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Natural selection on floral morphology can be influenced by climate

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1   **Natural selection on floral morphology can be influenced by climate**

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Abstract

13   Climate has the potential to influence evolution, but how it influences the strength or direction of

14   natural selection is largely unknown. We quantified the strength of selection on four floral traits

15   of the subalpine herb *Ipomopsis* ssp. in ten years that differed in precipitation, causing extreme

16   temporal variation in the date of snowmelt in the Colorado Rocky Mountains. The chosen floral

17   traits were under selection by hummingbird and hawkmoth pollinators, with hawkmoth

18   abundance highly variable across years. Selection for flower length showed environmental

19   sensitivity, with stronger selection in years with later snowmelt, as higher water resources can

20   allow translation of pollination success into fitness based on seed production. Selection on

21   corolla width also varied across years, favoring narrower corolla tubes in two unusual years with

22   hawkmoths, and wider corollas in another late snowmelt year. Our results illustrate how changes

23   in climate could alter natural selection even when the primary selective agent is not directly

24   influenced.

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Keywords

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1. Introduction

Recent climate change has been linked to changes in species distribution, local abundance, and phenology [1]. Whereas such ecological responses have been documented for many organisms, we know little about how climate change influences evolution. Evolutionary responses to climate are important to understand, especially because they may influence demographic impacts. For example, adaptation to new conditions could in theory rescue populations from negative demographic impacts imposed by the new environmental challenge [2, 3]. One important way that evolutionary responses are mediated is through changes in natural selection in response to the new climatic conditions. Variation in selection across years has been documented, but time series longer than five years are virtually unknown for plants [4]. Furthermore, variation in selection across years has only occasionally been linked to a specific environmental cause [5, 6]. Shifts in selection with climate are likely to be common and to involve indirect mechanisms that have hardly been considered, as well as changes in selection imposed directly by the thermal environment.

One potential indirect mechanism involves natural selection on flower traits by pollinators. Pollen vectors often exert strong selection for floral features and are thought to be critically important to the remarkable radiation of the angiosperms (reviewed in [7]). Pollinator-mediated selection could vary across years due to changes in pollinator abundance or composition [8], mismatches in flowering and pollinator phenology [9], or changes in resources such as soil moisture that affect the ability of plants to translate fertilized ovules into fitness, but these mechanisms have been studied only rarely [10]. We took advantage of long-term common garden studies with two species of the herb *Ipomopsis* in the Polemoniaceae family [11] in an

54　　area of the Colorado Rocky Mountains with long-term climatic records [12] to measure selection

55　　on multiple floral traits in 10 years that varied by 50 days in snowmelt date.

56　　　　A wealth of previous information on *Ipomopsis* and its pollinators allowed us to make

57　　specific predictions about selection on floral traits. We hypothesized selection patterns based on

58　　previous studies of selection mediated by hummingbirds and hawkmoths along with prior

59　　demonstrations that pollen receipt on the stigma relates to pollinator visitation [8] and seed set to

60　　pollen receipt [13, 14]. Hummingbirds are consistently common at our sites, but hawkmoths are

61　　not, leading us to make different predictions depending on their abundance in a given year. For

62　　years with abundant hawkmoths, we predicted selection would favor long, narrow corollas, as

63　　studies in the 1990s demonstrated that plants with those traits received higher hawkmoth

64　　visitation [15]. For years without hawkmoths, we predicted selection favoring long, wide

65　　corollas, more intensely red flowers, and high nectar production, as plants with those traits

66　　received higher hummingbird visitation [16-18]; Fig. 1A) and, for flower colour, made more

67　　seeds at a site with no hawkmoths [19]. We furthermore hypothesized that the strength of

68　　selection through female function would depend on water availability. We hypothesized more

69　　intense selection in wetter years with later snowmelt (Fig. 1B) if soil moisture is required before

70　　or during seed maturation to translate greater pollen receipt into higher seed production, as seen

71　　in related species of *Polemonium* [20]. If so, drought conditions could eliminate pollen-limitation

72　　of seed production and thereby weaken selection on floral traits.

73　**2. Materials and Methods**

74　**(a) Study site and common garden design**

75　　　　Natural selection was measured for plants growing from seeds of the herb *Ipomopsis*

76　　*aggregata*, its close relative *Ipomopsis tenuituba*, and F_2 hybrids that were planted into two

77 common gardens at Poverty Gulch, Gunnison County, CO. The use of both species and their

78 hybrids allowed us to present a wider range of phenotypic trait combinations than present in a

79 single natural population, yielding a more powerful test of selection and the ability to generalize

80 across multiple species. Plants of *Ipomopsis aggregata* spp. *aggregata* normally grow in the

81 valley bottom at elevations of 2900 m and below, plants of *I. tenuituba* spp. *tenuituba* grow on

82 steep slopes above 3100 m, and natural hybrids grow in between. Plants of both species are self-

83 incompatible monocarps that almost always flower during a single season (after 2-12 years with

84 a median of 5 years), set seed, and then die [21, 22]. This monocarpic life history allowed us to

85 measure lifetime female fitness.

86   Two sets of plantings were included. The first set has been previously described in [22].

87 Briefly, we collected parental plants of both species in 1995 and crossed them to produce F_1

88 progeny that served as the parents for F_2 and backcrosses that we planted as seed in the field

89 during 1997 and 1998. We planted seeds of *I. aggregata* (AA), *I. tenuituba* (TT), both reciprocal

90 F_1 hybrids (i.e. with both *I. aggregata* as the maternal plant and *I. tenuituba* as the maternal

91 plant), both reciprocal F_2 hybrids, reciprocal backcrosses of the F_1 to both parental species and

92 (in 1998 only) seeds from crosses between natural hybrids. We analysed only seeds planted into

93 the *I. aggregata* or hybrid site (sites L and I; [15]), as different patterns of selection are likely in

94 the *I. tenuituba* site. Most of these plants that bloomed did so during 2001 - 2006, providing us

95 with six years of data for selection analysis.

96   The second set of seeds was planted in 2007 and 2008 and has not been previously

97 described. In 2007, AA (*I. aggregata x I. aggregata*) seeds were generated from reciprocal

98 factorial crosses of 5 individual plants yielding 5 maternal half-sib families and supplemented

99 with one family of field-collected seeds, TT (*I. tenuituba x I. tenuituba*) seeds were generated

100   from reciprocal factorial crosses of 5 individual plants yielding 5 maternal half-sib families plus

101   one family of field-collected seeds, 5 families of AT (F1 with *I. aggregata* as the maternal

102   parent) and 5 families of TA (F1 with *I. tenuituba* as the maternal parent) were generated by

103   crosses between these same individuals, and 14 families of F_2 seeds were generated by crossing 7

104   independent pairs of F_1 plants in both reciprocal directions. Seeds from the 36 families were

105   planted into 20 blocks at the *I. aggregata* site and 20 blocks at the hybrid site, with each block

106   containing approximately 60 (average = 58) seeds chosen at random from the 36 families and

107   planted at 10 cm intervals into the top 6 rows of the 1 m x 1m block using a gridded planting

108   frame to indicate planting locations. In 2008 we generated another 6 families of F_2 seeds and

109   planted them into 10 blocks at the *I. aggregata* site, with each block containing 90 seeds chosen

110   at random from the 6 families and planted at 10 cm intervals. Existing vegetation was left intact

111   inside the planting area, so that competition with other plants was not modified, except that

112   flowering individuals of *Ipomopsis* were removed from the surrounding 1 m area to prevent drop

113   of seeds into the planting area. There is no seed bank, and seedlings typically emerge within 1

114   cm of the planted location [22]. Seedlings were censused in the year following planting using the

115   planting frame, and surviving individuals were given a numbered metal tag for easier

116   identification. Surviving plants bloomed in 2010 and later, giving us an additional four years of

117   selection data (2010-2013). In total from both sets of experiments, 7979 seeds were planted

118   individually, of which 1457 produced seedlings. Survivors were censused each succeeding year,

119   and floral traits were measured in the year of blooming. Due to a small sample of blooming

120   plants in 2013, we supplemented the data set with an additional 11 *in situ* flowering plants

121   growing within 10 m of an experimental block.

122   **(b) Measurement of floral traits and fitness**

123   At our study sites, the main pollinators in the majority of years are the hummingbirds

124   *Selasphorus platycercus* and *S. rufus* [11], and plants with longer and wider corollas, redder

125   flowers, and higher nectar production receive more hummingbird visits in at least some years

126   [16, 17, 19]. Because seed production is limited by pollen transfer [13, 23], the increased pollen

127   receipt associated with higher visitation in *Ipomopsis* [8] is expected to lead to higher seed

128   production. In earlier experiments, such an impact was seen in a single test for flower colour, in

129   one out of four years for corolla length, in one out of three years for corolla width, but not for

130   nectar production [13, 17, 19]. In rare years, hawkmoths (*Hyles lineata*) are observed to visit,

131   and visitation by hawkmoths is greater for plants with narrower corollas [15].

132   Based on these previous studies, we measured four floral traits: corolla length, corolla

133   width, flower colour (for the 2007-2008 plantings only), and 24-hour nectar production (for the

134   2007-2008 plantings only). Corolla length and width at the opening of the tube were measured

135   using calipers and averaged over 2-10 flowers per plant. Corolla colour was measured for 2-4

136   flowers per plant using an Ocean Optics (Ocean Optics Inc., Dunedin, FL, USA) Red Tide

137   USB650 reflectance spectrometer with a LS-I light source, standardized by a white reflectance

138   standard, and a fiber optic probe held at a 45-degree angle. Redness of the flower was quantified

139   by finding the average value for relative reflectance (R) in the red compared to the green: (R_{626-}

140   $_{700}$ - $R_{476-550}$)/($R_{410-700}$). Flowers of these species do not reflect in the ultraviolet [24]. Nectar

141   production was measured for 1 to 5 flowers per plant by covering elongated buds with straws to

142   prevent access of pollinators and returning 48 hours later to extract nectar with a 5-µL

143   microcapillary tube 32 mm in length [17]. The length of the nectar column in mm was multiplied

144   by 5 µL / (2 days * 32 mm) and averaged across flowers to determine 24-hour nectar production

145   rate in µL per day.

146   At the end of each summer, we collected fruits and the calyces from failed fruits every 2-

147   3 days to estimate number of flowers made by each plant that bloomed, the seeds per flower, and

148   total seed production (methods in [21]). Of the 279 plants that bloomed, 59 were *I. aggregata*, 24

149   were *I. tenuituba*, 54 were F_1 hybrids, 40 were backcrosses, and 102 were F_2 hybrids or offspring

150   of crosses between natural hybrids, in which correlations between traits are likely broken down

151   due to recombination.

(c) Statistical analysis

153   Analysis of temporal variation in selection proceeded in several steps. First, we examined

154   correlations among the four floral traits and between the traits and flower number. Because

155   correlations with flower number were weak or absent ($-0.16 \leq r \leq 0.20$, $N = 80$ to 279),

156   estimates of phenotypic selection were not confounded by general increases in plant vigor in

157   better microhabitats. Furthermore, corolla length, corolla width, and nectar production were

158   weakly or not at all correlated with each other ($-0.14 \leq r \leq 0.16$, all $P > 0.05$), justifying

159   univariate analyses of selection one trait at a time. Flower redness did correlate negatively with

160   corolla length ($r = -0.25$, $P < 0.05$) and positively with nectar production ($r = 0.37$, $P < 0.001$), so

161   univariate measures of selection on that trait cannot distinguish between direct selection and

162   indirect selection of the correlated traits.

163   We then proceeded to analyse selection on each of the four floral traits separately.

164   Selection could vary across years due to a change in the relationship of absolute fitness to a trait

165   (Fig. 1C) or because of the tendency for variance in relative fitness (opportunity for selection) to

166   decline as mean absolute fitness increases [25, 26]. We examined both aspects in this study,

167   because effects on absolute fitness are critical to demographic consequences of changes in

168   selection [2] and because all plants in an experimental planting were part of a single generation

169　under selection. First, to check for non-linear effects on absolute fitness, we analysed the

170　following generalized linear model as specified with a model statement in the GENMOD

171　procedure in SAS ver 9.2 (SAS Institute, Cary, NC, USA):

172　$$model\ W = Year + Trait + (Trait - \overline{Trait})^2 + Year * Trait + Year * (Trait - \overline{Trait})^2$$

173　where W is absolute fitness estimated as seed production, and year was treated as a class

174　variable. We used likelihood ratio tests for type 3 analysis that assumes all other effects are in the

175　model. We also specified normally distributed residuals, as doing so provided good fits based on

176　AIC and scaled deviance. In no case was the interaction of year with the quadratic term

177　significant (P = 0.92, 0.61, 0.37, and 0.94 for the four traits), indicating lack of evidence that

178　non-linear selection varied across years. We next dropped that term, and also found no

179　significant quadratic term that would indicate non-linear selection (P = 0.37, 0.28, 0.35, and

180　0.80). We then tested for variation in directional selection across years by analysing the

181　following model for absolute fitness:

182　$$model\ W = Year + Trait + Year * Trait$$

183　A significant year by trait interaction indicated variation in selection across years. If the

184　interaction was significant, we then estimated selection separately in each year using a model

185　with the factor of year and trait nested within year. If no interaction was detected, we estimated

186　average selection using a standard analysis of covariance model with year as the class variable

187　and trait as the continuous variable.

188　　　We then tested whether the slope of absolute fitness on the trait, the opportunity for

189　selection (variance in relative fitness [27]), or the standardized selection differential based on

190　relative fitness varied with environmental factors. Relative fitness was calculated by dividing

191　seed production by the mean for that year. The selection differential (covariance between relative

192   fitness and the trait) was standardized in units of SD for the trait [28]. For traits with ≥ 10 years

193   of data we examined the environmental sensitivity of selection [29] by calculating the correlation

194   between the selection measure and either of two environmental variables: (1) summer

195   precipitation during June 1 to July 15 at Gothic, CO (National Atmospheric Deposition Program

196   CO10), the period when *Ipomopsis* is developing inflorescences and prior to most of our floral

197   measurements in late July and early August or (2) the date of first snowmelt at Gothic, CO, 8 km

198   from our common gardens and at a similar elevation near 2900 m. Later snowmelt is associated

199   with higher soil moisture in mountainous regions, with effects lasting for 2-4 months after

200   snowmelt, at least in the Sierra Nevada [30]. We also compared selection in years with versus

201   without hawkmoths. The sites at Poverty Gulch were visited 2-3 times per week throughout each

202   three month season, but hawkmoths were observed only on 9 July 2001, 13 July 2001, 5 August

203   2010, and 11 August 2010, indicating that 2001 and 2010 were more abundant years for moths.

204   ## 3. Results

205   Seed production varied greatly among years (P < 0.0001 for year effect in ANOVA) and

206   tended to be greater in years with later snowmelt (r = 0.59, N = 10, P = 0.075). On average, a

207   flowering plant made only 26 seeds in 2012, which had the earliest snowmelt (April 23) in 40

208   years of record keeping (1974 to 2013; www.gothicwx.org/long-term-snow.html), compared

209   with 224 seeds in 2011, which had the latest snowmelt in our 10 year data set (June 7) and the

210   third latest out of 40 years. The overall opportunity for selection did not, however, correlate with

211   snowmelt date (r = -0.08, P = 0.82). Average flower morphology did not change with the

212   environmental feature of snowmelt date (r = 0.03, P = 0.92 for corolla length and r = -0.01, P =

213   0.97 for corolla width).

214   Two of the four traits (corolla width and flower redness) showed significant variation

215   across years in selection as judged from year by trait interactions on seed production (electronic

216   supplementary material, table S1). Directional selection favored narrow corollas in 2001

217   (standardized selection differential S' = -0.24; P = 0.0006), a year with abundant hawkmoths, and

218   wide corollas in 2011 (S' = 0.22; P = 0.0268; Fig. 2). The standardized selection differential for

219   corolla width in a given year did not correlate overall with snowmelt date (r = 0.13, N = 10, P =

220   0.72) or summer precipitation (r = -0.53, P = 0.12), but instead was the most strongly negative in

221   the two years with abundant hawkmoths (Fig. 3). Removing those two moth years from the

222   analysis, years with later snowmelt showed the predicted higher gains in absolute fitness with

223   corolla width (r = 0.65, N = 8, one-tailed P < 0.05), but that did not translate into higher

224   standardized selection differentials (Fig. 3). Thus selection on corolla width related most strongly

225   to pollinator availability.

226   As predicted because both hummingbirds and hawkmoths preferentially visit longer

227   flowers of *Ipomopsis*, the estimate of selection on corolla length was positive in most years (8 of

228   10). Whereas we did not detect a significant year by trait interaction for corolla length, both the

229   slope of absolute fitness on corolla length and the standardized selection differential correlated

230   positively with snowmelt date (Fig. 3A and 3B; r = 0.79 and 0.63, P = 0.006 and 0.05,

231   respectively), making selection on that trait more intense in years with late snowmelt.

232   Precipitation from June 1 to July 15 was, however, not associated with the standardized selection

233   differential (r = 0.16) or the slope of absolute fitness. The correlation of seed set with corolla

234   length was not explainable by indirect selection of corolla width or nectar production, as those

235   traits were weakly or not at all correlated, but could conceivably reflect some selection of the

236   correlated trait of flower redness (r = -0.25, P = 0.0166). The relationship of corolla length with

237   fitness was also not explainable by independent effects of the environment on the trait as well as

238   on seed production, as corolla length did not change with snowmelt. Instead the association of

239   selection strength with snowmelt can be explained by the possibility of translating pollen receipt

240   into higher seed production in years with later snowmelt.

241   Our specific predictions were not upheld for the other two traits: flower colour and nectar

242   production. Selection on flower colour varied significantly across years, judging from the year

243   by colour interaction on absolute fitness measured as seed production (table S1). Selection,

244   however, strongly favored whiter (less red) flowers in 2011 (S' = -0.39; P < 0.0001; Fig. 2),

245   despite the scarcity of hawkmoths and expectation that hummingbirds would select for redder

246   flowers in that case. Selection on nectar production was not detectable at all, with no evidence

247   either for variation across years (table S1).

4. Discussion

249   In this subalpine ecosystem, we found that the strength of selection on flower corolla

250   length was positively associated with snowmelt date. Later snowmelt leads to greater water

251   availability in the soil, presumably allowing higher hummingbird pollination experienced by

252   long flowers to translate into more seeds. In wet years, the standardized selection differential for

253   length accounted for a higher proportion of the overall opportunity for selection [26], so that it

254   also increased with snowmelt date. Summer precipitation from June 1 to July 15 was not

255   associated with selection intensity, possibly because the total amount (mean = 5.5 cm) during

256   this drier part of the summer season is too small to have much effect on soil moisture, whereas

257   advancing snowmelt by 2-3 weeks can decrease water storage in soil by about one third [30].

258   That time frame is equivalent to the difference between the earliest date and the mean date of

259   snowmelt in our data set. Alternatively, or in addition, seed production may depend mostly on

260   resources already accumulated in the taproot or leaves by the plant during early spring when

261   snowmelt saturates the soil.

262   Although we had predicted selection for redder flowers, in 2011 selection instead favored

263   whiter flowers, despite the scarcity of hawkmoths that year. One potential explanation for the

264   negative association of redness with seed production is the strong indirect selection on the

265   negatively correlated character of corolla length in that year (Fig. 3A). Nectar production is a

266   trait that is theoretically also expected to be under selection, as nectar is the primary reward for

267   both hummingbirds and hawkmoths. Yet we did not detect selection on that trait, nor evidence

268   for variation in selection across years for nectar (table S1). Although paradoxical from a

269   theoretical perspective, these results are consistent both with absence of detectable selection

270   through female function on nectar in an earlier study of *Ipomopsis* [17] and with a recent review

271   claiming nectar as the only studied floral trait for which direct selection through female function

272   has not been reported (N = 22 estimates; [7]).

273   Perhaps the most striking result of our study is that natural selection via pollinators on the

274   trait of corolla length is strongly predicted by the date of snowmelt. Even though the selection on

275   this floral trait is driven directly by pollinators, its intensity relates to snowmelt. Snowmelt is an

276   important annual wetting event in ecosystems with seasonal snowpack [30], and is one of the

277   physical variables most likely to be affected by climate change in the Rocky Mountains, where it

278   correlates with flowering phenology and abundance for several species [12, 31]. If the average

279   date of snowmelt continues to advance as it has over the last 40 years in this area by 3.5 days per

280   decade [32], we predict less intense pollinator-mediated selection shaping floral length in

281   *Ipomopsis*. Climate models for the region predict a higher frequency of extreme droughts in the

282   future [33], which could also produce greater variation in selection intensity.

283   Most work on natural selection in response to climate change has focused on a scenario

284   in which a change in selection can adaptively restore positive growth to a population in decline

285   [2]. The change in selection that we saw corresponds to a different scenario in which early

286   snowmelts (if they continued) would eliminate pollen limitation and weaken selection on flower

287   morphology. If longer corolla tubes had been favored by pollinator-mediated selection in the

288   recent past, *Ipomopsis* populations would have experienced ongoing evolution of longer tubes, as

289   corolla length shows a relatively high narrow sense heritabilityof 0.74 [18]. That evolutionary

290   response could have helped to maintain populations with positive population growth, as

291   witnessed in some natural *Ipomopsis* populations [34] With earlier snowmelt, however, selection

292   on corolla length would decline, slowing evolution. More variable snowmelt date would also

293   diminish the evolutionary response, as fluctuation in selection intensity is predicted to help

294   maintain variation in a floral trait and dampen the average response [35] to selection. Thus with

295   earlier or more variable snowmelt any positive demographic effect of selection on corolla length

296   that occurred in the recent past would no longer occur, perhaps even reducing maintenance of

297   viable populations. That prediction results from snowmelt having an indirect effect on selection

298   experienced by corolla shape, rather than a situation in which selection is imposed directly by the

299   thermal environment. It is of course possible that other traits, such as vegetative or physiological

300   traits, would experience direct selection for values that are highly adaptive under new climatic

301   conditions. In addition, climate might affect abundance of the hawkmoths, a topic little

302   understood [36], which would alter selection on corolla width, potentially also with demographic

303   impacts [37].

304   **5. Conclusion**

305   Our results indicate that the intensity of selection on a floral trait can vary with the

306   climatic environment, particularly the date of snowmelt. That finding potentially has two general

307   implications. First, given the importance of snowmelt date to phenology of other plants and

308   animals in this ecosystem [32] and others worldwide [1], we expect that natural selection on

309   traits in other organisms will be altered by future climate change in mountainous areas. Second,

310   our work suggests the need to examine potential selection responses not only in thermal

311   tolerance and phenological timing, traits likely to show increased selection with rapidly changing

312   climate [38], but also in floral traits that may show indirect changes in selection, either because

313   events such as low snowpack or drought modify outcomes of pollinator-mediated selection or

314   because climate affects abundance of pollinators. Global climate change may alter not only the

315   abundance, phenology and distribution of organisms [1, 32] but also their evolutionary

316   trajectories.

317

320   **Authors' contributions.** DC designed the study, collected field data, carried out the statistical

321   analyses and helped to write the manuscript. JP collected field data, participated in data analysis,

322   and helped write the manuscript.

334

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336   **References**

337   [1] Parmesan, C. 2006 Ecological and evolutionary responses to recent climate change. *Annual*

338   *Review of Ecology, Evolution and Systematics* **37**, 637-669.

339   [2] Gomulkiewicz, R. & Shaw, R.G. 2012 Evolutionary rescue beyond the models. *Philosophical*

340   *Transactions of the Royal Society B: Biological Sciences* **368**, 20120093.

341   [3] Hoffmann, A.A. & Sgro, C.M. 2011 Climate change and evolutionary adaptation. *Nature*

342   **470**, 479-485.

343   [4] Siepelski, A.M., DiBattista, D. & Carlson, M. 2009 It's about time: the temporal dynamics of

344   phenotypic selection in the wild. *Ecology Letters* **12**, 1261-1276.

345   [5] Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C.

346   2008 Adaptive phenotypic plasticity in response to climate change in a wild bird population.

347   *Science* **320**, 800-803.

348   [6] Grant, P.R. & Grant, B.R. 2002 Unpredictable evolution in a 30-year study of Darwin's

349   finches. *Science* **296**, 707-711.

350   [7] Harder, L.D. & Johnson, S.D. 2009 Darwin's beautiful contrivances: evolutionary and

351   functional evidence for floral adaptation. *New Phytologist* **183**, 530-545.

352   [8] Price, M.V., Waser, N.M., Irwin, R.E., Campbell, D.R. & Brody, A.K. 2005 Temporal and

353   spatial variation in pollination of a montane herb: a seven-year study. *Ecology* **86**, 2106-2116.

354   [9] Forrest, J.R.K. & Thomson, J.D. 2011 An examination of synchrony between insect

355   emergence and flowering in Rocky Mountain meadows. *Ecological Monographs* **81**, 469-491.

356   [10] Benitez-Vieyra, S., Glinos, E., Medina, A.M. & Cocucci, A.A. 2012 Temporal variation in

357   the selection on floral traits in *Cyclopogon elatus* (Orchidaceae).

358   [11] Campbell, D.R. 2004 Natural selection in *Ipompsis* hybrid zones: implications for

359   ecological speciation. *New Phytologist* **161**, 83-90.

360   [12] Inouye, D.W. 2008 Effects of climate change on phenology, frost damage, and floral

361   abundance of montance wildflowers. *Ecology* **89**, 353-363.

362   [13] Campbell, D.R. 1991 Effects of floral traits on sequential components of fitness in

363   *Ipomopsis aggregata*. *American Naturalist* **137**, 713-737.

364   [14] Kohn, J.R. & Waser, N.M. 1985 The effect of *Delphinium nelsonii* pollen on seed set in

365   *Ipomopsis aggregata*: a competitor for hummingbird pollination. *American Journal of Botany*

366   **72**, 1144-1148.

367   [15] Campbell, D.R., Waser, N.M. & Meléndez-Ackerman, E.J. 1997 Analyzing pollinator-

368   mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial

369   scales. *American Naturalist* **149**, 295-315.

370   [16] Campbell, D.R., Waser, N.M., Price, M.V., Lynch, E. & Mitchell, R. 1991 Components of

371   phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution*

372   **45**, 1458-1467.

373   [17] Mitchell, R.J. 1993 Adaptive significance of *Ipomopsis aggregata* nectar production:

374   observation and experiment in the field. *Evolution* **47**, 25-35.

375   [18] Campbell, D.R. 1996 Evolution of floral traits in a hermaphroditic plant: Field

376   measurements of heritabilities and genetic correlations. *Evolution* **50**, 1442-1453.

377   [19] Meléndez-Ackerman, E.J. & Campbell, D.R. 1998 Adaptive significance of flower color

378   and inter-trait correlations in an *Ipomopsis* hybrid zone. *Evolution* **52**, 1293-1303.

379   [20] Galen, C. 2000 High and dry: drought stress, sex-allocation trade-offs, and selection on

380   flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *American*

381   *Naturalist* **156**, 72-83.

382   [21] Campbell, D.R. 1997 Genetic and environmental variation in life-history traits of a

383   monocarpic perennial: a decade-long field experiment. *Evolution* **51**, 373-382.

384   [22] Campbell, D.R., Waser, N.M., Aldridge, G. & Wu, C.A. 2008 Lifetime fitness in two

385   generations of *Ipomopsis* hybrids. *Evolution* **62**, 2616-2627.

386   [23] Campbell, D.R. & Halama, K. 1993 Resource and pollen limitations to lifetime seed

387   production in a natural plant population. *Ecology* **74**, 1043-1051.

388   [24] Meléndez-Ackerman, E. 1997 Patterns of color and nectar variation across an *Ipomopsis*

389   hybrid zone. *American Journal of Botany* **84**, 41-47.

390   [25] Vanhoenacker, D., Ågren, J. & Ehrlén, J. 2013 Non-linear relationship between intensity of

391   plant-animal interactions and selection strength. *Ecology Letters* **16**, 198-205.

392   [26] Reiss, J.O. 2013 Does selection intensity increase when populations decrease? Absolute

393   fitness, relative fitness, and the opportunity for selection. *Evolutionary Ecology* **27**, 477-488.

394   [27] Arnold, S.J. & Wade, M.J. 1984 On the measurement of natural and sexual selection:

395   Theory. *Evolution* **38**, 709-719.

396   [28] Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E.,

397   Hoang, A., Gibert, P. & Beerli, P. 2001 The strength of phenotypic selection in natural

398   populations. *American Naturalist* **157**, 245-261.

399   [29] Chevin, L., Lande, R. & Mace, G.M. 2010 Adaptation, plasticity, and extinction in a

400   changing environment: Towards a predictive theory. *PLOS Biology* **8**, e1000357.

401   [30] Blankinship, J.C., Meadows, M.W., Lucas, R.G. & Hart, S.C. 2014 Snowmelt timing alters

402   shallow but not deep soil moisture in the Sierra Nevada. *Water Resources Research* **50**, 1448-

403   1456.

404   [31] Iler, A.M., Høye, T.T., Inouye, D.W. & Schmidt, N.M. 2013 Nonlinear flowering responses

405   to climate: are species approaching their limits of phenological change? *Philosophical*

406   *Transactions of the Royal Society B: Biological Sciences* **368**.

407   [32] CaraDonna, P.J., Iler, A.M. & Inouye, D.W. 2014 Shifts in flowering phenology reshape a

408   subalpine plant community. *Proceedings of the National Academy of Sciences (USA)*.

409   [33] Notaro, M., Mauss, A. & Williams, J.H. 2012 Projected vegetation changes for the

410   American Southwest: combined dynamic modeling and bioclimatic-envelope approach.

411   *Ecological Applications* **22**, 1365-1388.

412   [34] Waser, N.M., Campbell, D.R., Price, M.V. & Brody, A.K. 2010 Density-dependent

413   demographic responses of a semelparous plant to natural variation in seed rain. *Oikos* **119**, 1929-

414   1935.

415   [35] Hairston Jr, N., Ellner, S., Geber, M., Yoshida, T. & Fox, J. 2005 Rapid evolution and the

416   convergence of ecological and evolutionary time. *Ecology Letters* **8**, 1114-1127.

417   [36] Brunet, J. 2009 Pollinators of the Rocky Mountain columbine: temporal variation,

418   functional groups and associations with floral traits. *Annals of Botany* **103**, 1567-1578.

419   [37] Campbell, D.R. 2008 Pollinator shifts and the origin and loss of plant species. *Annals of the*

420   *Missouri Botanical Garden* **95**, 264-274.

421   [38] Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. 2012

422   Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in

423   response to climate change. *Proceedings of the Royal Society of London Series B* **279**, 3843-

424   3852.

425   [39] Ashman, T.L., Knight, T.M., Steets, J.A., Amaresekare, P., Burd, M., Campbell, D.R.,

426   Dudash, M.R., Johnston, M.O., Mazer, S.J., Mitchell, R.J., et al. 2004 Pollen limitation of plant

427   reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**, 2408-2421.

428

Figure legends

429

430	**Figure 1.** Potential effect of climate on pollinator-mediated selection of a floral trait. **(A)** In

431	pollinator-mediated directional selection, a floral trait has a linear effect on pollination success,

432	as illustrated by the influence of corolla width on hummingbird visit rate in experiments with

433	*Ipomopsis* (redrawn from Fig. 3 in [15]). The standardized selection differential corresponding to

434	the slope of this line is S' = 0.36. **(B)** Seeds usually increase non-linearly with pollination

435	success, as illustrated by the relationship between seeds and pollen receipt on the stigma for

436	*Ipomopsis* (from equation reported in [14]). **(C)** A higher resource asymptote or higher saturation

437	parameter [39] are theoretically predicted under wet conditions (blue line) than under dry

438	conditions (red line). Because pollinator visitation relates linearly to the trait value (part A), and

439	pollen on the stigma increases linearly with visitation in *Ipomopsis* [8], seeds are predicted to

440	increase with the trait value in a similar fashion to the way they increase with pollen. Directional

441	selection, as indicated by the slope of relative fitness (seeds divided by its mean) on the trait,

442	would be more intense in wet years, for populations like those of *Ipomopsis* where seed

443	production is pollen-limited [13] and pollen receipt usually below the level where seeds level off

444	(range of values within the gray box).

445	**Figure 2.** Seed production as a function of four floral traits. Lines show slopes on the trait

446	values, with significant ones for individual years (P < 0.05) labelled with the year number. (A)

447	Selection on corolla length across 10 years of study. (B) Selection on corolla width across 10

448	years of study. (C) Selection on nectar production across 4 years of study. (D) Selection on

449	flower colour across 4 years of study. Flower colour was quantified as $(R_{626\text{-}700} - R_{476\text{-}550})/(R_{401\text{-}700})$

450	$_{700}$), where $R_{626\text{-}700}$ = reflectance from 626 to 700 nm wavelength, $R_{476\text{-}550}$ = reflectance from 476

451   to 550 nm wavelength, and $R_{401-700}$ = total reflectance in the visible range. Flowers of *Ipomopsis*

452   do not reflect in the UV [24].

453   **Figure 3.** Selection changes with date of snowmelt and pollinators. (**A**) The slope of seeds on

454   corolla length correlates positively with day of snowmelt where 1 = January 1 (r = 0.79, P <

455   0.01). Closed circles: hawkmoths absent. Open circles: hawkmoths abundant. Dashed line

456   indicates no selection. (**B**) The standardized selection differential on corolla length also

457   correlates positively with snowmelt date (r = 0.63, P = 0.05). (**C**) The standardized selection

458   differential on corolla width is not correlated with snowmelt, but narrow flowers were favored

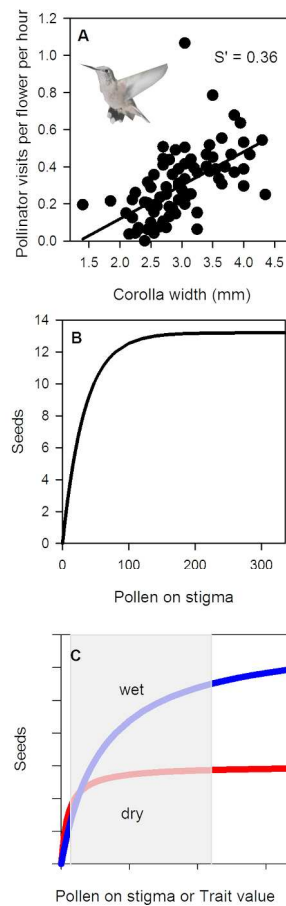459   only in the two years with hawkmoths (2001 and 2010).

Figure 1. Potential effect of climate on pollinator-mediated selection of a floral trait. **(A)** In pollinator-mediated directional selection, a floral trait has a linear effect on pollination success, as illustrated by the influence of corolla width on hummingbird visit rate in experiments with Ipomopsis (redrawn from Fig. 3 in [15]). The standardized selection differential corresponding to the slope of this line is S' = 0.36. **(B)** Seeds usually increase non-linearly with pollination success, as illustrated by the relationship between seeds and pollen receipt on the stigma for *Ipomopsis* (from equation reported in [14]). **(C)** A higher resource asymptote or higher saturation parameter [39] are theoretically predicted under wet conditions (blue line) than under dry conditions (red line). Because pollinator visitation relates linearly to the trait value (part A), and pollen on the stigma increases linearly with visitation in *Ipomopsis* [8], seeds are predicted to increase with the trait value in a similar fashion to the way they increase with pollen. Directional selection, as indicated by the slope of relative fitness (seeds divided by its mean) on the trait, would be more intense in wet years, for populations like those of *Ipomopsis* where seed production is pollen-limited [13] and pollen receipt usually below the level where seeds level off (range of values within the gray box).
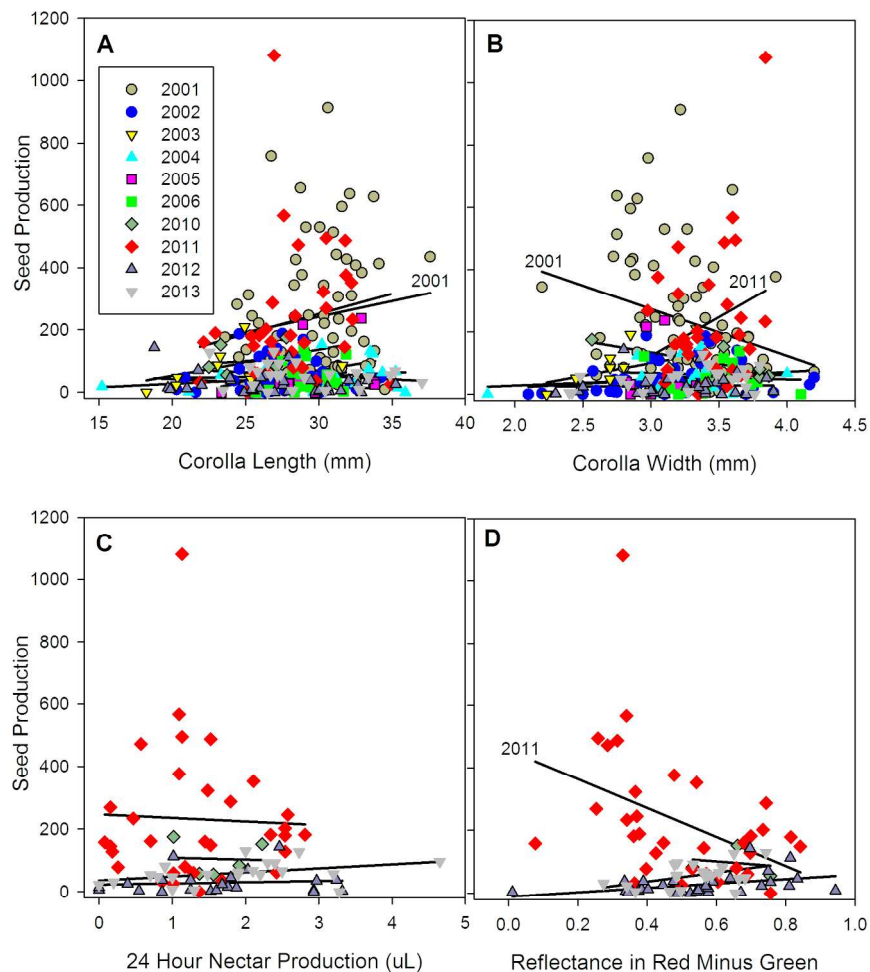
59x240mm (300 x 300 DPI)

Figure 2. Seed production as a function of four floral traits. Lines show slopes on the trait values, with significant ones for individual years ($P < 0.05$) labelled with the year number. (A) Selection on corolla length across 10 years of study. (B) Selection on corolla width across 10 years of study. (C) Selection on nectar production across 4 years of study. (D) Selection on flower colour across 4 years of study. Flower colour was quantified as $(R_{626-700} - R_{476-550})/(R_{401-700})$, where $R_{626-700}$ = reflectance from 626 to 700 nm wavelength, $R_{476-550}$ = reflectance from 476 to 550 nm wavelength, and $R_{401-700}$ = total reflectance in the visible range. Flowers of *Ipomopsis* do not reflect in the UV [24].
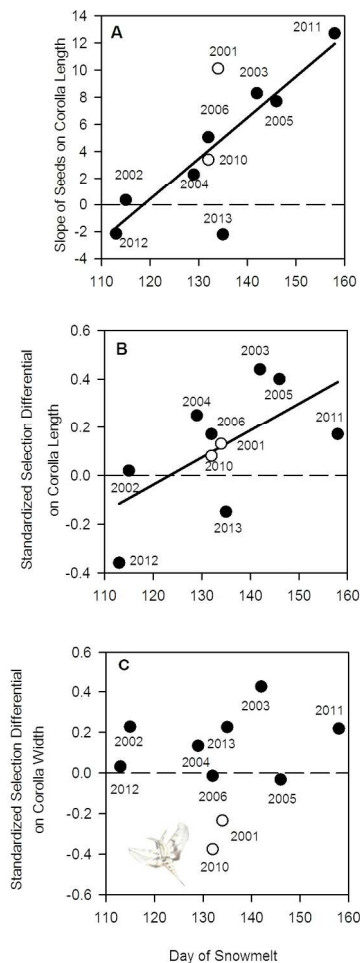177x240mm (300 x 300 DPI)

Figure 3. Selection changes with date of snowmelt and pollinators. **(A)** The slope of seeds on corolla length correlates positively with day of snowmelt where 1 = January 1 (r = 0.79, P < 0.01). Closed circles: hawkmoths absent. Open circles: hawkmoths abundant. Dashed line indicates no selection. **(B)** The standardized selection differential on corolla length also correlates positively with snowmelt date (r = 0.63, P = 0.05). **(C)** The standardized selection differential on corolla width is not correlated with snowmelt, but narrow flowers were favored only in the two years with hawkmoths (2001 and 2010).
69x226mm (300 x 300 DPI)