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Research Article

Variation in pollen limitation among reproductive modules points to likely resource reallocation in the alpine plant *Veratrum grandiflorum*

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Pollen limitation, a decrease in seed production due to insufficient pollen receipt, may influence plant demography and the evolution of sexual systems. Its empirical estimation of pollen supplementation of some of the flowers on an individual is well known to be prone to overestimation due to potential resource reallocation among the individual's resource pools, from unpollinated flowers and inflorescences. Despite this, studies that evaluate the effect of resource reallocation simultaneously across different modular levels of an individual remain rare, especially in alpine plants. Here, we estimated pollen limitation at three modular levels (i.e., flower, raceme, and whole plant) in the perennial alpine herb *Veratrum grandiflorum* (Maxim. Ex Baker) Loes in the eastern Himalayan Hengduan Mountains. We used fruit set and seed production as two proxies for female reproductive success. Based on seed production, we found that pollen limitation differed significantly among modular levels, with the highest and lowest values being at the flower and whole-plant levels, respectively. In contrast, fruit set was relatively insensitive to pollen supplementation, so estimates of pollen limitation were low and did not differ among modular levels. These results point to resource reallocation for seed production among modules within individuals and highlight the importance of multi-modular and multi-metric approaches for accurately assessing pollen limitation.

Keywords: mate limitation, Melanthiaceae, plant reproduction, pollinator, resource redistribution, supplemental pollination

Introduction

Pollen limitation, the reduction in seed production due to insufficient pollen receipt (Burd 1994, Larson and Barret 2000, Knight et al. 2005), may impact the evolution of floral characteristics and mating systems (Ashman et al. 2004, Fishman and Willis 2008) and even threaten the persistence of plant populations, with potential

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implications for plant conservation (Pattemore and Anderson 2013). Accordingly, pollen limitation has attracted substantial attention, both experimentally (Burd 1994, Larson and Barret 2000, Gómez et al. 2010, Wang et al. 2023) and theoretically (Ashman et al. 2004, Harder and Aizen 2010, Rosenheim et al. 2014, 2024). In empirical research, the standard approach has been to supplement a plant's stigmas with outcross pollen and to compare seed and/or fruit set with naturally pollinated plants. Higher reproductive output in pollen-supplemented plants then points to pollen receipt as a limiting factor for reproduction (Knight et al. 2006). According to a global database, the average effect size for pollen limitation is 0.49 (CI: 0.45–0.52) (Bennett et al. 2018), corresponding to a 63% increase in female reproductive success following pollen supplementation and implying that pollen limitation is likely common and prevalent in angiosperms (Bennett et al. 2020).

Nonetheless, most empirical studies of pollen limitation have been carried out at the flower or partial plant levels (2203 out of 2969 studies, as reviewed in Bennett et al. 2018). In these studies, pollen limitation may be overestimated (Knight et al. 2006, Wesselingh 2007). For example, if a single flower or section of a plant receives extra pollen, resources may be transferred to that growing fruit at the expense of other fruits or additional flower production, resulting in an overestimation of reproductive success of that flower. Consequently, the reproductive success of flowers that do not receive supplemental pollen may be underestimated, as resources may be preferentially reallocated to pollen-treated flowers, reducing resource availability for untreated flowers (Stephenson 1981, Zimmerman and Pyke 1988, Knight et al. 2006). Some studies have attempted to test experimental biases from resource reallocation by setting up two complementary controls, one from manipulated plants and the other from non-manipulated plants. This dual-control design provides a way to determine whether resources have been diverted among nearby reproductive modules (Wesselingh 2007, Gómez et al. 2010). While this approach can determine whether resource reallocation exists between modules by comparing two control groups, it remains insufficient for eliminating the impact of resource reallocation biases in pollen limitation assessments.

Another solution is to supplement all the stigmas of the whole plant with pollen, so that pollen limitation is truly assessed at the plant level. Knight et al. (2006) conducted a meta-analysis of the quantitative impacts of pollen supplementation experiments and found that the difference in reproductive success between treated and control flowers was significantly greater in experiments that used flowers or parts of plants than in those that conducted pollen supplementation to whole plants, pointing to the likely influence of resource reallocation on estimates of pollen limitation and confirming the effectiveness of whole-plant pollination experiments. This issue may be particularly important in alpine environments, where plant resource acquisition is constrained by factors such as low temperatures, short growing seasons, and limited soil fertility (Totland 2001, Hegland et al. 2009, Hong et al. 2015, Dai et al. 2017). These environmental limitations may increase the extent of intra-plant resource reallocation during pollen supplementation experiments, thereby potentially introducing estimation bias and reducing the reliability of pollen limitation assessments.

Importantly, Knight et al.'s (2006) meta-analysis was based on comparisons of pollen-supplemented pollination studies from various species carried out at different levels rather than comparisons of levels carried out in the same species. In the three studies conducted in the same species so far, two of them found no evidence for resource reallocation (Murphy and Vasseur 1995, Runquist and Moeller 2013), and the third found support for it (Ganger 1997). Moreover, while some research has been undertaken on supplemental pollination trials involving whole plants, the number of inflorescences and flowers on the investigated plants has tended to be small (Wesselingh 2007, Gielens et al. 2014, Jiang and Li 2017). Pollen supplementation experiments at multiple levels within the same species and with large sample sizes are thus sorely needed.

Another issue is that researchers evaluating pollen limitation have often taken into account one of a set of frequently assessed reproductive factors. In the most recent global dataset on pollen limitation, 1569 of the 2969 data points were based on fruit set as a proxy for reproductive success (Bennett et al. 2018), with the minority of studies using seeds per ovule, seeds per fruit, seeds per flower, and/or seeds per plant (Bennett et al. 2018). Knight et al. (2006) found a strong association between the degree of pollen limitation during fruit development and seed output across different plant species, and they thus concluded that fruit set may be a suitable and convenient substitution for investigating pollen limitation. However, some studies have found evidence of severe pollen limitation in terms of seed production and only modest evidence in terms of fruit set (Gómez et al. 2010, Runquist and Moeller 2013). More studies that estimate pollen limitation using different proxies of reproductive success in the same species are thus needed.

High levels of pollen limitation are often expected for plants growing in harsh environments, such as in alpine regions, which are often subject to unpredictable meteorological conditions (Carlson et al. 2008, Dai et al. 2017). Empirical studies on various alpine species have reported severe pollen limitation for different species in alpine regions, including alpine tundra (Elberling 2001), the European Central Alps (Gugerli 1998), the mountains of Andes (Muñoz and Arroyo 2006), the Himalayas (Ai et al. 2013), Kilimanjaro (Sawe et al. 2020), Alaska (Fulkerson et al. 2012) and northern Japan (Kudo 2022). A recent meta-analysis of studies conducted in the eastern Himalayan Hengduan Mountains found that pollen limitation increased with increasing elevation (Jiang and Xie 2020), while another meta-analysis found no notable differences in pollen limitation between alpine and lowland species (García-Camacho and Totland 2009). Although interesting, most of these studies focused solely on pollen limitation at the levels of individual flowers, inflorescences, or branches, and they thus suffer from the limitations identified above.

Here, we assessed evidence for pollen limitation on both fruit set and seed production in a population of the alpine herb *Veratrum grandiflorum* (Maxim. Ex Baker) Loes in the eastern Himalayan Hengduan Mountains, comparing the effect of pollen supplementation treatments at three modular levels: whole plant, raceme, and flower. We asked the following two questions.

1. Does resource reallocation affect estimates of pollen limitation? We expected to find an overestimate of pollen limitation when pollen was supplemented at partial-plant levels.
2. Does the choice of reproductive success metrics show consistent estimates of pollen limitation? We expected different proxies of reproductive success to show different magnitudes of pollen limitation.

Material and methods

Study species and study site

We assessed pollen limitation in a single population of *Veratrum grandiflorum* (Maxim. Ex Baker) Loes (Melanthiaceae) in 2022. The population was on an open slope of subalpine grassland covering an area of approximately 500 x 500 m at Balang Mountain in southwestern China (30°52′N, 102°57′E, 3513 m a.s.l.), part of the eastern Himalayan Hengduan Mountains and a hotspot of angiosperm diversity (Boufford 2014). At the time of sampling, the population comprised more than 500 flowering individuals. At an elevation of 2800 m at the Balang moutain, the average annual temperature is approximately 8.4°C, with average total yearly precipitation of 861.8 mm (Wolong Field Station), of which 68% occurs between May and September, the highest mean monthly temperature occurs in July (17.0°C), the lowest in January (−1.7°C) (Li et al. 2024).

Veratrum grandiflorum is a rhizomatous, andromonoecious perennial herb. It grows primarily in alpine and sub-alpine habitats in East Asia (Zomlefer et al. 2001, Liao et al. 2007), occupying a wide range of habitats at Balang Mountain at elevations of 3300–4000 m a.s.l. At the study site, the species flowers from the end of June to the end of July, producing white-green hermaphroditic and male flowers in paniculate inflorescences. In 2021, plants in the study population produced an average of 212.5 ± 84.2 flowers (n = 100), with an average of 126.9 ± 80.8 male flowers and 85.7 ± 49.4 hermaphroditic flowers. In 2022, plants had a mean plant height of 91.5 ± 13.2 cm (n = 63), producing an average of 10.1 ± 2.6 racemes (n = 63) per individual, and a mean of 88.9 ± 52.9 (n = 63) hermaphroditic flowers per plant (Fig. 1A). Hermaphroditic flowers, which have six stamens and three separate pistils, are often held on the main raceme and the bottom of lateral racemes. Male flowers, which have six stamens, are usually held on the lateral racemes (Fig. 1B, C). Racemes exhibited an acropetal flowering sequence. Phenological monitoring of 18 individuals in 2023 revealed a consistent flowering pattern: The main raceme had a flowering duration

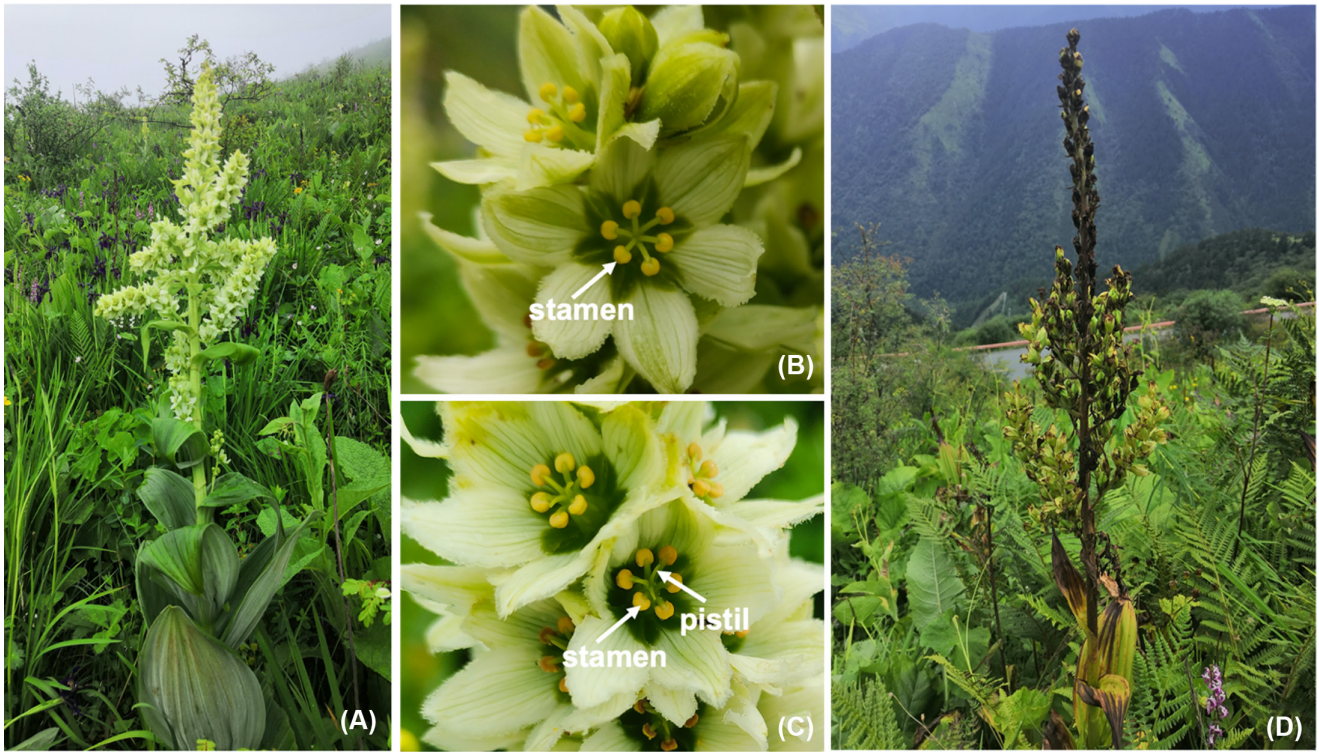


Figure 1. Morphological characteristics of *V. grandiflorum* at Balang Mountain. (A) A flowering plant in its natural habitat, (B) a male flower with six stamens, (C) a hermaphroditic flower with six stamens and three pistils, (D) mature capsules of the infructescence.

of 6.9 ± 2.7 days, on average, while the lateral racemes lasted for 12.9 ± 3.3 days. On average, anthesis in the main raceme occurred 6.0 ± 2.3 days earlier than in the lateral racemes. The flowering periods of the main and lateral racemes overlapped, resulting in a mean flowering duration of 15.8 ± 3.1 days per plant. Within individuals, hermaphroditic flowers consistently opened earlier than male flowers. Hermaphroditic flowers were protandrous: anther dehiscence preceded stigma receptivity, which was visually indicated by a transparent droplet at the stigma apex. The mating system of the study species is unknown, although many congeneric species have been reported to be self-incompatible (Kleijn and Steinger 2002, Kato et al. 2009, Weiherer et al. 2020). The floral visitors observed in the study population were mainly wasps and beetles in 2023 (Supporting information). Ovaries develop into capsules that dehisce at the end of August (Fig. 1D). Plants persist over winter as underground rhizomes.

Pollen supplementation experiment

We estimated pollen limitation at the whole-plant, raceme, and flower levels. Pollen supplementation was conducted in July throughout the flowering season of 2022. At the whole-plant level, all the hermaphroditic flowers of 33 arbitrarily chosen flowering individuals were supplemented by outcross pollen, whereas all the hermaphroditic flowers of another set of 33 individuals were left untreated and served as a control. At the raceme level, a different set of 33 individuals was selected. For each plant, two lateral racemes adjacent to the same node were arbitrarily chosen. All the hermaphroditic flowers of one of the racemes were subjected to pollen supplementation, while those on the other raceme were left untreated as control. At the flower level, again another set of 33 individuals was arbitrarily selected, and one lateral raceme was chosen from each plant. Four adjacent hermaphroditic flowers were marked on the chosen raceme, with two flowers subjected to pollen supplementation and the other two left untreated as control. Due to the loss of three samples, the final dataset was based on the remaining 129 labelled individuals. Pollen supplementation was conducted on 2610 hermaphroditic flowers from 65 individuals at the whole-plant level, 318 hermaphroditic flowers from 66 inflorescences at the raceme level, and 65 hermaphroditic flowers from 33 racemes at the flower level. At both the raceme and flower levels, supplemental pollination was performed on randomly selected hermaphroditic flowers, while all other hermaphroditic flowers on the individual were left untreated and exposed to natural pollination. The pollen used for supplementation was collected from plants located at least 5 meters away from the focal plants and was evenly applied to the stigmas using a brush. Pollen supplementation was repeated every other day to ensure successful pollination of all hermaphroditic flowers throughout the whole flowering season.

Measurements of female reproductive success

We used both fruit set and seed production as proxies for female reproductive success, with seed production here defined as seeds per fruit. During the flowering season, we recorded the number of hermaphroditic flowers at the whole plant, raceme, and flower levels. After the pollination manipulations, fruits were allowed to ripen on the plants and collected individually before dehiscence at the end of the season. At the whole-plant level, we randomly selected ten fruits to count the number of seeds per fruit. At the raceme level, if the number of harvested fruits exceeded ten, we used the same method as that at the whole-plant level; otherwise, we counted the seeds of all fruits. At the flower level, we counted the seeds for all fruits. Our analyses are based on a total sample of 1074 fruits, assessed at the end of August 2022.

Pollen limitation

We calculated pollen limitation as the log response ratio $\ln(x_{ps}/x_{nat})$, where x_{ps} and x_{nat} are the mean values for reproductive success (either fruit set or seed production) for the pollen supplementation and natural pollination treatments, respectively (Bennett et al. 2018, Siopa et al. 2023). Positive values imply pollen limitation. Pollen limitation was calculated at each reproductive level. To minimize variation associated with plant size, we analysed data at the whole-plant level by ranking individuals by size for each of the two treatments separately and then calculated pollen limitation for pairs of plants assigned together by their rank. Pairing was not needed for analysis at the raceme and flower levels, as comparisons here were conducted within each plant.

Statistical analyses

To test the effects of pollen supplementation on fruit and seed production, we fitted generalized linear mixed-effects models (GLMMs), with either fruit set or seed production as a response variable. Pollination treatment (pollen supplemented versus natural pollination) and reproductive levels (whole-plant, raceme, and flower levels), and their interactions were included as fixed effects. To account for possible effects of plant size, we included the total inflorescence number (a proxy for size) in the models as a random factor. To account for the non-independence of fruits collected from the same individuals and inflorescences, we also included the identity of individual plants as a random factor. We used a binomial distribution with a logit link function and a Poisson distribution with a log link function for fruit set and seed production, respectively. We tested the significance of fixed effects using type-II *F*-tests. Post-hoc pairwise comparisons using Tukey's honest significant difference (HSD) test were then conducted to explore differences among levels and treatments. We also used chi-square tests to assess differences in fruit set and seed production between treatments at different levels. In addition, we ran a one-way analysis of variance to test the effects of reproductive level on pollen limitation, followed by Tukey's HSD for pairwise comparisons. All analyses were carried out using R ver. 4.3.3 (www.r-project.org).

Results

Pollen limitation in terms of fruit set

We observed that both natural and supplemental pollination resulted in mature fruits at all three modular levels. At the

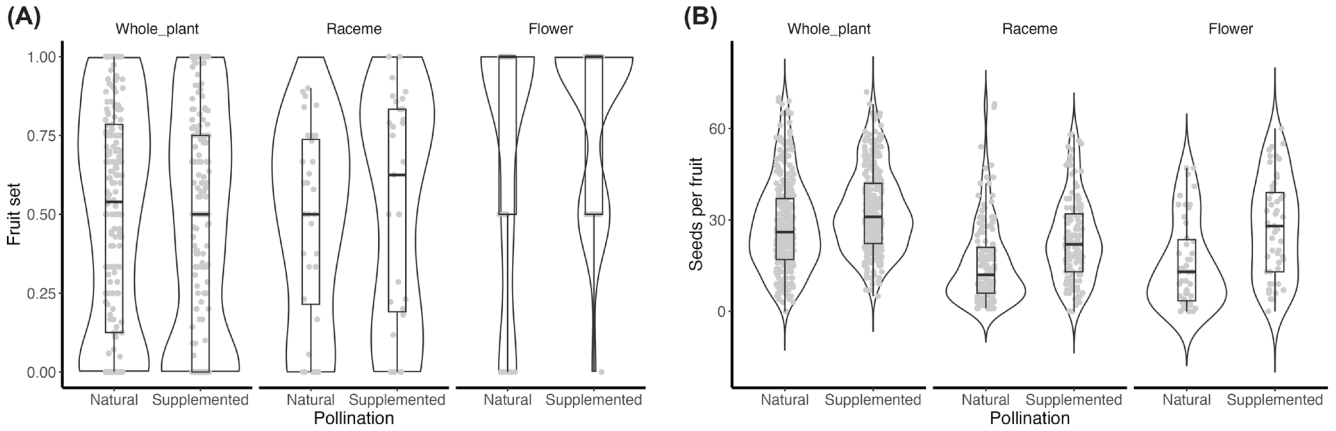


Figure 2. Fruit set and seed production across different pollination treatments and manipulation levels in *V. grandiflorum*. (A) The box plots represent the interquartile range (25th and 75th percentiles, top and bottom of the box) of fruit set, with the line inside the box indicating the median. Individual data points are shown as grey dots. The violin plots illustrate the distribution of fruit set at each manipulation level, (B) seed production under different pollination treatments and manipulation levels. The box plots represent the interquartile range (25th and 75th percentiles, top and bottom of the box) of seeds per fruit, with the line inside the box indicating the median. Individual data points are shown as grey dots. The violin plots illustrate the distribution of seed production at each manipulation level.

whole-plant level, estimates of fruit set for supplemental and natural pollination were 0.456 ± 0.364 and 0.492 ± 0.356, respectively. At the raceme level, fruit set for supplemental and natural pollination treatments were 0.517 ± 0.362 and 0.466 ± 0.309, respectively. At the flower level, fruit set for supplemental and natural pollination was 0.828 ± 0.273 and 0.712 ± 0.415, respectively (Fig. 2A). We found no difference in fruit set between supplemental and natural pollination treatments or among the different reproductive levels and there was no interaction effect between treatment and reproductive level (Table 1).

When fruit set was used as the response variable to assess pollen limitation at different reproductive levels, pollen limitation was weak and there was no evidence for resource reallocation in comparisons among treatments (Table 2). The average values of pollen limitation at each level were as follows: whole-plant level was 0.120 ± 0.522, raceme level was 0.069 ± 0.623, and flower level was 0.011 ± 0.467. Estimated values of pollen limitation did not differ among modular levels ($p = 0.654$).

Pollen limitation in terms of seed production

Among the 1074 fruits examined in the experiment, seed number per fruit ranged from 0 to 73. At the whole-plant level, the seed production for supplemental and natural pollination was 32.9 ± 13.5 and 28.3 ± 15, respectively. At the raceme level, the seed production for supplemental and natural pollination was 23.4 ± 13.5 and 15.2 ± 12.9, respectively. At the flower level, the seed production for supplemental and natural pollination was 27.2 ± 15.9 and 15.7 ± 14.2, respectively (Fig. 2B). Following pollen supplementation, seed production increased by approximately 16% at the whole-plant level, 53% at the raceme level, and 73% at the flower level. The chi-square analysis revealed significant effects of pollination treatment and modular levels on the seed production (Table 1). Pollination treatment exerted a strong influence on seed production ($\chi^2 = 361$ df = 1, $p < 0.001$), with supplemental pollination markedly enhancing seed output compared to natural pollination. Furthermore, seed production differed significantly across modular levels ($\chi^2 = 55.6$, df = 2, $p < 0.001$). Additionally, there was a significant interaction effect between pollination treatment and modular level ($\chi^2 = 22.1$, df = 2, $p < 0.001$).

There was strong evidence for differential pollen limitation on seed production at the different modular levels, pointing to the importance of resource reallocation across different treatments. Pollen limitation at the raceme and flower levels was approximately 3.5 and 6.1 times higher than at the whole-plant level, respectively. Estimated values of pollen limitation were significantly different ($p < 0.01$) among three modular levels (Tabel 2), the average pollen limitation at the whole-plant level was 0.129 ± 0.353, at the raceme level was 0.453 ± 0.717, and at the flower level was 0.787 ± 1.01 (Fig. 3). Pairwise comparisons revealed that only the comparison between the flower and whole-plant levels showed a statistically significant difference ($p < 0.001$), with the mean magnitude of pollen limitation at the flower level being significantly higher than at the whole-plant level (Fig. 3).

Discussion

We evaluated how resource reallocation may bias estimates of pollen limitation by conducting pollen supplementation simultaneously at three modular levels on the multi-flowered alpine plant species, *Veratrum grandiflorum*. Our results suggest that resource reallocation may lead to an overestimation of pollen limitation if only one or several flowers of the plants are treated, notably when seed production was used as the proxy for reproductive success. This is the first study to conduct pollen supplementation experiments on different modules of a multi-flowered alpine plant. Our results have

Table 1. Summary table of chi-squared tests assessing the effects on reproductive variables (fruit set and seed production) of pollination treatments ('Pollination': pollen supplemented versus natural), modular level ('Level': whole-plant, raceme, and flower levels), and their interaction on fruit set and seed production in *V. grandiflorum*.

Reproductive variables	Factors	χ^2	df	p-value
Fruit set	Pollination	0.004	1	0.951
	Level	4.49	2	0.106
	Pollination:Level	0.760	2	0.684
Seed production	Pollination	361	1	< 0.001
	Level	55.6	2	< 0.001
	Pollination:Level	22.1	2	< 0.001

important implications for interpretations of estimates of pollen limitation, especially those in alpine environments.

Biased estimates caused by resource reallocation among modules within plants

Through pollen supplementation experiments in *V. grandiflorum*, we found that the degree of pollen limitation measured as seed production was highest at the flower level, followed by the raceme level, and lowest at the whole-plant level. Our result is consistent with findings from a previous meta-analysis (Knight et al. 2006), which indicated that the reallocation of resources among modules may lead to a two- to eight-fold overestimation in estimates of pollen limitation. Our experiment demonstrates the pronounced existence of resource reallocation and its significant influence on the accuracy of pollen limitation measurements.

Research on pollen supplementation and resource reallocation has shown varying results across different species, emphasizing the influence of plant-specific reproductive traits. For instance, in *Clarkia xantiana* (Onagraceae), studies on pollen supplementation at both the flower and whole-plant levels found no evidence of resource reallocation in three reproductive components (fruit set, seeds per plant, seeds per fruit), likely due to the plants' reliance on autonomous self-pollination for reproductive assurance (Runquist and Moeller 2013). Similarly, a three-year study on *Hepatica acutiloba* (Ranunculaceae) reported no resource reallocation, possibly due to limited reproductive allocation, as the plant produced an average of only two flowers (Murphy and Vasseur 1995). These findings point to the importance of considering specific plant traits when assessing pollen limitation and resource reallocation. Our study adds to earlier work highlighting the importance of whole-plant level evaluations

Table 2. Summary table of one-way ANOVA results assessing the effects of pollen limitation across levels ('Level': whole-plant, raceme, and flower levels) on fruit set and seed production in *V. grandiflorum*.

Pollen limitation index	Resource	df	F value	p-value
Based on fruit set	Level	2	0.427	0.654
	Residuals	79	-	-
Based on seed production	Level	2	5.93	< 0.01
	Residuals	79	-	-

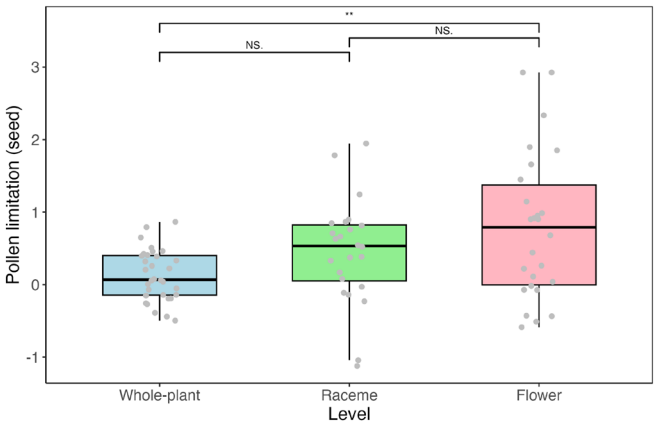


Figure 3. The degree of pollen limitation in seed production at different plant levels in *V. grandiflorum* (whole-plant, raceme, and flower level). The boxes show the median (line inside the box) and the interquartile range (25th and 75th percentiles, top and bottom of the box). Statistical comparisons were conducted with a Tukey test (NS: p = 0.05).

to better understand the dynamic allocation of resources across various plant modules.

Pollen limitation estimated in different proxies of reproductive success

Estimates of pollen limitation in *V. grandiflorum* varied depending on the response variable used. We found that pollen limitation was estimated to be negligible across modular levels when the fruit set was used as the proxy for reproductive success, but there was clear evidence for pollen limitation when seeds per fruit were used as the indicator. These findings are consistent with those found for *Erysimum mediohispanicum* (Brassicaceae), which reported stronger pollen limitation in terms of seed production than fruit production (Gómez et al. 2010), as well as with the results for *Clarkia xantiana* (Runquist and Moeller 2013).

Several factors may account for the greater sensitivity of seed production compared to fruit set to pollen supplementation treatments. First, based on ovule count data obtained from fruits collected within the same population during the 2023 field season, each flower of *Veratrum grandiflorum* was found to produce an average of 36 ovules. This high ovule number implies that even minimal pollen deposition on the stigma and a few fertilized ovules may suffice for fruit initiation. This makes fruit set an unreliable measure of reproductive success for this species. Second, using fruit set as a metric is more reliable for plants with a single flowering season (i.e., monocarpic) and flowers that have a fixed, small number of ovules, as multiple response variables (e.g., seed set, seeds per flower, seeds per fruit, and seeds per plant) tend to provide consistent information in such species. Burd (1994) noted that experiments often tend to report fruit set rather than seed production, which may have led to an underestimation of the effect of pollination on the number of seeds within each fruit. Seed production, as a direct measure of maternal fitness, of course, should provide a more accurate assessment of pollen limitation and plant reproductive success (Dudash and Fenster

1997, Ashman et al. 2004). Certainly, our study suggests that the use of seed production as a response variable is the more accurate measure of pollen limitation in V. grandiflorum.

Pollen limitation in alpine environments

We found weak pollen limitation on seed production in *V. grandiflorum*, when estimated at the whole-plant level. This may be partly explained by the generalist nature of its pollination system (Martén-Rodríguez and Fenster 2010, Lázaro et al. 2015, Kudo 2022), as evidenced by the wide diversity of floral visitors observed in the field (Supporting information). The large and showy floral display of *V. grandiflorum* likely attracts a broad range of pollinators, which may help buffer the species against pollen limitation by ensuring sufficient pollen delivery. This result contrasts with other studies on alpine plants, which have tended to find evidence of pollen limitation (Gugerli 1998, Arroyo et al. 2017, Sawe et al. 2020, Wang et al. 2023). While Jiang and Xie's (2020) meta-analysis of pollen limitation in the Hengduan Mountains revealed significant evidence for it, particularly at high elevation, most of the studies included in their analysis assessed pollen limitation at the levels of individual flowers, inflorescences, or branches rather than at the whole-plant level. Of those species manipulated at the whole-plant level, Roscoea schneideriana (Zingiberaceae) produces only one to four flowers per plant (Zhang and Li 2008), and Primula tibetica (Primulaceae) produces only one to six flowers per plant (Jiang and Li 2017).

Our study advances the understanding of pollen limitation in alpine species by using whole-plant manipulations, particularly in species with large floral displays – a common trait in alpine environments. By applying whole-plant pollen supplementation and seed-based reproductive metrics, we minimized biases caused by intra-plant resource reallocation, yielding more accurate estimates of pollen limitation. Our findings emphasize the need to account for resource reallocation when assessing pollen limitation, especially in perennial alpine species with large floral displays and constrained resource availability. Careful selection of experimental intensities and reproductive measures is therefore essential for reliable evaluation in such resource-limited settings.

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Conflict of interest – The authors declare no conflict of interest.

Author contributions

Xia Jiang: Data curation (lead); Formal analysis (equal); Investigation (lead); Visualization (equal); Writing – original draft (equal). **Kai-Hsiu Chen**: Conceptualization (equal); Formal analysis (equal); Visualization (lead); Writing – original draft (equal); Writing – review and editing (equal). **Shengman Lyu**: Formal analysis (lead); Visualization (lead). **Ling Liu**: Investigation (equal). **Meixian Yu**: Investigation (equal). **John R. Pannell**: Conceptualization (lead); Supervision (lead); Writing – review and editing (lead). **Guoxing Cao**: Conceptualization (equal); Funding acquisition (lead); Supervision (lead).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9s4mw6mw2 (Jiang et al. 2025).

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