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Rapid evolution of a family-diagnostic trait: artificial selection and correlated responses in wild radish, *Raphanus raphanistrum*

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Summary

- The mechanisms underlying trait conservation over long evolutionary time scales are poorly known. These mechanisms fall into the two broad and nonmutually exclusive categories of constraint and selection. A variety of factors have been hypothesized to constrain trait evolution. Alternatively, selection can maintain similar trait values across many species if the causes of selection are also relatively conserved, while many sources of constraint may be overcome over longer periods of evolutionary divergence. An example of deep trait conservation is tetradynamy in the large family Brassicaceae, where the four medial stamens are longer than the two lateral stamens. Previous work has found selection to maintain this difference in lengths, which we call anther separation, in wild radish, *Raphanus raphanistrum*.
- Here, we test the constraint hypothesis using five generations of artificial selection to reduce anther separation in wild radish.
- We found a rapid linear response to this selection, with no evidence for depletion of genetic variation and correlated responses to this selection in only four of 15 other traits, suggesting a lack of strong constraint.
- Taken together, available evidence suggests that tetradynamy is likely to be conserved due to selection, but the function of this trait remains unclear.

Introduction

Evolutionary biologists appropriately focus on variation within and among populations and species, but similarities among organisms are also a fundamental feature of biological diversity. While convergent evolution among groups and divergence within groups are common, major clades of organisms across the tree of life share trait similarities that help to define them. These similarities result from slower evolution within taxonomic groups compared to the rate of divergence among groups for these traits.

Reduced rates of trait evolution can occur either through some type of constraint or through stabilizing selection maintaining the current phenotypic value of a trait. There is a large and often confusing list of possible sources of evolutionary constraint, including physical or chemical laws and developmental processes (Maynard Smith *et al*., 1985; Gould, 1989; Antonovics & van Tienderen, 1991). In a within-species analysis, all of these are encapsulated in the **G**-matrix of quantitative genetics, comprised of additive genetic variances and covariances of a set of phenotypic traits. Only traits with additive genetic variation can evolve, so a lack of this variance will constrain evolution. Genetic covariances between two traits that are both under selection can also cause constraint, provided that selection is in opposite directions with a positive covariance or the same direction with a negative covariance (Conner & Hartl, 2004, table 6.7). However, strong constraints due to covariance seem to be uncommon (Agrawal & Stinchcombe, 2009), except perhaps when selection is acting simultaneously on many traits that covary (Blows & Hoffmann, 2005). In addition, both theory (Via & Lande, 1985) and past artificial selection studies (e.g. Bell & Burris, 1973; Weber, 1990; Wilkinson, 1993; Stanton & Young, 1994; Emlen, 1996; Conner *et al*., 2011) suggest evolution can proceed even with constraint caused by covariances, and **G**-matrices are themselves often not conserved even among closely related species (Steppan *et al*., 2002).

Alternatively, stabilizing selection may act to maintain the trait in its current form. Constraint and selection are not mutually exclusive, and they may differ in their ability to explain a lack of variance at different levels in the biological hierarchy. For example, while the **G**-matrix might constrain evolution within a single population or among closely related species, longer-term stasis and traits broadly shared across deeper phylogenetic groups have been thought to be more likely due to stabilizing selection or some other form of constraint (Stearns, 1994). However, tests of selection and constraint for conserved traits are rare. A complete lack of genetic variation has been found for cotyledon number (Conner & Agrawal, 2005 and references therein), clearly a deeply conserved trait, but this study also reported evidence for selection against plants with zero or one cotyledon.

Tetradynamy is a phylogenetically conserved trait across most of the large and important family Brassicaceae. Almost all of the *c.* 3700 Brassicaceae species have four long medial stamens and two shorter lateral stamens (Barnes, 2001); this trait is diagnostic for the family (Zomlefer, 1994), but the functional significance of this arrangement has not been widely studied. The presence of short stamens increased the duration of pollinator visits in *Brassica rapa* (Kudo, 2003). Previous work on wild radish, *Raphanus raphanistrum*, has shown that the short stamen anthers produce more pollen, but that more pollen is removed from long stamen anthers in single pollinator visits (Conner *et al.*, 1995). We use the term anther separation for the quantitative distance between the short and long stamen anthers; see Waterman *et al.* (2022) for other terms that have been used. In wild radish, decreased anther separation decreases single-visit pollen removal (Conner *et al.*, 2003, 2009), demonstrating that the trait affects stamen function, and stabilizing, disruptive, and negative directional selection on this trait have been found in different years (Conner *et al.*, 2003; Sahli & Conner, 2011; Waterman *et al.*, 2022). Therefore, there is clearly selection on anther separation in wild radish, but how the four long and two short stamens function in pollination and how this function affects fitness remains unclear.

Genetic constraint maintaining tetradynamy in wild radish is possible due to the extremely strong additive genetic correlation between the short and long filaments ($r_A = 0.91$; Conner & Via, 1993) caused by pleiotropy or extremely tight linkage (Conner, 2002). However, genetic correlations are not good indicators of constraint (Conner, 2012), and a half-sibling analysis found significant additive genetic variance for anther separation, with heritability = 0.25 (Karoly & Conner, 2000). This previous analysis was a snapshot of one generation; in this paper, we report results of five generations of artificial selection to verify additive genetic variance for anther separation as well as test for depletion of that variation by strong selection. Perhaps the best method to test for short-term constraints is artificial selection, because if a trait responds to artificial selection, it clearly can evolve, but if the trait does not respond, there is a constraint caused by a lack of genetic variation (Conner, 2003).

Artificial selection is also the strongest test for the presence of additive genetic covariances between traits, because even weak covariances will cause correlated responses over several generations in other traits not under selection. Thus, we also tested for constraints on the evolution of anther separation caused by correlated responses to selection in 15 other traits, most of which we have also estimated selection on; this is important because constraints due to correlated traits depend on the presence and the direction of selection on those traits (Agrawal & Stinchcombe, 2009). The earlier studies testing constraint using artificial selection have not been on traits conserved over long evolutionary time scales (except Conner & Agrawal, 2005), have not selected perpendicular to a strong correlation known to be caused by pleiotropy (but see Conner *et al.*, 2011 on the related trait of anther exsertion in wild radish), nor have they measured correlated responses on traits with previous measures of natural selection; our study was designed to fill these gaps in our knowledge.

Materials and Methods

Artificial selection was conducted in two replicate glasshouse sites: Reed College in Oregon (Reed, Rep1) and Kellogg Biological Station (KBS, Rep2). The parental population for each replicate was formed by growing one plant from each of 209 randomly selected maternal plants from the Binghamton, NY population of *R. raphanistrum* L. (Conner & Via, 1993); the random sampling was done separately for each replicate. The 20 plants with the smallest difference in natural log-transformed short and long filament lengths were chosen to create the selection lines in each replicate, and 20 other plants were chosen randomly to create the control lines. Log transformation was used to avoid selecting for overall smaller flowers. This results in selection perpendicular to the major axis of the short vs long filament genetic correlation and is thus in the direction of minimum genetic variance in bivariate space. This is similar to previous artificial selection studies (see Bell & Burris, 1973; Weber, 1990; Wilkinson, 1993; Stanton & Young, 1994; Emlen, 1996; Conner *et al.*, 2011, fig. 1) except that here we only selected in one direction, toward the ancestral condition of six filaments of equal length (i.e. no tetradynamy).

The overall design thus consisted of 80 maternal families in total, 20 in each of the two selection lines and 20 in each of the two control lines (Supporting Information Fig. S1). Within each generation and line, plants were mated randomly, with each plant used once as a male (pollen donor) and once as a female (seed production) without reciprocal crosses. In each of the four subsequent generations, 2–10 offspring (mean 7.4) were successfully grown from each of the 80 families, and short and long filament lengths were measured on two flowers on each plant. One individual from each family was chosen for mating – individuals with the lowest anther separation were chosen from each family in the selection lines, and an individual was chosen at random from each family in the control lines. Random crosses were performed within each line as described for the parental generation. This family selection design with equal fitness for each plant maximizes the effective population size and thus minimizes drift (Hill, 1980).

Offspring from the fifth round of selection were grown in a replicated glasshouse common-garden design to test for direct and correlated responses to selection. Four plants from each family from both replicates of each selection line were grown in a completely randomized design in two blocks, one at Reed College and the other at KBS ($n = 320$ per block). Each plant was assigned a number that did not identify the selection line of origin, so measurements were done blind. A total of 3437 plants were grown and measured across the six generations of the study.

We measured 12 floral and four fitness-related traits on each plant in the common gardens to test for correlated responses, all of which have been shown to be related to pollination or fitness in multiple plant species: days until first flower, petal length and width (limb), lengths of the corolla tube (claw), pistil, long and short filaments, pollen grain number (one anther from each position), pollen viability, ovule number per flower, nectar volume, nectar concentration, % mortality of germinants (KBS block only), total flower number, and total aboveground dry weight (these last two at Reed only). The floral traits were measured on one flower per plant; two petals and two short and two long stamens were measured on each flower at Reed and the mean used in analyses; only one of each was measured at KBS. Two composite traits were also created – anther separation (long filament minus short filament length) and anther exsertion (long filament length minus corolla tube length). Pollen counts were done with a Coulter counter as described in Rush *et al.* (1995). Nectar volume was measured with microcapillary tubes and concentration with a refractometer; there was too little nectar to measure concentration in 135 plants. Pollen viability was measured at KBS only by intercrossing one plant per family (79 plants total); each plant was used to pollinate one flower on an average of 10.5 other plants in the set (831 total flowers pollinated) with a small amount of pollen (transferred with a pin) and counting the resulting seeds. Previous work has shown no difference in seed set in crosses using pollen from the short and long stamen anthers (J. K. Conner, unpublished). All data used in this paper are included in Datasets S1 and S2.

To test for the linearity of the selection response, response values were regressed on the cumulative selection differentials (Falconer & Mackay, 1996). To remove the effects of environmental differences among generations in this analysis, values of the response in the selection lines were subtracted from the control values within each replicate (Hill, 1980). Because the pairs of selection and control lines for each generation within each replicate were grown simultaneously in the same glasshouse room, they represent the total genetic response to selection.

We tested for direct and correlated responses to the artificial selection using mixed-model ANOVA for these 18 traits with treatment, replicate nested within treatment (because families were unique in each replicate), and block as fixed factors (except for the four traits measured in one block only); also, family was nested within replicate and treatment as a random factor (except for pollen viability that was measured on only one plant per family).

Results

Artificial selection caused rapid evolution of anther separation (Fig. 1). Because the control and selected lines were grown together each generation within each replicate, the mean differences are due to genetic differentiation caused by the artificial selection. Environmental differences in the glasshouse across generations and possibly genetic drift caused anther separation to increase in the replicate 2 control lines in the first two generations, but the difference between control and selected increased each generation in both replicates and was highly significant in the final common garden (Treatment $P < 0.0001$; Table 1, S1).

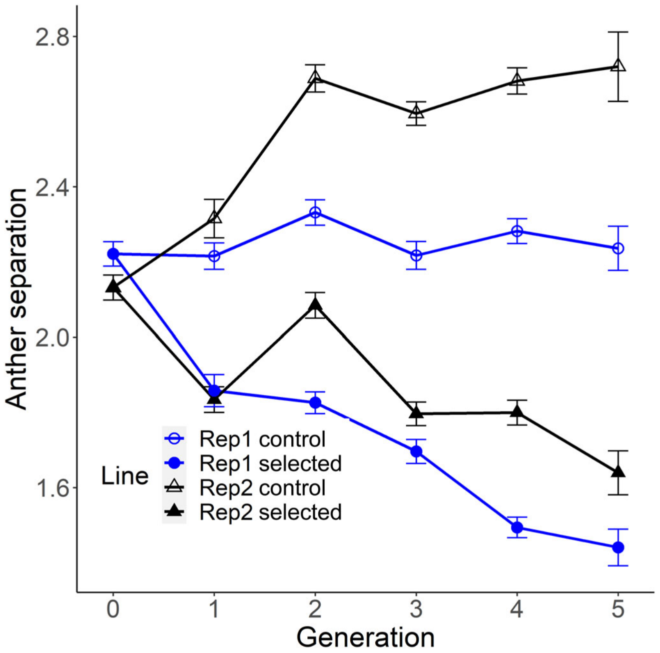

Fig. 1 Mean (±1 SE) anther separation across generations in the two lines selected for less anther separation (closed symbols) and the two randomly mated control lines (open symbols). *Raphanus raphanistrum* plants within each replicate and generation were grown simultaneously in the same glasshouse room, so differences between selected and control within replicate are due to evolved genetic differences. Each point is the mean of 209 plants in the parental generation (generation 0), 118–160 plants during generations 1–4, and 77–80 plants in generation 5. This last generation was the common-garden generation where all replicates were grown in both locations; to be consistent in this figure, only the plants that were grown at the location that the other generations were grown (Reed for Rep1 and KBS for Rep2) were plotted. Total $n = 3327$ plants (851 plants for Rep1 Selected, 893 for Rep2 Selected, 707 for Rep1 Control, and 876 for Rep2 Control).

These environmental differences across generations (but not differences due to genetic drift) can be removed by subtracting the control mean from the selected mean within each replicate and generation to produce the response to selection R. When R is regressed on the selection differential S summed across the generations, they reveal a very significant linear response to selection of very similar magnitude across the two replicates ($R^2 = 0.94$ and 0.99, both $P < 0.0001$; Fig. 2). The very high R^2 values for both replicates strongly suggest that drift was not a major contributor to the evolution of anther separation, because drift would have caused random fluctuations across the generations. The slope of these regressions are estimates of the heritability of filament length difference through a rearrangement of the breeder's equation (realized heritability; Falconer & Mackay, 1996). The estimates of heritability here are 0.35 and 0.37, somewhat higher than the previous heritability estimate of 0.25 (Karoly & Conner, 2000), which was from a half-sibling analysis on the base population used here. The highly linear response with very high R^2 values in both replicates strongly suggests no depletion of additive genetic variance over five generations of strong selection.

Plotting the individual family means in the glasshouse common gardens for each filament separately (Fig. 3) shows that the very similar response to selection for filament length difference in the two replicates occurred through different responses in each

Table 1 Summary statistics from the artificial selection.

	Rep1 control	Rep1 selected	Rep2 control	Rep2 selected
Cumulative *S* anther separation		−2.20		−2.55
LS mean anther separation	2.34 (0.06)	1.51 (0.06)	2.61 (0.06)	1.54 (0.06)
Cumulative *S* short filament		1.46		2.95
LS mean short filament length	8.92 (0.11)	8.86 (0.12)	9.10 (0.12)	10.17 (0.11)
Cumulative *S* long filament		−0.74		0.39
LS mean long filament length	11.26 (0.12)	10.38 (0.12)	11.71 (0.12)	11.71 (0.12)
Long vs short filament correlation	0.91 (0.78, 0.96)	0.93 (0.83, 0.97)	0.79 (0.53, 0.91)	0.86 (0.67, 0.94)

Cumulative *S* is the selection differential summed across all generations of selection. Least-squared means (SE; total $n = 630$) from the nested mixed model (see the Materials and Methods section) and correlations are from the final common-garden generation. Correlations are among the 20 full-sibling family means from each line and replicate in the common garden, so are estimates of the broad-sense genetic correlation r_G; 95% CI (in parentheses) are from the Z-transformation.

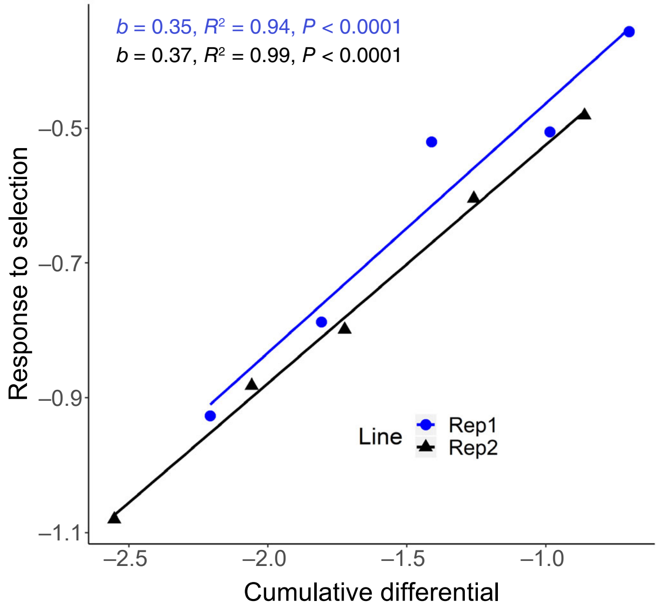

Fig. 2 Results of the *Raphanus raphanistrum* artificial selection experiment, plotted as the response of the composite trait (anther separation) regressed on cumulative selection differential. The slopes (*b*) represent the realized heritability of anther separation. Each point represents the difference in anther separation between the control and the selected lines (*R*) and the selection differential *S* summed over generations within each replicate. Due to the selection on separation being negative, generation 1 is in the upper right and generation 5 lower left in the figure. Because the control and the selected lines within each replicate were grown simultaneously in the same glasshouse room, differences in mean phenotype between them are due entirely to genetic change; by plotting these differences, phenotypic variation among generations that are caused by changes in the glasshouse environment over time are removed and the regressions reflect only true genetic evolution.

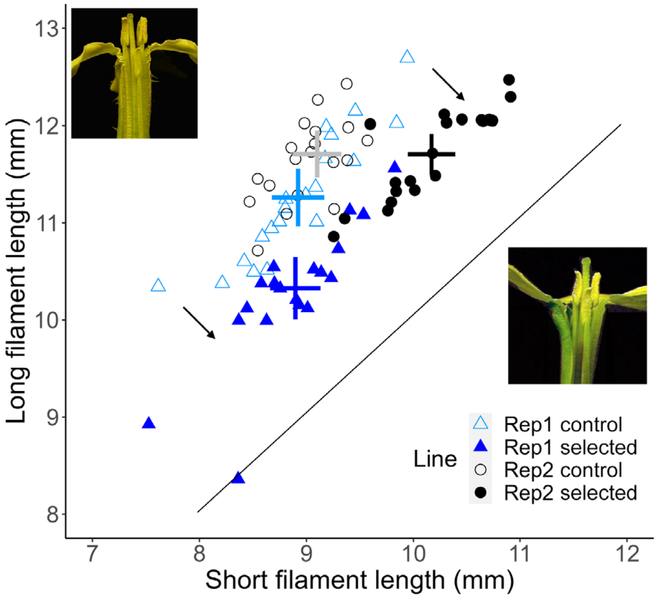

Fig. 3 Response to selection on anther separation of the individual traits short and long filament length. Each point represents the mean value for 8 *Raphanus raphanistrum* plants grown from each of the 80 families after five generations of selection. Two lines were selected for less separation and two were randomly mated controls. The crosses are the means and SE of each of the four lines in matching colors, with lighter tones for controls. The arrows depict the intended direction of the artificial selection perpendicular to the major axis of the correlation, and the line shows filaments of equal length. The pictures show examples of flowers from control (upper left) and selected (lower right) plants; these are side views with one petal and sepal removed so that the anthers of the short filament are clearly visible in the center.

component trait. In replicate 1, the long filament became shorter with little change in the mean short filament length (compare the blue crosses in Fig. 3), while in replicate 2, the short filament became longer with little change in the long filament (compare the gray and black crosses; also see least-squared means for the two filaments in Table 1). This matches the cumulative selection differentials on the individual traits for replicate 2 only, as most of the selection in replicate 2 was to increase the short filament length while there was selection in opposite directions on both filaments in replicate 1 (Table 1). These differences in selection on each filament resulted from chance differences in how selection on anther separation affected selection on each filament (cf. Conner, 1996). Over both replicates, the selected lines did diverge from controls along the minor axis of variation, in the direction of selection (denoted by arrows), but the selected lines also diverged from each other along the major axis of variation due to this difference in which filament responded in each replicate.

Fig. 3 and Table 1 also show that the difference between the two control replicates seen in Fig. 1 is halved when they are both

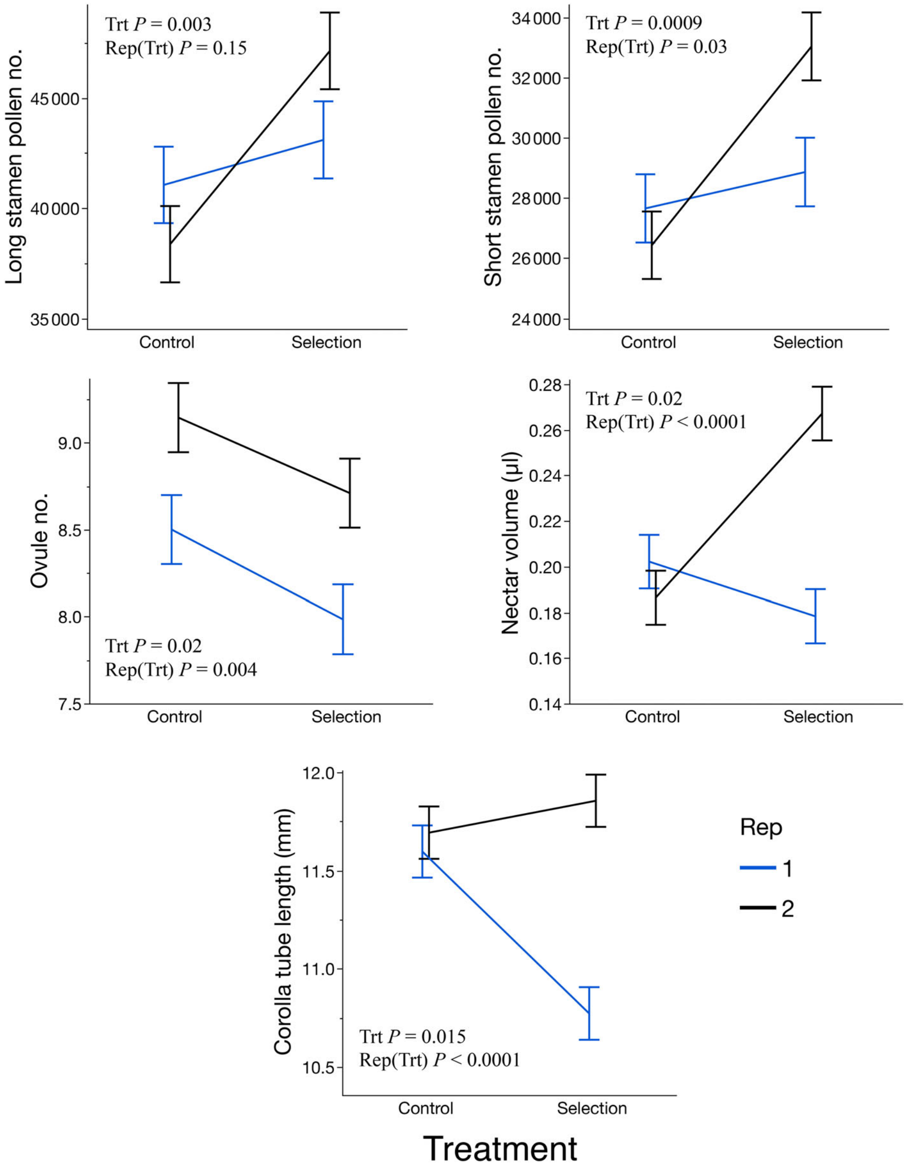

Fig. 4 Means (±SE) in each treatment and replicate for the five traits with significant correlated responses in *Raphanus raphanistrum* (see Supporting Information Table S1). As in all figures, replicate 1 is in blue and replicate 2 is in black. Significance values are shown for the treatment's main effect and replicate nested within treatment (because families were unique in each replicate).

grown in a common environment and that the artificial selection did not significantly change the correlation between the two filament lengths (highly overlapping 95% CI). This latter result matches theoretical expectations because we did not conduct correlational selection on the traits (Lande & Arnold, 1983), but rather conducted directional selection on the difference between the two traits, which caused evolution of the bivariate means.

Most of the correlated responses to our selection were not significant (Table S1). The correlated responses to selection that were significant (Fig. 4) suggest modest reallocation to male function, as long and short stamen anther pollen count increased, especially in replicate 2 where it increased by 24%, and ovule number decreased by about half an ovule on average in both replicates. Tube length decreased and nectar volume increased significantly due to the selection, but only in one of the replicates for each trait. The correlated response in nectar volume matches the patterns for short stamen evolution, with both decreasing slightly in replicate 1 and increasing strongly in replicate 2. This makes sense developmentally, because the main nectaries are located at the base of the short (lateral) stamens, and some have argued that together the short stamens and nectaries are in a separate floral whorl (reviewed in Nikolov, 2019).

Discussion

Our results provide little support for the hypothesis that long-term evolutionary constraint causes the conservation of tetradynamy across the Brassicaceae. Artificial selection produced a rapid response in anther separation, with only a few traits exhibiting correlated responses to this selection, suggesting a lack of strong constraint. Note that the genetic covariance between the short and long filaments certainly slowed the response, which would have been even faster without the covariance, but even with the covariance, there was adequate variation in anther separation for the rapid response. The linear response relative to selection (Fig. 2) demonstrates that additive genetic variance for anther separation was not depleted over five generations of strong selection. The lack of evidence for strong constraint on anther separation mirrors that of most artificial selection studies selecting perpendicular to the major axis of a covariance, that is, in the direction of minimum bivariate genetic variation (Bell & Burris, 1973; Weber, 1990; Wilkinson, 1993; Stanton & Young, 1994; Emlen, 1996; Conner *et al.*, 2011). However, few have selected against traits likely to be involved in a tradeoff; in a notable exception, Dorn & Mitchell-Olds (1991) were able to select for taller and earlier flowering plants in one generation. Another case where covariances might cause significant constraint is whether there are dimensions in multivariate space that lack variation (Blows & Hoffmann, 2005), although one artificial selection study found limited evidence for this (Hine *et al.*, 2014).

We have found evidence for natural selection in the field acting on three of the four traits showing significant correlated responses, but only one of these would likely cause additional constraint. We have shown significant selection for increased pollen and ovule number in two out of five field seasons (pollen number on both short and long stamens together; Conner *et al.*, 1996; Morgan & Conner, 2001; Waterman *et al.*, 2022), so the increase in pollen number due to the correlated response would augment rather than constrain the reduction in anther separation (Conner & Hartl, 2004; Agrawal & Stinchcombe, 2009). It is possible that the decrease of about a half an ovule in our selection lines (Table S1) could cause some constraint on the evolution of reduced anther separation in nature. However, given theory (Via & Lande, 1985) and the empirical results from our study and several others (see the Introduction section and above) that found rapid evolution perpendicular to genetic correlations, this would only slow the evolution of reduced anther separation, not prevent it. Selection on nectar volume has not been estimated in wild radish, but it seems more likely that the increased volume seen in replicate 2 would increase rather than decrease fitness and thus not constrain anther separation evolution. It is possible that unmeasured traits that are genetically correlated with anther separation and themselves under selection could cause constraint, but this explanation seems unlikely given the overall low level of correlated responses with other measured traits (Table S1). This low correlation with anther separation is due to the removal of overall flower size in this trait by subtracting the two filament lengths. While we only measured one vegetative trait, the low correlations between floral and vegetative traits in wild radish (Conner & Via, 1993; cf. Berg, 1960) make correlated responses from unmeasured vegetative traits less likely as well.

We have shown significant stabilizing (Conner *et al.*, 2003) and disruptive (Waterman *et al.*, 2022) selection on anther separation, both of which would maintain the current mean, but no evidence for directional selection that would alter the difference in stamen lengths. We are not aware of selection estimates on anther separation in species other than wild radish, so it is unclear how much this explanation holds across the family, but many Brassicaceae have generalized pollination similar to wild radish (fig. S2 in Gómez *et al.*, 2022). *Stanleya pinnata* has secondarily lost tetradynamy, which could have been caused by selection for its extreme anther exsertion (anthers far outside the corolla; Conner, 2006). Regardless of the mechanism, the secondary loss of tetradynamy in *Stanleya* and some other Brassicaceae is evidence for a lack of strong constraint. Thus, while the function of tetradynamy remains elusive (see the Introduction section; Waterman *et al.*, 2022), the available evidence suggests that while constraint could play a role, tetradynamy may be primarily maintained by selection. Studies of selection on anther separation in species other than wild radish as well as phylogenetic studies across the family testing for a correlation between the degree of anther separation, mating system, and effective pollinator taxa should be illuminating.

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Competing interests

None declared.

Author contributions

JKC and KK designed the research with input from FK, VAK and HS. JKC, KK, FK, VAK and HS performed the research and collected data. JKC, OIS and Z-GZ analyzed the data. JKC and OIS wrote the manuscript.

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Data availability

Data that support this study are openly available in Supporting Information.

References

Agrawal AF, Stinchcombe JR. 2009. How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences* **276**: 1183–1191.

Antonovics J, van Tienderen PH. 1991. Ontoecogenophyloconstraints? The chaos of constraint terminology. *Trends in Ecology & Evolution* **6**: 166–168.

Barnes MG. 2001. *Development and evolution of the tetradynamous stamen condition in the Brassicaceae.* Portland, OR, USA: Biology, Reed College, 69.

Bell AE, Burris MJ. 1973. Simultaneous selection for two correlated traits in *Tribolium. Genetics Research* **21**: 29–46.

Berg RL. 1960. The ecological significance of correlation pleiades. *Evolution* **14**: 171–180.

Blows MW, Hoffmann AA. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* **86**: 1371–1384.

Conner J, Via S. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* **47**: 704–711.

Conner JK. 1996. Understanding natural selection: an approach integrating selection gradients, multiplicative fitness components, and path analysis. *Ethology Ecology & Evolution* **8**: 387–397.

Conner JK. 2002. Genetic mechanisms of floral trait correlations in a natural population. *Nature* **420**: 407–410.

Conner JK. 2003. Artificial selection: a powerful tool for ecologists. *Ecology* **84**: 1650–1660.

Conner JK. 2006. Ecological genetics of floral evolution. In: Harder LD, Barrett SCH, eds. *Ecology and evolution of flowers*. New York, NY, USA: Oxford University Press, 260–277.

Conner JK. 2012. Quantitative genetic approaches to evolutionary constraint: how useful? *Evolution* **66**: 3313–3320.

Conner JK, Agrawal AA. 2005. Mechanisms of constraints: the contributions of selection and genetic variance to the maintenance of cotyledon number in wild radish. *Journal of Evolutionary Biology* **18**: 238–242.

Conner JK, Davis R, Rush S. 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* **104**: 234–245.

Conner JK, Hartl DL. 2004. *A primer of ecological genetics*. Sunderland, MA, USA: Sinauer Associates.

Conner JK, Karoly K, Stewart C, Koelling VA, Sahli HF, Shaw FH. 2011. Rapid independent trait evolution despite a strong pleiotropic genetic correlation. *American Naturalist* **178**: 429–441.

Conner JK, Rice AM, Stewart C, Morgan MT. 2003. Patterns and mechanisms of selection on a family-diagnostic trait: evidence from experimental manipulation and lifetime fitness selection gradients. *Evolution* **57**: 480–486.

Conner JK, Rush S, Jennetten P. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution* **50**: 1127–1136.

Conner JK, Sahli HF, Karoly K. 2009. Tests of adaptation: functional studies of pollen removal and estimates of natural selection on anther position in wild radish. *Annals of Botany* **103**: 1547–1556.

Dorn LA, Mitchell-Olds T. 1991. Genetics of *Brassica campestris*. 1. Genetic constraints on evolution of life-history characters. *Evolution* **45**: 371–379.

Emlen DJ. 1996. Artificial selection on horn length-body size allometry in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Evolution* **50**: 1219–1230.

Falconer DS, Mackay TFC. 1996. *Introduction to quantitative genetics*. Harlow, UK: Longman.

Gómez JM, González-Megias A, Narbona E, Navarro L, Perfectti F, Armas C. 2022. Phenotypic plasticity guides *Moricandia arvensis* divergence and convergence across the Brassicaceae floral morphospace. *New Phytologist* **233**: 1479–1493.

Gould SJ. 1989. A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. *Evolution* **43**: 516–539.

Hill WG. 1980. Design of quantitative genetic selection experiments. In: Robertson A, ed. *Selection experiments in laboratory and domestic animals*. Slough, UK: Commonwealth Agricultural Bureau, 1–13.

Hine E, McGuigan K, Blows MW. 2014. Evolutionary constraints in high-dimensional trait sets. *The American Naturalist* **184**: 119–131.

Karoly K, Conner JK. 2000. Heritable variation in a family-diagnostic trait. *Evolution* **54**: 1433–1438.

Kudo G. 2003. Anther arrangement influences pollen deposition and removal in hermaphrodite flowers. *Functional Ecology* **17**: 349–355.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Maynard Smith J, Burian R, Kauffman S, Alberch P, Campell J, Goodwin B, Lande R, Raup D, Wolpert L. 1985. Developmental constraints and evolution. *Quarterly Review of Biology* **60**: 265–287.

Morgan MT, Conner JK. 2001. Using genetic markers to directly estimate male selection gradients. *Evolution* **55**: 272–281.

Nikolov LA. 2019. Brassicaceae flowers: diversity amid uniformity. *Journal of Experimental Botany* **70**: 2623–2635.

Rush S, Conner JK, Jennetten P. 1995. The effects of natural variation in pollinator visitation on rates of pollen removal in wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* **82**: 1522–1526.

Sahli HF, Conner JK. 2011. Testing for conflicting and non-additive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution* **65**: 1457–1473.

Stanton M, Young HJ. 1994. Selecting for floral character associations in wild radish, *Raphanus sativus* L. *Journal of Evolutionary Biology* **7**: 271–285.

Stearns SC. 1994. The evolutionary links between fixed and variable traits. *Acta Palaeontologica Polonica* **38**: 215–232.

Steppan SJ, Phillips PC, Houle D. 2002. Comparative quantitative genetics: evolution of the G matrix. *Trends in Ecology & Evolution* **17**: 320–327.

Via S, Lande R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 505–522.

Waterman R, Sahli H, Koelling VA, Karoly K, Conner JK. 2022. Strong evidence for positive and negative correlational selection revealed by recreating ancestral variation. *Evolution* **77**: 264–275.

Weber KE. 1990. Selection on wing allometry in *Drosophila melanogaster*. *Genetics* **126**: 975–989.

Wilkinson GS. 1993. Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera, Diopsidae). *Genetical Research* **62**: 213–222.

Zomlefer WR. 1994. *Guide to flowering plant families*. Chapel Hill, NC, USA: University of North Carolina Press.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Filament lengths measured in each generation of all four selection lines as well as raw and relative fitness data for selected individuals in the selection lines.

Dataset S2 Data collected from the final glasshouse common-garden generation; see the Materials and Methods section for details.

Fig. S1 Schematic of the full experimental design.

Table S1 Means of all traits measured in the final common garden.