

1   **Title: Pollinator-Mediated Selection on Floral Traits in the Arctic Plant *Parrya***  
2   ***nudicaulis* (Brassicaceae)**

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6   **Running Title: Pollinator-Mediated Selection in an Arctic Plant**

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24   **Abstract**

25   The evolution of floral traits is largely attributed to pollinator-mediated selection;  
26   however, the importance of pollinators as selective agents in pollen-limited environments  
27   is poorly resolved. In pollen-limited arctic and subarctic regions, selection is expected to  
28   either favor floral traits that increase pollinator attraction or promote reproductive  
29   assurance through selfing. We quantified phenotypic selection on floral traits in two  
30   arctic and two subarctic populations of *Parrya nudicaulis*. Additionally, we measured  
31   selection in plants in both open-pollination and pollen-augmentation treatments to  
32   estimate selection imposed by pollinators in one population. Seed production was found  
33   to be limited by pollen availability and strong directional selection on flower number was  
34   observed. We did not detect consistently greater magnitudes of selection on floral traits in  
35   the arctic relative to the subarctic populations. Directional selection for more pigmented  
36   flowers in one arctic population was observed however. In some populations, selection on  
37   flower color was found to interact with other traits. We did not detect consistently  
38   stronger selection gradients across all traits for plants exposed to pollinator selection  
39   relative to those in the pollen-augmentation treatment; however directional selection  
40   tended to be higher for some floral traits in open-pollinated plants.

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42   *Key words:* *Parrya nudicaulis*; floral evolution; phenotypic selection; pollen limitation;  
43   Arctic; Alaska; flower color.

## 44 **Introduction**

45 Selection for improving the export and receipt of pollen to flowering plants by pollinators  
46 is recognized to have disproportionately resulted in the incredible diversification of floral  
47 form (Grant and Grant 1965; Fægri & van der Pijl 1979; Ashman & Morgan, 2004;  
48 Fenster *et al.*, 2004; Caruso *et al.* 2019). Pollinators have been shown to discriminate  
49 among differences in floral traits such as petal and spur length, inflorescence size, flower  
50 size, floral scent, and nectar production (Hodges *et al.* 2002; Fenster *et al.* 2004; Sandring  
51 & Ågren, 2009; Schiestl *et al.*, 2011; Campbell *et al.*, 2012). Flower color is also  
52 important for pollinator attraction and pollinator foraging intensity is commonly  
53 associated with variation in flower color (Schemske & Bradshaw, 1999; Jones & Reithel,  
54 2001; Hodges *et al.*, 2002; Medel *et al.*, 2003; Irwin & Strauss, 2005; Streisfeld & Kohn,  
55 2007). The fitness consequences to plants due to differential attraction to pollinators  
56 based on phenotypic variation can be severe (cf. Stanton & Preston, 1988; Schemske &  
57 Bradshaw, 1999; Alexandersson & Johnson, 2002; Parachnowitsch *et al.*, 2012; Caruso *et*  
58 *al.* 2019). Direct estimates of selection by pollinators on flower color and other floral  
59 traits are not common, despite the assumption that current patterns in adaptive evolution  
60 are in fact reflections of pollinator-mediated selection (Sandring & Ågren, 2009;  
61 Parachnowitsch & Kessler, 2010; Campbell *et al.*, 2012; Campbell *et al.*, 2013; see  
62 review in Caruso *et al.* 2019).

63         Selection on floral traits may also include non-pollinator agents. Campbell &  
64 Bischoff (2013) demonstrated that flower color in an alpine species was under selection,  
65 yet the agent of selection could not be due to pollinators. In some cases, herbivores have  
66 been shown to impose selection on a variety of floral traits including flower

67 pigmentation, floral architecture, and inflorescence size (Galen & Butchart, 2003;  
68 Gómez, 2004; Ashman *et al.*, 2004; Strauss *et al.*, 2004). Second, abiotic agents may also  
69 drive selection on floral traits. In particular, individuals with floral anthocyanin  
70 pigmentation often have greater fitness than individuals lacking floral pigmentation under  
71 stressful conditions of drought, heat, and competition (reviewed in Strauss & Whittall,  
72 2006). Fitness differences in these stressful conditions are likely operating through a  
73 shared biochemical pathway where enzymes that assemble anthocyanins also assemble  
74 flavonoid compounds that are responsible for diverse physiological roles (Close &  
75 Beadle, 2003; Rausher, 2008).

76         Selection on floral traits is expected to be greatest when plants are limited by  
77 pollen availability (Haig & Westoby, 1988). Pollen limitation is predicted to be strongest  
78 in habitats with low and stochastic pollinator availability (Ashman *et al.*, 2004; Burd *et*  
79 *al.*, 2009; Garcia-Camazho & Totland, 2009). The severe climate of wet, windy, and  
80 cooler temperatures limit the flying time and flower visitation rates of pollinators of  
81 arctic habitats and to a lesser extent, subarctic habitats (Hocking, 1968; Arroyo *et al.*,  
82 1985; Kevan *et al.*, 1993; Totland, 1994; Bergman *et al.*, 1996). Moreover, northern  
83 biomes have a low abundance and diversity of pollinators; this is particularly striking in  
84 the Arctic (Arroyo *et al.*, 1985; Kevan *et al.*, 1993; Totland, 1994; Bergman *et al.*, 1996;  
85 Elberling & Olesen, 1999). Strong pollen limitation can have important evolutionary  
86 consequences where species that have, or evolve, mechanisms for reproductive assurance  
87 are expected to persist over longer periods (Ashman *et al.*, 2004; Morgan & Wilson,  
88 2005; Porcher & Lande, 2005; Harder & Aizen, 2010). Selection is therefore expected to  
89 favor traits that increase selfing or enhance pollinator attractiveness to increase pollen

90 receipt (Johnston, 1991; Totland, 2001; Ashman & Morgan, 2004; Harder & Aizen,  
91 2010; Campbell & Bischoff, 2013).

92         While arctic plant-pollinator ecological relationships are studied to some extent  
93 (see Kevan, 1972; Molau, 1993; Lundgren & Olesen, 2005; Carlson *et al.*, 2008), the  
94 selective pressures and evolutionary processes have been largely overlooked. The lack of  
95 attention is possibly attributed to the arctic (and alpine) angiosperm flora typically being  
96 considered depauperate in terms of investment in animal pollination; the flora being  
97 composed of wind-pollinated, apomictic, and self-fertilizing plants, with pollinators often  
98 presumed to be of trivial importance to plant reproduction (Löve, 1959; Mosquin, 1966;  
99 Bliss, 1962; Billings & Mooney, 1968; Johnson, 1969; Billings, 1974; Lloyd, 1980;  
100 Richards, 1997). Flowers of arctic species have even been suggested to be vestigial  
101 organs; remnants of the evolutionary past, inherited from ancestors to the south (Löve,  
102 1959; Mosquin, 1966). Contrary to these assertions, pollinators have been shown to be  
103 necessary for seed production in numerous arctic and subarctic alpine plant species, and  
104 many tundra plants have mixed mating systems with some taxa possessing self-  
105 incompatible systems (Kevan, 1972; Tikhmenev, 1985; Bingham, 1999; Grundt *et al.*,  
106 2005; Fulkerson *et al.*, 2012). Furthermore, the arctic flora as a whole contains a  
107 relatively high percentage of anthocyanin-pigmented taxa with many capable of nectar  
108 and scent production (Whittall & Carlson, 2009; Jaakola & Hohtola, 2010), which is  
109 suggestive of maintained pollinator-mediated selection.

110         Here, we estimate the magnitude of phenotypic selection on floral traits, using  
111 probability of seed set and female fecundity as proxies for fitness in the arctic and  
112 subarctic mustard, *Parrya nudicaulis*. High within-population variation in flower size,

petal orientation, and pigmentation are common in *P. nudicaulis* (Fig. 1), which has also been shown to be pollinator dependent (i.e., protandry limits autodeposition of viable pollen) and severely pollen limited (Fulkerson *et al.*, 2012). We predict that the strength of pollinator-mediated selection is greater than non-pollinator-mediated selection on flower color and four other floral traits in *P. nudicaulis*. Additionally, we predict that pollinator-mediated phenotypic selection is linked to traits associated with increased pollinator attraction (greater flower number, petal size, and floral pigmentation). Last, as pollinator service is expected to be poorer in arctic relative to subarctic populations, we predict that phenotypic selection on floral traits is greater in the more northerly populations.

## **Materials and Methods**

### ***Study System***

*Parrya nudicaulis* L. Regel (Brassicaceae) is found from northeastern Asia, across Alaska and to the western Canadian Arctic Archipelago (Hultén, 1968; Al-Shehbaz 2010). Flowering occurs in late May to mid-June in subarctic sites in Alaska and several weeks later at on the Arctic Coastal Plain. At reproductive maturity, this perennial produces a single raceme of 8–14 flowers, which normally persists between 10–14 days with individual flowers senescing after three days. Flowers are protandrous; the upper anthers dehisce shortly after the flowers open, followed by the lower two anthers within approximately 12 hours, and the stigma becomes bilobed and receptive during the second day. Flower color of *P. nudicaulis* is highly variable among individuals in many populations (Butler *et al.*, 2014). While the hue is quite consistent, the lightness values

136 range dramatically among individuals. Flowers range from pure white, and produce no  
137 anthocyanins, to dark violet with substantial anthocyanin production (Dick *et al.*, 2011).  
138 Most flowers emit a sweet fragrance, comparable to *Syringa* species. Nectar is secreted at  
139 the base of the corolla and less than 4  $\mu$ L is produced in plants bagged for 24 hours  
140 (Fulkerson *et al.*, 2012). Floral visitors of *P. nudicaulis* at the studied populations in  
141 Alaska are infrequent (mean of 0.14 and 0.58 visits/flower/hour in 2009 and 2010), and  
142 although a diversity of visitors drink nectar and collect pollen on *P. nudicaulis*, muscid  
143 and syrphid flies make up the largest proportion of floral visitors (Fulkerson *et al.*, 2012).

144         This study was conducted at two arctic and two subarctic locations. The Galbraith  
145 site (68°27' N, 149°33' W, 880 m elevation) and Ivishak site (69° 20' N, 148° 45' W, 280  
146 m elevation) are located on the foothills of the Arctic Coastal Plain. Both the Galbraith  
147 and Ivishak sites are found within the Northern Alaska Arctic Floristic Province and are  
148 a graminoid tundra habitat dominated by tussock-sedge, dwarf shrubs, and moss and  
149 lichens (Raynolds *et al.*, 2005). The two subarctic sites were Eagle Summit (65° 28'N,  
150 145° 25'W, 1100 m elevation) and Twelve-Mile (65° 24' N, 145° 44' W, 680 m  
151 elevation) and are located in the White Mountains of interior Alaska and consist of mesic  
152 forb-ericaceous shrub tundra above treeline.

153         We stratified sampling of individuals by three broad color categories: white, light  
154 violet, and dark violet. The Eagle Summit and Twelve-Mile populations in the subarctic  
155 consisted of relatively equal proportions of individuals in each color category. The two  
156 arctic populations contained relatively few pure white individuals, thus all white  
157 individuals in these populations were sampled.

158 Phenotypic selection was estimated on a total of 41 individuals at Galbraith, 64  
159 individuals at Ivishak, and 42 individuals at Twelve-Mile in 2009. In 2010 57 individuals  
160 were sampled at Ivishak. We contrasted pollinator-mediated selection with non-pollinator  
161 selection at a single subarctic population (Eagle Summit) in 2010. At this site, plants  
162 were randomly assigned to open- (129 individuals) or pollen-augmentation treatments (83  
163 individuals), which is designed to remove the component of phenotypic selection  
164 imposed by pollinators (for more discussion of this approach see Sandring & Ågren,  
165 2009). Treatments and measurements of Eagle Summit occurred at the beginning of *P.*  
166 *nudicaulis* flowering season in early June. Infructescences were collected at the end of  
167 July, prior to seed dehiscence.

#### 168 ***Pollination Treatments***

169 To remove the component of phenotypic selection due to pollinator visitation, mixed  
170 pollen from at least ten haphazardly selected individuals that were >10 m distance the  
171 recipient were used to hand-pollinate flowers. Phenotypic selection was not estimated  
172 from plants that served as pollen donors. Manipulated flowers were marked with a small  
173 amount of “puffy paint” at the base of the pedicel. Every flower was hand-pollinated  
174 every day, until there were signs of flower senescence to ensure that stigma receptivity  
175 was not missed. Supplemental pollen added to the entire inflorescence reduces the chance  
176 of differential resource allocation interfering with the detection of pollen limitation  
177 (Zimmerman & Pyke, 1988; Ashman *et al.*, 2004; Knight *et al.*, 2006).

#### 178 ***Phenotypic Measurements***

179 We used the measurements of six floral traits that we expected could be under natural  
180 selection: flower number, petal width, petal length, corolla depth, anther height, and



181 flower color. Petal length was highly correlated to petal width and to reduce  
182 multicollinearity, petal length was not included in the analysis. Pistil height is correlated  
183 with corolla depth and the stigma becomes receptive when it nears the corolla opening;  
184 we did not measure pistil position to avoid contact or damage to the stigma. We counted  
185 the total number of flowers produced at the end of the flowering season. All other traits  
186 were measured at anthesis, when the flowers were fully open and anthers were accessible  
187 to pollinators. We measured the width and lengths of the largest petal, corolla depth, and  
188 height of the tallest anthers to the nearest 0.01 mm with a digital caliper at Eagle Summit,  
189 Galbraith and the 2009 Ivishak plants. To capture a large enough sample with limited  
190 time, at the Twelve-Mile and 2010 Ivishak populations, we measured corolla depth and  
191 anther height with digital calipers, but measured petal length and width using digital  
192 photographs of individual flowers with a scale bar; measurements were subsequently  
193 made in ImageJ image analysis software.

194       A Royal Horticultural Society Colour Chart (RHS 2007) was used to quantify the  
195 variation of flower color between plants at the time of anthesis. Using this chart,  
196 however, limits the factor of ‘color’ to categorical data. To determine lightness values of  
197 the color chips, we used the techniques followed by Fulkerson *et al.* (2012) to create CIE  
198 L\* values: L\* values range from 0 to 100, where ‘0’ is black or ‘near-black’ and ‘100’ is  
199 white or ‘near-white’ (see Voss 1992; Stephens *et al.*, 2007). *P. nudicaulis* petals fall  
200 within a narrow range of purple-violet of the RHS Colour Chart, and L\* is highly  
201 correlated with anthocyanin concentration (Whittall *et al.* unpublished data).

## 202 ***Selection Analysis***

203 The strength and direction of selection on the floral traits was measured using a  
204 multivariate regression analytic framework (Lande & Arnold, 1983). We used variance-  
205 standardized partial linear regression coefficients to estimate the strength of directional  
206 selection on traits independent of all other measured traits (i.e., selection gradients,  $\beta_\sigma$ )  
207 (Lande & Arnold, 1983). Additionally, we calculated mean-standardized selection  
208 coefficients ( $\beta_\mu$ ), as this metric has been shown to avoid the problem of conflating  
209 selection and variation, is particularly useful for summarizing the strength of selection for  
210 diverse traits, and for facilitating a more accurate estimate of response to selection (see  
211 Hereford et al., 2004). Mean-standardized results are presented in the supplemental  
212 tables. The number of individuals was not sufficient to measure nonlinear selection  
213 (convex or concave) for all populations, although the sample size approached  
214 recommended levels for Eagle Summit open-pollinated and pollen-augmentation  
215 treatments (see Walsh & Lynch, 2014). We therefore quantified nonlinear selection and  
216 correlational selection for variance-standardized traits at Eagle Summit between pairs of  
217 traits using quadratic ( $\gamma_{ii}$ ) and fifteen cross-product ( $\gamma_{ij}$ ) terms in the regression model  
218 (Sandring & Ågren, 2009). These regression coefficients were multiplied by two to  
219 derive the nonlinear selection coefficients (Stinchcombe *et al.*, 2008). Fitness was  
220 estimated by two separate values: the probability of producing seed and fecundity for  
221 those individuals which produced seed. These fitness values were relativized by dividing  
222 by the population mean. Multiple logistic regression was used to estimate selection on the  
223 probability of seed set due to the dichotomous nature of the fitness measure (Janzen &  
224 Stern, 1998). Binomial logistic regression coefficients were transformed into linear  
225 regression coefficients using the methods of Janzen & Stern (1998). Secondarily, we

226 measured selection gradients on those individuals that did set seed at the experimental  
227 population at Eagle Summit and at Ivishak using standard multiple regression methods.  
228 Contrasts in the magnitude and direction of selection gradients between open-pollinated  
229 and pollen-augmented treatments were compared with means and 95% confidence  
230 intervals to avoid the pitfalls of null-hypothesis significance testing (Anderson *et al.*,  
231 2000; Fidler *et al.*, 2006; Rinella & James, 2010). All analyses were conducted using R  
232 version 2.12 (R Development Core Team 2011).

233

## 234 **Results**

235 Pollen limitation was evidenced by a nearly four-fold increase in seed production in  
236 pollen-augmented plants at Eagle Summit compared to open-pollinated plants ( $10.96 \pm$   
237  $1.19$  SE seeds/plant in pollen-augmented plants relative to  $2.47 \pm 0.51$  SE seeds/plant in  
238 open-pollinated plants). Seed set in open-pollinated plants was similar in 2009 and 2010  
239 at Eagle Summit (Fulkerson *et al.*, 2012). The other subarctic population, Twelve-Mile,  
240 produced  $5.33 \pm 0.91$  SE seeds/plant. In 2009, the arctic sites at Galbraith and Ivishak  
241 produced  $2.00 \pm 0.59$  SE seeds/plant and  $6.26 \pm 0.87$  SE seeds/plant, respectively. In  
242 2010 seed production was high at the Ivishak population (mean of  $9.95 \pm 0.40$  SE  
243 seeds/plant).

## 244 ***Phenotypic Selection on Floral Characters***

245 Selection gradients for all traits, populations, and open-pollinated versus pollen-  
246 augmented treatments are summarized in Figures 2-5 and Tables 1-3. While we did not  
247 detect consistently stronger gradients across all traits in the pollinator-mediated selection  
248 treatment relative to the pollen-augmented treatment, we did observe a trend in stronger

directional selection on increased flower number for plants exposed to natural levels of  
pollination for both probability of seed set and fecundity (Fig. 2A, 2C, Table 1A).  
Additionally, the interaction between flower color and anther height was under disruptive  
selection in open- but not pollen-augmented plants (Fig. 2B, Table 1B). Open-pollinated  
plants with darker flowers and shorter anthers or plants with lighter flowers and more  
exserted anthers had higher probabilities of setting seed (Fig. 3). Contrary to our  
prediction of pollinator-mediated selection for increased pollinator attraction, we did not  
detect directional selection for petal size, pigmentation, or anther position in the open-  
pollinated treatment (Fig. 2A, 2C, Table 1A).

In the pollen-augmented treatment, the probability of seed set was greater for  
individuals with shorter corolla tubes (Fig. 2A, Table 1A). For those individuals that set  
seed in the pollen-augmentation treatment, fecundity was also lowest for individuals with  
intermediate anther position (Fig. 1D, Table 1B).

Our second prediction was that phenotypic selection gradients for open-pollinated  
plants would be of greater magnitude in arctic populations relative to subarctic  
populations. We find little support for this hypothesis, with strong directional selection  
observed for some traits in arctic sites and strong directional selection for other traits in  
subarctic sites in both 2009 and 2010 (Figs. 4 and 5; Tables 2 and 3). Flower number was  
under significant positive linear selection for the majority of populations (Fig. 5; Table  
3). In 2010 open-pollination treatments at the arctic Ivishak and subarctic Eagle Summit  
populations, selection gradients indicate consistent directional selection on greater flower  
number, and an indication for potential directional selection for shorter corolla tubes, and  
higher anther position (Fig. 4). When measuring fitness as the probability of seed set,

272 however, selection was not detectable in 2010 when 85% of flowering individuals at  
273 Ivishak set seed (Table 2). Strong directional selection was observed for increased flower  
274 number at Ivishak in 2010 when measuring fitness in terms of fecundity, however (Fig. 4,  
275 Table 3). Directional selection for darker flowers was observed for the arctic population  
276 (Ivishak), but not the subarctic population (Eagle) in 2010 (Fig. 4., Table 3). The arctic  
277 population displayed concave selection for the interaction of ‘flower color’ and ‘corolla  
278 depth’, with individuals of intermediate combinations of trait values displaying reduced  
279 fecundity.

280

## 281 **Discussion**

### 282 *Natural Selection Under Pollen-Limited Environments*

283 Selection on floral traits is predicted to be strongest under pollen-limited environments  
284 where traits that increase pollinator attractiveness or improve selfing are expected to be  
285 favored (Ashman & Morgan, 2004; Harder & Aizen 2010). Phenotypic selection for  
286 attractive floral traits in pollen-limited environments has been found in some systems  
287 (Johnston, 1991; Caruso, 2000; Totland, 2001), but not in others (Totland, 2004; Fishman  
288 & Willis, 2008). In a New Zealand alpine plant, the strength of selection on flower color  
289 (whiter flowers had greater fitness) was stronger under a lower pollination-limitation  
290 treatment than when pollen was more limiting (Campbell & Bischoff, 2013); however, in  
291 this case non-pollinator mediated selection was invoked. Selection on floral traits can  
292 also occur when pollen limitation is absent (Galen, 1996; Parachnowitsch & Kessler,  
293 2010). In this study of *P. nudicaulis*, selection gradients were generally stronger under  
294 the naturally pollen-limited environment relative to the pollen-augmentation treatment,

295 where pollinator-mediated selection should be largely removed. Further, selection  
296 gradients at the arctic Ivishak population were also stronger in the year with less  
297 favorable weather and much lower natural seed set. The 2009 flowering season at Ivishak  
298 was marked with a wet, windy, and cold climate that would limit insect flight time and  
299 pollinator availability (Totland, 1994; Bergman *et al.*, 1996). In contrast, the 2010 season  
300 was sunny with warmer temperatures and *ad lib* observations suggested increased  
301 pollinator activity. While we did not specifically test for pollen limitation at this  
302 population, seed set in 2010 was comparable to hand-pollination treatments in the  
303 subarctic alpine sites to the south, suggesting pollen and resource limitation are quite  
304 variable across years and that arctic populations are not universally more pollen limited.

305       Strong positive linear selection for a greater number of flowers was found for  
306 nearly all open-pollinated populations, as well as the pollen-augmentation treatment.  
307 Greater flower number may be influencing the probability of seed set by increased  
308 opportunities for pollen receipt through a reproductive season that typically has many  
309 days with unfavorable weather, as well as through attracting a greater number of  
310 pollinators to a larger and more rewarding floral display. Male fitness is also expected to  
311 increase with increasing number of flowers; unfortunately, selection on male fitness is  
312 rarely studied, despite its importance (Sutherland & Delph, 1984). Plants containing a  
313 greater number of ovules than are on average fertilized have been hypothesized to benefit  
314 from occasional “jackpot” chance visits in environments with highly stochastic pollinator  
315 visits (Ashman *et al.*, 2004; Burd *et al.*, 2009). Pollinator visits to *P. nudicaulis* in tundra  
316 habitats occur at much lower rates and depend on windows of favorable climate  
317 compared to plants in temperate habitats (Fulkerson *et al.*, 2012). Indeed, an increase in

318 ovule number would be beneficial for occasional pollinator visits, but an increase in  
319 flower number would further enhance the probability of seed set, as well as promoting  
320 pollen export (male fitness) presumably.

321        Selection gradients based on probability of seed set and fecundity appeared to be  
322 of greater magnitude for flower number in open-pollinated plants than in pollen-  
323 augmented plants, suggesting pollinators were either discriminating between  
324 inflorescence sizes or if larger inflorescences have receptive flowers for a longer time  
325 period, they are more likely to be visited. The fact that flower number was also under  
326 positive selection (though weak) in hand-pollinated plants, suggests that either hand-  
327 pollinations did not always deliver adequate amounts of viable pollen, smaller plants with  
328 fewer flowers are resource limited, or that plants with fewer flowers received pollen from  
329 a smaller total pool of sires, some of whom have low viability. However, we refreshed a  
330 vial of mixed pollen from more than ten individuals every hour and delivered pollen daily  
331 to every flower. Overall, phenotypic selection for a greater number of flowers in plants  
332 appears to be common in other floral selection studies and is expected as the trait is  
333 directly tethered to fitness metrics (reviewed in Harder & Johnson, 2009; Parachnowitsch  
334 & Kessler, 2010; Caruso *et al.* 2019).

335        The greater strength of pollinator-mediated, relative to non-pollinator-mediated  
336 selection on flower number is consistent with our prediction of selection favoring traits  
337 associated with enhanced pollinator attraction. Contrary to our prediction, however, we  
338 did not detect selection for larger petal size. Pollinators have been shown to prefer  
339 flowers with larger petals and corollas in a number of other studies (Galen, 1996;  
340 Campbell *et al.*, 1996; Gómez, 2003; Ashman *et al.*, 2004; Sandring & Ågren, 2009;

341 Parachnowitsch & Kessler, 2010; Sletvold & Ågren, 2010). However, the pollinator  
342 guilds of the arctic and subarctic habitats are diverse, generally dominated by flies, and  
343 dissimilar from previously studied regions (Fulkerson *et al.*, 2012) and phenotypic  
344 selection is typically higher in plants pollinated by bees, long-tongued flies, or birds  
345 (Caruso *et al.* 2019). Additionally, directional selection on the size of the corolla or  
346 pollination unit (e.g., capitulum in Asteraceae) has not always been detected (Andersson  
347 & Widén, 1992; Sletvold *et al.*, 2010; Parachnowitsch *et al.*, 2012). It is possible that  
348 some of the apparent phenotypic selection on flower size observed in these studies could  
349 be a product of covariation in ovule number (see Hansen *et al.*, 2003; however see  
350 Stanton & Preston, 1988). In *P. nudicaulis*, we suspect that flower size has a minor  
351 impact on the overall floral display perceived by pollinators and unmeasured traits such  
352 as scent production may be significantly more important in pollinator perception  
353 (Parachnowitsch *et al.*, 2012).

354         An alternative prediction to selection for enhanced pollinator attraction in pollen-  
355 limited environments could be selection for increased capacity for selfing. We did not,  
356 however, detect directional selection on reduced petal size, lower anther position, or  
357 reduced floral pigmentation in open-pollinated plants in either arctic or subarctic  
358 populations. In fact, the arctic population experienced directional selection for more  
359 darkly pigmented flowers; although as we did not pair a pollen-augmentation treatment  
360 with the open-pollinated plants at this population, we are not able to attribute the agent of  
361 selection to pollinators or another source. Anther-stigma separation was not measured to  
362 avoid accidental hand-pollination or damage to the stigma, but we measured corolla tube  
363 length which is correlated with stigma position (Fulkerson *et al.*, 2012) and anther height.



364 Our results suggest modest directional selection for shorter corolla tube length at both the  
365 arctic and subarctic populations and for higher anther position at the arctic site Ivishak.  
366 Shorter corolla tubes are expected to be associated with lower stigma position, below the  
367 top four anthers at anthesis and more likely to receive self-pollen; however, it is not clear  
368 that shorter-tube length is indeed associated with greater reproductive assurance in this  
369 species. Variation in proximity of anthers and stigmas, as well as corolla size, may have  
370 little effect on the capacity for self-fertilization in the absence of pollinators since this  
371 species is protandrous (Fulkerson *et al.*, 2012).

372         We observed selection acting on combinations of floral traits in the open-  
373 pollination treatment that were not observed in the pollen-augmentation treatment.  
374 Notably, fitness was greater in plants with darker flowers and less exserted anthers or in  
375 plants with lighter flowers and more exserted in the open-pollinated treatment. This is  
376 suggestive of divergent selective pressures imposed by different pollinator guilds; lighter  
377 colored flowers with higher anther position might be effective in attracting a greater  
378 number of one pollinator guild, such as syrphid or muscid flies, that generally do not  
379 probe the flowers deeply, while darker colored flowers may be more attractive to long-  
380 tongued pollinators. We have no coupled data on the pollinator community or pollinator  
381 behaviors to support this notion, however. In general, high within-population variation in  
382 floral traits could be maintained by divergent or fluctuating selection on combinations of  
383 partially unlinked traits, as may be the case for flower color and anther position in *P.*  
384 *nudicaulis*.

385         The pollen-augmentation treatment was intended to remove pollinator-mediated  
386 selection (Sandring & Ågren, 2009); however, we suggest that hand-pollinations in this

387 species at least, introduces unintended phenotypic selection on some floral traits. The  
388 probability of seed set was greater for hand-pollinated individuals with shorter corolla  
389 tubes. This pattern seems to be most likely explained by more effective hand pollinations  
390 when stigmas receive more pollen from its own anthers, better micro-environmental  
391 conditions for pollen germination and growth, or potentially due to shorter distance for  
392 pollen tubes to travel down styles.

393         We predicted that the magnitude of phenotypic selection would be greater on  
394 floral traits in arctic populations relative to subarctic populations. The strength of  
395 selection should increase with increasing pollen limitation and pollen limitation is  
396 believed to increase at higher latitudes as weather and climate appropriate for pollinator  
397 service declines. While in a number of cases we detected greater selection in the arctic  
398 populations; overall the strength of selection was inconsistent among populations, traits,  
399 and years. Often the direction of linear selection was divergent for traits (or trait  
400 combinations) between the arctic and subarctic populations. The arctic populations are  
401 approximately 400 km to the north of the subarctic populations and have substantially  
402 lower mean July temperatures on average (Dick *et al.*, 2011) that would be expected to be  
403 associated with reduced pollinator activity; however, the subarctic sites are at a higher  
404 elevation and are also often subjected to inclement weather. Year to year variation in  
405 weather is likely to make detection of regional patterns in selection gradients difficult to  
406 detect.

407         The relationship of the degree of pollen limitation to strength of selection  
408 appeared to depend on the fitness metric used. We measured phenotypic selection in one  
409 arctic population over two years, and in the first year, natural seed set was low and

410 similar to most other populations, while in the second year, seed set was nearly doubled  
411 and the majority of individuals set seed. All estimated selection coefficients were not  
412 statistically distinguishable from zero in the low pollen limitation year when using  
413 probability of seed set as the fitness proxy. Using fecundity as the fitness metric for this  
414 population and year of lower pollen limitation, however, revealed directional selection  
415 for darker flowers and more flowers. While we split female fitness into two components  
416 (probability of seed set and fecundity of those which set seed) to avoid violating  
417 analytical assumptions, this approach also facilitates estimation of selection to attract a  
418 minimum of visits for seed production, as well as selection on traits associated with  
419 increased pollen quantity and quality.

420         We provide modest evidence of stronger selection gradients for pigmentation at  
421 the higher latitudes compared to the lower latitude sites, where darker violet individuals  
422 had higher fecundity. Indeed, at a population level, anthocyanin pigmentation of *P.*  
423 *nudicaulis* increases in frequency with increasing latitude (Dick *et al.*, 2011). Flower  
424 color did not affect the probability of seed set at the Ivishak population, but selection  
425 coefficients for darker flower color was strongly significant with a greater number of  
426 flowers and marginally on its own. Flower color did not enhance the probability of seed  
427 set unless it interacted with another trait in the other sites. Selection on flower color can  
428 be a result of herbivores, pathogens, or abiotic factors directly acting on the trait but also  
429 be a result of indirect selection through correlated traits (Frey, 2004; Strauss & Whittall,  
430 2006; Rausher, 2008; Campbell & Bischoff, 2013). Selection on flower color appeared to  
431 be operating through interactions with other floral characters, suggesting flower color is

432 being indirectly selected by pollinators through correlated traits or other unmeasured  
433 traits or directly selected by abiotic responses not measured in this experiment.

434       Greater anthocyanin concentrations in higher latitudes and elevations would likely  
435 enhance growth and survivorship from the abiotic stresses associated with these habitats.  
436 Anthocyanins are important components for osmotic regulation in drought and frost-like  
437 conditions and protect plant cells from visible light by screening it through attenuation  
438 (Close & Beadle, 2003). A combination of many abiotic selective pressures interacting  
439 with genetic adaptations may be responsible for color variation between higher and lower  
440 latitude populations of *P. nudicaulis*. We envision future studies on phenotypic selective  
441 pressures to incorporate several years of measurements since reproductive success varied  
442 greatly between the years for the Ivishak site suggesting either resource limitation or  
443 pollinator reduction resulting in pollen limitation.

444

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454

455 **Data Accessibility:**

456 Data will be accessible to the public through our Alaska Center for Conservation Science,  
457 University of Alaska data portal (<https://accs.uaa.alaska.edu/> and  
458 <https://accscatalog.uaa.alaska.edu/search/type/dataset>), where we serve diverse sets of  
459 biological and conservation data for Alaska and the Arctic. Voucher specimens are  
460 deposited at the UAAH herbarium and are available digitally at  
461 <https://www.pnwherbaria.org/data/search.php>.

462

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668 **Figure Legends:**

669 **Figure 1.** *Parrya nudicaulis* flowers from Eagle Summit, showing the broad range of  
670 floral pigmentation and corolla size. Scale bar = 1 cm.

671 **Figure 2.** Variance-standardized linear (A and C) selection gradients ( $\beta_\sigma$ ) and nonlinear  
672 (B and D) selection gradients ( $\gamma_\sigma$ ) for plants subjected to pollen-mediated selection (black  
673 squares) and pollen-augmented plants (open squares) on probability of seed set and  
674 fecundity at Eagle Summit 2010. Bars display the 95% CI.

675 **Figure 3.** Relationship of relative fitness (probability of seed set) to flower color and  
676 anther position in pollinator-mediated selection treatment at Eagle Summit. Trait axes are  
677 in units of standard deviations. Darkly pigmented flowers are represented by negative  
678 values of greater magnitude, lighter pigmented and unpigmented flowers have positive  
679 values of greater magnitude. Positive anther positions indicate a higher and generally  
680 more exerted anther position relative to the base of the corolla tube; negative anther  
681 positions indicate plants with lower than average anther position. The probability of seed  
682 set was highest for dark pigmented flowers with short anther position and light flowers  
683 with exerted anthers.

684 **Figure 4.** Variance-standardized linear ( $\beta_\sigma$ ) (above) and nonlinear ( $\gamma_\sigma$ ) (below) selection  
685 gradients on fecundity for subarctic Eagle 2010 (black squares) and arctic Ivishak 2010  
686 (blue triangles) populations. Bars indicate 95% CI.

687 **Figure 5.** Variance-standardized linear selection gradients ( $\beta_\sigma$ ) on probability of seed set  
688 (above) and fecundity (below) for all populations in 2009. Gradients for the arctic sites  
689 are Galbraith (solid turquoise triangle) and Ivishak (open blue triangle). Gradients for the

690 subarctic sites are Eagle Summit (black squares) and 12-Mile (gray squares). Anther  
691 height was not measured in the arctic populations. Bars indicate 95% CI.





**Table 1A.** Variance-standardized linear ( $\beta_\sigma$ ) gradients (and 95% confidence intervals in parentheses) for open-pollinated and pollen augmentation treatments using logistic regression on probability of seed set, and multiple linear regression on fecundity (seed number) for those individuals that did set seed at Eagle Summit in 2010. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). The regression model included only the five traits without interactions. Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	<i>Probability of Seed Set</i>		<i>Fecundity</i>	
	$\beta_{open}$	$\beta_{augment}$	$\beta_{open}$	$\beta_{augment}$
L (Flower Color)	0.00 (-0.20, 0.20)	0.04 (-0.06, 0.15)	0.05 (-0.22, 0.31)	-0.03 (-0.22, 0.15)
Flower Number	<b>0.31**</b> <b>(0.09, 0.51)</b>	<b>0.13**</b> <b>(0.02, 0.23)</b>	<b>0.48**</b> <b>(0.18, 0.77)</b>	<b>0.29**</b> <b>(0.11, 0.48)</b>
Petal Width	0.09 (-0.15, 0.32)	0.08 (-0.04, 0.20)	-0.03 (-0.34, 0.27)	-0.08 (-0.30, 0.14)
Corolla Depth	0.12 (-0.14, 0.38)	<b>-0.17**</b> <b>(-0.32, -0.02)</b>	<b>-0.34·</b> <b>(-0.72, 0.03)</b>	-0.01 (-0.30, 0.28)
Anther Height	-0.01 (-0.28, 0.26)	0.04 (-0.07, 0.17)	0.10 (-0.27, 0.48)	0.06 (-0.23, 0.34)

**Table 1B.** Variance-standardized linear ( $\beta_{\sigma}$ ) and nonlinear ( $\gamma_{\sigma}$ ) selection gradients (and 95% confidence intervals in parentheses) for open-pollinated and pollen augmentation treatments using logistic regression on probability of seed set, and multiple linear regression on fecundity (seed number) for those individuals that did set seed at Eagle Summit in 2010. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998). Regression coefficients for  $\gamma$  matrix diagonals were multiplied by 2 to calculate concave and convex gradients. Positive  $\gamma$  values indicate concave (disruptive) selection and negative values indicate convex (stabilizing) selection. The regression model included all five traits and fifteen cross-product terms. Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	<i>Probability of Seed Set</i>				<i>Fecundity</i>			
	$\beta_{open}$	$\beta_{augment}$	$\gamma_{open}$	$\gamma_{augment}$	$\beta_{open}$	$\beta_{augment}$	$\gamma_{open}$	$\gamma_{augment}$
L (Flower Color)	-0.07 (-0.27, 0.13)	0.06 (-0.13, 0.26)	-0.08 (-0.46, 0.30)	0.28 (-0.10, 0.64)	0.01 (-0.41, 0.42)	-0.11 (-0.34, 0.13)	-0.22 (-0.57, 0.13)	-0.50 (-1.10, 0.10)
Flower Number	<b>0.33**</b> <b>(0.12, 0.54)</b>	<b>0.18·</b> <b>(-0.01, 0.38)</b>	-0.18 (-0.48, 0.14)	0.02 (-0.20, 0.22)	0.34 (-0.09, 0.77)	<b>0.25*</b> <b>(0.04, 0.47)</b>	0.34 (-0.32, 1.00)	<b>0.37·</b> <b>(-0.06, 0.80)</b>
Petal Width	0.12 (-0.10, 0.34)	-0.03 (-0.30, 0.25)	-0.004 (-0.40, 0.40)	0.26 (-0.12, 0.62)	-0.19 (-0.63, 0.24)	-0.12 (-0.40, 0.15)	0.15 (-0.22, 0.51)	-0.17 (-0.61, 0.27)
Corolla Depth	0.17 (-0.12, 0.46)	<b>-0.30**</b> <b>(-0.57, -0.02)</b>	-0.40 (-0.94, 0.16)	0.10 (-0.28, 0.50)	-0.15 (-0.89, 0.59)	-0.32 (-0.71, 0.08)	0.98 (-0.27, 2.23)	0.17 (-0.29, 0.63)
Anther Height	-0.07 (-0.34, 0.21)	<b>0.29**</b> <b>(0.01, 0.56)</b>	<b>-0.36·</b> <b>(-0.74, 0.02)</b>	<b>-0.26·</b> <b>(-0.54, 0.02)</b>	0.24 (-0.33, 0.82)	0.18 (-0.17, 0.52)	-0.31 (-0.88, 0.26)	<b>1.16**</b> <b>(0.77, 1.55)</b>
L × Flower Number			0.08 (-0.11, 0.28)	-0.18 (-0.42, 0.05)			-0.02 (-0.43, 0.39)	0.02 (-0.21, 0.26)
L × Petal Width			-0.17	0.22			-0.05	-0.14

	(-0.42, 0.08)	(-0.08, 0.51)	(-0.48, 0.38)	(-0.44, 0.16)
L × Corolla Depth	-0.01	-0.05	-0.35	<b>0.41*</b>
	(-0.27, 0.25)	(-0.31, 0.21)	(-0.96, 0.27)	<b>(0.02, 0.79)</b>
L × Anther Height	<b>0.37**</b>	0.04	0.45	-0.19
	<b>(0.07, 0.67)</b>	(-0.14, 0.22)	(-0.28, 1.18)	(-0.56, 0.18)
Flower Number × Petal Width	0.04	-0.19	-0.14	-0.08
	(-0.25, 0.33)	(-0.54, 0.15)	(-0.82, 0.55)	(-0.35, 0.19)
Flower Number × Corolla Depth	-0.11	-0.11	<b>-0.74*</b>	-0.17
	(-0.42, 0.19)	(-0.35, 0.13)	<b>(-1.42, -0.05)</b>	(-0.59, 0.25)
Flower Number × Anther Height	0.15	0.21	0.38	<b>0.31•</b>
	(-0.19, 0.49)	(-0.05, 0.47)	(-0.48, 1.24)	<b>(-0.04, 0.66)</b>
Petal Width × Corolla Depth	0.24	0.03	-0.21	<b>0.58•</b>
	(-0.17, 0.65)	(-0.38, 0.44)	(-1.10, 0.67)	<b>(-0.09, 1.24)</b>
Petal Width × Anther Height	-0.16	0.03	0.29	<b>-0.45•</b>
	(-0.57, 0.25)	(-0.15, 0.21)	(-0.60, 1.17)	<b>(-0.96, 0.06)</b>
Corolla Depth × Anther Height	0.22	-0.07	-0.24	-0.52
	(-0.13, 0.57)	(-0.37, 0.23)	(-0.33, 0.82)	(-1.25, 0.21)

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**Table 2.** Variance-standardized linear ( $\beta_o$ ) selection gradients (and 95% confidence intervals in parentheses) on probability of setting seed using logisitic regression for open-pollinated plants in arctic and subarctic regions. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Anther height was not recorded for Galbraith and Ivishak populations in 2009. Selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	$\beta_{open}$ Arctic			$\beta_{open}$ Subarctic	
	Galbraith (2009) <i>n</i> = 40	Ivishak (2009) <i>n</i> = 64	Ivishak (2010) <i>n</i> = 57	12 Mile (2009) <i>n</i> = 42	Eagle (2010) <i>n</i> = 129
L (Flower Color)	0.01 (-0.41, 0.43)	0.01 (-0.15, 0.18)	-0.03 (-0.16, 0.10)	0.01 (-0.21, 0.23)	0.00 (-0.20, 0.20)
Flower Number	0.03 (-0.38, 0.44)	<b>0.34**</b> <b>(0.12, 0.56)</b>	0.07 (-0.05, 0.18)	<b>0.42*</b> <b>(0.08, 0.77)</b>	<b>0.31**</b> <b>(0.09, 0.51)</b>
Petal Width	-0.03 (-0.53, 0.47)	0.02 (-0.15, 0.19)	-0.01 (-0.14, 0.12)	-0.14 (-0.38, 0.10)	0.09 (-0.15, 0.32)
Corolla Depth	0.01 (-0.46, 0.49)	-0.03 (-0.20, 0.13)	<b>0.18*</b> <b>(0.00, 0.35)</b>	0.27 (-0.15, 0.69)	0.12 (-0.14, 0.38)
Anther Height	-	-	0.10 (-0.06, 0.25)	-0.289 (-0.71, 0.16)	-0.01 (-0.3, 0.26)

**Table 3.** Variance-standardized linear ( $\beta_o$ ) selection gradients (and 95% confidence intervals in parentheses) on fecundity for open-pollinated plants in arctic and subarctic regions. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Anther height was not recorded for Galbraith and Ivishak populations in 2009. Selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	$\beta_{open}$ Arctic			$\beta_{open}$ Subarctic	
	Galbraith (2009) <i>n</i> = 14	Ivishak (2009) <i>n</i> = 42	Ivishak (2010) <i>n</i> = 50	12 Mile (2009) <i>n</i> = 26	Eagle (2010) <i>n</i> = 55
L (Flower Color)	-0.37 (-0.84, 0.10)	0.07 (-0.12, 0.26)	<b>-0.27*</b> <b>(-0.49, -0.05)</b>	0.05 (-0.20, 0.31)	0.04 (-0.22, 0.31)
Flower Number	-0.35 (-0.88, 0.17)	<b>0.39**</b> <b>(0.20, 0.58)</b>	<b>0.30**</b> <b>(0.09, 0.53)</b>	0.20 (-0.07, 0.47)	<b>0.48**</b> <b>(0.18, 0.77)</b>
Petal Width	0.34 (-0.36, 1.05)	0.08 (-0.11, 0.27)	-0.10 (-0.27, 0.071)	-0.14 (-0.42, 0.14)	-0.03 (-0.34, 0.27)
Corolla Depth	-0.22 (-0.80, 0.36)	-0.10 (-0.30, 0.10)	-0.10 (-0.36, 0.17)	-0.10 (-0.66, 0.92)	<b>-0.34·</b> <b>(-0.72, 0.03)</b>
Anther Height	-	-	0.10	0.00	0.10

$(-0.24, 0.34)$	$(-0.55, 0.55)$	$(-0.27, 0.48)$
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726 **Table 4.** Variance-standardized linear ( $\beta_{open}$ ) and nonlinear ( $\gamma_{open}$ ) selection gradients (and 95%  
 727 confidence intervals in parentheses) for open-pollinated plants using logistic regression on  
 728 probability of seed set, and multiple linear regression on fecundity (seed number) for those  
 729 individuals that did set seed at Ivishak in 2010. Regression coefficients for  $\gamma$  matrix diagonals were  
 730 multiplied by 2 to calculate concave and convex gradients. The regression model for fitness  
 731 estimated by probability of seed set included only the five traits, as most individuals set seed in  
 732 this year and site, limiting confidence in estimates of regression coefficients. The regression  
 733 model of fitness estimated through fecundity, however had sufficient sample size to include all  
 734 five traits and fifteen cross-product terms. Probability of seed set selection gradients are  
 735 transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Traits	Probability of Seed Set	Fecundity	
	$\beta_{open}$	$\beta_{open}$	$\gamma_{open}$
L (Flower Color)	-0.027 (-0.16, 0.10)	<b>-0.25<sup>•</sup></b> <b>(-0.50, 0.01)</b>	0.02 (-0.61, 0.66)
Flower Number	0.07 (-0.05, 0.18)	<b>0.56**</b> <b>(0.33, 0.80)</b>	0.06 (-0.34, 0.46)
Petal Width	-0.01 (-0.14, 0.12)	0.07 (-0.20, 0.32)	0.10 (-0.34, 0.24)
Corolla Depth	<b>0.18*</b> <b>(0.002, 0.35)</b>	-0.15 (-0.49, 0.19)	0.29 (-0.37, 0.95)
Anther Height	0.10 (-0.06, 0.25)	0.30 (-0.06, 0.66)	0.16 (-0.07, 0.40)
L × Flower Number			<b>-0.25<sup>•</sup></b> <b>(-0.54, 0.04)</b>
L × Petal Width			0.05 (-0.26, 0.33)
L × Corolla Depth			0.30 (-0.07, 0.67)
L × Anther Height			-0.30 (-0.66, 0.08)
Flower Number × Petal Width			0.16 (-0.19, 0.52)
Flower Number × Corolla Depth			0.11 (-0.26, 0.48)
Flower Number × Anther Height			0.09 (-0.29, 0.48)
Petal Width × Corolla Depth			-0.05

	(-0.50, 0.40)
Petal Width $\times$ Anther Height	0.00
	(-0.43, 0.44)
Corolla Depth $\times$ Anther Height	-0.12
	(-0.58, 0.34)

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**Supplemental Table 1.** Populations in arctic and subarctic regions in which phenotypic selection was measured for open-pollinated plants and pollen augmented plants. Sample size is indicated by “*n*”; “n/a” indicates that no phenotypic selection estimation was made for that population in the particular year).

Year	Arctic Populations		Subarctic Populations	
	Ivishak (69° 20' N, 148° 45' W, 280 m elev.)	Galbraith (68°27' N, 149°33' W, 880 m elev.)	Eagle Summit (65° 28' N, 145° 25' W, 1100 m elev.)	Twelve Mile Summit (65° 24' N, 145° 44' W, 680 m elev.)
2009	Open Pollination <i>n</i> = 64	Open Pollination <i>n</i> = 41	n/a	Open Pollination <i>n</i> = 42
2010	Open Pollination <i>n</i> = 57	n/a	Open Pollination <i>n</i> = 129	n/a
			Pollen Augmentation <i>n</i> = 83	

**Supplemental Table 2A.** Mean-standardized linear ( $\beta_\mu$ ) gradients (and 95% confidence intervals in parentheses) for open-pollinated and pollen augmentation treatments using logistic regression on probability of seed set, and multiple linear regression on fecundity (seed number) for those individuals that did set seed at Eagle Summit in 2010. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). The regression model included only the five traits without interactions. Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	<i>Probability of Seed Set</i>		<i>Fecundity</i>	
	$\beta_{open}$	$\beta_{augment}$	$\beta_{open}$	$\beta_{augment}$
L (Flower Color)	0.00 (-1.42, 1.42)	0.31 (-0.46, 1.08)	0.33 (-1.51, 2.18)	-0.26 (-1.64, 1.13)
Flower Number	<b>0.78**</b> <b>(0.24, 1.31)</b>	<b>0.37**</b> <b>(0.07, 0.67)</b>	<b>1.65**</b> <b>(0.64, 2.67)</b>	<b>0.84**</b> <b>(0.31, 1.37)</b>
Petal Width	0.58 (-0.97, 2.13)	0.60 (-0.29, 1.49)	-0.21 (-2.31, 1.89)	-0.63 (-2.26, 1.00)
Corolla Depth	1.18 (-1.33, 3.70)	<b>-1.16**</b> <b>(-3.04, -0.22)</b>	<b>-3.28·</b> <b>(-6.83, 0.27)</b>	-0.09 (-2.84, 2.66)
Anther Height	-0.11 (-2.348, 2.11)	0.41 (-0.73, 1.54)	0.93 (-2.34, 4.19)	0.52 (-2.07, 3.09)

**Supplemental Table 2B.** Mean-standardized linear ( $\beta_\mu$ ) and nonlinear ( $\gamma_\mu$ ) selection gradients (and 95% confidence intervals in parentheses) for open-pollinated and pollen augmentation treatments using logistic regression on probability of seed set, and multiple linear regression on fecundity (seed number) for those individuals that did set seed at Eagle Summit in 2010. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998). Regression coefficients for  $\gamma$  matrix diagonals were multiplied by 2 to calculate concave and convex gradients. The regression model included all five traits and fifteen cross-product terms. Probability of seed set selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	Probability of Seed Set				Fecundity			
	$\beta_{open}$	$\beta_{augment}$	$\gamma_{open}$	$\gamma_{augment}$	$\beta_{open}$	$\beta_{augment}$	$\gamma_{open}$	$\gamma_{augment}$
L (Flower Color)	-0.50 (-1.94, 0.94)	0.45 (-0.98, 1.88)	-4.1 (-23.3, 15.1)	15.1 (-5.1, 35.2)	0.07 (-2.9, 3.0)	-0.81 (-2.50, 0.95)	-21.7 (-56.2, 12.8)	-54.1 (-119, 10.8)
Flower Number	<b>0.85**</b> <b>(0.31, 1.38)</b>	<b>0.52·</b> <b>(-0.03, 1.38)</b>	-1.13 (-3.2, 0.9)	0.14 (-1.54, 1.83)	0.98 (-0.26, 2.22)	<b>0.71*</b> <b>(0.11, 1.33)</b>	5.66 (0.17, 11.3)	<b>5.99·</b> <b>(-0.97, 12.9)</b>
Petal Width	0.80 (-0.65, 2.26)	-0.21 (-2.23, 1.82)	-0.20 (-17.6, 17.2)	14.0 (-6.3, 34.3)	-1.31 (-4.33, 1.65)	-0.89 (-2.96, 1.11)	14.2 (-20.8, 48.1)	-18.6 (-66.9, 87.7)
Corolla Depth	1.62 (-1.18, 4.42)	<b>-2.79**</b> <b>(-5.43, -0.15)</b>	-36.6 (-87.8, 14.4)	9.6 (-26.2, 45.5)	-1.43 (-8.50, 5.64)	-3.05 (-6.76, 0.76)	179.0 (-63.9, 294)	30.8 (-52.6, 114)
Anther Height	-0.55 (-2.83, 1.73)	<b>2.63**</b> <b>(0.10, 5.17)</b>	<b>-24.1·</b> <b>(-50.8, 2.5)</b>	<b>-21.3·</b> <b>(-44.4, 1.8)</b>	2.10 (-2.88, 7.15)	1.64 (-1.56, 4.76)	-47.2 (-134, 39.5)	<b>194**</b> <b>(129, 260)</b>
L × Flower Number			3.0 (-4.1, 10.2)	-7.7 (-17.5, 2.1)			-0.81 (-17.4, 15.8)	0.84 (-8.79, 10.9)
L × Petal Width			-16.2 (-40.0, 7.6)	23.4 (-9.0, 55.8)			-4.82 (-46.3, 36.6)	-15.3 (-47.9, 17.4)
L × Corolla Depth			-1.1 (-37.1, 34.9)	-6.4 (-42.8, 30.0)			-46.9 (-128, 36.2)	<b>57.5*</b> <b>(2.8, 111)</b>
L × Anther Height			<b>43.8**</b>	5.6			55.1	-25.6

	<b>(9.0, 78.6)</b>	(-18.4, 29.7)	(-34.3, 144)	(-75.4, 24.2)
Flower Number ×	1.33	-8.2	-5.55	-3.37
Petal Width	(-8.6, 11.2)	(-22.8, 6.4)	(-32.5, 21.8)	(-14.7, 8.0)
Flower Number ×	-5.61	-6.1	<b>-40.8*</b>	-9.21
Corolla Depth	(-20.5, 9.3)	(-19.0, 6.9)	<b>(-78.3, -2.76)</b>	(-32.0, 13.6)
Flower Number ×	6.46	10.8	19.1	<b>16.1•</b>
Anther Height	(-7.9, 20.8)	(-2.7, 24.2)	(-24.1, 62.4)	<b>(-2.1, 34.4)</b>
Petal Width ×	30.7	4.2	-27.6	<b>81.8•</b>
Corolla Depth	(-20.1, 82.3)	(-53.5, 62.0)	(-144, 87.9)	<b>(-12.7, 175)</b>
Petal Width ×	-17.3	4.2	34.7	<b>-61.0•</b>
Anther Height	(-61.8, 27.1)	(-20.4, 28.8)	(-71.8, 140)	<b>(-130, 8.13)</b>
Corolla Depth ×	34.7	-12.5	-40.0	-90.7
Anther Height	(-20.7, 90.1)	(-64.8, 39.9)	(-165, 83.3)	(-217, 36.6)

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**Supplemental Table 3.** Mean-standardized linear ( $\beta_{\mu}$ ) selection gradients (and 95% confidence intervals in parentheses) on probability of setting seed using logistic regression for open-pollinated plants in arctic and subarctic regions. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Anther height was not recorded for Galbraith and Ivishak populations in 2009. Selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	$\beta_{open}$ Arctic			$\beta_{open}$ Subarctic	
	Galbraith (2009) <i>n</i> = 40	Ivishak (2009) <i>n</i> = 64	Ivishak (2010) <i>n</i> = 57	12 Mile (2009) <i>n</i> = 42	Eagle (2010) <i>n</i> = 129
L (Flower Color)	0.09 (-3.60, 3.79)	0.13 (-1.29, 1.54)	-0.24 (-1.36, 0.89)	0.04 (-1.44, 1.53)	0.00 (-1.42, 1.42)
Flower Number	0.13 (-1.86, 2.13)	<b>1.11**</b> <b>(0.40, 1.83)</b>	0.23 (-0.15, 0.63)	<b>1.32*</b> <b>(0.24, 2.40)</b>	<b>0.78**</b> <b>(0.24, 1.31)</b>
Petal Width	-0.19 (-3.11, 2.73)	0.10 (-0.79, 1.00)	-0.04 (-0.80, 0.72)	-0.88 (-2.41, 0.65)	0.58 (-0.97, 2.13)
Corolla Depth	0.06 (-3.52, 3.65)	-0.30 (-1.74, 1.14)	<b>1.69*</b> <b>(0.02, 3.35)</b>	3.01 (-1.72, 7.75)	1.18 (-1.33, 3.70)
Anther Height	-	-	1.11 (-0.63, 2.85)	-2.89 (-7.47, 1.68)	-0.11 (-2.348, 2.11)

**Supplemental Table 4.** Mean-standardized linear ( $\beta_{\mu}$ ) selection gradients (and 95% confidence intervals in parentheses) on fecundity for open-pollinated plants in arctic and subarctic regions. Gradients marginally and significantly different from zero are shown in bold ( $\cdot = p < 0.10 > 0.05$ ;  $* = p < 0.05 > 0.01$ ;  $** = p < 0.01$ ). Anther height was not recorded for Galbraith and Ivishak populations in 2009. Selection gradients are transformed from logistic regression coefficients using the method of Janzen & Stern (1998).

Trait	$\beta_{open}$ Arctic			$\beta_{open}$ Subarctic	
	Galbraith (2009) <i>n</i> = 14	Ivishak (2009) <i>n</i> = 42	Ivishak (2010) <i>n</i> = 50	12 Mile (2009) <i>n</i> = 26	Eagle (2010) <i>n</i> = 55
L (Flower Color)	-2.77 (-6.12, 0.78)	0.60 (-1.01, 2.21)	<b>-2.34*</b> <b>(-4.25, -0.41)</b>	0.47 (-1.70, 2.64)	0.33 (-1.51, 2.18)
Flower Number	-1.74 (-4.33, 0.84)	<b>1.43**</b> <b>(0.74, 2.12)</b>	<b>1.01**</b> <b>(0.30, 1.83)</b>	0.69 (-0.25, 1.64)	<b>1.65**</b> <b>(0.64, 2.67)</b>
Petal Width	2.02 (-2.19, 6.24)	0.42 (-0.58, 1.41)	-0.61 (-1.63, 0.43)	-0.85 (-2.52, 0.82)	-0.21 (-2.31, 1.89)
Corolla Depth	-1.59 (-5.73, 2.55)	-0.82 (-2.45, 0.81)	-1.00 (-3.69, 1.68)	-1.05 (-6.73, 4.64)	<b>-3.28·</b> <b>(-6.83, 0.27)</b>
Anther Height	-	-	1.06	-0.04	0.93

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 $(-2.46, 3.43)$  $(-5.66, 5.57)$  $(-2.34, 4.19)$ 

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