger floral display sizes can also increase the attractive capabilities of flowers to non-pollinators, which may be harmful to the visitation of pollinators (*i.e.* nectar thieves reduce nectar accumulation, thus reducing the visitation of pollinators) and therefore indirectly reduce fitness (Arizmendi *et al.* 1996; Maloof & Inouye 2000; Maloof 2001; Irwin & Brody 2011; Maruyama *et al.* 2015). Thus, biotic factors may mediate both the direct and indirect effects of floral traits on fitness and generate diverse selection pressures on floral traits. To fully understand and predict the evolutionary trajectories of floral traits, it is critical to disentangle the direct and indirect effects of floral traits on plant fitness in natural populations.

Nectar robbing widely exists in natural populations of tubular flowers (Irwin & Maloof 2002; Maruyama *et al.* 2015). In most cases, nectar robbing will cause a reduction in plant fitness by reducing the attractive capabilities of flowers to pollinators or changing the reproductive strategy of plants (Maloof & Inouye 2000; Deng *et al.* 2004; Irwin 2009; Greets 2016). It influences trait–fitness links and may generate selection pressures on floral traits to avoid, remove or weaken the disadvantage (Irwin *et al.* 2010). However, nectar robbers can offer the plant fitness benefits by indirectly influencing the behaviours of pollinators or directly increasing fitness production in some cases (Hernandez & Toledo 1979; Zhu *et al.* 2010). First, nectar robbers reduce the nectar accumulation of flowers, potentially increasing the foraging area of the pollinators to collect enough resources (Lara & Ornelas 2002) and reducing the time spent on each flower (Mayer *et al.* 2014; Zhang *et al.* 2014), thus increases the pollination efficiency per unit time, reduces geitonogamy and increases outcrossing. Second, nectar robbers will directly increase plant fitness (*i.e.* touching the sexual organs; Navarro 2000). For example, nectar robbers will promote pollen transfer from the L-morph flowers (long-style and short-anther phenotype) to the S-morph flowers (short-style and long-anther phenotype) in *Primula secundiflora* populations, thus directly increasing fitness benefits (Zhu *et al.* 2010, 2015). As a result, this specific floral visitor will have diverse and complex effects on plant fitness in natural populations, which may influence the trait–fitness relationships and mediate the direct and/or indirect effects of floral traits on fitness.

In natural populations of perennial plants with complex plant–visitor interactions, traits not only directly link to fitness production but also indirectly link through other paths in some cases (Conner 1996; Scheiner *et al.* 2000). At present, research indicates both the direct and indirect effects of floral traits on female fitness of some plant species using a path analysis model (Parachnowitsch & Caruso 2008; Valdés & Ehrlén 2017). The largest difference between path analysis and multiple regression analysis is that the former assumes a specific causal structure (direct and indirect) among the variables, while the latter assumes that all floral traits directly influence plant fitness (Scheiner *et al.* 2000).

Primula secundiflora is a distylous, self- and intra-morph incompatible perennial herb. Reproductive success of this species relies on insect pollen vectors (Richards 2014). Previous studies (Zhu *et al.* 2010, 2015) have implied that nectar robbers may influence the trait–fitness relationships and mediate the direct and/or indirect effects of floral traits on fitness. In addition, nectar robbers may generate differential effects on trait–fitness relationships between the L- and S-morph flowers because of typical floral architectures of distylous primrose species. In the present study, we experimentally quantified the phenotypic selection on floral traits through female fitness and estimated the casual effects of nectar robbing with different nectar robbing intensities on trait–fitness relationships in both the L- and S-morph flowers among *P. secundiflora* populations. In natural populations of *P. secundiflora*, nectar robbing is widespread, and the intensity of nectar robbing varies among populations (personal observation). We specifically examined whether (i) nectar robbers mediate indirect effects of floral traits on fitness; and if so, (ii) the effects vary between the L- and S-morph flowers and/or among populations with different nectar robbing intensities.

MATERIAL AND METHODS

Study species and sites

Primula secundiflora is a distylous [long-style and short-anther phenotype (L-morph); short-style and long-anther phenotype (S-morph)], 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 three to 43 flowers in a single umbel. The flowering period is from May to August, and the fruiting period is from August to September.

We conducted our experiments in five natural populations in Potatso National Park, Shangri-La County, Yunnan Province, southwest China (Pop 1: 99°54′34.557″ E, 27°47′55.390″ N, 3,435 m a.s.l), Pop 2: 99°54′44.785″ E, 27°47′50.213″ N, 3,406 m a.s.l, Pop 3: 99°59′56.814″ E, 27°51′31.415″ N, 3,633 m a.s.l, Pop 4: 99°54′35.546″ E, 27°48′42.789″ N, 3,435 m a.s.l and Pop 5: 99°59′50.964″ E, 27°51′37.803″ N, 3,662 m a.s.l). According to a previous study (Zhu *et al.* 2015) and our own observations, dominant floral visitors (bumblebees) to the primrose populations were composed of two main visitors: legitimate pollinators and nectar robbers. The legitimate pollinators, *Bombus convexus*, visited the flowers through the corolla tube entrance, whereas the nectar robbers, *B. lucorum*, visited the flowers by biting a hole in the corolla tube or using an existing hole (Figure S1). *Bombus richardsi* and *B. atrocinctus* always alternated roles between legitimate pollinators and nectar robbers during visitation (personal observation). When there was a hole in the corolla tube, *B. richardsi* and *B. atrocinctus* commonly used it to visit the flower; when there was no hole in the corolla tube, these two bees commonly visited the flower through the corolla tube entrance.

Field experiments and trait measurements

In late May 2017, we randomly marked 200 separate individuals (100 L-morph individuals and 100 S-morph individuals) at each of Pop 1, 2, 3 and 5 populations. Because of the limits of individuals in the Pop 4 population, we only marked 160 individuals, which included 80 L-morph individuals and 80 S-morph individuals.

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 base) 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 estimate the number of flowers that had experienced nectar robbing for each individual, we visited these populations twice per week throughout the flowering period. During each visitation, we recorded the number of nectar-robbed flowers only for the new open flowers for each individual. At the end of the flowering period, we added together each record to obtain the total number of nectar-robbed flowers for each individual. We used the number of nectar-robbed flowers per individual as a proxy for nectar robbing intensity. Although proportional nectar robbing was used to control for the effect of plant size, we used the absolute number of nectar-robbed flowers in the present study because we wanted to test for the effect of plant size (*i.e.* flower number) on nectar robbing.

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 used total seed production as a measurement of female fitness.

Statistical analysis

Two-way ANOVA was used to test the effects of population and morph (L-morph *versus* S-morph) on floral traits (flowering start, plant height, number of flowers, corolla tube length and corolla tube width), the number of nectar-robbed flowers and female reproductive success (fruit production, seeds per fruit and female fitness). To improve the normal distribution of the data, the flowering start, plant height, number of flowers, corolla tube length, corolla tube width, number of nectar-robbed flowers, fruit production, seeds per fruit and female fitness were $\log_{10}$ transformed prior to ANOVA.

To test the relationships between floral traits and number of nectar-robbed flowers (b_w), we regressed the number of nectar-robbed flowers on five floral traits using multivariate regression for each population and morph. In these analyses, as well as in the path analyses, we used the absolute number of nectar-robbed flowers rather than the proportion. Although proportional nectar robbing was used to control for the effect of plant size, we used the absolute number of nectar-robbed flowers in our analyses because we wanted to test for the effect of plant size (*i.e.* flower number) on nectar robbing.

Following the methods of Lande & Arnold (1983), we used multiple linear regression models to estimate directional selection gradients (β_i). In the regression models, we used the relative female fitness (individual female fitness/mean female fitness; using the original data, not the log-transformed data) and the standardised five floral traits (with a mean of 0 and a variance of 1; using the original data, not the log-transformed data) as the response variable and explanatory variables, respectively. We estimated the relative female fitness and standardised the traits separately for each population and morph, because of our interest in testing for morph-specific effects. We initially included cross-product terms (γ_{ij}, between floral traits) and quadratic terms (γ_{ii}) to quantify correlational selection, stabilising selection and disruptive selection. A few selection gradients were statistically significant, and the variance inflation factors (VIF) exceeded ten, which indicated substantial multicollinearity in these models (Quinn & Keough 2002). Consequently, we did not include the cross-product and quadratic terms in the regression models. To test for multicollinearity in these linear regression models, we calculated the VIF for the linear terms. All VIF were < 1.7, indicating no multicollinearity (Quinn & Keough 2002).

To test whether linear selection gradients of floral traits varied among populations and between morphs, we included data from five populations of plants in an ANCOVA. In the model, we used relative female fitness as the response variable and five standardised floral traits (flowering start, plant height, number of flowers, corolla tube length and corolla tube width), population, morph (L-morph *versus* S-morph), trait × population, trait × morph and trait × population × morph as the explanatory variables. Following the methods of Chapurlat *et al.* (2015), we used the trait × population interaction to test whether linear selection gradients of floral trait varied among populations, and the trait × morph interaction to test whether linear selection gradients of floral trait varied between morphs.

We used path analyses (Kingsolver & Schemske 1991) and structural equation modelling (SEM) to estimate the relative importance of direct and indirect effects of floral traits on female fitness in the populations where nectar robbers were present. The initial multiple linear regression analyses showed that plant height, number of flowers and corolla tube width (significant selection gradients) influenced female fitness. In addition, the number of flowers was related to the number of nectar-robbed flowers in all populations, and corolla tube width was related to the number of nectar-robbed flowers in some populations. As a consequence, we constructed three alternative, nested path models for each morph (Fig. 1). In model A, floral traits and nectar robbers directly influenced female fitness. In model B, the number of flowers had an indirect effect on female fitness through nectar robbers. In model C, both the number of flowers and corolla tube width had

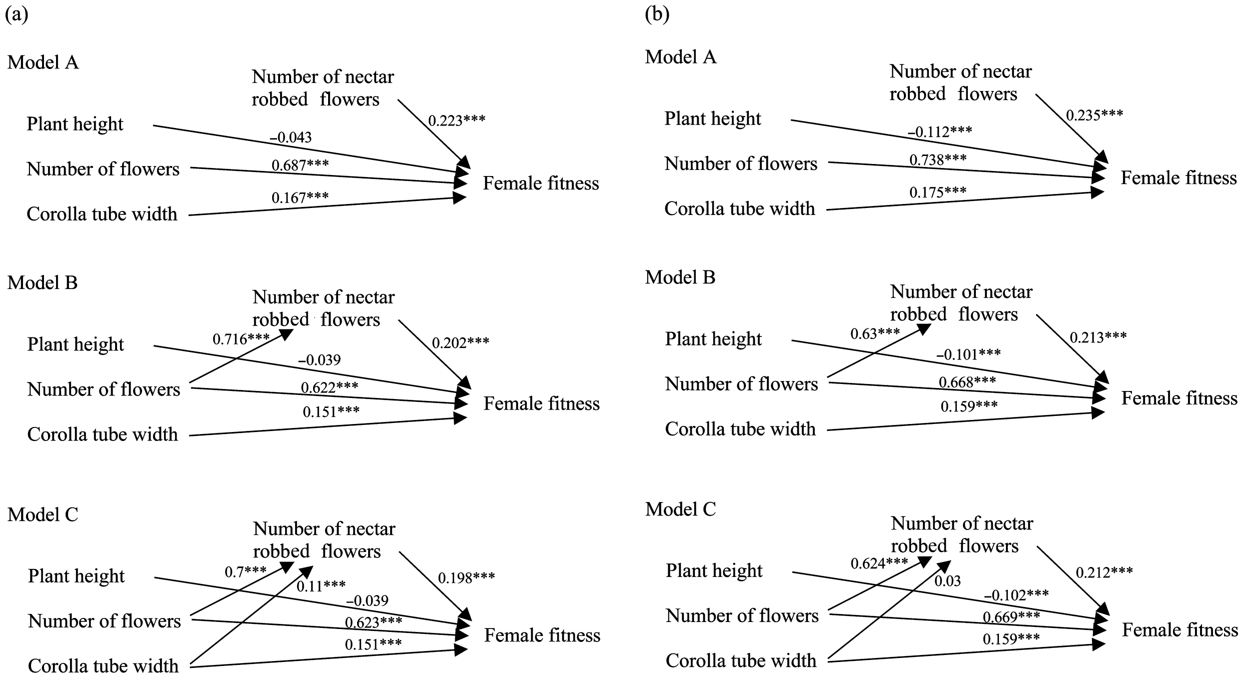

Fig. 1. Path analysis of the effects of standardised floral traits (plant height, number of flowers and corolla tube width) and standardised number of nectar-robbed flowers on relative female fitness in the L- (a) and S- (b) morph flowers of *Primula secundiflora*. In model A, floral traits and nectar robbers had only direct effects on female fitness. In model B, the number of flowers had an indirect effect on female fitness through nectar robbers. In model C, the number of flowers and corolla tube width had indirect effects on female fitness through nectar robbers. Path coefficients are represented with asterisks to show significance (***$P < 0.001$). Goodness of fit statistics for the three models are given in Table S3.

indirect effects on female fitness through nectar robbers. We statistically examined which model provided the better fit to our data using SEM (Mitchell 1992). A non-significant χ^2 value (except for $\chi^2 = 0$, this theoretically meant that the proposed causal model did not generate the observed data) and the lowest Akaike's information criterion (AIC) value better fit the data (Parachnowitsch & Caruso 2008). We selected the best model (see Results) and then used multigroup analysis (Grace 2006) to estimate the differences of path coefficients among populations and between morphs (ten 'groups', these groups including populations and morphs). First, we imposed equality constraints on all path coefficients among groups. Then, we imposed equality constraints on each path coefficient and examined the effect of these constraints on the overall model fit. The constraints for which the imposition of the equality assumption caused a significant decrease in the χ^2 value indicated that path coefficients differed among populations or between morphs (Valdés & Ehrlén 2017). Because the multigroup analyses indicated that the path coefficients varied among populations and between morphs (see Results), we fitted separate path models to each population and morph.

Path analyses, SEM and multigroup analyses were carried out in Amos 21 (SPSS, Chicago, IL, USA). All other analyses were performed with R software version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). We used Excel (2007) to generate the graphs.

RESULTS

Floral traits and female reproductive success

All five floral traits varied among populations (Tables S1 and S2). In the Pop 5 population, the plants were taller with a later flowering start, more flowers and longer corolla tube lengths than the plants in the other populations. The shortest corolla tube length was observed in the Pop 1 population. Corolla tube length and corolla tube width varied between morphs (Tables S1 and S2). Corolla tube length was shorter in L-morph flowers than in S-morph flowers, whereas corolla tube width was larger in L-morph flowers than in S-morph flowers in all populations except Pop 4 population. The number of nectar-robbed flowers varied among populations ($F_{4,834} = 264.75$, $P < 0.001$; Tables S1 and S2) and was highest in the Pop 5 population, followed by the Pop 4 population. The lowest number was observed in the Pop 1 population. The number of nectar-robbed flowers did not vary between the L- and S-morph flowers ($F_{1,834} = 0.002$, $P = 0.961$).

The plants in the Pop 5 population produced more fruits, more seeds per fruit (except for the S-morph flowers) and had higher female fitness than the plants in the other populations (Tables S1 and S2). In the Pop 1 population, plants produced fewer fruits, fewer seeds per fruit and had lower female fitness. Seeds per fruit varied between morphs ($F_{1,834} = 49.93$, $P < 0.001$; Tables S1 and S2). Plants produced more seeds per fruit in L-morph flowers than in S-morph flowers.

Relationship between number of nectar-robbed flowers and floral traits

Table 1. Relationships between number of nectar-robbed flowers and floral traits among five *Primula secundiflora* populations.

	L-morph		S-morph	
traits by sites	$b_w \pm$ SE	*P*	$b_w \pm$ SE	*P*
Pop 1				
flowering start	−0.111 ± 0.190	0.559	0.173 ± 0.204	0.400
plant height	0.020 ± 0.043	0.644	0.028 ± 0.037	0.455
number of flowers	0.303 ± 0.035	<**0.001**	0.152 ± 0.044	<**0.001**
corolla tube length	−0.430 ± 0.354	0.228	−1.091 ± 0.392	**0.007**
corolla tube width	2.235 ± 0.698	**0.002**	0.444 ± 0.946	0.640
Pop 2				
flowering start	−0.231 ± 0.207	0.269	−0.194 ± 0.230	0.401
plant height	−0.001 ± 0.045	0.987	0.057 ± 0.048	0.238
number of flowers	0.384 ± 0.045	<**0.001**	0.195 ± 0.052	<**0.001**
corolla tube length	−0.327 ± 0.409	0.427	0.044 ± 0.090	0.626
corolla tube width	1.575 ± 0.858	0.071	0.847 ± 1.060	0.427
Pop 3				
flowering start	0.137 ± 0.153	0.374	−0.153 ± 0.236	0.518
plant height	0.085 ± 0.048	0.082	−0.001 ± 0.047	0.991
number of flowers	0.278 ± 0.055	<**0.001**	0.412 ± 0.042	<**0.001**
corolla tube length	−0.965 ± 0.367	**0.010**	−0.814 ± 0.498	0.106
corolla tube width	2.451 ± 0.983	**0.015**	1.168 ± 1.290	0.368
Pop 4				
flowering start	0.464 ± 0.179	**0.012**	0.076 ± 0.299	0.800
plant height	0.033 ± 0.048	0.488	0.058 ± 0.049	0.242
number of flowers	0.339 ± 0.040	<**0.001**	0.439 ± 0.037	<**0.001**
corolla tube length	−0.193 ± 0.465	0.679	−1.425 ± 0.495	**0.006**
corolla tube width	0.873 ± 1.051	0.410	−1.641 ± 1.037	0.121
Pop 5				
flowering start	0.162 ± 0.345	0.639	0.247 ± 0.365	0.500
plant height	−0.030 ± 0.055	0.589	0.012 ± 0.052	0.821
number of flowers	0.512 ± 0.054	<**0.001**	0.599 ± 0.054	<**0.001**
corolla tube length	0.644 ± 0.514	0.214	0.120 ± 0.094	0.204
corolla tube width	−0.258 ± 1.126	0.819	1.993 ± 1.415	0.163

Significant estimates and *P*-values are indicated in bold.

The probability of being nectar-robbed was higher in plants with more flowers in all populations (Table 1). In L-morph flowers, the corolla tube width influenced the probability of being nectar-robbed in the Pop 1, 2 (marginally significant) and 3 populations. The corolla tube length also negatively influenced the probability of being nectar-robbed in S-morph flowers at the Pop 1 and 4 populations, and in the L-morph flowers at the Pop 3 population (Table 1). In addition, the flowering start positively influenced the probability of being nectar-robbed in the L-morph flowers in the Pop 4 population ($P = 0.012$).

Phenotypic selection

Directional selection on the number of flowers varied among populations and between morphs, as indicated by the significant number of flowers × population × morph interaction obtained with ANCOVA ($F_{4,779} = 10.287$, $P < 0.001$; Fig. 2, Table S3). A larger number of flowers was significantly selected in all five populations ($P < 0.05$; Fig. 2, Table S3). There was significant directional selection for wider corolla tubes in all five populations ($P < 0.05$; Fig. 2, Table S3). However, variations in selection of this trait among populations or between morphs were not detected ($F_{4,779} \times 0.107$, $P = 0.98$). In L-morph flowers, directional selection for taller plant height was observed in the Pop 5 population ($\beta = 0.073 \pm 0.032$, $P = 0.025$), whereas shorter plant height was selected in S-morph flowers of the Pop 1 population ($\beta = -0.129 \pm 0.053$, $P = 0.018$; Fig. 2, Table S3).

Direct and indirect effects of floral traits and nectar robbers on female fitness

Goodness-of-fit statistics showed that the χ^2 and *df* values of model A were 0 (Table S4), which theoretically meant that the proposed causal model (model A) did not generate the observed data. Model C had lower χ^2 (0.021 and 2.424 for the L- and S-morph flowers, respectively; not significant) and AIC values (4,753.399 and 4,688.531 for the L- and S-morph flowers, respectively; Table S4). Consequently, model C was the selected model. Multigroup analyses indicated significant among-population and between-morph differences in trait–fitness and nectar robber–fitness relationships ($\chi^2 = 378.049$, $df = 94$, $P < 0.001$; Tables S5 and S6). Models fitted for each population and morph revealed that effect sizes varied among populations ($\chi^2 = 178.665$, $P < 0.001$ and $\chi^2 = 181.125$, $P < 0.001$ for the L- and S-morph flowers, respectively;

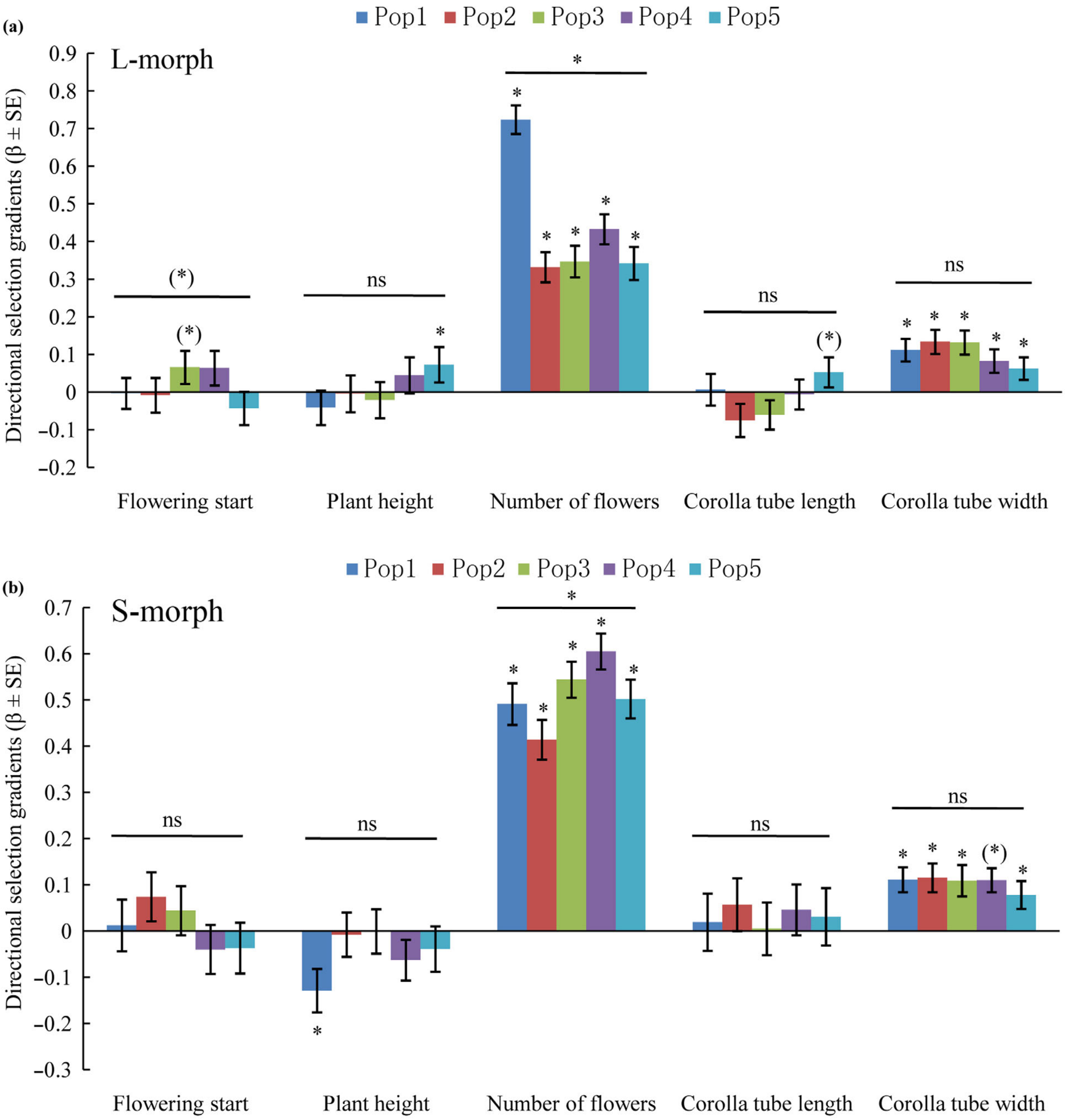

Fig. 2. Linear selection gradients ($\beta_i \pm$ SE) between the L- (a) and S- (b) morph flowers on flowering start, plant height, number of flowers, corolla tube length and corolla tube width in five *Primula secundiflora* populations. Symbols above individual bars indicate the level of significance of the gradient. Symbols above the lines spanning several gradients in the 'a' and 'b' graphs show whether directional selection varies among populations (significant trait × population term). *$P < 0.05$; (*)$P < 0.1$; ns, $P > 0.1$.

Table S5) and between morphs ($\chi^2 = 60.745$, $P < 0.001$, $\chi^2 = 55.338$, $P < 0.001$, $\chi^2 = 57.648$, $P < 0.001$, $\chi^2 = 31.402$, $P = 0.005$ and $\chi^2 = 77.248$, $P < 0.001$ for Pop 1, 2, 3, 4 and 5 populations, respectively; Table S6), but there were consistent direct and indirect effects of the number of flowers on female fitness (path coefficients were from 0.247 to 0.895; $P < 0.001$; Table 2). Path analyses indicated that more flowers and wider corolla tubes had positive effects on female fitness through effects of the intensity of nectar robbing (Table 2). However, indirect positive effects of corolla tube width on female fitness were only observed in L-morph flowers of the Pop 1, 2 and 3 populations. The direct and indirect effects of the number of flowers on female fitness were stronger (absolute value of path coefficients) in the S-morph flowers than in the L-morph flowers (Table 2). Plant height only had direct effects on female fitness, including negative effects in the Pop 1 and 3 populations (path coefficient = −0.136 and −0.068, respectively) and positive effects in L-morph flowers of the Pop 5 population (path coefficient = 0.085). Nectar robbers had direct positive effects on female fitness in L-morph flowers of the Pop 1, 3 and 5 populations (path coefficient = 0.146, 0.226 and 0.103, respectively), whereas their positive effects on female fitness in S-morph flowers were observed in the Pop 1, 2 and 3 populations (path coefficient = 0.181, 0.173 and 0.173, respectively; Table 2).

DISCUSSION

More flowers and wider corolla tubes had both direct and indirect positive effects on female fitness in five natural populations of *P. secundiflora*. Among these effects, the indirect effects were mediated by nectar robbers. In addition, nectar robbers had a direct positive effect on female fitness. In most populations, the strength of direct and/or indirect effects of floral traits and nectar robbers on female fitness was stronger in the S-morph flowers than in the L-morph flowers.

Biotic factors will mediate the direct and indirect effects of floral traits on fitness, thus influencing the evolutionary trajectory of floral traits (Parachnowitsch & Caruso 2008; Valdés & Ehrlén 2017). In the present study, more flowers and wider corolla tubes were significantly selected in the L- and S-morph flowers. However, path analysis clearly showed that only the number of flowers had consistent direct and indirect positive effects on female fitness in all five primrose populations. The indirect effect was mediated by nectar robbers, and the strength of this effect (absolute value of path coefficients) increased with increasing nectar robbing intensity. This implies that nectar robbing is one of the agents that influences the number of flowers–fitness relationships in this primrose species. In contrast, the indirect effect of corolla tube width on female fitness was only observed in the L-morph flowers and only in a few populations. These results demonstrate that this trait can partly influence plant female fitness through its effect on nectar robbing. The number of flowers determines the floral display sizes and influences the attractiveness of flowers to floral visitors. A larger number of flowers will produce visual advertisements and more visitation rewards for floral visitors (Conner & Rush 1996; Worley & Barrett 2000; Sandring & Ågren 2009), thus promoting the visitation of nectar robbers and increasing the fitness benefits. In contrast, corolla tube width is commonly favoured for improving the morphological fit between flowers and pollinators (Campbell *et al.* 1991, 1996). Indeed, selection for wider corolla tubes is attributed to legitimate pollinators in this primrose species (personal data, unpublished). Consistent direct effects of this trait on female fitness also confirm this hypothesis. At present, the few experimental data available restrict our understanding of why the indirect effect of corolla tube width on female fitness exists in the L-morph flowers but not in the S-morph flowers. Based on our own observations, this may reflect the indirect effect of nectar robbing on female fitness by influencing legitimate pollinators. Wider corolla tubes of L-morph flowers may simultaneously increase the visitation from nectar robbers and legitimate pollinators. Nectar robbing may reduce the nectar accumulation of flowers, potentially increasing the foraging area required by the pollinators to collect enough resources (Lara & Ornelas 2002) or will reduce the time spent on each flower (Mayer *et al.* 2014; Zhang *et al.* 2014). Thus increasing the pollination efficiency per unit time, reducing geitonogamy and increasing outcrossing is beneficial to female fitness.

In the present study, plant height only had a direct effect on female fitness, but its effect was only detected in a few populations and was not consistent (negative or positive). This may suggest weak links between plant stature and female reproductive success in the *P. secundiflora* populations. Field experiments suggest that pollinator-mediated selection on plant stature is stronger in tall plants than in short plants (Ehrlén

Table 2. Standardised path coefficients for the effects of floral traits and number of nectar-robbed flowers on female fitness in *Primula secundiflora*.

morph	population	plant height → fitness	no. flowers → fitness	corolla tube width → fitness	no. nectar-robbed flowers → fitness	no. flowers → no. nectar-robbed flowers	corolla tube width → no. nectar-robbed flowers
L-morph	Pop 1	−0.038	**0.622*****	**0.083***	**0.146****	**0.675*****	**0.215*****
	Pop 2	−0.029	**0.297*****	**0.134****	0.047	**0.693*****	**0.153***
	Pop 3	−**0.068***	**0.247*****	0.058	**0.226*****	**0.562*****	**0.165***
	Pop 4	0.018	**0.435*****	**0.089***	−0.002	**0.71*****	0.085
	Pop 5	**0.085****	**0.263*****	**0.066***	**0.103***	**0.718*****	−0.016
S-morph	Pop 1	−**0.136*****	**0.438*****	**0.118****	**0.181*****	**0.316****	−0.028
	Pop 2	−0.069	**0.353*****	**0.122****	**0.173*****	**0.426*****	0.112
	Pop 3	−0.022	**0.426*****	**0.09****	**0.173*****	**0.729*****	0.056
	Pop 4	−0.032	**0.749*****	**0.101***	−0.157	**0.895*****	−0.112
	Pop 5	−0.026	**0.491*****	**0.09*****	−0.003	**0.818*****	0.081

Significance estimates and *P*-values are indicated in bold.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

et al. 2002; Ågren *et al.* 2006). However, taller and shorter plant heights are selected in the L- and S-morph individuals in a few populations. This could suggest that some visitors (apart from nectar robbers) may generate selective pressures on this trait.

There was no selection on flowering phenology and corolla tube length. Path analysis also showed that these two traits had no direct or indirect effects on female fitness. These results indicate that these two traits were neither directly nor indirectly linked to fitness nor were they the target traits that were mediated by nectar robbers in the *P. secundiflora* populations. Accurate floral architectures are thought to be shaped by pollinators to improve the morphological fit between flowers and pollinators, thus increasing pollination efficiency (Harder & Johnson 2009; Boberg *et al.* 2014). In the present study, the absence of an effect of the corolla tube length on female fitness may have one common explanation: nectar robbers visit the flowers through a nectar robbing hole in the corolla tube. This mechanism may relax the effect of corolla tube length on female fitness. Above all, nectar robbers will influence the number of flowers–fitness relationship in this primrose species.

Nectar robbers can offer the plant fitness benefits by indirectly influencing the behaviours of pollinators or directly increasing fitness in some cases (Hernandez & Toledo 1979; Zhu *et al.* 2010). Our results show that nectar robbers have a direct positive effect on female fitness in the *P. secundiflora* populations. Indeed, nectar robbers always touch the reproductive organs during visitation (personal observation). In addition, the average effect (absolute value of path coefficients) is stronger in the S-morph flowers than in the L-morph flowers. In S-morph flowers, the positive effects of nectar robbers on female fitness are significant in populations with low nectar robbing intensity. However, the effects are not significant in populations with high nectar robbing intensity. This result suggests that the direct effect of nectar robbers on female fitness in the S-morph flowers is not positively limitless: low nectar robbing intensity improves female fitness, but high nectar robbing intensity does not. High nectar robbing intensity may lead to a lack of attractiveness to legitimate pollinators and thus may influence fitness for the S-morph flowers. Similar results were not observed in the L-morph flowers.

Using multiple regression analysis and path analysis, we have demonstrated the direct and indirect effects of floral traits on the female fitness of *P. secundiflora*; the indirect effects are mediated by nectar robbers. Nectar robbers have a direct positive effect on female fitness, but this effect varies between the L- and S-morph flowers. These results show the potential role of nectar robbers in influencing the trait–fitness relationships in this primrose species.

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SUPPORTING INFORMATION

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

Table S1. Effects of population and morph (L-morph *versus* S-morph) on floral traits, robbed flowers and female reproductive success analysed with two-way ANOVA.

Table S2. Floral traits, robbed flowers and female reproductive success (mean ± SD) for plants in the *Primula secundiflora* populations.

Table S3. Linear selection gradients ($\beta_i \pm$ SE) between the L- and S-morph flowers on flowering start, plant height, number of flowers, corolla tube length and corolla tube width in five *Primula secundiflora* populations.

Table S4. Goodness of fit statistics for the three path models in Fig. 1.

Table S5. Results of the multigroup path analyses for effects of floral traits and number of nectar-robbed flowers on female fitness among populations of *Primula secundiflora*.

Table S6. Results of the multigroup path analyses for effects of floral traits and number of nectar-robbed flowers on female fitness between the L- and S-morph flowers for each population of *Primula secundiflora.*

Figure S1. The legitimate pollination (a) and nectar robbing (b) of bumblebees.

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