Water-induced stress influences the relative investment in cleistogamous and chasmogamous flowers of an invasive grass, Microstegium vimineum (Poaceae)

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Background: Global climate change has the potential to shape evolutionary trajectories of invasive species *via* many routes, including through changes in mating systems. Many cleistogamous (CL) plants adjust investment in CL (selfed) vs. chasmogamous (CH, potentially outcrossed) progeny across environmental gradients. However, the details of such adjustments are lacking for highly invasive plant species.
Aims: We used a highly invasive grass, *Microstegium vimineum*, as a model for understanding how changes in water-induced stress (including potential associated changes in soil nutrient availability) might affect mating systems and thus evolutionary change in invasive species. We predicted that plants would respond to increased water-induced stress through a relative reduction in investment in CL vs. CH reproduction (i.e., a decrease in the CL:CH ratio).
Methods: Under greenhouse conditions, we measured fecundity (number of inflorescences and florets per plant) as well as relative investment in CL vs. CH florets (CL:CH ratios for number of inflorescences, florets per inflorescence, overall florets) in response to three watering treatments approximating mesic (low) to inundated (high) conditions.
Results: Plant biomass was significantly lower in high-watering treatment relative to intermediate and low treatments, indicating that the high-water condition was stressful. Contrary to expectations, stressed plants significantly increased relative investment in CL reproduction, a pattern associated with decreased inflorescence number and increased numbers of CL florets per inflorescence.
Conclusions: We conclude that changes in water-induced stress could strongly influence realised rates of outcrossing in this invasive plant, leading to mating system evolution, and altered invasiveness.

Keywords: cleistogamy; invasive species; mating system; phenotypic plasticity; soil moisture; water gradient

Introduction

A growing body of evidence suggests that genetic and evolutionary processes are key to determining whether invasive species can establish and spread. During the colonisation phase, high genetic diversity can result in improved success; e.g., high-diversity *Arabidopsis thaliana* founder populations had higher seed germination rates, higher biomass, longer flowering seasons, and were more fecund than low-diversity populations (Crawford and Whitney 2010). In the generations following colonisation, levels of genetic diversity are expected to be positively correlated with rates of evolutionary change (Baker 1965; Sakai et al. 2001; Lee 2002; Holt et al. 2005);e.g., invasive *Raphanus* lineages that contained more genetic diversity experienced more rapid phenotypic evolution than lineages with less genetic diversity (Campbell et al. 2009). In contrast, genetically depauperate populations may have a greater extinction risk (Newman and Pilson 1997) and slower rates of evolution (Wade et al. 1996). In general, traits important to invasiveness, such as competitive ability, growth rate, and propagule size, can evolve

rapidly with detectable changes over time periods of less than a century (Whitney and Gabler 2008). Given that large differences in climatic conditions may characterise the original species range relative to the area where it is introduced (Maurer et al. 2007), it is critical to understand how changes in water availability and other environmental variables might shape genetic variation and evolutionary potential in invasive species.

Mating systems are a major determinant of how genetic diversity is organised within species and of rates of evolution (Wright 1921; Allard et al. 1968; Hamrick et al. 1979; Schoen 1982; Loveless and Hamrick 1984; Holtsford and Ellstrand 1989; Hamrick and Godt 1996). Plant mating systems are characterised by the fertilisation rates of ovules by self vs. non-self or "outcross" pollen (Wright 1921). There is abundant evidence that mating systems should be sensitive to alterations in environmental conditions induced by climate change. For example, outcrossing rates are known to vary across moisture gradients (Marshall and Allard 1970; Hamrick and Allard 1972; Brown et al. 1974; Clegg 1980; Holtsford and Ellstrand

1989). Cleistogamous (CL) strategies, in which plants produce non-opening, self-pollinating flowers, have repeatedly evolved throughout the angiosperm phylogeny (700 spp., 50 families, 34–41 independent events), suggesting an adaptive advantage (Goodwillie et al. 2005; Culley and Klooster 2007; Oakley et al. 2007). In dimorphic CL species, which produce both self-pollinating CL flowers and potentially outcrossing chasmogamous (CH) flowers, the ratio of CL:CH reproduction is known to respond to light level (Wilken 1982; Cheplick and Fox 2011; Colbert 2015), nutrient availability (Le Corff 1993), and moisture level (Bell and Quinn 1987; Webster and Grey 2008). In particular, more favourable conditions have been shown to produce lower CL:CH ratios in several plant species (Brown 1952; Langer and Wilson 1965; Waller 1980; Wilken 1982; Minter and Lord 1983; Albert et al. 2011). Through self-pollination, CL flowers in many CL species may provide reproductive assurance (Mitchell-Olds and Waller 1985; Cheplick 2007; Albert et al. 2011). In contrast, openly pollinated CH flowers of CL species may serve to create more genetically diverse offspring, some of which may be more successful in stressful or unpredictable environments, and to provide greater dispersal opportunities as seeds of CL flowers are often locally dispersed while seeds of CH flowers often travel longer distances (Schmitt et al. 1985). However, we are unaware of any studies that have evaluated plasticity in mating systems in highly invasive plant species.

To explore these issues, we tested the effect of water-induced stress on the degree of cleistogamy in a highly invasive grass, *Microstegium vimineum*. In North America, invading *M. vimineum* creates dense, mono-specific stands, thereby reducing native plant diversity and productivity, inhibiting forest regeneration, and altering forest species composition and successional trajectories (Oswalt et al. 2007; Flory and Clay 2009a). While the species appears to harbour substantial plasticity for many traits (e.g., Droste et al. 2010), plasticity in its allocation to selfing vs. outcrossing flowers has not been evaluated. However, the relative allocation of resources to CH vs. CL seeds is often extremely plastic in grasses (Cheplick 2007). When growing under favourable conditions, *M. vimineum* plants are taller and thus produce more internodes per stem. Since CL flowers grow within the internodes and CH flowers are terminal, we predict taller plants will exhibit higher CL:CH ratios relative to shorter plants. Increased branching may also occur, allowing for more terminal (CH) inflorescences, but this is not likely to offset the increases in CL reproduction. Thus, we predicted that increasingly stressful conditions would adjust the ratio of CL:CH reproduction downward in this invasive species, thereby setting the stage for altered rates of microevolution, invasiveness, and impacts on native communities. We discuss our results and previous research in the context of invasive species and climate change.

Materials and methods

Study organism

M. vimineum, commonly known as Nepalese Browntop or Japanese Stiltgrass, is an annual, shade-tolerant, forest herb that thrives in disturbed areas (Winter et al. 1982; Barden 1987; Horton and Neufeld 1998; Cheplick 2005). Originating in Asia, *M. vimineum* was introduced to Tennessee, USA prior to 1919 (Fairbrothers and Gray 1972). It has since spread throughout the Eastern USA, *via* human and deer vectors, and currently resides in 25 states and Puerto Rico, including the north-east corner of Texas (Barden 1987; Mehrhoff 2000; Baiser et al. 2008; USDA 2015). *M. vimineum* invasions have had strong negative impacts on native plant diversity (Adams and Engelhardt 2009; Flory and Clay 2009b, 2010; Flory 2010), arthropod communities (Simao et al. 2010), soil microbes, and ecosystem processes (Ehrenfeld et al. 2001; Kourtev et al. 2002; Baiser et al. 2008; McGrath and Binkley 2009). In part, *M. vimineum* may be a successful coloniser because it exhibits a high degree of phenotypic plasticity (Claridge and Franklin 2003; Cole and Weltzin 2004; Droste et al. 2010), by, for instance, altering resource allocation towards vegetative (rather than reproductive) biomass in low-light conditions (Cheplick 2005).

M. vimineum sexually reproduces through openly pollinated, CH spikelets along emergent terminal racemes and self-fertilising, CL spikelets at nodes on axillary racemes enclosed in leaf sheaths (Tanaka 1975). Given that branching in *M. vimineum* produces terminal racemes, plants with more branches and tillers produce more CH spikelets. Mature seeds (caryopses) are produced by CH and CL spikelets simultaneously in early autumn with each tiller producing a few CH spikelets and many CL spikelets.

Seed source and propagation

In October 2006, 10 *M. vimineum*, (Trin.) A. Camus maternal half-sib families were collected from CH inflorescences from a population growing in Clark Forest, Indiana, USA. The population was located beside a small stream in a mature forest. Hundreds of seeds collected from each maternal plant were refrigerated until 15 seeds per maternal plant were planted into plug trays (12 plugs per 13.3 cm × 17.78 cm, Kord Containers Ltd., St. Louis, MO, USA) with a mixture of 75% Pro mix BX peat (Premier Horticulture Ltd., Rivière-du-Loup, Canada) and 25% sand on 16 October 2008. We planted 15 seeds per maternal plant by placing seeds on top of the soil mixture. At the two-leaf stage, we transplanted 12 seedlings of each maternal plant into individual pots (10.16 cm × 10.16 cm, Kord Containers Ltd., St. Louis, MO, USA) filled with 800 cm^3 of the same soil mixture.

Greenhouse experiment

Water availability and, more broadly, climate can dramatically influence plant growth and reproduction through both direct effects on plants, as well as indirect effects on nutrient availability (i.e., increased water may leach nutrients from the soil and create low-nutrient conditions). Since we were interested in tightly controlling water-induced stress, we grew experimental plants in a greenhouse. The greenhouse was located at Rice University (Houston, Texas) which has a different photoperiod than Indiana (the source of the seeds) and so we adjusted the photoperiod to induce flowering (described below). Importantly, we were most interested in assessing the general effect of soil water-induced stress on CL:CH ratios

of a CL plant, rather than modelling the specific effects of Indiana soil moisture variation. Plants responded well to the high-light greenhouse environment and were much larger than typical wild plants from shady habitats (Table 1), as reported in other low-competition, high-light environments (Cheplick and Fox 2011). We imposed a watering regime with three treatments: high (125 ml water day^{-1}), intermediate (watered when the measured soil volumetric water content (VWC) fell below 65% of the VWC of the high-water treatment), and low (watered when VWC fell below 35% of the high-water treatment); for measured VWC values, see Figure 1. The high-water treatment probably caused, through inundation, the loss of soil minerals and may have created anoxic conditions. The

Table 1. Average (SE) cleistogamous and chasmogamous floret production and plant architecture for 10 *Microstegium vimineum* families grown under varying levels of water availability.

		Watering treatment					
		Low ($N = 10$)		**Intermediate** ($N = 10$)		**High** ($N = 10$)	
Trait	Flower type	Mean	SE	Mean	SE	Mean	SE
Inflorescences	CH	275.06[a]	21.16	209.63[b]	18.86	137.47[c]	10.97
	CL	577.40[a]	45.39	518.77[a]	38.71	367.03[b]	24.81
	Total	852.46[a]	58.53	728.41[a]	51.13	504.50[b]	31.13
Florets/inflorescence	CH	17.90[a]	0.76	20.21[a]	1.02	20.51[a]	0.97
	CL	3.37[a]	0.19	3.98[a]	0.26	4.47[b]	0.21
Florets	CH	4890.38[a]	400.08	4454.60[a]	435.12	2625.49[b]	170.00
	CL	2024.95[a,b]	178.44	2181.81[a]	164.93	1653.26[b]	140.40
	Total	6915.33[a]	473.28	6636.41[a]	490.34	4278.75[b]	257.16
Above-ground biomass (g)		20.29[a]	0.59	22.91[a]	1.09	15.67[b]	0.88
Branches/g		10.84[a]	0.51	8.07[b]	0.59	7.07[b]	0.66
Nodes/g		59.95[a]	2.39	52.60[b]	3.27	45.00[c]	2.64

Note: Superscripts represent significant differences among watering treatments ($P < 0.05$, Sidak adjustment for multiple comparisons).
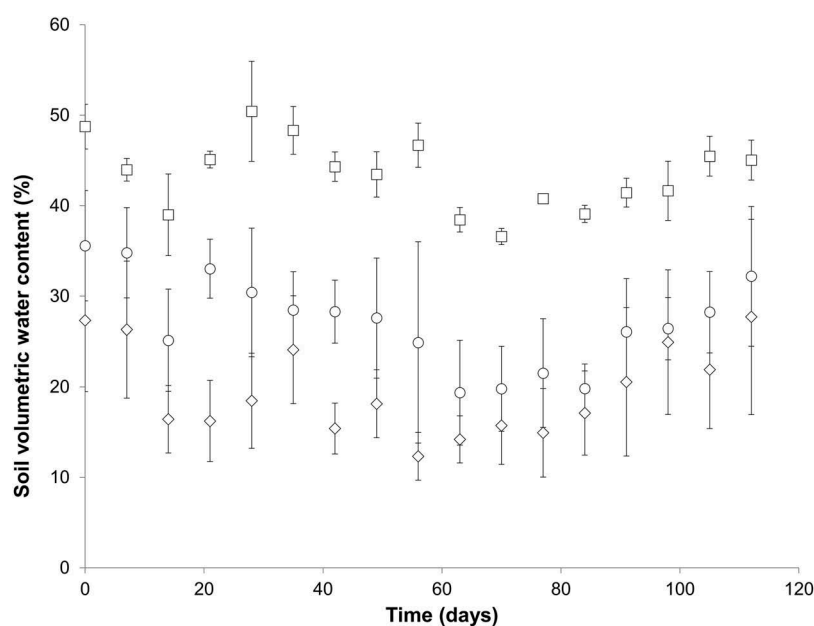

Figure 1. Average weekly soil volumetric moisture content of five monitored plants for three watering treatments: high (□), intermediate (○), and low (◊). Error bars represent 95% confidence intervals.

low-water treatment was still relatively mesic and ultimately did not cause drought stress (i.e., we did not see plant wilting or chlorosis).

Three or four seedlings from each maternal plant were assigned at random to each of the three watering treatments, for a total of ca. 35 seedlings per treatment, and 110 seedlings overall. Mean soil moistures within our experiment (low = 19.46%; intermediate = 27.10%; high = 43.48%) fell within the range naturally experienced by *M. vimineum*, as it is found in riverine forest shores and wetlands (Zampella and Laidig 1997; Luken and Bezold 2000) as well as upland areas that are drier (where VWC ranges between 13% and 46%) (Belote et al. 2004; Cole and Weltzin 2004). To ascertain the VWC of each treatment, we measured the VWC of five plants per watering treatment daily, using a soil moisture meter (Field Scout, TDR 100/200 Spectrum Technologies, Inc., Plainfield, IL, USA). These 15 "sentry" plants were excluded from analyses in case frequent disturbance by probes altered their growth. Watering treatments were maintained from 3 November 2008 to 1 March 2009. After 3 months of growing in these pots, most plants appeared pot bound; 2 of the 110 plants died prior to reproduction. Once 99% of surviving plants had reproduced (only one plant did not flower) and started to senesce, plants were watered as needed, rather than by treatment. Harvesting occurred between 1 and 15 March 2009.

On 13 November 2008, the plants were sprayed with insecticidal soap (2% Garden Safe, Schultz, Bridgeton, MO, USA) to control for thrips, and rinsed with 200 ml of water 24 h later. Plants were sprayed on 5 January and 9 February 2009 with 0.0005% Conserve SC to control thrips (Dow AgroSciences LLC, Indianapolis, IN, USA). On 3 and 27 December 2008, all plants were watered with ca. 125 ml of 3.77 g l^{-1} fertiliser (Miracle-Gro 24–8-16, Scotts Miracle-Gro Products, Inc., Marysville, OH). Our growing conditions and fertiliser application may have resulted in unusually large plants, relative to sizes reported in other studies (e.g., Gibson et al. 2002; see also Results and Discussion).

To account for any environmental variation within the greenhouse, plants were spatially randomised biweekly until anthesis. Greenhouse temperature was maintained between 20°C and 24 °C. To induce anthesis, day length was artificially reduced to 8 h by covering plants nightly with a black plastic sheet from 27 January to 3 March 2009. Flowering started from 18 February 2009.

Data collection

Because plants attained a large size, our response variables were measured on a subsample of each plant and then extrapolated to whole-plant values based on the ratio of subsample biomass to above-ground whole-plant biomass. We subsampled each plant by haphazardly choosing a substantial tiller (at least 34 cm long and, on average, 64 cm long) and cutting it above the first node with

roots. We measured the full length of the tiller and counted the number of branches and number of nodes. We counted the number of CH inflorescences, CL inflorescences, and number of CL florets per CL inflorescence (for a minimum of 10 inflorescences, if possible; otherwise as many as our subsample contained). We counted the number of CH florets per CH inflorescence from 10 haphazardly harvested CH inflorescences per plant. We quantified only floret (not seed) production because CL seed production may compensate for reduced CH production in the absence of pollen vectors (Redbo-Torstensson and Berg 1995; Berg and Redbo-Torstensson 1998; Albert et al. 2011). We measured dry biomass of each sampled tiller and of the remaining above-ground biomass of each plant.

Statistical analysis

Ratios of CL:CH florets may vary due to morphological adjustments at different hierarchical levels. Therefore, we calculated CL:CH ratios for number of inflorescences, number of florets per inflorescence, and number of florets. The latter was calculated as the CL:CH ratio for overall floret number (number of CL inflorescences times average of CL florets per inflorescence divided by number of CH inflorescences times average of CH florets per inflorescence). To estimate total number of CL and CH inflorescences and florets for each individual, we scaled the number of inflorescences or florets by the proportion of biomass sampled from the whole plant.

To test for differences among watering treatments, we ran linear univariate analyses of variance (ANOVAs) for the total number of inflorescences and florets per plant, and above-ground biomass of the plant where watering treatment was treated as a fixed factor and maternal plant (and its interaction with treatment) was treated as a random factor in the model. These measures of absolute fecundity were not included in the following multivariate analysis of variance (MANOVA) due to lack of independence of dependent variables. To test for differences in CL:CH ratios and plant architecture among watering treatments and families, we carried out a fixed-factor MANOVA for three response variables (CL:CH ratios for inflorescences, florets per inflorescence, and total florets), where watering treatment was treated as a fixed factor and maternal plant (and its interaction with treatment) was treated as a random factor in the model. To meet the assumptions of ANOVA, biomass and the absolute number of CL and CH inflorescences were natural logarithm transformed, and CL:CH ratios were arcsine square root transformed. All analyses were made with SPSS (v.17; SPSS Inc., Chicago, IL, USA).

Results

Effects of water-induced stress on growth and fecundity

The high-water treatment appeared to reduce biomass relative to that in the low or intermediate water treatment
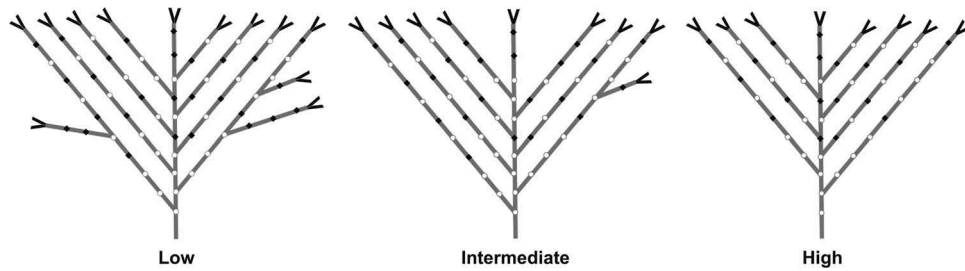
Figure 2. Diagrammatic representation of 1 g of *Microstegium vimineum* above-ground dry biomass after plants were grown under one of three watering treatments (low, intermediate, and high) in glasshouse conditions. Depictions of the number of branches, the number of nodes (empty circles), and the number of cleistogamous (black diamonds) and chasmogamous inflorescences (black dashes, paired at branch tips) are based on mean values reported in Table 1.

(Figure 2). Plants in the high-water treatment produced 29% and 46% less above-ground biomass than those in the low or intermediate treatments, respectively (Table 1; $F_{2,18} = 17,47$, $P < 0.0001$). Further, high-water treatment plants produced almost 40% fewer florets overall ($F_{2,18} = 12.342$, $P < 0.001$, Table 1). The number of branches and nodes per gram differed significantly among treatments (Table 1; branches per gram: $F_{2,18} = 12.671$, $P < 0.001$; nodes per gram: $F_{2,18} = 11.331$, $P < 0.001$), where plants that received less water had significantly more complex architecture (i.e., more branches and nodes per gram), than plants in the high-water treatment (Figure 2).

Effects of water-induced stress on relative investment in cleistogamy

Across the water treatment gradient (from low to high), plants invested more in selfing *via* CL floret production. Specifically, overall investment in CL vs. CH reproduction (ratio of CL:CH florets) increased by 7.5% from low water to intermediate and by 14.0% from intermediate to high water (Figure 3(c)); $F_{2,18} = 5.33$, $P = 0.008$). This was due to a significant increase in the ratio of CL:CH

inflorescences across the water gradient (Figure 3(b); $F_{2,18} = 5.24$, $P = 0.009$), as the ratio of CL to CH florets per inflorescence did not differ significantly among watering treatments (Figure 3(a); $F_{2,18} = 1.54$, $P = 0.22$).

Discussion

We predicted that increasingly stressful conditions would affect plant size and thus adjust the ratio of CL:CH reproduction downward in this invasive species, thereby setting the stage for altered rates of microevolution, invasiveness, and impacts on native communities. Here, we presented evidence that alteration of the degree of water-induced environmental stress, which might be common when an organism invades a new location, changes vegetative and reproductive architecture of *M. vimineum*. Ultimately, high-water conditions resulted in increased stress (based on plant biomass responses) and increased CL:CH ratios, thus decreasing potential rates of outcrossing in our experimental plants, assuming that outcrossing rates of CH florets would remain constant. The direction of change in CH:CL ratio was counter to our initial predictions but in fact matches studies of noninvasive plants, all of which show an increase of CL:CH ratios with increasing stress.
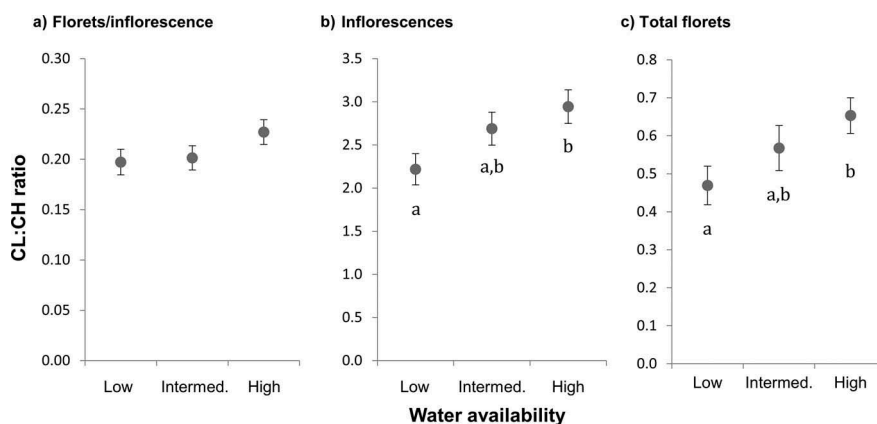

Figure 3. The relative investment in CL and CH florets in *Microstegium vimineum* grown under three watering treatments. Relative investment was assessed at the level of (a) florets per inflorescence, (b) number of inflorescences per plant, and (c) total florets per plant. Means and SE based on 10 maternal families. Letters indicate statistical differences ($P < 0.05$) among least mean squares, when present.

Changes to the mating system of *M. vimineum* may have consequences for the control and management of this invasive weed.

Plasticity in mating may have important effects on invasiveness

For plants to successfully invade, they must persist in and eventually adapt to new environmental stresses. Phenotypic plasticity may be key to the survival of invading plants before there is time to evolve (Yeh and Price 2004; Chevin and Lande 2011; Reed et al. 2011), especially phenotypic plasticity in mating strategy (Peterson and Kay 2015). In small founder populations, the flexibility to self-fertilise and/or clonally reproduce provides opportunities for reproduction despite the consequent loss of fitness due to inbreeding in mixed mating species (Baker 1965; Peterson and Kay 2015). However, evolution proceeds more slowly in populations with high rates of selfing or clonality compared with populations that have mixed mating (Charlesworth 1992) or high rates of out-crossing (Holeski and Kelly 2006) (although see Peterson and Kay (2015) for context dependent estimates of evolutionary rates). Further, increased self-fertilisation may lead to reduced fitness (e.g., in *Impatiens capensis*, Waller 1980; Schmitt and Gamble 1990), and therefore in invasive species, inbred offspring could result in reduced invasiveness. In fact, mating system plasticity (i.e., the allowance of some outcrossing with a standard ability to self-fertilise, as in CL plants) may provide adaptive advantages by promoting niche evolution through reduced extinction risk, by reducing maladaptive gene flow and by temporarily increasing genetic variance in traits under selection (Peterson and Kay 2015). In contrast, plants that only self-fertilise have limited adaptive potential.

Furthermore, many plants for which rapid evolution of invasive traits has been documented (Whitney and Gabler 2008) are obligate outcrossers (e.g., *Eschscholzia californica*, Leger and Rice 2007; *Hypochaeris radicata*, Ortiz et al. 2006; *Lythrum salicaria*, Eckert and Barrett 1992; *Pastinaca sativa*, Hendrix and Trapp 1992; *Rorippa austriaca*, Bleeker and Matthies 2005; *Sapium sebiferum*, Duke Forthcoming 1983; *Silene latifolia*, Desfeux et al. 1996; *Solidago altissima*, Gross and Werner 1983; *S. gigantea*, Melville and Morton 1982). In contrast, many invasive species for which we have little or no evidence of evolutionary change (*Alternanthera philoxeroides*, Geng et al. 2007; *Butomus umbellatus*, Kliber and Eckert 2005; *Caulerpa taxifolia*, Meusnier et al. 2002; *Eichhornia crassipes*, Li et al. 2006) appear to reproduce primarily by clonal reproduction (see Geng et al. 2007; Eckert et al. 2000; Benzie et al. 1997; Barrett 1980; respectively). These results highlight the disparate evolutionary consequences of divergent mating systems, and pave the way for future research on the role of mating system plasticity in promoting invasion in flowering plants.

Climate change may alter plant mating systems and thus genetic structure of populations

Although the effects of global climate change on plant mating systems have yet to be evaluated in the wild, we have demonstrated that CL:CH ratios of an invasive species vary across experimental moisture gradients. Evidence from naturally occurring gradients of moisture and temperature (Lambrecht and Dawson 2007) suggests that mating systems are sensitive to the environment in a variety of flowering plant species, including species that produce only CH flowers. For example, Clegg (1980) observed that typically selfing populations of grasses tended to outcross more in mesic than xeric environments (e.g., *Avena barbata*: Marshall and Allard 1970; *Bromus mollis*: Hamrick and Allard 1972; Brown et al. 1974; *Hordeum spontaneum*: 1974). Similarly, selfing populations of *Clarkia tembloriensis* tend to be found in hotter habitats where the soils dry earlier than those habitats that support populations that typically outcross (Holtsford and Ellstrand 1989).

Previous studies exploring the effects of soil moisture on the degree of cleistogamy have found a relatively uniform response: as soil moisture increased (and water stress apparently decreased), plants produced more CH flowers relative to CL flowers (Brown 1952; Langer and Wilson 1965; Waller 1980; Wilken 1982; Minter and Lord 1983; Webster and Grey 2008). That is, plants increased their outcrossing potential under wetter conditions. If all species behaved this way, it would be relatively simple to make standardised recommendations for conservation management, weed control, and agricultural breeding strategies. However, the patterns recorded for our population of *M. vimineum* were in the opposite direction: as soil moisture increased, *M. vimineum* produced more CL flowers relative to CH flowers and, therefore, experienced increased potential rates of selfing. High CL:CH ratios occurred under the most stressful conditions even though the nodes per gram of above-ground biomass (where CL inflorescences can be produced) were lower for plants that grew at high soil water content. This paradox is explained by floret production within a node: while the number of CL florets per node increased under high-stress conditions, CH floret production per branch appeared to be insensitive to water availability. Similar responses of CL:CH ratios to water availability have only been found in one other species, the perennial grass *Dichanthelium clandestinum* (Bell and Quinn 1987). Future studies, especially field studies, will be needed to determine the universality of these patterns across *M. vimineum* populations as well as other invasive species.

We suggest that stress, not water availability *per se*, determines relative investment in outcrossing vs. selfing in species that can plastically adjust their mating systems. Higher relative investments in CH flowers often correspond with larger total plant biomass, suggesting that plants growing in favourable conditions can afford to allocate more resources to CH flowers (Wilken 1982).

The reduced size and simpler architecture of *M. vimineum* plants growing at the highest moisture level may reflect more stressful conditions, due possibly to anoxia or loss of nutrients relative to lower moisture levels, despite our repeated nutrient fertilisation. Apparent higher stress in the higher-water treatments could also have arisen through plants being more root bound in these treatments, but this variable was not measured. Thus, from the viewpoint of stress, our results are consistent with other studies of CL plants finding reduced investment in outcrossing under stress (Waller 1980; Webster and Grey 2008). Therefore, we may be able to predict mating system responses by plant populations under climate change scenarios (e.g., Bates et al. 2008) if we understand the species-specific conditions that produce stress.

A knowledge of how mating systems may respond to environmental variation may provide insights into how genetic diversity is maintained within populations and their evolutionary trajectories. Both theory and empirical studies suggest that primarily outcrossing species will have greater allelic diversity, have higher levels of heterozygosity, and show less differentiation among populations than primarily self-fertilising species (e.g., Wright 1921; Schoen 1982; Hamrick and Godt 1996). Furthermore, CH flowers are expected to increase outcrossing rates (Goodwillie and Stewart 2013) and thus the relative frequency of CH flowers should have significant consequences for the organisation of genetic diversity within populations. If sustained changes in water availability were encountered during an invasion (or during the course of climate change) and were to result in sustained changes in plant mating systems, we would expect significant ecological and evolutionary consequences *via* effects on population genetic diversity (Newman and Pilson 1997; Hughes et al. 2008; Morran et al. 2009).

Conclusions

A sound understanding of the plastic responses of mating systems to environmental drivers will be vital to the development of models predicting evolutionary responses of species to larger scale phenomena such as global climate change (Eckert et al. 2009). Invasive plant species provide dynamic evolutionary and phenotypically plastic model systems for such studies. Despite a basic understanding of mating systems of invasive species, we have little knowledge of mating system variation after colonisation of new locations (but see Barrett et al. 2009; Ness et al. 2010) and even less knowledge of the influence of altered mating patterns on the evolutionary potential of colonising populations. Direct measurements of mating system parameters in native vs. invasive ranges, and mechanistic studies connecting outcrossing rates to rates of evolution and adaptation, would help to fill these gaps.

Notes on contributors

Lesley Campbell is a plant evolutionary ecologist interested in how plant mating systems affect the evolutionary trajectories of populations, especially focusing on weedy and invasive plants.

Loren Albert is a Ph.D. candidate in Ecology and Evolutionary Biology, studying plant ecological physiology with a focus on forest responses to climate change.

Esra Gumuser graduated from Rice University with a B.S. in EEB, is finishing her masters in biomedical science at Texas A&M and is planning to attend medical school in the near future.

Ken Whitney is an evolutionary ecologist with interests in the causes and consequences of interspecific hybridisation, maintenance and effects of genetic diversity, the evolution of genome size, and plant–animal interactions.

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