

Genotype \times environment interaction obscures genetic sources of variation in seed size in *Dithyrea californica* but provides the opportunity for selection on phenotypic plasticity

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Abstract

Premise: In many species, seed size influences individual fitness, but its heritability is low, impeding its evolution. In heterogeneous environments, even if heritability of seed size is low, genetic variation in phenotypic plasticity for seed size may provide the opportunity for selection, but this possibility has rarely been investigated in wild species. The evolutionary trajectory of seed size depends on whether additive, maternal, or non-additive genetic variance dominates; moreover, the expression of any of these sources of variance may be environment-dependent, reflecting genetic variation in plasticity. In this study, we examined these sources of variation in seed size and their response to drought in *Dithyrea californica*.

Methods: We used a diallel design to estimate variance components for seed size in three greenhouse-raised populations sampled from California and northern Mexico. We replicated diallels in two watering treatments to examine genetic parameters and genotype \times environment interactions affecting seed size. We estimated general (GCA) and specific (SCA) combining ability, reciprocal effects (RGCA and RSCA), and their interactions with water availability, and we sought evidence that sexual conflict influences seed size.

Results: Norms of reaction revealed genetic variation in plasticity for seed size in each population. Seed size in *D. californica* is determined by the combination of watering treatment, GCA and RGCA; parental identity and water availability do not consistently affect seed size, and we detected no evidence for sexual conflict.

Conclusions: Multiple sources of genetic variation in phenotypic plasticity for seed size have the potential to influence its evolutionary trajectory in heterogeneous environments.

KEYWORDS

Brassicaceae, diallel analysis, genotype \times environment interaction, phenotypic plasticity, seed size, water availability

In wild populations, how a trait evolves under natural selection is determined in part by the contributions of genetic variance, environmental variance, and the interaction between them to the trait's total phenotypic variance (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Combined with estimates of the environment-specific strength and direction of total selection on a given trait, measures of these sources of variation facilitate predictions regarding the trait's short-term evolution. An interaction

between genotype and environmental conditions (i.e., a $G \times E$ interaction) that determines individual phenotype indicates that the phenotypic ranks of, or the variance among, genotypes differ across environments. When sufficiently strong, $G \times E$ interactions can contribute to the maintenance of genetic variation in fitness-related traits because the identity of the genotype(s) with the highest mean fitness may change over space or time (Byers, 2005), reducing the rate at which natural selection can purge

populations of low-fitness genotypes. $G \times E$ interactions also make it difficult to predict the evolutionary trajectory of populations occupying heterogeneous environments when the heritability of individual traits, and of fitness itself, are environment-specific, such that the rate and direction of evolutionary change in these traits depends on the frequencies of alternative conditions. More generally, in heterogeneous environments, $G \times E$ interactions reduce the proportion of total variance in focal traits that is due to additive genetic variance (and therefore reducing their heritability) and indicate the presence of genetic variation in phenotypic plasticity, which itself may evolve if it is both heritable and the target of natural selection.

Genotype \times environment interactions in plants have been studied for a variety of traits including flowering time (Mazer and Schick, 1991; Pigliucci et al., 1995; Vogler et al., 1999; Quinn and Wetherington, 2002), germination time (Galloway, 2001a,b), and chemical defenses (Agrawal, 2001; Holeski et al., 2010; Schiestl et al., 2014; Wagner and Mitchell-Olds, 2018). Few studies, however, have sought evidence that $G \times E$ interactions affect individual seed size in wild plant species (Schmitt et al., 1992; Schmid and Dolt, 1994; Sultan, 1996; Agrawal, 2001). Seed size is a particularly complex trait for which to estimate $G \times E$ interactions because offspring size is influenced by a variety of genetic factors that are expressed independently in the parents and in the offspring. These genetic factors originate from three main sources: (1) additive genetic variation, determined by parental nuclear genetic contributions to a seed's genotype that influence its size; (2) non-additive variation, generated by genetic interactions between genes at the same locus (dominance) or at different loci (epistasis); and (3) maternal genetic effects, which refer to the influence of a sporophyte's diploid genotype or cytoplasmic genes on the phenotype of its offspring.

Among those species that have been studied, additive and non-additive sources of genetic variance in seed size are usually low compared to maternal and environmental influences (Antonovics and Schmitt, 1986; Mazer, 1987; Schwaegerle and Levin, 1990; Waser et al., 1995). In *Anthoxanthum odoratum*, a diallel analysis revealed little additive genetic variance in seed size, while maternal effects on mean seed size (both genetic and environmental) predominated (Antonovics and Schmitt, 1986). This finding led the authors to conclude that there is little opportunity for seed size to evolve in a species due to additive genetic effects expressed in the offspring. Similarly, in *Nemophila menziesii*, additive genetic variance was not significantly different from zero, but maternal genetic effects accounted for 20% of the total phenotypic variance in seed size. Under these conditions, seed size might evolve as a genetically determined maternal trait governed by the amount of resources allocated per seed, but not as a trait determined by an individual seed's nuclear genotype (Platenkamp and Shaw, 1993; Byers et al., 1997).

Genetic factors that affect seed size in wild species are often dominated by maternal effects (Roach and Wulff,

1987; Gutterman, 2001). While genetic contributions to an individual embryo from its male and female parents are equal in terms of nuclear contributions, there is ample evidence that the female parent has a greater extranuclear genetic influence than the male parent (Roach and Wulff, 1987). Maternal genetic contributions to seed size originate from more sources than those from male contributions, including the maternal sporophyte's diploid genotype, cytoplasmic genes, and potential genetic interactions between these genetic factors and the nuclear or endosperm genotypes of their developing seeds (Byers et al., 1997; Thiede, 1998; Galloway et al., 2009). Male contributions to fertilized ovules, however, may also play an important role in seed provisioning by influencing maternal resource allocation to developing seeds. This paternal effect may be accomplished through the phenomenon of genomic imprinting, which is an epigenetic modification that causes genes to be expressed, or not, depending on the parent of origin from which they were inherited (Haig and Westoby, 1989; House et al., 2010; Willi, 2013). In *Arabidopsis thaliana*, higher doses of paternally imprinted loci resulted in the provisioning of larger seeds by influencing the amount of resources devoted to the endosperm in which the paternal gene was expressed, while a higher dosage of maternally imprinted loci resulted in smaller seeds (Scott et al., 1998; Adams et al., 2000).

Given the potential for maternal, paternal, and offspring genotypes to influence seed size, the detection and measurement of all $G \times E$ interactions affecting this trait requires examining environmental effects on each source of genetic variance independently. The analysis of a diallel breeding design, in which reciprocal crosses are conducted among all pairs of a group of unrelated maternal and paternal genotypes, can isolate and estimate these distinct sources of genetic variance in seed size. Furthermore, if diallels are replicated in alternative environments, $G \times E$ interactions can be estimated for each variance component contributing to seed size, along with environment-specific values for each variance component and the genetic parameters (such as narrow- and broad-sense heritability) derived from them.

The current study was designed to detect, if present, genetic variation in the plasticity of seed size within populations of a widespread species, the winter annual herb *Dithyrea californica*. In *D. californica*, an observational field study found that seed provisioning was controlled in part by local biotic conditions (Larios and Venable, 2015). Naturally germinated plants of *D. californica* were monitored for one growing season in two localities; mean individual seed size produced by maternal plants that experienced different amounts of intraspecific competition were then compared. Maternal plants exposed to greater competition produced smaller seeds on average than those that experienced less competition. In addition, a parent-offspring regression revealed that offspring provisioning was determined not by genetic variation but by maternal plant size (which itself was determined by the

intensity of competition), suggesting that offspring seed size was influenced by a plastic response to the maternal plant's environment. However, this study was observational and neither controlled for genetic identity nor directly manipulated the strength of environmental interactions, preventing the estimation of the contribution of plasticity to total phenotypic variance in seed mass or the measurement of $G \times E$ interaction.

In the current investigation, we conducted a controlled greenhouse experiment using a diallel breeding design to investigate the genetic basis of seed size in *D. californica*, including the genetic influences of both maternal and paternal contributions as well as $G \times E$ interactions. Previous work on the genetic basis of seed size has suggested that seed size might evolve in wild populations through maternal effects (Antonovics and Schmitt, 1986; Byers et al., 1997; Galloway et al., 2009). The current study expands this scope by replicating a diallel breeding design in two watering treatments to detect environmentally induced changes in a greater number of quantitative genetic variance components that influence seed size. In addition, we explored the possibility of a sexual conflict in the determination of seed size by estimating the independent contributions to offspring size by cosexual individuals used as both sires (i.e., used as pollen donors, or fathers) and dams (i.e., used as pollen recipients, or maternal plants). We created >8000 individually measured, pedigreed seeds that completed their development under either wet or dry greenhouse conditions, replicating parental crosses in each treatment to estimate $G \times E$ interactions and the degree and direction of the genetically based correlation between the mean seed size produced by cosexual individuals as males and as females in each treatment. We asked the following questions: (1) Is seed size heritable in this species within watering treatments? If seed size has been under sustained directional selection in wild populations of *D. californica*, and if $G \times E$ interactions are negligible, then we expect additive genetic variance to be low due to the eroding effects of natural selection. (2) Do both the nuclear genetic identity of seeds and maternal genetic contributions significantly influence the size of seeds that they produce? If the nuclear identity of seeds contributes predominantly to seed size variation, then we expect to find significant levels of additive genetic variance in seed size. If maternal genetic effects predominantly determine seed size, then we expect to find significant extranuclear maternal variance in seed size; (3) Is there genetic variation for phenotypic plasticity in mean seed size? If the heritability of seed size is environment-specific or if the phenotypic values of genotypes markedly change ranks across environments, then we expect to find significant $G \times E$ interactions for additive genetic variance, extranuclear maternal and/or paternal sources of variance; (4) Do narrow- and broad-sense heritabilities differ for seed size? If maternal genetic effects have a larger influence on seed size than paternal effects, then broad-sense heritability will necessarily exceed narrow-sense heritability; and (5) Is there a sexual conflict in the determination of seed size? If

so, then we expect to find negative correlations between mean individual seed size produced by genotypes acting as sires vs. as dams in both watering treatments. In sum, this single experiment was designed to provide an unusually comprehensive assessment of both independent and interacting sources of phenotypic variance in seed size within and among multiple populations of a well-studied species.

MATERIALS AND METHODS

Study species

Dithyrea californica (Brassicaceae) is a self-incompatible winter annual native to sand dune habitats in the Mohave and Sonoran Deserts. *Dithyrea californica* germinates in response to late-fall or early-winter rains. Once germinated, it grows as a basal rosette for approximately 2 months, flowering and producing seeds in late February to early April, depending on the timing of precipitation. Flowers are hermaphroditic and protogynous, and if unpollinated, they typically remain open for 3–4 days. The pistil becomes receptive before the petals open, after which the anthers dehisce simultaneously with petal opening. Anthers protrude slightly from the distal end of the corolla, while pistils extend to a height that is no more than one quarter of the length of the petals, making the flowers easy to emasculate. Fruits consist of two subunits called mericarps, each of which contains one seed.

The geographic distribution of *D. californica* is broad, occurring in most arid sand dune systems in western North America (northern Mexico and southwestern United States). *Dithyrea californica* is an ideal species with which to investigate the selective dynamics of seed size in the field due to a special feature of the germinated seed. When a seed inside a mericarp germinates, the rim of the mericarp (the diameter of which provides a measure of seed size) remains attached to the root until plants become adults. This special feature has allowed us to track individual plants in the field and to relate seed size to both survival and fecundity, the main components of individual fitness (Larios et al., 2014; Larios and Venable, 2018). *Dithyrea californica* is also a suitable species in which to investigate quantitative genetic variation because it is easy to emasculate and is self-incompatible, preventing accidental pollinations when conducting controlled crosses to create pedigreed seeds.

Source populations

During the spring of 2017, seeds of *D. californica* were collected in three locations. The northernmost population is 800 km away from the southernmost location. A northern population (K population) located in the Mojave Desert was collected from the Kelso dunes at the Mojave National Preserve in California, United States (34.88°N, -115.77°W, altitude: 676 m a.s.l.). A central population (L population) was located in the Sonoran

Desert on a coastal dune near the town of Puerto Lobos, Sonora, Mexico (30.35°N, -112.81°W, altitude: 70 m a.s.l.). The most southern population (S population) is also located in the Sonoran Desert and was collected on a coastal dune in the town of Desemboque de los Seris in Hermosillo, Sonora, Mexico (29.51°N, -112.41°W, altitude: 10 m a.s.l.). Seeds were stored in an outdoor shed (protected from rainfall) but exposed to ambient temperature for 2 years in paper bags until the spring of 2019; this exposure to natural year-round conditions facilitated the loss of dormancy.

Breeding design

The experiment was performed in a greenhouse at the University of California, Santa Barbara campus in Goleta, California. In the greenhouse, we planted eight seeds representing each maternal line, one seed per pot, and selected two plants of each maternal line to be grown in two alternative watering treatments (one individual/treatment). Once plants became reproductive, we conducted controlled pollinations using 5 × 5 diallel breeding designs, performing reciprocal crosses and excluding self-pollinations. Because individuals of *D. californica* are cosexual, this breeding design allows us to estimate each individual's effect on seed size as both a maternal (seed-bearing) and paternal (pollen-donating) plant. We created three diallels from the K population, three diallels from the S population, and only one diallel from the L population. The number of diallels per population was determined by the number of field-collected maternal families from which seeds successfully germinated in the greenhouse. Pollinations from a given pollen donor were performed on a pollen recipient's single reproductive stem so we could have better control of the pedigree of resulting seeds. Pollinations were conducted by collecting entire dehiscent stamens and rubbing them against a receptive stigma using alcohol-sterilized forceps. We sterilized the forceps with rubbing alcohol after each flower was pollinated. To avoid self-pollen contamination, we emasculated all flowers before anther dehiscence by slicing off the tip of the flowers before bud opening (the short style prevented damage to the stigma).

Each diallel was replicated in two watering treatments (Figure 1); here, replication meant that each of the five individuals in a given diallel had a maternal sibling in a paired diallel exposed to the other treatment. All pots were watered using a MiracleGro (Marysville, OH, USA) nutrient solution (1 tablespoon of fertilizer/3.78 L of deionized water, as suggested by the manufacturer). Wet conditions were achieved by watering the pots every 3 days, which kept the soil saturated for the duration of the reproductive stage. In the wet treatment, plants were maintained at full turgidity throughout the experiment. Dry conditions were achieved by watering the pots each week with the nutrient solution and allowing the soil to dry until we observed the onset of

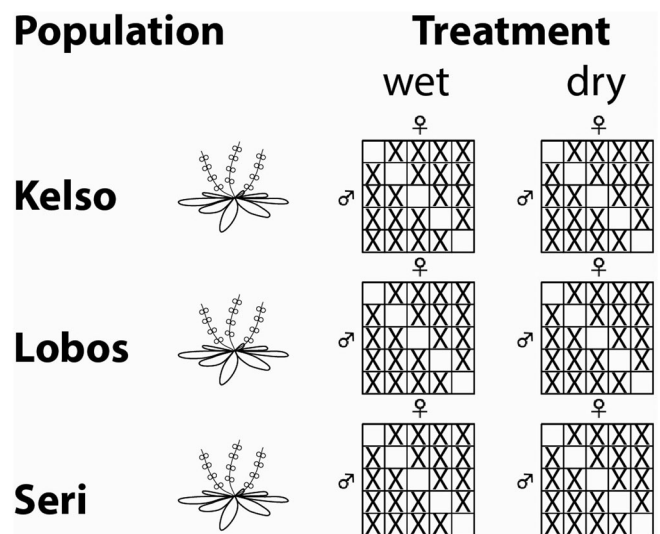


FIGURE 1 Diagram of the diallel breeding design using reciprocal crosses and no-self pollinations.

wilting, but plants retained the capacity to return to turgidity after watering. Pedigreed seeds resulting from these controlled pollinations were identified by color-coding each seed with small dots of acrylic paint to identify their maternal and paternal origin. Seeds were allowed to mature on the plant and were harvested when they became easy to detach.

Data analyses

The linear dimensions of each harvested seed were measured using the imaging software ImageJ (Schneider et al., 2012). *Dithyrea californica* seeds are shaped as flat ellipses; we estimated seed size as the area of one face of the ellipse, using the two major axes of each seed and the equation $(HW\pi)/4$, where H is the length of the longest axis and W the length of the longest axis perpendicular to H .

To estimate the variance components of seed size, we analyzed variation in ellipse area produced by crosses using the random diallel analysis that includes reciprocal crosses and no self-pollinations (diallel type III sensu Hayman, 1954; Griffing, 1956). From this analysis, we estimated the general combining ability (GCA), the specific combining ability (SCA), the reciprocal general combining ability (RGCA), the reciprocal specific combining ability (RSCA), and the interactions of each of these variance components with the watering treatment, for each population separately. The GCA refers to additive effects of genes (nuclear effects) and is represented as the mean deviation between the mean seed size produced by both parents and the diallel's grand mean (Hayman, 1954; Griffing, 1956). The SCA refers to non-additive effects of nuclear genes (dominance and epistatic effects) and is represented as the deviation of mean seed size of specific parental combinations from the parental seed size means. Reciprocal effects are deviations in

mean seed size due to the direction of a cross between two parents and due to the effects of the parents' functional gender on offspring genotype. In the Hayman (1954) model, reciprocal effects are partitioned into reciprocal general combining ability (RGCA), which is represented by the deviation in mean seed size produced by a parental genotype functioning as a male vs. as a female. The reciprocal specific combining ability (RSCA) is represented by the deviation in mean seed size between a given cross (between two parents) and its reciprocal. This model is defined by the following equation:

$$Y_{ijk} = \mu + G_j + G_k + S_{jk} + RG_j + RG_k + RS_{jk} + E_{ijk}, \quad (1)$$

where Y_{ijk} is the individual seed size produced by the combination of dam j and sire k , μ is the grand mean of seed size within a diallel, G_j and G_k are the GCAs of dam j and sire k , S_{jk} is the SCA of the j th and k th parent combination, RG_j and RG_k are the RGCA of the j th and the k th parents, RS_{jk} is the RSCA of the j th and k th parent combination, and E_{ijk} is the residual variance.

We analyzed the data using the R packages `lmDiallel` (Onofri et al., 2021) and `sommer` package to estimate random effects of variance components (Covarrubias-Pazarán, 2016). We first constructed design matrices that indicated the identity of dams and sires for each pedigreed seed with the `lmDiallel` package functions `GCA`, `SCA`, `RGCA`, and `RSCA`. We fitted generalized linear mixed-effects models where we used ellipse area (as a measure of seed size) as the response variable, watering treatment as a fixed explanatory variable, and `GCA`, `SCA`, `RGCA`, `RSCA`, `GCA × water`, `SCA × water`, `RGCA × water`, and `RSCA × water` as random effects. We tested the statistical significance of each random effect with likelihood-ratio tests comparing a model that contained all random predictor variables (the full model) with models from which we excluded the random variable to be tested. We tested for a change in mean seed size between watering treatments per population with likelihood-ratio tests between the full models that contained the watering treatment as a fixed factor and submodels that did not contain the watering treatment as the fixed factor.

Additionally, we performed the diallel analysis on each population and watering treatment separately to estimate environment-specific variance components of seed size and the significance of maternal and paternal genetic contributions to seed size in each treatment.

We estimated narrow-sense heritability per population and per watering treatment as:

$$\sigma_{GCA}^2 / (\sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_{RGCA}^2 + \sigma_{RSCA}^2 + \sigma_e^2). \quad (2)$$

Narrow-sense heritability is a proportion estimated as the additive genetic variance (σ_{GCA}^2) divided by total phenotypic variance (Falconer and Mackay, 1996).

We also estimated broad-sense heritability as:

$$(\sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_{RGCA}^2 + \sigma_{RSCA}^2) / (\sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_{RGCA}^2 + \sigma_{RSCA}^2 + \sigma_e^2) \quad (3)$$

Broad-sense heritability is a proportion estimated as total genetic variance ($\sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_{RGCA}^2 + \sigma_{RSCA}^2$) divided by total phenotypic variance.

We estimated norms of reaction of seed size using the residual variation of ellipse area within each diallel cross. Residual variation was estimated for the dams and sires separately by subtracting the diallel mean seed size of each dam or sire from the mean seed size produced by each of the dams or sires within a diallel. We pooled all dam and sire residuals by population and represented the norms of reaction by population rather than by individual diallel. By examining the norms of reaction for each population, we can observe the environmentally induced effect of watering treatment on the mean seed size and phenotypic rank of each maternal and paternal genotype. We tested for a change in variance in seed size between watering treatments with Levene's tests of homogeneity of variance per population where we compared the within-population variance among dams or sires in ellipse area of dry and wet treatments.

We examined the sex-specific performance of, and correlations between, cosexual individuals with respect to the size of seeds produced in each watering treatment. To analyze the correlation between sexes, we estimated Pearson's correlations to examine the bivariate relationship between the residual mean ellipse area produced by individuals as pollen donors (sires) and as pollen recipients (dams). Finally, to analyze the cross-environment correlation of sex-specific residual seed size, we also estimated Pearson's correlations to examine the bivariate relationship between the residual mean ellipse area produced by individuals as pollen donors (sires) in the dry and wet treatments and as pollen recipients (dams) in the dry and wet treatments. For each diallel matrix, we used the diallel mean ellipse area of all sires and dams to calculate sire- and dam-specific residuals. Then, all residuals for a given population were pooled before plotting the bivariate relationships presented here.

RESULTS

Variance components of seed size

Variance components for seed size differed between populations but were generally dominated by the interactions between watering treatment and genetic factors (particularly `GCA` and `SCA`), indicating that genetic variation for phenotypic plasticity in seed size has an additive component (the `GCA × water` interaction, likelihood ratio test: $P < 0.001$) and an extranuclear maternal

non-additive component (the SCA \times water interaction, likelihood ratio test: $P < 0.001$; Table 1). Significant interactions between variance components and watering treatment were consistent across populations. GCA \times water interactions were statistically significant in all populations (Table 1), indicating environment-dependent additive genetic effects on seed size. The SCA \times water interaction was statistically significant in the K population (Table 1), indicating that the expression of non-additive effects

depended on watering treatment in that population. And, the RSCA \times water interaction was significant in all populations (Table 1), indicating that the expression of non-additive maternal effects was also environment-dependent.

When analyzing the variance components expressed in each watering treatment separately, we found several cases of treatment-dependent genetic effects on seed size (Tables 2A and B). In Population S, SCA was significant in the dry treatment but not the wet treatment, indicating a treatment-

TABLE 1 Variance components of seed size of Seri, Lobos, and Kelso populations of *Dithyrea californica* grown in two watering treatments (W). Variance components were estimated with the diallel analysis of Hayman (1954), using the ellipse areas of 8530 pedigreed seeds (Seri: 4019 seeds, Kelso: 2635 seeds, Lobos: 1876 seeds). β : fixed effect coefficient. σ^2 : variance, GCA: general combining ability, SCA: specific combining ability, RGCA: reciprocal general combining ability, and RSCA: reciprocal specific combining ability. Asterisks denote significant factors at the 0.05 level.

Seri		Lobos		Kelso	
Fixed	β (SE)		β (SE)		β (SE)
Water	-0.268 (0.683)	Water	1.779 (0.982)	Water	0.427 (0.897)
Random	σ^2 (SE)		σ^2 (SE)		σ^2 (SE)
GCA	0.246 (0.335)	GCA	1.714 (1.71)	GCA	0 (0.497)
SCA	0.084 (0.123)	SCA	0.971 (0.701)*	SCA	0 (0.423)
RGCA	0 (0.18)	RGCA	3.763 (3.531)	RGCA	0 (0.215)
RSCA	0.335 (0.416)	RSCA	0.891 (1.351)	RSCA	-0.012 (0.78)
GCA \times W	0.803 (0.357)*	GCA \times W	0.571 (0.468)*	GCA \times W	1.27 (0.7)*
SCA \times W	0.155 (0.127)	SCA \times W	0.122 (0.165)	SCA \times W	1.007 (0.58)*
RGCA \times W	0.163 (0.214)	RGCA \times W	2.15 (1.753)	RGCA \times W	-0.104 (0.3)
RSCA \times W	0.98 (0.453)*	RSCA \times W	1.858 (1.259)*	RSCA \times W	2.7 (1.09)*
Residual	4.7 (0.106)	Residual	8.081 (0.266)	Residual	7.427 (0.209)

TABLE 2 Variance components of seed size for Seri, Lobos, and Kelso populations of *Dithyrea californica* analyzed by watering treatment separately. (A) Variance components expressed during the dry treatment. (B) Variance components of seed size expressed during the wet treatment. VC: variance components, GCA: general combining ability, SCA: specific combining ability, RGCA: reciprocal general combining ability, and RSCA: reciprocal specific combining ability. Asterisks denote significant factors at the 0.05 level.

Seri		Lobos		Kelso	
VC	σ^2 (SE)	VC	σ^2 (SE)	VC	σ^2 (SE)
A. Dry					
GCA	2.093 (0.818)*	GCA	1.086 (1.104)	GCA	1.529 (0.803)*
SCA	0.042 (0.056)	SCA	1.126 (0.811)*	SCA	0.897 (0.545)*
RGCA	0 (0.251)	RGCA	0.691 (0.832)	RGCA	0.061 (0.35)
RSCA	2.08 (0.783)*	RSCA	1.778 (1.207)*	RSCA	2.21 (0.993)*
Residual	5.59 (0.176)	Residual	8.394 (0.457)	Residual	7.7 (86.37)
B. Wet					
GCA	0.423 (0.292)*	GCA	3.446 (1.104)*	GCA	0.974 (0.585)*
SCA	0.678 (0.35)*	SCA	0.965 (0.694)*	SCA	1.139 (0.582)*
RGCA	0.208 (0.165)	RGCA	11.116 (7.72)	RGCA	0 (0.336)
RSCA	0.445 (0.249)*	RSCA	3.552 (2.33)*	RSCA	2.572 (1.034)*
Residual	3.7 (0.121)	Residual	7.907 (0.328)	Residual	7.242 (0.25)

dependent expression of non-additive genetic variance. In the L population, GCA was significant in the wet treatment but not the dry treatment, indicating treatment-dependent expression of additive genetic variance.

In contrast to GCA and SCA, reciprocal effects displayed no treatment-dependence. RGCA is not detectably different from zero in any population in either wet or dry conditions, while RSCA is significant in all populations in both watering treatments (Tables 2A and B).

In all populations and watering treatments, broad-sense heritability was higher than narrow-sense heritability (Table 3), reinforcing that maternal effects have more variance available for selection than paternal effects.

Effects of watering treatment on seed size

Across all genotypes in each population, mean individual seed size was not affected by watering treatment (Table 1, Figure 2). Within populations, however, the strength and the direction of the norms of reaction were genotype-dependent (Figures 3 and 4). Norms of reaction derived from the mean deviations of dam-specific mean individual

TABLE 3 Narrow-sense (h^2) and broad-sense (H^2) heritabilities of seed size for three populations of *Dithyrea californica*, derived from diallel crosses and replicated in two watering treatments (W: wet; D: dry). Asterisks indicate significant heritabilities, as inferred by the statistical significance of one or more variance components in the numerator of the ratio that comprised the heritability estimates (see section *Variance components of seed size*).

Population	h^2		H^2	
	Dry	Wet	Dry	Wet
Seri	0.213*	0.077*	0.429*	0.321*
Lobos	0.083	0.127*	0.358*	0.706*
Kelso	0.122*	0.081*	0.376*	0.392*

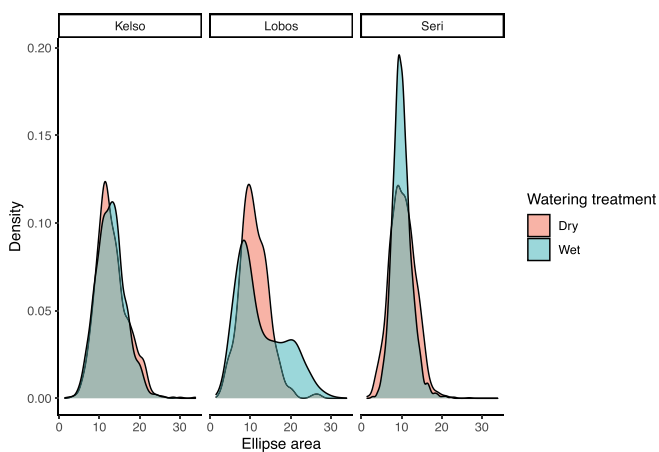


FIGURE 2 Density plots showing the frequency distribution of ellipse areas per watering treatment per population. Seed ellipse area ranged from 1.43 mm² to 33.91 mm².

seed size showed changes in rank among dams in each population (Figure 3). Changes in variance in dam-specific residual seed size in relation to watering treatment were significant for the S population (Levene's test: $P = 0.02$) while for the K and L populations were nonsignificant (Levene's test: K, $P = 0.5$; L, $P = 0.18$).

Norms of reaction derived from average deviations of sire-specific mean individual seed size showed a similar pattern to those derived from the dam residuals. Every population showed a change in genotypic ranks, but no population presented a change in variance between watering treatments (Levene's test: S, $P = 0.22$; K, $P = 0.36$; L, $P = 0.13$; Figure 4).

Cross-environment correlation

In all populations, mean seed size produced by maternal genotypes in the wet treatment was independent of the mean seed size produced by their siblings in the dry treatment (Figure 5, top graphs). Similarly, the mean seed size produced by genotypes as sires in the wet treatment was independent of the mean seed size produced by their siblings as sires in the dry treatment (Figure 5, bottom graphs). None of the bivariate relationships between mean ellipse area produced by genotypes as dams (or as sires) in the wet vs. dry treatments had slopes that differed from zero. The bivariate relationships among maternal genotypes in wet vs. dry treatments were Seri, $P = 1$; Kelso, $P = 0.7$; Lobos, $P = 0.08$ (Table 4A, Figure 5A). The bivariate relationships among paternal genotypes in wet vs. dry treatments for the three populations were S, $P = 0.73$; K, $P = 0.48$; L, $P = 0.18$ (Table 4A, Figure 5B).

Potential for sexual conflict

In all populations and in both treatments, the mean seed size produced by individuals as dams was independent of their mean seed size as sires, suggesting that there is no sexual conflict in the determination of seed size in this species (Figure 6). None of the bivariate relationships between mean ellipse area produced by individuals as sires vs. as dams in the dry treatment or in the wet treatment had slopes that differ from zero, except for correlations for the S and L populations in the wet treatment that are marginally nonsignificant (dry: S, $P = 0.11$; K, $P = 0.74$; L, $P = 0.92$; Table 4B, Figure 6A; Wet: S, $P = 0.06$; K, $P = 0.31$; L, $P = 0.09$; Table 4B, Figure 6B).

DISCUSSION

In the current study, seed size was determined primarily by $G \times E$ interactions, which were detected for all three components of variance that could be partitioned in an analysis of a diallel mating design. None of these sources of variance

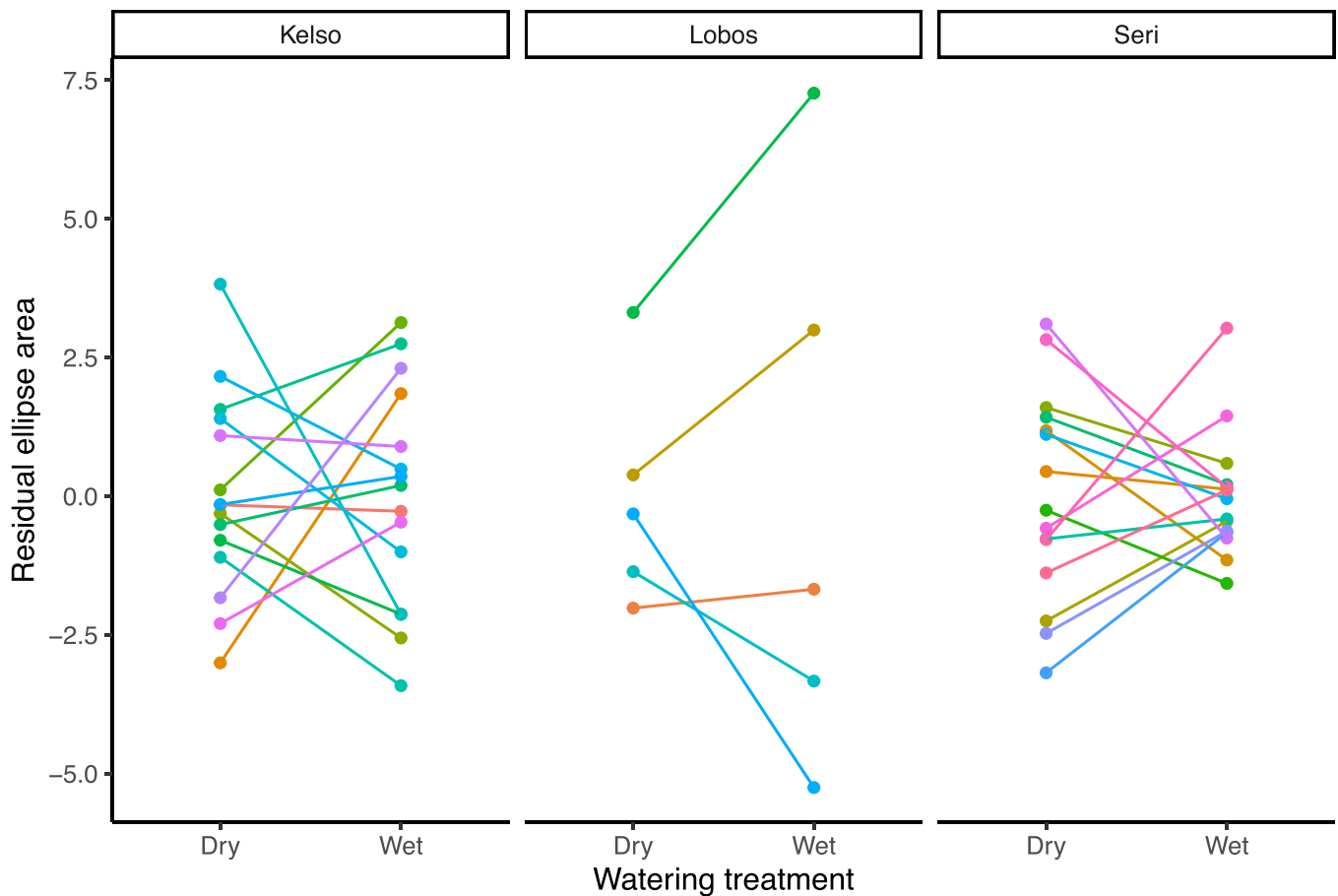


FIGURE 3 Norms of reaction of mean individual ellipse area in relation to water availability using residuals derived from the mean ellipse area from dams within diallels. Each graph represents a population that may include one to three 5×5 diallels.

(additive, maternal, and non-additive) in seed size were statistically significant as main effects in all three populations when watering treatments were pooled and the $G \times E$ interaction term was included in the model (Table 1). These patterns indicate that, under the conditions observed in this study, the opportunity for selection on seed size is predominantly through selection on phenotypic plasticity, which exhibits genetic variation in all three sampled populations. However, for seed size to evolve due to selection on plasticity would require plasticity to be correlated with both seed size and fitness. Estimating the strength or direction of phenotypic selection on plastic responses of seed size to drought was beyond the scope of this study, but to our knowledge, it is the first to detect genetic variation in phenotypic plasticity of seed size in multiple conspecific populations and for different sources of genetic variance.

Environment-specific genetic variation in seed size

Among greenhouse-raised genotypes sampled from three wild populations of *D. californica*, the three sources of genetic variance in seed size examined here were

undetectable when tested as main effects across watering treatments (with the exception of SCA in population L, Table 1). Neither a general parental effect (GCA) nor an extranuclear maternal effect (RGCA) was statistically significant in any of the populations sampled, indicating that narrow-sense heritability (see Eq. 3) was undetectable across the two watering treatments. However, the significant $G \times E$ interactions ($GCA \times W$: Table 1) found in all populations revealed that heritable variation in seed size was consistently environment-dependent. In the K and S populations, the genotypic ranks changed markedly between watering treatments without co-occurring changes in variance among genotypes (Figures 3 and 4). By contrast, in the L population (for which—due to mortality in the greenhouse—sample sizes were relatively low), we observed changes in both genotypic ranks and the variance among genotypes. In a heterogeneous environment in which the direction of selection on seed size is similar across environments, such changes in ranks can slow the response to selection because the identity of the genotype with the highest fitness is environment-dependent. Previous work on *Dithyrea californica*, however, has found that the direction and strength of selection on seed size depends on both water availability and conspecific density (Larios and Venable,

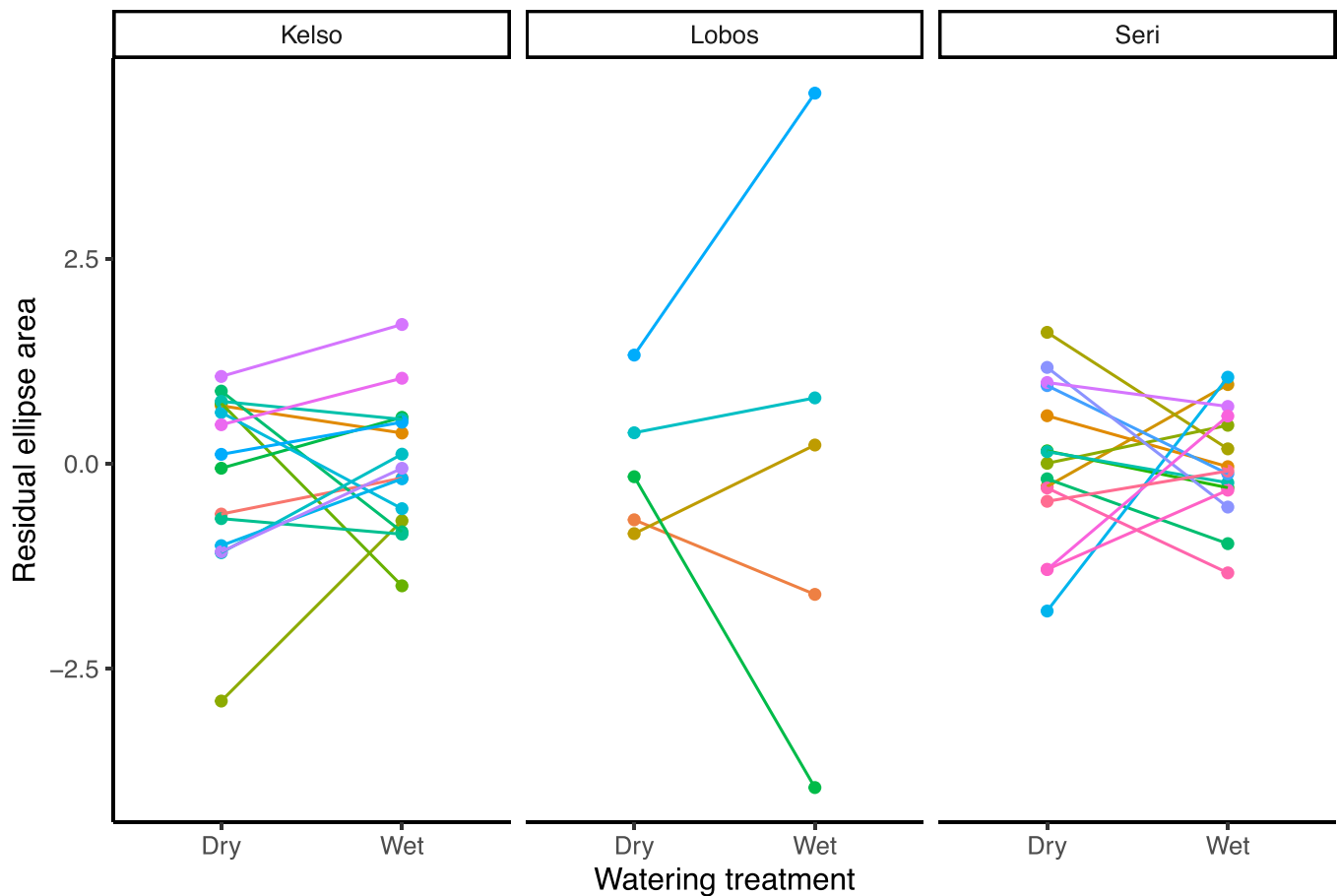


FIGURE 4 Norms of reaction of mean individual ellipse area in relation to water availability using residuals derived from the mean ellipse area from sires within diallels. Each graph represents a population that may include one to three 5×5 diallels.

2018), which makes the evolutionary trajectory of seed size difficult to predict in heterogeneous environments.

The $G \times E$ interactions detected in the current study for GCA, SCA, and RSCA (Table 1) demonstrate that the components of genetic variation on which natural selection depends are also environment-dependent, further complicating evolutionary forecasts. For example, SCA in S and GCA in L are significantly greater than zero under wet conditions but not under dry conditions (Table 2). Given that GCA reflects narrow-sense heritability in seed size, the opportunity for selection on this trait to generate an evolutionary response is higher in more mesic conditions. Moreover, the $G \times E$ interactions exhibited in Figures 3 and 4 reflect variation among genotypes in the direction of plastic responses to local water availability. These $G \times E$ interactions indicate that the expression of both GCA and RSCA in seed size are sensitive to drought. This genetically based variation in plasticity was expressed in all three populations and may be itself the target of natural selection (Schlichting, 1986; Schlichting and Pigliucci, 1998).

Only a few previous studies have attempted to measure $G \times E$ interactions in seed size (Schmitt et al., 1992; Sultan, 1996; Agrawal, 2001), and only one of them detected genetic variation in phenotypic plasticity in seed size. In *Raphanus raphanistrum*,

seed size exhibited genetic variation in phenotypic plasticity in response to herbivory (Agrawal, 2001). Second-generation maternal lines growing in greenhouse conditions were subjected to herbivory and control conditions and their resulting seed sizes measured. Similar to the current study, the direction of plasticity in seed size was genotype-specific; relative to control conditions, some maternal genotypes produced larger seeds under herbivory while others produced smaller seeds. The other two studies attempted to seek evidence for heritable variation in phenotypic plasticity but found no significant $G \times E$ interactions. In *Polygonum persicaria*, parental genotypes exposed to alternative levels of light, moisture, and nutrients maintained a relatively constant seed size in resource-limited conditions (Sultan, 1996). These genotypes exhibited little phenotypic plasticity in seed size, but responded to a reduction in abiotic resources by reducing fecundity, an unavoidable consequence of the trade-off between seed size and seed number (Smith and Fretwell, 1974). The author concluded that such maintenance of offspring quality over quantity, mediated by phenotypic plasticity in fecundity, is a mechanism of functional homeostasis that maintains individual seed quality in stressful environments. In *Plantago lanceolata*, seed size responded plastically to light availability when maternal plants were reciprocally planted between an abandoned field (low-light environment) and a

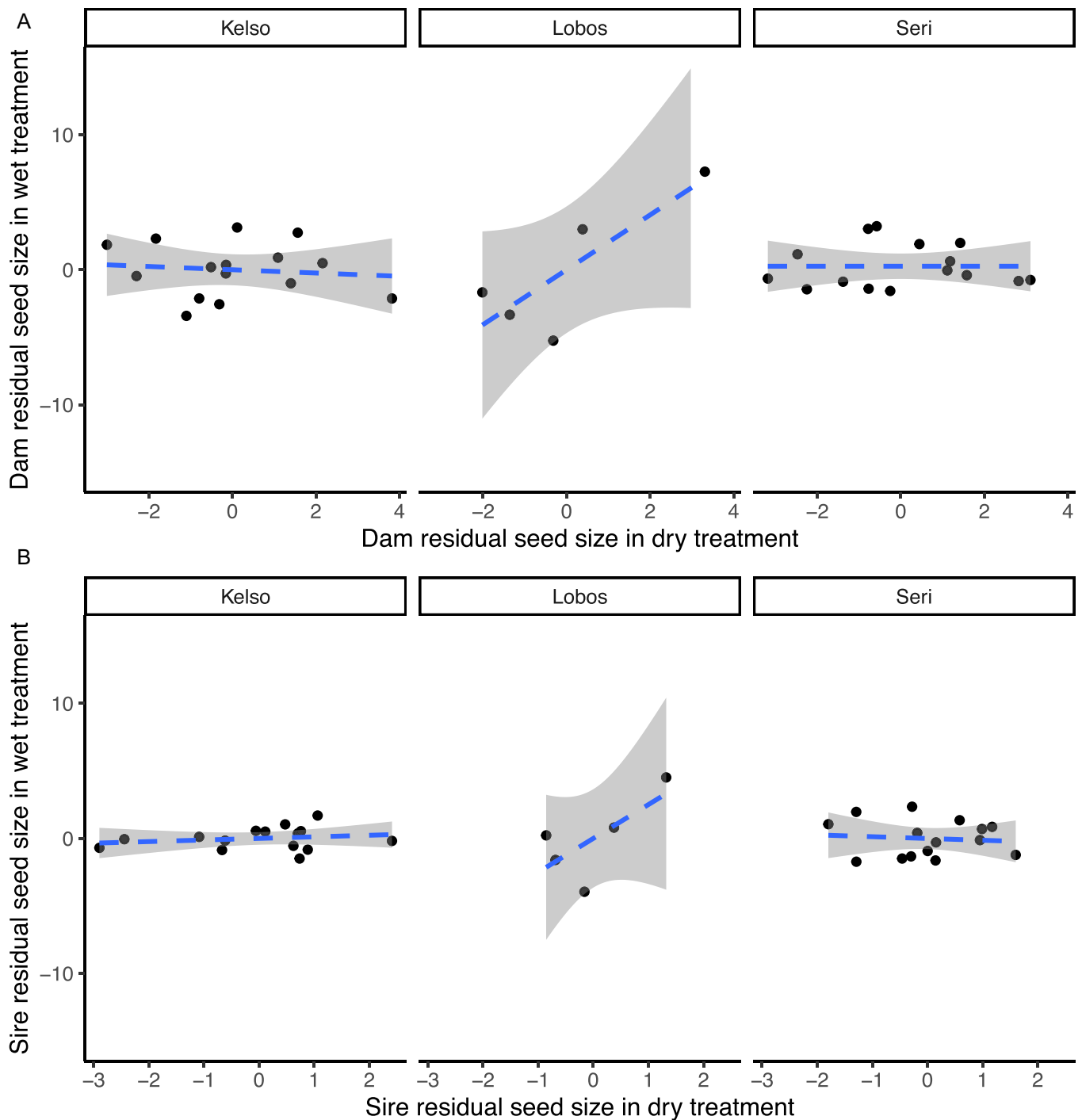


FIGURE 5 Bivariate plots showing the effect of watering treatment on dam and sire residuals for each of three populations of *Dithyrea californica*. (A) Top three graphs correspond to dams. (B) Bottom three graphs correspond to sires. Dashed fitted lines represent nonsignificant slopes; grey bands represent standard errors.

mowed lawn (high-light environment). Plants consistently produced heavier seeds when grown in the abandoned field than when grown in a mowed lawn, suggesting that low-light conditions induce an increase in offspring size (Schmitt et al., 1992). In a greenhouse experiment, the same maternal lines were grown in a factorial combination of two light and two nutrient environments. In this experiment, only with high

nutrients did seed size differ between light environments, and, in contrast to the field experiment described above, plants produced heavier seeds in high light, indicating a light \times nutrient treatment interaction but no $G \times E$ interaction. More studies are needed to elucidate the frequency among populations and species in which genetic variation in phenotypic plasticity in seed size is expressed.

TABLE 4 Parameters and test statistics derived from Pearson's correlations designed to test for (A) cross-environment correlations of mean individual residual ellipse area between watering treatments among parental genotypes functioning as dams and as sires; and (B) the potential for sexual conflict over seed size is assessed as the relationship between pollen donors (sires) and pollen recipients (dams) in alternative watering treatments for Seri, Lobos, and Kelso populations. *r*: Pearson's product-moment correlation coefficient of the bivariate relationship, CI: 95% confidence intervals.

A. Cross-environment correlations				
	<i>r</i>	CI	<i>t</i>	<i>P</i>
Seri				
Dam dry ~ Dam wet	0.00003	-0.512 to 0.512	0	1
Sire dry ~ Sire wet	-0.067	-0.578 to 0.439	-0.341	0.73
Lobos				
Dam dry ~ Dam wet	0.3	-0.203 to 0.988	2.55	0.08
Sire dry ~ Sire wet	0.19	-0.467 to 0.978	1.727	0.18
Kelso				
Dam dry ~ Dam wet	-0.09	-0.588 to 0.427	-0.393	0.7
Sire dry ~ Sire wet	0.324	-0.352 to 0.643	0.717	0.48
B. Sexual conflict				
	β	CI	<i>t</i>	<i>P</i>
Seri				
Sire dry ~ Dam dry	-0.83	-0.771 to 0.107	-1.708	0.11
Sire wet ~ Dam wet	0.594	-0.022 to 0.803	2.059	0.06
Lobos				
Sire dry ~ Dam dry	-0.13	-0.894 to 0.869	-0.097	0.929
Sire wet ~ Dam wet	-1.31	-0.987 to 0.245	-2.417	0.094
Kelso				
Sire dry ~ Dam dry	0.11	-0.441 to 0.576	0.33	0.747
Sire wet ~ Dam wet	-0.67	-0.692 to 0.272	-1.047	0.314

In *D. californica*, seed size has been found to respond plastically to two kinds of environmental conditions: water availability (this study) and conspecific density (Larios and Venable, 2015). The plastic responses to water availability were observed in controlled conditions between which genotypes were replicated and were not consistent in direction among sampled genotypes, precluding predictions of the effects of water availability on population mean seed size in genetically diverse populations. By contrast, the plastic responses to increased conspecific density were observed under field conditions, where maternal plants in a natural population responded to naturally occurring variation in density by altering the size of seeds produced; higher density resulted in lower mean seed size (Larios and Venable, 2015). The latter study did not replicate genotypes among density treatments, however, precluding the detection of genetic variation in phenotypic plasticity in seed size. Moreover, the effects of conspecific density on plant performance may be influenced by multiple physical factors that are modified by

crowding, including light and soil nutrient availability, so the specific factor driving the response of seed size to density could not be identified by Larios and Venable (2015).

Cross-environment correlations of mean seed size in alternative watering treatments

We found no evidence of significant cross-environment correlations between mean seed size produced by genotypes acting as dams or as sires (Table 4A, Figure 5). Maternal genotypes and paternal genotypes that produced relatively large (or small) seeds in the wet conditions did not produce relatively large (or small) seeds in the dry. The absence of such correlations suggests that natural selection on seed size that occurs under wet (or dry) conditions will not indirectly generate a correlated response of seed size expressed in dry (or wet) conditions.

Potential for sexual conflict in the determination of seed size

The norms of reaction for dams exhibited greater residual variance than those for sires in all populations and treatments, confirming that there is a greater opportunity for selection on seed size within dry or wet conditions based on maternal effects than on paternal effects (Figure 3 vs. Figure 4). This inference is consistent with the relative values of broad-sense and narrow-sense heritabilities shown in Table 3. We did not find, however, any evidence of a sexual conflict affecting mean seed size in *D. californica*, as suggested by the absence of a correlation between the mean seed size produced by genotypes as dams and as sires in both watering treatments (Table 4B, Figure 6). This result, however, may simply reflect the fact that in the samples of *D. californica* examined here, there is little variance in seed size due to either sire or dam identity alone (as shown by the nonsignificant effects of GCA; Table 1). With insufficient variation among sires or dams, it is not surprising that we did not detect a significant correlation (positive or negative) between the sexes, a result similar to that found in *Phlox drummondii* (Schlichting and Devlin, 1989). Nevertheless, outcrossing is thought to permit the evolution of sexual conflicts in plants. For hermaphroditic species (which characterize most angiosperm species), a conflict between the sexes can evolve because individual paternal donor genotypes benefit when their sired offspring acquire more resources than half-sibs (simultaneously developing within the same fruit or maternal individual), while maternal genotypes generally benefit when their resources are equally distributed among their offspring. Consistent with this inference, in *Dalechampia scandens*, sexual conflict increases with outcrossing rates (Raunsgard et al., 2018). In this study, Raunsgard et al. (2018) performed crosses between genotypes of *D. scandens* within and between four populations with different outcrossing histories. They found that maternal genotypes produced larger seeds than expected when pollinated by donors derived from highly outcrossing

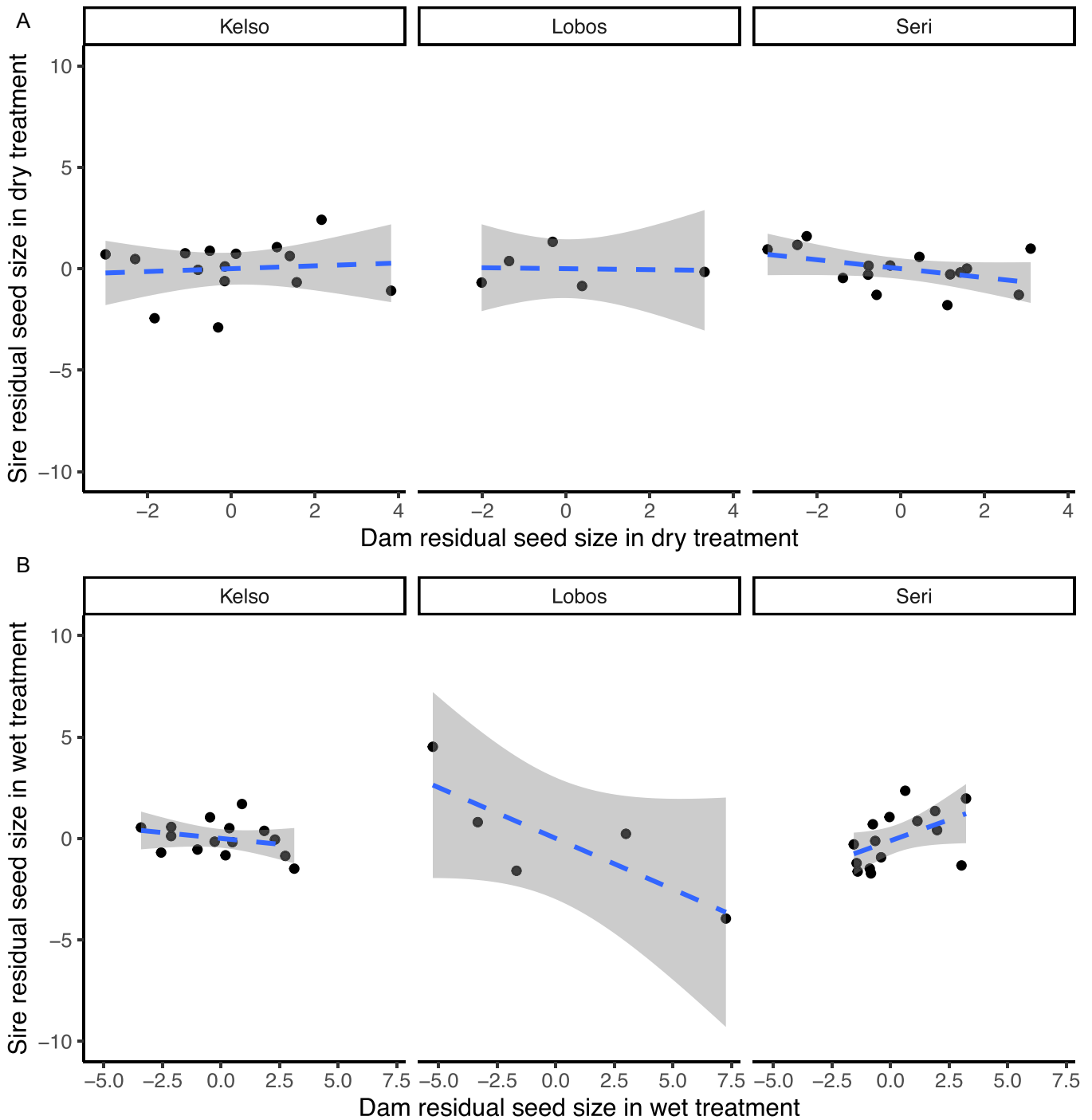


FIGURE 6 Bivariate plots showing the potential of sexual conflict on dam and sire residuals for each of three populations of *Dithyrea californica* and two watering treatments. (A) Top three graphs correspond to the dry treatment. (B) Bottom three graphs correspond to the wet treatment. Dashed fitted lines represent nonsignificant slopes; grey bands represent standard errors.

populations, which supports the hypothesis that high levels of outcrossing promote the evolution of paternal genotypes whose developing seeds can garner more nutrients from a maternal plant than paternal genotypes that evolved in populations with high levels of selfing. Similarly, when the maternal parent was derived from a more highly outcrossing population than that of the paternal genotype, the seeds produced by the maternal genotype were smaller than expected.

CONCLUSIONS

The strong interactions between several components of genetic variance and watering treatment observed in all three study populations of *D. californica* (Table 1) demonstrate that they harbor genetically based variation in both the direction and magnitude of phenotypic plasticity in seed size in response to water availability.

If the pattern of plasticity in seed size exhibited by a genotype influences its expected fitness in a heterogeneous environment, then phenotypic plasticity in seed size in *D. californica* has the potential to evolve by natural selection. Moreover, if the size of sown seeds has consistent effects on individual fitness across environmental conditions, then the environment-dependent additive genetic effects on seed size observed here in which genotypic ranks change between treatments (Figure 5) could slow the loss of additive genetic variation in seed size in a spatially or temporally heterogeneous environment (Schlichting, 1986). And, if the efficacy of natural selection on seed size is sufficiently constrained by the kinds of strong $G \times E$ interactions observed in *D. californica*, then both additive and non-additive genetic variance in seed size may be maintained in natural populations. Such sources of genetically based variance in seed size may contribute to the high phenotypic variance in seed mass routinely observed in this and other species, even in the absence of significant direct effects of environmental conditions on mean seed size.

AUTHOR CONTRIBUTIONS

E.L. collected seeds, carried out the experiment, and wrote the first draft of the manuscript. E.L. and S.J.M. contributed to the experimental design, analyzed data, and wrote the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data and analysis R code are available in the Dryad Digital Repository at <https://datadryad.org/stash/dataset/doi:10.25349/D9MW4M> (Larios and Mazer, 2022).

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