

The effects of maternal salinity and seed environment on germination and growth in *Iris hexagona*

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ABSTRACT

Seed germination and seedling establishment are determined by local environmental conditions as well as by the environment of the parental generation, resulting in a type of trans-generational phenotypic plasticity known as an inherited environmental effect. Parental effects should be adaptive in habitats where the selective environment is spatially and temporally predictable, and where parental effects confer a fitness advantage. Natural populations of the native wetland perennial *Iris hexagona* occupy saline habitats and plant performance is strongly impacted by salinity stress. We examined the capacity for environmental effects to act directly and indirectly on the germination responses of *I. hexagona* by (1) growing plants in three different maternal salinity levels in a common garden, (2) performing crosses through hand pollinations, and (3) planting the resulting seeds in four different salinity environments in a greenhouse study. Our results indicate that salinity has strong direct and indirect effects on seed germination. Direct exposure to salinity delayed and reduced seed germination and produced seedlings with 32% less mass than those grown in the freshwater treatment. Seeds produced by maternal plants growing in high salinity germinated earlier and in greater numbers than seeds from low salinity plants, demonstrating an indirect effect of the maternal environment. Maternal effects were no longer detectable 10 days after germination, indicating that the indirect effect of salinity was more influential on germination timing and success than on seedling growth. Furthermore, the maternal effect was only apparent in saline environments, suggesting that salinity-induced maternal effects are context dependent. These maternal effects may confer an advantage and be adaptive for *I. hexagona* in salt marsh populations.

Keywords: adaptive maternal effects, Louisiana, Marsh Island, saline marsh, salinity stress, survival analysis.

INTRODUCTION

During the transition from propagule to established individual, plants are susceptible to many environmental factors, resulting in strong selection at this stage (Harper, 1977; Cabin *et al.*, 1997; Meyer *et al.*, 1997). In some species, the allele frequencies of seeds and

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established seedlings differ (e.g. Cabin *et al.*, 1997), demonstrating that germination ability can have an effect on the genetic structure of plant populations. Because germination success (reviewed in Baskin and Baskin, 1998), growth rates (Cheplick and Sung, 1998) and tolerance to environmental stress (Jain and Bradshaw, 1966; Silander, 1985; Cobb *et al.*, 1994) vary among individuals within a population, germination and establishment can have a strong influence on the genetic variation and structure of local populations (Hamrick, 1983; Cabin *et al.*, 1997).

Maternally inherited environmental effects are a form of inter-generational phenotypic plasticity, whereby the environment of the maternal generation alters the quality of the offspring (Mousseau and Fox, 1998). Maternal effects can have large impacts on seed germination (Rossiter, 1996, 1998), and are considered adaptive when they enhance establishment success, thereby altering the genetic structure of the next generation (Roach and Wulff, 1987; Rossiter, 1996, 1998; Lacey, 1998; Mousseau and Fox, 1998). Maternal effects can increase genetic variance (Wade, 1998), and counteract selection by buffering normally strong selective agents (Kirkpatrick and Lande, 1989). Conversely, they can strengthen responses to selection if maternally induced adaptive traits are correlated with other favourable traits (Kirkpatrick and Lande, 1989).

Maternal plants influence seeds by contributing organelles, endosperm, seed coat and other structures, all of which play important roles in determining seed dormancy, dispersal and establishment (reviewed in Roach and Wulff, 1987; Baskin and Baskin, 1998). There are many different environmental factors with the potential to alter seed germination success that can be transmitted through the maternal plant (reviewed in Gutterman, 1992; Wulff, 1995; Baskin and Baskin, 1998). In general, larger plants produce bigger seeds that exhibit higher germination success and seedling survival (reviewed in Wulff, 1995; Baskin and Baskin, 1998); therefore, factors that affect plant size can potentially cause maternal effects. For example, in the alpine perennial *Ranunculus adoneus*, plants in sites where snow melts earlier produce larger seeds that have higher survival and greater probability of emergence than seeds from plants in later melting sites (Stanton and Galen, 1997). Similarly, individual *Nemophila menziesii* grown in competition with *Bromus diandrus* produce smaller, more dormant seeds than those grown without competitors (Platenkamp and Shaw, 1993). Other examples of strong environmental effects on seed morphology and germination profiles are shown in studies of conspecific plants growing at different elevations (Dorne, 1981), levels of water stress (Sawhney and Naylor, 1982) or temperatures (Lacey *et al.*, 1997).

Maternal environmental effects are predicted to be advantageous in systems where the seedling environment is spatially or temporally variable but in a somewhat predictable manner (Donohue and Schmitt, 1998; Lacey, 1998). For example, seeds produced by maternal plants grown in low red : far red wavelength micro-environments (caused by neighbour shading) require exposure to a particular light quality to stimulate germination (Cresswell and Grime, 1981; Schmitt *et al.*, 1992; Van Hinsberg, 1998). This requirement is used as a germination cue to indicate that competition for light has been reduced (Ballaré *et al.*, 1990). Maternal environmental effects may also be adaptive when they confer a fitness advantage to offspring through increased recruitment, survival or the ability to pre-empt resources from competitors (Donohue and Schmitt, 1998). Early germination is a potentially adaptive trait, since adult fitness can be higher in early emerging than later emerging seedlings in the presence of competitors (Kalisz, 1986). Similarly, parental temperature can affect offspring fitness components such as germination, spike production and the

probability of flowering (Lacey and Herr, 2000), demonstrating that maternally influenced traits can have important fitness consequences for the next generation.

The conditions for adaptive maternal effects are met in salt and brackish marshes. These habitats are spatially and temporally heterogeneous, with seasonally predictable variation in competition, light and water availability, and salinity (Adam, 1990). In salt marsh wetlands, perennials spread largely by vegetative propagation and flowering and seedling establishment is rare (Adam, 1990; Ungar, 1991). Therefore, factors that augment the establishment of individuals via increased germination can disproportionately influence the distribution, viability and evolutionary potential of populations (Bertness *et al.*, 1992; Shumway and Bertness, 1992). Furthermore, in saline and brackish marshes, the environment to which a seed is dispersed has a strong effect on germination and establishment. Even salt-adapted plants have higher germination rates in lowered salinities (Ungar, 1991, 1995; Baskin and Baskin, 1998), making germination and seedling establishment more likely in seasons or locations with lower salinity. There is also limited evidence that the maternal salinity environment can modify germination success. Individual plants from high salinity sites produce seeds that germinate more successfully in high salinities than seeds from maternal plants in low salinity (Choudhuri, 1968; Bazzaz, 1973; Bülow-Olsen, 1983; Ungar, 1991). However, many of these studies concluded that these differences were more likely due to the formation of different ecotypes than to adaptive maternal effects. Therefore, the importance of salinity as an environmentally induced maternal effect has not been conclusively established.

In this study, we used a combination of common garden and greenhouse experiments to examine the direct and indirect (i.e. maternal) effects of salinity on the germination rate of *Iris hexagona* seeds and the establishment of seedlings. Because *I. hexagona* populations in southern Louisiana have become exposed to salinity stress within the last 2000–5000 years (Van Zandt and Mopper, 2003), we expected salinity to be a particularly influential factor affecting maternal seed provisioning and therefore germination ability. Specifically, we addressed four questions: (1) What is the effect of the maternal environment on the germination success of seeds and subsequent growth of seedlings? That is, do seeds from maternal plants grown in high salinity exhibit differential germination from low salinity derived seeds? (2) How does the salinity of the seed's environment affect germination and growth of seedlings? (3) If maternal effects are detected, is there an interaction between maternal salinity and germination salinity that would suggest that maternal effects are adaptive? (4) Do seeds derived from separate populations differ in germination rate and seedling growth in response to both maternal and germination salinity?

METHODS

Study species and habitat

Iris hexagona Walter (Iridaceae) is a long-lived, wetland perennial common to freshwater lakes, bayous and coastal areas of southern Louisiana (Viosca, 1935; Bennett, 1989). Irises also occur in a brackish marsh community on Marsh Island (29.79°N, 91.78°W, Iberia Parish, Louisiana, USA), a 33,000-hectare moderately saline marsh located in the Gulf of Mexico 10 km from the south Louisiana mainland. Erosion and other geological processes separated the island from the mainland over the past 5000 years, changing the habitat from freshwater to brackish-salt marsh (Orton, 1959; Törnqvist *et al.*, 1996). In this island

habitat, soil salinity may reach 10–15 ppt within established *I. hexagona* populations, but typical salinity levels are 0–8 ppt (Van Zandt and Mopper, 2002, 2003). Reproduction in *I. hexagona* occurs clonally via rhizomes and sexually by large seed capsules containing 30–60 seeds. The main pollinators are bumblebees (*Bombus* sp.) and hummingbirds (Cruzan and Arnold, 1993). On Marsh Island, *I. hexagona* occurs in discrete populations separated by large expanses of marsh vegetation. Populations range in size from 1 to 40 m in diameter and contain numerous clonal genets interconnected by extensive rhizome systems. Both RAPD (randomly amplified polymorphic DNA) and SSR (single sequence repeats, or microsatellites) DNA markers indicate that *I. hexagona* populations on Marsh Island are genetically differentiated (unpublished data, Jerry He and Susan Mopper, University of Louisiana at Lafayette, and Alan Meerow, USDA-ARS-SHRS, National Germplasm Repository, respectively).

Parental generation

We collected *I. hexagona* plants from 10 different populations on Marsh Island in March 1997 and planted them in an array of thirty 227-litre Rubbermaid™ tubs filled with Mississippi alluvial topsoil at the University of Louisiana Research Center in CarenCro, Louisiana. While salinity levels vary among these 10 populations, any differences are swamped by considerable intra-annual variation (Van Zandt and Mopper, 2002). The common garden was 80 km away from Marsh Island and over 10 km away from any other populations of *I. hexagona*. An average of five rhizomes from each of three populations was planted per tub. Plants were grown in constantly hydrated soils and exposed to one of three salinity treatments for 12 months before flowering. Treatment salinities were maintained by multiple applications of Instant Ocean™ (Aquarium Systems, Inc., Mentor, OH, USA) in solution to the tubs (complete methods in Van Zandt *et al.*, 2003). Because long-term exposure to salinity is more stressful than short-term pulses (Flynn *et al.*, 1995; Howard and Mendelsohn, 1999a,b), we were conservative in establishing salinity levels in order to avoid mortality or excessive stress, particularly during plant reproduction. Weekly salinity readings (taken with an Orion 125™ meter, Orion Research Inc., Beverly, MA) during the 20-month experiment indicated that salinity in the control, 2 ppt and 4 ppt treatments averaged 0.2 ± 0.4 (standard error), 2.0 ± 1.2 and 3.7 ± 1.8 ppt, respectively, over the experimental period, and reflected typical levels during flowering and seed development on Marsh Island (Van Zandt and Mopper, 2002). Even low salinity has strong effects on iris performance and reproduction of this freshwater species (Van Zandt *et al.*, 2003). Each source population $\times$ salinity combination was replicated three times for a total of 90 population $\times$ salinity replicates, and each salinity level was represented by 10 tubs.

We obtained seeds produced by hand and bee pollinations of 144 plants from 9 to 26 April 1998. We hand-pollinated flowers that were enclosed in nylon mesh to exclude pollinators, and used pollen from one of 10 pollen donors. These donors included unreplicated representatives from different populations and salinity levels; therefore, a significant pollen source effect could represent either of these effects or their interaction. Pollen from the same individual for each pollen donor was used throughout the 18-day flowering period. Bee-pollinated flowers were left uncovered. We harvested 236 mature capsules yielding 5626 seeds from June to September 1998. We counted, weighed and stored the seeds in paper envelopes at room temperature (20°C) for approximately 10–24 weeks, depending on harvest date.

Offspring generation

In March 1999, we planted seeds in individual cells within one of four germination salinity treatments (0, 3, 6 and 9 ppt) in vermiculite-filled planting flats. Treatment salinities reflect the levels that occur in natural populations during flowering, seed development and germination. The cells had holes in the bottom to allow water to saturate the vermiculite. Flats were randomly assigned to one of three benches in a greenhouse. We used a randomization program to assign each of the seeds, from all capsules and maternal salinities, to flats and cells within flats, assigning them equally into each of the four germination salinities (Table 1). We planted one seed per cell at a depth of 5–10 mm. Each capsule was represented by a single seed in each flat, and each maternal population was represented a maximum of eight times per flat. There were 22 flats per germination salinity level (total number of flats = 88), and each germination salinity level received an equivalent number of seeds. Flats were watered as necessary to maintain constant salinity and moisture, and rotated weekly to minimize position effects on germination. Salinity was monitored periodically throughout the experiment. We recorded germination (characterized by emergence of the cotyledon from the surface of the vermiculite) daily until February 2000 (341 days), when greater than 51% of seeds had germinated.

Statistical analysis

We used survival analysis (Allison, 1995; Fox, 2002) to compare differences in germination timing between salinity treatments, maternal populations and pollen donors. We classified seeds that failed to germinate as censored observations (Fox, 2002). We used a Cox proportional hazards regression model (PROC PHREG; SAS, 2001), where the dependent variable was the number of days until germination. Cox regression estimates the change in the hazard function or, in this case, the age-specific probability of a seed germinating, given the experimental treatments. The influence of predictor variables (germination salinity, maternal salinity, maternal population, pollen source, seed mass, bench) was evaluated in the model for each factor with a χ^2 test with one degree of freedom (Allison, 1995; SAS, 2001). PROC PHREG also produces a hazard ratio statistic that allows an estimate of the magnitude of each predictive factor (Allison, 1995); however, hazard ratios of main effects are inappropriate for models with significant interaction terms (P. Savarese, personal communication). Therefore, we included all pairwise interactions between seed salinity, population and maternal salinity, but did not report hazard ratios for main effects when significant interactions were detected. A significant germination salinity $\times$ maternal

Table 1. Planting design and number of seeds in each salinity treatment

Maternal salinity	Germination salinity				Total
	0 ppt	3 ppt	6 ppt	9 ppt	
Low	289	289	291	292	1161
Medium	555	551	546	552	2204
High	570	565	566	560	2261
Total	1414	1405	1403	1404	5626

population interaction would indicate that populations varied in their tolerance for germinating in salinity, whereas a germination salinity $\times$ maternal salinity interaction would suggest a potentially adaptive influence of a maternal effect. An interaction between maternal salinity and population would indicate variation in the way populations express salinity-induced maternal effects.

Seeds that are non-viable should not be included in the analysis of germination studies. Commonly, seed viability is assessed with a tetrazolium test (Kearns and Inouye, 1993), but as an alternative we performed the survival analysis both with and without censored (non-germinated) observations to determine if there were any patterns of differential seed viability. Qualitative differences between the two analyses would suggest that differential viability resulted in an underlying pattern of censoring that could confound our results (Fox, 2002).

Once the experiment was terminated, we analysed germination success of all seeds with logistic regression (PROC GENMOD: Allison, 1999; SAS, 2001). Logistic regression produces an odds ratio for each level of every factor adjusted for other terms in the model, which indicates the odds (or likelihood) of the response occurring. Odds ratios are statistically compared to 1 (equal likelihood of the event occurring in each case); therefore, values less than 1 indicate decreased odds and values greater than 1 indicate increased odds relative to controls (0 ppt salinity).

Seed size is often an important factor in germination success and seedling growth. We used the average mass of a seed from each capsule as an estimate of the initial seed mass before planting and used it as a covariate, except when noted otherwise.

Seedling growth

To estimate the influence of population or treatment effects on seedling growth, we measured 1002 randomly selected seedlings at 10 days after emergence. These seedlings were evenly distributed among the four germination salinity treatments. We dried and weighed each seedling at 70°C, and analysed total seedling mass with a mixed-model analysis of covariance (ANCOVA) using the same independent variables as above.

Recovery from salinity stress

Salinity can delay germination by reducing imbibition by the seed or it can kill the embryo through ion toxicity (Ungar, 1995). To determine the effect of salinity on *I. hexagona* seeds, we conducted a salinity stress recovery experiment using non-germinating seeds from the germination experiment. In May 2000, we randomly selected trays from the 3, 6 and 9 ppt treatments and either lowered the salinity level of the tray by flushing it with freshwater ($n = 31$ trays), or left salinity unchanged ($n = 35$ trays). We then monitored germination for 30 weeks after this manipulation. We analysed the date of germination using survival analysis with current salinity (lowered or unchanged), previous salinity (3, 6 or 9 ppt) and maternal salinity (control, 2 and 4 ppt) as main effects.

RESULTS

When we terminated the experiment on 25 February 2000, a total of 2878 seeds (51.2%) had germinated. The first germination occurred in August 1999, 146 days after planting. The greatest number of germinations (36% of 2878 seeds) occurred during November 1999.

The survival analyses with and without censored observations were qualitatively similar; therefore, we report only the analysis using the complete data set (which includes censored seeds).

The effect of seed mass on seedling emergence and growth

Heavier seeds germinated faster and in higher proportions (Table 2) than small seeds. Heavier seeds also produced heavier seedlings measured at 10 days (Table 3). Because seed size is an important factor in germination success and seedling growth in *I. hexagona*, we included seed mass as a covariate in all analyses.

Table 2. Results from survival analysis (Rate) and logistic regression (Success) of *Iris hexagona* seed germination using individual seeds as replicates

Factor	Rate		d.f.	Success	
	χ^2	<i>P</i>		χ^2	<i>P</i>
Maternal salinity	18.65	<0.0001	2	61.86	<0.0001
Seed mass	36.52	<0.0001	1	76.91	<0.0001
Germination salinity	20.50	<0.0001	3	118.39	<0.0001
Population	0.038	0.85	9	104.59	<0.0001
Pollen source	17.98	<0.0001	1	0.62	0.43
Table	26.68	<0.0001	2	16.16	0.0003
Germination salinity $\times$ population	6.94	0.008	27	24.87	0.58
Germination salinity $\times$ maternal salinity	1.00	0.32	6	9.85	0.13
Pollen source $\times$ population	15.07	0.0001	9	48.72	<0.0001
Maternal salinity $\times$ population	9.59	0.002	18	124.81	<0.0001

Note: For survival analysis, the degrees of freedom = 1 for each factor.

Table 3. Maximum likelihood ANCOVA for *Iris hexagona* seedling mass 10 days after germination

Factor	Type	d.f.	<i>F</i> or χ^2	<i>P</i>
Maternal salinity	fixed	2,739	0.22	0.80
Seed mass	covariate	1,739	8.18	0.0044
Germination salinity	fixed	3,739	17.18	<0.0001
Population	random	1	0.5	0.48
Pollen source	random	1	14.4	0.0001
Germination salinity $\times$ population	random	1	<0.1	>0.99
Germination salinity $\times$ maternal salinity	fixed	6,739	1.30	0.25
Pollen source $\times$ population	random	1	7.7	0.006
Maternal salinity $\times$ population	random	1	0.6	0.44

Note: Seedlings were removed from the germination trays with roots intact, dried, measured and weighed. Significance was assessed for fixed effects with an *F*-ratio test with type III sums of squares, and for random effects with a likelihood ratio χ^2 test with one degree of freedom.

The effect of maternal and germination salinity on seedling emergence and growth

Seeds from the three maternal salinity treatments began germinating at approximately the same time, but germination proceeded at different rates (Fig. 1a; Table 2). Seeds produced by 4 ppt maternal plants germinated faster than seeds from either the 2 ppt ($\chi^2_1 = 7.1$,

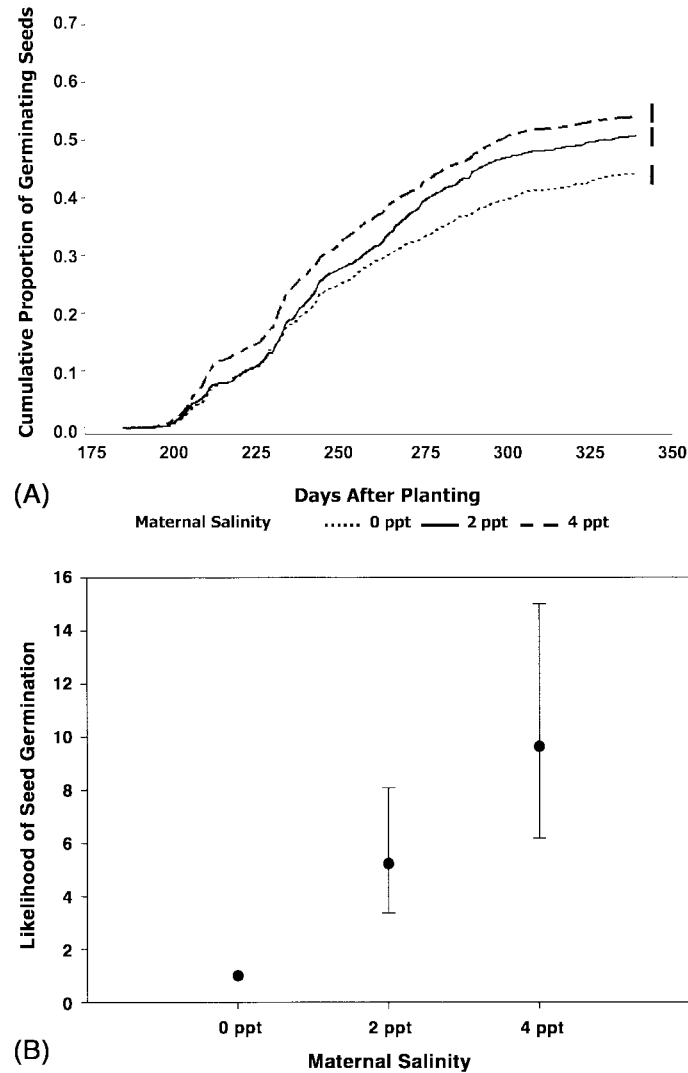


Fig. 1. Germination of seeds obtained from maternal plants in three different salinity environments: control, 2 ppt and 4 ppt. (A) The cumulative proportion of germinated seeds, which is equivalent to the inverse of the hazard function derived from the proportional hazards survival analysis. Germination curves connected by a line at the end are not different from one another at $\alpha = 0.05$. (B) The likelihood of germination for seeds from two maternal salinities relative to controls. Data are odds ratios from logistic regression analysis of data from Table 2. Odds ratios greater than one indicate higher odds of germinating compared to controls.

$P = 0.007$) or control ($\chi^2_1 = 36.8$, $P < 0.0001$) salinity plants. Seeds from 2 ppt plants also germinated faster than those from controls ($\chi^2_1 = 15.3$, $P < 0.0001$). The final proportion of seeds germinating was strongly affected by maternal salinity (Table 2). Germination success was highest among seeds produced by plants grown in high salinity (Fig. 1b). Maternal salinity had no effect on seedling mass at 10 days (Table 3).

Germination salinity strongly delayed germination (Fig. 2a; Table 2). Each level of

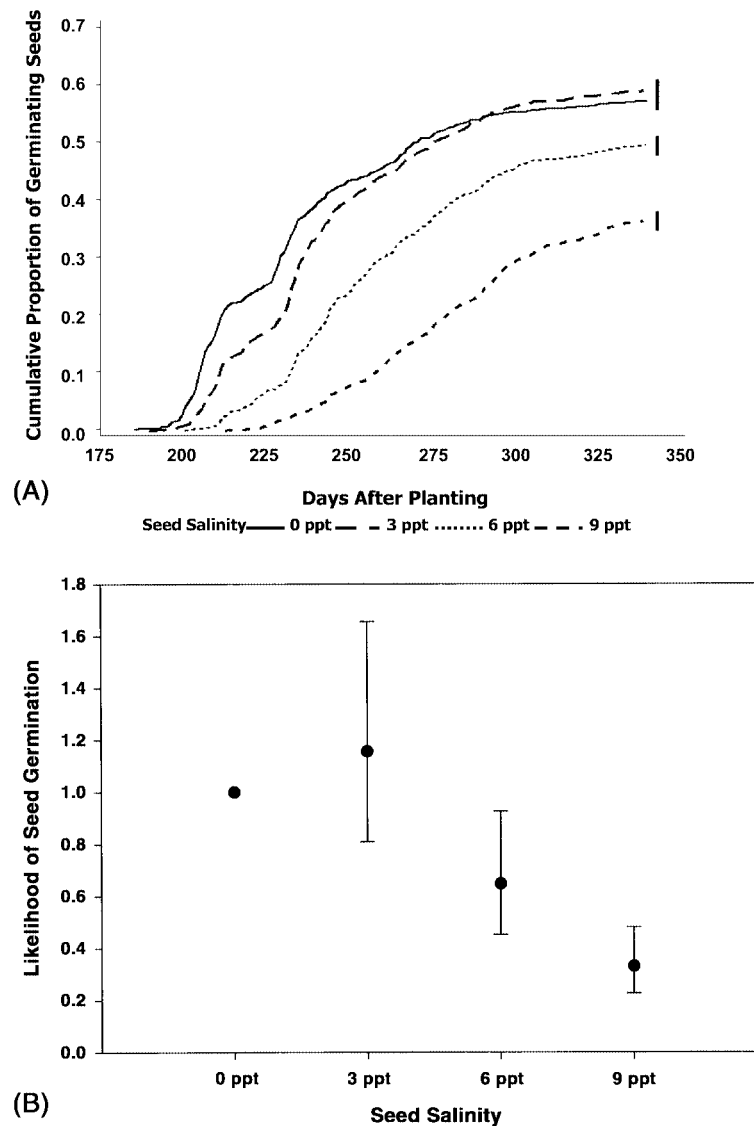


Fig. 2. Seed germination for seeds planted in four different salinity environments measured as (A) the cumulative proportion of germinated seeds and (B) the likelihood of germination. Seeds from different maternal salinities and populations were planted equally in the four germination salinity treatments. Data are presented as in Fig. 1.

germination salinity differed significantly, except the 0 and 3 ppt treatments (0 ppt vs 3 ppt: $\chi^2_1 = 1.1$, $P = 0.29$; 0 ppt vs 6 ppt: $\chi^2_1 = 55.9$, $P < 0.0001$; 0 ppt vs 9 ppt: $\chi^2_1 = 225.8$, $P < 0.0001$; 3 ppt vs 6 ppt: $\chi^2_1 = 41.8$, $P < 0.0001$; 3 ppt vs 9 ppt: $\chi^2_1 = 198.4$, $P < 0.0001$; 6 ppt vs 9 ppt: $\chi^2_1 = 79.7$, $P < 0.0001$). Germination salinity also strongly reduced the total proportion of seeds that germinated (Fig. 2b; Table 2). Germination salinity strongly reduced seedling mass at 10 days (Fig. 3; Table 3).

Effects of maternal population and pollen donors on seedling emergence and growth

Despite considerable variation among populations in germination rate (Fig. 4), the main effect of maternal population was not significant (Table 2). This is probably due to strong interactions between population identity and other main effects. Populations differed strongly in final germination success, despite substantial interactions with other factors (Table 2). Maternal population identity did not significantly affect seedling growth (Table 3). Seeds fertilized from different pollen sources varied in their germination rates but not germination success (Table 2), indicating that there is the potential for a paternal contribution to seed quality. Pollen source also had strong effects on seedling mass (Table 3).

Interactions with direct and maternal effects

The interaction between maternal salinity and germination salinity was not significant for germination timing (Table 2). Seeds from all three maternal salinities germinated more slowly with increasing germination salinity, but seeds from high-salinity maternal

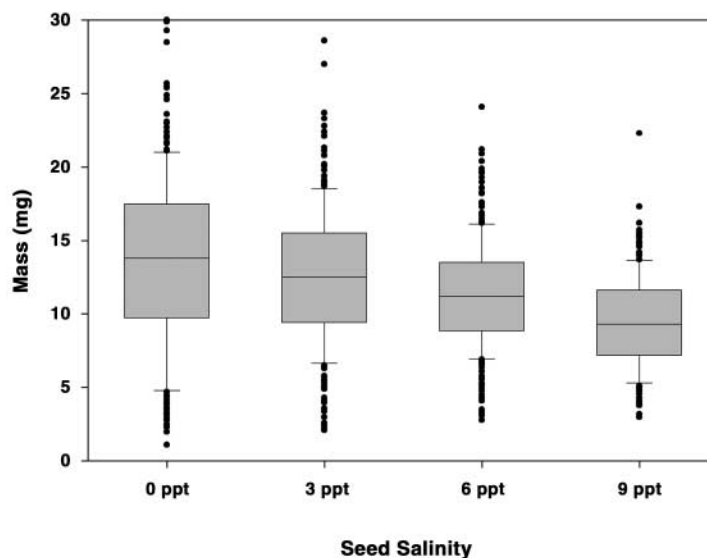


Fig. 3. Seedling mass as a function of germination salinity treatments. Seeds were measured 10 days after germination. Statistics are presented in Table 3.

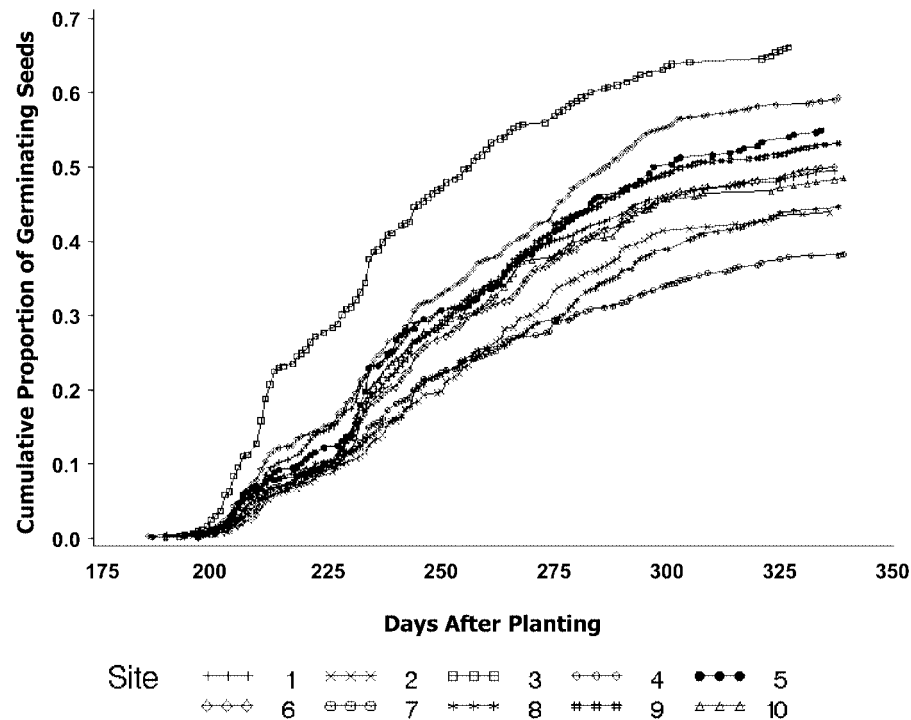


Fig. 4. Germination timing for seeds from the 10 populations used in this experiment across all maternal and germination salinities.

plants were impacted the least by increases in germination salinity (Fig. 5). Also, seeds from high-salinity maternal plants germinated the earliest in all four germination salinities, but the difference was greatest in the 9 ppt treatment (Fig. 5). The germination salinity $\times$ maternal salinity interaction was not significant for final germination success (Table 2) or for seedling growth (Table 3).

The germination salinity $\times$ population interaction was significant for germination rate but not for overall germination success (Table 2), indicating that populations germinated at different times, but were equally viable across salinity treatments. The interaction between maternal salinity and population was highly significant for germination date and for germination success (Table 2). The population $\times$ pollen source interaction was highly significant for germination timing, as well as for germination success and seedling growth (Tables 2 and 3). No other interactions significantly affected seedling mass at 10 days (Table 3).

Bench effects

There were substantial effects of table positioning on germination rates and germination success (Table 2), suggesting that the microclimate of table locations within the greenhouse affected germination rates. There were no detectable effects of table position on seedling growth (mass: $F_{2,740} = 0.94$, $P = 0.39$), or significant interactions between table position and any other factors for any responses.

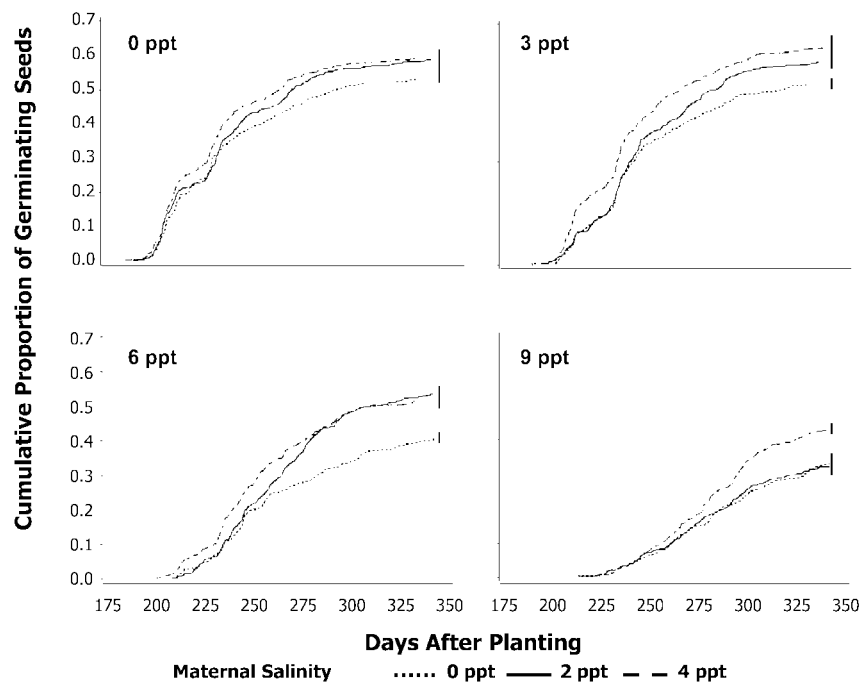


Fig. 5. Germination of seeds from three maternal salinity levels when planted in each of four different salinity treatments. For each panel, germination curves connected by a line are not different from one another at $\alpha = 0.05$.

Recovery from salinity and subsequent maternal effects

Seeds in trays with both lowered and unaltered salinity levels were still viable during the second germination season (Fig. 6). Germination during the recovery experiment began in October 2000, approximately 155 days after germination salinity was lowered, and continued until the experiment was terminated ($n = 526$ seeds). Seeds in the 3, 6 and 9 ppt reduced-salinity treatments germinated faster than those with unchanged salinity (Wald $\chi^2_1 = 50.5$, $P < 0.0001$) (Fig. 6). Maternal salinity effects were still strong (Wald $\chi^2_1 = 20.3$, $P < 0.0001$), and seeds from elevated-salinity plants still germinated more readily than seeds from low-salinity plants (control vs 2 ppt: Wald $\chi^2 = 29.4$, $P < 0.0001$; control vs 4 ppt: $\chi^2 = 25.9$, $P < 0.0001$). However, the two elevated-maternal-salinity treatments did not differ from one another (2 ppt vs 4 ppt: Wald $\chi^2 = 0.14$, $P = 0.70$).

Seeds in all salinity treatments remained equally viable, because seeds from all germination salinity levels exhibited similar germination responses in the recovery experiment. The previous salinity treatment that seeds experienced was marginally significant (Wald $\chi^2_1 = 3.5$, $P = 0.06$), and *post-hoc* comparisons among germination salinity treatment levels did not detect any differences (3 ppt vs 6 ppt: Wald $\chi^2_1 = 1.44$, $P = 0.23$; 3 ppt vs 9 ppt: Wald $\chi^2_1 = 3.1$, $P = 0.078$; 6 ppt vs 9 ppt: $\chi^2 = 0.32$, $P = 0.57$).

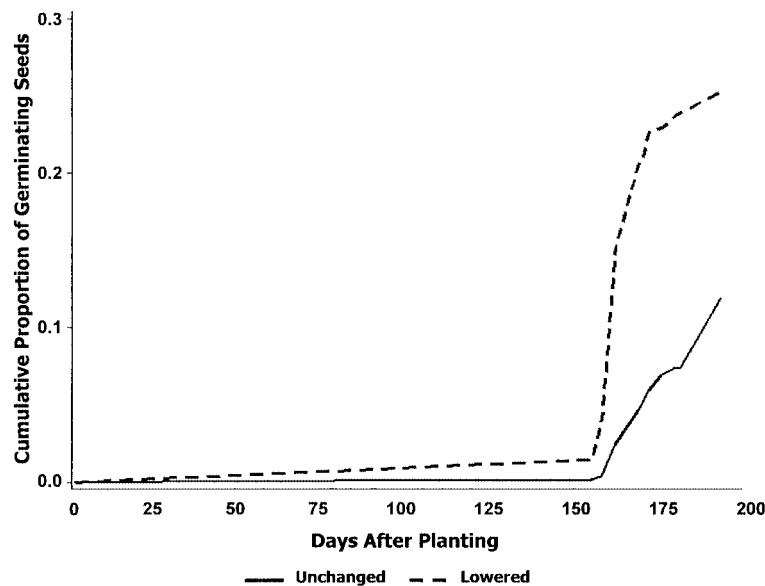


Fig. 6. Germination of seeds in the recovery experiment. The salinity level of each germination tray was either lowered by flushing the tray with fresh water (lowered) or unchanged, in which case the original germination salinity (3, 6 or 9 ppt) was maintained (unchanged).

DISCUSSION

Indirect and direct effects of salinity on germination

Salinity had strong direct and indirect effects on seed germination in *I. hexagona*. Germination was delayed in seeds that were planted in high salinity, but high maternal salinity accelerated germination. Seeds from high-salinity maternal plants germinated earlier than seeds from medium- or low-salinity plants regardless of germination salinity (Fig. 1). Although the main effects of both maternal and germination salinity were significant, quantitative estimates of the impacts of each could not be obtained due to significant interactions. However, inspection of the germination curves within each level of germination salinity (Fig. 5) suggests that differences in germination timing of up to 60 days might separate seeds from low- and high-salinity plants. Furthermore, seeds from high-salinity mothers were over 9% more likely to germinate than seeds from low-salinity mothers by the end of the germination experiment (Fig. 1b). Taken together, these salinity-induced effects could result in large fitness differences due to the maternal environment. Previous work (Bülow-Olsen, 1983; Ungar, 1991; Krauss *et al.*, 1998) demonstrated that seeds from maternal plants grown in high salinity germinated more readily in high salinity compared with seeds from low-salinity plants. However, those studies did not separate maternal salinity, site and population effects. Only one other study has indicated evidence for a maternally induced environmental effect of salinity. Uchiyama (1987) determined that the salinity environment of *Atriplex nummularia* maternal plants profoundly affected the germination response of their seeds. Only 4% of the seeds from maternal plants grown in the 0% NaCl controls germinated in 2% NaCl, while 41% of the seeds from plants cultivated

in the 2% NaCl treatment germinated at 2% NaCl, demonstrating the possible adaptive advantage of a maternal environmental effect.

In the present study, salinity also had a strong direct effect on *I. hexagona* germination, in agreement with previous work (Choudhuri, 1968; Ungar, 1991, 1995; Krauss *et al.*, 1998; Dodd and Donovan, 1999; Houle *et al.*, 2001). We found that high germination salinity delays the timing of emergence as well as the likelihood of seed germination. Seeds in the 9 ppt treatment were about one-third as likely to germinate as those in the 0 ppt treatment, indicating substantial reductions in viability for seeds in high-salinity environments. Under similar conditions, seeds planted in 12 ppt salinity failed to germinate (P.A. Van Zandt, unpublished data). Therefore, it appears that the salinity tolerance for germination in *I. hexagona* is between 9 and 12 ppt.

Individual and population effects

Iris seeds exhibited considerable variation in germination among populations and individual pollen donors. Seeds from different populations initiated germination at the same time, but differed in rates and final germination success. The strong population effects on germination timing and success, combined with population differences in growth and reproduction (Van Zandt and Mopper, 2003) and molecular data (see Introduction), demonstrate that Marsh Island *I. hexagona* populations are genetically differentiated. The significant population $\times$ germination salinity interaction for germination rate illustrates population differences in sensitivity to salinity. However, the population $\times$ germination salinity interaction was not significant for the final proportion of seeds germinating. Although timing of germination may be genetically variable, there appears to be no genetic variation in germination success among populations in different salinity regimes.

Although we did not replicate pollen donors within parental populations and salinity treatments, pollen source affected seed germination rates and success. Furthermore, pollen source was also a significant factor for seedling mass, whereas maternal population and maternal environment were not (Table 3). Therefore, both total seed germination and the growth rate of seedlings are affected by the source of pollen. While many parental effects are mediated through maternally derived tissues such as the seed coat (Roach and Wulff, 1987), the strong effect of pollen donor on germination rates indicates a potential paternal influence in germination. In irises, variation in seed and seedling success from different pollen donors could have been caused by differences in the paternal environment, individual genetic variation among paternal donors, pre- or post-zygotic selection, or gametic selection (Mazer and Gorchov, 1996). Few studies have investigated paternal effects (Roach and Wulff, 1987; Shaw and Byers, 1998; Galloway, 2001; Etterson and Galloway, 2002), and in most cases these effects have been rather weak (Antonovics and Schmitt, 1986; Shaw and Byers, 1998; Lacey and Herr, 2000). However, in one study, *Solidago altissima* exhibited soil-induced maternal and paternal environmental effects on germination rates, as well as persistent paternal effects for growth characters mediated by environmentally driven pollen quality differences (Schmid and Dolt, 1994). Unfortunately, our experiment cannot distinguish genetic from environmental differences in pollen quality. At present, little is known about the impact and magnitude of paternal environmental effects in plants, but *I. hexagona* appears to be a promising species for investigating variation in pollen quality.

Seed mass, germination and salinity

Seed size and germination success are often positively correlated (Wulff, 1995; Baskin and Baskin, 1998; Mazer and Wolfe, 1998). Seed size can also be very important in elevated salinity conditions. Large *Atriplex triangularis* seeds germinate more readily at higher salinities than do small seeds (Khan and Ungar, 1984; Ungar, 1995). Mohammed and Sen (1988) predicted that plants in high-salinity environments acquire fewer resources to provision seeds, and therefore produce smaller propagules with reduced germination success. Although larger seeds germinated more readily than smaller seeds in our study, the results do not support Mohammed and Sen (1988), because iris seeds from high-salinity maternal plants germinated more readily than seeds from low-salinity plants, contrary to their prediction. Furthermore, salinity had no significant effect on iris seed mass, but significantly increased seed production (Van Zandt and Mopper, 2003).

Seed recovery from salinity stress

Seeds remained viable after 14 months of exposure to salinity. Prior salinity had no effect on viability, and seeds germinated more readily once salinity was lowered (Fig. 6). Our salinity treatments presumably produced an osmotic environment that was unsuitable for seed germination, but it was reversible and not lethal to seeds (Ungar, 1995; Baskin and Baskin, 1998). The ability of seeds to germinate rapidly once salinity declines is common in salt-tolerant plant species, but rare for salt-intolerant plants in which salinity can be toxic (Ungar, 1991, 1995).

The potential mechanisms of salinity-induced effects

The increase in seed germination following maternal exposure to salinity may be mediated by the plant stress hormone, abscisic acid (ABA), which is often associated with seed dormancy in other species (Bewley and Black, 1994; Karssen, 1995; Baskin and Baskin, 1998). Typically, seeds increase in ABA content during the middle stages of development, but these concentrations are reduced by the time seeds mature (Karssen, 1995). Mutant ABA-deficient *Arabidopsis thaliana* and *Zea mays* that have reduced dormancy do not exhibit a developmental peak in ABA production (Karssen, 1995; White *et al.*, 2000), suggesting that lower concentrations of ABA during seed maturation can increase germination rates. However, seeds of *I. hexagona* contain higher concentrations of ABA if produced by plants in high salinity (Wang *et al.*, 2001), yet exhibit reduced dormancy.

In other iris species, dormancy is enforced physically through differences in seed coat thickness, or chemically by germination inhibitors in the endosperm (Arditti and Pray, 1969; Blumenthal *et al.*, 1986). In *I. hexagona*, removing the seed coat near the embryo and hydrating the seeds stimulates radicle emergence within 2 weeks, whereas removing the seed coat from other areas does not (P.A. Van Zandt, unpublished data), suggesting that inhibition of germination is associated likely due to physical constraints on the embryo.

Our results demonstrated the presence of environmentally induced effects, but the design of the study did not distinguish non-additive genetic components (gametic or gametophytic selection) from non-genetic components (altering seed quality or differential parental gene expression) (Case *et al.*, 1996; Mazer and Gorchov, 1996; Roff, 1998). Therefore, the salinity-induced maternal effects found for *I. hexagona* may be referred to as statistical

parental effects (*sensu* Mazer and Gorchov, 1996), or as environmentally induced parental effects in the broad sense (Lacey, 1998). Similarly, while the significant effects of pollen donor identity on seedling mass could be interpreted as evidence for paternal additive genetic variation for these traits, we cannot rule out the possibility of gametic or gametophytic selection (Mazer and Gorchov, 1996; Mazer and Wolfe, 1998). In an earlier study (Van Zandt and Mopper, 2003), iris plants in high salinity produced 2.7 times more seeds than those in low salinity, which suggests that selection either among pollen or developing ovules does not occur in high-salinity plants.

Persistence of maternal effects

Frequently, maternal effects are strongest in the seed and seedling stages, becoming less influential as plants mature. However, while strong maternal effects can persist for multiple generations (Alexander and Wulff, 1985; Miao *et al.*, 1991; Case *et al.*, 1996), in field conditions the magnitude and persistence of these effects is weakened (Thiede, 1998; Lacey and Herr, 2000). We found strong main and interaction effects of the maternal salinity environment on both germination rate and total proportion of seeds germinating. However, 10 days after germination there were no detectable effects of maternal environment on seedling growth. Therefore, the advantage conferred to seedlings from high-salinity maternal plants does not lie in a faster growth rate, but in faster germination and a greater probability of germinating.

Are salinity-induced maternal effects adaptive?

Environmental maternal effects can have a strong impact on plant fitness (Lacey and Herr, 2000). If maternal effects are adaptive, they should confer a fitness advantage in environments similar to those experienced by the parental generation (Donohue and Schmitt, 1998; Rossiter, 1996, 1998), and should be particularly important in stressful environments (Wulff, 1995). Early germination is potentially adaptive for *I. hexagona*, since earlier emergence can provide a competitive advantage over inter- or intraspecific competitors (Harper, 1977). The pattern of germination timing (Fig. 5) suggests that the maternal effect is more strongly expressed in high-salinity than in freshwater environments; however, the maternal salinity $\times$ seed salinity interaction was not significant for either germination timing or success. Therefore, while the strong maternal salinity $\times$ population interaction for both germination timing and success indicates that populations differ in the magnitude of their maternal effects, these data do not provide evidence that these effects are adaptive for these irises.

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