



Research

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Natural selection on floral morphology can be influenced by climate

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Climate has the potential to influence evolution, but how it influences the strength or direction of natural selection is largely unknown. We quantified the strength of selection on four floral traits of the subalpine herb *Ipomopsis* sp. in 10 years that differed in precipitation, causing extreme temporal variation in the date of snowmelt in the Colorado Rocky Mountains. The chosen floral traits were under selection by hummingbird and hawkmoth pollinators, with hawkmoth abundance highly variable across years. Selection for flower length showed environmental sensitivity, with stronger selection in years with later snowmelt, as higher water resources can allow translation of pollination success into fitness based on seed production. Selection on corolla width also varied across years, favouring narrower corolla tubes in two unusual years with hawkmoths, and wider corollas in another late snowmelt year. Our results illustrate how changes in climate could alter natural selection even when the primary selective agent is not directly influenced.

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 5 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 owing 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 area of the Colorado Rocky Mountains with long-term climatic records [12] to measure selection on multiple floral traits in 10 years that varied by 50 days in snowmelt date.

A wealth of previous information on *Ipomopsis* and its pollinators allowed us to make specific predictions about selection on floral traits. We hypothesized selection patterns based on previous studies of selection mediated by hummingbirds and hawkmoths (*Hyles lineata*), along with prior demonstrations

that pollen receipt on the stigma relates to pollinator visitation [8] and seed set to pollen receipt [13,14]. Hummingbirds are consistently common at our sites, but hawkmoths are not, leading us to make different predictions depending on their abundance in a given year. For years with abundant hawkmoths, we predicted that selection would favour long, narrow corollas, as studies in the 1990s demonstrated that plants with those traits received higher hawkmoth visitation [15]. For years without hawkmoths, we predicted selection favouring long, wide corollas, more intensely red flowers and high nectar production, as plants with those traits received higher hummingbird visitation ([16–18]; figure 1*a*) and, for flower colour, made more seeds at a site with no hawkmoths [19]. We furthermore hypothesized that the strength of selection through female function would depend on water availability. We hypothesized more intense selection in wetter years with later snowmelt (figure 1*b*) if soil moisture is required before or during seed maturation to translate greater pollen receipt into higher seed production, as seen in related species of *Polemonium* [20]. If so, drought conditions could eliminate pollen-limitation of seed production and thereby weaken selection on floral traits.

2. Material and methods

(a) Study site and common garden design

Natural selection was measured for plants growing from seeds of the herb *Ipomopsis aggregata*, its close relative *Ipomopsis tenuituba*, and F_2 hybrids that were planted into two common gardens at Poverty Gulch, Gunnison County, CO, USA. The use of both species and their hybrids allowed us to present a wider range of phenotypic trait combinations than present in a single natural population, yielding a more powerful test of selection and the ability to generalize across multiple species. Plants of *I. aggregata* spp. *aggregata* normally grow in the valley bottom at elevations of 2900 m and below, plants of *I. tenuituba* spp. *tenuituba* grow on steep slopes above 3100 m, and natural hybrids grow in between. Plants of both species are self-incompatible monocarps that almost always flower during a single season (after 2–12 years with a median of 5 years), set seed and then die [21,22]. This monocarpic life history allowed us to measure lifetime female fitness.

Two sets of plantings were included. The first set has been previously described in [22]. Briefly, we collected parental plants of both species in 1995 and crossed them to produce F_1 progeny that served as the parents for F_2 and backcrosses that we planted as seed in the field during 1997 and 1998. We planted seeds of *I. aggregata* (AA), *I. tenuituba* (TT), both reciprocal F_1 hybrids (i.e. with both *I. aggregata* as the maternal plant and *I. tenuituba* as the maternal plant), both reciprocal F_2 hybrids, reciprocal backcrosses of the F_1 to both parental species and (in 1998 only) seeds from crosses between natural hybrids. We analysed only seeds planted into the *I. aggregata* or hybrid site (sites L and I; [15]), as different patterns of selection are likely in the *I. tenuituba* site. Most of these plants that bloomed did so during 2001–2006, providing us with 6 years of data for selection analysis.

The second set of seeds was planted in 2007 and 2008 and has not been previously described. In 2007, AA (*I. aggregata* × *I. aggregata*) seeds were generated from reciprocal factorial crosses of five individual plants yielding five maternal half-sib families and supplemented with one family of field-collected seeds, TT (*I. tenuituba* × *I. tenuituba*) seeds were generated from reciprocal factorial crosses of five individual plants yielding five maternal half-sib families plus one family of field-collected seeds, five families of AT (F_1 with *I. aggregata* as the maternal

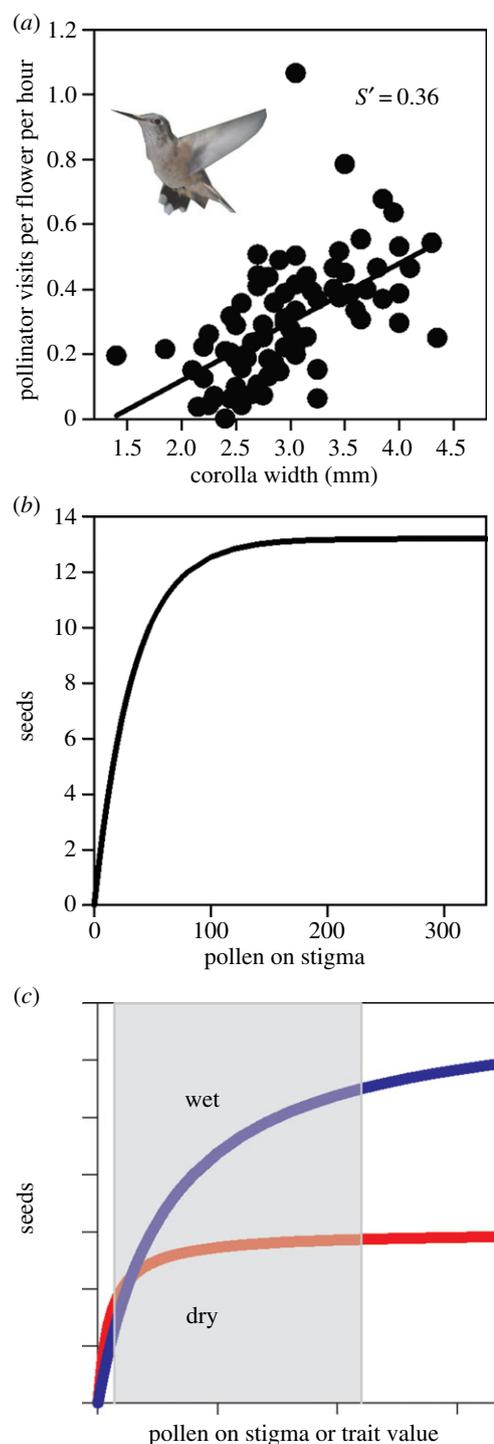


Figure 1. Potential effect of climate on pollinator-mediated selection of a floral trait. (a) In pollinator-mediated directional selection, a floral trait has a linear effect on pollination success, as illustrated by the influence of corolla width on hummingbird visit rate in experiments with *Ipomopsis* (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 nonlinearly 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 [25] are theoretically predicted under wet conditions (blue line) than under dry conditions (red line). Because pollinator visitation relates linearly to the trait value (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 grey box). (Online version in colour.)

parent) and five families of TA (F_1 with *I. tenuituba* as the maternal parent) were generated by crosses between these same individuals, and 14 families of F_2 seeds were generated by crossing seven independent pairs of F_1 plants in both reciprocal directions. Seeds from the 36 families were planted into 20 blocks at the *I. aggregata* site and 20 blocks at the hybrid site, with each block containing approximately 60 (average = 58) seeds chosen at random from the 36 families and planted at 10 cm intervals into the top six rows of the 1×1 m block using a gridded planting frame to indicate planting locations. In 2008, we generated another six families of F_2 seeds and planted them into 10 blocks at the *I. aggregata* site, with each block containing 90 seeds chosen at random from the six families and planted at 10 cm intervals. Existing vegetation was left intact inside the planting area so that competition with other plants was not modified, except that flowering individuals of *Ipomopsis* were removed from the surrounding 1 m area to prevent drop of seeds into the planting area. There is no seed bank, and seedlings typically emerge within 1 cm of the planted location [22]. Seedlings were censused in the year following planting using the planting frame, and surviving individuals were given a numbered metal tag for easier identification. Surviving plants bloomed in 2010 and later, giving us an additional 4 years of selection data (2010–2013). In total from both sets of experiments, 7979 seeds were planted individually, of which 1457 produced seedlings. Survivors were censused each succeeding year, and floral traits were measured in the year of blooming. Owing to a small sample of blooming plants in 2013, we supplemented the dataset with an additional 11 *in situ* flowering plants growing within 10 m of an experimental block.

(b) Measurement of floral traits and fitness

At our study sites, the main pollinators in the majority of years are the hummingbirds *Selasphorus platycercus* and *Selasphorus rufus* [11], and plants with longer and wider corollas, redder flowers and higher nectar production receive more hummingbird visits in at least some years [16,17,19]. Because seed production is limited by pollen transfer [13,23], the increased pollen receipt associated with higher visitation in *Ipomopsis* [8] is expected to lead to higher seed production. In earlier experiments, such an impact was seen in a single test for flower colour, in 1 out of 4 years for corolla length, in 1 out of 3 years for corolla width, but not for nectar production [13,17,19]. In rare years, hawkmoths are observed to visit, and visitation by hawkmoths is greater for plants with narrower corollas [15].

Based on these previous studies, we measured four floral traits: corolla length, corolla width, flower colour (for the 2007–2008 plantings only) and 24-h nectar production (for the 2007–2008 plantings only). Corolla length and width at the opening of the tube were measured using callipers and averaged over two to ten flowers per plant. Corolla colour was measured for two to four flowers per plant using an Ocean Optics (Ocean Optics Inc., Dunedin, FL, USA) Red Tide USB650 reflectance spectrometer with a LS-I light source, standardized by a white reflectance standard, and a fibre optic probe held at a 45° angle. Redness of the flower was quantified by finding the average value for relative reflectance (R) in the red compared to the green: $(R_{626-700} - R_{476-550}) / (R_{401-700})$. Flowers of these species do not reflect in the ultraviolet [24]. Nectar production was measured for one to five flowers per plant by covering elongated buds with straws to prevent access of pollinators and returning 48 h later to extract nectar with a 5- μ l microcapillary tube 32 mm in length [17]. The length of the nectar column in millimetres was multiplied by 5 μ l / (2 days \times 32 mm) and averaged across flowers to determine 24-h nectar production rate in microlitres per day.

At the end of each summer, we collected fruits and the calyces from failed fruits every 2–3 days to estimate the

number of flowers made by each plant that bloomed, the seeds per flower and total seed production (methods in [21]). Of the 279 plants that bloomed, 59 were *I. aggregata*, 24 were *I. tenuituba*, 54 were F_1 hybrids, 40 were backcrosses and 102 were F_2 hybrids or offspring of crosses between natural hybrids, in which correlations between traits are likely broken down owing to recombination.

(c) Statistical analysis

Analysis of temporal variation in selection proceeded in several steps. First, we examined correlations among the four floral traits and between the traits and flower number. Because correlations with flower number were weak or absent ($-0.16 \leq r \leq 0.20$, $N = 80-279$), estimates of phenotypic selection were not confounded by general increases in plant vigour in better microhabitats. Furthermore, corolla length, corolla width and nectar production were weakly or not at all correlated with each other ($-0.14 \leq r \leq 0.16$, all $p > 0.05$), justifying univariate analyses of selection one trait at a time. Flower redness did correlate negatively with corolla length ($r = -0.25$, $p < 0.05$) and positively with nectar production ($r = 0.37$, $p < 0.001$), so univariate measures of selection on that trait cannot distinguish between direct selection and indirect selection of the correlated traits.

We then proceeded to analyse selection on each of the four floral traits separately. Selection could vary across years owing to a change in the relationship of absolute fitness to a trait (figure 1c) or because of the tendency for variance in relative fitness (opportunity for selection) to decline as mean absolute fitness increases [26,27]. We examined both aspects in this study, because effects on absolute fitness are critical to demographic consequences of changes in selection [2] and because all plants in an experimental planting were part of a single generation under selection. First, to check for nonlinear effects on absolute fitness, we analysed the following generalized linear model as specified with a model statement in the GENMOD procedure in SAS v. 9.2 (SAS Institute, Cary, NC, USA):

$$\text{model } W = \text{year} + \text{trait} + (\text{trait} - \overline{\text{trait}})^2 + \text{year} \times \text{trait} + \text{year} \\ \times (\text{trait} - \overline{\text{trait}})^2,$$

where W is absolute fitness estimated as seed production, and Year was treated as a class variable. We used likelihood ratio tests for type 3 analysis that assumes all other effects are in the model. We also specified normally distributed residuals, as doing so provided good fits based on AIC and scaled deviance. In no case was the interaction of year with the quadratic term significant ($p = 0.92, 0.61, 0.37$ and 0.94 for the four traits), indicating lack of evidence that nonlinear selection varied across years. We next dropped that term, and also found no significant quadratic term that would indicate nonlinear selection ($p = 0.37, 0.28, 0.35$ and 0.80). We then tested for variation in directional selection across years by analysing the following model for absolute fitness:

$$\text{model } W = \text{year} + \text{trait} + \text{year} \times \text{trait}.$$

A significant year-by-trait interaction indicated variation in selection across years. If the interaction was significant, we then estimated selection separately in each year using a model with the factors of year and trait nested within year. If no interaction was detected, we estimated average selection using a standard analysis of covariance model with year as the class variable and trait as the continuous variable.

We then tested whether the slope of absolute fitness on the trait, the opportunity for selection (variance in relative fitness [28]) or the standardized selection differential based on relative fitness varied with environmental factors. Relative fitness was calculated by dividing seed production by the mean for that year. The selection differential (covariance between relative fitness and the trait) was standardized in units of SD for the

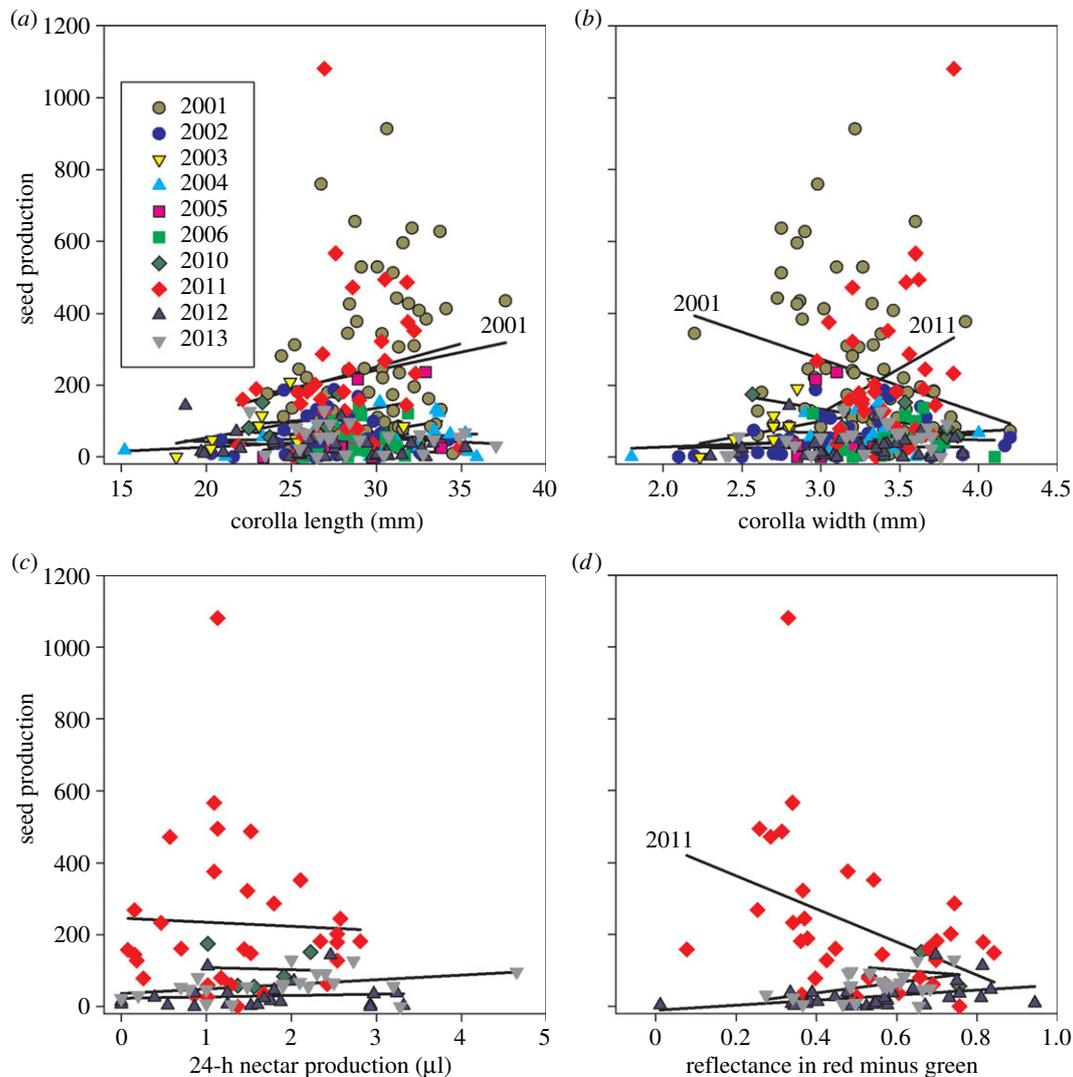


Figure 2. Seed production as a function of four floral traits. Lines show slopes on the trait values, with significant ones for individual years ($p < 0.05$) labelled with the year number. (a) Selection on corolla length across 10 years of study. (b) Selection on corolla width across 10 years of study. (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]. (Online version in colour.)

trait [29]. For traits with 10 or more years of data, we examined the environmental sensitivity of selection [30] by calculating the correlation between the selection measure and either of two environmental variables: (i) summer precipitation between 1 June and 15 July at Gothic, CO (National Atmospheric Deposition Program CO10), the period when *Ipomopsis* is developing inflorescences and prior to most of our floral measurements in late July and early August, or (ii) the date of first snowmelt at Gothic, CO, 8 km from our common gardens and at a similar elevation near 2900 m. Later snowmelt is associated with higher soil moisture in mountainous regions, with effects lasting for two to four months after snowmelt, at least in the Sierra Nevada [31]. We also compared selection in years with versus without hawkmoths. The sites at Poverty Gulch were visited two to three times per week throughout each three-month season, but hawkmoths were observed only on 9 July 2001, 13 July 2001, 5 August 2010 and 11 August 2010, indicating that 2001 and 2010 were more abundant years for moths.

3. Results

Seed production varied greatly among years ($p < 0.0001$ for year effect in ANOVA) and tended to be greater in years with

later snowmelt ($r = 0.59$, $N = 10$, $p = 0.075$). On average, a flowering plant made only 26 seeds in 2012, which had the earliest snowmelt (23 April) in 40 years of record keeping (1974–2013; www.gothicwx.org/long-term-snow.html), compared with 224 seeds in 2011, which had the latest snowmelt in our 10 year dataset (7 June) and the third latest out of 40 years. The overall opportunity for selection did not, however, correlate with snowmelt date ($r = -0.08$, $p = 0.82$). Average flower morphology did not change with the environmental feature of snowmelt date ($r = 0.03$, $p = 0.92$ for corolla length and $r = -0.01$, $p = 0.97$ for corolla width).

Two of the four traits (corolla width and flower redness) showed significant variation across years in selection as judged from year-by-trait interactions on seed production (electronic supplementary material, table S1). Directional selection favoured narrow corollas in 2001 (standardized selection differential $S' = -0.24$; $p = 0.0006$), a year with abundant hawkmoths, and wide corollas in 2011 ($S' = 0.22$; $p = 0.0268$; figure 2). The standardized selection differential for corolla width in a given year did not correlate overall with snowmelt date ($r = 0.13$, $N = 10$, $p = 0.72$) or summer precipitation ($r = -0.53$, $p = 0.12$), but instead was the most

strongly negative in the 2 years with abundant hawkmoths (figure 3). Removing those two moth years from the analysis, years with later snowmelt showed the predicted higher gains in absolute fitness with corolla width ($r = 0.65$, $N = 8$, one-tailed $p < 0.05$), but that did not translate into higher standardized selection differentials (figure 3). Thus selection on corolla width related most strongly to pollinator availability.

As predicted, because both hummingbirds and hawkmoths preferentially visit longer flowers of *Ipomopsis*, the estimate of selection on corolla length was positive in most years (eight out of 10). Whereas we did not detect a significant year-by-trait interaction for corolla length, both the slope of absolute fitness on corolla length and the standardized selection differential correlated positively with snowmelt date (figure 3*a,b*; $r = 0.79$ and 0.63 , $p = 0.006$ and 0.05 , respectively), making selection on that trait more intense in years with late snowmelt. Precipitation from 1 June to 15 July was, however, not associated with the standardized selection differential ($r = 0.16$) nor the slope of absolute fitness. The correlation of seed set with corolla length was not explainable by indirect selection of corolla width or nectar production, as those traits were weakly or not at all correlated, but could conceivably reflect some selection of the correlated trait of flower redness ($r = -0.25$, $p = 0.0166$). The relationship of corolla length with fitness was also not explainable by independent effects of the environment on the trait as well as on seed production, as corolla length did not change with snowmelt. Instead, the association of selection strength with snowmelt can be explained by the possibility of translating pollen receipt into higher seed production in years with later snowmelt.

Our specific predictions were not upheld for the other two traits: flower colour and nectar production. Selection on flower colour varied significantly across years, judging from the year-by-colour interaction on absolute fitness measured as seed production (electronic supplementary material, table S1). Selection, however, strongly favoured whiter (less red) flowers in 2011 ($S' = -0.39$; $p < 0.0001$; figure 2), despite the scarcity of hawkmoths and expectation that hummingbirds would select for redder flowers in that case. Selection on nectar production was not detectable at all, with no evidence either for variation across years (electronic supplementary material, table S1).

4. Discussion

In this subalpine ecosystem, we found that the strength of selection on flower corolla length was positively associated with snowmelt date. Later snowmelt leads to greater water availability in the soil, presumably allowing higher hummingbird pollination experienced by long flowers to translate into more seeds. In wet years, the standardized selection differential for length accounted for a higher proportion of the overall opportunity for selection [27], so that it also increased with snowmelt date. Summer precipitation from 1 June to 15 July was not associated with selection intensity, possibly because the total amount (mean = 5.5 cm) during this drier part of the summer season is too small to have much effect on soil moisture, whereas advancing snowmelt by two to three weeks can decrease water storage in soil by about one-third [31]. That time frame is equivalent to the difference between the earliest date and the mean date of snowmelt in our dataset. Alternatively, or in addition, seed production may depend mostly on

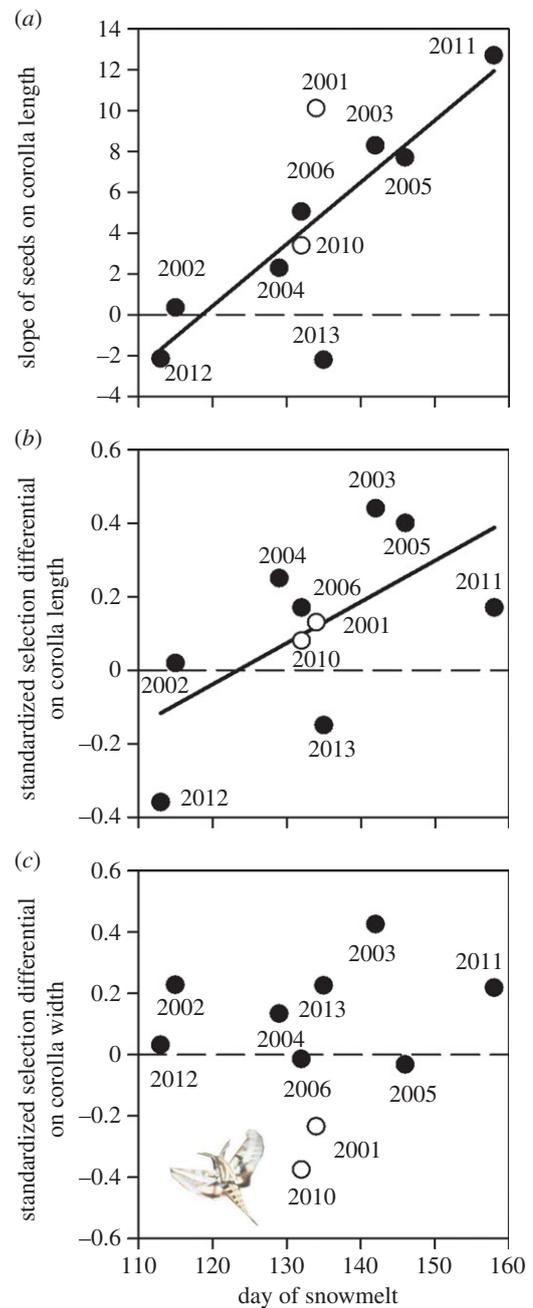


Figure 3. Selection changes with date of snowmelt and pollinators. (a) The slope of seeds on corolla length correlates positively with day of snowmelt where 1 = 1 January ($r = 0.79$, $p < 0.01$). Filled circles: hawkmoths absent. Open circles: hawkmoths abundant. Dashed line indicates no selection. (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 favoured only in the 2 years with hawkmoths (2001 and 2010). (Online version in colour.)

resources already accumulated in the taproot or leaves by the plant during early spring when snowmelt saturates the soil.

Although we had predicted selection for redder flowers, in 2011 selection instead favoured whiter flowers, despite the scarcity of hawkmoths that year. One potential explanation for the negative association of redness with seed production is the strong indirect selection on the negatively correlated character of corolla length in that year (figure 3*a*). Nectar production is a trait that is theoretically also expected to be under selection, as nectar is the primary reward for both hummingbirds and hawkmoths. Yet, we did not detect selection on

that trait, nor evidence for variation in selection across years for nectar (electronic supplementary material, table S1). Although paradoxical from a theoretical perspective, these results are consistent both with absence of detectable selection through female function on nectar in an earlier study of *Ipomopsis* [17] and with a recent review claiming nectar as the only studied floral trait for which direct selection through female function has not been reported ($N = 22$ estimates; [7]).

Perhaps the most striking result of our study is that natural selection via pollinators on the trait of corolla length is strongly predicted by the date of snowmelt. Even though the selection on this floral trait is driven directly by pollinators, its intensity relates to snowmelt. Snowmelt is an important annual wetting event in ecosystems with seasonal snowpack [31] and is one of the physical variables most likely to be affected by climate change in the Rocky Mountains, where it correlates with flowering phenology and abundance for several species [12,32]. If the average date of snowmelt in this area continues to advance as it has over the past 40 years by 3.5 days per decade [33], we predict less intense pollinator-mediated selection shaping floral length in *Ipomopsis*. Climate models for the region predict a higher frequency of extreme droughts in the future [34], which could also produce greater variation in selection intensity.

Most work on natural selection in response to climate change has focused on a scenario in which a change in selection can adaptively restore positive growth to a population in decline [2]. The change in selection that we saw corresponds to a different scenario in which early snowmelts (if they continued) would eliminate pollen limitation and weaken selection on flower morphology. If longer corolla tubes had been favoured by pollinator-mediated selection in the recent past, *Ipomopsis* populations would have experienced ongoing evolution of longer tubes, as corolla length shows a relatively high narrow sense heritability of 0.74 [18]. That evolutionary response could have helped to maintain populations with positive population growth, as witnessed in some natural *Ipomopsis* populations [35]. With earlier snowmelt, however, selection on corolla length would decline, slowing evolution. More variable snowmelt date would also diminish the evolutionary response, as fluctuation in selection intensity is predicted to help maintain variation in a floral trait and dampen the average response [36] to selection. Thus with earlier or more variable snowmelt, any positive demographic effect of selection on corolla length that occurred in the recent past would no longer occur, perhaps even reducing maintenance of viable populations. That prediction results from snowmelt having an indirect effect on selection

experienced by corolla shape, rather than a situation in which selection is imposed directly by the thermal environment. It is of course possible that other traits, such as vegetative or physiological traits, would experience direct selection for values that are highly adaptive under new climatic conditions. In addition, climate might affect abundance of the hawkmoths, a topic little understood [37], which would alter selection on corolla width, potentially also with demographic impacts [38].

5. Conclusion

Our results indicate that the intensity of selection on a floral trait can vary with the climatic environment, particularly the date of snowmelt. That finding potentially has two general implications. First, given the importance of snowmelt date to phenology of other plants and animals in this ecosystem [33] and others worldwide [1], we expect that natural selection on traits in other organisms will be altered by future climate change in mountainous areas. Second, our work suggests the need to examine potential selection responses not only in thermal tolerance and phenological timing—traits likely to show increased selection with rapidly changing climate [39]—but also in floral traits that may show indirect changes in selection, either because events such as low snowpack or drought modify outcomes of pollinator-mediated selection or because climate affects abundance of pollinators. Global climate change may alter not only the abundance, phenology and distribution of organisms [1,33] but also their evolutionary trajectories.

Data accessibility. Selection on floral traits: Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.bk175>.

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Authors' contributions. D.R.C. designed the study, collected field data, carried out the statistical analyses and helped to write the manuscript. J.M.P. collected field data, participated in data analysis and helped write the manuscript.

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