

Maternal Effects of Drought Stress and
Inbreeding in *Impatiens capensis*

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Abstract

Increased inbreeding and environmental stress are two factors that may significantly affect plant population persistence in the future. However, little is known about the way in which maternal environmental and inbreeding effects combine to influence progeny. We conducted a greenhouse experiment with genetic lines from two populations, differing in inbreeding history and in soil moisture, of the herbaceous annual *Impatiens capensis* (Balsaminaceae) to determine the effects of maternal drought stress and inbreeding on progeny fitness traits, physiology, and response to drought stress. Significant maternal treatment effects were detected, particularly in the typically moist, highly outcrossing population. Overall, maternal drought stress reduced the fitness of progeny. Significant maternal inbreeding effects were also detected, depending on population and life-history stage. In general, inbreeding depression was not exacerbated by maternal environmental stress, but rather was apparent only when maternal environmental conditions were benign.

Introduction

Climate change scenarios for the 21st century predict an increase in climate variability, including more variable temperature and precipitation conditions (IPCC, 1998). As a result, plant population persistence may depend upon the ability of plants to cope with more frequent and more variable drought conditions. Habitat fragmentation due to human encroachment may place an additional stress on natural populations by reducing population sizes, which may in turn increase the frequency with which inbreeding depression is expressed among plant populations (Heschel and Paige, 1994; Lande, 1998). An understanding of ecological and evolutionary responses to drought stress and inbreeding depression is necessary in order to predict the persistence of plant populations. While studies have addressed the potential for populations to acclimate plastically (Aronson et al., 1993; Silva et al., 1999) and to evolve tolerance to a stress (Hoffman and Parsons, 1991; Heschel et al., in review), few have considered the role of maternal effects in these responses (Wolfe, 1993; Montalvo, 1994).

Maternal effects (see Roach and Wulff, 1989 for review) have both an environmental and a genetic component. Maternal environmental effects comprise a form of phenotypic plasticity such that environmental cues in the parental generation will influence the offspring phenotype (Schmitt et al., 1992; Sultan, 1996). If maternal environmental effects are adaptive, offspring fitness may be maintained in unfavorable environmental conditions via maternal provisioning, particularly if the maternal environment predicts the offspring environment (Donohue and Schmitt, 1996; Sultan, 1996). On the other hand, there may be non-adaptive, detrimental effects of maternal environment stress, such that progeny fitness is reduced as a result of maternal stress (Donohue and Schmitt, 1996).

Maternal genotype can also affect the degree to which a maternal plant provisions its offspring (Schmitt et al., 1992). Because of their ability to alter an organism's phenotype, maternal genotype effects can have an important effect on evolutionary responses to natural selection (Kirkpatrick and Lande, 1989). If the maternal genotype effect is positive, the phenotype of the offspring can be exaggerated above the effect of

the genotype alone, thereby accelerating evolution. Conversely, if the maternal genotype effect is negative, it can retard the evolution of a character.

In most plants, the maternal environment and maternal genotype affect the ability of maternal plants to provision their offspring with seed resources (Roach and Wulff, 1987; Sultan, 1996). Water availability is a crucial part of the maternal environment, and there can be substantial plastic and genetic variation in the response to drought stress within populations of plants (Bennington and McGraw, 1995; Dudley, 1996b; Heschel et al., in review). Thus, drought stress in plants provides an excellent opportunity to study the way in which maternal provisioning affects both plastic and evolutionary responses to environmental stress.

One possible mechanism for the effect of maternal drought on progeny is the production of the phytohormone abscisic acid (ABA) (Larcher, 1995), which can be induced by drought conditions (Quarrie and Jones, 1979; Benech-Arnold et al., 1991; Larcher, 1995). The correlated effects of ABA include reduced transpiration due to stomatal closure (Larcher, 1995), growth retardation (Quarrie and Jones, 1977), and changes in germination rate among offspring of plants that exhibit elevated levels of ABA (Sawhney and Naylor, 1982; Benech-Arnold et al., 1991). Plants that have a higher concentration of endogenous ABA also tend to exhibit an increased water-use efficiency ($WUE = \text{rate of carbon assimilation (A) per unit water loss via stomatal conductance (g)}$) (Wong et al, 1979; Voesenek and Blum, 1996). Since increased WUE allows a plant to maximize carbon gain while minimizing water loss due to transpiration, it is an important determinant of a plant's fitness under drought conditions (Dudley, 1996a; Heschel and Hausmann, in review).

Plants that experience drought stress are known to produce seeds with elevated levels of ABA in their endosperm (Goldbach and Goldbach, 1977; Benech-Arnold et al., 1991), potentially provisioning their offspring in anticipation of drought conditions. This maternal environmental effect, however, may vary according to the maternal genotype (Karssen et al., 1983) and its plasticity in ABA production under drought conditions. Natural plant populations are known to differ in morphological and physiological characters that confer drought tolerance (Bennington and McGraw, 1995; Dudley, 1996b), and crop studies have demonstrated the heritability of ABA production (Quarrie,

1981; Conti et al., 1994), suggesting that populations may differ in ABA production and sensitivity in response to drought stress (Heschel and Hausmann, in review). This, in turn, may yield population differentiation in the maternal provisioning of offspring to drought tolerance.

Maternal provisioning is also affected by the degree of inbreeding in the maternal plant. Fitness advantages of outcrossing have been documented in field conditions (Barrett and Kohn, 1991; Montalvo, 1994; Heschel and Paige, 1995) and in greenhouse experiments (Dudash, 1990; Agren and Schemske, 1993; Norman et al., 1995). Since maternal fitness and genotype play a role in maternal provisioning, the level of maternal inbreeding can have fitness consequences for the progeny (del Castillo, 1998). These maternal effects of inbreeding have been shown to decrease the fitness among the offspring of selfed (inbred) plants relative to the offspring of outcrossed plants (del Castillo, 1998). Since maternal effects can persist throughout the life of a plant (Roach and Wulff 1987; Campbell, 1997), the maternal effects of inbreeding may depress the fitness of inbred individuals. On the other hand, maternal environmental effects may be of greater importance than the effects of progeny and maternal inbreeding. Particularly in the seedling stage of development, adaptive maternal environmental effects can override the negative effects of inbreeding (Schaal, 1984; Wolfe, 1993; Montalvo, 1994).

Studies have demonstrated that inbreeding depression can be exacerbated by adverse environmental conditions (Dudash, 1990; Wolfe, 1993; Pray et al., 1994; Miller, 1994; Norman et al., 1995) such that the fitness advantage of outcrossed individuals, heterosis, is stress-dependent. Previous studies have shown that drought can amplify the magnitude of inbreeding depression (Heschel and Paige, 1995; Hauser and Loeschcke, 1996). However, the interaction among drought stress, degree of inbreeding, and maternal provisioning is unknown (Meyer and Allen, 1999). A reduction in fitness due to drought stress and inbreeding could reduce the maternal plant's ability to provision its offspring for drought tolerance, thus decreasing the offspring fitness and accelerating the demise of a population. Alternatively, the environmental maternal effects of drought stress could pre-condition offspring for drought tolerance, overriding the negative fitness effects of inbreeding and maternal effects of inbreeding. Thus an understanding of the

way in which environmental stress, inbreeding, and maternal effects interact is necessary in predicting the fate of a natural population.

To investigate these interactions, we conducted a greenhouse experiment on the annual herb, *Impatiens capensis* Meerb. (Balsaminaceae). *I. capensis* provides a good system in which to examine the maternal effects of drought stress and inbreeding. Natural populations are common in floodplains, stream banks, and wet woods throughout North America, but populations can also persist at drier woodland sites (Leck, 1979; Leck, 1996). Previous studies of *I. capensis* have shown population differences in drought tolerance (Heschel and Hausmann, in review), sensitivity to ABA (Heschel and Hausmann, in review), and in WUE plasticity across drought and non-drought treatments (Heschel et al., in review). Jewelweed exhibits a mixed mating system, producing both self-fertilizing cleistogamous and obligately outcrossing chasmogamous flowers, thus facilitating manipulations of inbreeding coefficient. In addition, inbreeding depression is well documented in this species (Waller, 1979, 1980, 1984; Schmitt and Erhardt, 1990; Schmitt and Gamble, 1990; McCall et al., 1994).

In this paper we address the following questions: (1) Does maternal drought stress confer offspring drought tolerance, or, alternatively, does maternal drought stress have a negative impact on progeny fitness? (2) Is there evidence for inbreeding depression in maternal provisioning? (3) Is there evidence for stress-dependent inbreeding depression in maternal provisioning to drought tolerance? (4) Is there genetic variation within and between populations with different histories of selection on drought tolerance and inbreeding for response to maternal drought stress and maternal inbreeding?

Materials and Methods

Maternal genotypes

Randomly selected seedlings were collected from each of two natural populations of *I. capensis* in May, 1995. These populations, located on Brown University's Haffenreffer Reserve in Bristol, RI, are separated by less than one kilometer, but the sites differ in light and moisture conditions. One site is moist and sunny, while the other is dry and shaded (see Heschel and Hausmann, in review for detailed description). In addition, the populations differ in levels of outcrossing. The moist, sunny population (hereafter referred to as the "Wet" population) exhibits a higher degree of outcrossing than does the "Dry" population, as evidenced by the significantly higher rate of chasmogamous flower production among the Wet population (Heschel et al., in prep.).

Each seedling was used to establish an inbred line over 6 generations of single-seed descent. Lines were maintained under uniform conditions in the Brown University greenhouse so as to remove maternal environmental effects, which can persist for several generations (Roach and Wulff, 1987). Seeds of each generation were stratified at 4°C for 4 months after collection. Nine lines from each population were used for this experiment.

Maternal environments

After 6 generations of selfing, lines were both selfed for a 7th generation and crossed with a line representing a neighbor in the natural population. We did this in order to produce progeny of the same parent that differed in inbreeding coefficient. Crosses were made with randomly chosen lines whose progenitor in the natural population was within a 5 m radius, the typical pollination distance of *I. capensis* (Waller, 1984). Eight selfed and 8 outcrossed offspring of each line were randomized over 8 blocks, 4 of which received a drought stress treatment one week in duration (Heschel et al., in prep.). All blocks were bottom-watered daily, except the drought blocks during the drought treatment, so as to insure uniform soil moisture conditions. Beginning 2.5 weeks after the drought treatment, seeds from each of the 288 maternal

plants were collected. Seed collection took place from mid-May through early June of 1999, and date of collection was recorded for each seed.

Experimental design

We conducted a greenhouse experiment to assess the effects of maternal environment and genotype in the combination of drought stress and inbreeding. Seeds from the 288 maternal plants were collected, weighed, and stratified at 4°C for 4 months. Seed were collected from cleistogamous flowers so as to insure that all progeny were selfed. This design allowed us to examine the effects of maternal inbreeding, but it has the limitation of confounding maternal inbreeding with progeny inbreeding. Progeny were either inbred for 1 generation or for 7 generations, whereas plants in the maternal generation were inbred for 0 generations or 6 generations. The single generation of outcrossing followed by a generation of inbreeding to produce the maternally outcrossed progeny in this study may have caused co-adapted gene complexes to be broken apart in the progeny generation where they were not separated in the maternal generation.

Seeds were planted in late September of 1999 into 3-inch square pots. The soil matrix was composed of 2 parts perlite for every 1 part Scott's Metromix 360 (Scotts-Sierra Horticultural Products Co., Marysville, OH) in order to create a slow and uniformly drying soil environment and to facilitate measurements of root allocation. Eight replicates per maternal treatment per line (576 plants total) were randomized over 8 blocks, with one offspring of each maternal plant in a drought treatment block and one in a non-drought block. We censused and recorded emergence among the 576 experimental plants and 288 extra plants every second day. Those individuals which did not emerge within 20 days were replaced with an extra seedling from the same maternal plant wherever possible. Where fewer than 2 offspring of a given maternal plant emerged, substitutions were made with seedlings of the same maternal treatment and genotype. All plants were grown in the Brown University greenhouse, bottom-watered daily, and fertilized weekly beginning after the emergence census was ended.

Morphological and physiological measurements were made after 4 weeks of growth, at which point most plants had one set of fully expanded leaves. Morphological measurements included height, number of nodes, cotyledon length and width, and the

length and width of the most recently fully expanded leaf. For plants which had no fully expanded leaves, we measured height alone. Physiological measurements were made on 4 blocks, 2 treatment and 2 control blocks, and included carbon assimilation (A) and stomatal conductance (g) (see below). Additionally, we measured the cotyledon and leaf length, width, and area from 40 extra plants. These plants represented 20 from each population and 5 from each maternal treatment (drought, crossed; drought, selfed; non-drought, crossed; non-drought, selfed) within each population. Cotyledons and leaves were traced, the traces scanned, and area measurements made with NIH-Image software (Macintosh version, Scion Corp.). Areas for both cotyledons and leaves were highly correlated (cotyledon: $R^2=0.92$; leaf: $R^2=0.94$) with the product of their length and width measurements in a linear model.

We applied a drought treatment to 4 blocks after 5 weeks of seedling growth. The treatment lasted 7 days and stressed the plants to just above the permanent wilting point. During the 4th-7th days of the treatment we repeated the physiological measurements on the same subset of individuals. Height was also measured to control for effects of size on physiology.

Plants were allowed to recover from the drought treatment and grow for 3 more weeks. We rotated the plants after 1.5 weeks of growth to eliminate neighbor effects. A final set of morphological measurements, including height, number of nodes, number of branches, and length and width of the most recently fully expanded leaf, was conducted at the time of harvest in early December. All shoots were oven-dried at 70°C for one week and weighed. Roots from the 4 blocks on which we performed physiological measurements were washed, oven-dried at 70°C for one week, and weighed.

Physiological measurements

Carbon assimilation rate ($A = \mu\text{M CO}_2\text{m}^{-2}\text{s}^{-1}$) and stomatal conductance ($g = \text{M H}_2\text{O m}^{-2}\text{s}^{-1}$) were measured with an ADC Infrared Gas Analyzer (IRGA) model LCA 4. All IRGA measurements were taken between 9 AM and 3 PM. The LCA 4 had an adjustable PAR light source, and light levels were kept between 790 and 800 $\mu\text{moles m}^{-2}\text{s}^{-1}$. The Parkinson Leaf Chamber was kept over ice, and a fan was used to push air over the chamber. Chamber temperature was kept between 19 and 26°C, with variations

within each day in the range of 2-3°C. Ambient humidity, varying from 40 to 50%, was used for all measurements. We estimated boundary-layer conductances with moist Whatman Filter Paper leaf mimics (Parkinson, 1985). To correct for different leaf areas in the Parkinson Leaf Chamber during measurements, we calculated individual leaf areas (estimated as the product of leaf length and width) which the IRGA then used for each measurement.

Carbon assimilation and stomatal conductance were adjusted for date and time by adding the residuals of a two-way ANOVA (date and time as fixed factors and carbon assimilation or stomatal conductance as response variables) to the grand mean unadjusted carbon assimilation or stomatal conductance (Farris and Lechowicz, 1990).

Statistical Analysis

All statistical analyses were performed with JMP (version 3.1, SAS Institute, 1994). Three-way ANOVAs were used to test for population, maternal treatment, and maternal cross-type differences in seed mass, emergence date, height, and carbon assimilation and stomatal conductance measurements made before the progeny drought treatment was administered. Population, maternal treatment, and maternal cross-type were fixed factors, while line (nested within population) and block were random factors (mixed model—Type III sums of squares). Due to higher order interactions in the three-way ANOVAs, data were sorted by maternal treatment and analyzed using two-way ANOVAs with population and maternal cross-type as fixed factors and line and block as random factors.

Four-way ANOVAs were used to test for population, maternal treatment, maternal cross-type, and progeny treatment differences in carbon assimilation, stomatal conductance, and biomass. Population, maternal treatment, maternal cross-type, and progeny treatment were fixed factors, while line (nested within population) and block (nested within progeny treatment) were random factors. Due to the observation of significant higher order interactions in the four-way ANOVAs, data were sorted by maternal treatment and progeny treatment, and analyzed using two-way ANOVAs with population and maternal cross-type as fixed factors and line and block as random factors.

Linear contrasts were calculated on model Least Square Means to ascertain where differences lie within interaction terms. All models were examined for normality of residuals and homoscedasticity. Emergence date was log-transformed to normalize residuals.

Results

Seed Traits

Seed mass did not differ between the two maternal treatments (Table 1). However, there was a marginally significant population by maternal treatment effect (Table 1). Contrasts within the interaction revealed that seed mass of plants from the Wet population differed depending on whether or not the maternal plant was subject to drought stress, whereas seed mass in the Dry population did not depend on maternal treatment (Figure 1). This result suggests that the Wet population seeds suffer negative repercussions when the mother plant experiences a drought stress, whereas the Dry population seeds are not affected. Much of the variation for seed mass, however, was dependent upon genotype, as significant genetic variation for the effect of cross-type on seed mass (significant line*maternal cross-type terms) was detected for both maternal non-drought and maternal drought treatments (Figure 2; Table 2).

For emergence date, there was no significant effect of maternal treatment, although there was a significant interaction between maternal treatment and maternal cross-type (Table 1). When the model was broken down by maternal treatment, emergence date among maternal non-drought seeds was revealed to depend upon maternal cross-type (Table 2); maternally outcrossed seeds tended to emerge earlier than maternally selfed seeds (Figure 3). There was, however, no difference in emergence date between maternally inbred and outcrossed seeds in the maternal drought treatment. Within the maternal non-drought treatment, there was also a significant effect of population such that Dry population seeds emerged significantly earlier than Wet population seeds (Dry population LSM=0.633 days (natural log transformed), Wet population LSM=0.744 days (natural log transformed), SE=0.033), while there was no statistically significant population difference within the maternal drought treatment (Table 2). Additionally, there was a significant line*maternal cross-type interaction among seeds from drought treatment mothers (Figure 4; Table 2), indicating genetic variation for response to outcrossing and selfing when the mother plant has experienced a drought stress.

Seedling Height

Four weeks after planting, the maternal non-drought treatment seedlings were taller than the maternal drought treatment plants (Table 1) (maternal drought treatment LSM=5.60 cm, maternal non-drought treatment LSM=5.82 cm, SE=0.06). Dry population seedlings were taller than Wet population seedlings in both maternal treatments (Table 2) (Dry population LSM=6.31 cm, Wet population LSM=5.11 cm, SE=0.26).

In addition to a main effect of population, there was a marginally significant population by maternal cross-type effect (Table 1). Contrasts within the interaction showed that maternally selfed plants in the Dry population were shorter than maternally outcrossed plants ($t=3.07$, $p=0.007$), while there was no difference between maternal cross-types within the Wet population ($t=0.43$, $p=0.673$) (Figure 5). Finally, there was a significant interaction between maternal cross-type and maternal treatment (Table 1). When the model was broken down by maternal treatment, maternal cross-type affected height for both populations only within the maternal non-drought plants (Figure 5; Table 2). In the maternal non-drought treatment, maternally outcrossed seedlings of both the Dry and Wet populations tended to be taller than maternally selfed seedlings, while in the maternal drought treatment, maternally outcrossed plants were taller only in the Dry population. These findings suggest that there was inbreeding depression for seedling height in both populations in non-drought conditions, but only the Dry population showed inbreeding depression in drought conditions.

The observed trends in height are in part attributable to the effects of population, maternal cross-type, and maternal treatment on emergence date. Emergence was a highly significant predictor of height when used as a covariate ($F=277.97$, $df=1$, $p<0.001$), and the trend for earlier emergence among outcrossed, maternally non-drought treated seedlings explains the significant effects of maternal cross-type and maternal treatment on height (Table 3). In addition, emergence date as a covariate reduces the significance of population as a predictor of height (Table 3); the later emergence of Dry population seedlings explains, in part, the reduced height among Wet population individuals relative to Dry population individuals.

Physiology

Maternal treatment did not affect carbon assimilation prior to the progeny drought treatment (Table 4). Within the maternal non-drought treatment, population had a significant effect on carbon assimilation (Table 5); Wet population seedlings had higher carbon assimilation when the mother experienced a non-drought treatment (Wet population LSM=12.30, Dry population LSM=11.44, SE=0.16), while there was no population difference in carbon assimilation if the mother experienced a drought treatment (Wet population LSM=12.15, Dry population LSM=11.63, SE=0.23). This is in part due to the effects of maternal treatment on emergence date, which is a significant predictor of carbon assimilation when used as a covariate ($F=7.49$, $df=1$, $p=0.007$). Emergence was delayed among seedlings of the Wet population relative to those of the Dry population in the maternal non-drought treatment, but seedlings of both populations emerged concurrently in the maternal drought treatment. When used as a covariate for carbon assimilation among maternally non-droughted progeny, emergence date reduced the significance of population as a predictor ($F=8.78$, $df=1$, $p=0.008$).

Maternal cross-type alone did not affect carbon assimilation within either maternal treatment (Table 5). In the maternal drought treatment, however, there was genetic variation for carbon assimilation response to maternal cross-type (Figure 6; Table 5), which was not detected for the maternal non-drought treatment.

There were no statistically significant effects of maternal treatment, maternal cross-type, or population on stomatal conductance in the pre-treatment physiological measurements (Table 4; Table 5).

During the progeny drought treatment, there was no significant effect of maternal treatment or progeny treatment on carbon assimilation (Table 6), although there was a significant line*maternal cross-type*progeny treatment interaction. When the model was examined by maternal treatment and progeny treatment due to this significant higher order interaction, population was found to be a significant predictor of carbon assimilation for the plants in the maternal drought, progeny non-drought treatment combination (Table 7); in all other combinations of maternal and progeny treatment, carbon assimilation did not depend on population. For the maternal drought, progeny

non-drought treatment combination, the Wet population had significantly higher carbon assimilation than the Dry population (Wet population LSM=10.91, Dry population LSM=10.17, SE=0.22). This represents a reversal of the pre-treatment trend for Wet population carbon assimilation to be greater than Dry population carbon assimilation only in the maternal non-drought treatment. As with the first set of physiological trait measurements, emergence date accounts for some of the population differences in carbon assimilation. Emergence date was a significant covariate to carbon assimilation during the progeny drought treatment ($F=12.74$, $df=1$, $p<0.001$), and within the maternal drought, progeny non-drought treatment combination, emergence date removed the effect of population on carbon assimilation ($F=1.93$, $df=1$, $p=0.184$; Table 7).

Maternal cross-type did not affect carbon assimilation during the progeny drought treatment (Table 7), although there was a significant line*maternal cross-type interaction for the maternal non-drought, progeny non-drought treatment combination (Figure 7, Table 7). This interaction indicates that there is genetic variation for carbon assimilation response to maternal cross-type when there is neither a maternal nor a progeny drought treatment, while response is constrained when there is a drought stress in either the maternal or progeny generation.

The progeny drought treatment greatly reduced stomatal conductance among the progeny drought treatment plants relative to the progeny non-drought treatment plants (progeny drought LSM=0.060, progeny non-drought LSM=0.177, SE=0.007) (Table 8), indicating that the treatment effectively stressed the plants. Maternal treatment, however, was not a significant predictor of stomatal conductance (Table 8). Breaking the model down by maternal and progeny treatment combinations (Table 9) revealed a significant effect of population in the maternal drought, progeny non-drought combination, wherein the Wet population had greater stomatal conductance than the Dry population (Wet population LSM=0.195, Dry population LSM=0.160, SE=0.007). This trend is in accordance with the trend for greater carbon assimilation in the Wet population for this combination of maternal and progeny treatments.

Maternal cross-type was also a significant predictor of stomatal conductance when both maternal and progeny generations experienced a drought stress (Table 9). Maternally selfed individuals of both populations had higher stomatal conductance in this

treatment combination than did maternally outcrossed individuals (maternally outcrossed LSM=0.048, maternally selfed LSM=0.075, SE=0.008). There was no effect of maternal cross-type on stomatal conductance for any of the other maternal and progeny treatment combinations.

We do not report the results for water-use efficiency (A/g) because the trends for carbon assimilation and stomatal conductance tended to be very similar in both sets of physiological trait measurements, with the result that the population, maternal treatment, maternal cross-type, and progeny treatment effects on water-use efficiency could be better explained through their effects on carbon assimilation and stomatal conductance individually.

Fitness

Final above-ground biomass provides a good indicator of overall fitness among *I. capensis* individuals (Waller, 1979). For biomass, Wet population plants were larger than Dry population plants across all maternal and progeny treatments (Table 10; Table 11). Maternal treatment was also a predictor of biomass (Table 10); plants with the maternal drought treatment were less fit than plants with the maternal non-drought treatment (maternal drought treatment LSM=652.11 mg, maternal non-drought treatment LSM=672.91 mg, SE=6.33). This response to maternal treatment, however, varied by population (Figure 8); contrasts for the significant population*maternal treatment term showed that Wet population biomass decreased in the maternal drought treatment relative to the maternal non-drought treatment ($t=3.28$, $p=0.005$), while the Dry population fitness did not change across maternal treatments ($t=0.008$, $p=0.994$). This trend is consistent with the reduced seed mass among the maternally drought-stressed Wet population individuals relative to maternal non-drought treatment Wet population individuals. Seed mass was a significant predictor of biomass when used as a covariate ($F=5.81$, $df=1$, $p=0.016$). Nonetheless, when holding the effect of seed mass constant, the effect of maternal drought on biomass within the Wet population was still significant (contrast within pop*maternal treatment term: $t=2.84$, $p=0.012$).

Maternal cross-type had an effect on biomass within the Dry population. Contrasts within the significant population*maternal cross-type interaction term revealed that Dry population biomass decreased among maternally outcrossed individuals relative to maternally selfed individuals ($t=2.61$, $p=0.020$), while the Wet population biomass did not vary between maternally inbred and outcrossed individuals ($t=0.35$, $p=0.734$) (Figure 8). Maternal cross-type, however, did affect all plants in the maternal non-drought, progeny non-drought treatment combination, independent of population (Table 11); maternally outcrossed plants were larger than maternally selfed plants when there was no drought treatment in either the maternal or progeny generation, but outcrossed and inbred individuals did not differ in all other treatment combinations. This result suggests that maternal inbreeding only reduced the fitness of progeny across both populations when there was no drought stress in either the maternal or progeny generation.

Discussion

Despite the implications of drought stress and inbreeding depression for the persistence of natural plant populations, little is known about the potentially interactive maternal effects of drought stress and inbreeding. In this study of *Impatiens capensis*, we found that the maternal effects of drought stress and inbreeding interact in complex ways, with their outcome depending upon population, genetic line, trait, and timing in the life-history. The effects of maternal drought may override some of the negative effects of inbreeding among progeny, but, overall, mothers which experienced a drought did not maintain the fitness of their progeny, nor did they pre-condition their offspring against the effects of an early life-history selection event in the progeny generation such as a drought. Maternal inbreeding did reduce the fitness of progeny, but the effects of maternal inbreeding differed across populations. In general, however, inbreeding depression was not exacerbated by maternal or progeny drought stress. Instead, inbreeding depression manifested itself predominantly when conditions were benign, a result that runs counter to predictions based on theory and other empirical studies. Although there was little variation among genotypes for response to maternal drought, there was significant variation for response to inbreeding and the combination of inbreeding and maternal drought, suggesting that the relationship among the two factors is not fixed within these two populations of *I. capensis*.

Maternal Drought Effects on Progeny

The overall effect of a maternal generation drought treatment was to reduce the fitness of progeny, contrary to the theory of adaptive maternal effects. The specific nature of these fitness effects, however, depended upon the population from which the genetic line was derived.

A maternal drought treatment reduced seed mass among Wet population progeny but not among Dry population progeny (Figure 1), which in part accounts for the decreased height and biomass among maternally drought treated Wet population individuals relative to maternally non-drought treated individuals. Wet population seeds

also tended to emerge later than Dry population seeds, which could put them at a significant disadvantage in terms of reproductive output by delaying reproductive maturity in field conditions (Kalisz, 1986; Heschel and Riginos, in prep.). The effects of maternal drought stress on the Wet population seed mass, compounded by the overall later emergence in this population, could have significant fitness consequences for progeny in the scenario of a progeny generation drought stress. Thus the maternal effects of drought do not appear to confer functional homeostasis to drought stress (Sultan, 1996) among progeny of the Wet population.

While the Dry population did not show any significant response to maternal treatment in terms of seed mass or emergence date, individuals among this population appeared to be more sensitive to maternal treatment in terms of physiology. In the second set of physiological trait measurements, Dry population individuals had reduced carbon assimilation in the maternal drought treatment relative to the maternal non-drought treatment, while Wet population individuals did not differ across maternal treatments. This increased sensitivity to the effects of maternal treatment on carbon assimilation among the Dry population is not surprising given that selection appears to have constrained the variance of response in early life-history carbon assimilation among the Wet population; no heritability for carbon assimilation was detected among seedlings of the Wet population in a separate experiment (Heschel and Riginos, in prep.).

Similarly, the Dry population had lower stomatal conductance than the Wet population when there was a maternal drought treatment, but the populations did not differ when there was no maternal drought treatment. This result is consistent with the greater sensitivity of the Dry population to exogenous ABA relative to the Wet population (Heschel and Hausmann, in review). Taken together, the increased sensitivity of the Dry population to the maternal effects of drought may suggest that maternal drought has a developmental effect, perhaps via ABA (Hansen, 2000), on physiology among individuals of the Dry population. It is unlikely that ABA acts directly on progeny of drought stressed mother plants, since the hormone has a short duration of residence (Harris and Outlaw, 1991) and would not have persisted through the 4 months dormancy of *Impatiens capensis* seeds. Additionally, had ABA been present in the germinating seeds of maternally drought stressed individuals, we would have expected

the Dry population seeds to have emerged later than the Wet population seeds, due to the increased sensitivity to ABA among the Dry population (Heschel and Hausmann, in review) and the germination-retarding effects of the hormone (Sawhney and Naylor, 1982; Benech-Arnold et al., 1991).

The overall lower rate of carbon fixation and water loss among the Dry population due to a maternal drought treatment may be beneficial only if slower growth confers greater fitness among individuals in a population (Givnish, 1979). Progeny of plants which experienced a drought stress conserved water relative to progeny of plants which experienced benign conditions at the cost of slower growth. This reduction in carbon assimilation would confer a fitness advantage on progeny during a late season drought stress (Heschel et al., in review; Heschel et al., in prep.), but may not be favorable in the situation where drought results in high mortality early in the life-history of the progeny. In an early season drought scenario, faster growth, and thus higher reproductive output, would favor progeny which did not receive a maternal drought stress or progeny which were minimally sensitive to a maternal drought stress, since a high rate of carbon assimilation is selectively advantageous in this scenario (Heschel and Riginos, in prep.). Thus the fitness consequences of maternal drought effects among the Dry population are dependent upon the conditions experienced among progeny.

These findings, however, are equivocal. While the second survey of physiological characters showed a population difference in carbon assimilation and stomatal conductance only when the mother experienced a drought treatment, the first set of physiological trait measurements showed a population effect only when the mother did not experience a drought treatment (carbon assimilation) or no population effect at all (stomatal conductance). This discrepancy between the two sets of physiological measurements is in part due to the overall later emergence among maternally droughted individuals. Maternally drought treated individuals may have been at a similar developmental stage in the second physiological trait survey as were the maternally non-drought treated individuals at the first survey of physiological traits. As a result, developmental stage may be confounding the effects of maternal treatment on physiological traits.

Maternal Inbreeding Effects on Progeny

Maternal inbreeding did reduce progeny fitness for a number of traits. In non-drought conditions, inbreeding depression was detected across both populations. The negative effects of inbreeding, however, were present in the Dry population, which experiences a high degree of selfing in the natural population, regardless of maternal treatment. Maternal inbreeding reduced seedling height (Figure 5) and biomass (Figure 8) in the Dry population relative to maternally outcrossed seedlings, while there was no effect of maternal inbreeding on the Wet population height and biomass. These results do not support the prediction that inbreeding depression is reduced in populations that have a high degree of selfing, such as the Dry population, due to the purging of deleterious recessive alleles (Charlesworth and Charlesworth, 1987), suggesting instead that inbreeding depression may persist in a population despite high levels of selfing (Byers and Waller, 1999).

This result is also counter to the findings among the maternal generation, in which the Dry population showed no effects of inbreeding, while the Wet population had strong inbreeding depression for morphological, physiological, and fitness traits (Heschel et al., in prep.). The attenuation of inbreeding depression later in the lifetime of the Dry population suggests that there may be relaxed selection on the Dry population early in the lifetime, whereas stronger selection later in the lifetime results in the purging of deleterious recessive alleles and thus the absence of inbreeding depression in the Dry population (Heschel and Hausmann, in prep.). Strong selective pressures are typically exerted upon the Dry population later in the lifetime due to increased water stress in field conditions, whereas early season soil moisture conditions tend to be benign (Heschel and Hausmann, in review). In the Wet population, on the other hand, there is strong competition for light early in the season (J. Schmitt, pers. comm.), thus potentially removing any negative effects of inbreeding early in the plants' lifetime.

Alternatively, the absence of an early life-history effect of inbreeding on the Wet population height and biomass may be due to the significant maternal treatment effect on seed mass in this population. Inbreeding effects can be masked by the effects of maternal environment for seedlings (Schmitt and Ehrhardt, 1990; Montalvo, 1994). This may explain why the Dry population, which did not have strong maternal treatment effects for

seed mass, exhibited inbreeding depression for height and biomass while the Wet population did not.

A potentially complicating factor in examining the effects of inbreeding in this study is the design that confounds maternal inbreeding with progeny inbreeding. The single generation of outcrossing followed by a generation of inbreeding that produced the maternally outcrossed progeny may have caused co-adapted gene complexes to be broken apart in the progeny generation where they were not separated in the maternal generation. This may in part explain the differences in inbreeding effects observed between the maternal and progeny generations. This may also explain the differences in inbreeding depression between the Dry and Wet populations, since the Dry population is highly structured genetically, with strong local adaptation (Schmitt and Gamble, 1990), while the Wet population does not show this degree of genetic structuring (Knight and Waller, 1987).

The fitness consequences of inbreeding in *Impatiens capensis* appear to vary according to life-history stage (Dudash, 1990; Byers and Waller, 1999). Given that inbreeding depression was seen in the Dry population during the seedling and young adult life-history stages, an early season selective pressure, such as a drought, could have a significant impact on this predominantly selfing population, since inbred individuals in the population may not have achieved reproductive maturity. A late-season selection event, on the other hand, would be more detrimental to the Wet population, where inbreeding depression is significant among adult plants (Heschel and Riginos, in prep.).

Stress-Dependent Heterosis and Maternal Effects

Maternal inbreeding was exacerbated by a stressful environment for stomatal conductance; selfed individuals had higher stomatal conductance relative to outcrossed individuals in the maternal drought, progeny drought treatment combination. This higher stomatal conductance results in increased water loss, and thus a potential fitness decrease, among selfed individuals in drought conditions. The effects of inbreeding were only apparent when both the mother and progeny experienced a drought stress, suggesting that inbreeding depression may become more severe with an increasing number of generations of environmental stress. For species such as *Impatiens capensis*, which tends

to produce fewer chasmogamous seeds under stressful environmental conditions (Waller, 1980), multiple generations of drought stress conditions could cause further inbreeding, accelerating the demise of a natural population in a positive feedback.

With the exception of height and stomatal conductance, however, maternal inbreeding only negatively affected the progeny when the maternal environmental conditions were benign. Emergence, for instance, was delayed among maternally inbred seedlings relative to maternally outcrossed seedlings only in the maternal non-drought treatment (Figure 3). Likewise for biomass, inbreeding depression was maternal treatment-dependent, such that inbreeding only reduced biomass when there was a maternal non-drought treatment. In no cases other than stomatal conductance was the effect of inbreeding dependent upon the progeny treatment. These findings are reversed from the predictions based on theory (Lloyd, 1980) and other studies (Dudash, 1990; Pray et al., 1994) which suggest that inbreeding depression is exacerbated by adverse environmental conditions. Our results indicate that in some situations the negative effects of inbreeding depression and environmental stress do not interact.

Genetic Variation in Responses

Although we found population differences for response to maternal drought stress, we did not find any significant genetic variation within the two populations for response to maternal drought. This suggests that the maternal effects of drought stress are fixed within each population, with little potential for future evolution of response to maternal drought (Sultan, 1996). Maternal effects may thus mask any genotypic variation in progeny response to a stress in the progeny generation. Since maternal drought had a detrimental effect on progeny for several traits, the constrained response to maternal drought treatment may jeopardize a population in future scenarios of more frequent and more variable droughts.

Likewise, there was little genetic variation in progeny response to maternal inbreeding. With the exception of seed mass, none of the traits examined showed significant line variation across maternal cross-types. This is surprising, given that significant genetic variation in response to inbreeding was detected in the maternal

generation (Heschel et al., in prep.). The variation in seed mass, however, is significant given the strong effect of seed mass on later traits such as biomass.

Although early life-history responses to maternal drought treatment and inbreeding appear to be constrained for most traits, the interaction between inbreeding and maternal drought does not appear to be fixed. We found significant genetic variation for response to inbreeding within maternally drought treated individuals for early life-history characters such as seed mass and emergence date. This suggests that the potential for evolution in response to environmental stress may still be present even within populations that exhibit a high degree of inbreeding, such as small or highly fragmented populations. However, multiple generations of environmental stress may act to fix phenotypic responses, possibly constraining the interaction between drought stress and inbreeding.

Conservation Implications

Our findings suggest that the maternal effects of drought stress and inbreeding have important effects on progeny fitness. In most cases, these effects are detrimental to the progeny generation. Mother plants did not maintain the fitness of progeny when subjected to a drought treatment, but instead delayed their emergence and retarded their growth. The reduced growth rate and water loss caused by maternal drought treatment could be beneficial to the progeny if they experience a drought stress later in life, but an early life-history selection event could cause high mortality before the progeny of drought-stressed mother plants have reached reproductive maturity. Likewise, maternal inbreeding in this study reduced the fitness of progeny early in their life, although inbreeding was not exacerbated by environmental stress.

Based on these findings, maternal environment and inbreeding history are important considerations in selecting seeds for seed banks or for restoration purposes. Care should be taken to observe the history of environmental stresses and the degree of inbreeding in a potential source population. This may pose a problem for plant conservation, since many rare or threatened species of plants persist in small populations that have been subjected to recent environmental stresses. Wherever possible, however, seeds should be collected from larger populations and from populations that are less

likely to have experienced recent adverse environmental conditions. Particularly since maternal effects can persist for multiple generations (Alexander and Wulff, 1985; Campbell, 1997), it is important to consider the past stresses to which a population has been exposed if re-population efforts are to be successful.

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TABLE 1. ANOVAs for population, line, maternal cross-type, and maternal treatment on seed traits and morphology.

Progeny Trait	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Seed mass (model $r^2 = 0.338$)	Population	104.80	1	2.84	0.111
	Line[Pop]	590.09	16	2.04	0.093
	Maternal Cross-Type	24.13	1	1.70	0.211
	Maternal Treatment	14.22	1	1.29	0.272
	Mat. Cross*Mat. Trt	6.83	1	0.96	0.343
	Pop*Mat. Cross	31.97	1	2.25	0.153
	Pop*Mat. Trt	41.88	1	3.81	0.069
	Pop*Mat. Trt*Mat. Cross	1.45	1	0.20	0.658
	Line[Pop]*Mat. Cross	227.44	16	1.99	0.090
	Line[Pop]*Mat. Trt	175.70	16	1.54	0.199
	Line[Pop]*Mat. Cross*Mat. Trt	114.23	16	1.39	0.147
	Residual	2611.01			
	Days to emergence (model $r^2 = 0.387$)	Population	1.75	1	4.39
Line[Pop]		6.39	16	4.03	0.007
Maternal Cross-Type		0.26	1	3.49	0.080
Maternal Treatment		0.10	1	1.55	0.232
Block		0.19	7	0.71	0.667
Mat. Cross*Mat. Trt		0.26	1	6.20	0.024
Pop*Mat. Cross		0.06	1	0.76	0.395
Pop*Mat. Trt		0.00	1	0.00	0.996
Pop*Mat. Trt*Mat. Cross		0.06	1	1.46	0.245
Line[Pop]*Mat. Cross		1.20	16	1.79	0.127
Line[Pop]*Mat. Trt		1.05	16	1.56	0.190
Line[Pop]*Mat. Cross*Mat. Trt		0.67	16	1.10	0.353
Residual		19.01			
Height (model $r^2 = 0.415$)	Population	206.52	1	10.99	0.004
	Line[Pop]	300.78	16	11.90	0.002
	Maternal Cross-Type	10.81	1	6.13	0.025
	Maternal Treatment	7.09	1	5.97	0.027
	Block	112.11	7	7.85	<0.001
	Mat. Cross*Mat. Trt	4.64	1	3.39	0.084
	Pop*Mat. Cross	6.15	1	3.49	0.080
	Pop*Mat. Trt	0.23	1	0.19	0.666
	Pop*Mat. Trt*Mat. Cross	0.73	1	0.53	0.476
	Line[Pop]*Mat. Cross	28.19	16	1.29	0.310
	Line[Pop]*Mat. Trt	18.98	16	0.87	0.611
	Line[Pop]*Mat. Cross*Mat. Trt	21.90	16	0.67	0.823
	Residual	1013.27			

TABLE 2. ANOVAs for population, maternal cross-type, and line on seed traits and morphology, by maternal treatment.

Progeny Trait	Maternal Treatment	Source of Variation	SS	df	<i>F</i>	<i>p</i>	
Seed mass	Non-drought	Population	7.09	1	0.43	0.519	
		Line[Pop]	261.21	16	1.88	0.109	
		Maternal Cross-Type	2.64	1	0.30	0.589	
		Pop*Mat. Cross	9.90	1	1.14	0.302	
		Line[Pop]*Mat. Cross	139.13	16	1.70	0.051	
		Residual	1305.85				
		Drought	Population	139.58	1	4.43	0.052
	Line[Pop]	504.58	16	2.49	0.039		
	Maternal Cross-Type	28.31	1	2.24	0.154		
	Pop*Mat. Cross	23.52	1	1.86	0.192		
	Line[Pop]*Mat. Cross	202.59	16	2.46	0.002		
	Residual	1305.15					
	Days to emergence	Non-drought	Population	0.8789	1	5.48	0.033
			Line[Pop]	2.5646	16	4.79	0.002
Maternal Cross-Type			0.5233	1	15.65	0.001	
Block			0.2259	7	0.86	0.538	
Pop*Mat. Cross			0.1189	1	3.56	0.078	
Line[Pop]*Mat. Cross			0.5348	16	0.89	0.580	
Residual			9.1852				
Drought		Population	0.8751	1	2.87	0.110	
Line[Pop]		4.8813	16	3.63	0.007		
Maternal Cross-Type		0.0000	1	0.00	0.996		
Block		0.3113	7	1.15	0.333		
Pop*Mat. Cross		0.0000	1	0.00	0.986		
Line[Pop]*Mat. Cross		1.3431	16	2.17	0.007		
Residual		9.4816					
Height	Non-drought	Population	96.49	1	10.77	0.005	
		Line[Pop]	143.39	16	7.40	<0.001	
		Maternal Cross-Type	14.81	1	12.23	0.003	
		Block	92.06	7	6.74	<0.001	
		Pop*Mat. Cross	1.32	1	1.09	0.312	
		Line[Pop]*Mat. Cross	19.38	16	0.62	0.866	
		Residual	477.86				
	Drought	Population	110.26	1	10.00	0.006	
	Line[Pop]	176.37	16	5.74	<0.001		
	Maternal Cross-Type	0.64	1	0.33	0.571		
	Block	41.05	7	2.79	0.008		
	Pop*Mat. Cross	5.56	1	2.89	0.108		
	Line[Pop]*Mat. Cross	30.71	16	0.914	0.553		
	Residual	514.40					

TABLE 3. ANCOVA for population, line, maternal cross-type, and maternal treatment on height with emergence date as a covariate.

Progeny Trait	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Height (model $r^2 = 0.625$)	Population	67.35	1	4.40	0.052
	Line[Pop]	261.95	16	10.02	0.002
	Maternal Cross-Type	1.08	1	0.51	0.483
	Maternal Treatment	1.60	1	2.33	0.146
	Block	94.85	7	10.35	<0.001
	Mat. Cross*Mat. Trt	0.01	1	0.01	0.943
	Pop*Mat. Cross	2.04	1	0.97	0.340
	Pop*Mat. Trt	0.236	1	0.35	0.565
	Pop*Mat. Trt*Mat. Cross	3.74	1	3.26	0.090
	Line[Pop]*Mat. Cross	33.61	16	1.83	0.118
	Line[Pop]*Mat. Trt	10.93	16	0.59	0.845
	Line[Pop]*Mat. Cross*Mat. Trt	18.34	16	0.88	0.598
	Log (days to emergence)	363.92	1	277.97	<0.001
	Residual	649.35			

TABLE 4. ANOVAs for population, line, maternal cross-type, and maternal treatment on pre-treatment physiological traits.

Progeny Trait	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Carbon assimilation (model $r^2 = 0.394$)	Population	31.42	1	8.49	0.010
	Line[Pop]	59.30	16	2.09	0.290
	Maternal Cross-Type	2.02	1	0.76	0.396
	Maternal Treatment	0.03	1	0.02	0.888
	Block	17.76	3	3.42	0.018
	Mat. Cross*Mat. Trt	3.24	1	1.29	0.273
	Pop*Mat. Cross	0.39	1	0.15	0.706
	Pop*Mat. Trt	2.10	1	1.29	0.273
	Pop*Mat. Trt*Mat. Cross	0.08	1	0.03	0.862
	Line[Pop]*Mat. Cross	42.56	16	1.06	0.454
	Line[Pop]*Mat. Trt	25.99	16	0.65	0.804
	Line[Pop]*Mat. Cross*Mat. Trt	40.18	16	1.45	0.122
	Residual	349.86			
	Stomatal conductance (model $r^2 = 0.300$)	Population	0.0010	1	0.31
Line[Pop]		0.0508	16	1.21	0.437
Maternal Cross-Type		0.0022	1	0.76	0.397
Maternal Treatment		0.0001	1	0.03	0.864
Block		0.0239	3	3.33	0.021
Mat. Cross*Mat. Trt		0.0003	1	0.13	0.726
Pop*Mat. Cross		0.0020	1	0.70	0.416
Pop*Mat. Trt		0.0001	1	0.03	0.871
Pop*Mat. Trt*Mat. Cross		0.0105	1	4.37	0.053
Line[Pop]*Mat. Cross		0.0468	16	1.22	0.346
Line[Pop]*Mat. Trt		0.0336	16	0.88	0.601
Line[Pop]*Mat. Cross*Mat. Trt		0.0383	16	1.00	0.459
Residual		0.4836			

TABLE 5. ANOVAs for population, maternal cross-type, and line on physiological traits, by maternal treatment.

Progeny Trait	Maternal Treatment	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Carbon assimilation	Non-drought	Population	25.99	1	14.88	0.001
		Line[Pop]	27.96	16	1.75	0.136
		Maternal Cross-Type	0.07	1	0.07	0.791
		Block	12.93	3	2.77	0.046
		Pop*Mat. Cross	0.06	1	0.06	0.807
		Line[Pop]*Mat. Cross	15.96	16	0.64	0.843
		Residual	157.18			
	Drought	Population	8.98	1	2.53	0.131
		Line[Pop]	57.00	16	0.86	0.614
		Maternal Cross-Type	5.27	1	1.28	0.274
		Block	19.21	3	3.52	0.018
		Pop*Mat. Cross	0.48	1	0.12	0.737
		Line[Pop]*Mat. Cross	65.99	16	2.27	0.007
		Residual	178.29			
Stomatal Conductance	Non-drought	Population	0.0009	1	.47	0.505
		Line[Pop]	0.0308	16	0.91	0.570
		Maternal Cross-Type	0.0021	1	1.0	0.332
		Block	0.0113	3	1.43	0.240
		Pop*Mat. Cross	0.0017	1	0.82	0.378
		Line[Pop]*Mat. Cross	0.0336	16	0.797	0.686
		Residual	0.2666			
	Drought	Population	0.0003	1	0.10	0.752
		Line[Pop]	0.0526	16	1.02	0.487
		Maternal Cross-Type	0.0004	1	0.14	0.716
		Block	0.0211	3	3.30	0.024
		Pop*Mat. Cross	0.0106	1	3.29	0.088
		Line[Pop]*Mat. Cross	0.0518	16	1.52	0.108
		Residual	0.2086			

TABLE 6. ANOVA for population, line, maternal cross-type, maternal treatment, and progeny treatment on carbon assimilation.

Source of Variation	SS	df	<i>F</i>	<i>p</i>
Population	0.87	1	0.19	0.669
Line[Pop]	73.55	16	3.62	0.801
Maternal Cross-Type	3.79	1	0.63	0.441
Maternal Treatment	1.18	1	0.28	0.604
Progeny Treatment	464.45	1	380.13	0.127
Block[Prog. Trt]	1.99	2	0.18	0.835
Mat. Cross*Mat. Trt	23.78	1	4.45	0.051
Mat. Cross*Prog. Trt	3.59	1	0.43	0.523
Mat. Trt*Prog. Trt	2.19	1	0.56	0.464
Mat. Cross*Mat. Trt*Prog. Trt	0.55	1	0.18	0.673
Pop*Mat. Cross	5.92	1	0.98	0.338
Pop*Mat. Trt	16.03	1	3.80	0.069
Pop*Prog. Trt	1.46	1	0.25	0.621
Pop*Mat. Trt*Mat. Cross	3.28	1	0.61	0.445
Pop*Mat. Cross*Prog. Trt	5.05	1	0.60	0.451
Pop*Mat. Trt*Prog. Trt	0.01	1	0.00	0.960
Pop*Mat. Cross*Mat. Trt*Prog. Trt	5.69	1	1.93	0.184
Line[Pop]*Mat. Cross	97.04	16	0.56	0.875
Line[Pop]*Mat. Trt	67.53	16	0.67	0.776
Line[Pop]*Prog. Trt	91.61	16	0.61	0.832
Line[Pop]*Mat. Cross*Mat. Trt	85.58	16	1.82	0.122
Line[Pop]*Mat. Cross*Prog. Trt	135.07	16	2.87	0.021
Line[Pop]*Mat. Trt*Prog. Trt	62.34	16	1.32	0.291
Line[Pop]*Mat. Cross*Mat. Trt*Prog. Trt	47.14	16	0.54	0.924
Trt				
Residual	780.97			

TABLE 7. ANOVAs for population, line, and maternal cross-type on carbon assimilation, by maternal and progeny treatment.

Maternal Treatment	Progeny Treatment	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Non-Drought	Non-drought	Population	0.96	1	0.33	0.575
		Line[Pop]	46.88	16	0.48	0.922
		Maternal Cross-Type	4.17	1	0.69	0.419
		Block	0.70	1	0.27	0.610
		Pop*Mat. Cross	0.15	1	0.02	0.879
		Line[Pop]*Mat. Cross	97.23	16	2.32	0.019
		Residual	91.72			
	Drought	Population	4.36	1	0.77	0.394
		Line[Pop]	91.06	16	0.77	0.697
		Maternal Cross-Type	0.79	1	0.11	0.749
		Block	3.45	1	0.47	0.498
		Pop*Mat. Cross	0.06	1	0.01	0.930
		Line[Pop]*Mat. Cross	118.41	16	1.01	0.474
		Residual	257.69			
Drought	Non-drought	Population	9.76	1	5.75	0.029
		Line[Pop]	27.17	16	0.67	0.781
		Maternal Cross-Type	4.39	1	1.74	0.206
		Block	1.29	1	0.49	0.487
		Pop*Mat. Cross	0.04	1	0.01	0.905
		Line[Pop]*Mat. Cross	40.36	16	0.97	0.511
		Residual	91.38			
	Drought	Population	3.30	1	0.41	0.533
		Line[Pop]	129.93	16	1.19	0.364
		Maternal Cross-Type	22.36	1	3.29	0.089
		Block	2.35	1	0.25	0.623
		Pop*Mat. Cross	19.70	1	2.90	0.108
		Line[Pop]*Mat. Cross	108.83	16	0.71	0.763
		Residual	334.40			

TABLE 8. ANOVA for population, line, maternal cross-type, maternal treatment, and progeny treatment on stomatal conductance.

Source of Variation	SS	df	<i>F</i>	<i>p</i>
Population	0.0038	1	1.02	0.328
Line[Pop]	0.0599	16	0.50	0.887
Maternal Cross-Type	0.0004	1	0.08	0.784
Maternal Treatment	0.0003	1	0.11	0.746
Progeny Treatment	0.9927	1	124.41	<0.001
Block[Prog. Trt]	0.0113	2	1.46	0.236
Mat. Cross*Mat. Trt	0.0126	1	6.23	0.024
Mat. Cross*Prog. Trt	0.0103	1	2.36	0.144
Mat. Trt*Prog. Trt	0.0001	1	0.03	0.860
Mat. Cross*Mat. Trt*Prog. Trt	0.0013	1	0.39	0.540
Pop*Mat. Cross	0.0010	1	0.19	0.669
Pop*Mat. Trt	0.0108	1	3.64	0.075
Pop*Prog. Trt	0.0082	1	1.32	0.267
Pop*Mat. Trt*Mat. Cross	0.0011	1	0.53	0.475
Pop*Mat. Cross*Prog. Trt	0.0001	1	0.01	0.906
Pop*Mat. Trt*Prog. Trt	0.0015	1	0.41	0.529
Pop*Mat. Cross*Mat. Trt*Prog. Trt	0.0016	1	0.51	0.486
Line[Pop]*Mat. Cross	0.0821	16	1.64	0.316
Line[Pop]*Mat. Trt	0.0475	16	1.24	0.480
Line[Pop]*Prog. Trt	0.0993	16	1.31	0.356
Line[Pop]*Mat. Cross*Mat. Trt	0.0322	16	0.62	0.824
Line[Pop]*Mat. Cross*Prog. Trt	0.0698	16	1.35	0.279
Line[Pop]*Mat. Trt*Prog. Trt	0.0579	16	1.12	0.414
Line[Pop]*Mat. Cross*Mat. Trt*Prog. Trt	0.0518	16	0.84	0.640
Trt				
Residual	0.5482			

TABLE 9. ANOVAs for population, line, and maternal cross-type on stomatal conductance, by maternal and progeny treatment.

Maternal Treatment	Progeny Treatment	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Non-Drought	Non-drought	Population	0.0000	1	0.00	0.952
		Line[Pop]	0.1023	16	1.57	0.188
		Maternal Cross-Type	0.0098	1	2.40	0.141
		Block	0.0039	1	1.16	0.288
		Pop*Mat. Cross	0.0006	1	0.14	0.718
		Line[Pop]*Mat. Cross	0.0652	16	1.21	0.309
		Residual	0.1179			
	Drought	Population	0.0022	1	0.95	0.345
		Line[Pop]	0.0374	16	0.63	0.820
		Maternal Cross-Type	0.0011	1	0.29	0.596
		Block	0.0029	1	0.74	0.396
		Pop*Mat. Cross	0.0006	1	0.17	0.688
		Line[Pop]*Mat. Cross	0.0598	16	0.95	0.523
		Residual	0.1371			
Drought	Non-drought	Population	0.0218	1	10.84	0.005
		Line[Pop]	0.0321	16	0.42	0.953
		Maternal Cross-Type	0.0005	1	0.107	0.748
		Block	0.0050	1	1.37	0.250
		Pop*Mat. Cross	0.0003	1	0.05	0.823
		Line[Pop]*Mat. Cross	0.0761	16	1.31	0.246
		Residual	0.1271			
	Drought	Population	0.0003	1	0.06	0.815
		Line[Pop]	0.0928	16	2.65	0.030
		Maternal Cross-Type	0.0131	1	6.01	0.026
		Block	0.0002	1	0.05	0.822
		Pop*Mat. Cross	0.0023	1	1.07	0.317
		Line[Pop]*Mat. Cross	0.0350	16	0.46	0.949
		Residual	0.1653			

TABLE 10. ANOVA for population, line, maternal cross-type, maternal treatment, and progeny treatment on above-ground biomass.

Source of Variation	SS	df	<i>F</i>	<i>p</i>
Population	3309534	1	25.09	<0.001
Line[Pop]	2110146	16	2.69	0.040
Maternal Cross-Type	76013	1	2.56	0.130
Maternal Treatment	62311	1	5.39	0.034
Progeny Treatment	453990	1	1.26	0.300
Block[Prog. Trt]	2049803	6	18.94	<0.001
Mat. Cross*Mat. Trt	23270	1	1.30	0.271
Mat. Cross*Prog. Trt	3808	1	0.32	0.582
Mat. Trt*Prog. Trt	236	1	0.02	0.881
Mat. Cross*Mat. Trt*Prog. Trt	3807	1	0.30	0.592
Pop*Mat. Cross	129633	1	4.36	0.053
Pop*Mat. Trt	61688	1	5.34	0.034
Pop*Prog. Trt	25987	1	0.74	0.403
Pop*Mat. Trt*Mat. Cross	10329	1	0.58	0.458
Pop*Mat. Cross*Prog. Trt	8	1	0.00	0.980
Pop*Mat. Trt*Prog. Trt	2857	1	0.28	0.605
Pop*Mat. Cross*Mat. Trt*Prog. Trt	9726	1	0.76	0.395
Line[Pop]*Mat. Cross	475513	16	1.73	0.227
Line[Pop]*Mat. Trt	184924	16	0.75	0.703
Line[Pop]*Prog. Trt	564211	16	3.67	0.126
Line[Pop]*Mat. Cross*Mat. Trt	286160	16	1.40	0.252
Line[Pop]*Mat. Cross*Prog. Trt	192785	16	0.95	0.543
Line[Pop]*Mat. Trt*Prog. Trt	164619	16	0.81	0.663
Line[Pop]*Mat. Cross*Mat. Trt*Prog. Trt	203715	16	0.71	0.789
Trt				
Residual	7684982			

TABLE 11. ANOVAs for population, line, and maternal cross-type on above-ground biomass, by maternal and progeny treatment.

Maternal Treatment	Progeny Treatment	Source of Variation	SS	df	<i>F</i>	<i>p</i>
Non-Drought	Non-drought	Population	1183019	1	20.24	<0.001
		Line[Pop]	930613	16	3.77	0.006
		Maternal Cross-Type	76084	1	4.94	0.041
		Block	348104	1	5.66	0.001
		Pop*Mat. Cross	6615.11	1	0.43	0.522
		Line[Pop]*Mat. Cross	246553	16	0.75	0.735
		Residual	2151463			
	Drought	Population	960237	1	31.77	<0.001
		Line[Pop]	483580	16	1.61	0.176
		Maternal Cross-Type	23230.8	1	1.24	0.283
		Block	772853	1	20.08	<.001
		Pop*Mat. Cross	31358.5	1	1.67	0.215
		Line[Pop]*Mat. Cross	300962	16	1.47	0.127
		Residual	1347090			
Drought	Non-drought	Population	797003	1	10.79	0.005
		Line[Pop]	1181791	16	3.61	0.007
		Maternal Cross-Type	3792.51	1	0.19	0.673
		Block	439107	1	6.34	<.001
		Pop*Mat. Cross	79289.2	1	3.87	0.067
		Line[Pop]*Mat. Cross	327420	16	0.89	0.586
		Residual	2422978			
	Drought	Population	459808	1	17.19	<0.001
		Line[Pop]	427916	16	1.51	0.209
		Maternal Cross-Type	3791.48	1	0.21	0.650
		Block	523974	1	10.61	<.001
		Pop*Mat. Cross	32433	1	1.83	0.195
		Line[Pop]*Mat. Cross	283238	16	1.07	0.388
		Residual	1729215			

Figure Legends

Figure 1. Population-dependent effect of maternal drought treatment on seed mass. Least-square means ± 1 S.E. are shown.

Figure 2. Reaction norms for maternally outcrossed vs. inbred progeny seed mass in the (a) maternal non-drought treatment, and (b) maternal drought treatment. Wet population genotypes are denoted by closed squares, and Dry population genotypes are denoted by open circles. Genotype by maternal cross-type interactions are significant in both maternal treatments.

Figure 3. Inbreeding depression is dependent on maternal treatment for emergence date. Least-square means ± 1 S.E. are shown.

Figure 4. Reaction norms for maternally outcrossed vs. inbred progeny emergence date in the (a) maternal non-drought treatment, and (b) maternal drought treatment. Wet population genotypes are denoted by closed squares, and Dry population genotypes are denoted by open circles. Genotype by maternal cross-type interaction is significant only in the maternal drought treatment.

Figure 5. Effects of maternal inbreeding and population on height across maternal treatments. Least-square means ± 1 S.E. are shown.

Figure 6. Reaction norms for maternally outcrossed vs. inbred progeny carbon assimilation in the (a) maternal non-drought treatment, and (b) maternal drought treatment. Wet population genotypes are denoted by closed squares, and Dry population genotypes are denoted by open circles. Genotype by maternal cross-type interaction is significant only in the maternal drought treatment.

Figure 7. Reaction norms for maternally outcrossed vs. inbred progeny carbon assimilation in the (a) maternal non-drought, progeny non-drought treatment combination; (b) maternal non-drought, progeny drought treatment combination; (c) maternal drought, progeny non-drought treatment combination; and, (d) maternal drought, progeny drought treatment combination. Wet population genotypes are denoted by closed squares, and Dry population genotypes are denoted by open circles. Genotype by maternal cross-type interaction is significant only in the maternal non-drought, progeny non-drought treatment combination.

Figure 8. Effects of maternal inbreeding and population on biomass across maternal treatments. Least-square means ± 1 S.E. are shown.

Figure 1

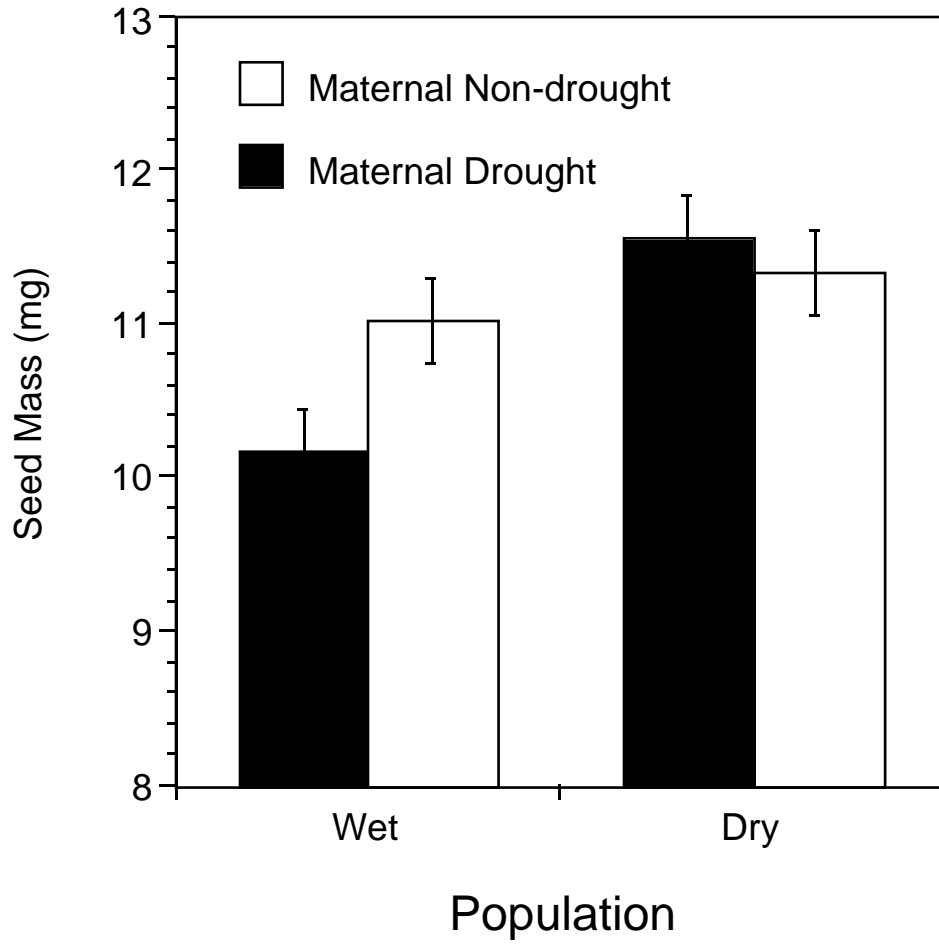
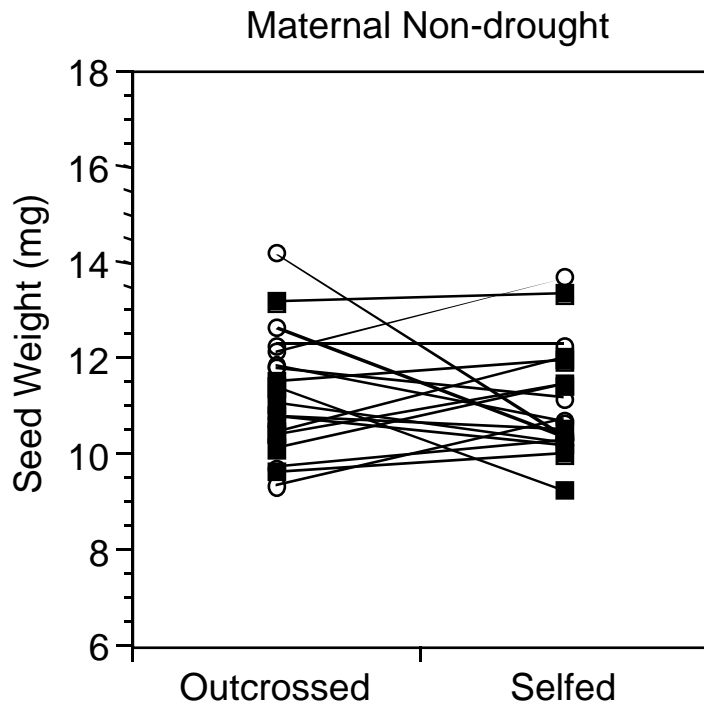


Figure 2

A.



B.

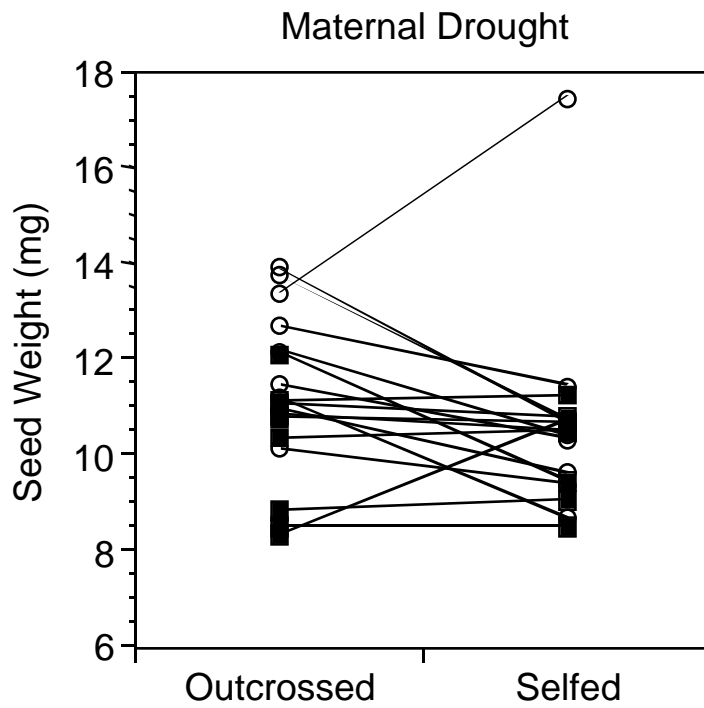


Figure 3

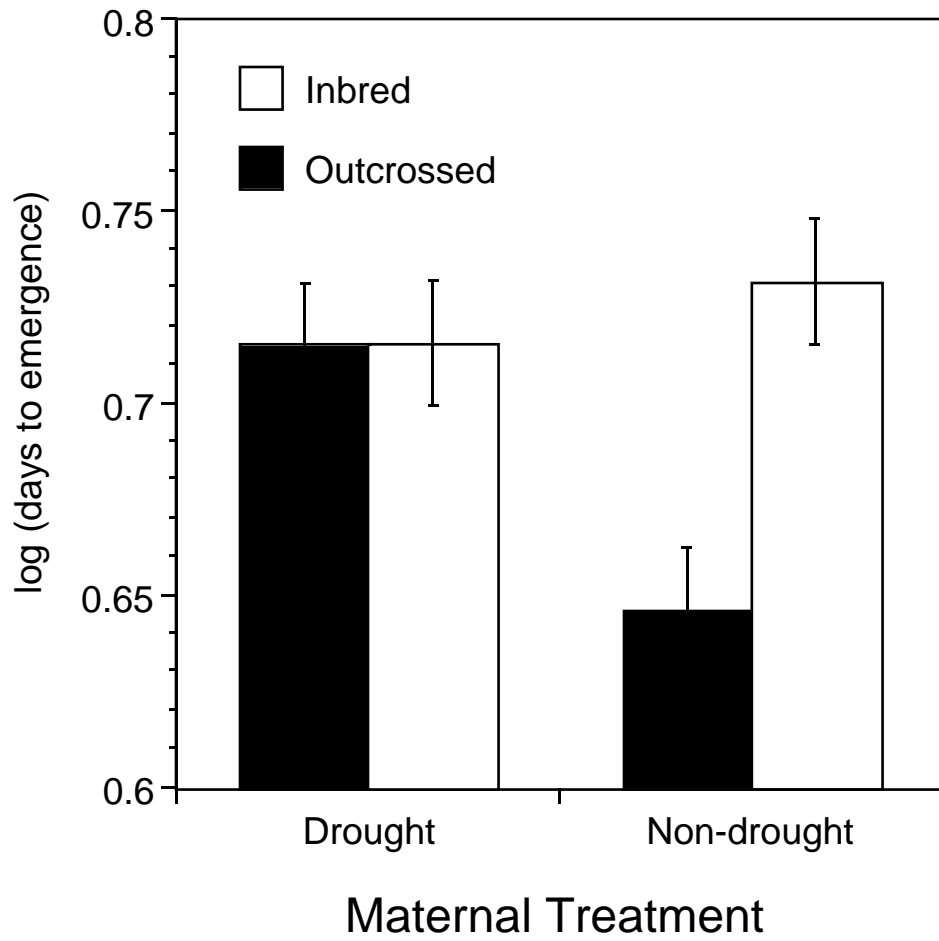
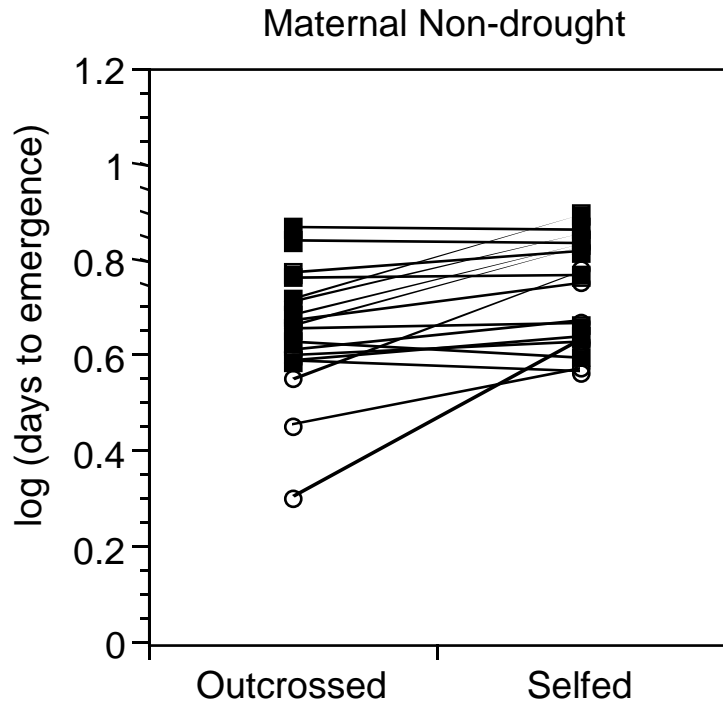


Figure 4

A.



B.

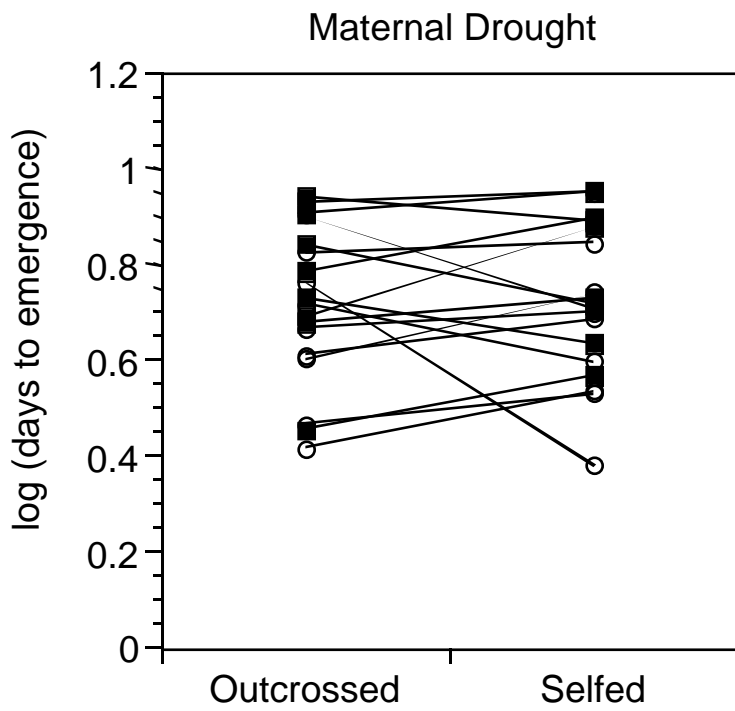


Figure 5

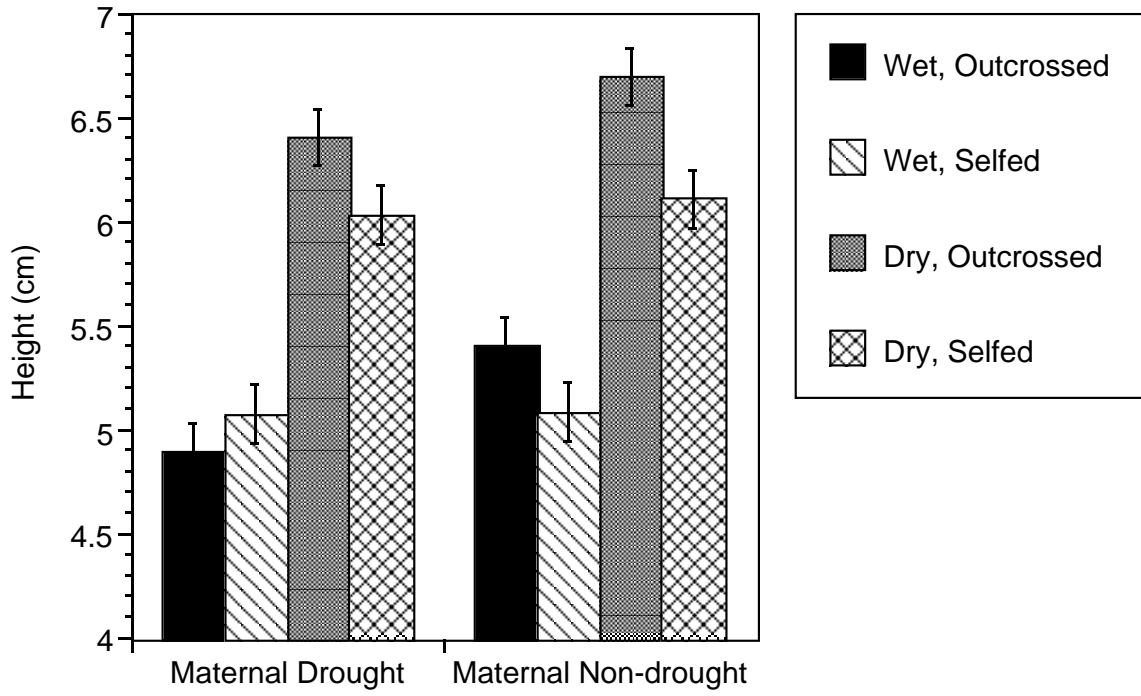
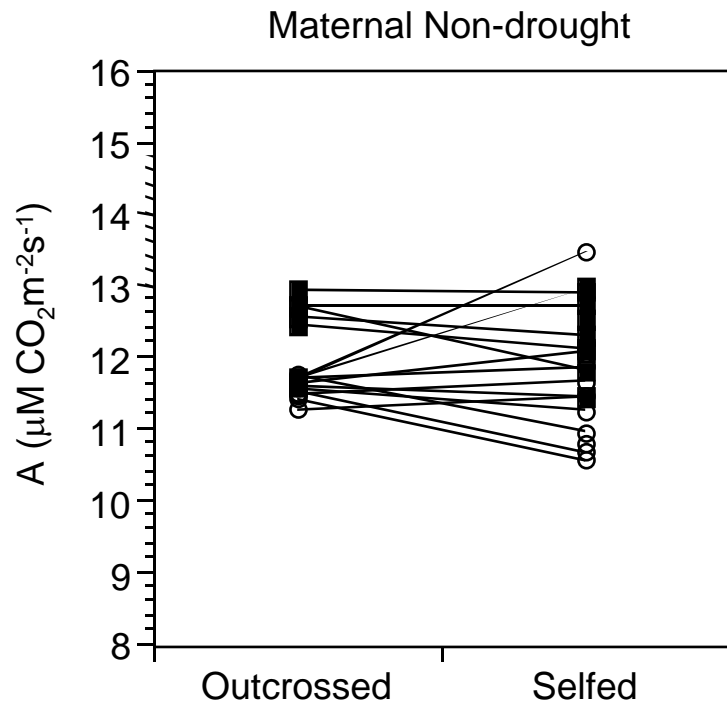


Figure 6

A.



B.

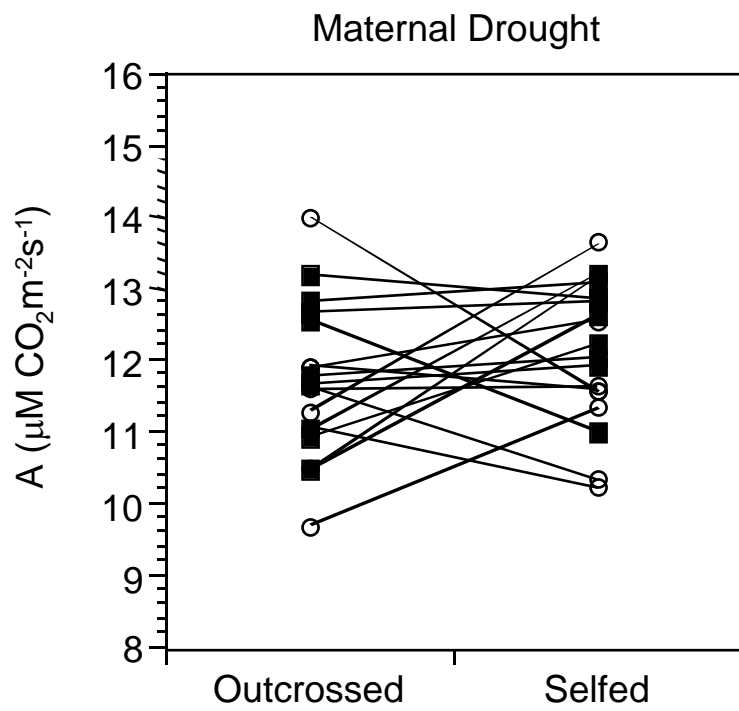
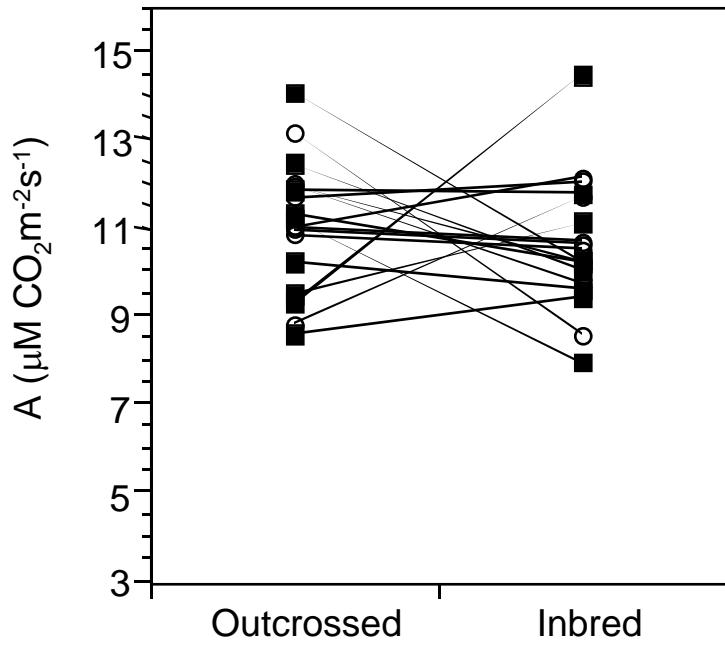


Figure 7

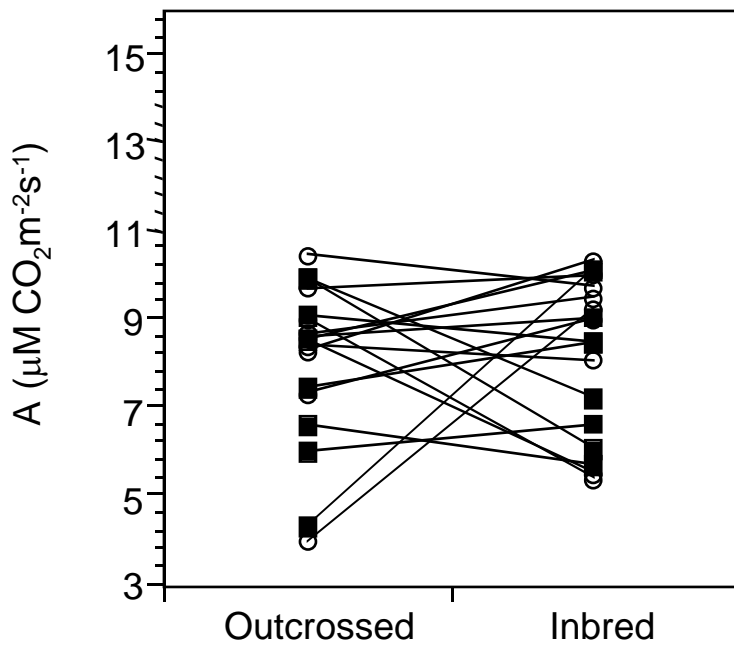
A.

Maternal Non-drought, Progeny Non-drought



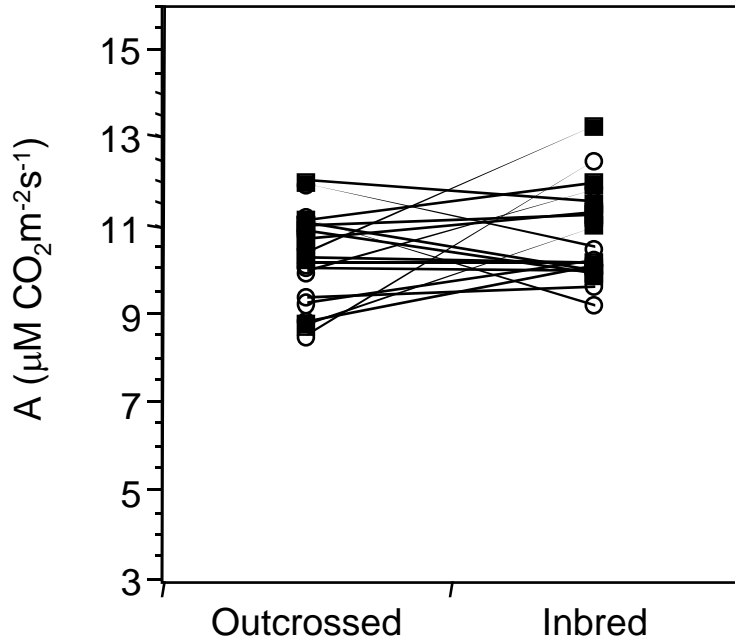
B.

Maternal Non-drought, Progeny Drought



C.

Maternal Drought, Progeny Non-drought



D.

Maternal Drought, Progeny Drought

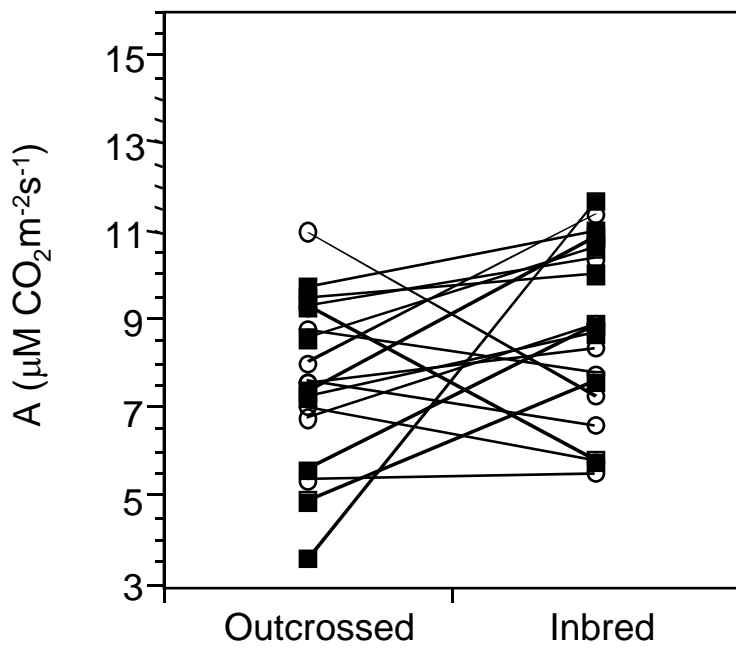


Figure 8

