

**SALINITY TOLERANCE AND GENETIC VARIABILITY IN  
FRESHWATER AND BRACKISH *IRIS HEXAGONA* COLONIES<sup>1</sup>**

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- *Premise of the study:* Saltwater intrusion is one of the most widespread environmental threats to freshwater wetlands. *Iris* species worldwide are important members of these plant communities. Wetland irises reproduce clonally and sexually, which permits populations to spread and disperse in benign and stressful conditions. The ability of iris populations to tolerate and adapt to elevated salinity can play an important role in determining the long-term health of wetland ecosystems.
- *Methods:* We used microsatellite markers to evaluate population structure and genetic diversity, and we performed a common garden experiment to examine the effect of salinity on the growth and reproduction of wild *Iris hexagona* collected from freshwater and brackish wetlands.
- *Key results:* Colonies were genetically distinct, with average to high heterozygosity (0.55–0.66) for a clonal species. Salinity had negative linear effects on leaf mass (g), clonal growth (g), root mass (g), and flower numbers, and it had nonlinear effects on seed numbers and seed mass (mg). The greatest sexual reproduction occurred in the intermediate-salinity (4 parts per thousand) treatment. Flowering phenology was delayed for 5 days in the highest-salinity treatment.
- *Conclusions:* We hypothesized that irises from brackish habitats would tolerate salinity better than freshwater irises would, but no difference in iris performance existed between the two habitats. The observed salinity tolerance and genetic diversity of *I. hexagona* indicate that populations will persist despite moderate increases in environmental salinity.

**Key words:** adaptation; clonal reproduction; environmental disturbance; flowering phenology; Iridaceae; *Iris hexagona*; microsatellites; plant performance; population structure; salinity stress.

Salinity is a severe environmental stress that leads to habitat and biodiversity loss in freshwater ecological communities (Yeo, 1998; Rogers and McCarty, 2000). Many factors cause elevated salinity, including sea level rise (Wigley and Raper, 1987; Zuo and Oerlemans, 1997; Smith and Wigley, 2000), geological subsidence (Watts, 1982), and petroleum exploration (Galbraith et al., 2002). Halophytic plants have mechanisms to regulate salt uptake, such as succulence, salt excretion, and sequestration of salt ions (Hale and Orcutt, 1987). But glycophytes have no such adaptations and exhibit pronounced symptoms of physiological stress even at low salt concentrations (Wang et al., 2001; Van Zandt et al., 2003). Even minute amounts of salinity can inhibit photosynthesis, suppress tissue development, prevent nutrient assimilation, and suppress cell growth in freshwater plants (Hasegawa et al., 2000).

The long-term persistence of wetland plant populations may require tolerance of and adaptation to increasing levels of environmental salt. Plants are renowned for their ability to cope with stressful conditions (Jones, 1971; Dudley, 1996). *Anthoxanthum odoratum* growing in heavy metals (Antonovics and Bradshaw, 1970), herbivore-tolerant *Quercus rubra* (Sork et al., 1993), and low-nutrient adapted *Potamogeton pectinatus* (Hangelbroek et al., 2003) illustrate the evolutionary plasticity of plants. Despite numerous examples of local adaptation to abiotic conditions (Linhart and Grant, 1996; Leimu and Fischer, 2008), surprisingly few records exist of evolved salinity tolerance in nonhalophytic plants (Mopper et al., 2004; Richards et al., 2005). Local adaptation is promoted by strong selection and is disrupted by dispersal, migration, and drift (Slatkin, 1987; Mopper et al., 2000; Leimu and Fischer, 2008). Furthermore, the clonal reproduction employed by many wetland plants (Mitsch and Gosselink, 2000) ostensibly limits genetic diversity (Ellstrand and Roose, 1987) and perhaps constrains rapid adaptations to environmental change.

*Iris hexagona* is the most abundant native iris in central North America and is an important component of Gulf Coast plant communities. Environmental salinity exerts strong selection pressure on *I. hexagona* populations and affects plant physiology (Wang et al., 2008), phytohormone production (Wang et al., 2001), herbivory (Schile and Mopper, 2006), florivory (Tobler et al., 2006), clonal growth (Van Zandt et al., 2003), and sexual reproduction (Van Zandt and Mopper, 2004). We have shown that brackish-marsh irises have different performance responses to salinity (Van Zandt et al., 2003). Phenology also has a strong genetic component (Mopper, 2005) and is altered in *I. hexagona* exposed to salinity (Van Zandt and Mopper, 2002).

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In this study, we investigated the potential for *I. hexagona* to tolerate and adapt to salinization of freshwater wetlands. We used microsatellite genetic markers (Hedrick, 1999; Meerow et al., 2005, 2007) to characterize population structure and genetic variation of iris colonies and performed a common garden experiment to compare how irises from freshwater and brackish natal colonies respond to salinity stress. We hypothesized that irises from brackish habitats perform better in the presence of salinity than do plants from freshwater sites. And finally, we examined the effect of salt on floral phenology because temporal variation in flowering could affect gene flow, reproductive isolation, and genetic structure among iris populations. This research provides insight into the ecological responses and adaptive potential of plant populations inhabiting rapidly changing wetland environments.

MATERIALS AND METHODS

**Natural history**—*Iris hexagona* is indigenous to the US Gulf Coast. In Louisiana, large populations are distributed throughout freshwater coastal wetlands (Gough and Grace, 1998). Marshes with intermediate to brackish salinity (3–22 parts per thousand [ppt]), (Odum, 1988) contain small and isolated *I. hexagona* colonies (Van Zandt and Mopper, 2002). This herbaceous perennial reproduces clonally through belowground rhizomes and sexually via hydrochorous seeds that remain afloat and viable for extended periods (Goranova, 2010). Flowers are pollinated primarily by *Bombus* spp. bumblebees and are self-compatible when hand-pollinated (Van Zandt and Mopper, 2004). Low levels of salinity increase seed production and germination success but reduce clonal growth (Van Zandt et al., 2003; Van Zandt and Mopper, 2004).

Three study colonies were located on Marsh Island, a 300-km<sup>2</sup> wildlife refuge in Vermilion Bay (29°37'N, 91°52'W). This land mass separated from the mainland approximately 5000 yr ago when it was still a freshwater cypress swamp (Orton, 1959). Salinity fluctuates with tide, season, year, and hurricanes (Van Zandt and Mopper, 2002). In this brackish marsh, levels typically range from 2–8 ppt but can exceed 20 ppt during large tropical storms. Iris colonies were small (100–200 ramets) and isolated from each other by at least 2 km (Van Zandt and Mopper, 2002).

Our three freshwater iris colonies occurred at Cypremort Point (29°44'N, 91°51'W), a 200-km<sup>2</sup> wetland on the Louisiana mainland. This wetland has low and stable salinity (0–2 ppt) and supports much larger colonies (500–1000 ramets) than the brackish Marsh Island habitat does. These colonies were isolated from one another by at least 0.5 km. The Vermilion Bay separates the brackish

Marsh Island and freshwater Cypremort Point study sites, which are about 20 km apart.

**Microsatellite analysis**—We collected fresh, undamaged leaves from 20 individuals growing in each of the Marsh Island and Cypremort Point locations. Irises are highly clonal, so we maximized the distance among samples to obtain the best estimate of genetic variation with colonies. Samples were stored at –80°C until genetic analysis. We screened nine dinucleotide microsatellite loci: IH37, IH42, IH56, IH63, IH73, IH122, IH153, IH155, and IH178. Primers were characterized from natural *I. hexagona* populations and were developed at the USDA-ARS-SHRs, National Germplasm Repository, Miami, Florida USA (Meerow et al., 2005, 2007). Microsatellites were amplified with 6-FAM or HEX end-labeled forward primers (Invitrogen, Carlsbad, California, USA). The polymerase chain reaction (PCR) was performed in a 10-μL reaction mixture containing 10–15 ng genomic DNA, 0.25 μL each of fluorescently labeled 10-μM forward and reverse primers, 200 μM dNTP, 1 μL 10× PCR reaction buffer with 20 mM MgCl<sub>2</sub> (Promega, Madison, Wisconsin, USA), 0.25 units *Taq* polymerase (New England Biolabs, Ipswich, Massachusetts, USA), and 7.25 μL deionized sterile water.

PCR began with 5-min denaturation at 94°C, followed by 40 cycles at 94°C for 50 s, followed by 50 s at annealing temperatures (50°C for IH42, IH63, IH73, IH153, IH178; 60°C for IH56; and 65°C for IH37, IH122, IH155), then 72°C for 1 min 30 s, ending with a final cycle at 72°C for 8 min. The PCR products were detected as fluorescent signals with an ABI-3100 genetic analyzer with a 36-cm capillary array (Applied Biosystems, Carlsbad, California, USA). A 1-μL aliquot was mixed with 10 μL Hi-Di formamide (Applied Biosystems), a 0.5-μL GeneScan 500 ROX-labeled size standard, and deionized water. Microsatellite alleles were identified and scored using ABI prism GeneMapper software (version 3.0, Applied Biosystems) and confirmed by visual inspection.

**Genetic structure of freshwater and brackish iris colonies**—We used GENEPOP version 4.0 (Raymond and Rousset, 1995) and GENALEX (Peakall and Smouse, 2006) to estimate colony genetic variability and population structure ( $\Phi_{ST}$ ). Probability values were calculated with the molecular variance analysis (AMOVA) in the GENALEX program. We estimated  $F_{ST}$  values (FSTAT, Goudet, 1995) and examined correlations between genetic and geographic distance among iris colonies using Isolation by Distance (version 3.16, Jensen et al., 2005).

**Common garden experiment**—In 2001, we collected more than 30 mature plants with rhizomes intact from each of the Marsh Island and Cypremort Point colonies. Plants were established in the experimental gardens at the Center for Ecology and Environmental Technology (CEET) at the University of Louisiana in Lafayette, Louisiana, USA. Rhizomes of individual plants were labeled with colorfast nylon thread and planted in thirty 227-L containers (Rubbermaid

TABLE 1. Number of alleles ( $N_a$ ) and the expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity for nine *Iris hexagona* microsatellite loci in brackish (B) and freshwater (F) colonies in Marsh Island and Cypremort Point, Louisiana, USA. Twenty individuals were analyzed in each colony.

Colony	Characteristics measured	Loci									Average
		IH37	IH42	IH56	IH63	IH73	IH122	IH153	IH155	IH178	
1 B	$N_a$	8	3	7	7	2	10	10	9	10	7
	$H_e$	0.79	0.45	0.76	0.76	0.18	0.86	0.85	0.88	0.86	0.71
	$H_o$	0.45	0.55	0.75	0.45	0.20	0.85	0.84	0.43	0.75	0.59
2 B	$N_a$	8	4	9	8	5	8	9	5	6	7
	$H_e$	0.85	0.59	0.85	0.85	0.54	0.72	0.82	0.79	0.76	0.75
	$H_o$	0.60	0.50	0.80	0.90	0.45	0.55	0.68	0.42	0.90	0.64
3 B	$N_a$	6	3	9	8	3	6	7	6	6	6
	$H_e$	0.74	0.19	0.77	0.73	0.14	0.76	0.67	0.66	0.73	0.60
	$H_o$	0.70	0.15	0.90	0.40	0.05	0.85	0.58	0.50	0.79	0.55
1 F	$N_a$	6	5	13	6	4	6	8	15	10	8
	$H_e$	0.73	0.49	0.89	0.36	0.28	0.75	0.82	0.93	0.86	0.68
	$H_o$	0.50	0.41	0.80	0.25	0.20	0.95	0.78	0.89	0.81	0.62
2 F	$N_a$	3	4	15	8	5	8	8	10	9	8
	$H_e$	0.64	0.51	0.92	0.84	0.32	0.80	0.82	0.86	0.87	0.73
	$H_o$	0.45	0.45	0.90	0.60	0.25	1.00	0.76	0.68	0.85	0.66
3 F	$N_a$	4	6	14	8	1	4	9	10	8	7
	$H_e$	0.51	0.60	0.92	0.86	0.00	0.76	0.82	0.87	0.84	0.69
	$H_o$	0.20	0.75	0.61	0.84	0.00	1.00	0.63	0.50	0.65	0.58

TABLE 2. Estimates of colony genetic variability and population structure ( $\Phi_{PT}$ ; Raymond and Rousset, 1995; Peakall and Smouse, 2006) for brackish (B) and freshwater (F) *Iris hexagona* colonies in Marsh Island and Cypremort Point, Louisiana, USA ( $P \leq 0.002$ ).

Colony	Colony pairwise $\Phi_{PT}$ values				
	B1	B2	B3	F1	F2
B2	0.066				
B3	0.111	0.182			
F1	0.133	0.159	0.157		
F2	0.118	0.133	0.191	0.068	
F3	0.154	0.180	0.218	0.105	0.081

Products, Wooster, Ohio, USA) with deltaic topsoil. Each colony received a unique color code that was identifiable when the experiment ended and the plants were excavated. Two irises from three freshwater or three brackish colonies were placed in every container, for a total of six plants per container. Marsh Island and Cypremort Point irises did not share the same container, but different colonies from the same habitat were intermixed within containers. The freshwater and brackish colonies were replicated in 15 containers each, which allowed us to test colony-level differences.

Containers were randomly assigned to either a low-, medium-, or high-saltwater treatment, which was maintained throughout the experiment. The salinity solutions were created with the synthetic sea salt Instant Ocean (Aquarium Systems, Mentor, Ohio, USA). We monitored NaCl concentration with an Orion 125 conductivity meter (Orion Research, Beverly, Massachusetts, USA). Experimental salinity levels averaged  $0.2 \pm 0.003$  SE ppt (low-salinity treatment),  $4.0 \pm 0.04$  SE ppt (medium-salinity treatment), and  $6.3 \pm 0.04$  SE ppt (high-salinity treatment), which are similar to levels in coastal freshwater and brackish marsh. We had five replicates for each salinity-colony combination ( $N = 30$  containers). In March–May 2003, we measured flower production and flowering phenology daily. In July 2004, we excavated the containers and separated and weighed fresh leaves, flower stalks, capsules, rhizomes, and roots. We counted and weighed the mature seeds contained in each fruit capsule.

**The effect of salinity and colony on iris performance**—We evaluated the factorial effects of salinity and iris colony on plant growth and reproduction using MANOVA and ANOVA with pairwise adjusted Tukey comparisons (SAS 9.1.3, SAS Institute, Cary, North Carolina, USA). Dependent variables included leaf mass (g), clonal rhizome mass (g), root mass (g), and number of seeds. Containers with missing data were removed before the MANOVA tests were run, as required by the analysis. We conducted contrasts to determine whether plant performance differed between brackish and freshwater habitats. Data were log-transformed to meet the assumptions of parametric analysis and back-transformed for presentation in figures, text, and tables.

**Flowering phenology**—We evaluated iris flowering phenology with the equation,

$$F(x) = \alpha \times \exp(-\exp(-K \times (x - \gamma)))$$

where  $F(x)$  is the number of flowers on  $x$  day,  $\alpha$  is the total number of flowers produced over the growing season,  $\gamma$  is the day on which flowering peaked,  $K$  is a constant, and  $\exp$  is the exponential function, subject to the conditions  $\alpha > 0$ ,

TABLE 3. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) (Nei, 1978) among *Iris hexagona* colonies in Marsh Island and Cypremort Point, Louisiana, USA.

Colony	Colonies					
	B1	B2	B3	F1	F2	F3
B1	****	0.8548	0.8514	0.7113	0.7644	0.7144
B2	0.1568	****	0.7368	0.6524	0.7359	0.6523
B3	0.1609	0.3054	****	0.7369	0.7122	0.6739
F1	0.3407	0.4271	0.3053	****	0.8407	0.7672
F2	0.2686	0.3067	0.3394	0.1735	****	0.8489
F3	0.3363	0.4273	0.3946	0.2650	0.1639	****

$k > 0$ , and  $\gamma > 0$  (Winsor, 1932; d'Onofrio, 2005). We added 1 to each value before fitting the curve using the software program Minitab (Minitab, State College, Pennsylvania, USA). For this analysis, we pooled colonies within freshwater and brackish habitats and used ANOVA to test differences in flower number and phenology.

## RESULTS

**Genetic structure of freshwater and brackish iris colonies**—We detected 1–15 alleles per locus and an average colony heterozygosity of 0.55–0.66. In each colony, the observed heterozygosity was slightly lower than the expected heterozygosity, but it was within the normal range for clonal plants (Table 1). Significant genetic structure existed among colonies ( $\Phi_{PT, 5, 114} = 0.138$ ,  $P = 0.001$ , Table 2), and those in closest proximity were typically the most similar genetically (Table 3). A positive correlation existed between colony genetic and geographic distance (Mantel's  $Z = 21.22$ ,  $r = 0.68$ ,  $P = 0.04$ ).

**Effect of salinity and colony on iris performance**—Salinity strongly affected iris rhizome mass (g), root mass (g), seed numbers, and seed mass (mg). All variables were highly correlated (Table 4). Salinity had negative linear effects on vegetative and clonal mass (Fig. 1). However, salinity had nonlinear effects on sexual reproduction, with the intermediate-salinity (4 ppt) treatment producing the most seeds with the largest individual seed mass.

A significant effect of salinity on the combined growth and reproduction variables existed (Pillai's trace = 0.65,  $F_{10,120} = 5.8$ ,  $P < 0.0001$ ). Univariate tests confirmed strong salinity responses among individual variables: leaf mass ( $F_{2,63} = 9.3$ ,  $P = 0.0003$ ), rhizome mass ( $F_{2,63} = 9.9$ ,  $P = 0.0002$ ), root mass ( $F_{2,63} = 12.9$ ,  $P = 0.0001$ ), seed mass ( $F_{2,63} = 10.6$ ,  $P = 0.0001$ ), and seed numbers ( $F_{2,63} = 6.7$ ,  $P = 0.002$ ).

In adjusted Tukey pairwise comparisons, leaf mass (g) was similar in 0- and 4-ppt salinity ( $P = 0.09$ ), and both were greater than 6-ppt salinity ( $P = 0.0002$  and  $P = 0.053$ , respectively, Fig. 1A). Rhizome mass (g) also was similar in the 0- and 4-ppt salinity treatments ( $P = 0.57$ ), with both greater than in 6-ppt salinity ( $P = 0.0002$  and  $P = 0.003$ , respectively, Fig. 1B). The same pattern held for root mass (g), with similarity between 0- and 4-ppt salinity ( $P = 0.21$ ), and both were greater than 6-ppt salinity ( $P = 0.0001$  and  $P = 0.0025$ , respectively, Fig. 1C).

TABLE 4. Correlation among plant performance variables for *Iris hexagona* colonies in Marsh Island and Cypremort Point, Louisiana, USA. Pearson's  $r$ ,  $P$  value, and sample size are shown.

Performance variable	Correlation measured	Performance variables			
		Rhizome mass (g)	Root mass (g)	Seed mass (mg)	Number of seeds
Leaf mass (g)	$r$	0.88	0.63	0.50	0.52
	$P$	<0.0001	<0.0001	<0.0001	<0.0001
	Sample	81	81	81	81
Rhizome mass (g)	$r$	1	0.76	0.58	0.57
	$P$		<0.0001	<0.0001	<0.0001
	Sample		81	81	81
Root mass (g)	$r$		1	0.63	0.65
	$P$			<0.0001	<0.0001
	Sample			81	81
Seed mass (mg)	$r$			1	0.94
	$P$				<0.0001
	Sample				81

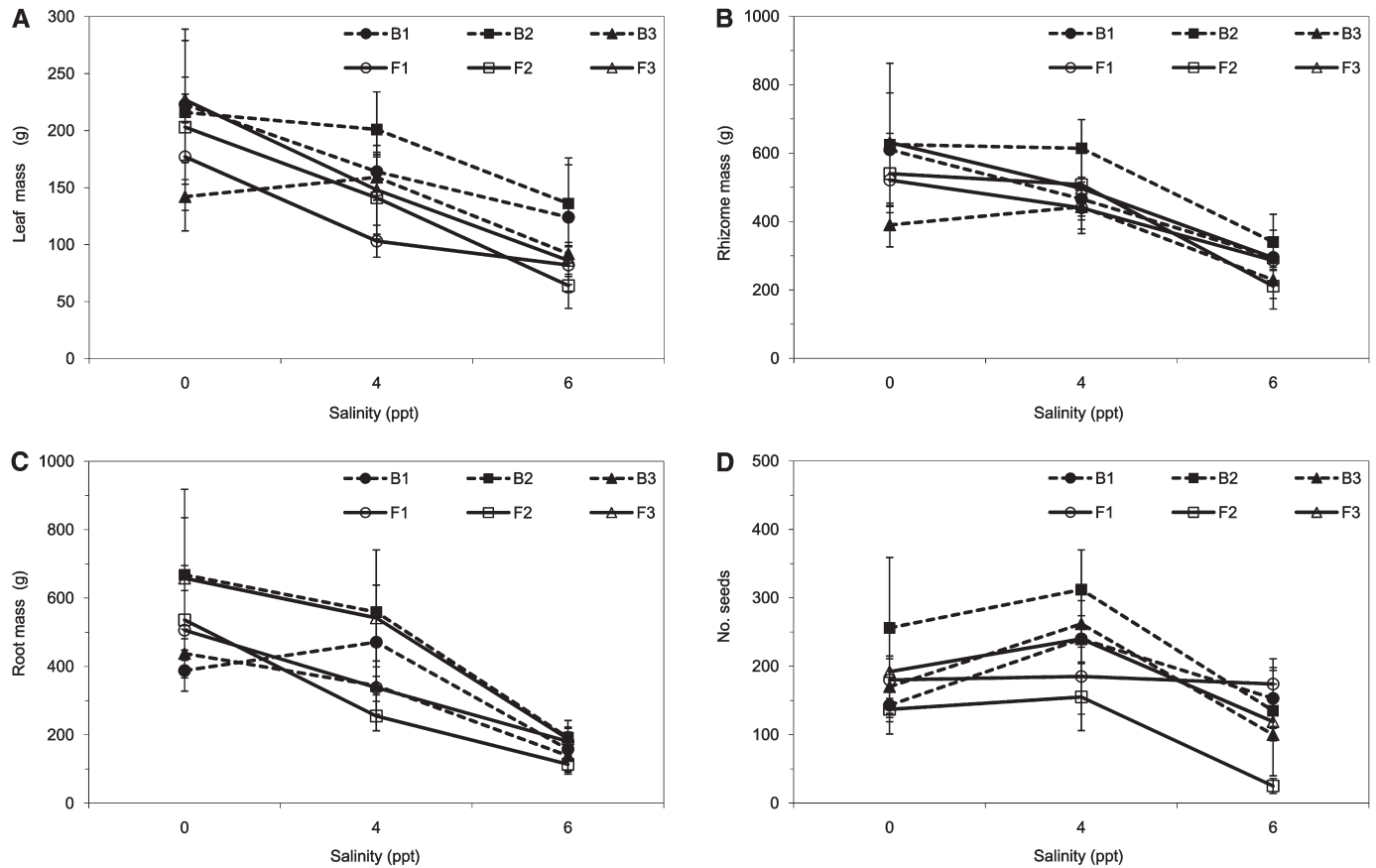


Fig. 1. The effect of salinity on leaf mass (A), rhizome mass (B), root mass (C), and seed numbers (D) on *Iris hexagona* colonies from freshwater (solid lines) and brackish habitats (hatched lines) in Cypremort Point and Marsh Island, Louisiana, USA. Data points shown are means  $\pm$  1 SE. Figure abbreviations: B, brackish colony; F, freshwater colony; ppt, parts per thousand.

Salinity had a nonlinear effect on sexual reproduction. The most seeds were produced in the 4-ppt treatment (Fig. 1D), which was significantly greater than the results in the 6-ppt

treatment ( $P = 0.0015$ ) but did not differ significantly from the results in the 0-ppt treatment ( $P = 0.16$ ). Results in the 0-ppt and 6-ppt treatments were similar ( $P = 0.13$ ). Individual seed

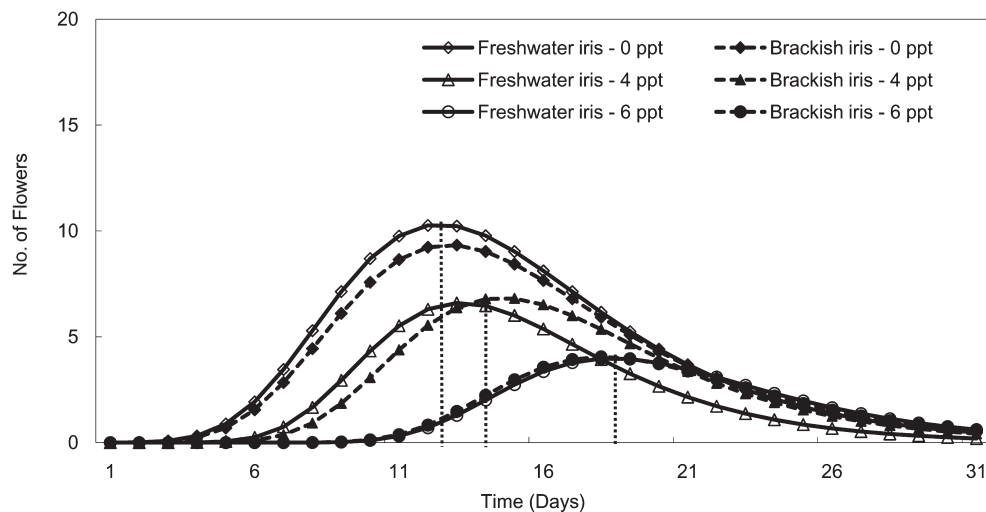


Fig. 2. The effect of salinity on the flowering phenology of *Iris hexagona* colonies from freshwater (solid line) and brackish (hatched line) habitats in Cypremort Point and Marsh Island, Louisiana, USA. The peak flowering period for each salinity level is indicated by the vertical dotted lines. Figure abbreviation: ppt, parts per thousand.

mass (data not shown) was highly correlated with seed numbers ( $r = 0.94$ ) and was greatest in the 4-ppt treatment, which was significantly different from the results in the 6-ppt treatment ( $P = 0.0001$ ) but was similar to results in the 0-ppt treatment ( $P = 0.15$ ).

A significant overall effect of iris colony on the combined leaf, rhizome, root, and seed response variables existed (Pillai's trace = 0.5,  $F_{25,315} = 1.5$ ,  $P = 0.053$ ), but no individual variables exhibited significant differences among colonies: leaf mass ( $F_{5,63} = 1.2$ ,  $P = 0.31$ ), rhizome mass ( $F_{5,63} = 1.0$ ,  $P = 0.45$ ), root mass ( $F_{5,63} = 1.2$ ,  $P = 0.33$ ), seed mass ( $F_{5,63} = 1.4$ ,  $P = 0.23$ ), and seed numbers ( $F_{5,63} = 1.8$ ,  $P = 0.12$ ). No colony-salinity interaction effect on the combined variables existed (Pillai's trace = 0.4,  $F_{50,315} = 0.6$ ,  $P = 0.99$ ).

The a priori contrast analysis that grouped colonies from brackish and freshwater environments did not detect significant differences in plant performance between the two habit types: leaf mass ( $F_{1,75} = 0.98$ ,  $P = 0.33$ ), rhizome mass ( $F_{1,75} = 0.01$ ,  $P = 0.9$ ), root mass ( $F_{1,75} = 0.04$ ,  $P = 0.84$ ), seed mass (not run), and seed numbers ( $F_{1,75} = 2.2$ ,  $P = 0.14$ ).

**Flowering**—Salinity reduced flower production ( $F_{2,24} = 36.9$ ,  $P < 0.001$ ) with 0-ppt treatments producing the most flowers, followed by 4- and 6-ppt salinity (Fig. 2). Habitat had no effect on the number of flowers produced ( $F_{1,24} = 0.04$ ,  $P = 0.5$ ), and no interaction existed between salinity and habitat ( $F_{2,24} = 0.6$ ,  $P = 0.6$ ). Elevated salinity delayed flowering phenology ( $F_{2,24} = 25.2$ ,  $P < 0.001$ ). The 0-ppt and 4-ppt salinity treatments displayed similar flowering phenology ( $P = 0.14$ ), but flowering was delayed by about 5 d in the 6-ppt salinity treatment ( $P < 0.0001$ ).

## DISCUSSION

Saltwater intrusion is spreading in coastal wetland communities. To survive and persist, freshwater plant populations will have to tolerate moderate levels of salinity. This requires genetic variability and physiological plasticity, which we observed in *I. hexagona* colonies from freshwater and brackish habitats. Although *I. hexagona* heterozygosity levels were lower than expected for outcrossing plants, they were moderate to high compared with those of other clonal species (Ellstrand and Roose, 1987; Brzyski, 2010). Furthermore, genetic variability was independent of colony size or habitat, and colonies from the same habitat were genetically distinct. These results indicate that even the small brackish-marsh colonies may contain sufficient genetic variation to adapt to changing environments. Performance data from a previous experiment support the genetic results: irises collected from 10 separate brackish-marsh colonies and grown in a common garden displayed significant differences in growth and reproduction (Van Zandt and Mopper, 2002; Van Zandt et al., 2003).

Salinity is a potentially strong source of selection because it affects clonal growth and sexual reproduction. But contrary to our prediction, plants collected from brackish and freshwater *I. hexagona* colonies exhibited similar responses to salinity. Regardless of their natal habitat, all irises performed best in fresh water, but they also grew and reproduced in the highest level of sustained experimental salinity (6 ppt). This suggests that freshwater and brackish iris populations have a similar physiological tolerance of salt. Because plant populations cannot easily relocate when disturbance occurs, such physiological

plasticity to environmental stress can be highly beneficial (Bradshaw, 1965; Schlichting, 1986; Ackerly et al., 2000; Sultan, 2000; Gimeno et al., 2008).

Physiological adaptations to salinity must include energetic strategies and metabolic activities that maximize physiological recovery and tolerance (Munns and Tester, 2008). In salt-stressed irises, energy is needed to excrete and sequester excess  $\text{Na}^+$  ions and to synthesize organic osmolytes, which balance plant water potential during water deficits (Wang et al., 2008). Salt can also affect growth and reproduction by inducing or suppressing plant hormones that regulate normal metabolic processes in *I. hexagona* (Wang et al., 2001). These diverse and powerful selection pressures may help to sustain genetic variation among and within iris colonies.

Phenology is a genetically controlled trait that can strongly influence mating and genetic isolation (Mopper, 2005). *Iris hexagona* flowers are receptive to pollinators for only about 2 d (Van Zandt and Mopper, 2004), thus the 5-d flowering delay caused by 6-ppt salinity represents a strong barrier to interbreeding. Asynchronous flowering leading to restricted pollen transfer probably occurs between freshwater and brackish populations. In coastal Louisiana, freshwater wetlands are typically restricted to 0–2 ppt NaCl, but in brackish habitats salinity varies widely seasonally, and saltwater pulses from hurricanes can attain 22 ppt NaCl or higher (Van Zandt and Mopper, 2002). Furthermore, salinity gradients also occur within brackish wetlands (Van Zandt and Mopper, 2002); thus, colonies in relatively similar habitats may experience restricted gene flow from flowering delays.

Since 2002, several large hurricanes have impacted the region (e.g., Lili, Katrina, Rita, Ike, Gustav). Mathematical models predict that their concomitant salt pulses can alter life-history traits and long-term demographic trajectories of iris populations (Pathikonda et al., 2009). On the basis of the findings reported here, we propose that *I. hexagona* populations contain sufficient genetic variation and physiological plasticity for continued persistence as saltwater intrusion alters their historically freshwater wetlands.

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