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The Role of Reproductive Assurance in the Arctic: a Comparative Study of a Homostylous and Distylous Species Pair

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Abstract

Pollinator-dependent reproductive systems are predicted to be disadvantageous in the Arctic due to the unreliability of pollinator services. Observations in northwestern Alaska appear to support this prediction, where the common *Primula eximia* possesses reproductive assurance through homostyly and is outpacing the reproduction of its rare, distylous, and pollinator-dependent congener, *P. tschuktschorum*. We examined the reproductive ecology of these two taxa to determine if *P. tschuktschorum* is fully distylous with self and intramorph incompatibility, if *P. eximia* is homostylous with an advantage in fecundity over the rare species, and if there are reproductive interactions between the species. Our results indicate that while *P. tschuktschorum* is morphologically distylous, it displays only weak incompatibility reactions, but nonetheless has significant reproductive limitations relative to the fecundity of *P. eximia*, especially in the face of herbivory, which reduces the pool of compatible mates. Some hybridization is occurring between the species. These results support previous suggestions that reproductive assurance may be an important factor limiting the distribution of distyly where pollinator service is unreliable. Homostylous species are likely to be more successful and outcompete distylous progenitors, and selection pressures for reproductive assurance may lessen strong self and intramorph incompatibility reactions in distylous species.

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Introduction

The frequency of obligately outcrossing plants is believed to decline dramatically in the Arctic (Lloyd, 1980; Bierzychudek, 1985). This idea is largely based on the paucity of pollinators and the predominantly cold and windy weather that limits the time insect pollinators can be active; observations indicate most bisexual plants in the Arctic autonomously set seed in the absence of pollinators, even while offering entomophilous attractants that provide an opportunity for facultative outcrossing (Kevan, 1972; Brochmann and Steen, 1999). Reproductive strategies that rely solely on insect visitation are probably rare at high latitudes and likely to suffer long-term fitness consequences when pollinator service fails.

Distyly is a reproductive system highly dependent on pollinator service (Washitani et al., 1994) where diallelic sporophytic incompatibility allows reproduction only between two different genotypes that are typically manifested as long-style (''pin'') and short-style (''thrum'') floral morphs (Fig. 1.). Populations are comprised of the two mating types that differ in morphology; these are generally highly intramorph and self incompatible (Darwin, 1877; Ganders, 1979; Barrett, 2002). A strict diallelic incompatibility system in distylous species ensures that on average an individual may mate successfully with only half the other individuals in a population.

Conditions of reduced pollinator numbers or activity should severely curtail reproduction in distyles (Ishihama et al. 2006) and give self-fertile homostyles (recombinant distylous forms that produce anthers and stigmas at the same height and are self-fertile) a fitness advantage (Charlesworth and Charlesworth, 1979; Piper et al., 1984). Fertility assurance can allow homostyles to become established, perhaps to outcompete their less fecund progenitors or to spread into new territory when colonization opportunity exists, a scenario present in arctic regions at various times during and since the last glaciation (Elias et al., 1996). The example of the distylous *Primula tschuktschorum* and the homostylous *P. eximia*, its sister taxon, in *Primula* sect. *Crystallophlomis* (Mast et al. 2001), appears to illustrate the reproductive assurance model of homostyle success.

Primula tschuktschorum is a rare species of the Bering Strait region of northwestern Alaska and northeastern Asia (Kelso, 1987a, 1987b) with small populations, few flowers per plant, and low seed set. It is listed as globally rare (G3) by the Alaska Natural Heritage Program, University of Alaska Anchorage (see http://aknhp.uaa.alaska.edu/Botany_tracking_page.htm). Previous studies (Kelso, 1987a, 1987b) have shown *P. tschuktschorum* to be morphologically distylous with classical manifestations of the syndrome that include dimorphic pollen sizes, stigmatic papillae, and dimorphic androecia and gynoecia; no detailed analysis of breeding system function, however, had been performed for this species. In comparison, homostylous *P. eximia* is relatively widespread in western Alaska and northeastern Asia, covering a region also only recently ice-free (Manley and Kaufman, 2002). Previous studies have shown that populations of *P. eximia* are typically large and plants are robust, floriferous, and well marked genetically and morphologically (Kelso, 1987b; Mast et al., 2001). Both species have rose-magenta colored corollas. *Primula eximia* appears to have exploited opportunities in open terrain such as

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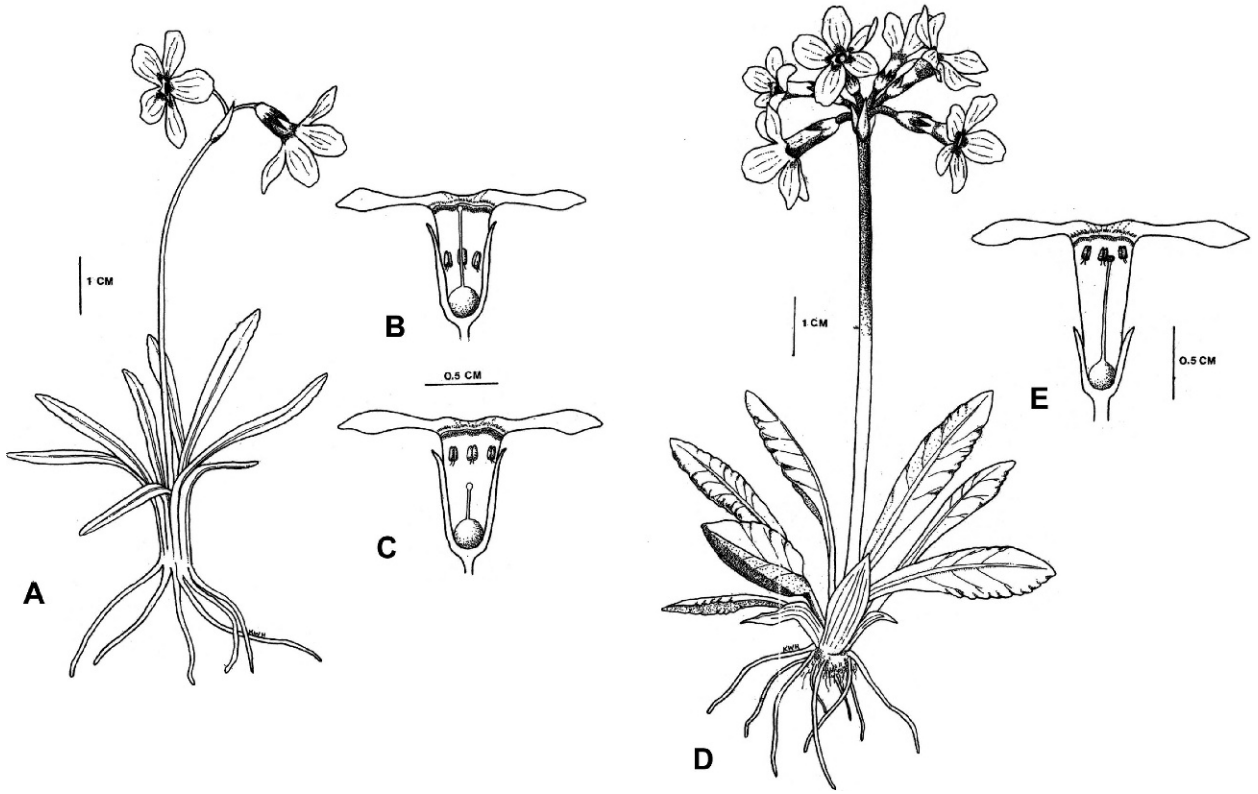


FIGURE 1. (A) *Primula tschuktschorum*, (B) long-style "pin" flower morph, (C) short-style "thrum" flower morph, (D) *Primula eximia*, (E) homostyle flower morph. Illustration modified from Kelso (1987b) and used by permission from *Brittonia*.

deglaciated high latitude mountain ranges and is perhaps now locally outcompeting its distylous presumptive progenitor, *P. tschuktschorum* (M. Carlson, T. Kaye, personal observations), where they co-occur around the Bering Strait.

Our study examined the reproductive ecology of these two taxa in northwestern Alaska, where they co-occur and flower simultaneously, to determine (1) if *P. tschuktschorum* is fully distylous with self and intramorph incompatibility that could place it at a reproductive disadvantage under arctic conditions of pollinator limitation, (2) if *P. eximia* is homostylous with self fertility, (3) if *P. eximia* has a reproductive advantage over the rare distylous species with respect to actual or potential fecundity, and (4) if there are reproductive interactions between the species that provide the potential for hybridization or other forms of reproductive interference, adding further reproductive stress on the rarer taxon.

Materials and Methods

We conducted our study during the summer of 2004 at Kuzitrin Lake in the Bendeleben Mountains on the Seward Peninsula in northwestern Alaska. The study site was located in the Bering Land Bridge National Preserve, ca. 140 km northeast of Nome. The study populations occurred along the southwestern margin of the lake at 435 m elevation for *P. eximia*, and up the slope at 610 m for *P. tschuktschorum*. The two species co-occur at the lake margin, but due to the low number of *P. tschuktschorum* individuals here we used *P. tschuktschorum* individuals from the nearest definable population.

We made observations of insect visitors to both species and collected two to three specimens of each visitor. The voucher specimens are on loan to the arthropod collection at Oregon State University.

ASSESSMENT OF REPRODUCTIVE SYSTEM

To quantify the extent of morphological dimorphism in *P. tschuktschorum* and the potential for reproductive interference between the species due to similar floral morphologies, we measured 10 characters from randomly selected individuals, collected a minimum of 2 m apart. Measurements were made to the nearest 0.01 mm, using digital calipers under 10× magnification from 40 *P. eximia* and 47 *P. tschuktschorum* specimens that were carefully pressed and dissected. We measured total flower number (buds + flowers + fruits), leaf length and width, corolla diameter, aperture width in the corolla throat where the tube expands to the petals, corolla tube length, calyx length, stigma position (height relative to the base of the ovary), and proximal and distal positions of the anthers (height relative to the base of the ovary).

Ovule and pollen counts were made from dissected buds under 20×. The buds were collected from one flower per individual and preserved in alcohol in the field. Numbers and proportions of ovules and pollen were used to determine potential maximum female and male fitness and to estimate the relative degree of outcrossing following Cruden (1977).

ASSESSMENT OF REPRODUCTIVE COMPATIBILITY AND POLLEN LIMITATION

To determine the nature of self, intra, and intermorph compatibilities in *P. tschuktschorum,* we emasculated in bud and subsequently pollinated approximately 15 flowers each for six treatments: (1) short-style self, (2) long-style self, (3) short-style × short-style, (4) long-style × long-style, (5) short-style♂ × long-style♀, and (6) long-style♂ × short-style♀. Short-style morphs are "thrum" and long-style morphs are "pin" in classical terminology

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TABLE 1

Comparative morphology of *P. eximia* and the long- and short-styled morph of *P. tschuktschorum*. Means are followed by (SE); $N = 40$, 29, and 18 for *P. eximia*, LS, and SS, respectively. Homogeneous groups (Student-Newman-Keuls) are represented by shared letters, following 1-way ANOVA. Stigma position is measured as the distance from the base of the ovary to the stigmatic surface. "Anther pos a" is the distance from the base of the ovary to the top of the anther, and "Anther pos b" is the distance to the bottom of the anther.

Character	*P. eximia*	LS *P. tschuktschorum*	SS *P. tschuktschorum*
Flower/plant	4.38 (0.27) a	2.79 (0.20) b	2.28 (0.18) b
Leaf ln	33.27 (1.46) a	71.71 (3.58) b	65.08 (3.53) b
Leaf wd	6.85 (0.41) a	5.65 (0.31) b	5.48 (0.30) b
Corolla wd	15.54 (0.30) a	21.21 (0.43) b	20.79 (0.56) b
Aperture wd	4.41 (0.10) a	5.18 (0.17) b	5.25 (0.11) b
Tube ln	9.69 (0.15) a	8.77 (0.15) b	9.35 (0.13) b
Calyx ln	5.59 (0.11) a	7.75 (0.16) b	6.95 (0.20) c
Stigma pos	8.39 (0.27) a	7.50 (0.13) b	4.92 (0.13) c
Anther pos a	8.89 (0.15) a	5.71 (0.10) b	8.10 (0.11) c
Anther pos b	7.87 (0.15) a	4.61 (0.15) b	6.90 (0.11) c

for distylous systems; typically "legitimate" (fertile) pollinations are those between morphs, i.e., treatments 5 and 6 only.

For *P. eximia,* self compatibility and autonomous seed set was determined by exposing three receptive flowers to one of three treatments: (1) unmanipulated and bagged, (2) self pollinated, and (3) cross pollinated. Self and cross pollinated flowers were emasculated in bud in late June and pollinated 24 to 36 hours following emasculation. During this time period, intact buds of the size we emasculated opened and began dehiscing pollen, thus indicating that the buds were likely to be mature. Self pollination was conducted by within-plant (geitonogamous) crosses. Plants were caged with window screen material around wire frames to prevent insect visitation.

The level of pollen limitation in both species was determined by exposing flowers of a single plant to unmanipulated, open pollination, and to pollen augmentation where pollen was transferred to a stigma in one individual by using pollen from another to coat the ball-tip of an insect pin (see Miller et al., 1994). A second control group was used in *P. eximia* to test for the effect of the pollen transfer technique alone. This treatment involved touching the stigma with a clean ball-tip of an insect pin and leaving the flower open for pollination. All treatments were marked using colored nylon string tied around the pedicels. Fruits of all treatments were collected four weeks following pollination when capsules were becoming dry, but remained closed.

To test for compatibility between species, we made 10 hand pollinations for 4 interspecific crosses: (1) *P. eximia*♂ × *P. tschuktschorum* short-style♀, (2) *P. tschuktschorum* short-style♂ × *P. eximia*♀, (3) *P. eximia*♂ × *P. tschuktschorum* long-style♀, and (4) *P. tschuktschorum* long-style♂ × *P. eximia*♀. Considering *P. eximia* to have thrum (short-style) anthers with a pin (long-style) stigma as per the classical model of a homostyle *Primula* (Mast and Conti, 2006), "legitimate" crosses would be interspecific crosses (2), (3), and (4) only. All pollen recipients were emasculated in bud and stigmas were brushed with a mature dehiscing anther, and capsules were collected four weeks following pollination.

Results

FLORAL VISITORS

For six days during peak flowering, the large floral displays of both species only attracted a few floral visitors despite consistently warm and still weather. We typically observed one of the three species of bumblebees (*Bombus hyperboreus*, *B. frigidus*, and *B. balteatus*) visiting one to three flowers per plant for only a few (ca. 2–5) plants in a patch before flying to another *Primula* patch. Floral visitation rates for a patch of approximately 1000 plants were approximately 0.5 per hour. Most of the visits were observed

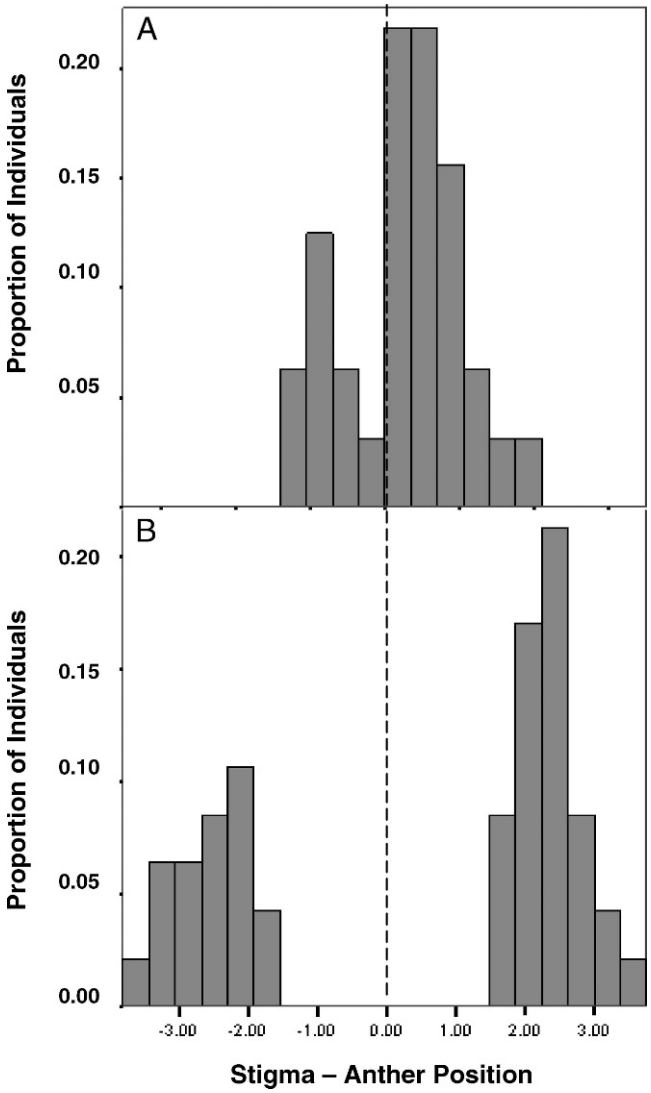


FIGURE 2. Frequency distributions of the difference in stigma and anther position for (A) *P. eximia* and (B) *P. tschuktschorum* individuals. No difference in position is indicated with the dashed line.

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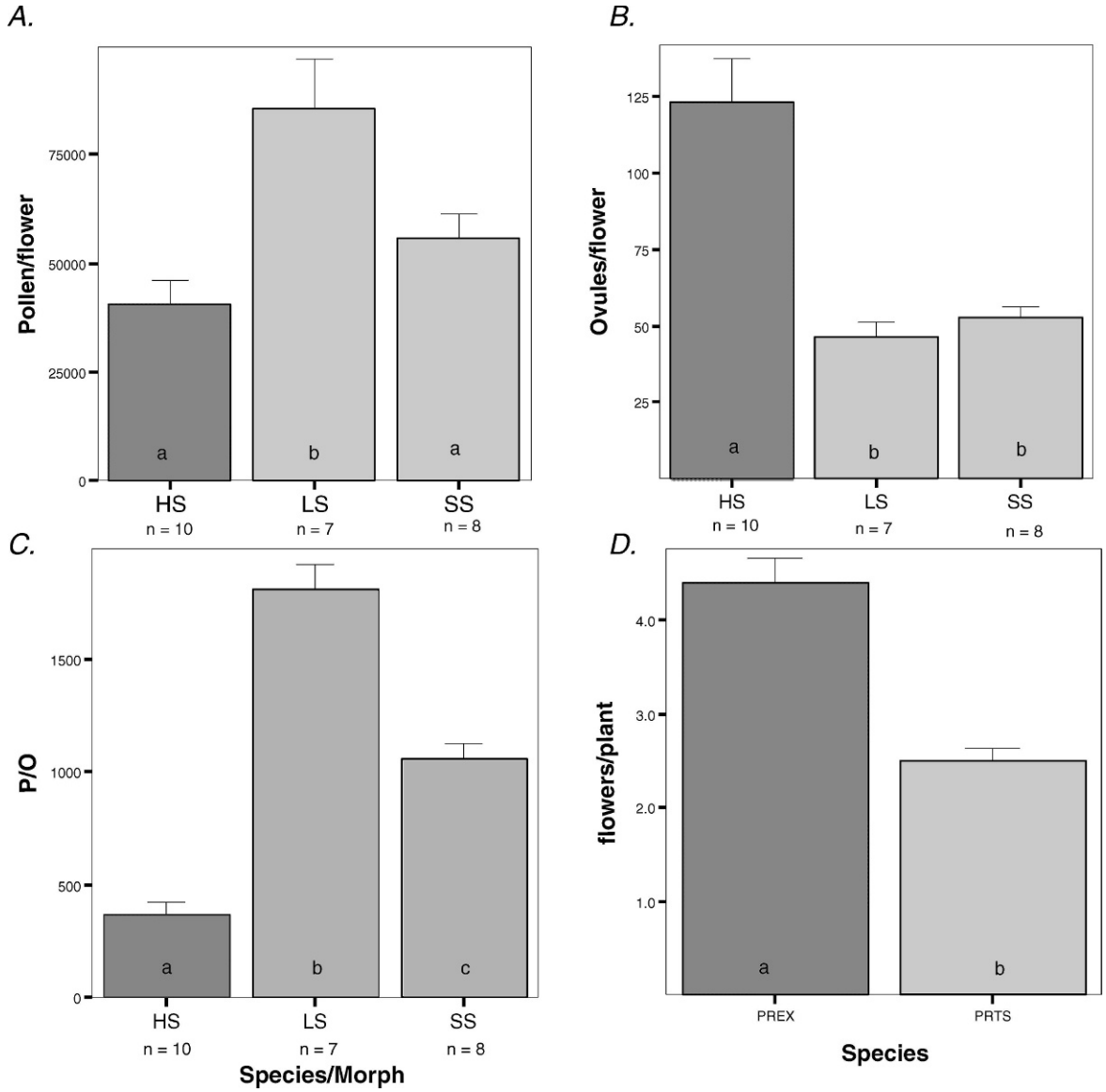


FIGURE 3. Reproductive investment for *Primula eximia* (PREX) homostyles (HS) and the long-styled (LS) and short-styled (SS) morphs of *P. tschuktschorum*. (A) Pollen per flower, (B) ovules per flower, (C) pollen/ovule ratio, (D) flowers per plant. Homogeneous groups (Student-Newman-Keuls) following 1-way ANOVA are represented by shared letters. Error bars indicate 1 SE.

as occurring on *P. eximia*, which has a more prolific floral display. An unidentified syrphid fly and an Empid fly (*Empis* sp.) were also observed on *P. tschuktschorum*.

REPRODUCTIVE BIOLOGY

As expected from previous studies, *P. eximia* is morphologically homostylous, with a unimodal distribution of stigma and anther positions, while the bimodal stigma-anther positions of *P. tschuktschorum* clearly distinguish it as morphologically distylous (Table 1, Fig. 2.). All three morphs (*P. eximia* homostyles, *P. tschuktschorum* short-styles, and *P. tschuktschorum* long-styles) are similar in variation of stigma and anther placement (Table 1).

Primula eximia shows significant differences from both morphs of *P. tschuktschorum* in all traits except amount of pollen per flower, where it is similar to the short-styled morph of *P. tschuktschorum* and roughly half that of the long-styled morph (Fig. 3). Similarity in androecium characters to the short-style morph would be expected for a homostyle with anther type and position derived from the short-style morph. Flower diameter is considerably smaller in *P. eximia*, but the corolla depth (as measured by corolla tube length) is longer. Leaves of *P. eximia* are significantly shorter and wider. Interestingly, the positions of stigmas and anthers of *P. eximia* are placed more distally and are not equivalent to those of the long-styled morph stigma position and short-styled morph anther position (Table 1).

In *Primula tschuktschorum*, aside from stigma and anther positions, only calyx length and pollen number are different between the two morphs. The long-styled morph has a significantly longer calyx and produces nearly twice the number of pollen grains as the short-style morph (Table 1, Fig. 3). The difference in pollen production between morphs is consistent with the results of Ornduff (1979, 1980) and Schou (1983), who found anthers in long-style *Primula* morphs to contain 1.6–3 times as much pollen as anthers in short-style morphs. This difference also supports the comparable pollen production of *P. eximia* (with "short-style pollen") and short-style *P. tschuktschorum*.

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Primula eximia has nearly twice the number of flowers per plant as *P. tschuktschorum*, and 2–3 times the number of ovules per flower (Table 1, Fig. 3). Greater ovule production may be associated with the longer corolla tubes in *P. eximia* and suggests that maximum seed production per plant would be 538.7 in *P. eximia*, but just 128.3 for the long-styled and 120.8 for the short-styled *P. tschuktschorum* morphs.

Primula eximia has a pollen-ovule ratio of 367, while P/O ratio in *P. tschuktschorum* is 1811 and 1055 for the long-styled and short-styled morphs, respectively (Fig. 3). These ratios strongly support the profile of an outcrossing (*P. tschuktschorum)* and selfing (*P. eximia*) species pair as portrayed by Cruden (1977). Change in P/O ratio, along with the increase in ovule number and in number of flowers per inflorescence indicate a substantial reallocation of reproductive investment has occurred in *P. eximia*, along with, or more likely following, the mutation to homostyly.

REPRODUCTIVE COMPATIBILITY AND POLLEN LIMITATION

Unlike the majority of distylous species, *P. tschuktschorum* does not have a strong self and intramorph incompatibility. Self pollination produced fruits and seeds in both morphs, and short-style intramorph crosses also produced fruits and seeds (Fig. 4). Only long-style × long-style crosses did not produce fruits. Fruit and seed set tended to be greater, but were not significantly different between "illegitimate" (self and intramorph) and "legitimate" (intermorph) pollinations (Table 2). Emasculation in bud appeared to depress fruit and seed set relative to open-pollination or pollen-augmentation treatments as the two legitimate intermorph hand-pollinations had 7.1 and 2.6 seeds per fruit, while open-pollinated and pollen augmented treatments produced between 18.1 and 28.1 seeds per fruit.

Primula eximia is fully self compatible and capable of autonomous seed set (i.e., seed set without insect visitation). Mean fruit set was 0.54, 0.40, and 0.73 for autonomous, hand-self pollinated, and outcrossed treatments, respectively, and not significantly different from one another ($p = 0.19$, Kruskal-Wallis Test). Mean seed set was 59.5, 62.5, and 43.5 for autonomous, hand-self pollinated, and outcrossed treatments, respectively ($p = 0.42$, Kruskal-Wallis Test).

Although our data do not demonstrate overall pollen limitation for *P. eximia* or *P. tschuktschorum* (Fig. 5), there was a trend for pollen limitation for long-styled morphs of *P. tschuktschorum*, where mean seed number per fruit was 18.1 in open-pollinated plants and 28.1 in plants with pollen-augmentation treatments. However, this difference is not statistically significant ($p = 0.59$, Wilcoxon Signed Ranks Test). Nearly half of the paired treatments were damaged or their capsules had dehisced prior to seed recovery, thus unexpectedly reducing sample sizes. Additional studies with larger samples are needed before this suggestion of pollen limitation can be confirmed or rejected.

We found the two species to be partially cross compatible, but only from short-styled ♂ *P. tschuktschorum* to *P. eximia* ♀ (a legitimate cross between thrum anthers and pin stigma); no other cross produced fruits (Fig. 6). Half of the 10 short-styled ♂ *P. tschuktschorum* to *P. eximia* ♀ pollinations set fruit, with an average of 22.0 seeds per fruit. Additionally, individuals possessing a mosaic of both species' traits were observed near the lake margin and a number of individuals were shown to possess both *P. eximia* and *P. tschuktschorum* DNA molecular markers (J. B. Whittall, unpublished data).

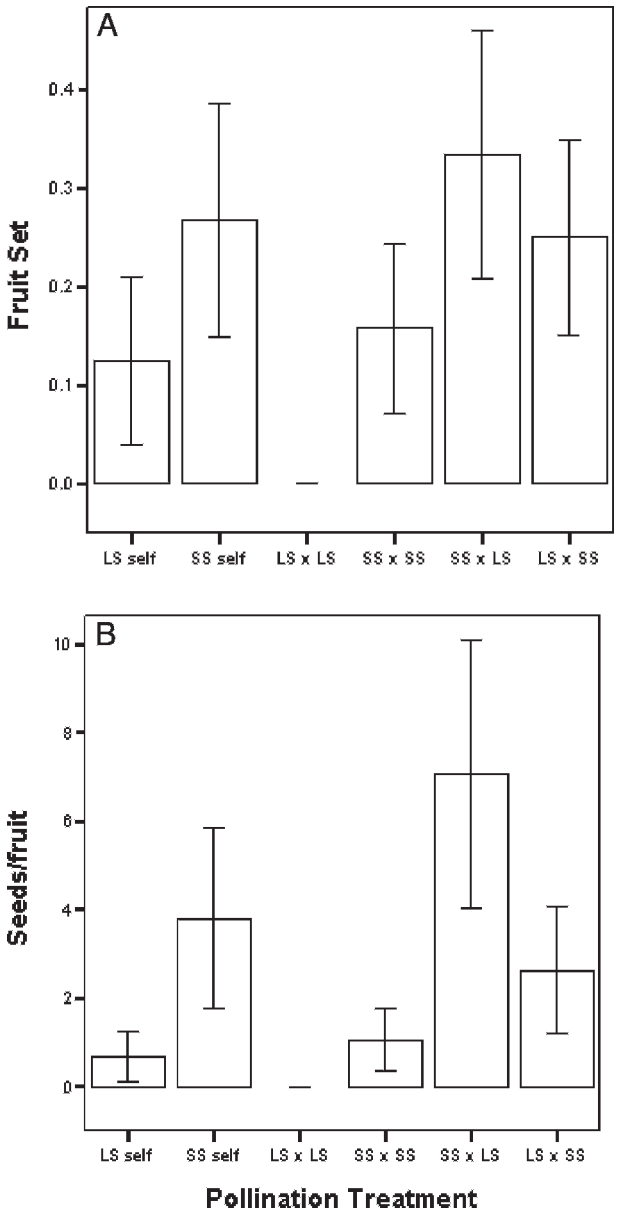


FIGURE 4. Seeds per fruit for self, intra-, and intermorph hand pollination treatments in both morphs of *P. tschuktschorum*; intermorph ("legitimate") crosses are indicated in black. Horizontal lines at zero indicate that no seeds were produced for any of the hand pollinations of that cross type. Error bars indicate 1 SE.

Discussion

DISTYLY-HOMOSTYLY

Primula eximia and *P. tschuktschorum* are well documented as an arctic distylous-homostylous species pair that share similar habitats, but diverge greatly in range size (Kelso, 1987a, 1987b; Kelso, 1992; Mast et al., 2001). Despite the considerable distributional, taxonomic, and genetic attention given to these species, quantitative field data are necessary to evaluate the functioning of their reproductive systems, relative degrees of reproductive assurance, and potential for interspecific reproductive interference. Such information can inform debates regarding rare species' ecology, especially those with obligate interactions with mutualists, and those with potential interactions with more aggressive congeners. This study provides support for the concept of arctic reproductive assurance of homostyly, with its fertility

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TABLE 2

Fruit and seed set between "legitimate" intermorph and "illegitimate" self and intramorph pollinations in *P. tschuktschorum*. $N = 103$ and 102 for fruit set and seed set, respectively.

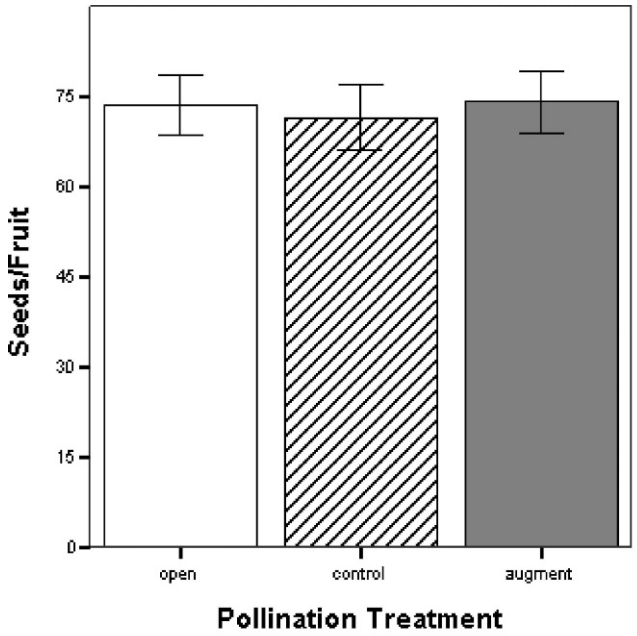
	Legitimate pollination mean (SE)	Illegitimate pollination mean (SE)	Mann-Whitney U	Z	*p*
Fruit set	0.26 (0.077)	0.13 (0.041)	1007.5	−1.89	0.059
Seed set	4.59 (1.58)	1.29 (0.52)	983.0	−1.85	0.065

advantage, that may put distylous, insect-dependent congeners at a substantial disadvantage on multiple levels.

Morphologically the two species conform to the norms of distylous and homostylous primulas. *Primula tschuktschorum* shows reciprocal positioning of mean anther and stigma position and moderate precision of organ placement (see Eckert and Barrett, 1994). Stigma positions between the two morphs in *P. tschuktschorum* do rarely overlap, but this is likely due to very recently opened long-styled flowers without fully elongated styles, as these individuals also had shorter corolla tubes and anther positions. This is a tendency seen in many *Primula* species for the corolla tube to elongate to greater or lesser degrees throughout anthesis and thus change the degree of herkogamy between anthers and stigma (S. Kelso, unpublished data).

Primula eximia lacks stigma-anther separation and was found to be highly self-fertile and capable of seed set without visitation, as expected based on its morphology, and as modeled for the typical homostyle *Primula*. While *P. eximia* has long styles and stamens placed high in the corolla tube, the styles are 10% longer than those of the long-styled morph and 10% longer than the stamens of the short-styled morph of *P. tschuktschorum*. This along with the restricted inter-taxon fertility and differences in vegetative characteristics (not likely part of the distyly supergene that is known to impact only reproductive traits in the gynoecium and androecium) suggest that the two taxa have diverged considerably and merit the current species-level taxonomic recognition (Kelso 1987b). While these taxa differ significantly in all the traits we measured, the differences are not large and it appears that pollinators do not discriminate when they forage between them in nearby or parapatric populations.

The clear morphological distinction between long-styled and short-styled *P. tschuktschorum* morphs implies self and intra-



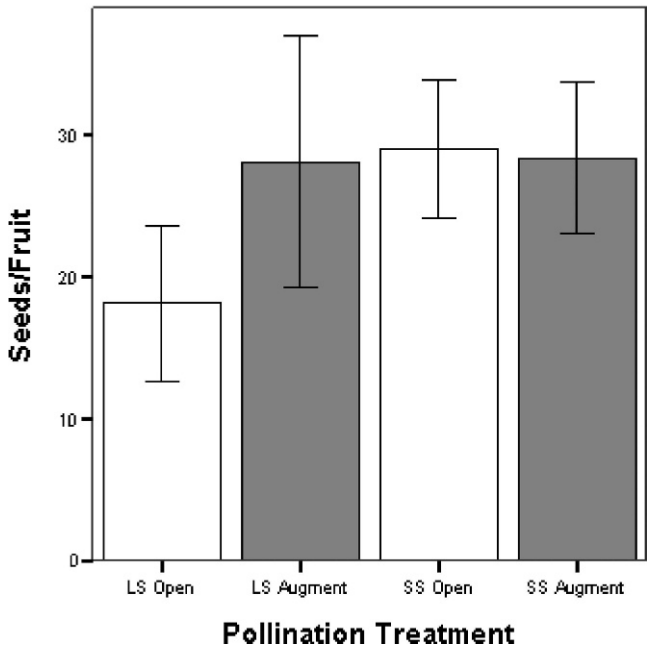


FIGURE 5. Seeds per fruit for under open, control, and pollen augmentation for (A) *Primula eximia* and (B) the long-styled and short-styled morphs of *P. tschuktschorum*. Error bars indicate 1 SE.

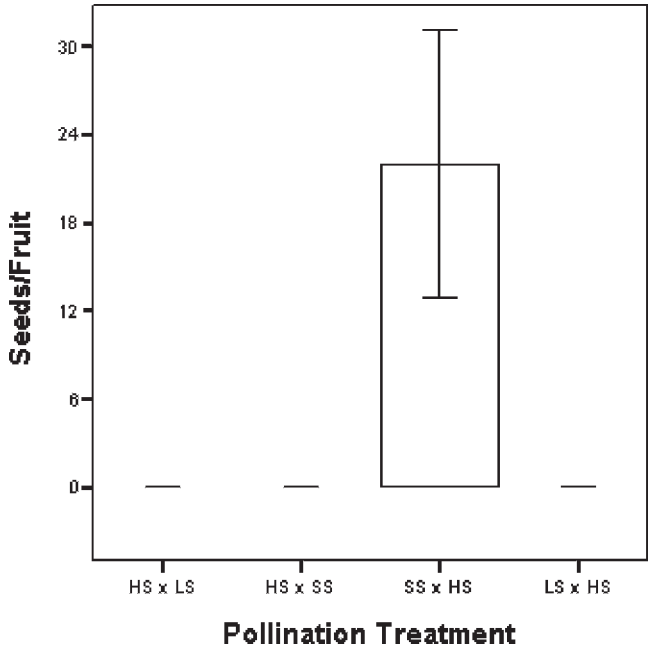


FIGURE 6. Seeds per fruit for between species crosses. Horizontal lines at zero indicate that no seeds were produced for any of the hand pollinations of that cross type. Error bars indicate 1 SE.

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morph incompatibilities as observed in other *Primula* species (Kelso, 1992; Miller et al., 1994; Richards, 2003). However, our hand-pollinations indicate that incompatibility is weak within *P. tschuktschorum* since moderate levels of fruit and seed production occurred after "illegitimate" self and short-style intramorph pollinations. The only pollination type that did not produce seeds was long-style × long-style. These results suggest that in this environment, where pollinator service was or has become unreliable, selection pressure for reproductive assurance may have weakened a previously strong incompatibility that typically (but not universally) occurs in other species in the genus (Darwin, 1877; Wedderburn and Richards, 1990; Richards, 2003), or incompatibilities are following the evolution of the morphological dimorphism (see Lloyd and Webb, 1992). *Primula tschuktschorum* appears to have a mechanism for maintaining at least modest seed set in the context of low pollen flow. Variability in the strength of inhibition and the location of inhibition in general (Wedderburn and Richards, 1990) indicate more evolutionary lability of this component of distyly than is generally acknowledged. Ecological conditions (e.g., pollen limitation) and levels of inbreeding depression should produce strong selection for compatibility (see Crosby, 1949; Bodmer, 1960; Charlesworth and Charlesworth, 1979; Lloyd, 1979); in the case of *Primula*, it appears that sufficient variation may be present to respond to this selection. This may be particularly the case in environments such as the Arctic where pollinators may be limited or unreliable (Murray, 1987; Totland and Sottocornola, 2001, and references therein), or where profound episodic climatic changes have impacted insect faunas with respect to diversity, numbers, or timing.

REPRODUCTIVE LIMITATION

Despite unusually favorable weather for pollinators (10–20°C and little wind) in July 2004 on the Seward Peninsula, Alaska, and extensive displays of thousands of *P. eximia* and *P. tschuktschorum* flowers, we observed only a few visits to these species by bumblebees and flies. The paucity of pollen vectors should therefore put fully distylous taxa at a severe competitive disadvantage relative to self-compatible homostylous sister species in conjoining or overlapping habitats. This difference in reproductive system has been suggested to explain the broader geographic range of the presumably derivative homostylous *P. eximia* (Kelso 1987b, 1992) and the rarity of distyly in the Arctic in general, where only 10% of arctic *Primula* are distylous relative to roughly 90% for the genus as a whole (cf. Mast et al., 2001; Guggisberg et al., 2006).

Primula eximia does indeed appear to be capable of much greater seed production and population growth and is capable of producing four times more seeds per plant than *P. tschuktschorum*. Additionally, while *P. eximia* produces less pollen per flower relative to the long-styled *P. tschuktschorum* morph, a reallocation typical of a switch to autogamy from xenogamy (Cruden, 1977, and references within), total pollen production per plant is similar between the species since flower number is greater in *P. eximia*. Therefore, *P. tschuktschorum* appears to have neither a female nor male fitness advantage. These results indicate that if seed production is not resource limited, which did not appear to be the case in 2004, the fitness advantage of a *P. tschuktschorum* individual would need to be fourfold over a *P. eximia* individual to match the greater reproductive output of its homostylous relative. Further, this assumes that pollen is not limiting. If pollinator activity is so low that pollen limitation occurs, *P. tschuktschorum* would have an even greater fitness disadvantage.

Other studies have shown that reduced pollination can cause reproductive distress of distylous species, but that under these conditions, fecundity of the self-fertile homostyle morphs can be several times greater (summarized in Washitani et al., 2005). These results parallel those of Piper et al. (1986) for *Primula vulgaris* and are in alignment with general conclusions about the detrimental effects of diminishing population sizes and habitat fragmentation on European primulas (e.g., Kéry et al., 2000; Jacquemyn et al., 2002; van Rossum et al., 2002; Lienert and Fischer, 2003; Brys et al., 2004). Conjoined factors produce negative reproductive impacts quickly, while extended impacts may induce local extirpation or promote the establishment of mutant homostyles. Loss of reliable pollinators plus decreasing distyle population sizes, coupled with opportunities for migration and ecological expansion, may be a potent recipe for recombinant homostyle success. Although neither of these species has particular adaptations for dispersal, should dispersal occur through zoogenic or hydrologic vectors, *P. eximia* would have a profound founder advantage over its distylous counterpart due to its selfing capacity.

Reproductive disparity may explain the apparent simultaneous decline in *P. tschuktschorum* and increase in *P. eximia* populations over the last 10 years along Kuzitrin Lake. Many thousands of *P. tschuktschorum* individuals were observed here in 1995 (S. Kelso, personal observation), but we were unable to locate these large populations in 2004. Herbivory may play an additional role in these contrasting population trends; lakeside plants are subject to intense grazing by molting Canada geese that forage nearly exclusively on *Primula* and *Carex* here (Carlson, 2006). The geese remove the reproductive scapes of *Primula* and reduce fecundity by over 50%; this means that for a distylous species with partial incompatibility, even fewer individuals in the population can serve as a pollen source or recipient. The importance of population density and the spatial arrangement of morphs to reproduction was highlighted in another rare *Primula* (Ishihama et al., 2006). In contrast, the self-fertile homostyle would not have the compounding impact of a reduced mating pool since it can serve as its own pollen donor and recipient. Grazing pressure is thus likely to affect the two species to different degrees, with *P. tschuktschorum* receiving more negative impact on its reproductive system from reduced effective population size.

REPRODUCTIVE INTERACTIONS

These two species have very similar ecological requirements for moist, low-competition habitats, co-flower in the Bering Strait region, and appear to share floral visitors. Reproductive interactions are therefore quite likely. These interactions could be mutualistic (e.g., increasing visitation rates by attracting more pollinators), neutral, or competitive (e.g., loss of pollen to foreign stigmas and loss of ovules to heterospecific pollen), and greater study is needed to determine the relative costs and benefits of coflowering to both species.

Negative interactions through hybridization are probable. Hand pollinations indicate the two species are partially interfertile and suggest they are recently diverged (see Edmands, 2002). However, only crosses between short-styled pollen donors and *P. eximia* pollen recipients resulted in fruit or seed set, via a cross that is effectively "legitimate", since short-styled pollen grains are exposed to a long-styled gynoecium and should not be inhibited. When long-styled pollen grains are exposed to the incompatible long-styled gynoecium of the homostyle, no seeds are produced. We observed plants possessing trait mosaics of each species where both species commingled, and a number of these were clearly

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hybrids based on ITS molecular data (J. B. Whittall, unpublished data). Therefore, we conclude that pollinators do not consistently distinguish between the two taxa, and pollen and ovules appear to be lost to heterospecific sources. The fitness, fertility, and fate of these hybrids are unknown.

Conclusions

The picture that emerges from these two arctic taxa is one of competition and relaxed incompatibilities for both species in favor of reproductive assurance. Reproductive interactions have been suggested to drive divergence in reproductive systems towards selfing and outcrossing extremes (Fishman and Wyatt, 1999) and this may be occurring for these two taxa. The pollen-ovule ratios fall into the facultative autogamous (*P. eximia*) and facultative xenogamous (*P. tschuktschorum*) mating systems as reported by Cruden (1977).

The narrowly endemic *Primula tschuktschorum* is morphologically distylous but lacks corresponding strong self and intramorph incompatibility, which increases its pool of potential mates and can boost seed set in an environment where pollinators may not be reliable. Its homostylous sister species is fully self compatible and capable of high seed output regardless of the presence of pollinators, since ovule and flower numbers are higher than in *P. tschuktschorum*. When the additional ecological impacts of small population sizes or herbivory (which reduce the abundance of potential mates) is added, fully self-compatible, auto-pollinating homostyles are at a clear advantage. In this situation, *Primula tschuktschorum* is likely to be threatened by negative ecological interactions with *P. eximia*.

Primula tschuktschorum is indeed a species at risk and should be of conservation concern, with an unusual risk factor being its lack of competitive ability against its sister taxon, a presumed descendent from a successful mutation that converted a primarily xenogamous breeding system to an at least faculatively autogamous one. *Primula eximia* has more reliable and fecund reproduction and a porous species barrier that appears to allow introgression in favor of its more fertile genome. More broadly, this species pair provides a comparative view of homostyle success over a distylous relative, and strong support for the role of reproductive assurance in breeding system evolution in environments like the Arctic where destabilization of ecological relationships may be occurring.

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References Cited

Barrett, S. C. H., 2002: The evolution of plant sexual diversity. *Nature Reviews Genetics*, 3: 274–284.

Bierzychudek, P., 1985: Patterns of plant parthenogenesis. *Experientia*, 41: 1255–1264.

Bodmer, W. F., 1960: The genetics of homostyly in populations of *Primula vulgaris*. *Philosophical Transactions of the Royal Society of London, Series B. Biological Sciences*, 242: 517–549.

Brochmann, C., and Steen, S. W., 1999: Sex and genes in the flora of Svalbard—Implications for conservation biology and climate change. *Det Norske Vitenskaps-Akademi. I. Matematisk Naturvitenskapelig Klasse, Skrifter, Ny Serie*, 38: 33–72.

Brys, R., Jacqueyn, H., Endels, P., van Rossum, F., Hermy, M., Triest, L., DeBruyn, L., and Blust, G. D. E., 2004: Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. *Journal of Ecology*, 92: 5–14.

Carlson, M. L., 2006: Natural threats to the rare arctic primrose, *Primula tschuktschorum*: goose grazing and reproductive interference with its sister species. Anchorage: Alaska Natural Heritage Program, 20 pp.

Charlesworth, D., and Charlesworth, B., 1979: The maintenance and breakdown of distyly. *American Naturalist*, 114: 499–513.

Crosby, J. L., 1949: Selection of an unfavourable gene-complex. *Evolution*, 3: 212–230.

Cruden, R. W., 1977: Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution*, 31: 32–46.

Darwin, C., 1877: *The Different Forms of Flowers on Plants of the Same Species*. London: John Murray, 352 pp.

Eckert, C. G., and Barrett, S. C. H., 1994: Tristyly, self-incompatibility, and floral variation in *Decodon verticilliatus* (Lythraceae). *Biological Journal of the Linnean Society*, 53: 1–30.

Edmands, S., 2002: Does parental divergence predict compatibility? *Trends in Ecology and Evolution*, 17: 520–527.

Elias, S. A., Short, S. K., Nelson, C. H., and Birks, H. H., 1996: Life and times of the Bering land bridge. *Nature*, 382: 60–63.

Fishman, L., and Wyatt, R., 1999: Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Carylophyllaceae). *Evolution*, 53: 1723–1733.

Ganders, F., 1979: The biology of heterostyly. *New Zealand Journal of Botany*, 17: 607–635.

Guggisberg, A., Mansion, G., Kelso, S., and Conti, E., 2006: Evolution and biogeographic patterns, ploidy levels, and breeding systems in a diploid-polyploid species complex of *Primula*. *New Phytologist*, 171: 617–632.

Ishihama, F., Ueno, S., Tsumura, Y., and Washitani, I., 2006: Effects of density and floral morph on pollen flow and seed reproduction of an endangered heterostylous herb, *Primula sieboldii*. *Journal of Ecology*, 94: 846–855.

Jacquemyn, H., Brys, R., and Hermy, M., 2002: Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia*, 130: 617–625.

Kelso, S., 1987a: Systematics and biogeography of the arctic and boreal species of *Primula*. Ph.D. dissertation. University of Alaska Fairbanks.

Kelso, S., 1987b: *Primula tschuktschorum* and *Primula eximia* (Primulaceae: Section Crystallophlomis): a distylous species and its homostylous derivative from the Bering Strait Region, Alaska. *Brittonia*, 39: 63–72.

Kelso, S., 1992: The genus *Primula* as a model for evolution in the Alaskan flora. *Arctic and Alpine Research*, 24: 82–87.

Kéry, M., Matthies, D., and Spillman, H.-H., 2000: Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology*, 88: 17–30.

Kevan, P. G., 1972: Insect pollination of high arctic flowers. *Journal of Ecology*, 60: 831–847.

Lienert, J., and Fischer, M., 2003: Habitat fragmentation affects the common wetland specialist *Primula farinosa* in north-east Switzerland. *Journal of Ecology*, 91: 587–599.

Lloyd, D. G., 1979: Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist*, 113: 67–79.

Lloyd, D. G., 1980: Demographic factors and mating patterns in Angiosperms. *In* Solbrig, O. T. (ed.), *Demography and evolution in plant populations*. *Botanical Monographs,* 15: 67–88.

Lloyd, D. G., and Webb, C. J., 1992: The evolution of heterostyly. *In* Barrett, S. C. H. (ed.), *Evolution and function of heterostyly*. New York: Springer-Verlag, 151–178.

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Manley, W. F., and Kaufman, D. S., 2002: Alaska paleoglacier atlas. Boulder: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado (http://instaar.colorado.edu/Qgisl/ak_paleoglacier_atlas/).
Mast, A. R., and Conti, E., 2006: The primrose path to heterostyly. *New Phytologist*, 171: 439–442.
Mast, A., Kelso, S., Richards, A. J., Lang, D. J., Feller, D. M. S., and Conti, E., 2001: Phylogenetic relationships in *Primula* L. and related genera (Primulaceae) based on noncoding chloroplast DNA. *International Journal of Plant Sciences*, 162: 1381–1400.
Miller, J., Litvak, M., Kelso, S., and Vargo, A., 1994: Comparative reproductive biology of two alpine primrose species. *Arctic and Alpine Research*, 26: 297–303.
Murray, D. F., 1987: Breeding system in the vascular flora of arctic North America. *In* Urbanksa, K. M. (ed.), *Differentiation patterns in higher plants*. London: Academic Press, 239–262.
Ornduff, R., 1979: Pollen flow in a population of *Primula vulgaris* Huds. *Botanical Journal of the Linnean Society*, 78: 1–10.
Ornduff, R., 1980: Heterostyly, pollen composition, and pollen flow in *Hedyotis caerulea*. *American Journal of Botany*, 67: 95–103.
Piper, J. G., Charlesworth, B., and Charlesworth, D., 1984: A high rate of self-fertilization and increased seed fertility of homostyle primroses. *Nature*, 310: 50–51.
Piper, J. G., Charlesworth, B., and Charlesworth, D., 1986: Breeding system evolution in *Primula vulgaris* and the role of reproductive assurance. *Heredity*, 56: 207–217.
Richards, A. J., 2003: *Primula*. Portland: Timber Press, 386 pp.
Schou, O., 1983: The distyly in *Primula elatior* (L.) Hill (Primulaceae), with a study of flowering phenology and pollen flow. *Botanical Journal of the Linnean Society*, 86: 261–274.
Totland, Ø., and Sottocornola, M., 2001: Pollen limitation of reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. *American Journal of Botany*, 88: 1011–1015.
van Rossum, F., Echchgadda, G., Szabadi, I., and Triest, L., 2002: Commonness and long-term survival in fragmented habitats: *Primula elatior* as a study case. *Conservation Biology*, 16: 1286–1295.
Washitani, I., Osawa, R., Namai, H., and Niwa, M., 1994: Patterns of female fertility in heterostylous *Primula sieboldii* under severe pollinator limitation. *Journal of Ecology*, 82: 571–179.
Washitani, I., Ishihama, F., Matumura, C., Nagai, M., Nishihiro, J., and Nishihiro, M. A., 2005: Conservation of *Primula sieboldii*: synthesis of information about the prediction of the genetic/demographic fate of a population. *Plant Species Biology*, 20: 3–15.
Wedderburn, F., and Richards, A. J., 1990: Variation in the within-morph incompatibility inhibition sites in heteromorphic *Primula* L. *New Phytologist*, 116: 149–162.

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