Title: A resurrection experiment reveals reduced adaptive potential in a common agricultural weed

Authors: Sasha G.D. Bishop[1], John R. Stinchcombe[2], Regina S. Baucom[1]

[1]Department of Ecology and Evolutionary Biology, University of Michigan; Ann Arbor, 48109, USA.
[2]Department of Ecology and Evolutionary Biology, University of Toronto; Toronto, M5S 3B2, Canada.

ABSTRACT

Adaptive evolution is critical to population response to anthropogenic global change. Despite research suggesting adaptation commensurate with the rate of contemporary environmental change is possible, mismatches between expected and realized evolution in natural systems are widespread. Using a combination of quantitative genetics and resurrection ecology, we show that covariances among pollination and mating system related traits in the common morning glory (*Ipomoea purpurea*) constrain the rate of evolution and that such constraints are strengthening over time, dramatically reducing adaptive potential. Pollinator-attracting traits evolved on a rapid time scale of less than ten years, but genetic covariances between traits limited the adaptive rate and evolutionary trajectory of flowering phenology, a trait with widely recognized importance in adapting to climatic shifts.

Introduction

Understanding whether evolutionary adaptation can keep pace with rapid environmental shifts is critical to understanding current biodiversity declines and the continued resilience of ecosystems to global change (*1*, *2*). Species can respond to rapid environmental changes by shifting their geographic range, plastic trait responses, and *in situ* adaptation. Geographic range shifts can be limited by large-scale habitat fragmentation and destruction (*3*, *4*) whereas compensatory responses via plasticity have been shown to demonstrate a limiting upper bound (*5*, *6*). Taken together, this suggests that long-term, sustained responses to climatic changes rely heavily on *in situ* adaptation. A major unresolved question relating to adaptive evolution is how adaptive potential, defined here as genetic variation that can lead to a response to selection, may be changing in wild populations facing contemporary environmental pressures.

Given environmental change, a population's adaptive capacity depends on the presence and availability of genetic variation, as well as how efficiently selection acts on phenotypic variation. Trait-trait covariances can slow or prohibit evolutionary response if directional selection on genetically linked traits is misaligned with the sign of the trait covariance (*7–9*).The presence of trait covariance in wild populations (*10*, *11*) as well as the possibility for these covariances to induce lags in adaptive rate in response to artificial selection (*12–14*) are both well documented. However, genetic correlations can change over time in response to novel environments (*9*, *15*), with one of the strongest driving causes being conditions of fluctuating correlational selection (*16*, *17*). Global change is exposing wild populations to multiple, simultaneous shifts in abiotic and biotic variables. Evidence suggests that historically coupled selective agents may be decoupling (*18*), creating a context in which misaligned selection or changing correlational selection is becoming increasingly probable. Characterizing how genetic correlations may change in the face of global change, however, remains a largely unmet challenge. As such, the causes and consequences of constraint are open questions that pertain directly to projections of adaptive rate and population viability in the face of global change.

The potential for constraints on climate change adaptation induced by among-trait genetic correlations has been assessed using a space-for-time approach (*7*), but spatial gradients of climatic change can be misleading proxies for climatic change over time or population response to that change (*19*). Non-climatic variables may decouple species response from climatic changes, or adaptation to local conditions can result in localized declines given climatic shifts rather than adaptation to spatially predicted trait means (*20*). Resurrection ecology mitigates these issues by comparing temporally sampled lineages from the same locations, thus removing spatial differences among populations altogether. While resurrection ecology is increasing in popularity for measuring phenotypic shifts, it has yet to be applied to assess the real-time evolution of constraints. Here, we combine a resurrection ecology approach and quantitative genetics to examine temporal changes in adaptive constraints on floral traits in wild morning glory (*Ipomoea purpurea*) populations, and address the question of what underlies constraint evolution by evaluating trait specific trade-offs and pollinator visitation.

Approach

We conducted a resurrection experiment using replicate maternal lines of *Ipomoea purpurea* sampled at two time points (2003 and 2012; hereafter ancestral and descendant, respectively) as

seed from seven wild populations (Fig. S1). Replicates of each maternal line were planted into field conditions at the Matthaei Botanical Gardens (Ann Arbor, MI) on June 4, 2022. During the course of the experiment we recorded trait values for five floral traits (date of first flower, corolla width, corolla length, anther-stigma distance, and nectar sucrose content (°Bx)) along with plant size (leaf count) and fitness (total seed count). These measurements were used to calculate heritable genetic variation per trait and a multivariate statistic, ***R,*** which compares the expected rate of adaptation given a selection gradient, β**,** and variance-covariance matrix, ***G***, to the rate of adaptation with the same selection gradient but a variance-covariance matrix with all trait covariances constrained to zero (*21*). Estimates of $\boldsymbol{R} < 1$ demonstrate evolutionary constraint such that the rate of adaptation is slower than expected without covariance. Conversely, $\boldsymbol{R} > 1$ would indicate evolutionary facilitation. We then investigated each trait individually by comparing predicted evolutionary change with ($\boldsymbol{\Delta\bar{z}}$) and without ($\boldsymbol{\Delta\bar{z}_{\mathrm{nc}}}$) trait covariance to identify which floral traits influence the rate of adaptation (***R***). We further dissected temporal patterns of selection and potential genetic correlations between traits to detect instances where new or emerging pairwise trait correlations act in an antagonistic direction to selection, indicating evolution of constraints or trade-offs between traits (*22*). Finally, we used pollinator visitation surveys and structural equation modeling to determine if fitness costs and benefits associated with traits are mediated through pollinator visitation frequency and thus whether adaptation in pollinator-attracting traits may constrain the response to other possible selective agents.

Results

Trait Covariance is Causing a Rapid Decline in Adaptive Rate Over Time

In both the ancestral and descendant populations, across traits, the overall rate of adaptation is lower when genetic covariances between traits are included. Notably, however, evolutionary constraint is much stronger in the descendant populations with $\boldsymbol{R} = 0.09$ compared to $\boldsymbol{R} = 0.76$ in the ancestral populations (99.85% significance threshold; Fig. 1A). Thus, in the ancestral populations, accounting for trait covariances results in an adaptive rate that is 76% as fast as predictions without trait covariances, but this predicted rate drops precipitously to 9% in the descendant populations, indicating that constraints have increased over time. An alteration to the ***R*** statistic, ***R'*** (with values >1 indicating greater adaptive potential in ancestral populations)**,** that compares the ancestral and descendant populations directly shows that predicted rates of adaptation without covariance are largely sustained over time, though perhaps marginally faster in ancestral populations, $\boldsymbol{R_{nc}'} = 1.81$. However, when accounting for trait covariance, $\boldsymbol{R'} = 23.61$, demonstrating that, contrary to univariate predictions, the adaptive rate was 23x faster in ancestral populations - *i.e.* over nine years the populations lost 96% of their adaptive potential. These findings indicate that constraints among floral traits evolve on a rapid time scale of less than ten years and are acting to limit adaptive potential, overall highlighting that consideration of multi-trait dynamics is critical to understanding the impact of global change on patterns of biodiversity.

We next compared the expected adaptation with and without covariance ($\boldsymbol{\Delta\bar{z}}$ to $\boldsymbol{\Delta\bar{z}_{\mathrm{nc}}}$) to identify which traits underlie observed shifts in ***R.*** We found significant constraint on the rate of adaptation in corolla width as well as a change in the direction of evolution for corolla length in both the ancestral and descendant populations (corolla width, ancestral: $\boldsymbol{\Delta}z_{\mathrm{cw}} = 0.02$, $\boldsymbol{\Delta}z_{\mathrm{nc_cw}} =$

0.13, descendent: $\Delta z_{cw} = 0.01$, $\Delta z_{nc_cw} = 0.09$; corolla length, ancestral: $\Delta z_{cl} = 0.01$, $\Delta z_{nc_cl} = -0.07$, descendent: $\Delta z_{cl} = 0.01$, $\Delta z_{nc_cl} = -0.07$; Table 1, Fig. 1B). Comparatively, for anther-stigma distance (ASD) and day of first flowering, we find that genetic covariance between traits in ancestral populations results in adaptive facilitation such that expected adaptation of smaller ASD ($\Delta z_{ASD} = -0.07$, $\Delta z_{nc_ASD} = -0.002$; 96.55% significance threshold) and earlier flowering ($\Delta z_{DoFF} = -0.14$, $\Delta z_{nc_DoFF} = -0.07$; 99% significance threshold) is faster than without covariance. Descendant populations, however, display no effect of covariances on ASD, and show adaptive constraint on flowering phenology such that the adaptive rate of flowering time is slowed by covariance with other traits ($\Delta z_{DoFF} = -0.03$, $\Delta z_{nc_DoFF} = -0.05$; 92% significance threshold; Table 1, Fig. 1B). A comparison of Δz between years shows that there is a decrease over time in expected rate of adaptation toward earlier flowering (Table 1, 99.85% significance threshold) and plant size (ancestral $\Delta z_{size} = 0.19$, descendant $\Delta z_{size} = 0.01$; 99.95% significance threshold).

Constraints Result in New Trait Optima

Two commonly proposed mechanisms for lack of evolutionary change are an absence of heritable genetic variation or trait trade-offs in the form of negative genetic correlations or covariances (*9*); either, or both, of these mechanisms could explain the results on adaptive rate. However, we found each of the floral traits to exhibit heritable genetic variation across collection years (Table 1). This means that lack of appreciable genetic variation within individual traits will not induce evolutionary constraint, further corroborated by variation maintained in $\boldsymbol{W_{nc}}$ but not in $\boldsymbol{W}$ (Fig. 1A). Analysis of genetic correlations showed that, while trade-offs between traits were present and generally took the form of negative genetic correlations between floral traits and plant size, most remained similar in sign and strength between the ancestral and descendent populations (Fig. 1C, Fig. S3, S5). Two notable exceptions are the strengthening over time of the correlation between corolla width or corolla length and date of first flower (corolla width and date of first flower: ancestral $r = -0.005$, $p = 0.481$, descendant $r = 0.34$, $p < 0.0001$; corolla length and date of first flower: ancestral $r = 0.02$, $p = 0.627$, descendant $r = 0.22$, $p = 0.003$). The emergence of a positive correlation between corolla size and flowering date in descendant populations indicates that a new trade-off has evolved between larger flowers and earlier flowering.

We likewise identified different trait optima in the descendent versus ancestral populations favoring evolutionary investment in pollinator-attracting traits at the cost of evolvability in flowering phenology. In the common garden setting, a change in selection coefficient is representative of a new trait optimum not due to selection regime itself changing, but of a differing fitness response for a given trait to shared environmental conditions. As such, we report comparisons of total and direct selection on traits to show shifts in adaptive capacity.

We found that the adaptive capacity of date of first flower differed between ancestral and descendant populations (ANCOVA contrasting slopes: $F = 5.39$, $p = 0.003$, Table S1). In ancestral populations, we uncovered higher fitness in earlier flowering plants ($S = -0.16$, $p = 0.03$; Table S1) as well as a phenotypic shift to earlier flowering in descendant populations by ~1 day (t-ratio = 1.78, $p = 0.07$; Fig. S4, Table S3, *also shown in greenhouse conditions*, (*23*)). In contrast, in line with the constraint indicated by Δz_{DoFF} and a trade-off with flower size, earlier flowering dates are no longer favored in descendant populations, and instead highest fitness is found for intermediate flowering dates ($\gamma = -0.10$, $p = 0.05$; Table S1). Descendent populations

also displayed the emergence of new correlational selection between date of first flower and three other traits: corolla width (γ = -0.34, p = 0.071), nectar sucrose content (γ = -0.14, p = 0.067), and corolla length (γ = 0.39, p = 0.045, Fig. 2), all featuring a fitness ridge favoring intermediate values of flowering time.

Despite constraints on the rate of adaptation of corolla width, trends in trait response to selection in the common garden on both corolla width and °Bx indicate an adaptive pathway toward investment in pollinator-attracting traits of larger floral display and higher sugar content in nectar. Specifically, corolla width displays significant positive selection in both the ancestral and descendent populations (S = 0.11, p = 0.001 ancestral; S = 0.12, p < 0.001 descendant; Table S1); further, the continued evolution of broader corollas is corroborated by a significant increase in mean trait value from ancestral to descendant populations by ~1mm (t-ratio = -2.53, p = 0.01; Fig S4, Table S3). Although selection differentials in descendant populations display positive selection for corolla length (S = 0.05, p = 0.06), the selection gradient shows negative selection on corolla length (β = -0.37, p = 0.04). Thus, the positive total selection on corolla length likely arises from the strong correlation between corolla length and width (r = 0.92, p < 0.001) and the overall positive selection on corolla width. For nectar, ancestral populations display a pattern of disruptive selection where both high and low levels of sucrose are favored (γ =0.06, p = 0.02; Fig. S4, Table S1), however, there is a nearly-significant change in the quadratic relationship between fitness and °Bx in descendant populations (ANCOVA contrasting slopes: F = 3.40, p = 0.066, Table S1) such that lower sucrose values are no longer favored and instead there is a trend toward higher fitness at high values of °Bx (S = 0.05, p = 0.12, Table S1).

Overall, these data suggest that the evolution of earlier flowering times in descendant populations is limited by selection for increased corolla width (Fig. 1B & 1C, Tables S1 & S2), with a concomitant increase in nectar sucrose. These changes in adaptive direction and degree are additionally accompanied by a decrease in relative fitness of descendant populations compared to ancestral (ancestral = 1.06, descendant = 0.93; p = 0.001), indicating that, at least in some environments, reduced adaptive capacity is also resulting in lowering fitness. Beyond the development of linear trade-offs, our results show that constraints due to changes in genetic architecture have evolved within the nine-year period studied, and that the covariance structure—particularly between floral traits and the timing of flowering—may remain unstable as global changes continue.

Pollinators as Agents of Selection

We next examined the potential that pollinators impose selection on both corolla width and nectar sucrose content in *Ipomoea purpurea* using structural equation modeling. Our best supported model (Table S4, S5) indicates that increases in corolla width and nectar sucrose content between ancestral and descendant populations are driven by pollinator preference, whereas changes in flowering phenology are not responsive to pollinator foraging frequency, but rather some other environmental factor. Specifically, we find a positive effect of corolla width on frequency of pollinator floral approaches (r = 0.242, p = 0.02) and pollinator forages (r = 0.286, p = 0.004) as well as a positive relationship between nectar sucrose content and pollinator approaches (r = 0.149, p = 0.002), meaning that large corolla widths and high sucrose content in nectar attracts more pollinators to a flower (Fig. 3, Table S6). An additional high conversion of pollinator approach to foraging (r = 0.366, p < 0.0001) and positive relationship between

pollinator foraging and plant fitness ($r = 0.173$, $p = 0.032$) results in an indirect relationship between corolla width and nectar on plant fitness where high values of each are favored by pollinators and result in higher plant fitness. Conversely, earlier dates of first flower have a positive effect on plant fitness ($r = 0.321$, $p = 0.005$), but do not impact pollinator foraging behavior, meaning that selection on flowering phenology is driven by something other than pollinators, an observation corroborated by numerous studies showing that flowering phenology is core to adaptation to global change factors such as temperature (*24*, *25*) and precipitation (*26*). Overall, and in conjunction with the development of constraints on flowering phenology due to trade-offs with corolla width and first flower, our results highlight an adaptive pathway that is driven by investment in pollinator attraction at the cost of evolvability in flowering phenology, a shift that has occurred within a nine-year period.

Conclusions

One of the driving questions in the study of ecological resilience to global change is whether the rate of adaptive evolution will keep pace with the rate of environmental change. Recent studies find chronic lags in realized compared to modeled evolution, highlighting a disconnect between predictions of evolutionary change compared to actual rates of adaptation in the wild (*2*). Using a resurrection ecology approach, we show that constraints among five floral traits in *Ipomoea purpurea* have increased in strength over time, leading to a decline in the potential rate of adaptation by 96% over nine years. This finding demonstrates that rapid shifts in covariance between traits can slow adaptation, offering a plausible explanation for the observed lags in adaptation across other systems.

Prior studies assessing global change adaptation in plants discuss three major adaptive pathways–either the evolution of increased selfing (*Viola praemorsa*, (*27*); *Viola arvensis*, (*25*)), investment in pollinator attraction due to reduced pollinator abundance (*Lobelia siphilitica* (*28*)), or climatically-induced shifts in flowering phenology (*29*). We highlight an adaptive pathway in *Ipomoea purpurea* in which plants are increasing investment in pollinator-attracting traits over time, but at the cost of evolvability in flowering time.

Recent decades have seen significant reductions in pollinator populations (*30–32*), with unprecedentedly high declines of bumblebee species in North America specifically between the years of 2000-2014 (*33*) when these experimental *Ipomoea purpurea* populations were collected. While the evolution of flowering time is well established as a key mechanism for flowering plants to adapt to climatic changes in temperature and precipitation (*34–37*), our research suggests that declining pollinator populations could undermine resilience to climate change through adaptive constraint–specifically, the diminished adaptive potential in flowering phenology is at odds with projected climatic shifts that continue to alter temperature and precipitation patterns (*38*). These findings thus highlight the necessity of considering multiple dimensions of global change, and emphasize that adaptation to one factor, such as reduced pollinator populations, could conflict with the adaptive responses required for other factors, like climate-induced shifts in temperature and precipitation.

Acknowledgements

The authors thank Michael Palmer and Jeremy Moghtader at the Matthaei Botanical Gardens for assistance with experiment logistics and field preparation. We also thank Grace Zhang and the

team of undergraduate field assistants who helped with field maintenance and trait measurements. We give particular thanks to field technicians Anah Soble, Alice Eliott, and Ruby Barron for assistance with data collection and pollinator observations, and to Adam Kuester for the 2012 seed collections. Management of plants in a second growth room resurrection experiment used to look at plasticity was done primarily by Lily MacKrell and Alex Husted. Finally, we thank Drs. Rachel Spigler, Timothy James, and Meha Jain for their invaluable feedback on experimental design and analysis.

Author Contributions

S.B. and R.S.B. conceived and designed the study with R.S.B. acting as advisor. S.B. collected and analyzed the data and J.S. provided critical review and analysis recommendations. S.B. and R.S.B drafted the initial manuscript with all authors contributing to the final version.

References

1. B. Pujol, S. Blanchet, A. Charmantier, E. Danchin, B. Facon, P. Marrot, F. Roux, I. Scotti, C. Teplitsky, C. E. Thomson, I. Winney, The Missing Response to Selection in the Wild. *Trends Ecol. Evol.* **33**, 337–346 (2018).
2. R. A. Martin, C. R. B. da Silva, M. P. Moore, S. E. Diamond, When will a changing climate outpace adaptive evolution? *Wiley Interdiscip. Rev. Clim. Change*, doi: 10.1002/wcc.852 (2023).
3. J. Lenoir, R. Bertrand, L. Comte, L. Bourgeaud, T. Hattab, J. Murienne, G. Grenouillet, Species better track climate warming in the oceans than on land. *Nature Ecology & Evolution* **4**, 1044–1059 (2020).
4. E. Hamann, D. Denney, S. Day, E. Lombardi, M. I. Jameel, R. MacTavish, J. T. Anderson, Review: Plant eco-evolutionary responses to climate change: Emerging directions. *Plant Sci.* **304**, 110737 (2021).
5. C. M. Sgrò, J. S. Terblanche, A. A. Hoffmann, What Can Plasticity Contribute to Insect Responses to Climate Change? *Annu. Rev. Entomol.* **61**, 433–451 (2016).
6. J. M. Cohen, M. J. Lajeunesse, J. R. Rohr, A global synthesis of animal phenological responses to climate change. *Nat. Clim. Chang.* **8**, 224–228 (2018).
7. J. R. Etterson, R. G. Shaw, Constraint to adaptive evolution in response to global warming. *Science* **294**, 151–154 (2001).
8. L. F. Galloway, R. H. B. Watson, H. R. Prendeville, Response to joint selection on germination and flowering phenology depends on the direction of selection. *Ecol. Evol.* **8**, 7688–7696 (2018).
9. J. Heblack, J. R. Schepers, Y. Willi, Evolutionary potential under heat and drought stress at the southern range edge of North American Arabidopsis lyrata. *J. Evol. Biol.* **37**, 555–565 (2024).
10. G. A. Henry, J. R. Stinchcombe, Strong selection is poorly aligned with genetic variation in Ipomoea hederacea: implications for divergence and constraint. *Evolution* **77**, 1712–1719 (2023).
11. I. Ćalić, S. C. Groen, J. Y. Choi, Z. Joly-Lopez, E. Hamann, M. A. Natividad, K. Dorph, C. L. U. Cabral, R. O. Torres, G. V. Vergara, A. Henry, M. D. Purugganan, S. J. Franks, The influence of genetic architecture on responses to selection under drought in rice. *Evol. Appl.* **15**, 1670–1690 (2022).

12. L. A. Dorn, T. Mitchell-Olds, Genetics of Brassica campestris. 1. Genetic Constraints on Evolution of Life-History Characters. *Evolution* **45**, 371 (1991).
13. Ø. H. Opedal, L. S. Hildesheim, W. S. Armbruster, Evolvability and constraint in the evolution of three-dimensional flower morphology. *Am. J. Bot.* **109**, 1906–1917 (2022).
14. M. T. J. Johnson, A. A. Agrawal, J. L. Maron, J.-P. Salminen, Heritability, covariation and natural selection on 24 traits of common evening primrose (Oenothera biennis) from a field experiment. *J. Evol. Biol.* **22**, 1295–1307 (2009).
15. Y. Sakata, S. Utsumi, T. P. Craig, J. K. Itami, M. Ikemoto, T. Ohgushi, Environmentally triggered variability in the genetic variance-covariance of herbivory resistance of an exotic plant Solidago altissima. *Ecol. Evol.* **10**, 3103–3111 (2020).
16. L. J. Revell, The G matrix under fluctuating correlational mutation and selection. *Evolution* **61**, 1857–1872 (2007).
17. I. do O, M. C. Whitlock, The evolution of genetic covariance and modularity as a result of multigenerational environmental fluctuation. *Evol. Lett.* **7**, 457–466 (2023).
18. S. M. Wadgymar, J. E. Ogilvie, D. W. Inouye, A. E. Weis, J. T. Anderson, Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. *New Phytol.* **218**, 517–529 (2018).
19. D. L. Perret, M. E. K. Evans, D. F. Sax, A species' response to spatial climatic variation does not predict its response to climate change. *Proc. Natl. Acad. Sci. U. S. A.* **121**, e2304404120 (2024).
20. M. L. DeMarche, G. Bailes, L. B. Hendricks, L. Pfeifer-Meister, P. B. Reed, S. D. Bridgham, B. R. Johnson, R. Shriver, E. Waddle, H. Wroton, D. F. Doak, B. A. Roy, W. F. Morris, Latitudinal gradients in population growth do not reflect demographic responses to climate. *Ecol. Appl.* **31**, e2242 (2021).
21. A. F. Agrawal, J. R. Stinchcombe, How much do genetic covariances alter the rate of adaptation? *Proc. Biol. Sci.* **276**, 1183–1191 (2009).
22. D. A. Roff, D. J. Fairbairn, The evolution of trade-offs under directional and correlational selection: Selection on a trade-off. *Evolution* **66**, 2461–2474 (2012).
23. S. G. D. Bishop, S.-M. Chang, R. S. Baucom, Not just flowering time: a resurrection approach shows floral attraction traits are changing over time. *Evol Lett* **7**, 88–98 (2023).
24. W.-K. Dai, G. M. Amboka, E. L. Kadiori, Q.-F. Wang, C.-F. Yang, Phenotypic plasticity of floral traits and pollination adaption in an alpine plant Pedicularis siphonantha D. Don when transplanted from higher to lower elevation in Eastern Himalaya. *J. Mt. Sci.* **14**, 1995–2002 (2017).
25. P.-O. Cheptou, E. Imbert, M. Thomann, Rapid evolution of selfing syndrome traits in Viola arvensis revealed by resurrection ecology. *Am. J. Bot.* **109**, 1838–1846 (2022).
26. V. P. Chand, A. Sukumaran, V. P. Khanduri, Reproductive phenology and floral visitors in Rhododendron arboreum (Ericaceae) in a temperate forest of Garhwal Himalaya. *Sci. Forum* **50** (2022).
27. N. T. Jones, B. C. Husband, A. S. MacDougall, Reproductive system of a mixed-mating plant responds to climate perturbation by increased selfing. *Proc. Biol. Sci.* **280**, 20131336 (2013).
28. K. S. Brown, C. M. Caruso, The effect of experimental pollinator decline on pollinator-mediated selection on floral traits. *Ecol. Evol.* **13**, e10706 (2023).

29. J. Freimuth, O. Bossdorf, J. F. Scheepens, F. M. Willems, Climate warming changes synchrony of plants and pollinators. *Proc. Biol. Sci.* **289**, 20212142 (2022).
30. R. Winfree, I. Bartomeus, D. P. Cariveau, Native Pollinators in Anthropogenic Habitats. *Annu. Rev. Ecol. Evol. Syst.* **42**, 1–22 (2011).
31. M. Thomann, E. Imbert, C. Devaux, P.-O. Cheptou, Flowering plants under global pollinator decline. *Trends Plant Sci.* **18**, 353–359 (2013).
32. C. A. Hallmann, M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, H. de Kroon, More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One* **12**, e0185809 (2017).
33. P. Soroye, T. Newbold, J. Kerr, Climate change contributes to widespread declines among bumble bees across continents. *Science* **367**, 685–688 (2020).
34. D. L. Byers, Studying plant-pollinator interactions in a changing climate: A review of approaches. *Appl. Plant Sci.* **5** (2017).
35. M. Gérard, M. Vanderplanck, T. Wood, D. Michez, Global warming and plant-pollinator mismatches. *Emerg Top Life Sci* **4**, 77–86 (2020).
36. S. S. Renner, C. M. Zohner, Climate Change and Phenological Mismatch in Trophic Interactions Among Plants, Insects, and Vertebrates. *Annu. Rev. Ecol. Evol. Syst.* **49**, 165–182 (2018).
37. N. C. Soares, P. K. Maruyama, V. G. Staggemeier, L. P. C. Morellato, M. S. Araújo, The role of individual variation in flowering and pollination in the reproductive success of a crepuscular buzz-pollinated plant. *Ann. Bot.* **127**, 213–222 (2021).
38. H.O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.), Climate change 2022: Impacts, adaptation, and vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. *IPCC 2022 Cambridge University Press,* 3056 pp (2022).
39. Y. García, B. S. Dow, A. L. Parachnowitsch, Water deficit changes patterns of selection on floral signals and nectar rewards in the common morning glory. *AoB Plants* **15**, lad061 (2023).
40. L. Galetto, G. Bernardello, Floral nectaries, nectar production dynamics and chemical composition in six ipomoea species (convolvulaceae) in relation to pollinators. *Ann. Bot.* **94**, 269–280 (2004).
41. D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
42. R Core Team, R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, (2024).
43. D. Nychka, S. Bandyopadhyay, D. Hammerling, F. Lindgren, S. Sain, A Multiresolution Gaussian Process Model for the Analysis of Large Spatial Datasets. *J. Comput. Graph. Stat.* **24**, 579–599 (2015).
44. K. Bollen, J. Pearl, Eight Myths About Causality and Structural Models, *Handbook of Causal Analysis for Social Research* **Chapter 15**, 301-328 (2013).
45. J. S. Lefcheck, piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.* **7**, 573–579 (2016).

46. Y. A. Wang, M. Rhemtulla, Power Analysis for Parameter Estimation in Structural Equation Modeling: A Discussion and Tutorial. *Advances in Methods and Practices in Psychological Science* **4**, 2515245920918253 (2021).
47. R. V. Lenth, Estimated Marginal Means, aka Least-Squares Means [R package emmeans version 1.11.1]. (2025).

Tables & Figures

	Year	Δz_i	SE	Δz_{nci}	SE	h^2	Trait Mean
Corolla Width (mm)	A	**0.02**	**1.86E-05**	**0.13**	**2.23E-05**	**0.11**	**48.0**
	D	**0.01**	**5.37E-06**	**0.09**	**1.54E-05**	**0.11**	**48.7**
Corolla Length (mm)	A	**0.01**	**1.70E-05**	**-0.07**	**1.36E-05**	**0.09**	53.4
	D	**0.01**	**4.50E-06**	**-0.07**	**1.12E-05**	**0.05**	53.4
ASD (mm)	A	-0.07	2.11E-05	-0.002	3.16E-07	**0.08**	0.37
	D	-0.02	7.14E-06	-0.01	2.13E-06	**0.09**	0.55
Nectar Sucrose (°Bx)	A	-0.006	1.88E-05	-0.04	8.77E-06	**0.12**	3
	D	0.004	4.76E-06	-0.005	1.07E-06	**0.11**	3
Flowering Phenology (Julian Date)	A	-0.14	1.91E-05	-0.07	8.33E-06	**0.35**	**218**
	D	**-0.03**	**7.11E-06**	**-0.05**	**4.02E-06**	**0.40**	**217.3**
Plant Size (Leaf Count)	A	0.19	3.32E-05	0.16	3.01E-05	**0.09**	**37.2**
	D	0.01	6.05E-06	-0.001	1.24E-07	**0.04**	**33**

Table 1. The adaptive potentials of floral traits are limited by trait-trait covariances. For each trait, the mean and standard error for Δz_i and Δz_{nci}, with those displaying significant evolutionary constraint marked in bold. Significance is defined as the percent of bootstrap samples where Δz_i - Δz_{nci} is either above or below zero, depending on the expected difference calculated from the actual data. Δz is calculated with standardized traits such that variance(maternal line) = 1. Significant broad sense heritabilities (h^2) for each trait are also shown in bold. Estimated marginal mean trait values are shown for corolla width, length and ASD in cm, nectar sucrose in °Bx, flowering phenology as Julian Date, plant size as leaf count, and relative fitness as seed count. Ancestral and descendent populations are noted as A or D, respectively. Bolded values represent traits with a significant change in trait value between ancestral and descendant populations (Table S3).

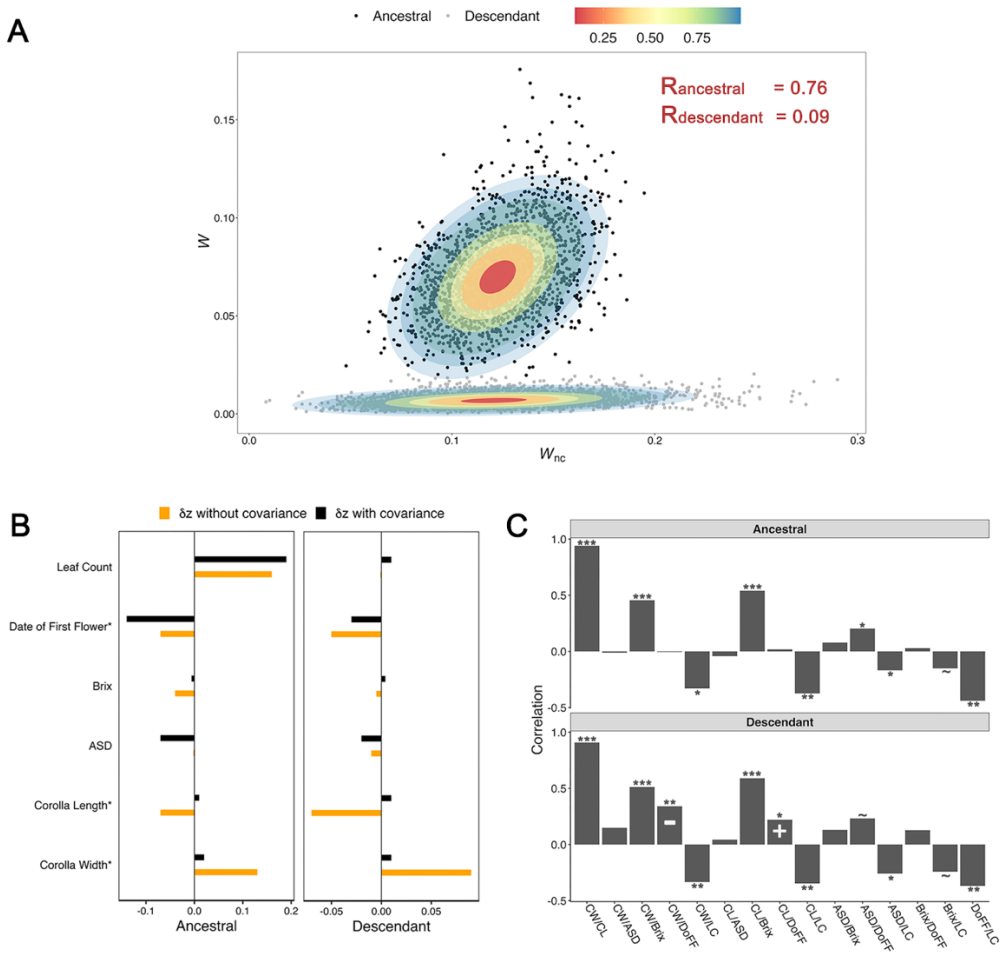

Figure 1. Adaptive potential decreases over time due to a trade-off between corolla size and flowering phenology. (A) Bootstrap estimates of ***R***, plotted as the two component parts of the ratio ΔWz and ΔWnc(z). Black points indicate resampled ***R*** estimates for ancestral populations, and grey points indicate resampled ***R*** estimates for descendant populations. Ellipses correspond to confidence intervals of 10, 34, 50, 68, 90, 95, and 98% with the respective color gradient ranging from red to light blue. Actual ***R*** estimates are reported in the top right. (**B)** The expected response to selection with (Δz_i) and without (Δz_{nci}) trait covariances (black and orange bars, respectively) for each trait. Traits with an asterisk had a significant difference between Δz_i and Δz_{nci} based on bootstrap resampling. **C)** Pairwise correlations between traits for both ancestral and descendant populations calculated as Pearson's correlation coefficient. Traits displaying a significant change in correlation over time based on bootstrap resampling are shown with a "-" indicating that the direction of joint selection is antagonistic to the direction of correlation or a "+" indicating that the direction of selection is reinforcing to the direction of trait correlation. Asterisks next to each bar indicate a significant correlation within year ($p < 0.1$~, $p < 0.05$*, $p < 0.01$**, and $p < 0.001$***).

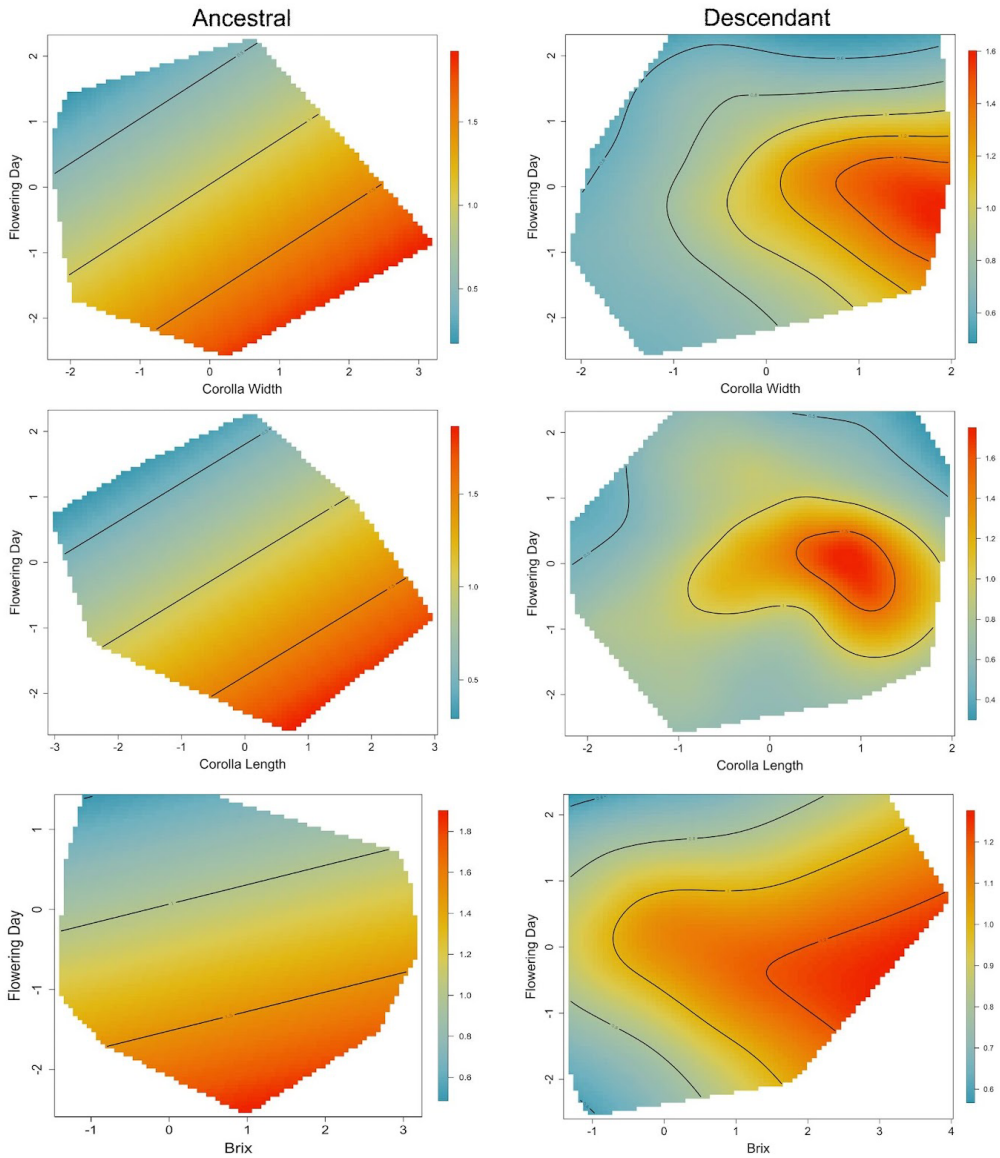

Figure 2. Correlational selection is present in descendent but not ancestral populations. Fitness surface for correlational selection in ancestral and descendant populations acting upon date of first flower and three other floral traits tested using selection gradients including all linear, non-linear, and interacting terms (multivariate selection). Relative fitness is depicted by the color gradient with red being the highest fitness, yellow intermediate fitness, and blue lowest fitness. In ancestral populations, no indication of correlational selection was present. All traits were included in the selection gradient, only those with selection gradients indicative of correlational selection in descendant populations are shown: corolla width ($\gamma = -0.34$, $p = 0.071$), corolla length ($\gamma = 0.39$, $p = 0.045$), and °Brix ($\gamma = -0.14$, $p = 0.067$).

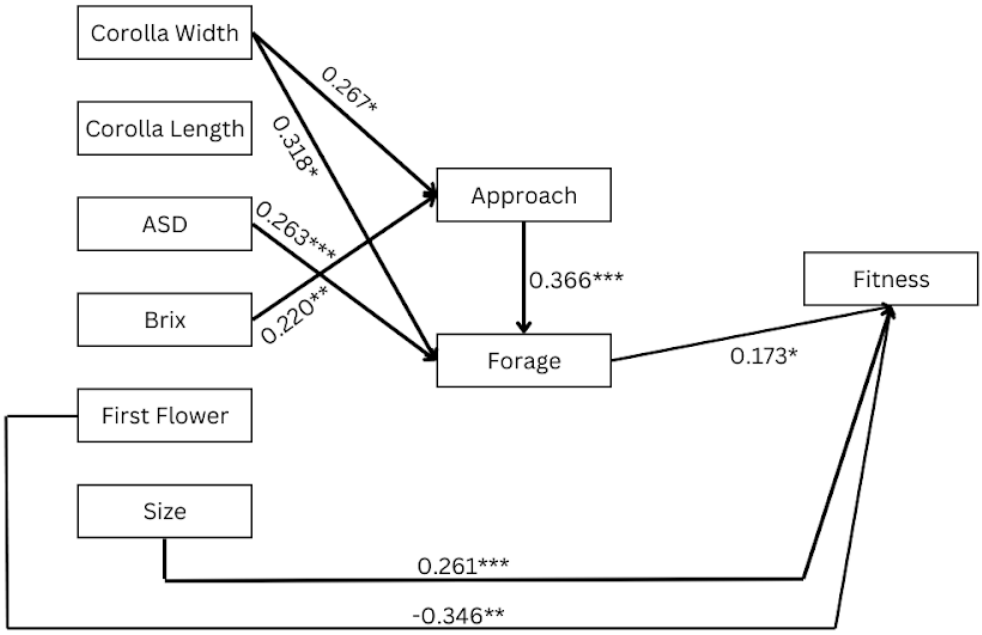

Figure 3. Pollinator foraging behavior mediates fitness effects of floral size and quality traits. Best-fit SEM showing the direct and indirect effects determining pollinator visitation rate and fitness. Standardized path coefficients are shown for each significant path where * indicates a p-value < 0.05, ** indicates p < 0.01, and *** indicates p < 0.001. See Table S6 for a full listing of direct, indirect, and total effect sizes of functional traits on fitness.