

Elevation-related variation in the population characteristics of distylous *Primula nivalis* affects female fitness and inbreeding depression

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ABSTRACT

The population characteristics of distylous species are highly sensitive to stochastic natural selection pressure. Therefore, populations growing under different environmental conditions may vary in floral morph ratios, potentially affecting female fitness and leading to inbreeding depression. However, the variation in offspring quality among populations as a result of inbreeding depression is poorly understood in distylous species. This study investigates variations in plant density, seed mass, seed viability, female fitness, and post-dispersal inbreeding depression in both sexual morphs (long-styled and short-styled plants) of the distylous *Primula nivalis* that were subjected to different pollination treatments along an elevational gradient from 1657 to 2704 m a.s.l. Population characteristics (morph plant density and ratio) and fruit set were significantly affected by sexual morph and elevation. Plant density and fruit set frequencies were lower for short-styled than for long-styled plants at 2704 m a.s.l. The seeds from the cross-pollinated flowers of both morphs were higher in quality than those of self-pollinated flowers. The female fitness of seeds from cross-pollinated flowers of both morphs was higher than that of seeds from open-pollinated and self-pollinated flowers. The female fitness of seeds from long-styled flowers was higher than that of seeds from short-styled flowers at all elevations. Inbreeding depression increased with elevation among plants with short-styled flowers but not among those with long-styled flowers. Variation in the elevation-dependent mating system might influence female fitness and affect inbreeding depression in both floral morphs. In conclusion, the low quality of seeds from short-styled flowers at high elevations might decrease short-styled flower frequency, affecting population characteristics.

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1. Introduction

Darwin proposed that heterostyly (distylous and tristylous), the condition of having styles of different lengths relative to the stamens in individual plants, promotes outcrossing rather than self-fertilization. Nevertheless, some heterostylous species self-pollinate under harsh conditions that prevent effective pollinator

activity or reduce plant density (Barrett, 1990; Ferrer et al., 2009). When environmental conditions deviate from those found in the main distributional range of a heterostylous species, a morph can disappear from some populations (Meeus et al., 2012; Weber et al., 2012; Santos-Gally et al., 2013). Among distylous species that have long-styled and short-styled floral morphs (hereafter referred to as L-morphs and S-morphs), low pollinator activity, small population size, and the low frequency of one floral morph might inhibit legitimate pollination and increase illegitimate pollination, especially in harsh environments. The loss of self-incompatibility in distylous species, which include L-morph and S-morph plants (also called pin and thrum plants), might alter morph ratio and/or floral trait evolution (Barrett, 1990; Kohn et al., 1996; Weber et al., 2012;

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Bryson and Jacquemyn, 2015). But high self-compatibility in distylous species affects inbreeding depression and female fitness, and reduces changes in the long- and short-styled floral morph ratio and plant density (Barrett and Eckert, 1990; Sampson and Krebs, 2012; Weber et al., 2013), as variations in style morph frequency in distylous plants are highly sensitive to stochastic natural selection pressures in different populations (Thompson et al., 2003; Santos-Gally et al., 2013; Papuga et al., 2015).

Inbreeding depression is an important selection pressure against the production of self-progeny in cross-pollinated heterostylous species. Self-incompatible distyly within a population might influence the evolution of mating systems (Charlesworth and Charlesworth, 1987; Weber et al., 2012; Delmas et al., 2014). Inbreeding depression increases with the self-fertilization rate at both the population and species levels (Weller et al., 2005; Goodwillie and Knight, 2006). The effects of inbreeding depression have been explored in populations with different style morph frequencies (Weber et al., 2013) and in relation to habitat fragmentation (Nguyen et al., 2015). However, the effects of morph ratio variation within a population on female fitness and inbreeding depression in distylous species remain poorly understood (Endels et al., 2002; Spigler and Chang, 2009; De Vere et al., 2009).

Mating patterns and population characteristics may reduce the compatibility of a plant species with its pollinators, which, in turn, influence the evolution of distyly (Weber et al., 2012). Therefore, the decrease in effective pollinators with increasing elevation (up to the alpine zone) might affect the breeding system and population characteristics of distylous species growing at various elevations (Wirth et al., 2010). Seed susceptibility to predators, morph ratio, and female fitness might mediate variations in elevation-dependent environmental factors (temperature or pollinator activity) (Baeten et al., 2010; Straka and Starzomski, 2015). Furthermore, resource availability, flowering time, pollinator preference, abundance or effectiveness of pollinators, and mating system could all influence variation in seed quantity, viability, and fruit abortion rate (Baker, 1972; Forrest and Thomson, 2008; Rafferty and Ives, 2012; Straka and Starzomski, 2015). Reduced pollinator service might reduce offspring quality via inbreeding depression. Therefore, the absence of pollinators in harsh environments could increase selfing rates (Barrett and Eckert, 1990; Van Etten et al., 2015). Consequently, species growing in alpine zones, which are generally characterized by low air temperatures, low species diversity, and short growing seasons (Körner, 1999; Vittoz et al., 2009), might have undergone significant changes in mating type and plant recruitment (Fay and Schultz, 2009; Sun et al., 2014).

Our preliminary observations indicated that, in the distylous species *Primula nivalis* (Primulaceae), pollinator visitation frequency decreased and L-morph frequency increased with increasing elevation. Therefore, we hypothesized that elevation-dependent pollinator limitation and high selfing levels in both sexual morphs affect population characteristics and female offspring fitness. To test this hypothesis, we investigated (1) whether the population characteristics and fruit set of *P. nivalis* vary at different elevations; (2) the effects of pollination treatment and elevation on seed mass and viability; and (3) the relationship between female fitness and post-dispersal inbreeding depression in the two floral morphs at different elevations.

2. Material and methods

2.1. Material and study site

P. nivalis is a spring-flowering herbaceous perennial distylous species that grows in forest and grassland habitats in northwestern Xinjiang at elevations between 1600 and 3000 m. *P. nivalis* plant

density increases from low to high elevation. In high-elevation grassland populations, the frequency of L-morphs is greater than that of S-morphs; furthermore, at high elevations, the stamen-stigma distance of L-morphs is shorter (Abdusalam, 2017). Plant height, length of scapes, and length of leaves all decrease from low to high elevation; in addition, flowering and fruiting occur later in populations at high elevations than in populations at low elevations (Abdusalam and Li, 2018).

To reduce human interference, field experiments were conducted during the flowering seasons of 2014 and 2015 (May to August) in populations of *P. nivalis* at three different elevations (1650 m, 2403 m, and 2704 m) on the northern slope of the Tianshan Mountains in the Kunas area of northwestern Xinjiang (Fig. 1). These elevations allowed us to evaluate natural populations in forests (1650 m) and grasslands (2403 and 2704 m). Mean annual temperature, precipitation, sunshine, and habit characteristics of each study population are presented in Table 1.

2.2. Population and fruit set characteristics

To determine elevational variation in plant density and floral morph ratios, we randomly selected 35 plots, each 1 m × 1 m (selecting 5 plots in each 20-m² sample), for each of the three different elevation study populations of *P. nivalis* at the peak flowering period (Fig. 1). The distance between plots was ≥ 10 m. The total number of L-morph, S-morph, and all flowering plants per plot was determined. The percentage of individuals of each sexual morph per plot was calculated as follows: $P = [1 - (N/T)] \times 100$, where P is the percentage of L-morph or S-morph individuals per plot, N is the number of L-morph or S-morph plants per plot, and T is the total number of flowering individuals (L-morphs + S-morphs) per plot. Flowering plants of both morphs were marked in each plot, and the fruit set of each individual plant and the number of flowers per inflorescence were determined for each population at fruit maturity.

2.3. Effect of pollination treatment on seed mass and viability

To explore the effects of elevation-dependent mating patterns on seed quality, in flowering time, we selected 150 soon-to-open *P. nivalis* flowers from 50 inflorescences of both L-morph and S-morph individuals at each population and covered them with a paper bag. After all flowers were freshly opened, they were divided into three treatments: (1) open-pollination, (2) intra-morph hand self-pollination (all flowers were hand self-pollinated using pollen from flowers on the same plant and then re-bagged with paper bags), and (3) inter-morph hand cross-pollination (all flowers were covered with paper bags after removal of all stamens, and then flowers were hand cross-pollinated (from 10 m away) with pollen from another plant of the opposing morph and flowers re-bagged with paper bags) in each of the three study populations in 2015. One month after pollination, the fully matured fruits were collected, and the seed set per fruit was determined for all the treatments. Seed quality was assessed from viability and mass. To determine seed viability, three replicates of 30 mature, fully developed seeds from each treatment of both flower types were soaked in water for 24 h at 30 °C. The seed coat was then opened to expose the embryo. Seeds were placed in 1% TTC (2, 3, 5-triphenyl tetrazolium chloride) and incubated in the dark for 24 h at 30 °C (Baskin and Baskin, 2014). If the embryo turned pink, it was scored as viable. No color change indicated that the embryo was nonviable. To determine seed mass, 10 replications of 1000 seeds from each treatment, morph, and elevation were weighed to a precision of ±0.0001 g using a Sartorius BS210S electronic analytical balance (Göttingen, Germany).

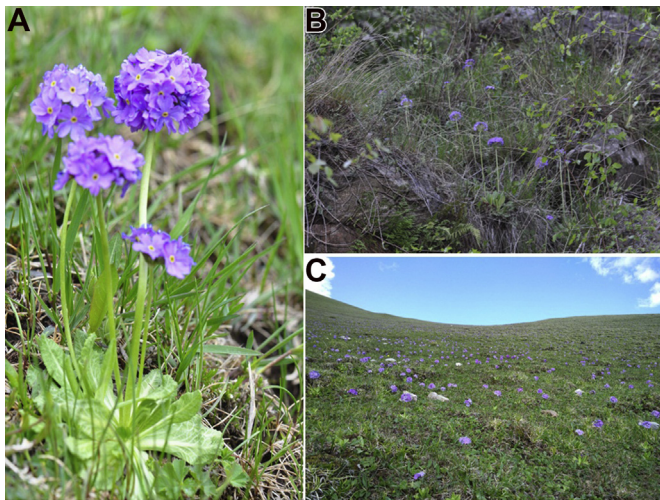


Fig. 1. Plant individuals (A) and different elevation of natural populations of *Primula nivalis* in forests (1650 m) and grasslands (2704 m) environments (B, C) in north-western Xinjiang, China.

2.4. Female fitness and post dispersal inbreeding depression

To investigate the influence of pollination treatments on female fitness and post-dispersal inbreeding depression, seed germination data for each treatment, morph, and elevation were used. First, eight replicates of 50 seeds generated by each morph in each treatment and elevation were placed in Petri dishes on two sheets of filter paper moistened with distilled water. All seeds were cold-stratified with moist air at 0 °C in darkness for five weeks (Washitani and Kabaya, 1988). The seeds were then incubated under optimal germination conditions (12 h:12 h fluorescent light:dark at 15/30 °C) for four weeks. Germination was scored as a visible shoot protruding from the top of the seed.

Female fitness was determined following the methods of Ferrer et al. (2009), where $ff = f_d s_s s_v$ (ff is female fitness, f_d is floral display, s_s is seed set, and s_v is the percentage of seed germination). Floral display (floral longevity) is 6–7 d for both morph flowers and it did not significantly differ among the three elevations (unpublished data). Therefore, any variation in female fitness was attributed to differences in seed set and viability. Post-dispersal inbreeding depression was determined for the seeds of both sex morphs produced by the three pollination treatments at the three elevations. Inbreeding depression (δ) was determined from the relative performance (RP). The inbreeding benefits of flowering plants increased with decreasing RP, calculated as follows: $RP = 1 - (W_o -$

$W_s)/W_{max}$, where W_o and W_s represent the fitness of self- and out-crossed progeny, respectively, and W_{max} is the larger of the two values (Baskin and Baskin, 2015).

2.5. Data analysis

All data were analyzed using SPSS v. 18.0. Figures were drawn using SigmaPlot v. 12.5. Data were first tested for normality and homogeneity of variances before the analysis. Fruit set, seed viability, seed mass, female fitness, and inbreeding depression data were not normal, so they were arcsine-, \log_{10} -, or square root-transformed before analysis to ensure homogeneity of variance. Pre-analysis normality of variance was determined to ensure that the data met the requirements for MANOVA (generalized linear model, GLM) or one-way ANOVA. A generalized linear model was used to evaluate elevation-dependent population density, the percentage of each sexual morph, seed mass, seed viability, female fitness, and inbreeding depression. If ANOVA results indicated significant differences for the same elevation or sexual morph, Tukey's HSD test was performed for multiple comparisons to determine significance ($p < 0.05$) among treatments.

3. Results

3.1. Population and fruit set characteristics

Population characteristics were significantly affected by sexual morph ($F_{1,180} = 11.589$, $p < 0.001$) and elevation ($F_{2,180} = 6.059$, $p < 0.05$), but not by the interaction of elevation and sexual morph ($F_{2,180} = 1.651$, $p > 0.05$). The percentage of L-morph individuals was higher than that of S-morph individuals at high elevation (Fig. 2A). Plant density was significantly affected by sexual morph ($F_{1,178} = 21.651$, $p < 0.001$), elevation ($F_{2,172} = 15.171$, $p < 0.001$), and the interaction of elevation and sexual morph ($F_{2,170} = 5.403$, $p < 0.01$). The density of both sexual morphs increased with increasing elevation; however, the density of the L-morphs was higher in each population than that of S-morphs (Fig. 2B).

The fruit set was significantly affected, both at the individual plant and flower levels, by sexual morph ($F_{1,234} = 8.059$, $p < 0.001$; $F_{1,189} = 6.203$, $p < 0.001$), elevation ($F_{2,235} = 16.660$, $p < 0.001$; $F_{2,188} = 12.039$, $p < 0.001$), and the interaction of elevation and sexual morph ($F_{1,234} = 4.213$, $p < 0.001$; $F_{1,180} = 3.008$, $p < 0.05$). The effect of elevation on fruit set was greater on individual plants of both morphs than on flowers. The fruit set for each individuals and flowers was lower in the S-morph than in the L-morph (Fig. 3).

Table 1

Characteristics of the three study populations of *Primula nivalis* in the Tianshan Mountains, China. Data for mean annual temperature, mean annual precipitation, and annual sunshine difference of each population were downloaded from IPCC (<http://www.ipcc-data.org>) and WorldClim (<http://www.worldclim.org>). Mean average daily air temperature and rainfall data for the population at 1657 m were obtained from the local Meteorological Bureau in the Kunas area of NW Xinjiang Province, China.

Parameter	Population 1	Population 2	Population 3
Elevation (m)	1657 ± 8	2423 ± 11	2704 ± 10
Longitude (E)	84° 17' 29.05"	84° 22' 54.82"	84° 21' 22.43"
Latitude (N)	43° 16' 02.86"	43° 23' 52.91"	43° 08' 53.00"
Habitat	Forest-grassland	Grassland	Grassland
Mean temperature (°C)	0.180	-3.340	-4.430
Mean annual precipitation (mm)	226	276	289
Annual sunshine (h)	9.141	8.467	8.233
Growing season length each year	130–160 d	120–155 d	120–150 d
Flowering time	Early May	Late May	Early June
Fruit mature time	Middle July	Late July	Early Aug
Floral display	5–6 d	6–7 d	6–7 d

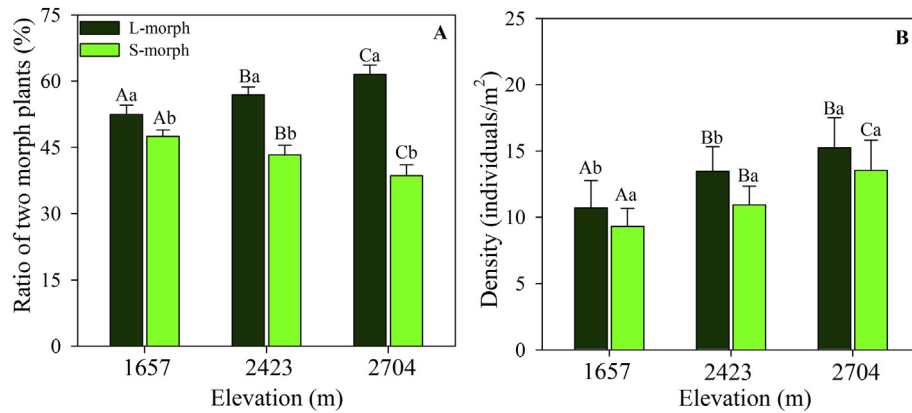


Fig. 2. Effect of elevation on the percentage (A) and density (B) of long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* in a natural population at three elevations (mean + SE). Bars with different lowercase letters indicate significant differences between the two morphs at the same elevation, and different uppercase letters, significant differences between the same morphs at different elevations.

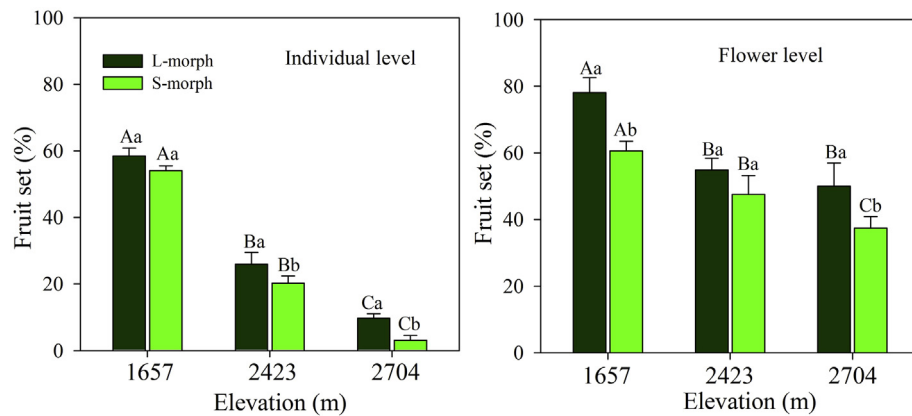


Fig. 3. Individual (A) and flower (B) level fruit set of *Primula nivalis* plants with long-styled (L-morph) and short-styled (S-morph) morphs in a natural population at three elevations (mean + SE). Bars with different lowercase letters indicate significant differences between the two morphs at the same elevation, and different uppercase letters, significant differences between the same morphs at different elevations.

3.2. Effect of pollination treatment on seed mass and viability

Seed mass was significantly influenced by pollination treatment ($F_{2,102} = 63.745$, $p < 0.001$), elevation ($F_{2,102} = 18.506$, $p < 0.001$), sexual morph ($F_{1,102} = 6.118$, $p < 0.05$), the interaction of treatment and elevation ($F_{4,102} = 14.608$, $p < 0.001$), and the interaction of treatment and sexual morph ($F_{2,102} = 3.636$, $p < 0.05$), but it was not affected by the interaction of sexual morph and elevation ($F_{2,102} = 0.118$, $p > 0.05$) or by treatment, sexual morph, and elevation ($F_{4,102} = 0.482$, $p > 0.05$). However, the seed mass from the three pollination treatments of both sexual morph flowers differed significantly ($F_{2,102} = 121.482$, $p < 0.001$). The rank order of seed mass was cross-pollination > open-pollination > self-pollination. The seed mass of S-morphs was greater than that of L-morphs (Fig. 4).

Seed viability was significantly affected by treatment ($F_{2,180} = 29.345$, $p < 0.001$), sexual morph ($F_{1,180} = 6.118$, $p < 0.05$), elevation ($F_{2,180} = 11.501$, $p > 0.05$), and the interaction of treatment and elevation ($F_{4,180} = 9.279$, $p < 0.05$), but it was not influenced by the interaction of treatment and sexual morph ($F_{2,180} = 1.206$, $p > 0.05$); sexual morph and elevation ($F_{2,180} = 0.378$, $p > 0.05$); or treatment, elevation, and sexual morph ($F_{4,180} = 0.482$, $p > 0.05$). Viability of seeds from self-pollinated flowers of both sexual morphs was lower than that of seeds from open-pollinated and cross-pollinated flowers, and viability of seeds from self-pollinated

L-morph flowers was higher than that of seeds from self-pollinated S-morph flowers (Fig. 5). Viability of seeds from self-pollinated S-morph flowers decreased with increasing elevation, but it was not significantly affected by elevation in L-morph flowers ($F_{2,30} = 0.462$, $p > 0.05$, Fig. 5). However, seed viability was higher for open-pollinated S-morph flowers than for open-pollinated L-morph flowers, whereas seed viability of cross-pollinated flowers did not differ significantly for either of the two morphs ($F_{1,60} = 0.509$, $p > 0.05$, Fig. 5).

3.3. Female fitness and post dispersal inbreeding depression

Seed germination was significantly affected by pollination treatment ($F_{2,54} = 13.977$, $p < 0.01$, Fig. 6), sexual morph ($F_{1,54} = 5.835$, $p < 0.05$), and the interaction of treatment and sexual morph ($F_{1,54} = 2.808$, $p < 0.05$). The seed germination rates of cross-pollinated flowers were higher than those of self- and open-pollinated flowers. The seed germination rate of L-morph flowers was higher than that of S-morph flowers; and the rate of self-pollinated flowers increased with increasing elevation.

Female fitness was affected by treatment ($F_{2,18} = 21.645$, $p < 0.01$), sexual morph ($F_{1,18} = 23.743$, $p < 0.01$), elevation ($F_{2,18} = 10.780$, $p < 0.001$), and the interaction of sexual morph and treatment ($F_{2,18} = 5.373$, $p < 0.05$). However, female fitness was not significantly affected by the interactions of sexual morph and

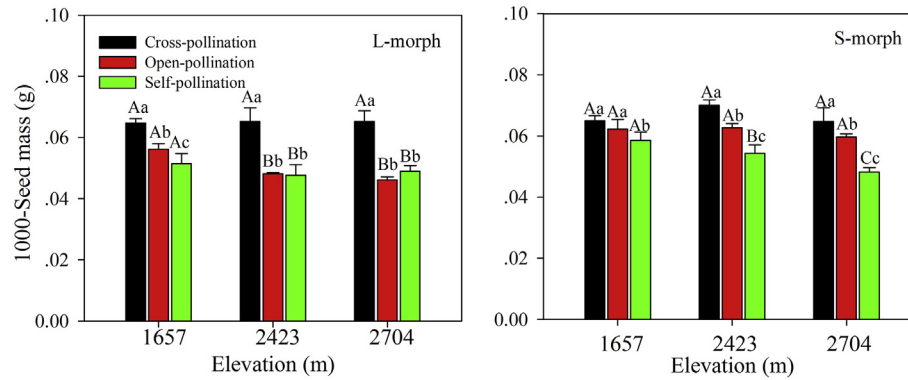


Fig. 4. Mass of seeds resulting from different pollination treatments of long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* in a natural population at three elevations (mean + SE). Bars with different lowercase letters indicate significant differences between the two morphs at the same elevation, and different uppercase letters, significant differences between the same morphs at different elevations.

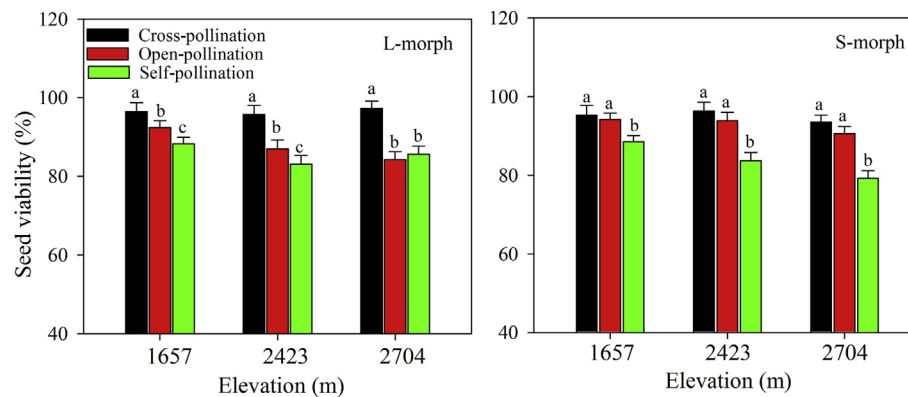


Fig. 5. Viability of seeds resulting from different pollination treatments of long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* in a natural population at three elevations (mean + SE). Bars with different lowercase letters indicate significant differences between the same elevations within different treatments flowers.

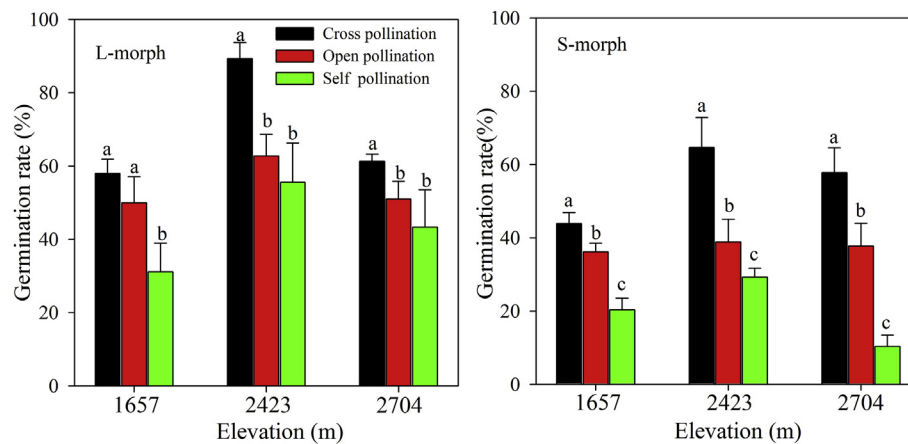


Fig. 6. Seed germination rate of different pollination treatments of long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* in a natural population at three elevations (mean + SE). Bars with different lowercase letters indicate significant differences between the same elevations within different treatments flowers.

elevation ($F_{2,18} = 0.163$, $p > 0.05$); treatment and elevation ($F_{4,18} = 0.054$, $p > 0.05$); or treatment, elevation, and sexual morph ($F_{4,18} = 0.030$, $p > 0.05$, Fig. 7). Interestingly, fitness in the different treatments differed significantly for both sexual morphs ($F_{2,18} = 13.072$, $p < 0.01$). The rank order of seed fitness was cross- > open- > self-pollination. The fitness of L-morphs was higher in each population than that of S-morphs. Female fitness in the three

pollination treatments of L-morphs and in the cross-pollination treatment of S-morphs increased with increasing elevation (Fig. 7), but it did not differ significantly for naturally or self-pollinated S-morphs at any of the three elevations (Fig. 7).

Post-dispersal inbreeding depression was positively influenced by sexual morph ($F_{1,18} = 17.203$, $p < 0.001$), elevation ($F_{2,18} = 23.091$, $p < 0.001$), and the interaction between elevation and sexual morph

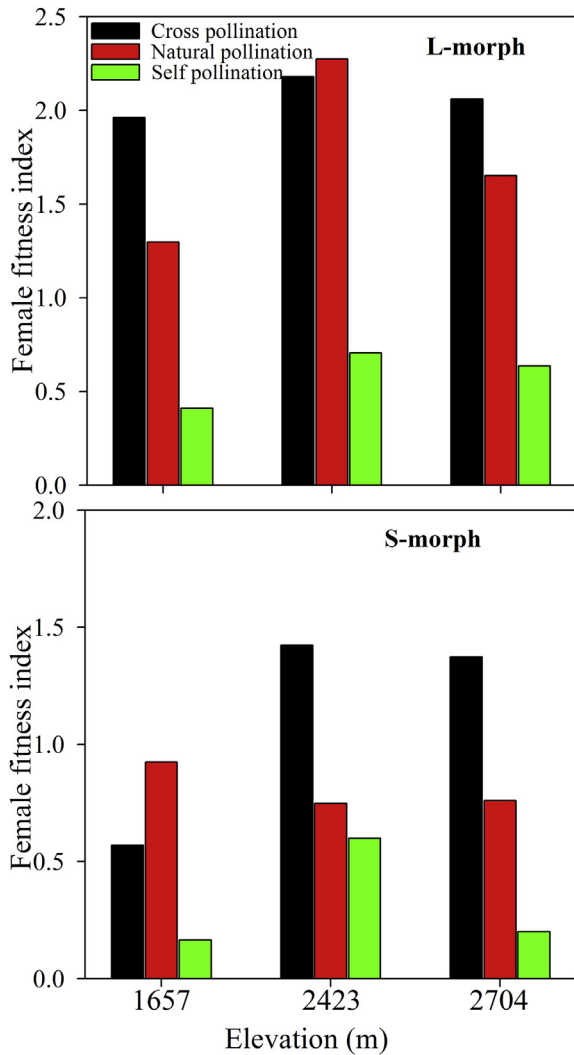


Fig. 7. Female fitness resulting from self-, open-, and cross-pollination treatments on long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* among populations at three elevations (mean \pm SE).

($F_{2,18} = 4.048$, $p < 0.01$, Fig. 8). The inbreeding depression index for S-morphs increased with increasing elevation, but was lower in the population at 2423 m than in the populations at 1657 and 2704 m. The inbreeding depression index was higher for S-morphs than for L-morphs in the populations at 2423 and 2704 m, but it was lower for S-morphs than for L-morphs in the population at 1657 m.

4. Discussion

Both the sexual morph frequency and density of *P. nivalis* were significantly affected by elevation, with the percentage of L-morph plants being higher than that of S-morph plants in each of the three populations (Fig. 2). Many biotic and abiotic factors influence morph frequency in populations of distylous species (Jacquemyn et al., 2001; Endels et al., 2002), including population size, density of individuals, low pollinator activity, mating type, and inbreeding depression (Kéry et al., 2000, 2003). For instance, in the distylous perennial *Primula veris*, demographic stochasticity causes the morph ratio to deviate from unity in populations at high elevations (Kéry et al., 2003). In the present study, the percentage of L-morphs was higher than that of the S-morphs, regardless of elevation (Fig. 3A); however, the density of each morph increased

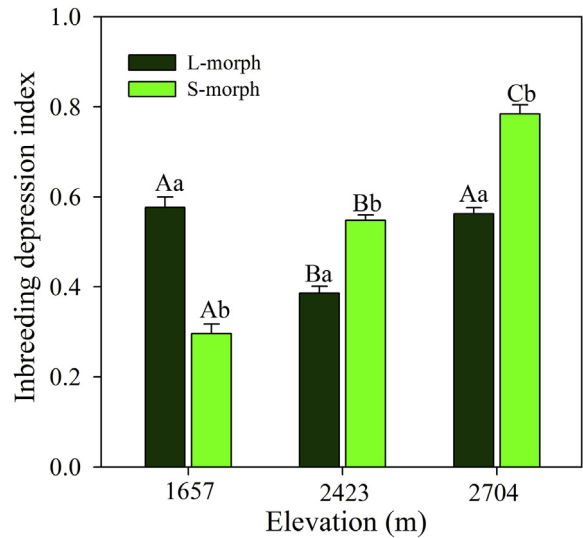


Fig. 8. Post-dispersal inbreeding depression of seeds from long-styled (L-morph) and short-styled (S-morph) morphs of *Primula nivalis* among populations at three elevations (mean \pm SE). Bars with different lowercase letters indicate significant differences between the two morphs at the same elevation, and different uppercase letters, significant differences between the same morphs at different elevations.

with elevation (Fig. 2B). Therefore, in *P. nivalis*, elevation-dependent increases in density in both morphs and the relatively high percentage of L-morphs affect offspring fitness (female fitness and inbreeding depression).

The fruit set of individual plants and flowers of both sexual morphs was affected by elevation, i.e., fruit set decreased with increasing elevation (Fig. 3), which might be due to type of pollination, low pollinator efficiency, and/or low temperature in the harsh alpine environment (Knight et al., 2005; Bernareggi et al., 2015; Qi et al., 2015). In addition, the increase in the percentage of L-morph plants at high elevation had a further negative effect on the fruit set of individual plants and flowers in these pollinator-limited high-elevation populations. Specifically, the highly compatible individual plants and flowers of L-morphs produced more mature fruits than did the S-morphs. Although the fruit set of open-pollinated flowers decreased with increasing elevation in both morphs, the selfing rate of L-morphs increased with increasing elevation (unpublished data). Our results suggest that elevation-dependent variation in the mating type of both sexual morphs and high plant density cause the level of fruit set to decline in both morphs among high-elevation populations.

Seed mass and viability of *P. nivalis* individuals were more strongly affected by the type of pollination than by elevation (Figs. 4 and 5), and these values were lower in self-pollinated than in cross- and open-pollinated flowers of both sexual morphs. The level of pollination has varying effects on seed quantity (number) and quality (mass) (Agren et al., 2006; Baskin and Baskin, 2014, 2015), while the seed mass of cross-pollinated flowers is greater than that of self-pollinated plants. However, some studies suggest that selfed seeds are larger than crossed seeds (Agren et al., 2006; Susko and Clubb, 2008; Baskin and Baskin, 2015) and may therefore contain more resources and be considered higher in quality. For example, seeds from cross-pollinated flowers of *Clarkia tembloriensis* (Holtsford and Ellstrand, 1990), *Primula farinosa* (Galletto et al., 2000), and *Erythrina crista-galli* (Agren et al., 2006) have greater mass than those from open or self-pollinated flowers. However, seeds from the self-pollinated flowers of *Hesperis matronalis* have greater mass than those from cross-pollinated flowers (Susko and Clubb, 2008). The mass and viability of seeds from selfed S-

morphs and open-pollinated L-morphs decreased significantly with increasing elevation in the present study. Seed mass was mostly affected by the type of pollination at high elevations, where pollinator activity was low (Figs. 4 and 5), because seed viability decreases at high elevation due to inbreeding related to population size (Goodwillie and Knight, 2006; Susko and Clubb, 2008; Weber et al., 2012) in different populations.

The “stress tolerance” hypothesis suggests that seed mass increases with increasing elevation, because large seeds have an advantage over small seeds during seedling establishment in stressful high elevation environments (Pluess et al., 2005; Qi et al., 2015). In contrast, the “energy constraint” hypothesis suggests that seed mass is negatively correlated with elevation, because low temperatures and the short growing season at high elevations might reduce photosynthetic rates, thus reducing the energy available for seed development and seed provisioning (Baker, 1972; Guo et al., 2010). In *P. nivalis*, seed mass and seed viability were affected more by pollination treatment than by other factors, suggesting that these factors are restricted by mating type and by low legitimate pollination, as seed development time increases with elevation. Thus, our data do not support either the stress tolerance or the energy constraint hypothesis.

Seed mass has been shown to play an important role in seed dispersal, germination, and fitness (Pluess et al., 2005; Guo et al., 2010; Baskin and Baskin, 2015). In general, we found that seeds from the cross-pollinated flowers of both sexual morphs at all elevations had more mass and higher viability than those from self-pollinated flowers of both sexual morphs at each elevation (Fig. 6A). In both of the sexual morphs of *P. nivalis*, the female fitness of offspring from cross-pollinated heavy seeds was higher than that of offspring from self-pollinated seeds (Figs. 6A and 7). Thus, variation in pollination-dependent seed mass between the L-morphs and S-morphs at different elevations might affect seedling establishment in both morphs.

Seed germination rate and female fitness in L-morphs were higher than those in S-morphs at all three elevations (Figs. 6A and 7). Likewise, seeds from L-morphs had a higher mean germination rate than those from S-morphs in *Primula cusickiana* (Rayburn et al., 2013). Davidson and Wolf (2011) concluded that the major difference between LS and SS flowers (which they describe as pin and thrum flowers, with pin being more prevalent) could be reduced by high levels of legitimate pollination. In the present study, *P. nivalis* female fitness decreased while inbreeding depression increased in S-morphs at high elevations. The decrease in the mass and viability of seeds from S-morphs could result in the decreased growth of seedling progeny from S-morphs in a population. Furthermore, the lower inbreeding depression of seeds from L-morphs at high elevations, relative to that of seeds from S-morphs, might alter the population characteristics and dynamics of *P. nivalis*, impacting the breeding system and floral morph ratio.

Reduction in the fitness of progeny due to the type of mating might also be important in the population dynamics of *P. nivalis*. Regardless of elevation, a bias in the self-compatibility and mating patterns of L-morphs would reduce effective offspring fitness, thus increasing inbreeding depression in S-morphs, which, in turn, would affect the function and structure of populations at different elevations (Fig. 8). Furthermore, natural selection driven by sexual morph frequency and mating patterns in a harsh environment should result in a decrease in legitimate pollination levels and the offspring fitness of S-morph plants in high elevation populations.

5. Conclusions

Variation in elevation-dependent mating might influence the female fitness and inbreeding depression of flowers of both sexual

morphs in *P. nivalis*. Low levels of adaptation of seeds from short-styled flowers might cause the frequency of individuals with short-styled flowers to decrease at high elevations. The lack of a 1:1 sexual morph frequency at each elevation probably reflects a difference in the natural selection pressure on the two morphs in the different environments, leading to variations in population characteristics and reproductive mechanisms.

Author contribution

Aysajan A and Qing-jun Li designed experiments; Aysajan A carried out experiments and analyzed experimental results. Aysajan A and Qing-jun Li wrote the manuscript.

Conflicts of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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