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Causes and Consequences of Seed Size Variation in the California-native annual species, *Nemophila menziesii*

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by

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ABSTRACT

Causes and Consequences of Seed Size Variation in the California-native Annual Species, *Nemophila menziesii*

by

Lisa H. Kim

I.

Seed size is a critical factor in determining various aspects of a plant's life cycle, such as germination rate, seedling size, and survival. The sources of variation in the trait are essential to understand its adaptive potential to changing environments. This study evaluates the genetic and environmental influences on seed size variation within and among four wild populations of *N. menziesii*, a widespread California annual herb. By leveraging a greenhouse breeding experiment with a nested half-sibling mating design, this study examines the magnitude of additive genetic variance and heritability of seed size within populations, the concordance between different heritability estimation methods, and the contribution of the maternal and paternal parents to seed size. Mean seed size differed significantly among populations, regardless of growing location, while the magnitude of plasticity in response to environmental conditions was similar among populations. The study revealed a disparity between the two methods used to estimate heritability – parent-offspring regression and the nested half-sibling analysis. We found evidence for maternal variance in

seed size in all four populations, but significant additive genetic variance was only present in two populations.

II.

Seed size is also a critical trait that affects life-history attributes, reproductive output, and overall fitness of adult plants. Despite the competitive advantages of larger seeds, seed size variation persists within and among plant populations due to additive genetic effects, non-additive genetic effects, environmental influences, and differential seed provisioning by the maternal sporophyte. This study investigates the factors contributing to seed size variation and its fitness consequences in the California-native annual species, *Nemophila menziesii*, across multiple conspecific populations that were reintroduced to their home environments following a one-generation greenhouse breeding experiment. Seeds produced in greenhouse conditions were generally larger than those produced in the field, and environmental factors were significant determinants of offspring seed size across populations. Parental genotype and phenotype effects on seed size were population-specific, with maternal influences tending to exceed paternal effects. The impact of maternal seed size on reproductive traits and fitness was also population-specific, with larger maternal seed size associated with increased fruit production in three of the four populations, and increased fecundity and reproductive yield in two of the four populations. Tradeoffs between seed mass and the number of seeds produced per fruit by a reproductive individual were detected in two of the four populations.

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I. Sources of variation in seed size within and among four populations of the California-native annual species, *Nemophila menziesii* (Boraginaceae)

A. Introduction

The mass of an individual seed produced by a maternal plant (hereafter, seed size) has been demonstrated to be strongly correlated with many attributes of the seed's life cycle (Baskin and Baskin, 1998) including germination rate (Harper et al., 1970; Cruden, 1974; Stanton, 1984; Biere, 1991), time to germination (Biere, 1991), seedling size (Wulff, 1986b; Roach and Wulff, 1987; Biere, 1991; Zhang and Maun, 1991; Swanborough and Westoby, 1996), seedling survival (Eriksson, 1999; Larson et al., 2020), and other functional traits such as vegetative biomass, lifetime fecundity, offspring mean seed size, and mean seed dispersal distance (Westoby et al., 2002). Larger seeds typically have higher germination rates and produce larger seedlings, providing a competitive advantage over smaller seeds. Smaller seeds, however, often have greater dispersibility (Harper et al., 1970; Primack, 1987) and greater persistence in the seed bank (Leishman et al., 2000), which may allow them to establish in new habitats and escape competition (Leishman et al., 2000; Westoby et al., 2002). These correlations indicate that the size of a sown seed often determines the resulting individual's lifetime fitness (Stanton, 1984; Mazer, 1987a; Westoby et al., 1992, 1996; Moles and Leishman, 2008). However, seed size itself is influenced by complex interactions between genetic and environmental factors attributed to both the maternal (Van Sanford and Matzinger, 1982; Lande and Arnold, 1983) and paternal individuals (Kempthorne, 1957; (Willham, 1963, 1972).

Quantitative genetic studies have traditionally partitioned the sources of seed size variation into its contributing components, which together make up the total phenotypic variance (Thomas and Rutledge, 1986; Mazer, 1987b; Schwaegerle and Levin, 1991; Platenkamp and Shaw, 1993; Shaw and Platenkamp, 1993; Shaw et al., 1995; Byers et al., 1997; Thiede, 1998; Cheplick, 2001). The phenotypic variance and covariance result from environmental as well as genetic components, which are made up of additive, dominance, and interaction effects of loci (Falconer and Mackay, 1981). The potential for adaptation to occur by natural selection on seed size depends on additive genetic (co)variance in this trait, which in turn determines its narrow sense heritability (h^2) (Lande and Arnold, 1983; Falconer and Mackay, 1981). The h^2 of a trait is defined as the proportion of its total phenotypic variation that is due to the effects on phenotype of nuclear genes, excluding the effects of genetic interactions (e.g., dominance and epistasis). h^2 is the proportion of phenotypic variation that can be attributed to additive genetic factor. In the case of seed size, h^2 provides an estimate of the potential for selection to cause evolutionary change in seed size.

The contributions of the maternal plant to seed size variation are complex and include both genetic and environmental effects (Cohen, 1966). Maternal effects on offspring seed size can be generated by nuclear genetic transmission of the maternal genotype (Mendelian transmission), extranuclear (cytoplasmic) transmission of genes, by differential resource allocation among seeds, by environmental variation, and by interactions between the seed genotype and the maternal plant (Roach and Wulff, 1987). In comparison, the paternal effect on seed size is typically small, and in some cases negligible (Alexander and Wulff, 1985; Antonovics and Schmitt, 1986; Roach and Wulff, 1987; Biere, 1991; Schwaegerle and Levin, 1991; Platenkamp and Shaw, 1993; Shaw and Platenkamp, 1993; Byers et al., 1997; Baskin

and Baskin, 2019). Since the paternal effect is largely attributed to nuclear genetic transmission and the contribution of the maternal and paternal genotype to offspring nuclear genotype are assumed to be roughly equal, paternal variance can be used to estimate additive genetic variance in seed size. Additive genetic variance (V_A) is the mean effect of a parent's nuclear genes transmitted to its offspring or, in this case, the direct contribution of the nuclear genotype to seed size. In half-sibling breeding designs such as the one used in the current study, it is estimated as four times the value of paternal variance (Fisher, 1930; Falconer and Mackay, 1981; Schwaegerle and Levin, 1991; Platenkamp and Shaw, 1993; Byers et al., 1997). Additive genetic variance is used as a parameter to estimate heritability using the following equation:

$$h^2 = \frac{4\sigma^2_{Pat}}{\sigma^2_{Tot}} = \frac{V_A}{V_{Tot}} = \frac{Additive\ genetic\ variance}{Total\ phenotypic\ variance}$$ [Eq. 1] (Falconer and Mackay, 1981)

Accordingly, when the paternal effect is not detected, it indicates that additive genetic variance in the observed trait, and by extension, heritability, are also not detected. High heritability in a trait indicates that variation within a population is strongly influenced by parental lineage such that offspring closely resemble parents. When the signal for additive genetic variance and heritability is weak, however, it is inferred that the primary causes of seed size variation are a combination of one or more of the following: non-nuclear genetic transmission, gene interaction (Kojima and Kelleher, 1961), the biotic or abiotic environment of the maternal plant, and the environment or position of seeds as they develop within the maternal plant ((Wulff, 1986a; Cheplick and Sung, 1998; Susko and Lovett-Doust, 2000; Guo et al., 2010).

Phenotypic plasticity – the change in phenotype in response to environmental conditions – has been shown to be a significant contributor to variation in seed size (Baskin and Baskin, 1998). The persistence of phenotypic variation among offspring produced from the same parental lineage (same mother and father) highlights the importance of considering both genetic and environmental factors in understanding the mechanisms underlying seed size variation and predicting the potential for evolutionary change. Environmental variation experienced by individuals prior to and during fruit development increases variation in seed size among related individuals (Roach and Wulff, 1987). It has been frequently demonstrated under experimentally controlled conditions that mean seed size decreases in stressful environments and increases in favorable ones, which are influenced by temperature (McWilliams et al., 1968; Alexander and Wulff, 1985; Wulff, 1986a; b), competition (Mazer and Wolfe, 1992; Shaw et al., 1995; Larios and Venable, 2015; Germain et al., 2019), and resource availability (Morse and Schmitt, 1985; Antonovics and Schmitt, 1986; Lawrence Venable, 1992; Thiede, 1998).

While environmental effects have been frequently demonstrated to have a strong influence on seed size (Roach and Wulff, 1987; Quarles and Roach, 2019), the potential for adaptation to occur by natural selection depends on whether there is genetic variation on which selection can act. For seed size to adapt to a population's local environment, seed size must be variable, heritable, and result in differential fitness among seed size phenotypes. High phenotypic plasticity may allow a population to respond more quickly to changing environmental conditions, but it may lower heritability [Eq. 1], thereby impeding the process of local adaptation by seed size evolution. On the other hand, high heritability – i.e., when the difference between total phenotypic variance and additive genetic variance for a trait is

small – indicates that variation in a trait is largely due to (additive) genetically based differences among individuals in the population, rather than differences in the environment. High h^2 can facilitate rapid evolution of a trait in response to selection because the offspring of individuals with higher fitness (which will represent higher proportions of the population in successive generations) are more likely to express similar phenotypes. Somewhat counterintuitively, theory suggests that fitness-related traits that have undergone directional selection over a long period should retain little genetic variance because the frequency of genotypes associated with lower fitness is steadily reduced (Fisher, 1930; Harper et al., 1970). However, if total phenotypic variation is reduced proportionally to additive genetic variance, heritability would remain high and retain the potential for adaptive evolution.

In addition to heritability, the strength and pattern of selection among genotypes within and among populations determines the potential for traits to evolve. Divergence of mean seed size among populations may indicate not only that selection has operated in the past, but also that the environmental conditions that populations experience cause differential patterns of selection to act on this functional trait. However, when natural selection on a trait leads to adaptive changes in mean seed size over successive generations, it may also reduce additive genetic variance, and thereby heritability, of the trait. Using quantitative genetic methods, we may partition total phenotypic variance of individuals with known pedigree into the distinct genetic and environmental factors that determine seed size expression.

This thesis is one of few studies (Larios and Mazer, 2022) that measures both maternal and paternal genetic influences on seed size variation – as well as environmental effects – in multiple wild populations. Chapter 1 focuses on estimating genetic sources of variation in seed size within and among four wild populations of *N. menziesii*. I address the following

questions to identify the most important sources of variation in seed size within and among populations and to make inferences concerning its adaptive capacity:

1. What is the magnitude of additive genetic variance relative to total phenotypic variance (i.e., the narrow-sense heritability, h^2) in seed size within populations?
2. Do different methods for estimating heritability (nested breeding design vs parent-offspring regression) yield concordant values?
3. Does the maternal environment and/or non-nuclear genetic transmission contribute to variation in seed size?

I examine the relative magnitude of parental genotype and the environment in shaping seed size variation in *Nemophila. menziesii* and I estimate heritability and phenotypic plasticity to evaluate the patterns of local adaption and adaptive potential of *N. menziesii* populations.

This work leverages a greenhouse breeding experiment in which, for each of four wild populations of a widespread California annual herb (*N. menziesii*), offspring of known parentage (or pedigree) were produced according to a nested mating design. I assess the potential of natural selection to operate on seed size by measuring the environmental and nuclear genetic effects on seed size. Studies have documented the evolutionary response of seed size to natural selection across intraspecific plant populations, (Ginwal et al., 2005; Saklani et al., 2012; Lönnberg and Eriksson, 2013; Sõber and Ramula, 2013; Christie et al., 2022), but a deeper understanding of the roles genetic sources of variation in fitness-related traits, and of the roles of natural selection and phenotypic plasticity in its evolution are essential to understanding the long-term dynamics of wild plant populations.

B. Materials and Methods

Study Species

Nemophila menziesii Hook. & Arn. (Boraginaceae), commonly known as Baby Blue Eyes, is a small annual herb endemic to the California Floristic Province, occupying the foothills and mountain ranges surrounding the Central Valley of California, the Transverse ranges in southern California, and the Coastal ranges from Tijuana to Oregon. It is typically found in grassy meadows and canyons in communities including wildflower species such as *Eschscholzia californica, Dichelostemma capitatum,* and species of *Clarkia, Sidalcea, Brodiaea., Calochortus,* and succulents such as *Dudleya sp.* (Jepson Flora Project). Populations have been observed at elevations from sea level up to 2000 m in its native range. This region experiences a Mediterranean climate in which summers are hot and dry, and winters are cool and wet; late spring to summer is typically the driest time of year, coinciding with the timing of senescence in this species. Seeds germinate in the winter and surviving individuals begin to flower in early spring. The growing season typically terminates in late spring following the production of mature fruit. The capsules produced by this species dehisce at maturity. Seeds are small; mean individual seed mass among the populations sampled here is 3.21 ± 1.43 mg (standard deviation) (n = 4 populations, 510 maternal families) in the wild and 4.64 ± 2.32 mg (n = 4 populations, 2550 seeds) in the greenhouse. Seed dispersal is passive (the seeds produce no accessory structures that promote their dispersal by wind or by animals) and therefore limited in range. The species is purported to be strictly gynodioecious, whereby, within populations, some individuals produce only bisexual flowers while others are only female, with the sex ratio varying among populations (Ganders, 1978; Barr, 2004). However, we have observed variation in sex expression among

flowers within greenhouse-raised individuals of all four populations sampled here (data not shown), suggesting that the breeding system may be less stable than previously reported. Individuals are self-compatible but self-pollination is avoided by protandry and dichogamy (Cruden, 1972; Andersson, 1994). Byers et al. 1997 reported that, among genotypes sampled from a single population of *N. menziesii* in southern California, variation in seed mass due to maternal effects were substantial, while paternal effects and interactions between parents were slight.

Sample Populations

From May to June 2018, seeds from at least 200 naturally pollinated maternal plants were collected from each of four wild populations of *N. menziesii* located in ecological reserves administered by the University of California's Natural Reserve System: Angelo Coast Range Reserve (Mendocino County), Bodega Marine Reserve (Sonoma County), Blue Oak Ranch Reserve (San Jose County), and Hastings Natural History Reservation (Monterey County) - hereafter referred to as "AC", "BB", "BO", and "HR" respectively (*Table 1.1*). Collectively, the populations that occur at these sites occupy a broad climatic gradient across the species' natural geographic range with respect to annual precipitation and mean annual temperature (*Table 1.1*). The two former sites (AC and BB) are more northern and occur in cooler and wetter climates, and the latter two (BO and HR) are more southern sites that are relatively warmer and drier (*Table 1.1*) (Wang et al., 2016). At each of the reserves, a population was selected for sample collection that was highly locally abundant, occupied a large contiguous area, and easy access. These wild populations of *N. menziesii* occupied open areas (AC and HR: in small meadows; BB: an oceanside bluff) or in oak woodland (BO).

At each of the sites, seeds were collected from individuals that were at least one square meter apart (to reduce the likelihood of sampling related genotypes) and assigned a unique numerical identifier (i.e., the maternal family). In rare cases in which population densities were extremely high, seeds assigned to a single maternal family may have been unintentionally collected from multiple overlapping and entangled individuals. When seeds were sown for use as maternal or paternal genotypes in the breeding experiment, however, only one (or infrequently, two) seed(s) per one-square-meter plot were used (described below) such that each maternal family used to produce pedigreed seeds was usually represented by only one individual. This sampling procedure facilitated the accurate estimation of population means for all traits subsequently measured and to meet the assumption that the genotypes used in the study represented a random sample of those found in the wild population.

Greenhouse Cultivation of Field-collected Seeds

From August 2018 to October 2019, the collected seeds (F0) were used in a breeding experiment to produce pedigreed F1 seeds under greenhouse conditions. The goal was for each population to be represented by 50 paternal sibships (200 paternal families total), with 3 maternal families nested within each paternal family. Constraints in space, time, and labor in the greenhouse made it necessary to raise subsets of the field-collected seeds from each population in successive cohorts. Each cohort included a maximum of 40 paternal families across all four populations. All populations were represented in each of the cohorts used in this portion of the study and individuals were bred only with members of their own

population. Environmental variation (such as variation in temperature and in ambient diurnal light cycles) that occurred among cohorts was therefore shared by all populations.

The breeding experiment was conducted across seven cohorts in total but only the first six cohorts were used for the analyses shown here because not all the populations were represented in the seventh cohort. Survival rates differed among populations, resulting in different numbers of paternal families represented in each cohort; the seventh cohort was created to achieve the intended number of families representing AC and HR (*Table 1.2*).

Field collected seeds were first treated with a 2.5% gibberellic acid solution to promote germination and then sown in a DI water-saturated coconut coir plug. The saturated plugs were autoclaved in a gravity cycle prior to seed introduction for at least 60 minutes to minimize contamination by mold and fungi. The seeds were germinated in these sterilized plugs in a dark cold room (maintained at ~ 5°C) and transferred to a refrigerated bench in the greenhouse when seedlings emerged. Plants experienced ambient photoperiod in greenhouse conditions. Seedlings developed in the alpine chilling-bench maintained at ~ 18-20°C and were transplanted into 8 x 6 cm diameter pots when the first true leaves emerged. They were then transplanted to 15 x 11.5 cm diameter pots when at least two pairs of true leaves were observed and transferred to a ~ 39 ± 5 [standard deviation] °C greenhouse. Among cohorts, the mean temperature was lowest in Cohort 3 (35 ± 4 °C) and highest in Cohort 1 (42 ± 3 °C) (*Table 1.2*).

The soil mix used consisted of coconut coir, perlite, vermiculite, and a slow-release organic fertilizer at a by-volume ratio of 4:8:3:0.005, respectively. Individuals in 15 cm pots were fertilized every two weeks with 200 mL of a dilute solution of 1 tbsp. MaxSea Bloom Plant Food (3-20-20 [N-P-K]) and 2 tsp. Peters Professional General Purpose Fertilizer (20-

20-20) per two gallons of DI water throughout their lifetime. Regular fertilization ensured that individuals were not resource-limited and were able to produce viable offspring in sufficient numbers for the field experiment described in the next chapter.

Nested Breeding Design

Each individual used in the breeding design was the progeny of a distinct field-collected maternal family. As individuals flowered in the greenhouse, healthy F0 individuals were selected to serve as either pollen donors (sires) or pollen recipients (dams) and randomly assigned to mating groups according to a nested breeding design (*Figure 1.1*). Occasionally, individuals selected to serve as pollen donors had to be reassigned to function as pollen recipients because they produced insufficient pollen. A single sire donated fresh pollen to three dams, such that three maternal families were nested within one paternal family. The offspring produced by different maternal plants within a paternal family were therefore half-siblings, while the offspring produced by a single maternal plant were full siblings. All pollinations occurred in the absence of insect pollinators.

The nested mating design is well-suited for this study because it offers a balance between feasibility and large sampling of genotypes within each population. It allows for the use of larger sample sizes relative to diallel mating and reciprocal cross designs, and results in less potential bias due to maternal effects relative to offspring-parent regressions. Importantly, the nested paternal half-sibship breeding design allows estimation of both paternal and maternal effects on progeny phenotype (Comstock and Robinson, 1948; Cockerham, 1963; Falconer and Mackay, 1981). In this design, if paternal sibships (a paternal sibship is defined as all F1 progeny that share a pollen donor, or father) differ with respect to a trait (i.e., the mean mass

of individual F1 seeds produced), this is defined as a "paternal effect" on offspring phenotype. Significant paternal effects (i.e., significant variance among paternal sibship means) on progeny phenotype indicate significant additive genetic variance among the paternal genotypes in the parental generation due to the transmission of paternally inherited alleles through the pollen (assuming no gene interactions and no paternal environmental effects). Similarly, a "maternal effect" on offspring phenotype is detected when the progeny of greenhouse-grown maternal families (i.e., all F1 progeny derived from the same seed-bearing dam in the greenhouse generation) differ significantly with respect to a trait.

Prior to pollination, the hermaphroditic flowers of dams were emasculated (anthers removed) within the first two days of the flower buds' opening – when stigmas were not yet receptive, and the anthers had not yet dehisced – to reduce the possibility of self-pollination. The pollen was then transferred by hand directly from the anthers of a pollen donor's (i.e., sire's) flower onto all receptive stigmas of the dams' flowers. A stigma was considered to be receptive when its tip was forked and the two stigma branches were recurved, darkly pigmented and slightly swollen at their tips, and sticky. Over the course of two to three weeks, approximately 50-100 flowers per maternal plant were hand-pollinated with pollen from its assigned sire. Hand-pollinated flowers were labeled by placing a strip of colored tape on the stem beneath the pedicel and then allowed to mature on the maternal plant. Any flowers or developing ovaries that were not marked with tape were removed. Fruits were usually collected directly prior to dehiscence when all or most of the capsule was dry, the fruit wall had changed from green to purple, the calyx was dry and brown, and the peduncle was rigid and curved. Fruits were placed in manila coin envelopes stored in desiccant to continue to ripen and dry at ambient temperature.

Seeds were then separated from the surrounding fruit tissue and examined for viability. Seeds were determined to be viable when they were filled, and the seed coat was convexly rounded and did not have a deflated or wrinkled appearance. For each dam, mean seed mass, and the individual mass of five arbitrarily selected seeds were measured. The five seeds were weighed singly on a Cahn C-30 Microbalance to the nearest 10 µg to obtain individual seed mass. In addition, 25 – 100 seeds (97 ± 13 [mean ± SD] seeds) per maternal plant were arbitrarily chosen to be batch weighed to the nearest 0.1 mg to obtain estimates of mean seed mass produced by each F1 individual. For each of the maternal plants, approximately 30 F1 seeds were arbitrarily chosen to contribute to the F1 generation (see next chapter). The seeds were cleaned, placed in glassine envelopes within airtight plastic bags with desiccant, and then transferred to a cold room (~ 5°C) for storage until used in subsequent experiments.

Ultimately, AC was represented by 37 paternal sibships each with two or three maternal plants nested within (n= 98 maternal families); BB was represented by 50 paternal and 149 maternal families; BO was represented by 48 paternal and 140 maternal families; and HR was represented by 42 paternal and 123 or 124 maternal families (*Table 1.2*). For HR, the number of maternal families used in the analyses of the effects of growing location on mean individual seed size and in the estimation of heritability were 123 and 124, respectively, because the mean size of the field-produced seeds (F0) was not measured in one maternal family. Across the six cohorts, no sire or dam was duplicated in the breeding design except in rare cases when a maternal family that had been used previously as a dam was reused in another cohort as a sire or vice versa. The only exception to this was in the BB population, in which one maternal family was unintentionally duplicated in two separate cohorts. In this case, the two individuals were each assigned different sires.

Data Structure and Analysis

Two datasets were assembled and analyzed for this study. The first dataset was used to determine, for each population, whether field-collected (F0) seeds differ in mean individual seed mass from those produced in the greenhouse (F1) following cross-pollination of the adults grown from field-collected seeds. The second dataset was used to estimate genetic and environmental components of variation in individual seed mass and included only the F1 seeds produced in the greenhouse. Each of these datasets are described in detail, as follows.

Effect of Seed Source (Field vs. Greenhouse) on Seed Mass: Dataset

The first dataset was used to quantify the effect of growing location – field (F0) vs. greenhouse (F1) – on mean individual seed size (n = 510 individuals representing four populations). The number of maternal families differed among source populations (AC: n = 98; BB: n = 149; BO: n = 140; HR: n = 123) (*Table 1.2*, *Figure 1.2*). This dataset was composed of individual plant means based on the seeds that were bulk weighed for each maternal family. All viable seeds of a given maternal family (2-10 seeds) collected directly from the source populations were bulk weighed to determine mean seed size in the F0 generation. For the F1 seeds that were produced in the greenhouse, 25-100 seeds were bulk weighed to determine the mean seed size of each maternal family. Maternal families that produced fewer than 25 seeds in the F1 generation were excluded from the study because there were not enough replicates for use in the field study described in Chapter 2 of this thesis. Only the maternal families for which mean individual seed size was recorded in both

the F0 (source seeds) and F1 (greenhouse-produced seeds) generations were used in the analyses presented here.

Genetic and Environmental Sources of Variation in Individual Seed Mass: Dataset

In the second dataset, five viable F1 seeds from each greenhouse-raised maternal family were weighed individually to estimate heritabilities of seed size in each population. A total of 2585 seeds were weighed among the four populations: AC was represented by 37 paternal families and 98 maternal families (n = 490 seeds), BB was represented by 50 paternal and 150 maternal families (n = 745 seeds), BO was represented by 48 paternal and 143 maternal families (n = 700 seeds), and HR was represented by 42 paternal and 124 maternal families (n = 620 seeds) (*Table 1.2*).

Effect of Seed Source (Field vs Greenhouse) on Seed Mass: Analysis

We examined the mean seed size of the four populations in each generation by conducting an analysis of variance (ANOVA). When untransformed, maternal family mean seed sizes were non-normally distributed and right-skewed in both the F0 (Shapiro-Wilk: W = 0.975, P = 1.23e-7) and F1 (Shapiro-Wilk: W = 0.965, P = 1.223e-9) generations for all populations. For all analyses, seed size was thus square root transformed which greatly improved the normality of residuals, but the distributions remained non-normal (F0: W = 0.990, P = 0.002; F1: W = 0.993, P = 0.02) (but see Scheffe, 1999; Bradley, 1960; Falconer and Mackay, 1981; Mitchell-Olds and Shaw, 1987 for approaches to violations of normality assumptions). Two-way ANOVAs with post-hoc Tukey HSD tests using the *stats* package in R version 4.2.1 (R Development Core Team 2022) were used to detect statistically

significant differences among populations in mean seed size within the two generations of seeds produced. Then, within populations, ANOVAs were conducted to detect significant effects of growing environment on mean seed size. Additionally, in a bivariate linear regression using the *lm* function in R, we determined the significance and magnitude of growing environment in each population.

Genetic and Environmental Variance Components in Individual Seed Mass: Analysis

Heritability values were estimated separately for each population because its value depends on many factors that may differ among populations, including population size (which influences the potential for genetic drift), mating system, history of natural selection, and gene flow from other populations. Within populations, heritability of seed mass was estimated as the proportion of total phenotypic variance attributed to additive genetic variance in seed mass observed in the sampled genotypes. Additive genetic variance was estimated as four times the paternal variance component for each population [Eq. 1]. Mean seed size significantly differed among cohorts and the change in seed size was not consistent among populations. Thus, individual seed size was centered by the mean of each population and cohort combination to account for variation in seed size among cohorts. Variance components were estimated from a mixed effects linear regression using maximum likelihood (ML) for parameter estimation using the function *lmer* (package *lme4* version 1.1-31). Paternal family and maternal family (nested within paternal family) were included as random effects and individual seed mass was treated as the response variable. The effect of F0 mean seed size on F1 mean seed size was also included as a fixed effect when it significantly improved the model fit in comparison to the reduced model. Model comparisons

were made using Likelihood Ratio Tests with the function *anova* (package *stats* version 4.2.1) to compare models with added parameters to the reduced model which only included an intercept and the maternal effect nested within paternal effect as a random effect. The full model for the mixed effects linear regression is:

$$Y_{ijk} = \mu + p_i + m_{ij} + s_{ij} + e_{ijk}$$

where Y_{ijk} is the individual seed mass of the kth offspring of the ith paternal parent and the ijth maternal parent, μ is the population mean, p_i is the effect of the ith paternal parent, m_{ij} is the effect of the ijth maternal parent mated to the ith paternal parent, s_{ij} is the ijth effect of F0 mean seed mass of the maternal family, and e_{ijk} is the experimental error.

C. Results

Differences in mean seed mass among field-collected populations

Collectively, the mean seed mass of maternal families collected directly from source populations (F0) varied over 40-fold, ranging from 0.21 to 9.07 mg (3.21 ± 1.43 mg, n = 510 maternal plants). The mean seed mass of plants growing at the two northern, mesic sites, AC (1.51 ± 0.63 mg) and BB (4.06 ± 1.24 mg) were respectively the smallest and largest-seeded populations of the four examined, despite having similar mean annual temperature and greater annual precipitation relative to the two southern sites. The population mean seed mass of AC was significantly lower than all other populations. The population mean seed mass of the two drier southern sites, BO (3.89 ± 1.05 mg) and HR (2.79 ± 1.10 mg) differed significantly from each other, but only by a factor of 1.4. The population mean seed mass of BB and BO did not differ significantly from each other (*Table 1.3, Figure 1.3A*) and were the largest-seeded populations of the four examined.

Differences in mean seed mass among greenhouse-raised populations

In the breeding experiment, the variation among greenhouse produced seeds was roughly half that of the field-collected maternal families of F0 seeds; the mean F1 seed mass of maternal plants varied by 19-fold, ranging in magnitude from 0.72 to 13.83 mg (4.64 ± 2.32 mg, n = 510 maternal plants). The population mean seed mass produced by genotypes collected from the relatively mesic populations (AC and BB) were 2.10 ± 1.22 mg and 5.20 ± 2.06 mg, respectively. The population mean seed mass produced by genotypes from the relatively xeric populations (BO and HR) were 5.78 ± 2.13 mg and 4.69 ± 1.99 mg,

respectively (see *Table 1.3* for square root transformed values). Similar to the case for F0 seeds, the F1 mean seed mass of the AC population was significantly lower than all other populations. While the F1 mean seed mass of the BO and HR populations differed significantly from each other, that of BB did not differ from either (*Figure 1.3B*); and BO remained the largest-seeded population of the four examined.

Differences in mean seed mass between generations

The maternal mean mass of seeds of the F0 generation (3.21 ± 1.43 mg) differed from the mean mass of seeds produced in the F1 generation (4.64 ± 2.32 mg) (Welch Two-Sample t-test: $N = 1020$, df = 944.14, $T = -11.165$, $P = < 0.001$). Seeds produced in the greenhouse tended to be larger than seeds produced in the home environment and there was no significant difference in magnitude among populations. The estimated change in back-transformed mean seed size per population was 0.167 ± 0.02 mg (mean ± standard error), 0.170 ± 0.01 mg, 0.017 ± 0.02 mg, 0.078 ± 0.02 mg, 0.518 ± 0.003 mg for each population, AC, BB, BO, HR, and with all populations pooled, respectively (*Table 1.4, Figure 1.4B*).

Genetic and environmental variance components for individual seed mass

The mass of five seeds per maternal family, weighed individually, provided replicate measures within maternal families. A total of 2250 pedigreed seeds were weighed (AC: $N = 490$, BB: $N = 745$, BO: $N = 700$, HR: $N = 615$). Individual seed sizes were centered by their corresponding population and cohort means. Seed size varied most among populations (40% of variation) and least among paternal sibships (half-siblings) nested within populations (7%

of variation). Variation among maternal genotypes nested within paternal sibships was 29%. The residual variation (i.e., variation within plants) was 24%.

The AC and BB populations showed no significant additive genetic variance (estimated by the paternal variance component) (*Table 1.5),* indicating that heritability was low or negligible (*Table 1.6).* The populations BO and HR showed significant additive genetic variance and estimates of heritability, indicating that seed mass is in part, determined by nuclear genetic transmission from parent to offspring in these populations. Additive genetic variance accounted for, respectively, 32.1% and 48.1% of total phenotypic variance in BO and HR. However, relative to the contribution of the maternal parent to seed mass, the paternal effect was small. In all populations, the maternal variance component was significantly greater than zero. In AC and BB, the effect of F0 seed size, or the previous generation's mean maternal seed size, positively influences the individual seed sizes of offspring. In BO and HR, this effect was not significant, and was removed from the models reported in Table 1.6 to estimate variance components.

D. Discussion

In this study, I investigated the sources of seed size variation and heritability in four populations of *N. menziesii*, examining the influence of maternal effects, paternal effects, and the growing environment. The results showed that maternal effects had a stronger influence on seed size than other genetic effects. In the northern mesic populations (AC and BB) no evidence of additive genetic variance was detected. In the southern xeric populations (BO and HR), both maternal and additive genetic variance was observed, indicating substantial genetic variation in seed size for selection to act upon in these populations.

Mean seed size differs among populations regardless of growing location

The comparison of F0 and F1 seeds was conducted to assess whether differences in mean seed size among population observed in the field were maintained when they were bred under greenhouse conditions. Except for populations BB and BO (which did not differ from each other), mean seed size differed among all populations (*Figure 1.3A)* when grown in the same greenhouse environment. We then compared mean seed size among populations produced in the greenhouse to determine whether a significant genetic component accounts for phenotypic divergence among populations when cultivated in shared environmental conditions. While populations AC, BO, and HR differed significantly in seed size regardless of growing location, BB was similar to both BO and HR (*Figure 1.3B)*. The relative mean seed sizes of the four populations did not differ qualitatively between the F0 and F1 generations (based on post-hoc Tukey HSD tests).

The magnitude of plasticity in seed size is similar among growing locations

On average, maternal families across all populations produced larger seeds in the greenhouse, where they were neither water- nor nutrient-limited. The mean change in seed size did not differ among populations (*Table 1.4, Figure 1.4B*) indicating that the plastic response of maternal families (genotype-environment interaction) was similar among populations (Kojima, 1961; Cockerham, 1963). Similar magnitudes of change in maternal mean seed size among growing locations (mean slope of pooled populations = 0.83 ± 0.09, *N = 510; F* = 209.1; *P* = < 0.001***) suggest that variation in seed size phenotype is maintained at least in part through plasticity and that the magnitude of plasticity is similar among the four populations. Furthermore, these results indicate that the mean seed size of a maternal family (F0) has a significant and positive effect on the mean seed size of its offspring (F1) in three of the four populations. This effect was only significant in two of the four populations however when analyzing the data comprised of individual seed size (*Table 1.5 & Table 1.6)*. Maternal mean seed size has a transgenerational effect on individual seed size in two of the four populations, but this pattern was detected in three of the four populations when examining the relationship among inter-generational maternal family means.

Parent-offspring regression vs Half-sibling analysis

Two methods described and used extensively in the literature were used in the current study to estimate genetic covariance and heritability: parent-offspring regression and half-sibling nested-breeding (Robinson and Comstock, 1949; Kempthorne and Tandon, 1953; Falconer and Mackay, 1981; Scheiner and Lyman, 1989; Mazer and Schick, 1991; Larios et

al., 2023). The strength of the effect of F0 seed mass on F1 seed mass based on the parent-offspring regression (*Table 1.4*) and the single-generation analysis of F1 seeds alone with the maternal effect included as a random effect (*Table 1.6*) show a disparity among the two methods for estimating heritability in seed size. The parent-offspring regression indicated that the maternal effect was positively correlated to F1 seed size in populations BB, AC, and HR (ordered by highest to lowest slope estimates) and was not significant in BO. While the half-sibling analysis found evidence for the heritability of seed size in populations HR and BO (ordered by highest to lowest maternal variance estimates), but none in AC and BB. The discrepancy between the two methods in estimating the heritability of seed size highlights the complexity and challenges in accurately assessing genetic covariance and heritability in plant populations.

Maternal effects more strongly influence seed size than any other genetic effects

In our study of the heritability of seed size in four populations of *N. menziesii*, we found significant maternal variance in all populations but no evidence of additive genetic variance in the populations sampled from the two northern mesic sites (AC and BB). In populations AC and BB, 66% and 57%, respectively, of the total phenotypic variance was attributed to the maternal genotype. While there is significant genetic variation in seed size within these populations, it is attributed to the maternal genotype (including the additive effects of its diploid sporophyte genotype as well as potential non-nuclear, cytoplasmic genetic effects on offspring seed size), rather than to additive genetic variance. This is generally consistent with previous research on *N. menziesii* (Platenkamp and Shaw, 1993; but see Byers et al., 1997). Byers et al. (1997) used a reciprocal factorial mating design to estimate the additive genetic

variance component for seed size in a single population as 0.5% of total phenotypic variance, while the maternal variance component estimated in the study was 15% of total phenotypic variance. The disparity between maternal and additive genetic variance estimates from the current study may, in part, be attributed to overestimates of paternal and maternal variance components by the nested breeding design. However, our results corroborate the findings of Byers et al.'s (1997), that seed size is largely determined by the maternal environment and genotype, and that substantial genetic variation among maternal plants is present for selection to act upon in these populations. Our findings indicate that seed size may be largely determined by the maternal sporophyte genotype and by extra-nuclear transmission of non-additive genetic effects.

In contrast, we observed significant maternal variance and additive genetic variance (and paternal variance) for seed size in the southern xeric populations, BO and HR. In populations BO and HR, 8% and 12%, respectively, of total phenotypic variance were attributed to paternal variance and 32% and 53%, respectively, were attributed to maternal variance. Similar to the findings of Byers et al. (1997), these populations exhibit substantial maternally and paternally inherited genetic variation in seed size on which selection may operate. As a result, population BO had a heritability estimate of 0.321 and HR had a heritability estimate of 0.481 (*Table 1.6*). This is much higher than previously published estimates of the heritability of seed size in other wild species. For example, in single population studies, Antonovics and Schmitt (1986) reported that $h^2 = 0.04$ in *Anthoxanthum odoratum*, Mitchell-Olds and Bergelson, (1990a) reported that $h^2 = 0.27$ in *Impatiens capensis*, Biere (1991) reported that $h^2 = 0.02$ in *Lychnis flos-cuculi*, Platenkamp and Shaw (1993) reported $h^2 = 0.04$ in *N. menziesii*, Schwaegerle and Levin (1991) reported that $h^2 = 0.02$ in *Phlox drummondii*),

and Mazer (1987b) reported that h^2 = 0.03 and 0.08 in *Raphanus raphanistrum*). Etterson (2004) found similar estimates of heritability for life history traits (i.e., reproductive stage and leaf number) in one of two populations examined using a nested breeding design. And Larios and Mazer (2022) found that h^2 varied from 0.083-0.213 among three populations of *Dithyrea californica.* Estimates of h^2 derived from other wild species are reviewed in Larios et al. (2023). The relatively high heritability estimates determined in the current study suggest that heritability estimates are highly sensitive to growing conditions, which is more closely examined in the following chapter of this thesis. Despite this, while comparing heritability estimates among populations in the current study, we may conclude that the estimates are effectual and robust. These findings indicate that strong selective pressures have not acted in the past to eliminate additive genetic variance for this trait and adaptive potential for seed size is maintained.

E. Conclusion

Our study found that mean seed size has diverged significantly among wild populations of *Nemophila menziesii*, but the plastic response to environmental conditions did not differ significantly among populations. This suggests that variation in seed size is maintained through plasticity and strongly regulated by maternal genetic components present in multiple populations. Our analysis of heritability showed that while there was significant maternal variance in seed size in all four populations, significant nuclear genetic transmission (additive genetic variance) was only present in two. These findings have important implications for scientists studying the mechanisms that enable species to persist and adapt to their environments. Identifying genetic factors that contribute to seed size may allow for the targeting of conservation and restoration efforts toward populations with higher adaptive potential. While our study examined only four populations of *N. menziesii*, in a study of 19 native populations of *N. menziesii* aggr. (*N. atomaria, N. menziesii, and N. integrifolia*) across California, Cruden (1974) found that adaptations in germination responses were ecoclinal with respect to both latitude and elevation. Furthermore, these ecoclinal differences were characterized by differences in temperature, light availability, and water availability. While investigation of these climatic characteristics was not feasible in the scope of the current study, our findings do not contradict the inference that adaptation in life-history traits may be ecoclinal. Future research identifying the sources and causes of intraspecific variation in life-history traits across a greater number of populations occupying a broader geographic range could provide valuable insights into the adaptive potential of species in a changing climate.

F. Tables and Figures

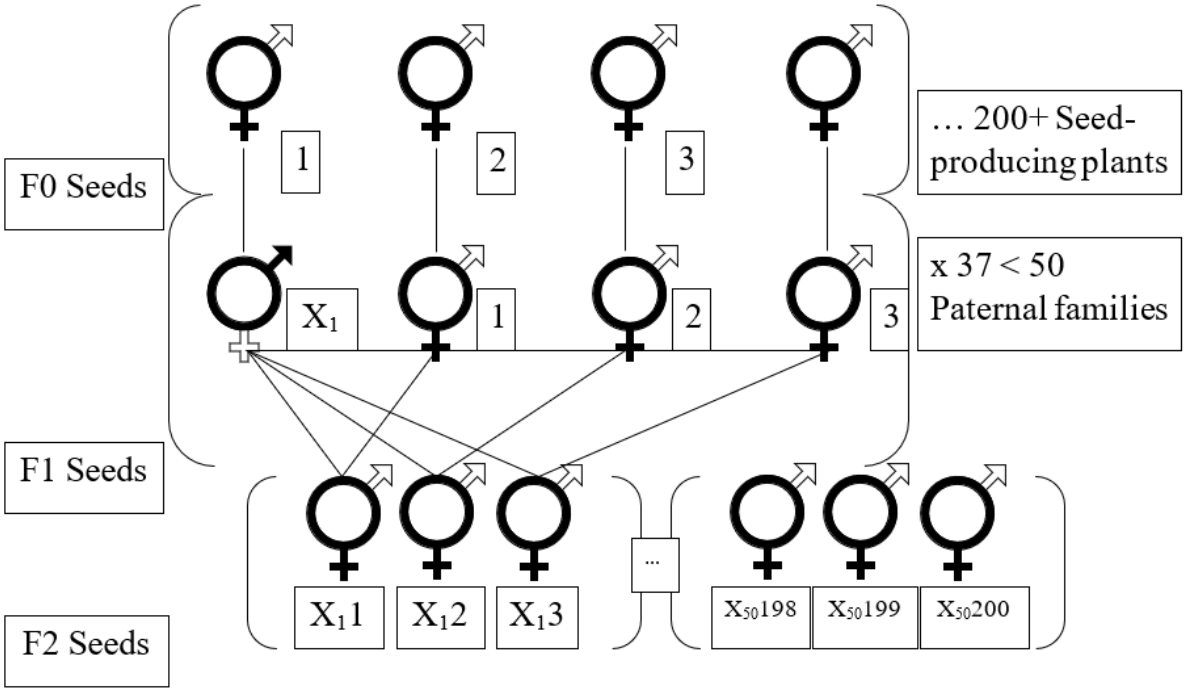


Figure 1.1: Design of one population of the three-generation design for one population. Illustrates some of the seed-producing crosses and cross identities (maternal and paternal family) for a single population. The plants are hermaphroditic with their functional sex indicated by wide-lined symbols.

Site	Latitude	Longitude	Annual PPT (mm)	Spring PPT (mm)	Annual T (°C)	Spring T (°C)	Elev. (m)
AC	39.7469	-123.6380	2135.6 ± 637.1	541.4 ± 255.8	12.3 ± 0.58	10.5 ± 1.1	567
BB	38.3146	-123.0694	910.5 ± 262.9	222.7 ± 118.6	12.43 ± 0.53	11.7 ± 0.9	2
BO	37.3870	-121.7158	670.4 ± 203.5	185.0 ± 101.7	14.77 ± 0.58	12.4 ± 1.1	728
HR	36.3783	-121.5662	729.9 ± 222.5	187.5 ± 100.9	14.78 ± 0.59	12.4 ± 1.0	603

Table 1.1: Study sites in California, U.S. Angelo Coast Range Reserve (AC), Bodega Marine Reserve (BB) Blue Oak Ranch Reserve (BO), Hastings Natural History Reservation (HR). Included are geographic coordinates of each respective site, elevation, and 30-year climate normals (means) (± SD) of mean annual precipitation (PPT), mean winter PPT, mean spring PPT, mean annual temperature (T), mean winter T, and mean spring T observed from 1991 – 2020 (Climate NA).

			Population							
			AC		**BB**		**BO**		**HR**	
Cohort:	Sow Date:	Mean temp (°C) ± SD	Sires	Dams	Sires	Dams	Sires	Dams	Sires	Dams
1	Aug 2018	41.7 ± 3.5	6	18	9	27	7	21	7	19/20
2	Sept 2018	38.5 ± 4.7	5	11	9	27	9	27	5	14
3	Dec 2018	35.1 ± 3.5	8	21	10	30	4	11	9	27
4	Feb 2019	35.6 ± 3.3	4	10	10	30	5	15	7	21
5	Mar 2019	35.4 ± 5.2	10	29	9	27	13	37	8	24
6	Apr 2019	36.3 ± 3.8	4	9	3	8	10	29	6	18
		Total	**37**	**98**	**50**	**149***	**48**	**140**	**42**	**123/124**

Table 1.2: Number of paternal and maternal families in each cohort for each population. See Table 1.1 for population abbreviations. Seed sowing date and mean greenhouse temperature ± standard deviation for each cohort is shown. *In BB, one maternal family was repeated as a maternal individual (dam) in two separate cohorts, resulting in 149 dams but only 148 distinct maternal families. In HR, the number of dams shown before the backslashes represents the number used in the comparative analyses between growing locations while the numbers following the backslashes show the number of dams (maternal families) used to estimate heritabilities. The number of dams differs due to data missing from one of the field-collected seeds.

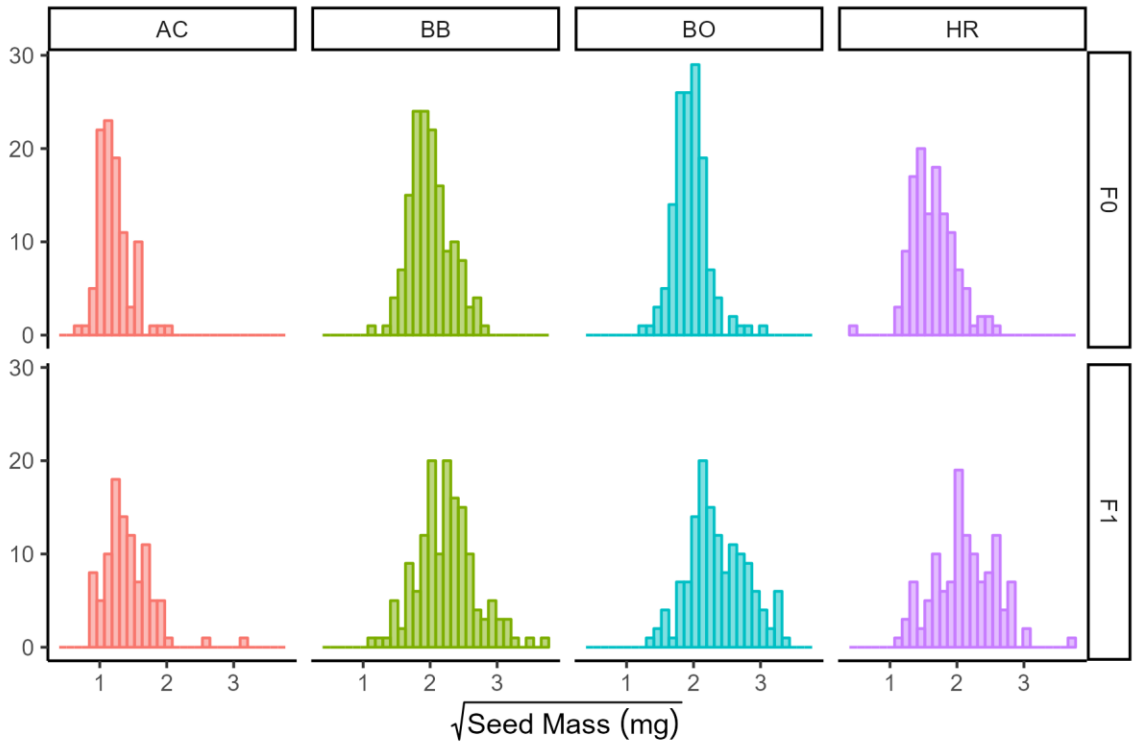

AC
BB
BO
HR
F0
F1
30
20
10
0
1
2
3
√Seed Mass (mg)

Figure 1.2: Histograms showing the distribution of square root transformed means of individual seed mass of maternal families for each population. (A) mean size of seeds collected directly from the source populations (B) mean seed size of seeds produced in the greenhouse breeding study.

Population: AC					
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**
Generation	2	1.996	22.24	< 0.001	0.098
Error	194	17.415			
Mean seed mass	***N***	**Est.**	**SE**	***T***	**Grouping**
F0 (Intercept)	98	1.205	0.03	39.83	b
F1	98	0.202	0.043	4.716	a
Population: BB					
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**
Generation	1	4.5	31.59	< 0.001	0.093
Error	296	42.13			
Mean seed mass	***N***	**Est.**	**SE**	***T***	**Grouping**
F0 (Intercept)	149	1.993	0.03	64.474	b
F1	149	0.246	0.044	5.621	a
Population: BO					
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**
Generation	1	11.54	89.61	< 0.001	0.241
Error	278	35.8			
Mean seed mass	***N***	**Est.**	**SE**	***T***	**Grouping**
F0 (Intercept)	140	1.957	0.03	64.515	b
F1	140	0.406	0.043	9.466	a
Population: HR					
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**
Generation	3	14.25	91.62	< 0.001	0.27
Error	244	37.94			
Mean seed mass	***N***	**Est.**	**SE**	***T***	**Grouping**
F0 (Intercept)	123	1.64	0.036	46.118	b
F1	123	0.481	0.05	9.572	a

Table 1.3: Type I ANOVA to determine change in mean seed size among generations, F0 and F1 seed size as the dependent variables (sqrt-transformed). Analysis was conducted separately for each of the populations. Linear regression models were used to obtain coefficients, standard errors, t-values, significance level, and adjusted R^2.

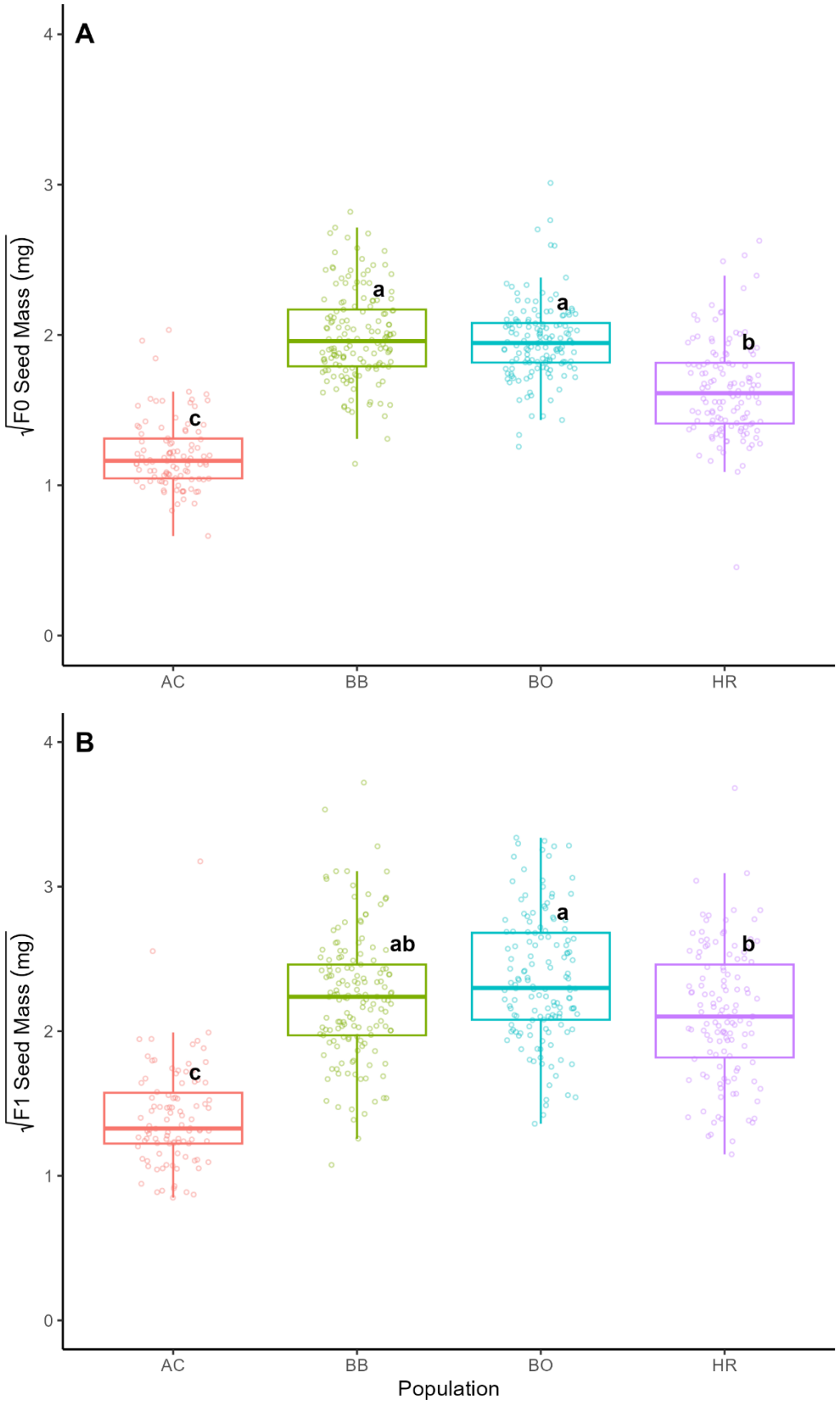

A
√F0 Seed Mass (mg)
4
3
2
1
0
a
a
b
c
AC
BB
BO
HR
B
√F1 Seed Mass (mg)
4
3
2
1
0
ab
a
b
c
AC
BB
BO
HR
Population

Figure 1.3: Each point shows the square root transformed mean seed size value of one maternal family. For each population, the box shows sample quartiles with the center line indicating the population median. The bars show the standard deviation of each population and letters identify which group means are significantly different from another as determined by a Tukey HSD test for the pooled population dataset. (A) shows F0 (field-produced) mean seed size and distributions; (B) shows F1 (greenhouse-produced) mean seed size and distributions.

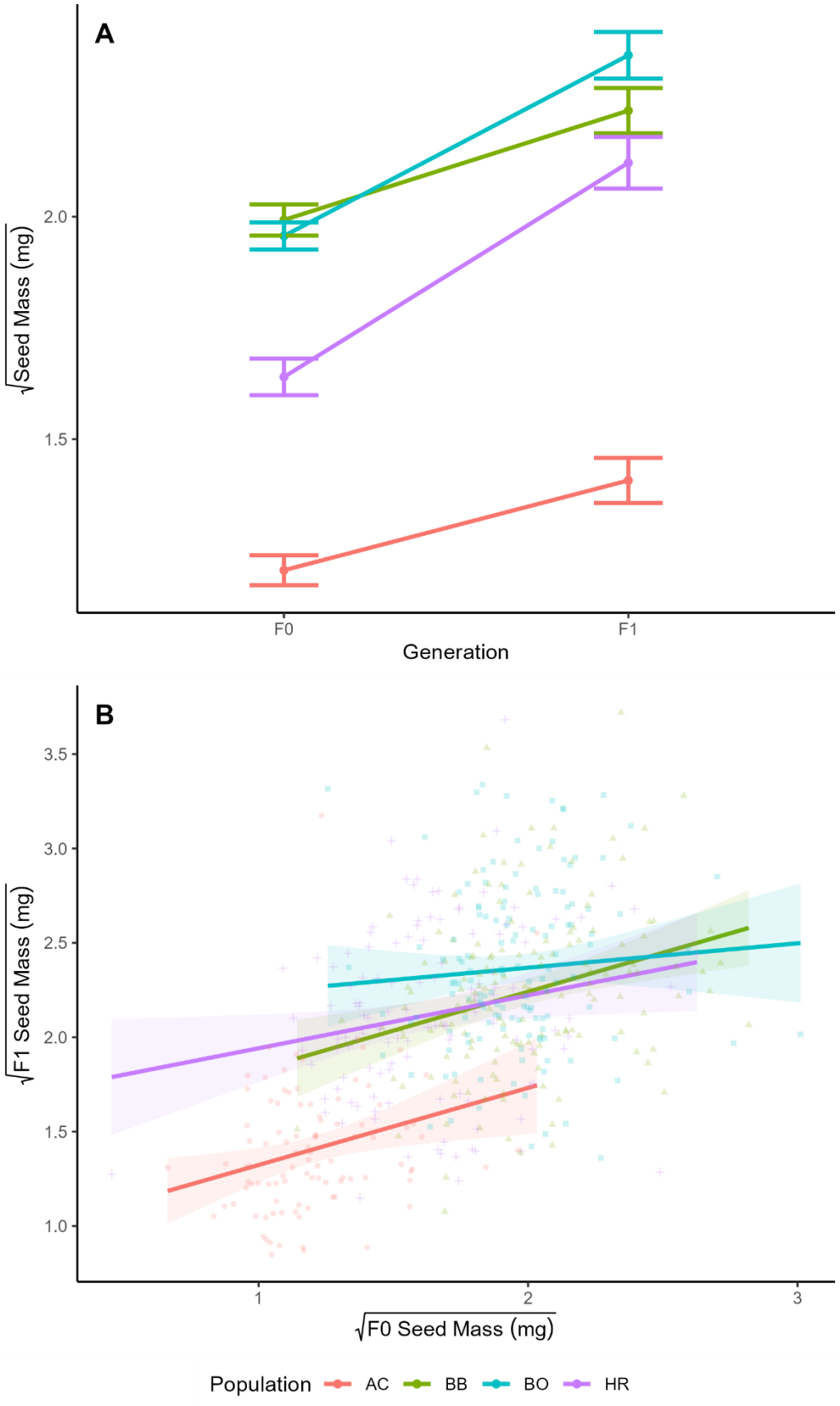

A
√Seed Mass (mg)
2.0
1.5
F0
F1
Generation
B
√F1 Seed Mass (mg)
3.5
3.0
2.5
2.0
1.5
1.0
1
2
3
√F0 Seed Mass (mg)
Population
AC
BB
BO
HR

Figure 1.4: (A) Population means and standard errors of seed size (sqrt-transformed in both A and B) in each generation. Solid lines show the change from one generation to another but do not show significance. Band widths show the standard error. (B) Parent-offspring regression of F1 seed mass on F0 seed mass. Each point indicates a maternal family. Differences in mean seed size among populations and between generations and are significant (Table 1.3); the slopes displayed in (B) do not differ among populations. Band widths show 95% confidence interval.

							Intercept			F0 Seed Size		
Pop	**Source**	***N***	**df**	**MS**	***F***	**Adj. R^2**	**Est. ± SE**	***T***	***P***	**Est. ± SE**	***T***	***P***
AC	F0	98	1	0.89	7.59	0.064	0.915 ± 0.18	5.03	< 0.001	0.408 ± 0.15	2.76	< 0.01
	Error		96	0.117								
BB	F0	149	1	2.30	12.84	0.074	1.417 ± 0.23	6.11	< 0.001	0.412 ± 0.12	3.58	< 0.001
	Error		147	0.179								
BO	F0	140	1	0.15	0.77	~ 0	2.110 ± 0.29	7.27	< 0.001	0.129 ± 0.15	0.88	0.381
	Error		138	0.194								
HR	F0	123	1	1.00	4.96	0.031	1.662 ± 0.21	7.90	< 0.001	0.280 ± 0.13	2.23	< 0.05
	Error		121	0.201								
All	F0	510	1	44.41	209.1	0.29	0.828 ± 0.09	9.27	< 0.001	0.720 ± 0.05	14.46	< 0.001
	Error		508	0.21								

Table 1.4: Summary statistics of bivariate linear regressions conducted within populations. F1 seed size as the response and F0 seed size as the predictor variable where both variables were square root transformed. Note the low R-squared values, which indicate weak correlations between F0 and F1 seed sizes within populations despite significant p-values. The higher R-squared when pooling all populations reflects the strong correlation between population means for F0 and F1, suggesting a correlation between them among populations, but not within them.

Population: AC					
Model: F1 Seed mass ~ F0 Seed mass + Maternal Family				Pseudo R^2 = 0.68	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	0.000	0.031	96	0.000	1.000
F0 Seed mass	0.460	0.134	96	3.419	< 0.001
Random	**Variance**	**SD**			
Maternal family	0.087	0.295			
Residual	0.046	0.213			
Population: BB					
Model: F1 Seed mass ~ F0 Seed mass + Maternal family				Pseudo R^2 = 0.59	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	-0.003	0.034	146.018	0.000	0.937
F0 Seed mass	0.382	0.114	145.962	3.356	0.001
Random	**Variance**	**SD**			
Maternal family	0.151	0.388			
Residual	0.115	0.340			
Population: BO					
Model: F1 Seed mass ~ (Paternal family | Maternal family)				Pseudo R^2 = 0.4	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	~ 0	0.038	47.845	-0.009	0.993
Random	**Variance**	**SD**			
Paternal family	0.024	0.155			
Maternal family (nested)	0.094	0.307			
Residual	0.181	0.425			
Population: HR					
Model: F1 Seed mass ~ (Paternal family | Maternal family)				Pseudo R^2 = 0.65	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	~ 0	~ 0	41.02	0.019	0.985
Random	**Variance**	**SD**			
Paternal family	**0.029**	**0.358**			
Maternal family (nested)	0.128	0.358			
Residual	0.084	0.290			

Table 1.5: Summaries of mixed effects linear models examining genetic seed size determinants within populations. ANOVA of individual mass of seeds produced in the nested breeding design. Final models were selected by basis of AIC, log-likelihood, and Likelihood Ratio tests. The reduced model that included the random effect(s) only-maternal effect (nested within paternal effect when applicable) and paternal effect – or the full model including the random effects and F0 seed size as a fixed effect (Appendix A) were compared to test for best fit. The analyses were conducted separately for each of the four populations.

	Component	AC	BB	BO	HR
Model Estimates	Paternal	N/A	N/A	0.024 (8.08%)	0.029 (11.91%)
	Maternal (nested)	0.087 (65.64%)	0.151 (56.68%)	0.094 (31.50%)	0.128 (53.19%)
	Residuals	0.046 (34.36%)	0.115 (43.32%)	0.181 (60.43%)	0.084 (34.9%)
Calculated Estimates	Additive genetic	N/A	N/A	0.096	0.116
	Total phenotypic	0.133	0.266	0.299	0.241
Heritability		N/A	N/A	0.321	0.481

Table 1.6: REML estimates and calculated values [Eq. 1] from mixed effects linear regressions of the quantitative genetic components of variance for seed size (square transformed mg and mean centered by cohort). In parentheses are percentages of the total variance, only significant variance components, as determined by the fit of ANOVA (Table 1.5), are shown.

II. Sources of variation and fitness consequences of seed size within and among four populations of the California-native annual species, *Nemophila menziesii* (Boraginaceae)

A. Introduction

Seed size is a critical trait affecting vital life-history attributes, and consequently, overall fitness (Leishman, 2000). Seed size influences germination (Stanton, 1984; Stanton, 1985;Morse and Schmitt, 1985; Weller, 1985; Roach, 1987; Leishman and Westoby, 1994; Andersson, 1996; Eriksson, 1999; Baskin and Baskin, 2019), resource acquisition (Roach, 1987; Winn, 1988), seedling survival (Pitelka et al., 1983; Stanton, 1984a, b; Wulff, 1986; Winn, 1988; Eriksson, 1999; Moles and Westoby, 2004a, b, 2006, Susko and Cavers, 2008), stress resilience (Howell, 1981; Weller, 1985; Marshall et al. 1986; Larson et al., 2020), and reproductive rates (Stanton, 1984a, b; Wulff, 1986a, b; Mazer, 1987a; Winn, 1988; Kalisz, 1989) within populations. Numerous studies have documented the competitive advantages of plants grown from larger seeds relative to those derived from smaller seeds (Roach, 1987; Stanton, 1984b; Mazer, 1987a, b; Moles and Westoby, 2004a, b; Moles and Westoby, 2006; Susko and Cavers, 2008; Metz et al., 2010; reviewed in Westoby et al., 1992). These findings suggest that natural selection should favor larger seed sizes, leading to low levels of genetically based seed size variation within wild populations (Harper et al., 1970), as corroborated in the previous chapter.

It would be expected that seed size variation within species should be relatively low compared to other fitness-related traits, as seed mass should be targeted by natural selection

such that the mean phenotype is directionally increased (Harper et al., 1970) and phenotypic variation reduced. However, despite the advantages associated with larger seeds, variation in seed size persists within and among many plant populations. This apparent paradox (Silvertown, 1989) can be observed in the high phenotypic variation of seed size within most wild populations (Stanton 1984a, b; Thompson, 1984; Wolf et al., 1986; Wulff, 1986a, Winn, 1988). As discussed in the previous chapter, the primary factors contributing to this variation are environmental influences, differential seed provisioning, and non-additive genetic effects transmitted through the seed-producing (sporophytic) plant (Mazer, 1987a, b; Roach and Wulff, 1987; Mazer and Gorchov, 1996; Byers et al., 1997; Galloway, 2005; Donohue, 2009).

Seed size also differs among conspecific populations, which may be the result of either (a) local adaptation in response to environmental differences in the strength or direction of natural selection on seed size and/or correlated traits (Baker, 1972; Guo et al., 2010; Cochrane et al., 2015) or (b) environmentally induced variation in seed size (i.e., phenotypic plasticity) (Baker, 1972; Salisbury, 1974; Schlichting, 1986; Marshall et al., 1986; Mazer, 1989; Byers et al., 1997; Wright and Westoby, 1999; Leishman, 2001; Larson et al., 2020). The current study was conducted to determine whether populations of *Nemophila menziesii* that differ in mean seed size experience different patterns or intensities of natural selection on seed size.

Seed mass evolution has been widely studied in plants, particularly in annual species, due to its potential to impact plant fitness and ecological interactions (Salisbury, 1942; Westoby and Lord, 1996; Leishman et al., 2000; Moles and Westoby, 2006). However, the influence of seed size on lifetime fitness has been rarely evaluated among multiple conspecific

populations across multiple years in a field setting (but see Metz et al., 2010). In this study, we investigate both the sources of variation in seed mass in the California-native annual species, *Nemophila menziesiii,* and the potential impact of these influences on plant fitness and population dynamics in a field setting to predict responses to environmental changes, such as those associated with climate change (Cochrane et al., 2011; Walck, et al., 2011; Fernandez-Pascual et al., 2019).

To gain a comprehensive understanding of these factors contributing to seed size variation and its fitness consequences in *Nemophila menziesii*, this study seeks to address the following questions:

1. How does the environment influence mean seed size when seed-producing plants sharing genetic material (described in Chapter 1) are raised in different environments? And is the relationship between growing environment and mean seed size consistent among populations?
2. Within populations, is the mean seed size of offspring determined by parental genotype and/or parental seed size phenotype? And does the influence of these determinants remain consistent across generations (and growing environments)?
3. Does maternal mean seed size influence offspring reproductive traits and fitness?

These research questions aim to explore the extent of genetic influences on seed size, the capacity of seed size to change in response to environmental conditions, the fitness consequences of parental mean seed size, and to identify potential trade-offs between seed size and other reproductive traits. In this chapter, I use a three-generation, multi-population study to examine intraspecific variation in, first, the role of plasticity in determining seed size, the role of heritability in determining seed size, and finally, the consequences of seed

size on reproductive fitness. The results of this study can inform the development of strategies to preserve plant biodiversity in a changing environment by assessing the adaptive significance of seed size variation. Despite the influence that seed size may have on reproductive fitness, whether or not natural selection has the capacity to drive evolutionary change in seed size depends on its heritability. In other words, if seed size is not heritable, then it cannot evolve by natural selection even if seed size affects fitness. By understanding the role of seed size in a species' adaptation to environmental changes, conservation efforts can prioritize the protection and management of populations with seed size traits that enhance fitness under changing local conditions. Understanding the factors that generate and/or maintain seed size variation within populations is crucial for predicting how plant populations may respond to changing environments and for the development of strategies to preserve plant biodiversity.

B. Materials and Methods

Field Design

In the fall of 2021, pedigreed seeds produced in a common greenhouse environment using the nested mating design described in the previous chapter (*Figure 1.1*) were sown in their respective home environments. Seeds from each of the maternal plants pollinated in the greenhouse were sown; 107 maternal families were sown at the Angelo Coast Range Reserve (AC), 147 maternal families at Bodega Bay Marine Laboratory (BB), 132 maternal families at Blue Oak Ranch Reserve, and 137 maternal families at the Hastings Natural History Reservation (HR). From each maternal family, 30 seeds were sown at AC and 24 seeds were sown for BB, BO, and HR. At each of the field sites, the study area was divided into three blocks in which 8 seeds (for BB, BO, and HR) or 10 seeds (for AC) of every maternal family were sown. Each family's seeds were sown in a one-meter segment; these linear segments were arranged within blocks, as follows. At AC, BO, and HR, each block was comprised of three 20- to 40-meter transects such that any effects on the traits expressed by the field-grown plants due to environmental variation among transects and/or blocks could be detected and controlled statistically. At BB, it was not feasible to distribute the one-meter segments in transects because of the heterogeneity of the site in terms of its terrain and existing vegetation. Instead, two to four line segments were arranged around a central "location" (mean = ~ 3.1 maternal families per location for 142 locations total), each within a 2-meter radius from it. Each location was assigned a sequential number identifier, with the number increasing along an environmental gradient (along a slight elevational gradient), such that locations that were close in number were also close in proximity. Block 1 locations were generally lowest in elevation on a cliffside, and Block 3 locations were highest. To maintain

consistency across all population analyses, only the block effect was used to control for environmental effects on plant phenotype.

Seeds were first sown under laboratory conditions at the University of California, Santa Barbara, in Aero^{NT} plugs (20 mm diameter x 30 mm height) (Quick Plug North America) at a depth of 1 cm and then transplanted within 72 hours (in the plugs) to locations that were randomly assigned for each maternal family. At each location, 8 or 10 plugs (depending on the population, as described above), each containing one seed from the same maternal family, were evenly spaced, and planted at 10 cm intervals along a linear 1-meter segment. (*Figure 2.1)*. Across the length of each segment, and at a width of ~20 cm, the soil surface was scraped and lightly tilled to create a homogenous substrate free of vegetation. Plugs were then buried such that the top surface of the plug was aligned with the soil surface. When more than half of the surviving plants of a given segment began to flower, one healthy individual from that segment was randomly selected for monitoring of fitness-related traits (hereafter referred to as the "fitness individual").

In this design, each maternal family within each block was represented by one fitness individual except when no individuals of a maternal family in a given block survived to produce viable seeds. Populations were monitored at intervals of one to two weeks once flowering was first observed among the sown individuals. The temporal resolution of the recorded life-history traits depended on the frequency of monitoring, which differed among the sites. The AC population was least frequently monitored with seven visits across 74 days, BB was monitored 13 times across 89 days, BO was monitored 12 times across 75 days, and HR was monitored 12 days across 75 days. Ultimately, the AC population was comprised of 277 flowering plants representing 96 maternal families, BB was comprised of 107 flowering

plants from 82 maternal families, BO was comprised of 136 flowering plants from 89 maternal families, and HR was represented by 345 flowering plants from 118 maternal families (*Table 2.1*). Populations BB and BO had a relatively low proportion of fitness individuals that survived to produce seeds (*Table 2.1*). In both populations, the onset of phenological stages (i.e., day of first flower) of the study plants were observed to be later than those that were adjacent to the study plots. In addition, there was also notable levels of herbivory and frugivory of fitness individuals. These factors likely negatively impacted both survivorship and the ability of the study plants to obtain and retain resources – particularly, water – that are essential for reproductive growth. In some cases, all the fruits of a fitness individual may have either dehisced or been eaten prior to collection, and if no intact fruits could be collected from a fitness individual, it was excluded from the analysis.

Data Collection

Grandmaternal (F0) and maternal (F1) mean seed size

As described in Chapter 1, F0 seeds were collected from four wild populations of *N. menziesii* in 2018. At each population, seeds were collected from at least 200 maternal plants that were spaced at least 1 square meter apart. For each maternal family, 2-117 of the collected seeds were bulk weighed on a Cahn TA 4200 Analytical Scale to the nearest 10 mg to obtain the mean individual seed mass of the F0 generation. These maternal families were used in a greenhouse-based nested breeding design (also described in Chapter 1) in which randomly selected groups of three maternal plants (pollen recipients) were each assigned to a single paternal individual (a pollen donor) from which pollen was used to hand-pollinate flowers on each of the recipients. Each of the maternal plants used in this design were raised

from the seed produced by a different maternal family initially collected from the field (*Figure 1.1*). For each hand-pollinated maternal plant, 25-100 viable seeds (the F1 generation) were bulk weighed to obtain F1 maternal family mean individual seed mass. In sum, the F2 seeds produced in this field experiment were the grand-offspring of the F0 seeds collected from the source populations in the spring of 2018 and the offspring of the F1 seeds that were produced in the greenhouse breeding study conducted from August 2018 to October 2019 and then sown at their home sites in Fall 2021. All maternal lines in which F0, F1, and F2 mean seed size were measured were included in the analysis.

Maternal mean seed size and offspring fitness

In the field study conducted from October 2021 to June 2022, the F1 seeds were sown at their home population field site as described above, and, as the fitness individual produced fruits and seeds, the ripe fruits were harvested at approximately weekly intervals and stored in labeled coin envelopes maintained at room temperature at UCSB. Fruits were collected at maturity, typically when the peduncle was rigid, the calyx was brown, and the pericarp (fruit wall) had begun to dry out. Fruits that had not yet dehisced ("intact" fruits) were stored separately from fruits that had begun to dehisce ("open" fruits) so that we could estimate total fecundity as the mean number of seeds per fruit among the intact fruits multiplied by the total number of fruits (intact and open) produced by an individual plant during its lifetime.

All viable F2 seeds produced by each fitness individual were counted and then bulk weighed on an OHAUS Pioneer PX85 Microbalance to the nearest 10 µg. F2 maternal mean individual seed mass was estimated as the mean value for mean individual seed mass among the seed-producing fitness individuals belonging to a maternal family (across the three

blocks). Reproductive output of an individual was therefore measured based on three variables - the total number of fruits produced, mean number of seeds per intact fruit, and the mean seed mass of fitness individuals. Reproductive fitness was estimated in two ways – fecundity and reproductive yield. Total fecundity was estimated as the product of the mean number of seeds per fruit and the total number of fruits produced (including both intact and dehisced fruits collected from each fitness individual). Reproductive yield was estimated as the product of total fecundity and mean individual mass of F2 seeds. When a maternal family was not represented by a fitness individual in a block because no individuals survived to produce seed, the individual did not contribute to the recorded maternal family means.

Data Structure and Statistical Analysis

All statistical analyses were conducted in R version 4.2.1 (R Development Core Team 2022). All the reproductive traits evaluated in the study, grandmaternal (F0) mean seed size, maternal (F1) mean seed size, offspring (F2) mean seed size, the number of fruits produced, the mean number of seeds produced per fruit, fecundity, and reproductive yield, were right skewed and were log (base-10) transformed prior to analyses to approach a normal distribution.

Mean seed size among generations

To examine whether mean seed size significantly differed among the three generations, F0, F1, and F2, I used separate one-way ANOVA tests (*stats* package, function 'aov' and 'lm' to obtain coefficients) for each population, with generation as an independent variable. The mean seed mass of a maternal family in each respective generation was the

response variable (AC: n = 321, BB: n = 252, BO: n = 270, HR = 405) (*Table 2.1*). F2 mean seed mass was estimated as the mean of the maternal family across all blocks in the field design. Comparisons of seed mass among the three generations were conducted to determine whether, within populations, an inter-generational change in mean seed mass could be attributed to environmental variation among the growing environments.

To compare the mean change in seed size among generations across populations, a post-hoc Tukey Honest Significance Difference test was conducted on a dataset in which all the populations were pooled. Populations, generations, and the population x generation interaction were included as predictor variables in the two-way ANOVA using Type II Sum of Squares significance testing (*car* package, function 'Anova'; *emmeans* package, function 'emmeans' using Sidak correction to obtain estimated marginal means; *multcomp* package, function 'cld' to obtain seed size class) (*Appendix B*). And to determine whether the magnitude and direction of change was shared among the populations.

Grandparental (F0)- and parental (F1) influence on seed size

For each population, mixed effects linear regression models (*lme4* package, function 'lmer') were constructed to determine the overall effect of the parental genotype (the greenhouse-raised pollen donor and maternal family) and the grandmaternal (F0) and maternal (F1) phenotype on F2 seed size (AC: n = 278, BB: n = 109, BO: n = 136, HR: n = 340). In the full model, block, paternal individual, and maternal family (nested within paternal individual, when applicable) were included as random effects, and F0 seed mass and F1 seed mass were included as fixed effects. The fit of all nested models (i.e., excluding one or more parameters) were compared to that of the full models by comparison of Akaike

information criterion (AIC) values and, the significance of factors in the most parsimonious model were determined with Likelihood Ratio Tests (*stats* package, function 'anova') (*Appendix C*) Pseudo-R^2 values were extracted for the final models using the function, 'r.squaredGLMM' in the *MuMIn* package.

Maternal seed size and offspring reproductive traits

Multiple linear regression models (*stats* package, function 'lm) were constructed to determine the effect of maternal (F1) mean seed mass on several reproductive traits expressed in the field-raised F1 generation - the total number of fruits produced, the mean number of seeds produced per fruit, and mean F2 seed mass. This analysis allowed for a comprehensive evaluation of the relationship between maternal mean seed size and each reproductive trait, while controlling for covariation among the reproductive traits. In these models, each reproductive trait was evaluated as a response to F1 seed size and the two remaining reproductive traits that were measured (Models 1-3). Then, to examine the effect of maternal mean seed size on offspring reproductive fitness, bivariate linear regression models (*stats* package, function 'lm') were constructed with either estimated fecundity or estimated reproductive yield as response variables (Models 4-5). Multiple linear regressions were first conducted for each population separately by the following equations:

Model 1:

$$F_2\ seed\ mass \sim \beta_0 + \beta_1 F_1\ seed\ mass + \beta_2 fruit\ number + \beta_3 mean\ seeds\ per\ fruit + \varepsilon$$

Model 2:

$$Mean\ seeds\ per\ fruit \sim \beta_0 + \beta_1 F_1\ seed\ mass + \beta_2 F_2\ seed\ mass + \beta_3 fruit\ number + \varepsilon$$

Model 3:

$$Fruit\ number \sim \beta_0 + \beta_1 F_1\ seed\ mass + \ \beta_2 F_2\ seed\ mass + \beta_3 mean\ seeds\ per\ fruit + \ \varepsilon$$

Model 4:

$$Fecundity \sim \beta_0 + \beta_1 F_1\ seed\ mass + \ \varepsilon$$

Model 5:

$$Reproductive\ yield \sim \beta_0 + \beta_1 F_1\ seed\ mass + \ \varepsilon$$

The values for each of these traits were measured on each fitness individual and then mean centered within populations by the block mean. This value (i.e., the deviation between an individual's phenotype and its block mean) was then used to calculate the mean deviation for each maternal family (from 1-3 fitness individuals) (AC: n = 107, BB: n = 84, BO: n = 90, HR: n = 135). Within each population, F1 mean seed mass was centered by cohort means (see previous chapter) and F0 seed mass was centered by population mean. All variables were respectively transformed and centered around the respective means, to control for the environmental factors that otherwise overparameterized the model. For the pooled population analysis examining the effect of F1 seed mass on reproductive traits, the data were subsequently scaled by 1 standard deviation to allow for comparison among populations.

C. Results

Mean seed size among generations

Population BB (F0: 4.09 ± 1.27 (mg) (mean ± SD), F1: 5.12 ± 2.07, F2: 4.12 ± 2.62) and BO (F0: 3.93 ± 1.12, F1: 5.54 ± 2.03, F2: 3.76 ± 1.58) were the largest-seeded populations on average, followed by population HR (F0: 2.78 ± 1.04, F1: 4.56 ± 1.98, F2: 2.73 ± 0.75) and AC (F0: 1.51 ± 0.63, F1: 2.01 ± 1.21, F2: 1.90 ± 0.42) (*Table 2.2, Figure 2.2*). Within each population, mean seed mass differed among the three generations produced in the field and in the greenhouse (*Table 2.3)*. Populations BB, BO, and HR, all produced the largest seeds in the F1 generation, when cultivated in the greenhouse. On average, seeds produced in the field were smaller, and were not statistically different between the F0 and F2 generations in these populations (*Figure 2.4*). However, in AC, the smallest seeded generation was F0, and the F1 and F2 generations were larger and similar in mass.

Grandparental and parental influence on offspring seed size

This analysis investigated the relationship between offspring (F2) seed mass and parental (F0 and F1) seed mass while controlling for parental identity and environmental effects. The final regression model parameters differed among populations (*Table 2.4*) as determined by model comparison of AIC and Likelihood Ratio Tests (*Appendix C*). For population AC, the final model included only the block effect and non-nested maternal family as random effects. For population BB, the model with block effect, pollen donor (paternal) effect, and maternal family nested within paternal individual, included as random effects had better fit than additive models but led to heteroscedasticity. The reduced model with only block as a

random effect did not violate assumptions of homoscedasticity. For population BB and BO, only the block effect was included in the model as a random effect as no other examined parameters significantly influenced F2 seed mass. For population HR, F1 seed mass was included as a fixed effect, and block, paternal individual, and maternal family nested within paternal individual were included as random effects. Addition of F0 seed mass as a parameter led to convergence issues in this population and was not included in the final model. Assumptions of the final specified models were checked by examination of the residual plots and no violations were observed.

Similar to the findings of the previous chapter (*Table 1.6*), no evidence of statistically significant paternal variance or additive genetic variance in individual seed mass was found in population AC (*Table 2.4*). The effect of maternal family (shown by maternal variance) was the only significant genetic effect in determining F2 seed mass and accounted for 14% of variation in offspring phenotypes (*Table 2.4*). Neither maternal (F1 seed size) nor grandmaternal (F0 seed size) phenotype had an influence on offspring phenotype, indicating that the transgenerational effects observed across the F0 and F1 generation (*Table 1.5*) did not persist to the F2 generation.

In populations BB and BO, there was no evidence that F2 seed mass was influenced by either parental genotype (paternal individual and maternal family), nor by maternal phenotype (F0 and F1 seed size). However, high rates of mortality in these populations may have confounded or obscured these relationships (*Table 2.1*). In the previous chapter, I reported that, in the greenhouse environment, F1 seed size was not heritable (in the narrow sense, based on the variance among paternal sibships in mean seed size) but there was a positive correlation among maternal family means between F0 and F1 seed size in population

BB. That is, the two-generation analysis indicated that seed size was not heritable, while the mother-offspring regression detected significant broad-sense heritability. The converse was true for population BO, where, in the greenhouse environment, F1 seed size was heritable but there was no significant mother-offspring regression between F0 and F1 seed size (*Table 1.5*). Among the F2 generation of seeds, seed size was neither heritable nor influenced by the maternal parent's (F1) mean see size in either population. Transgenerational effects (the effect of F1 seed mass on F2 seed mass) detected in the greenhouse environment (the effect of F0 seed mass on F1 seed mass) for population BB (*Table 1.5*) did not persist to the F2 generation.

In population HR, F1 seed size of the maternal parent, as well as both the paternal individual and the maternal family (nested within paternal individual) contributed to F2 seed size phenotype. Paternal variance was in fact, greater than maternal variance, as estimated by the model, accounting for 12.9% of variance in F2 seed size, while maternal variance accounted for only 0.7% (*Table 2.4*). 17.5% of total variance in F2 seed size was explained by the model, including both fixed and random effects. The significant positive correlation of F1 seed size on F2 seed size in HR conflicts with the pattern reported in Chapter 1(*Table 1.5*) in which there was no evidence that the maternal mean seed size of field-collected seeds (F0) determined offspring F1 seed size among greenhouse-raised plants.

Maternal seed size and offspring reproductive traits

Sources of variation in lifetime fruit production

Greenhouse-raised maternal families that produced relatively large seeds tended to produce offspring that, when raised in the field, had relatively high lifetime fruit production.

In three of the four populations, the mean number of fruits produced by the offspring of a maternal family in the F2 generation was positively influenced by F1 seed size. Populations AC (slope = 0.375 ± 0.124 [SE]; T = 3.018; P = < 0.05), BB (slope = 0.426 ± 0.18; T = 2.359; P = < 0.05), and HR (slope = 0.344 ± 0.114; T = 3.005; P = < 0.05) (*Table 2.5, Figure 2.5*). The slope estimates (values were mean centered and scaled by 1 standard deviation) were similar across the three populations, indicating that the positive relationship between the two traits is conserved across these conspecific populations (*Figure 2.5*). There was a significant positive effect of the mean number of seeds produced per fruit on the number of fruits produced, but only in population AC (slope = 0.376 ± 0.14, T = 2.684; P = < 0.05) (*Table 2.5*).

Sources of variation in mean number of seeds per fruit

None of the populations exhibited a significant effect among maternal family means of F1 seed mass on the mean number of seeds produced per fruit (*Table 2.6, Figure 2.6*). However, in populations AC, BB, and HR, a tradeoff between the mean number of seeds per fruit and mean F2 seed mass was detected; maternal families that produced relatively many F2 seeds per fruit tended to produce relatively small F2 seeds. This relationship was also negative in population BO, but the effect was statistically non-significant (slope = -0.05 ± 0.156; T = -0.32; P = 0.75). In population AC, among maternal families, the mean number of seeds per fruit was also positively correlated with the mean number of fruits produced (slope = 0.174 ± 0.065; T = 2.684; P = < 0.05) (*Table 2.6*).

Sources of variation in F2 seed mass

Maternal family mean F2 seed mass was significantly and positively associated with F1 seed mass only in population HR (slope = 0.116 ± 0.049, T = 2.389; P = < 0.05) (*Table 2.7, Figure 2.7*). However, the mean number of seeds per fruit (in F1 adults) was negatively correlated with F2 seed mass in populations BB (slope = -0.236 ± 0.088; T = -2.785; P = < 0.05), and HR (slope = -0.130 ± 0.033; T = -3.99; P = < 0.05) (*Table 2.7, Figure 2.8*), which was also indicated by the model examining mean number of seeds per fruit as a response variable (*Table 2.6*). In population AC (slope = -0.129 ± 0.056; T = -2.291; P = < 0.05), the p-value (0.147) for the overall model for population AC indicated that the model as a whole was not a good fit for the data. While this relationship was also negative in population BO, the model and slope were not significant (slope = -0.024 ± 0.075; T = -0.32; P = 0.75) (*Table 2.7*).

Fecundity and Reproductive Yield

F1 seed size was positively correlated with both lifetime fecundity and reproductive yield in populations AC (fecundity: slope = 0.549 ± 0.171 [SE], T = 3.213, P = < 0.05; yield: slope = 0.567 ± 0.171, T = 3.306, P = < 0.05) and HR (fecundity: slope = 0.355 ± 0.178, T = 1.991, P = < 0.05;yield: slope = 0.564 ± 0.176, T = 3.201, P = < 0.05) but not in populations BB and BO (*Table 2.8-9*).

D. Discussion

This study investigated the environmental effects and genetic, maternal and paternal, effects on seed size and reproductive traits in four populations of *N. menziesii*. Seeds produced in greenhouse conditions were generally larger than those produced in the field, with environmental factors being a significant determinant to offspring seed size across populations. Parental genotype and phenotype effects on seed size were population-specific, with maternal influences tending to exceed paternal effects. The impact of maternal seed size on reproductive traits and fitness was also population-specific, with larger maternal seed size associated with increased fruit production in three of the four populations, and increased fecundity and reproductive yield in two of the four populations. Tradeoffs between seed mass and the number of seeds produced per fruit were detected in two of the four populations.

Environmental effects consistently contribute to offspring seed size across populations

It was expected that seeds produced in the greenhouse would tend to be larger than those produced in the field (Bradshaw, 1965; Marshall et al., 1986; Sugiyama and Bazaaz, 1997; Mal and Lovett-Doust, 2005), given that neither the seed-producing (maternal) nor the pollen donor (paternal) plants experienced competition or resource limitation (see greenhouse cultivation methods described in Chapter 1). In three of the four populations, BB, BO, and HR, this trend was observed such that seeds produced in field conditions (F0 and F2) were generally smaller than those produced in the greenhouse (F1) (*Table 2.3, Figure 2.4*). The largest seeded group was BO in the F1 generation (5.54 [mean] ± 2.03 mg [standard

deviation]; 1.85 < 11.13 mg [min < max]), and the smallest seeded group was the F0 generation of AC (1.51 ± 0.63 mg; 0.44 < 4.13 mg) (*Table 2.2, Figure 2.4, Appendix B*).

In population AC, the F0 generation was the smallest seeded, while generations F1 and F2 were larger and not significantly different from one another (*Table 2.3, Figure 2.4*). The increase in mean seed mass observed between the F0 and F2 generations suggests either that environmental conditions were more favorable in the F2 generation than in the F1 generation for seed development; transgenerational plastic effects were transmitted from the F1 generation to offspring; and/or that the effect of paternal parent was significantly positive for both the F1 and F2 generations. Further analyses (discussed in the next section) suggest that the change in mean seed size phenotype from the F0 to the F2 generation may be best explained by a more favorable field environment in the F2 generation, rather than an interaction between the growing environment and parental (F1) phenotype.

The effect of parental genotype and phenotype on seed size are population-specific

In this study, the effects of parental genotype and ancestral phenotype (F0 and F1 seed size) on F2 seed size (*Table 2.4*) differed among populations, indicating that genetic influences and other parental effects on seed size are not uniform across the range of *N. menziesii*. Nevertheless, maternal influences on offspring seed size tended to exceed paternal effects.

Population AC

When included as predictors in the model evaluating sources of variation in F2 mean seed size in AC, neither F1 seed mass, nor the paternal pollen donor significantly improved the

model. In Chapter 1 of this thesis, F0 seed size was observed to positively influence F1 seed size in this population (*Table 1.5*), suggesting that transgenerational plasticity contributed to offspring phenotype (Donohue, 2009). However, these effects did not persist to the F2 generation, which indicates that transgenerational plasticity, either negative (maladaptive) or positive (adaptive), were only transmitted in the greenhouse environment, where resources were not limited (Galloway, 2005; Zas et al., 2013), and did not significantly contribute to mean seed size in F2 seeds.

In the F1 and F2 generations, there was no significant effect on mean seed mass of paternal pollen donor in this population, indicating that additive genetic variance, and thereby, heritability, were negligible (*Table 2.4*) (Falconer and Mackay, 1983). The non-additive genetic maternal effect, which may be disseminated through non-nuclear genetic transmission, by environmental conditions experienced by a maternal plant during fruit and seed development, by patterns of maternal resource allocation per seed determined by the maternal sporophyte's genotype, and/or by differential competitive ability for resource acquisition among maternal families (Alexander and Wulff, 1985; Mazer and Wolfe, 1992; Platenkamp and Shaw, 1993; Galloway, 2001), accounted for most of the total phenotypic variance in seed mass in this population. This is consistent with the results of the previous chapter (*Table 1.6*) and with a previous study conducted by Byers et al. 1997. The absence of strong paternal effects on seed size suggests that the mean seed size produced by individuals and by maternal families is determined largely by a combination of the maternal sporophyte genotype, by extra-nuclear genetic transmission, and by environmental effects within this population.

Populations BB and BO

In populations BB and BO, there was no significant effect of parental genotype (maternal family or paternal pollen donor) or ancestral phenotype (F0 and F1 seed mass) on F2 seed size. Block effects accounted for only, respectively, 6.9% and 0.6% of total phenotypic variance (*Table 2.4*). The remaining variance was attributed to environmental factors that were not controlled for by the block effect. The absence of strong paternal effects, maternal effects, and transgenerational effects in the F2 generation indicates that environmental effects largely determine seed size in these populations when they are grown in their natural environment. Seed size was highly variable in these populations relative to AC and HR, but heritability was negligible, indicating that, at their home environments, population BB and BO may not undergo adaptive change via natural selection acting directly on seed size, and variation is largely maintained by phenotypic plasticity.

Population HR

The final model for population HR included paternal pollen donor, maternal family nested within paternal individual, and F1 seed size, as independent variables. Paternal pollen donor accounted for 12.9% of total phenotypic variance in F2 mean seed size, maternal family accounted for 0.7%, and the block effect accounted for 1.6% (*Table 2.4*). Maternal effects on mean seed size were much larger among seeds that were produced in the greenhouse (F1) than in the field (F2) (*Table 1.5* and *Table 2.4*). This finding was, in fact, consistent across all populations and supports the inference that extra-nuclear genetic effects and differential resource allocation per seed by the maternal sporophyte, are more likely to be expressed in favorable environments than in stressful ones (Zas et al., 2013). The significant

effect of F1 seed size on F2 seed size in HR contradicted the results of the previous chapter (*Table 1.5*), which found no significant effect of the previous generation's (F0) maternal mean seed size on F1 mean seed size (discussed further in the next section). In population HR, F2 seed size was significantly influenced by the paternal genotype, maternal genotype, and by F1 seed size, providing evidence for both heritability and transgenerational effects in determining seed size.

Maternal seed size effects on reproductive traits are population-specific

I investigated the effects of maternal seed size (F1) on reproductive traits and reproductive fitness of F1 adults grown in the field environment (*Tables 2.4-9, Figures 2.5-8*). Overall, the findings indicate that the effect of maternal seed size is not uniform across the range of *N. menziesii*¸ Larger maternal seed size in population AC was associated with increased fruit production, fecundity, and reproductive yield. Populations BB and BO, despite having the largest seeds on average, exhibited the lowest survivorship, fecundity, reproductive yield, and overall mean fitness among the four populations. Maternal seed size had negligible effects on reproductive fitness in these populations. In population HR, maternal seed size was positively correlated with fruit production, seed size of the next generation, fecundity, and reproductive yield.

Population AC

Population AC produced significantly smaller seeds than any other population in every generation of the study (*Table 2.3, Figure 2.4*). Of the four populations, it had the highest survival rate (86.6%) (*Table 2.1*) and the highest mean fecundity (*Table 2.2*).

Moreover, phenotypic selection favored larger-seeded maternal families; maternal mean F1 seed size had strong and positive effects on reproductive fitness in this population. Maternal mean (F1) seed size contributed positively to the number of fruits produced by F1 individuals, but no other reproductive traits predicted fruit production in this population (*Table 2.5*). However, when examining reproductive fitness, maternal mean F1 seed size was significantly positively correlated with both fecundity and reproductive yield (*Tables 2.8-9*). The effect size of maternal mean seed size on reproductive fitness was greater in population AC than in any of the other three populations.

Populations BB and BO

Although populations BB and BO were, on average, the largest-seeded populations, and the largest seeded groups in the F1 generation (BO was marginally larger than BB), they had the lowest survivorship to adulthood and the lowest mean reproductive yield in the field-raised F1 generation (*Table 2.1*, *Table 2.2*). Consequently, populations BB and BO had the lowest overall mean fitness (including survival rate) among populations. In population BO, maternal mean F1 seed size had no detectable effect on reproductive traits (i.e., number of fruits produced, mean number of seeds per fruit, or seed mass) nor on estimated reproductive fitness (i.e., fecundity and reproductive yield) (*Tables 2.5-9*). In population BB, maternal mean F1 seed size affected only the number of fruits produced by fitness individuals (*Table 2.5*). Neither fecundity nor reproductive yield, both of which are positively correlated with the number of fruits produced, were positively correlated with F1 maternal mean seed size in BB (*Tables 2.8-9*). The current analysis shows that maternal mean seed size had a negligible

effect on either individual reproductive traits or overall reproductive fitness of fitness individuals in populations BB and BO.

Population HR

Population HR produced smaller seeds than populations BB and BO and larger seeds than population AC (*Table 2.2, Figure 2.4*). HR had survival rates similar to those of population AC (83.5%) (*Table 2.1*). On average, individuals had lower fecundity than in population AC, but had higher reproductive yield than all other populations (*Table 2.2*). Maternal F1 mean seed size contributed positively to the number of fruits produced (*Table 2.5*) and to F2 mean seed size (*Table 2.7*). When examining reproductive fitness of the fitness individuals, maternal F1 seed size was significantly positively correlated with both fecundity (*Table 2.8*) and reproductive yield *(Table 2.9*), although the magnitude of the effect was not as great as in population AC.

Fruit production is greater in maternal families with larger seeds

In populations AC, BB, and HR, maternal F1 mean seed size positively influenced fruit production. The slope estimates and standard errors for this effect were remarkably similar across all three populations. This shared effect across populations suggests that larger seeded maternal families have greater fruit production than smaller seeded ones. However, the effect on reproductive fitness, measured by either fecundity or reproductive yield, was significant only in populations AC and HR (*Tables 2.8-9*). The competitive advantage of larger seeds in population BB may have been mitigated and offset by tradeoffs with other reproductive traits

(Venable, 1992; Leishman et al., 1995). The effect of maternal mean F1 seed mass on fruit production was non-significant in population BO (*Table 2.5, Figure 2.5*).

Seed mass constrains the number of seeds produced in a fruit (or vice versa)

In all populations, a tradeoff between mean individual seed mass (F2) and the mean number of seeds produced per fruit was detected, although in population BO, the effect was not significant (*Tables 2.6-7, Figure 2.8*). The slope of this effect was nearly identical for populations AC and BB in the standardized model including all the populations.

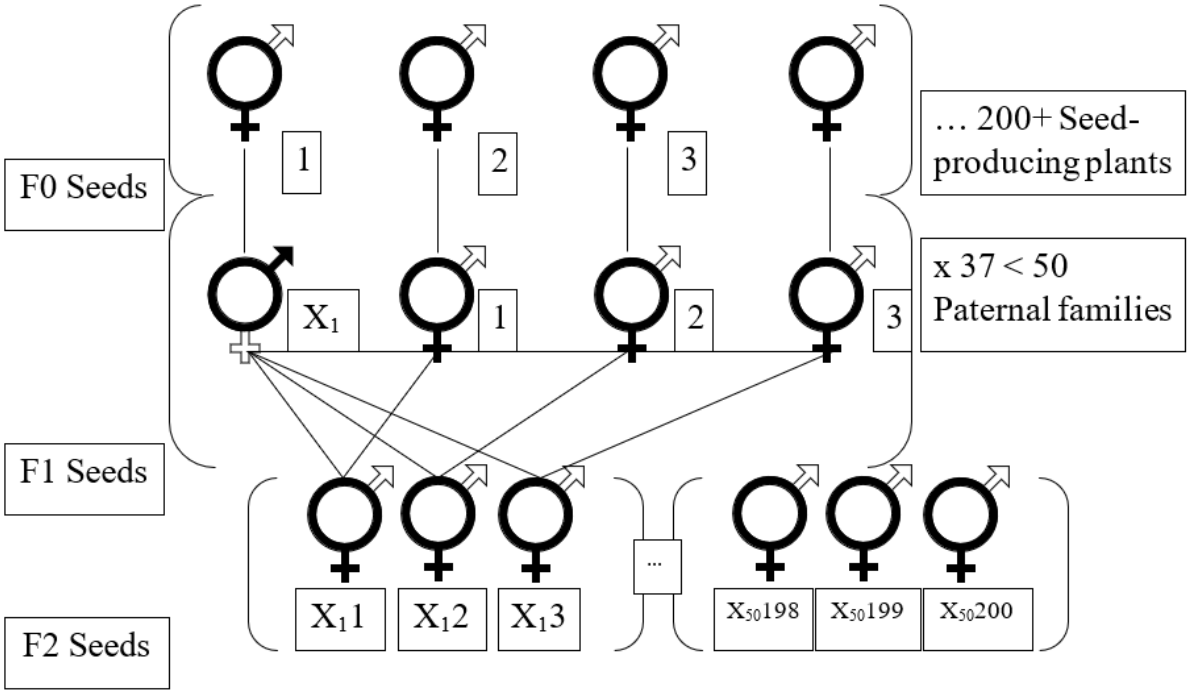
E. Conclusion

This chapter has demonstrated that environmental and maternal factors play a significant role in shaping seed size within and among populations of *N. menziesii*. The study revealed that seeds produced in favorable greenhouse conditions were generally larger than those produced in the field, with some variation among the populations. The effect of parental genotype on offspring seed size was typically small in all the populations that were included in the study. In two of the four populations (BB and BO), both the maternal and paternal effect were negligible. However, in the populations for which maternal seed size had significant and positive correlations on reproductive fitness (AC and HR), parental effects (the maternal effect in AC, and the maternal effect, paternal effect, and transgenerational effect in HR) were also detected. Moreover, while the tradeoff between offspring seed size and the mean number of seeds per fruit was significant, larger seeded maternal families consistently had higher reproductive fitness in these populations. Both the sources of variation in seed size and the consequences of maternal seed size on reproductive fitness were observed to be both population-specific and environment- specific.

While environmental effects play a large, and sometimes predominant, role in determining seed size across all populations, the capacity for natural selection to act directly on seed size is dependent on whether the trait is heritable. The findings of this study contribute valuable insights into the complex interplay of genetic, maternal, and environmental factors in determining seed size and its consequences on fitness. The broader implications of this study extend beyond the specific species and populations examined here. Understanding how these factors shape seed size is critical for predicting whether plant

populations may respond to changing environmental conditions by adaptive change in seed size. By elucidating the role of seed size in reproductive fitness, we infer the direction of evolutionary change in the trait that would convey a competitive advantage over conspecific individuals within a population. Ultimately, these findings emphasize the importance of considering the intricate relationships between genetic and environmental factors when assessing plant population dynamics and developing strategies to preserve native plant species in the face of a changing environment.

F. Tables and Figures



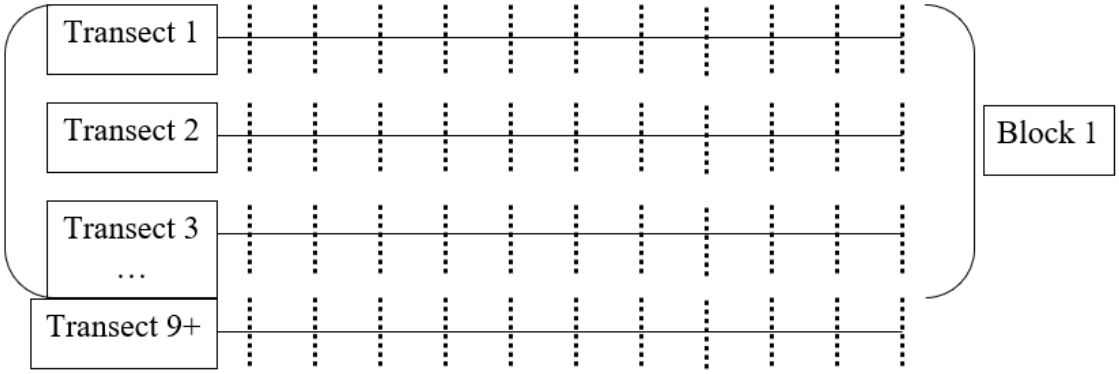


Figure 2.1: Design of the three-generation design for one population. The upper portion illustrates some of the seed-producing crosses and cross identities (maternal and paternal individual) for a single population. The plants are hermaphroditic with their functional sex indicated by wide-lined symbols. The lower portion illustrates the design at populations AC, BO, and HR, of a single block in the field portion of the study where F1 seeds were planted and F2 seeds were produced by F1 adults. Each dotted line along a transect represents one segment in which 8-10 seeds (depending on the population) from a single maternal family were planted. There were three blocks in which each maternal family was replicated (although some maternal families did not yield reproductive adults) for each population.

	Sowing Date	Transfer Date	Block	Transect	Transect Length	Selected Fitness Individuals	Surviving fitness ind. (% Survival)
AC	Oct. 27-28, 2021	Nov. 4-5, 2021	1	1	40 m	36	32
				2	40 m	36	33
				3	40 m	35	31
			2	4	40 m	36	31
				5	40 m	36	32
				6	40 m	35	31
			3	7	40 m	36	34
				8	40 m	36	30
				9	40 m	35	24
			Total			321	278 (86.6%)
BB	Nov. 29, 2021	Dec. 2-4, 2021	1	NA	NA	147	47
			2	NA	NA	147	23
			3	NA	NA	147	39
			Total			441	109 (24.7%)
BO	Nov. 11-12, 2021	Nov. 17-18, 2021	1	1	33 m	44	12
				2	25 m	33	15
				3	25 m	33	6
				4	23 m	22	1
			2	5	33 m	44	10
				6	23 m	30	12
				7	20 m	26	10
				8	22 m	32	16
			3	9	23 m	29	13
				10	30 m	40	15
				11	30 m	40	16
				12	25 m	23	10
			Total			396	136 (34.3%)
HR	Nov. 10-11, 2021	Nov. 19-20, 2021	1	1	50 m	46	39
				2	50 m	46	39
				3	50 m	45	39
			2	4	50 m	46	38
				5	50 m	46	35
				6	50 m	45	37
			3	7	50 m	46	38
				8	50 m	46	37
				9	50 m	45	41
			Total			411	343 (83.5%)

Table 2.1: Field experimental design at each of the population sites: Angelo Coast Range Reserve (AC), Bodega Marine Reserve (BB) Blue Oak Ranch Reserve (BO), Hastings Natural History Reservation (HR). Geographic coordinates of field sites are shown in Table 1.1.

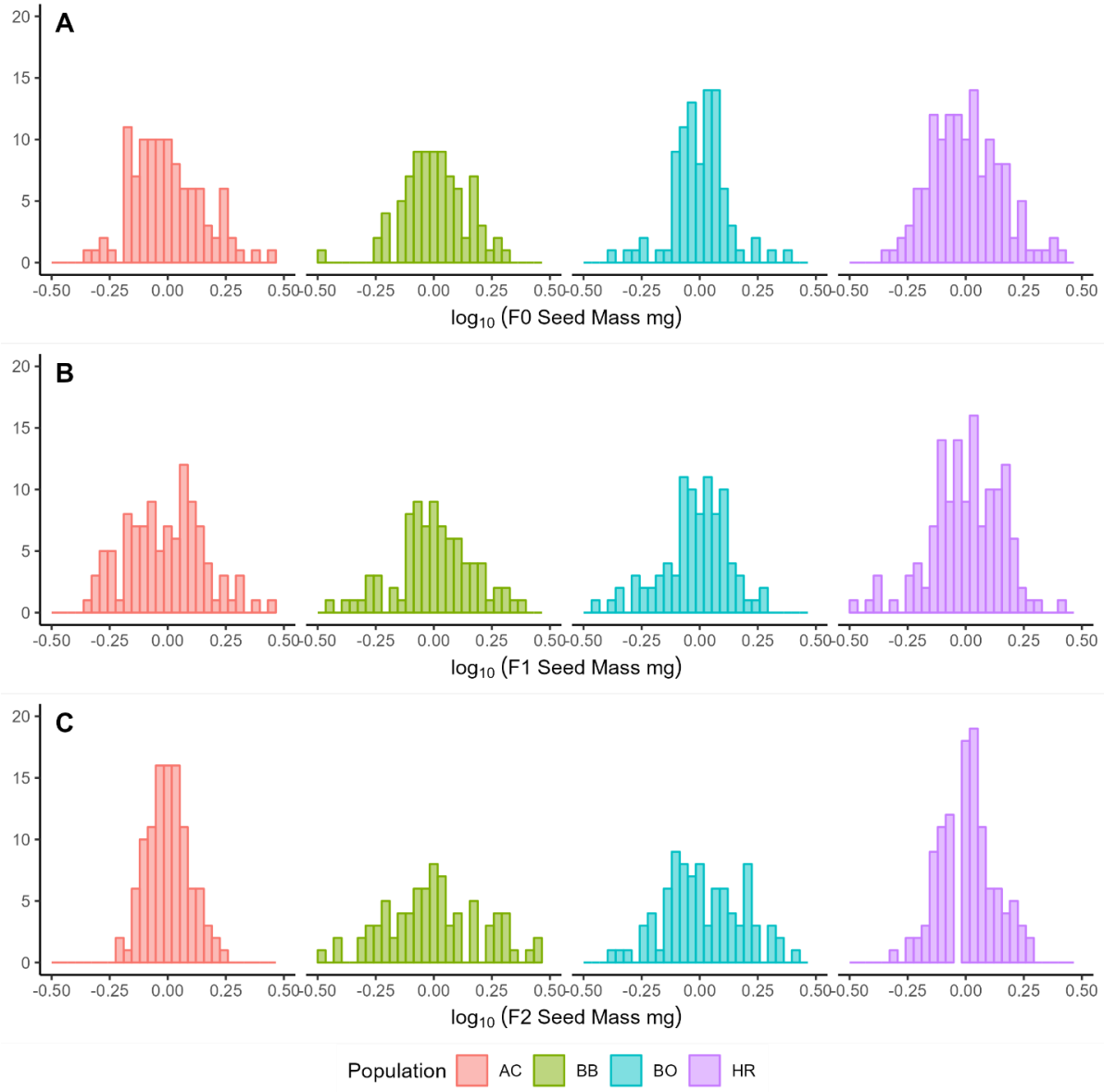

A
B
C
20
15
10
5
0
-0.50
-0.25
0.00
0.25
0.50
log10 (F0 Seed Mass mg)
log10 (F1 Seed Mass mg)
log10 (F2 Seed Mass mg)
Population
AC
BB
BO
HR

Figure 2.2: Histograms showing the distribution of log-transformed means of individual seed size by maternal family for each population. (A) mean size of seeds collected directly from source populations (B) mean size of seeds produced in the greenhouse breeding study (C) mean size of seeds produced in the field study.

	F0 seed size ± SE	F1 seed size ± SE	F2 seed size ± SE	Number of fruits ± SE	Number of seeds per fruit ± SE	Fecundity ± SE	Reproductive yield ± SE
AC	1.51 ± 0.16	2.01 ± 0.30	1.90 ± 0.10	10.42 ± 1.49	2.34 ± 0.22	27.18 ± 5.41	49.02 ± 9.76
BB	4.09 ± 0.34	5.12 ± 0.55	4.12 ± 0.70	2.77 ± 0.60	3.68 ± 0.67	10.96 ± 3.88	39.29 ± 12.58
BO	3.93 ± 0.29	5.54 ± 0.53	3.76 ± 0.42	6.80 ± 1.94	1.85 ± 0.31	11.49 ± 4.22	45.28 ± 18.44
HR	2.78 ± 0.25	4.56 ± 0.47	2.73 ± 0.18	14.41 ± 1.56	1.39 ± 0.18	20.56 ± 3.83	53.26 ± 9.53

Table 2.2: Mean and standard error for mean individual seed mass, reproductive traits– the number of fruits produced (fruit production) and the number of seeds per fruit – and reproductive fitness – fecundity (total number of seeds produced per individual) and reproductive yield (fecundity times mean F2 seed mass) recorded in each generation and population

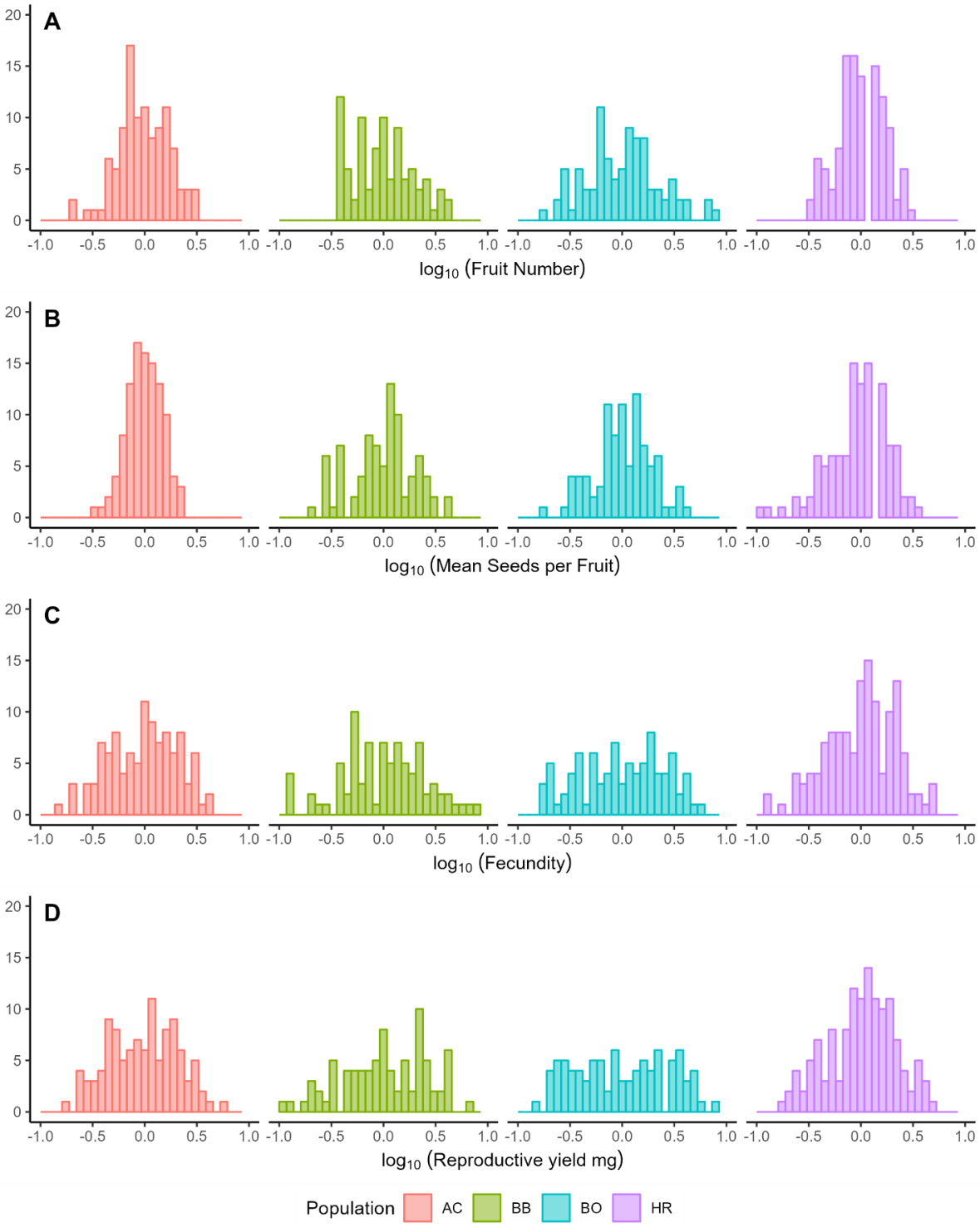

A
B
C
D
log10 (Fruit Number)
log10 (Mean Seeds per Fruit)
log10 (Fecundity)
log10 (Reproductive yield mg)
Population
AC
BB
BO
HR

Figure 2.3: Histograms showing the distribution of log-transformed means of maternal family traits by population. (A) fruit production of F1 individuals (B) mean number of seeds produced per fruit by F1 individuals (C) fecundity (total number of seeds produced) of F1 individuals (D) reproductive yield (fecundity * F2 mean seed mass) of F1 individuals.

Population: AC						
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**	
Generation	2	0.818	15.37	< 0.001	0.082	
Error	318	8.465				
Mean seed mass	***N***	**Est.**	**SE**	***T***	***P***	**Grouping**
F0	107	0.147	0.016	9.303	< 0.001	a
F1	107	0.102	0.022	4.553	< 0.001	b
F2	107	0.112	0.022	5.015	< 0.001	b
Population: BB						
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**	
Generation	2	0.763	10.23	< 0.001	0.068	
Error	249	9.283				
Mean seed mass	***N***	**Est.**	**SE**	***T***	***P***	**Grouping**
F0	84	0.591	0.021	28.053	< 0.001	def
F1	84	0.083	0.030	2.783	< 0.01	fg
F2	84	-0.051	0.030	-1.696	0.0912	de
Population: BO						
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**	
Generation	2	1.685	32.11	< 0.001	0.188	
Error	267	7.006				
Mean seed mass	***N***	**Est.**	**SE**	***T***	***P***	**Grouping**
F0	90	0.578	0.017	33.84	< 0.001	de
F1	90	0.136	0.024	5.624	< 0.001	g
F2	90	-0.0515	0.024	-2.132	< 0.05	d
Population: HR						
Source	**df**	**SS**	***F***	***P***	**Adj. R^2**	
Generation	2	3.757	77.52	< 0.001	0.275	
Error	402	9.742				
Mean seed mass	***N***	**Est.**	**SE**	***T***	***P***	**Grouping**
F0	135	0.418	0.013	31.21	< 0.001	c
F1	135	0.200	0.019	10.549	< 0.001	ef
F2	135	-0.009	0.019	-0.453	0.651	c

Table 2.3: One-way ANOVA with F2 seed size as the dependent variable and F0 and F1 seed size as independent variables (all log-transformed). Analysis was conducted separately for each of the populations. Linear regression models were used to obtain coefficients, standard errors, t-values, significance level, and adjusted R^2. The grouping class, denoted as a letter (from a-f) was determined by a two-way ANOVA (Appendix B) for the pooled dataset with all populations, generations, and interactions as independent variables and provides mean seed size class across all populations.

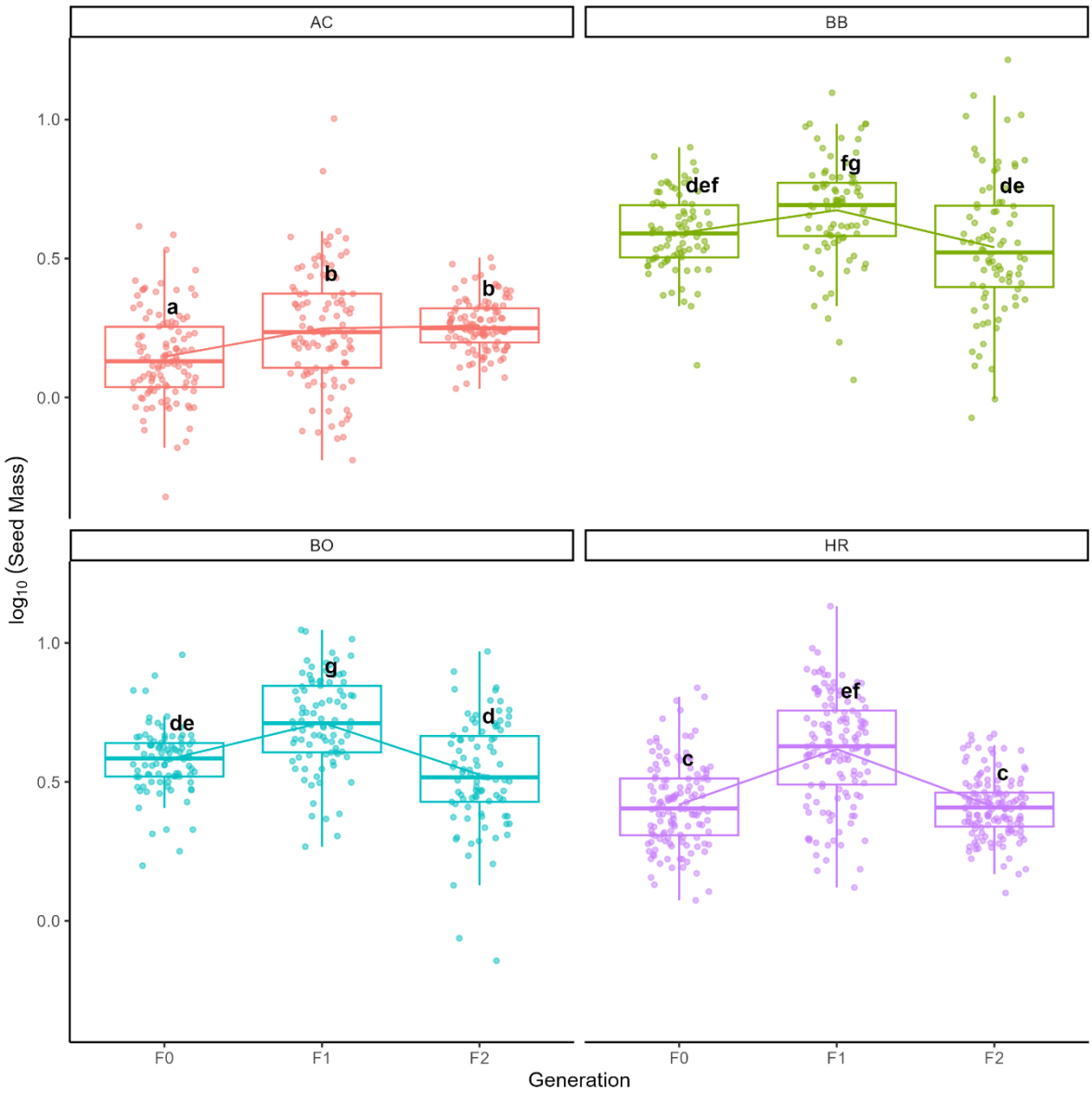

AC
BB
BO
HR
a
b
b
def
fg
de
de
g
d
c
ef
c
1.0
0.5
0.0
log10 (Seed Mass)
F0
F1
F2
Generation

Figure 2.4: Each point represents the mean of the maternal family in each generation (log-transformed). The upper and lower bounds of the box show the first and third quartiles, center lines show medians of each generation with respect to population. Path lines connect means of each generation. The bolded letters show compact letter display groupings of seed size as determined by the two-way ANOVA (Appendix B)

Population: AC					
Model: F2 Seed mass ~ Block + Maternal family				Pseudo-R^2 = 0.163	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	0.26	0.01	2.54	18.08	< 0.001
Random	**Variance*1000**	**SD*1000**			
Block	0.39 (2.3%)	19.8			
Maternal Family (non-nested)	2.39 (14%)	48.86			
Residual	14.3 (83.7%)	119.45			
Population: BB					
Model: F2 Seed mass ~ Block				Pseudo-R^2 = 0.069	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	0.52	0.04	1.79	11.86	< 0.001
Random	**Variance*1000**	**SD*1000**			
Block	4.06 (6.9%)	63.68			
Residual	54.77 (93.1%)	234.02			
Population: BO					
Model: F2 Seed mass ~ Block				Pseudo-R^2 = 0.006	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	0.53	0.02	2	25	< 0.001
Random	**Variance*1000**	**SD*1000**			
Block	0.3 (0.6%)	17.33			
Residual	46.66 (99.4%)	216			
Population: HR					
Model: F2 Seed mass ~ F1 Seed mass + (Paternal Individual | Maternal family)				Pseudo-R^2 = 0.175	
Fixed	**Slope**	**SE**	**df**	***T***	***P***
Intercept	0.33	0.03	46.3	10.2	< 0.001
F1 Seed Mass	0.13	0.05	119.89	2.68	< 0.05
Random	**Variance*1000**	**SD*1000**			
Block	0.37 (1.6%)	19.15			
Paternal Individual	2.91 (12.9%)	53.93			
Maternal Family (nested)	0.17 (0.7%)	12.93			
Residual	19.04 (84.7%)	138			

Table 2.4: Summaries of mixed effects linear regression models, including summary statistics and estimates for slope (for fixed effects) and variance (for random effects) to determine the sources of variation in seed size in each population. Pseudo R^2 shows the variance explained by both fixed and random effects of the entire model.

Population: AC	***N***	**df**	**RSE**	***F***	***P***
Model	107	103	0.227	6.396	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.022	-0.342	0.733
F1 seed mass		0.375	0.124	3.018	< 0.05
F2 seed mass		0.073	0.247	0.295	0.768
Mean seeds per fruit		0.376	0.14	2.684	< 0.05
Adj. R^2		0.133			
Population: BB	***N***	**df**	**RSE**	***F***	***P***
Model	84	80	0.272	2.018	0.118
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.03	-0.061	0.951
F1 seed mass		0.426	0.18	2.359	< 0.05
F2 seed mass		0.064	0.133	0.479	0.633
Mean seeds per fruit		0.028	0.106	0.263	0.793
Adj. R^2		0.035			
Population: BO	***N***	**df**	**RSE**	***F***	***P***
Model	90	86	0.358	0.558	0.644
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.038	0.023	0.982
F1 seed mass		-0.086	0.265	-0.325	0.746
F2 seed mass		0.027	0.199	0.136	0.892
Mean seeds per fruit		-0.168	0.136	-1.233	0.221
Adj. R^2		~ 0			
Population: HR	***N***	**df**	**RSE**	***F***	***P***
Model	135	131	0.201	5.141	< 0.01
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.005	0.017	0.264	0.792
F1 seed mass		0.344	0.114	3.005	< 0.01
F2 seed mass		0.242	0.172	1.403	0.163
Mean seeds per fruit		-0.003	0.068	-0.046	0.963
Adj. R^2		0.085			
Pooled populations	***N***	**df**	**RSE**	***F***	***P***
Model	416	412	0.973	7.8	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.004	0.048	0.074	0.941
F1 seed mass		0.214	0.048	4.423	< 0.001
F2 seed mass		0.064	0.05	1.288	0.199
Mean seeds per fruit		0.037	0.049	0.752	0.453
Adj. R^2		0.047			

Table 2.5: Summary of multiple linear regressions to examine the covariates of fruit production among maternal families (number of fruits produced) conducted first, within populations, and then across all populations ("pooled"). F1 seed size, F2 seed size, and the mean number of seeds per fruit are included as fixed effect independent variables.

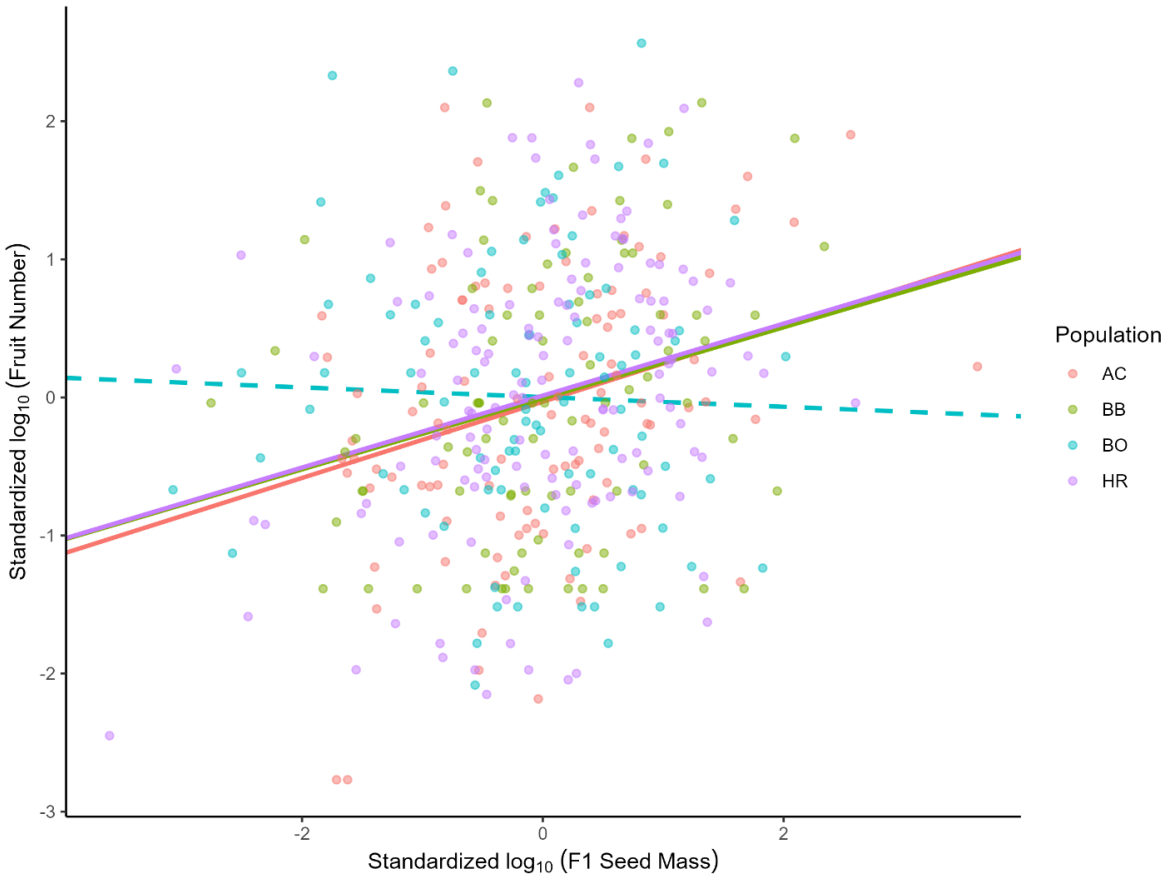

Standardized log10 (Fruit Number)
Standardized log10 (F1 Seed Mass)
Population
AC
BB
BO
HR

Figure 2.5: Linear regression of lifetime fruit production on F1 mean seed size. (Each point represents the mean seed size of maternal families (log-transformed and standardized by population). Slopes that differ significantly from zero ($\alpha < 0.05$) are shown by solid lines. Slope estimates are based on the results of the multiple linear regressions shown in Table 2.5.

Population: AC	***N***	**df**	**RSE**	***F***	***P***
Model	107	103	0.154	5.024	< 0.01
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.015	-0.049	0.961
F1 seed mass		0.058	0.088	0.664	0.508
F2 seed mass		-0.376	0.164	-2.291	< 0.05
Fruit production		0.174	0.065	2.684	< 0.01
Adj. R^2		0.102			
Population: BB	***N***	**df**	**RSE**	***F***	***P***
Model	84	80	0.288	3.045	< 0.05
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.004	0.032	0.141	0.888
F1 seed mass		-0.172	0.197	-0.872	0.386
F2 seed mass		-0.375	0.135	-2.785	< 0.01
Fruit production		0.031	0.118	0.263	0.793
Adj. R^2		0.069			
Population: BO	***N***	**df**	**RSE**	***F***	***P***
Model	90	86	0.281	0.554	0.647
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.006	0.03	0.192	0.848
F1 seed mass		0.023	0.208	0.109	0.913
F2 seed mass		-0.05	0.156	-0.32	0.75
Fruit production		-0.104	0.084	-1.233	0.221
Adj. R^2		~ 0			
Population: HR	***N***	**df**	**RSE**	***F***	***P***
Model	135	131	0.257	5.442	< 0.05
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.022	-0.276	0.783
F1 seed mass		0.138	0.151	0.913	0.363
F2 seed mass		-0.836	0.21	-3.99	< 0.001
Fruit production		-0.005	0.112	-0.046	0.963
Adj. R^2		0.091			
Pooled populations	***N***	**df**	**RSE**	***F***	***P***
Model	416	412	0.972	8.046	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.048	-0.116	0.908
F1 seed mass		0.028	0.05	0.568	0.570
F2 seed mass		-0.236	0.048	-4.888	< 0.001
Fruit production		0.037	0.049	0.752	0.453
Adj. R^2		0.049			

Table 2.6 Summary of multiple linear regressions to examine the covariates of the mean number of seeds produced per fruit (among maternal families) conducted first, within populations, and then across all populations ("pooled"). F1 seed size, F2 seed size, and fruit production are included as fixed effect independent variables.

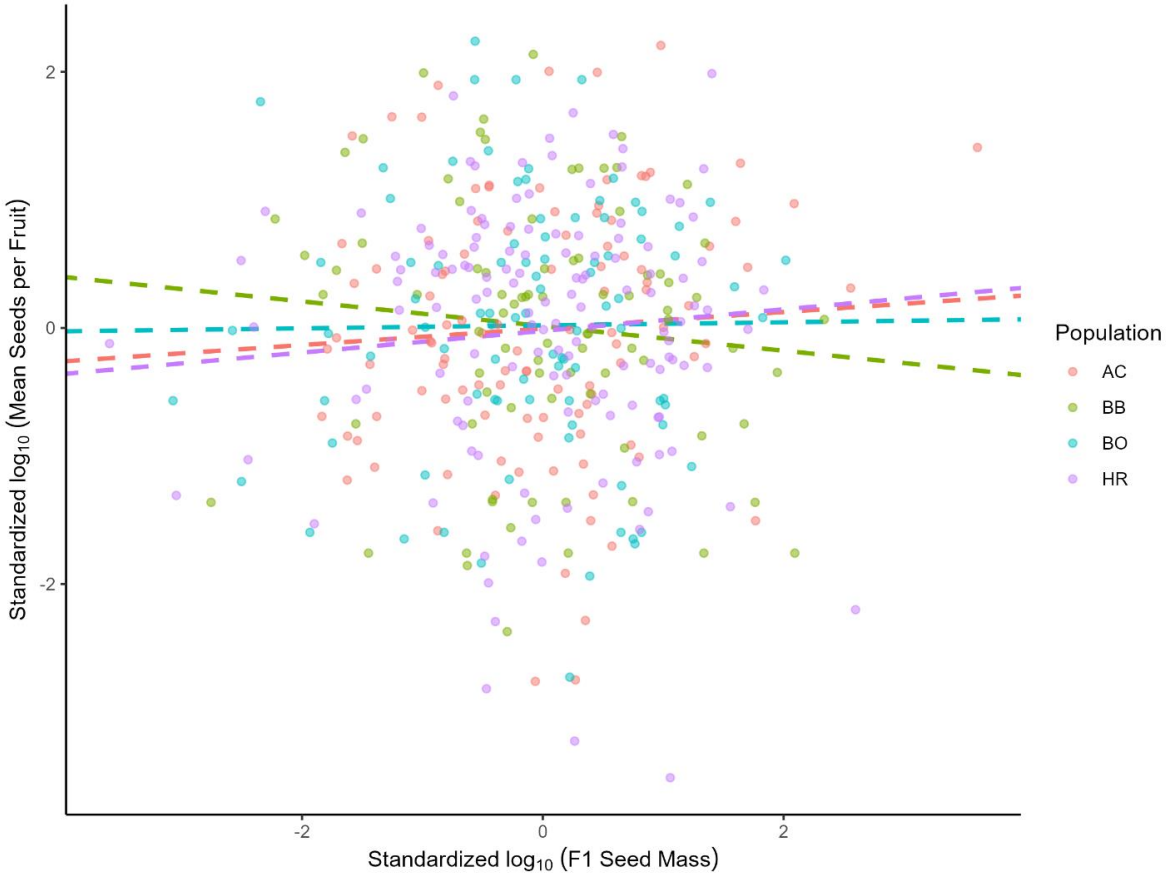

Standardized log10 (Mean Seeds per Fruit)
2
0
-2
-2
0
2
Standardized log10 (F1 Seed Mass)
Population
AC
BB
BO
HR

Figure 2.6 Linear regression of the mean number of seeds produced per fruit on F1 mean seed mass. Each point represents the mean seed size of a maternal family (log-transformed and standardized by population). None of these slopes differ significantly from zero ($\alpha < 0.05$) Slope estimates are based on the results of the multiple linear regressions shown in Table 2.6.

Population: AC	***N***	**df**	**RSE**	***F***	***P***
Model	107	103	0.09	1.824	0.147
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.010	0.017	0.986
F1 seed mass		0.029	0.052	0.562	0.575
Fruit number		0.012	0.039	0.295	0.768
Mean seeds per fruit		-0.129	0.056	-2.291	< 0.05
Adj. R^2		0.023			
Population: BB	***N***	**df**	**RSE**	***F***	***P***
Model	84	80	0.228	3.058	< 0.05
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.013	0.025	0.518	0.606
F1 seed mass		0.094	0.156	0.603	0.548
Fruit number		0.045	0.094	0.479	0.633
Mean seeds per fruit		-0.236	0.088	-2.785	< 0.01
Adj. R^2		0.069			
Population: BO	***N***	**df**	**RSE**	***F***	***P***
Model	90	86	0.194	0.047	0.986
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.001	0.021	0.049	0.961
F1 seed mass		-0.01	0.144	-0.067	0.946
Fruit number		0.008	0.059	0.136	0.892
Mean seeds per fruit		-0.024	0.075	-0.32	0.75
Adj. R^2		~ 0			
Population: HR	***N***	**df**	**RSE**	***F***	***P***
Model	135	131	0.101	11.05	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		~ 0	0.009	-0.021	0.983
F1 seed mass		0.18	0.057	3.147	< 0.01
Fruit number		0.061	0.044	1.403	0.163
Mean seeds per fruit		-0.13	0.033	-3.99	< 0.001
Adj. R^2		0.18			
Pooled populations	***N***	**df**	**RSE**	***F***	***P***
Model	416	412	0.963	10.88	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.018	0.047	0.381	0.703
F1 seed mass		0.116	0.049	2.389	< 0.05
Fruit number		0.063	0.049	1.288	0.199
Mean seeds per fruit		-0.232	0.047	-4.888	< 0.001
Adj. R^2		0.067			

Table 2.7 Summary of multiple linear regressions to examine the covariates of F2 mean seed size (among maternal families) conducted first, within populations, and then across all populations ("pooled"). F1 seed size, the mean number of seeds produced per fruit, and fruit production are included as fixed effect independent variables.

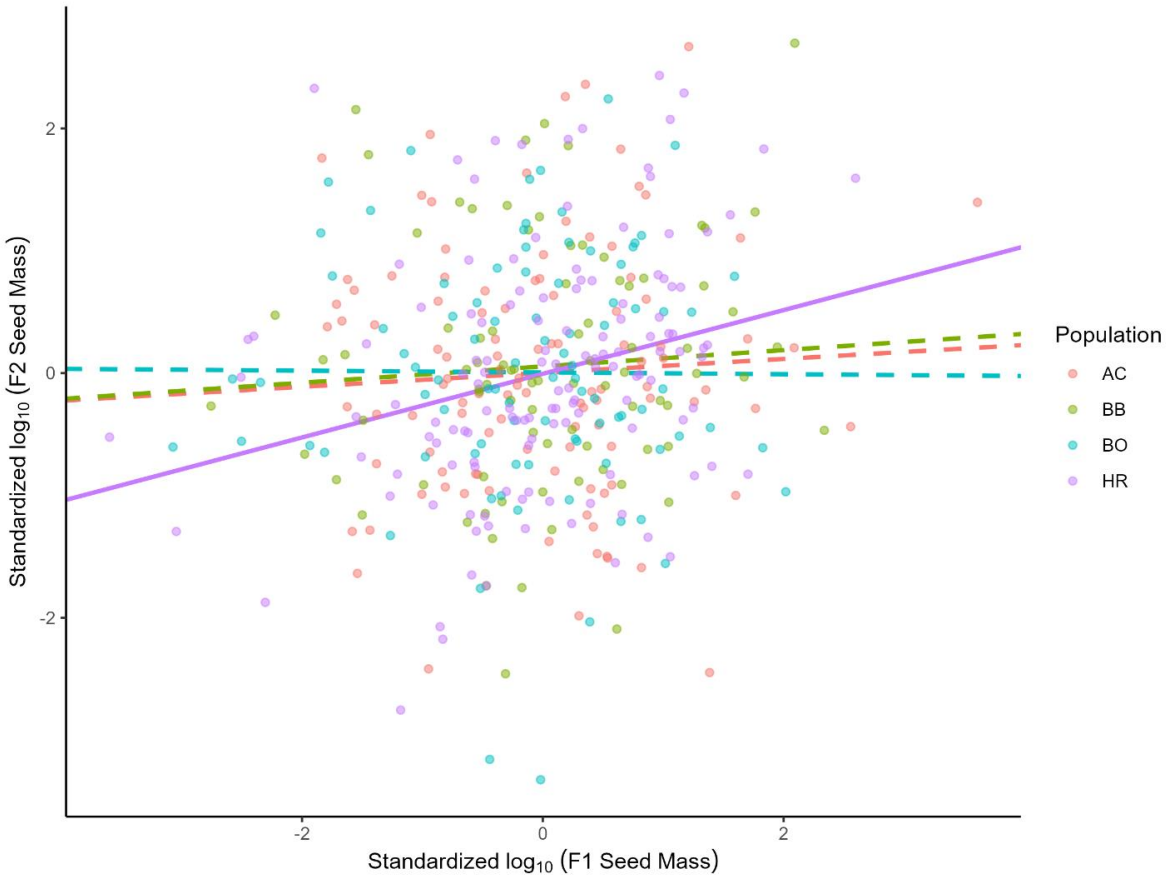

Standardized log10 (F2 Seed Mass)
2
0
-2
-2
0
2
Standardized log10 (F1 Seed Mass)
Population
AC
BB
BO
HR

Figure 2.7: Linear regression of F2 mean seed size on mean seed size of parent (F1). Each point represents the mean seed size of a maternal family (log-transformed and standardized by population). Slopes that differ significantly from zero ($\alpha < 0.05$) are shown by solid lines. Slope estimates are plotted based on the results of the multiple linear regressions shown in Table 2.7.

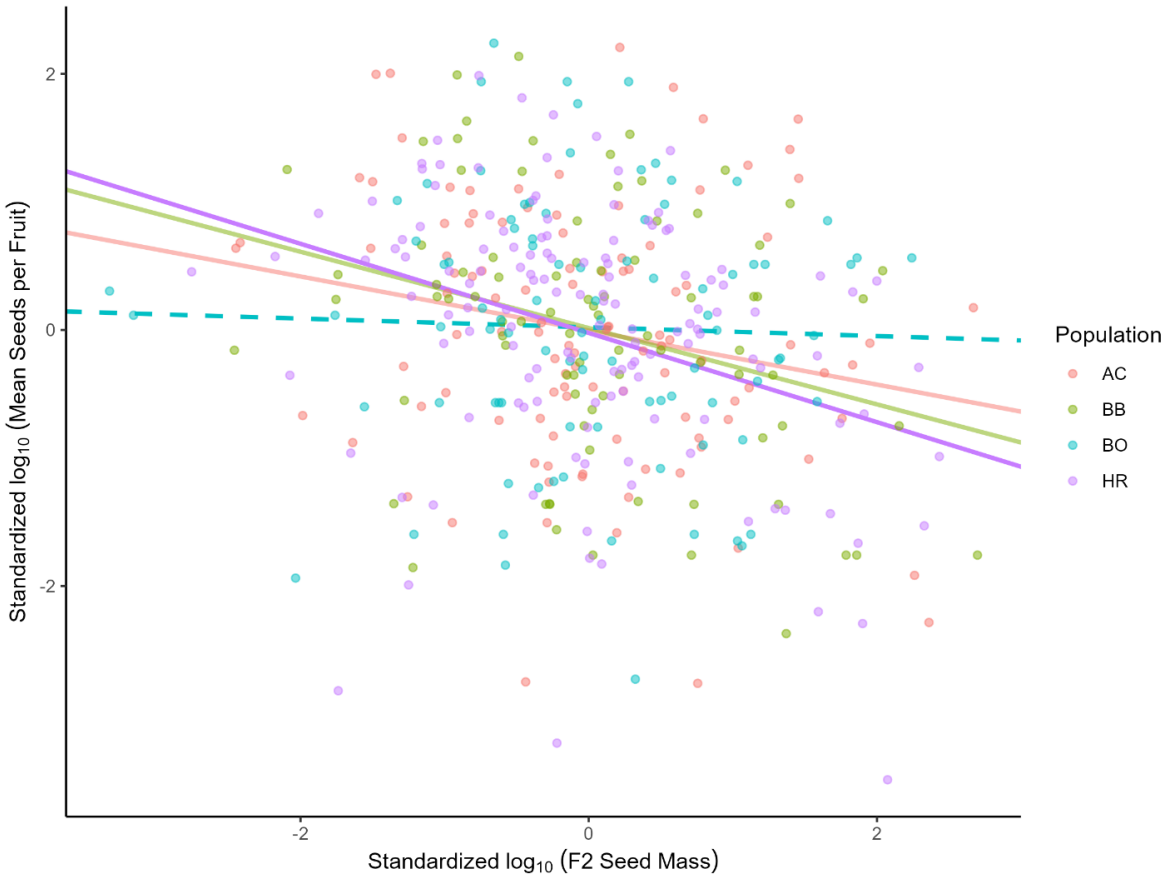

2
0
-2
Standardized log10 (Mean Seeds per Fruit)
-2
0
2
Standardized log10 (F2 Seed Mass)
Population
AC
BB
BO
HR

Figure 2.8: Linear regression mean number of seeds produced per fruit on F2 mean seed mass. Each point represents the mean seed size of a maternal family (log-transformed and standardized by population). Slopes that differ significantly from zero ($\alpha < 0.05$) are shown by solid lines. Slope estimates are plotted based on the results of the multiple linear regressions shown in Table 2.6. The slopes and intercepts overlap for populations AC and BB and is shown by the dark green line.

Population: AC	***N***	**df**	**RSE**	***F***	***P***
Model	107	105	0.316	10.33	< 0.01
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.011	0.031	-0.349	0.728
F1 seed mass		0.549	0.171	3.213	< 0.01
Adj. R^2		0.081			
Population: BB	***N***	**df**	**RSE**	***F***	***P***
Model	84	82	~ 1	0.626	0.431
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.001	0.044	-0.033	0.974
F1 seed mass		0.21	0.265	0.791	0.431
Adj. R^2		~ 0			
Population: BO	***N***	**df**	**RSE**	***F***	***P***
Model	90	88	0.424	0.036	0.851
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.006	0.045	0.125	0.901
F1 seed mass		-0.06	0.313	-0.189	0.851
Adj. R^2		~ 0			
Population: HR	***N***	**df**	**RSE**	***F***	***P***
Model	135	133	0.329	3.964	< 0.05
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.002	0.028	-0.076	0.94
F1 seed mass		0.355	0.178	1.991	< 0.05
Adj. R^2		0.022			
Pooled populations	***N***	**df**	**RSE**	***F***	***P***
Model	416	414	0.019	8.907	< 0.01
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.005	0.048	0.112	0.911
F1 seed mass		0.145	0.049	2.984	< 0.01
Adj. R^2		0.019			

Table 2.8: Summary statistics of bivariate linear regressions to examine the correlation between mean fecundity of F2 maternal families and maternal mean seed size (F1) conducted first, within populations, and then across all populations ("pooled").

Population: AC	***N***	**df**	**RSE**	***F***	***P***
Model	107	105	0.317	10.93	< 0.01
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.01	0.031	-0.336	0.737
F1 seed mass		0.567	0.171	3.306	< 0.01
Adj. R^2		0.086			
Population: BB	***N***	**df**	**RSE**	***F***	***P***
Model	84	82	0.428	1.783	0.185
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.012	0.047	0.246	0.806
F1 seed mass		0.375	0.281	1.335	0.185
Adj. R^2		0.185			
Population: BO	***N***	**df**	**RSE**	***F***	***P***
Model	90	88	0.464	0.0421	0.8379
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.007	0.049	0.133	0.895
F1 seed mass		-0.07	0.343	-0.205	0.838
Adj. R^2		~ 0			
Population: HR	***N***	**df**	**RSE**	***F***	***P***
Model	135	133	0.326	10.24	< 0.001
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		-0.001	0.028	-0.04	0.968
F1 seed mass		0.564	0.176	3.201	< 0.01
Adj. R^2		0.065			
Pooled populations	***N***	**df**	**RSE**	***F***	***P***
Model	416	414	0.0793	15.62	< 0.05
Independent variable		**Estimate**	**SE**	***T***	***P***
Intercept		0.004	0.048	0.082	0.935
F1 seed mass		0.191	0.048	3.952	< 0.001
Adj. R^2		0.034			

Table 2.9: Summary statistics of bivariate linear regressions to examine the correlation, among maternal family means, between reproductive yield and maternal family mean seed size (F1) conducted first, within populations, and then across all populations ("pooled").

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Appendix

Population: AC								
Models:								
AC_reduced: F1 Seed mass ~ (1 | Maternal family)								
AC_mod: F1 Seed mass ~ F0 Seed mass + (1 | Maternal family)								
	npar	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Df**	**Pr(>Chisq)**
AC_reduced	3	122.69	135.27	-58.343	116.69			
AC_mod	4	113.43	130.21	-52.714	105.43	11.258	1	< 0.001
Population: BB								
Models:								
BB_reduced: F1 Seed mass ~ (1 | Maternal family)								
BB_mod: F1 Seed mass ~ F0 Seed mass + (1 | Maternal family)								
	npar	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Df**	**Pr(>Chisq)**
BB_reduced	3	819.27	833.11	-406.64	813.27			
BB_mod	4	810.28	828.73	-401.14	802.28	10.993	1	< 0.001
Population: BO								
Models:								
BO_reduced: F1 Seed mass ~ (1 | Paternal family/ Maternal family)								
BO_mod: F1 Seed mass ~ F0 Seed mass + (1 | Paternal family/ Maternal family)								
	npar	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Df**	**Pr(>Chisq)**
BO_reduced	4	995.7	1013.9	-493.85	987.7			
BO_mod	5	997.4	1020.1	-493.7	987.4	0.3046	1	0.581
Population: HR								
Models:								
HR_reduced: F1 Seed mass ~ (1 | Paternal family/ Maternal family)								
HR_mod: F1 Seed mass ~ F0 Seed mass + (1 | Paternal family/ Maternal family)								
	npar	**AIC**	**BIC**	**logLik**	**deviance**	**Chisq**	**Df**	**Pr(>Chisq)**
HR_reduced	4	512.31	529.99	-252.15	504.31			
HR_mod	5	512.56	534.66	-251.28	502.56	1.7499	1	0.1859

Appendix A: Mixed effects linear regressions to assess the sources of variation in F1 seed size. Multiple models are tested for best fit in each population. The additive model that includes only random effects is shown as "XX_reduced" and the full model is shown as "XX_mod". Models including paternal family as a random effect also include maternal family nested within paternal family as a random effect. Models with only maternal family as a random effect are not nested. F0 seed mass included in the full model ("XX_mod") as a fixed effect.

ANOVA Table (Type III tests)				
Source	**df**	**SS**	***F***	***P***
(Intercept)	1	2.304	82.551	< 0.001
Population	3	12.686	151.515	< 0.001
Generation	2	0.818	14.656	< 0.001
Population x Generation	6	1.98	11.822	< 0.001
Residuals	1236	34.496		

Population	Generation	emmean	SE	df	Lower CL	Upper CL	Size Class
AC	F1	0.248	0.016	1236	0.202	0.295	b
AC	F2	0.259	0.016	1236	0.212	0.305	b
AC	FO	0.147	0.016	1236	0.1	0.193	a
BB	F0	0.591	0.018	1236	0.539	0.643	def
BB	F1	0.674	0.018	1236	0.622	0.726	fg
BB	F2	0.54	0.018	1236	0.488	0.593	de
BO	F0	0.578	0.018	1236	0.527	0.628	de
BO	F1	0.714	0.018	1236	0.663	0.764	g
BO	F2	0.526	0.018	1236	0.476	0.577	d
HR	F0	0.418	0.014	1236	0.377	0.459	c
HR	F1	0.618	0.014	1236	0.577	0.659	ef
HR	F2	0.41	0.014	1236	0.368	0.451	c

Appendix B: The upper portion shows a Type III two-way ANOVA summary table for testing significant differences in seed size among populations and generations. The lower portion shows the estimated marginal means of log-transformed seed mass for each population and generation, the lower and upper confidence levels, and size class shown by compact letter displays, obtained by Sidak adjustment.

```
# only Block as random effect
lmods1_paroff_reg <- list()
for (g in groups) {
  lm_paroff1 <- lmer(log_f2 ~ (1|Block),
                     data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods1_paroff_reg[[g]] <- lm_paroff1
}

# Block and Recipient as random effects
lmods2_paroff_reg <- list()
for (g in groups) {
  lm_paroff2 <- lmer(log_f2 ~ (1|Block) + (1|Recipient),
                     data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods2_paroff_reg[[g]] <- lm_paroff2
}

# Block, Donor, and nested Recipient as random effects
lmods3_paroff_reg <- list()
for (g in groups) {
  lm_paroff3 <- lmer(log_f2 ~ (1|Block) + (1|Donor/Recipient),
                     data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods3_paroff_reg[[g]] <- lm_paroff3
}

# model comparisons to determine random effects that improve model fit
anova(lmods1_paroff_reg$AC, lmods2_paroff_reg$AC, lmods3_paroff_reg$AC)
```

F0 (log_f0) and F1 (log_f1) seed mass are fixed effects

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$AC: log_f2 ~ (1 | Block)
## lmods2_paroff_reg$AC: log_f2 ~ (1 | Block) + (1 | Recipient)
## lmods3_paroff_reg$AC: log_f2 ~ (1 | Block) + (1 | Donor/Recipient)
##                      npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$AC    3 -341.60 -330.71 173.80  -347.60
## lmods2_paroff_reg$AC    4 -343.76 -329.25 175.88  -351.76 4.1619  1    0.04134
## lmods3_paroff_reg$AC    5 -343.45 -325.31 176.72  -353.45 1.6918  1    0.19336
##
## lmods1_paroff_reg$AC
## lmods2_paroff_reg$AC *
## lmods3_paroff_reg$AC
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lmods1_paroff_reg$BB, lmods2_paroff_reg$BB, lmods3_paroff_reg$BB)
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$BB: log_f2 ~ (1 | Block)
## lmods2_paroff_reg$BB: log_f2 ~ (1 | Block) + (1 | Recipient)
## lmods3_paroff_reg$BB: log_f2 ~ (1 | Block) + (1 | Donor/Recipient)
##                       npar    AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$BB    3 1.3236  9.3977 2.3382  -4.6764
## lmods2_paroff_reg$BB    4 2.4537 13.2191 2.7732  -5.5463 0.8699  1    0.35098
## lmods3_paroff_reg$BB    5 1.0944 14.5511 4.4528  -8.9056 3.3593  1    0.06683 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lmods1_paroff_reg$BO, lmods2_paroff_reg$BO, lmods3_paroff_reg$BO)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$BO: log_f2 ~ (1 | Block)
## lmods2_paroff_reg$BO: log_f2 ~ (1 | Block) + (1 | Recipient)
## lmods3_paroff_reg$BO: log_f2 ~ (1 | Block) + (1 | Donor/Recipient)
##                       npar     AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$BO    3 -25.301 -16.5632 15.651  -31.301
## lmods2_paroff_reg$BO    4 -23.959 -12.3081 15.979  -31.959 0.6575  1     0.4174
## lmods3_paroff_reg$BO    5 -22.947  -8.3839 16.474  -32.947 0.9885  1     0.3201
```

```
anova(lmods1_paroff_reg$HR, lmods2_paroff_reg$HR, lmods3_paroff_reg$HR)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$HR: log_f2 ~ (1 | Block)
## lmods2_paroff_reg$HR: log_f2 ~ (1 | Block) + (1 | Recipient)
## lmods3_paroff_reg$HR: log_f2 ~ (1 | Block) + (1 | Donor/Recipient)
##                       npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$HR    3 -319.37 -307.88 162.68  -325.37
## lmods2_paroff_reg$HR    4 -322.25 -306.93 165.12  -330.25 4.8784  1   0.027194
## lmods3_paroff_reg$HR    5 -326.92 -307.78 168.46  -336.92 6.6757  1   0.009773
##
## lmods1_paroff_reg$HR
## lmods2_paroff_reg$HR *
## lmods3_paroff_reg$HR **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Block as random effect, f1 seed mass as fixed effect
lmods1a_paroff_reg <- list()
for (g in groups) {
  lm_paroff1a <- lmer(log_f2 ~ log_f1 + (1|Block),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods1a_paroff_reg[[g]] <- lm_paroff1a
```

```
}

# Block as random effect, f0 seed mass as fixed effect
lmods1b_paroff_reg <- list()
for (g in groups) {
  lm_paroff1b <- lmer(log_f2 ~ log_f0 + (1|Block),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods1b_paroff_reg[[g]] <- lm_paroff1b
}

# Block as random effect, f0 and f1 seed mass as fixed effects
lmods1c_paroff_reg <- list()
for (g in groups) {
  lm_paroff1c <- lmer(log_f2 ~ log_f0 + log_f1 + (1|Block),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods1c_paroff_reg[[g]] <- lm_paroff1c
}

# Block and Recipient as random effects, f1 seed mass as fixed effect
lmods2a_paroff_reg <- list()
for (g in groups) {
  lm_paroff2a <- lmer(log_f2 ~ log_f1 + (1|Block) + (1|Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods2a_paroff_reg[[g]] <- lm_paroff2a
}

# Block and Recipient as random effects, f0 seed mass as fixed effect
lmods2b_paroff_reg <- list()
for (g in groups) {
  lm_paroff2b <- lmer(log_f2 ~ log_f0 + (1|Block) + (1|Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods2b_paroff_reg[[g]] <- lm_paroff2b
}

# Block and Recipient as random effects, both f0 and f1 seed mass fixed effects
lmods2c_paroff_reg <- list()
for (g in groups) {
  lm_paroff2c <- lmer(log_f2 ~ log_f0 + log_f1 + (1|Block) + (1|Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods2c_paroff_reg[[g]] <- lm_paroff2c
}

# Block, Donor, and nested Recipient as random effects, f1 seed mass as fixed effect
lmods3a_paroff_reg <- list()
for (g in groups) {
  lm_paroff3a <- lmer(log_f2 ~ log_f1 + (1|Block) + (1|Donor/Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods3a_paroff_reg[[g]] <- lm_paroff3a
}

# Block, Donor, and nested Recipient as random effects, f0 seed mass as fixed effect
lmods3b_paroff_reg <- list()
for (g in groups) {
```

```
  lm_paroff3b <- lmer(log_f2 ~ log_f0 + (1|Block) + (1|Donor/Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods3b_paroff_reg[[g]] <- lm_paroff3b
}

# Block, Donor, and nested Recipient as random effects,
#both f0 and f1 seed mas as fixed effects
lmods3c_paroff_reg <- list()
for (g in groups) {
  lm_paroff3c <- lmer(log_f2 ~ log_f0 + log_f1 + (1|Block) + (1|Donor/Recipient),
                      data = na_merge_fitdf[na_merge_fitdf$Population == g, ])
  lmods3c_paroff_reg[[g]] <- lm_paroff3c
}
```

```
## boundary (singular) fit: see help('isSingular')
```

```
# model comparisons when including fixed effects, final model selection
anova(lmods2_paroff_reg$AC, lmods2a_paroff_reg$AC,
      lmods2b_paroff_reg$AC, lmods2c_paroff_reg$AC)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods2_paroff_reg$AC: log_f2 ~ (1 | Block) + (1 | Recipient)
## lmods2a_paroff_reg$AC: log_f2 ~ log_f1 + (1 | Block) + (1 | Recipient)
## lmods2b_paroff_reg$AC: log_f2 ~ log_f0 + (1 | Block) + (1 | Recipient)
## lmods2c_paroff_reg$AC: log_f2 ~ log_f0 + log_f1 + (1 | Block) + (1 | Recipient)
##                       npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods2_paroff_reg$AC     4 -343.76 -329.25 175.88  -351.76
## lmods2a_paroff_reg$AC    5 -343.02 -324.88 176.51  -353.02 1.2571  1     0.2622
## lmods2b_paroff_reg$AC    5 -342.72 -324.59 176.36  -352.72 0.0000  0
## lmods2c_paroff_reg$AC    6 -341.58 -319.81 176.79  -353.58 0.8548  1     0.3552
```

```
anova(lmods1_paroff_reg$BB, lmods1a_paroff_reg$BB,
      lmods1b_paroff_reg$BB, lmods1c_paroff_reg$BB)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$BB: log_f2 ~ (1 | Block)
## lmods1a_paroff_reg$BB: log_f2 ~ log_f1 + (1 | Block)
## lmods1b_paroff_reg$BB: log_f2 ~ log_f0 + (1 | Block)
## lmods1c_paroff_reg$BB: log_f2 ~ log_f0 + log_f1 + (1 | Block)
##                       npar    AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$BB     3 1.3236  9.3977 2.3382  -4.6764
## lmods1a_paroff_reg$BB    4 1.7119 12.4773 3.1440  -6.2881 1.6117  1     0.2043
## lmods1b_paroff_reg$BB    4 2.1292 12.8946 2.9354  -5.8708 0.0000  0
## lmods1c_paroff_reg$BB    5 3.0484 16.5051 3.4758  -6.9516 1.0809  1     0.2985
```

```
anova(lmods1_paroff_reg$BO, lmods1a_paroff_reg$BO,
      lmods1b_paroff_reg$BO, lmods1c_paroff_reg$BO)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods1_paroff_reg$BO: log_f2 ~ (1 | Block)
## lmods1a_paroff_reg$BO: log_f2 ~ log_f1 + (1 | Block)
## lmods1b_paroff_reg$BO: log_f2 ~ log_f0 + (1 | Block)
## lmods1c_paroff_reg$BO: log_f2 ~ log_f0 + log_f1 + (1 | Block)
##                        npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods1_paroff_reg$BO      3 -25.301 -16.563 15.651  -31.301
## lmods1a_paroff_reg$BO     4 -24.010 -12.359 16.005  -32.010 0.7089  1     0.3998
## lmods1b_paroff_reg$BO     4 -23.395 -11.744 15.697  -31.394 0.0000  0
## lmods1c_paroff_reg$BO     5 -22.097  -7.534 16.049  -32.097 0.7028  1     0.4018
```

```
anova(lmods3_paroff_reg$HR, lmods3a_paroff_reg$HR,
      lmods3b_paroff_reg$HR, lmods3c_paroff_reg$HR)
```

```
## refitting model(s) with ML (instead of REML)

## Data: na_merge_fitdf[na_merge_fitdf$Population == g, ]
## Models:
## lmods3_paroff_reg$HR: log_f2 ~ (1 | Block) + (1 | Donor/Recipient)
## lmods3a_paroff_reg$HR: log_f2 ~ log_f1 + (1 | Block) + (1 | Donor/Recipient)
## lmods3b_paroff_reg$HR: log_f2 ~ log_f0 + (1 | Block) + (1 | Donor/Recipient)
## lmods3c_paroff_reg$HR: log_f2 ~ log_f0 + log_f1 + (1 | Block) + (1 | Donor/Recipient)
##                        npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## lmods3_paroff_reg$HR      5 -326.92 -307.78 168.46  -336.92
## lmods3a_paroff_reg$HR     6 -331.99 -309.02 172.00  -343.99 7.0706  1   0.007836
## lmods3b_paroff_reg$HR     6 -329.68 -306.71 170.84  -341.68 0.0000  0
## lmods3c_paroff_reg$HR     7 -334.03 -307.23 174.01  -348.03 6.3467  1   0.011760
##
## lmods3_paroff_reg$HR
## lmods3a_paroff_reg$HR **
## lmods3b_paroff_reg$HR
## lmods3c_paroff_reg$HR *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix C (pg. 128-132): Mixed effects linear regressions to assess the sources of variation in F2 seed size. Multiple models are tested for best fit in each population. See R documentation on Dryad.