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What determines the evolutionary trajectories of wild plant species? Approaches to the study of quantitative fitness-related traits

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Abstract

Wild plant species provide excellent examples of qualitative traits that evolve in response to environmental challenges (e.g., flower color, heavy metal tolerance, cyanogenesis, and male sterility). In addition to such discrete characters, a dazzling array of continuously distributed, quantitative traits are expressed at every phase of the life cycle. These traits are known or suspected to have evolved by natural selection because they are heritable, differ among populations or closely related taxa occupying distinct habitats, and have individual phenotypes associated with survival and reproductive success. This special issue [*American Journal of Botany* 109(11)] focuses on the tools and approaches for detecting or inferring the ecological and genetic factors contributing to changes in genetically based variation of quantitative traits within or among populations, or causing their divergence among taxa. The assembled articles use one or more of three primary approaches to detect the process or outcome of natural selection on morphological, life history, reproductive, chemical, and physiological quantitative traits: the analysis of phenotypic or artificially imposed selection to detect direct and indirect selection on traits whose function is well-understood; common garden experiments, including reciprocal transplants and "resurrection" experiments; and quantitative genetic analyses designed to detect and to estimate the environmental and genetic sources of phenotypic variation or to forecast short-term evolutionary change. Together, these articles examine and reveal the adaptive capacity of quantitative traits and the genetically based constraints that may limit their directional evolutionary change, thereby informing and testing inferences, hypotheses, and predictions concerning the evolutionary trajectories of wild plant species.

KEYWORDS
Adaptation, adaptive capacity, artificial selection, correlated selection, functional traits, genetic variance, genotype by environment interaction, mating system evolution, phenology, phenotypic plasticity, phenotypic selection, quantitative genetics

The process of adaptive evolution depends on a variety of population-level parameters that are hard to measure and that may interact in idiosyncratic ways that accelerate or constrain adaptation. These parameters include the heritabilities of and genetically based correlations between fitness-related traits, the heritability of fitness itself, the covariance between individual phenotype and fitness, and estimates of the effects of biotic and abiotic conditions on each of these parameters within and among wild populations. Not surprisingly, there are few wild species in which all of these parameters are well understood for even a few traits and populations.

Within populations, genetic variation in fitness drives natural selection and the pace of local adaptation (Falconer and Mackay, 1996; Shaw and Etterson, 2012; Shaw and Shaw, 2013; Shaw, 2019). The estimation of individual fitness and its genetic basis in wild and experimental populations is therefore central to studies designed to detect the opportunity for selection to increase population mean fitness and, ideally, to predict values of mean fitness in the future by applying Fisher's fundamental theorem of natural selection (Fisher, 1930; Kulbaba et al., 2019; Shaw, 2019). Estimating the fitness of individuals and genotypes is also fundamental to studies of the evolutionary consequences of

heritable variation in traits other than fitness itself. Given that individual fitness and many of the ecological and functional traits that contribute to it exhibit continuous frequency distributions, the tools of quantitative genetics—the study of traits whose phenotypes are continuously distributed and influenced by multiple genes each of relatively small effect—are uniquely well suited to the study of the process and outcome of natural selection in wild and experimental populations. Plants are especially amenable to the study of local adaptation and patterns of selection in quantitative traits because their (sessile) populations can be monitored and manipulated to estimate the genetic basis of phenotypic variation and to detect and to measure both biotic and abiotic drivers of selection (Anderson et al., 2011), including stressors such as drought, which are intensifying due to climate change (e.g., Franks et al., 2007).

The joint estimation of the heritabilities of morphological, life history, and physiological traits; the genetically based correlations among them (one component of genetic architecture); and their quantitative effects on survival and fecundity (through the application of selection gradient analyses) can be used in two primary ways. First, these parameters are essential for predicting the direction and rate of phenotypic change in traits of interest between generations in populations occupying stable environmental conditions. Second, they inform inferences concerning the selective mechanisms that drive phenotypic divergence between populations or closely related higher taxa. In sum, measurements of the heritability of fitness-related traits, their genetic architecture, and the genetic basis of fitness itself are key to predicting their evolution.

Plant evolutionary ecologists have long recognized that many fitness-related traits such as seed size (Mazer, 1987), germination time (Kalisz, 1986; Stratton, 1992), flower size (Gilbert et al., 1996), anther dimorphism (Conner et al., 2003), flowering time (Stanton et al., 2000; Munguía-Rosas et al., 2011), leaf size and plasticity (Tucic et al, 1998), specific leaf area (Steinger et al., 2003), leaf hair density (Roy et al., 1999), and many other traits are continuously distributed and often correlated with other functionally important traits, calling for the use of multivariate analyses to detect and to measure their direct, indirect, and at times environment-dependent effects on fitness (Rausher, 1992; Scheiner et al., 2002). This fascination with the evolutionary significance of variation in quantitative traits continues to blossom, leading to new discoveries about the evolutionary process in plants that we are delighted to highlight in this special issue of the *American Journal of Botany*. The papers assembled here report recent research designed to measure or to anticipate the outcome of historical and ongoing natural selection on ecologically important traits within and among wild populations and species of plants.

Natural selection has captivated the interest and imagination of biologists ever since Charles Darwin and Alfred Russel Wallace recognized its ubiquity and its role in generating and maintaining biological diversity. Today, however, given the detrimental effects of urbanization, development, extreme weather events, wildfires, and a rapidly changing climate on wild species, the study of local adaptation (and phenotypic plasticity) takes on a pressing urgency for two reasons. First, given that wild species evolved for a much longer period in the absence of anthropogenic disturbance than in its presence, for most species, we are unable to study the evolutionary process under the (human-free) conditions in which most of their evolution has occurred. In other words, the window of opportunity required to characterize—under relatively pristine conditions—the direction and strength of selection on the traits and the types of species that are most frequently targeted by selection is rapidly closing. Second, to predict whether and how wild populations will adapt to rapid climate change requires understanding the effects of environmental stressors on the process of, and the evolutionary response to, selection (Franks et al., 2007; Atkins and Travis, 2010; Shaw and Etterson, 2012; Aitken and Whitlock, 2013; Anderson and Wadgymar, 2020; Lortie and Hierro, 2022). At a time when many educational institutions are eliminating field courses due to the high cost of teaching time-intensive (and necessarily low-enrollment) courses, we risk failing to educate the next generation of biologists on the use of the tools of quantitative genetics to understand how natural selection operates in the wild.

We designed this special issue to showcase studies of the approaches being used by plant biologists to develop and to test predictions concerning the process of natural selection and its outcome. The papers assembled here represent three primary approaches used to reveal the causes of variation in fitness-related traits and their influence on components of fitness (survivorship and fecundity): the analysis of phenotypic or artificially imposed selection; common garden experiments, including reciprocal transplants and "resurrection" experiments; and quantitative genetic analyses designed to detect and to estimate the environmental and genetic sources of phenotypic variation. To the credit of the contributing authors, many of the papers in this special issue encompass more than one of these approaches. Consequently, we recognize that the classification of the papers in the following summaries is, in some cases, a bit forced or regrettably narrow.

MANIPULATIVE AND OBSERVATIONAL STUDIES OF PHENOTYPIC SELECTION

Phenotypic selection gradient analysis is a statistical method based on linear regression that estimates the independent, direct effects on fitness of variation among individuals or genotypes in multiple traits (i.e., their phenotypes) and each trait's indirect effect on fitness due to its potential genetically based correlations with other traits that directly affect fitness (Lande and Arnold, 1983). This approach also

allows the inclusion of extrinsic factors as predictors of fitness, such as population, environmental treatment, or interactions between any of the intrinsic or extrinsic predictors. It also permits the detection and measurement of the three distinct modes of selection on individual traits: linear, stabilizing (or optimizing), and disruptive selection. In this issue, many of the investigators used selection analysis to examine or to infer the process of selection on traits such as germination time (Muir et al., 2022), flowering phenology (Johnson et al., 2022, MacTavish and Anderson, 2022, Valdés et al., 2022), flower size and height (Chen and Pannell, 2022), plant size at flowering (MacTavish and Anderson, 2022), specific leaf area and plant height (Waterton et al., 2022), and physiological performance (Mazer et al., 2022; see also Johnson et al., 2022).

When the relationships between individual phenotype and fitness are measured under alternative, controlled environmental conditions, we can glean how the direction and strength of natural selection on specific traits may be affected by local environmental conditions. Such context-dependent selection provides a mechanism for population and species divergence, and, ultimately, taxonomic diversity. In wild populations, however, environmental manipulations are not always practical or permitted; in this case, phenotypic selection gradient analyses may be limited to observations of individuals or genotypes as they naturally occur. This constraint precludes the unambiguous identification of the causal environmental factor(s) that generates geographic or temporal variation in the strength or direction of selection on the focal traits. One way to overcome this limitation, however, is to compare patterns of phenotypic selection in multiple populations of closely related taxa that are exposed to different environmental conditions. If the phenotypic divergence between taxa is consistent with differences between them in the direction of selection observed on the focal traits, then this concordance corroborates the hypothesis that the genetically based differences between the taxa are the result of selection operating differently in the conditions that they chronically occupy.

Johnson et al. (2022) used a resurrection approach along with an experimental drought treatment to compare an ancestral population of rapid-cycling *Brassica rapa* (Brassicaceae) to descendant populations that each experienced four generations of experimentally induced moist vs. dry conditions and were then grown concurrently in both watered and dry conditions. The resulting comparisons were used to assess short-term evolutionary change, phenotypic plasticity, and adaptation with respect to flowering time. They hypothesized that even though the accession of *B. rapa* used in this study had been bred for early flowering and a rapid life cycle, the ancestral population may still harbor sufficient genetic variation to allow the evolution of even earlier flowering time in response to exposure to drought. Their comparison of the post-selection populations corroborated the prediction that drought induced the evolution of earlier flowering and

lower water-use efficiency relative to watered conditions, consistent with the interpretation that in this species, drought imposes effective selection for traits that promote escape from drought.

Valdés et al. (2022) investigated whether mean flowering time, mean fitness, and the pattern of selection on flowering time in the carnivorous common butterwort, *Pinguicula vulgaris* (Lentibulariaceae), are influenced by geothermally heated local soil temperature, experimentally manipulated pollination intensity, and prey availability. In addition, they assessed whether the plastic response of flowering time to environmental conditions was maladaptive based on the environment-specific patterns of selection on this trait. They found that higher soil temperature induced earlier flowering and resulted in lower mean fitness (in the absence of supplemental pollination) in spite of the observation that phenotypic selection favored late-flowering individuals in heated soil and early-flowering individuals in unheated soil. In pollen-supplemented plants, however, mean fitness increased with soil temperature. The combination of examining the responses of both mean phenotype and the direction of selection to elevated soil temperatures supported the interpretation that the observed phenological response to elevated soil temperatures is maladaptive in this system. In addition, the authors demonstrated that pollen availability rather than resource (prey) availability mediates the pattern of selection on flowering time in this environment. This study provides an excellent case study of the way in which geothermally heated environments may generate maladaptive phenological mismatches between (sessile) plant populations and their volant insect pollinators.

MacTavish and Anderson (2022) performed a greenhouse-based selection experiment using 35 accessions of the perennial mustard *Boechera stricta* (Brassicaceae) derived from populations sampled across an elevational gradient in the Rocky Mountains (USA) and cultivated under six combinations of water and nutrient availability. Within each treatment combination, they measured lifetime seed production, the onset of flowering and its duration, and plant size and the number of leaves borne at first flower, and then estimated the selection coefficients for, and the genetic correlations between, these traits. They found that some patterns of selection were consistent across treatments; early and longer flowering, and greater height at flowering were associated with relatively high fitness in most conditions. Environment-specific selection, however, was also observed for some traits; for example, selection favored low leaf production (at first flower) under drought conditions but high leaf production when water was not limiting. By using an experimental population comprised of a wide range of genotypes sampled from and potentially adapted to different conditions in the wild, the authors were also able to detect evidence of local adaptation among the low-elevation genotypes, which (in nature) are exposed to greater drought stress than those sampled from high-elevation populations. In this experiment, genotypes sampled from low-elevation populations exhibited higher fitness under drought than

genotypes sampled from high-elevation populations. This kind of multifactorial design exemplifies how replicating a genetically diverse population under alternative treatment combinations and examining phenotypic selection in each one can reveal the direct effects of single environmental factors on the direction and strength of natural selection on traits for which the fitness consequences may well be altered by upcoming climate change.

Chen and Pannell (2022) investigated context-specific selection on the height at which flowers are borne on inflorescences of the andromonoecious alpine anemone, *Pulsatilla alpina* (Ranunculaceae). While inflorescence height has been studied previously in the context of its positive effects on the dispersal of pollen and seeds under wind dispersal, its effects on fitness in populations of animal-pollinated and animal-dispersed plants is less understood. The authors detected disruptive selection on inflorescence height when estimating its effects on three events that influence maternal seed production: fertilization, seed predation, and rates of seed maturation. However, when fitness was estimated by seed production itself, total selection on inflorescence height was directional, favoring individuals with greater inflorescence height, indicating that the mode of selection on proxies for maternal reproductive success may differ from the mode of selection directly on fecundity. Inflorescence height was also sensitive to resource depletion; experimental leaf removal reduced inflorescence height, suggesting that tall inflorescences are costly to produce. These and other results from this work suggest that inflorescence height is driven mainly by its effects on female function, but also that it is highly plastic and that its contribution to fitness may be influenced by the responses of both pollinators and predators.

In another study that revealed how biotic interactions may influence developmental phases that contribute to maternal fitness, Cardel and Koptur (2022) conducted a greenhouse experiment to measure the effects of self- and cross-pollination and simulated herbivory on ovule abortion and seed quality in the perennial legume *Centrosema virginianum* (Fabaceae). They found that self-pollinated flowers and defoliated plants had higher rates of ovule abortion than cross-pollinated flowers and non-defoliated plants, respectively. Moreover, there were strong positional effects within fruits that influenced the likelihood of ovule abortion, and these positional effects were mediated by the type of pollination and the degree of defoliation. Within developing legumes, basal ovules were more likely to abort than distal ovules, and this positional effect was stronger in self-pollinated flowers and in plants with high levels of defoliation. The advantages of cross pollination and the avoidance of herbivory were also evident for seed mass. Although Cardel and Koptur's experiment did not include measures of phenotypic selection on the intrinsic plant or floral traits that may directly affect ovule abortion and seed quality, their results echo those of Chen and Pannell in that the traits of *C. virginianum* that influence pollinator visitation or behavior (and consequently the deposition

of self vs. outcross pollen) or herbivore attraction (and subsequent defoliation) will likely have direct effects on several key components of maternal reproductive success, including seed maturation rates, fecundity, and seed size.

In another experimental approach designed to determine how and whether patterns of selection on functional traits are context-specific, Waterton et al. (2022) investigated the effects of nitrogen (N) enrichment on phenotypic selection in replicated experimental populations of the invasive annual grass *Setaria faberi* (giant foxtail; Poaceae). This study is particularly interesting given that anthropogenic N supplementation can alter both biotic and abiotic conditions through its effects on soil nutrients, the density and height of ground cover (and therefore the light received by plants), and community composition. Waterton et al. took advantage of a long-term N enrichment experiment at the Kellogg Biological Station Long-term Ecological Research Site to measure the effect of N on the direction and strength of selection on two traits influencing light acquisition: plant height and specific leaf area (SLA). They hypothesized that N enrichment would intensify directional selection on attributes that promote light acquisition (such as greater height or relatively high SLA). In addition to measuring phenotypic selection in the giant foxtail population in each N treatment, they measured properties of the vegetation in which these focal populations were embedded to identify the community-level features that may have contributed to context-specific patterns of selection. As expected, N enrichment increased primary productivity and light asymmetry, and lowered species diversity. Contrary to their predictions, however, N enrichment did not influence the opportunity for selection in giant foxtail by altering the variance in relative fitness, nor did it strengthen selection for increased height. Perhaps most surprising, the addition of N intensified selection favoring *lower* SLA, making one wonder whether N addition may generally disrupt ecological and evolutionary processes in ways that we have not yet anticipated.

Mazer et al. (2022) measured phenotypic selection in multiple field populations of four taxa to determine whether the physiological divergence observed between taxa was consistent with the direction of selection on these traits. They compared the strength and the direction of selection on gas exchange traits in each of two pairs of winter annual sister taxa in *Clarkia* (farewell-to-spring; Onagraceae) in which the members of each pair differ in mating system and gas exchange rates. In each pair of sister taxa, the autogamous selfers flower earlier (independent of variation in local climatic conditions, revealed by their analysis of herbarium specimens) and generally exhibit higher rates of photosynthesis and transpiration than their outcrossing counterparts. Mazer et al. hypothesized that the selection gradients observed in natural populations for these traits and for instantaneous WUE would be consistent with the phenotypic differences observed between sister taxa. Concordance with this prediction, however, was highly context-specific; patterns of selection on the physiological

traits were consistent with the direction of phenotypic divergence observed between sister taxa in just one third of the 18 comparisons conducted. Moreover, the observed patterns of selection (and the concordance between the directions of selection and divergence between sister taxa in each trait) depended on whether individuals were measured for gas exchange rates during vegetative growth (when soils were still relatively moist and temperatures cool in early spring) or during flowering (when soil moisture was depleted and temperatures rose during late spring). These results underscore the need for replication over time and among populations when investigating patterns of selection on traits known to be highly plastic, such as gas exchange rates and instantaneous WUE.

COMMON GARDEN AND RECIPROCAL TRANSPLANT EXPERIMENTS

Common garden experiments are often used to detect genetic differentiation in fitness-related traits and/or evidence of local adaptation by comparing genetically distinct populations or higher taxa grown under the same conditions (Mazer and Lebuhn, 1999; Cheplick, 2015). Such experiments are a rich source of evolutionary insights as they permit the partitioning of phenotypic variance in fitness-related traits among genotypes, among populations or higher taxa, and among environmental conditions. The gold standard for common garden studies designed to detect local adaptation is a reciprocal transplant experiment in which genotypes sampled from distinct populations are reciprocally raised in both their own "home" environment (or in conditions that simulate it) and in the natural or simulated environments of alternative populations. A history of local adaptation is then inferred where the mean fitness of genotypes in their home environments consistently exceeds that observed when they are grown in novel or "foreign" environments. In this special issue, one of the most exciting elements of the studies that used common gardens was to see this approach combined with analyses of the heritabilities of the focal traits and/or estimates of selection to test hypotheses or provide inferences concerning the evolutionary history or the evolutionary potential of the focal species or traits.

In this issue, common gardens or reciprocal transplants were used to examine a broad array of fitness-related traits, including floral scent (Eisen et al., 2022; Campbell et al., 2022a), SLA, WUE, trichome density, and floral morphology (Campbell, 2022a), germination time (Muir et al., 2022), flowering time (Cheptou et al., 2022), and physiological traits (Volk et al., 2022). Campbell et al. (2022a) combined variance component analysis with a common garden experiment comprising multiple generations to detect differentiation between taxa in floral vs. vegetative traits and to compare the current evolutionary potential of these

traits in *Ipomopsis aggregata* (Polemoniaceae), *I. tenuituba*, and their hybrids raised in three common gardens. Consistent with the high phenotypic plasticity often observed in vegetative traits, SLA and WUE were highly sensitive to garden location and exhibited low genetic differentiation among populations. By contrast, floral traits related to flower size showed high population differentiation and higher heritabilities than the focal vegetative traits. Interestingly, floral volatiles were influenced by sources of variation similar to those affecting the vegetative traits; heritabilities were generally low and environmentally induced variation high. Vegetative traits, however, displayed potentially adaptive phenotypic plasticity—an attribute that is less common among the more developmentally stable floral traits. This multigenerational study provides, to our knowledge, the most complete assessment and comparison to date of the evolutionary potential and plasticity of floral volatiles relative to morphological floral and vegetative traits. The authors also note that strong site by year interactions affecting SLA and trichome density call for caution when using geographic variation in fitness-related traits and in climate ("space-for-time substitution") to make projections concerning the effects of climate change on population-level phenotype.

In a complementary study of floral scent (including 40 volatile compounds), floral morphology, and floral rewards for pollinators, Eisen et al. (2022) studied the genetic basis and covariation among these traits in the fragrant evening primrose *Oenothera cespitosa* subsp. *marginata* (Onagraceae) and the degree of differentiation among populations. Under greenhouse conditions, they grew individuals from five populations sampled among sites across the species' range in the western United States and measured trait variation within and among individuals and populations. All three categories of floral traits exhibited variation at all ecological levels; and, although within-plant variation in floral scent was common, floral volatile production was nevertheless generally a repeatable trait. Collectively, populations were more highly differentiated with respect to the morphological traits and rewards that are associated with some pollinator syndromes (in this case, long-tongued hawkmoths) than with respect to floral scents, but the population-level signal for multivariate profiles of scent was comparable to that for morphology and rewards. Moreover, the variation in floral scents within populations generally exceeded that for morphological traits and rewards, indicating the high evolutionary potential of floral scents. The ability to distinguish and to measure very large numbers of volatile compounds indicates that floral scents may be a relatively untapped resource with which to test with rigor and great taxonomic breadth the idea that pollinator syndromes are distinct and can be reliably defined.

One of the strengths of common garden experiments is their potential role in detecting (or ruling out) local adaptation as an explanation for geographic variation in fitness-related traits. To this end, Muir et al. (2022) explored

a cline in germination time in the widely distributed scarlet monkey flower (*Mimulus cardinalis*; Phrymaceae) in California using a combination of selection analysis and reciprocal transplants. Germination time is a challenging trait to analyze given its (often) non-Gaussian distribution, so the methods used in this study will be of broad interest to others faced with analyzing non-normally distributed traits. Muir et al. assessed whether the cline in germination time can be explained by local adaptation by testing the adaptive hypothesis that variation among populations in the pattern of selection on germination time is concordant with the observed cline. While they found that germination time is heritable and seeds sampled from southern populations germinate faster and have higher early survival than northern populations, this geographic pattern could not be attributed to selection on the time to germination observed within populations. They proposed that the cline was generated by non-adaptive processes such as indirect selection on germination time due to its correlation with other traits.

Some common garden studies are conducted with the aim of using the expression of population differentiation and within-population genetic variation in functional traits to inform decisions regarding the provenance of seeds to be used in restoration efforts. Here, Volk et al. (2022) used a common garden experiment to explore physiological trait differentiation among 22 populations of the perennial *Geum triflorum* (prairie smoke; Rosaceae) sampled from the thin-soiled alvar ecoregions of the Great Lakes and Manitoba (characterized by predictable extremes in seasonal water availability) and from the prairie ecoregion (where populations experience unpredictable water availability). The patterns observed hold implications for the adaptive potential of populations of seeds sampled from these regions when introduced to novel sites targeted for restoration. Seeds derived from alvar populations developed into plants with higher WUE, smaller stomata, and higher stomatal densities than those from prairie populations, and also exhibited higher genetic variances and heritabilities of these quantitative traits. The authors offer the cautionary note, however, that genetic variances and heritabilities do not necessarily predict the evolvability (the additive genetic variance of a trait standardized by the mean value of the trait) of these traits, which were low for all the traits and ecoregions and may be a more important predictor of the capacity for future evolutionary change. Under these conditions, rather than simply selecting for restoration efforts seeds from populations with particularly high levels of genetic variation in functional traits, it may be more successful to use seeds collected from "pre-adapted" populations—those that evolved under or currently thrive in environmental conditions that are most similar to the current or projected conditions of restoration sites—or to create (through cross breeding) a synthetic but highly genetically diverse population of seeds with which to restore degraded sites.

As described above, the resurrection approach comprises a common garden study of multiple genotypes sampled from the same population but at different times (i.e., ancestral and descendant genotypes) permitting the detection of short-term evolutionary change. Ideally, to minimize maternal effects on offspring phenotype, the experimental design includes a "refresher" generation grown in a common garden prior to raising the "test" generation in which ancestral and descendant populations are directly compared. Cheptou et al. (2022) used this approach to study the evolution of flower size, the date of anthesis, floral longevity, the propensity for autonomous selfing, flower color, floral display, and total flower production across two decades (1991–2012) in the mixed-mating wild pansy (*Viola arvensis*; Violaceae). Within this short period, the population evolved earlier flowering, smaller flowers, higher autonomous selfing rates, and an increase in the frequency of pale (relative to purple) flowers. Cheptou et al. proposed that these changes are the result of a reduction in pollinator efficacy in agroecosystems, but note that resurrection studies must be interpreted with care due to the possibility that seed storage itself may generate phenotypic shifts between ancestors and descendants due to non-random, genetically based mortality among the seeds of the ancestral population (a phenomenon known as the invisible fraction; Weider et al., 2018).

The contributors of these common garden studies were consistently mindful to point out the potential for maternal genetic, non-additive, or environmental effects on offspring phenotype to obscure or to magnify phenotypic differences among genotypes or populations and to suggest ways to control these sources of variance statistically or experimentally.

QUANTITATIVE GENETIC ANALYSIS TO ESTIMATE THE HERITABILITY AND COMPONENTS OF VARIANCE OF FITNESS-RELATED TRAITS AND FITNESS

The analysis of quantitative traits in wild species leverages the joint power of plant breeding (the production, cultivation, and, sometimes, the artificial selection of pedigreed seeds) and statistics to explore the evolutionary potential of fitness-related traits, and of fitness itself, in wild populations of plants and animals. Due to the sessile nature of plants and the ability to cultivate them in great numbers, they are particularly well suited to controlled breeding and to the detection of subtle sources of quantitative genetically based variation that require large sample sizes. Accordingly, several papers assembled in this special issue apply the tools of quantitative genetics to address a variety of questions regarding the distribution and type of genetic variation on which natural selection may operate. What kinds of traits most reliably harbor high levels of heritable variation in spite of their strong effects on fitness, which may be

expected to lead to fixation? Which traits are most likely to be influenced by non-additive genetic effects, such as cytoplasmic inheritance, dominance, epistasis, or the effects of maternal growing conditions on offspring phenotype? Which traits are highly correlated—through antagonistic pleiotropy or genetic linkage—in ways that make them more resistant to evolution through natural selection? Which traits are subject to strong genotype × environment (G × E) interactions, generating selection on phenotypic plasticity itself or contributing to environment-specific evolutionary trajectories? Knowledge of the genetic architecture of traits, along with their independent and their indirect quantitative effects on fitness, is critical for predicting their evolutionary trajectory.

For some traits or populations, the genetic variation needed to fuel an evolutionary response to selection is not available as additive genetic variance expressed in a uniform environment but rather as a genotype by environment interaction. Larios and Mazer (2022) investigated this possibility in a study of the evolutionary potential of seed size (and the plasticity of seed size) in the perfect-flowered, winter annual *Dithyrea californica* (spectacle pod; Brassicaceae). Given the strong, positive effects of the size of sown seeds on germination and seedling survival in many species, the high phenotypic variance in seed size exhibited in *D. californica* is somewhat counterintuitive. Among genotypes sampled from each of three wild populations, Larios and Mazer performed reciprocal crosses in a diallel breeding design, replicating diallels (in two of the three populations) in two watering treatments, and then analyzing sources of variation in the seed size of the resulting progeny. The diallel design allowed them to partition and to estimate additive and non-additive components of genetic variance in seed size, while the watering treatments allowed them to estimate the magnitude of G × E interactions for each genetic component of variance. In this system, additive genetic variance (and narrow-sense heritability) of seed size was low (but often statistically significant) while genotype by watering treatment interactions were strong in all populations, indicating the potential for plasticity of seed size to evolve. The breeding design used here also allowed them to test for sexual conflict among parental plants; they found no evidence that the tendency of plants to produce large (or small) seeds as maternal plants was correlated with their tendency to produce large (or small) seeds as paternal plants.

Reed et al. (2022) investigated the evolutionary potential of flowering phenology by estimating the narrow-sense heritability of both the onset and duration of flowering in the herbaceous perennial *Echinacea angustifolia* (narrow-leaved purple coneflower; Asteraceae). This objective is particularly challenging in a long-lived species due to the potential for the magnitude of additive genetic variance in fitness-related traits to differ among years. This work provided the added value of estimating heritability using two methods (offspring–midparent regressions and restricted maximum likelihood) and of using two

approaches for the assignment of paternity. Reed et al. detected significant additive genetic variance in both phenological traits, but also a genotype by year interaction for the onset of flowering, indicating age-related or environmentally induced phenological shifts. While studies of multiple populations of annuals demonstrate that the magnitude of components of genetic variance may vary over space or between environmental treatments (e.g., Larios and Mazer, 2022), a sustained study of selection in a pedigreed population of perennial plants is perhaps the best way to demonstrate that the same principle may apply across the lifetime of a population.

Heywood et al. (2022) conducted a comprehensive quantitative genetic analysis designed to detect the potential for mating system evolution in the long-lived perennial herb *Ruellia humilis* (Acanthaceae), a species with a mixed mating system associated with the production of both chasmogamous (CH) and cleistogamous (CL) flowers. In some populations of *R. humilis* in the tallgrass prairies of southwestern Missouri, the primary pollinators of its CH flowers (hawkmoths) have been lost and at the same time, predispersal seed predators preferred seeds of CH flowers, indicating two mechanisms by which selection may favor elevated production of CL flowers in these populations. Heywood et al. assessed the possible evolutionary trajectory of the relative production of the two floral forms following pollinator loss by estimating the additive genetic variances of 11 CH floral traits, the genetic and phenotypic correlations between them, inbreeding depression, and a variety of fitness-related traits in a greenhouse population derived from a single wild population. They used a partial diallel crossing design to produce pedigreed seeds that were sown and raised in the greenhouse for 5 years. In this experimental population, floral traits of CH flowers were measured, and their effects on or correlations with architectural, phenological, and reproductive fitness were measured. Heywood et al. found significant additive genetic variance and moderate to high evolvability for most traits (e.g., measures of floral size and shape, total flower number, phenology of flowers and shoots, and shoot length), all consistent with the potential for a rapid response to selection. Based on previous field observations indicating strong selection by the seed predator favoring the production of CL flowers and a correlated decrease in mean stigma–anther separation (increasing the opportunity for prior selfing) the authors provide persuasive evidence that in the absence of evolutionary constraints (or opposing directions of selection) that were not detected in their study, this population is expected to experience the rapid evolution of higher percentages of CL flowers.

So, Sibolibane, and Weis (2022) explored how the nature of genetic variance might change depending on the environment, and specifically, the possibility that non-additive variance (e.g., dominance variance, V_D) could be converted to additive genetic variance (V_A) and thus subject to selection, depending on the environment. Using a pedigreed population of *Brassica rapa* grown for three

generations with a design that allowed them to separate dominance variance from maternal effect variance, plants were grown in two environments (field conditions mimicking the agricultural conditions of the original population vs. presumably more benign greenhouse conditions) to estimate additive, dominance, and maternal components of genetic variance using Bayesian models. They also used parental breeding values to estimate genetic correlations across environments, which revealed G × E interactions; the genotypes with relatively high trait values differed between the two environments. Traits measured included height at flowering and the numbers of leaves, inflorescences, and fruits. In the greenhouse, additive genetic variance was elevated, but they found no consistent patterns in the field or greenhouse indicating conversion of dominance variance, perhaps because of low genetic variance and broad confidence intervals for the variance estimates.

The importance of plant–pollinator interactions for the evolution of floral phenotypes has long been recognized, but until recently, few studies have addressed the evolvability of flowers from a multivariate perspective, including interactions among different selective pressures, genetic correlations, and genetic constraints all acting on the three-dimensional shape of flowers. Opedal et al. (2022) examined how standing genetic variation may be reduced with phenotypic selection on interacting and genetically correlated traits. The evolvability, genetic correlations, and constraints on the evolution of the three-dimensional morphology of flowers of *Dalechampia scandens* (Euphorbiaceae) were measured in the greenhouse. A diallel crossing design was used to estimate the **G** matrix for traits important for successful insect pollination or self-pollination, based on measurements of the relative positions of male (anthers) and female (stigmas) reproductive parts, and the position of the gland that secretes the pollinator reward (i.e., gland–anther distance, gland–stigma distance, and distance between anthers and stigmas). The **G** matrix was then used to estimate how genetic variation was distributed in multivariate space and thus to identify areas of high multivariate evolvability and areas of potential genetic constraints. Expanding the use of this matrix, the evolutionary divergence of populations and species within the genus was examined. Their results suggest that the evolvability of traits varies such that some traits exhibit greater independent evolutionary potential, while others are subject to strong genetic constraints that limit the joint evolution required for an optimal floral phenotype, whether for the fit between pollinators and the positions of floral organs in outcrossing species, or for optimal self-pollination in other species.

The evolution of separate sexes from hermaphroditism via an intermediate step of gynodioecy is common, but the genetic constraints influencing the transition are not well understood. Campbell et al. (2022b) investigated models for the evolution of dioecy from hermaphroditism in *Schiedea* (Caryophyllaceae), an endemic Hawaiian genus with species expressing wide variation in reproductive systems. They assessed genetic variances and covariances of floral traits and inflorescence traits in the gynodioecious *S. salicaria*, a species presumably under selection for increased representation of females based on measures of self-fertilization and inbreeding depression. In addition, two generations of artificial selection for increased female function (carpel and fruit biomass) in females and increased male function (stamen biomass) in hermaphrodites were used to determine how well the measures of genetic variance and covariance predicted changes resulting from artificial selection using replicate selection and control lines. Biomass was measured as a common currency because of its use in theoretical models for sex allocation. After two generations of artificial selection, stamen biomass in hermaphrodites and carpel and fruit biomass in females responded significantly to selection, supporting a fundamental assumption of models for the evolution of dioecy. Although responses to selection were predicted reasonably well by initial estimates of heritabilities and genetic correlations, deviations were generally in the direction of larger responses than expected based on genetic variances and covariances measured in the baseline generation (the initial generation before selection). Because estimates of the heritability of male biomass changed between generations, new predictions were made using heritabilities estimated in the first rather than baseline generation. These new predictions did a better job of predicting the response to selection in the second generation. Results indicate the power of artificial selection and the utility of multivariate selection models for improving our understanding of breeding system evolution. They also suggest (see also Reed et al., 2022) that single-generation estimates of variances and covariances may not lead to accurate estimates of the response to selection.

Model systems, especially those that focus on diverse life histories of species occurring in the wild and possessing a well-defined ecological context, offer the prospect for a deeper understanding of evolutionary processes. When genomic tools are also available for such systems, the details of the genetics underlying evolutionary change may become evident and provide new perspectives and opportunities for novel approaches. One such model system, the genus *Boechera* (Brassicaceae), is an example of this approach (Rushworth et al., 2022). The many genomic tools available for closely related *Arabidopsis thaliana* can also be used in *Boechera*, leading to the potential for rapid progress on many fronts. *Boechera* differs from most model systems in that some species are perennial, and while self-fertilization is the most common reproductive system in the genus, outcrossing is possible and is associated with the occurrence of apomixis. In common garden experiments the authors find that apomictic lineages have higher overwinter survival than sexual lineages, while sexual lineages have lower herbivory and higher survival in subsequent years. In addition to the numerous advances

in our understanding of apomixis made possible by studies of *Boechera*, the authors suggest the possibility that identification of the genes associated with apomixis might be useful in agriculture to ensure crop productivity in the face of pollinator decline. Among the many studies of local adaptation in *Boechera* (including a contribution to this special issue), a reciprocal transplant study of *B. stricta* and simulation of climate change through snow removal demonstrated that limited genetic variation in complex traits was a greater limitation to adaptation than genetic correlations across traits. *Boechera stricta* represents a classic example of response to climate change, as flowering has advanced by more than 2 weeks in the last 50 years. Gene flow among populations of this largely selfing species may be essential for providing the genetic variance permitting population persistence in the face of climate change. These and other studies employing this genus suggest the advantages of model systems with diverse life histories and where the ecological context is well understood.

CLOSING THOUGHTS

Readers of this special issue will discover a variety of newly detected features and outcomes of the selective process within and among wild populations. The authors' findings and interpretations have invigorated our enthusiasm for the application of both observational and experimental approaches to the study of selection in fitness-related traits, constraints on their evolution, and their contribution to local adaptation in heterogeneous and rapidly changing environments. For this, we are very grateful to the contributors. We hope that these papers will inspire a new generation of botanists to take on the labor-intensive hand pollinations, biomass measures of tiny dried stamens, kneeling and bending under difficult field circumstances, careful labeling of seed envelopes, and wrestling with statistical interpretation that ultimately lead to the highly gratifying work of observing and illuminating the evolutionary process, not only in the relatively few plant species that are well known, but also in the vast majority that remain unstudied.

AUTHOR CONTRIBUTIONS

Susan J. Mazer: Conceptualization; Project administration; Writing – original draft; Writing – review & editing. **Ann K. Sakai**: Conceptualization; Project administration; Writing – original draft; Writing – review & editing. **Stephen G. Weller**: Conceptualization; Project administration; Writing – original draft; Writing – review & editing. **Eugenio Larios**: Conceptualization; Project administration; Writing – original draft; Writing – review & editing.

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