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Spread of invasive *Phragmites australis* in estuaries with differing degrees of development: genetic patterns, Allee effects and interpretation

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Summary

1. The distribution of genetic variation can be interpreted to understand the timing and mechanisms of invasive species spread. Allee effects, positive relationships between fitness and density or number of conspecific individuals, can play a substantial role in determining the time lag between initial introduction and invasive spread and can produce genetic patterns in invading populations that can be interpreted to learn about factors affecting invasion mechanisms.

2. We examined the distribution of genetic variation in the invasive wetland grass *Phragmites australis* in the Chesapeake Bay, USA. We used microsatellite analysis to examine the reproductive mode (clonal vs. seed) by which the invasive haplotype of *P. australis* has spread and the distribution of genetic variation within and among brackish wetlands in nine subestuaries of the Chesapeake Bay. Watersheds associated with the subestuaries were dominated by forests, anthropogenic development or mixed forests and development.

3. Our results suggest that the invasive haplotype of *P. australis* has spread primarily sexually by seed, rather than clonally, and genetic diversity of patches within subestuaries increased while genetic similarity decreased with increasing development in the surrounding watershed.

4. This suggests a pattern whereby greater genetic diversity of patches may promote more rapid spread due to recruitment of multiple seedlings into a disturbed patch.

5. *Synthesis*. Evaluation of patterns of genetic distribution can help to identify factors affecting invasion in different environments and so inform management.

Key-words: Allee effect, Chesapeake Bay, genetic diversity, invasion ecology, invasive species, microsatellite markers, *Phragmites australis*

Introduction

As the substantial environmental, economic and ecological impacts of invasive species have become particularly apparent in recent years, factors affecting the likelihood of introduced plant species becoming invasive have received increased experimental and theoretical attention (e.g. Le Roux & Wieczorek 2009). The need to overcome the initial negative genetic and reproductive effects of small population size is common to nearly all invasions and can produce a temporal lag between species introduction and subsequent invasive spread (e.g. Crooks & Soulé 1999; Davis *et al.* 2004; Crooks 2005). How quickly this lag is overcome may differ among environments. Understanding the processes contributing to the lag phase is important for assessing which management approach is likely to be the most effective and for predicting which environments are most vulnerable to invasion.

One way to understand the release from the lag phase and subsequent rapid expansion of an invasive species is to look for differences in genetic structure among environments that have been invaded to different degrees. Using the distribution of genetic variation to understand population processes has a long history in population biology (e.g. Wright 1978; Hamrick & Godt 1990; Ouborg, Piquot & van Groenendael 1999) but has rarely been used to understand invasion processes beyond identifying invasive origin (Le Roux & Wieczorek 2009). Allee effects, 'positive relationships between fitness and density or number of conspecific individuals' (Allee 1931; Stephens, Sutherland & Freckleton 1999; Dennis 2002), have recently

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begun to receive attention for their potential to create the lag between introduction and spread characterizing many, if not most, biological invasions (e.g. Cappuccino 2004; Davis *et al.* 2004; Barney 2006; Elam *et al.* 2007). Allee effects encompass a range of mechanisms including mate finding, pollen limitation and inbreeding that can slow the spread of invasive species (e.g. Cappuccino 2004; Berec, Angulo & Courchamp 2007; Elam *et al.* 2007; Courchamp, Luděk & Gascoigne 2008). Such mechanisms can also produce differences in the distribution of genetic variation within and among populations, which can be interpreted to understand what factors promote greater invasion of some environments than others (Tobin *et al.* 2007).

In many invasive plants, clonality and self-compatibility are thought to overcome many effects of small population size, suggesting Allee effects may be negligible in explaining lags in invasive spread. However, while persistence through clonal growth can alleviate strong Allee effects, weak Allee effects, which delay population growth but do not result in a lower limit to population size below which population growth becomes negative, can still have substantial effects on population dynamics.

Phragmites australis (Cav.) Trin. ex Steud. is one of the most widespread perennial grasses in the world. It grows on every continent except Antarctica and has been rapidly expanding into freshwater and brackish wetlands across North America (Marks, Lapin & Randall 1994). Although *P. australis* is native to this region, its recent rapid spread has been attributed to a non-native haplotype of *P. australis* that was likely introduced from Europe in the late 1700s or early 1800s (Saltonstall 2002, 2003a; b; Lelong *et al.* 2007). This non-native *P. australis* haplotype has dramatically altered the composition and functionality of many estuarine and freshwater wetland communities throughout the United States, particularly along the Atlantic coast and Chesapeake Bay regions (Saltonstall 2003b). Nearly all of the *P. australis* in the mid-Atlantic region now belong to the non-native haplotype M (Saltonstall 2002).

While many plants reproduce primarily sexually, *P. australis*, like most aquatic (Cronk & Fennessy 2001) and many invasive species (Barrett, Eckhert & Husband 1993), can reproduce both clonally and sexually. This species forms dense mats of rhizomes, each of which has multiple nodes from which new ramets can sprout, and from which rhizome pieces can break off and float in the water. These rhizome fragments can become lodged in wrack piles, rafts of floating vegetation fragments that wash up into wetlands, where they can sprout and start new patches (Minchinton 2002). Wind-dispersed seeds may also be secondarily dispersed in water and wrack (Minchinton 2002). *Phragmites australis* is wind-pollinated and partially self-compatible, meaning some viable seeds are formed from self-fertilized inflorescences but many fewer than are formed with outcrossed pollen (Ishii & Kadono 2002; Lambert & Casagrande 2007). Clonal spread produces multiple patches with identical multi-locus haplotypes, while spread by sexually produced seeds usually produces unique multilocus haplotypes among, and sometimes within, patches. These genetic signatures may also differ as invasions progress and may distinguish pathways of invasion and document progress of invasions in different environments (e.g. Tobin *et al.* 2007).

Assessing genetic distribution in environments with different invasion dynamics can tell us about the mechanisms promoting invasion and help to target management (e.g. Hastings, Hall & Taylor 2006; Tobin *et al.* 2007; Belzile *et al.* 2010). In the Chesapeake Bay, King *et al.* (2007) found that the invasive *P. australis* was more abundant in subestuaries with watersheds dominated by developed land, where relative nitrogen availability was also higher, compared to wetlands in subestuaries dominated by forests. Similarly, the abundance of the invasive *P. australis* in coastal wetlands of Rhode Island was also positively related to development and eutrophication (Bertness, Ewanchuk & Silliman 2002; Minchinton & Bertness 2003; Silliman & Bertness 2004). However, exactly how development and eutrophication contribute to spread of the invasive haplotype is unclear. Studies have found that eutrophication results in greater seedling growth of the invasive haplotype (Chambers, Meyerson & Saltonstall 1999; Saltonstall & Stevenson 2007), suggesting that greater seedling success in developed subestuaries could lead to more rapid accumulation of within-patch genetic variation, and thus to explosive spread, than would be accomplished under oligotrophic conditions.

To determine whether the development of watersheds surrounding subestuaries affected the invasion strategy of *P. australis*, we studied patterns of genetic variation in wetland patches of *P. australis* in nine subestuaries of the Chesapeake Bay. We measured microsatellite variation to assess the distribution of genetic variation within subestuaries and the proportion of spread that could be attributed to clonal vs. sexual recruitment. Specifically, we asked the following questions: (i) what does the pattern of genetic variation within and among patches imply about how the invasive haplotype is spreading and whether patches are established sexually by seed or asexually by rhizomes? (ii) does the distribution of genetic variation differ among subestuaries that have watersheds with differing degrees of development? (iii) what can differences in the distribution of genetic variation at each of these scales tell us about the processes that have promoted *P. australis* invasion in subestuaries in the Chesapeake Bay that have been developed to different degrees? We then used the answers to these questions to propose a model of *P. australis* spread.

Materials and methods

STUDY AREA

This study was conducted in brackish tidal wetlands of nine subestuaries of the Chesapeake Bay on the United States Atlantic coast. The subestuaries covered a range of degrees of development from developed (Back River, Curtis Creek, Elizabeth River), mixed-developed (South River, Severn River, Mill Creek), to forested (Parkers Creek, Battle Creek, Saint Mary's River) (Fig. 1). Development of the watersheds surrounding each of these subestuaries was calculated by King *et al.* (2005), except for Parkers Creek, which was not part of their study. We calculated the percentage of the watershed that was forested or developed for Parkers Creek using their methods.

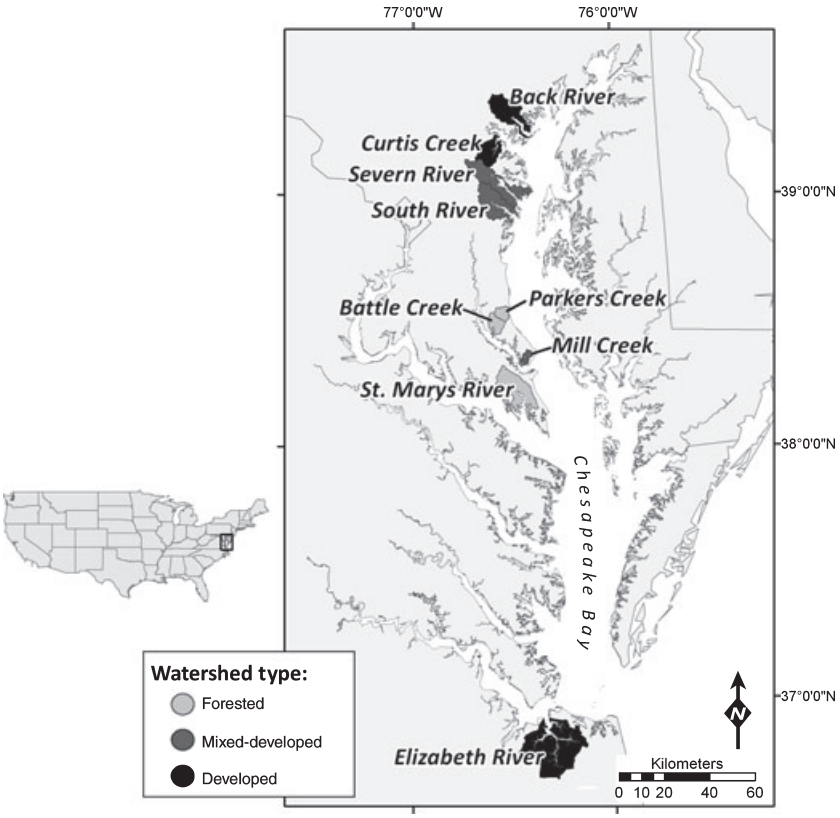

Fig. 1. Map of the nine subestuaries studied in the Chesapeake Bay, USA. The three most forested subestuaries are coloured light grey, three moderately developed subestuaries are coloured medium grey and the three most developed subestuaries are coloured black. Prevailing wind direction (not shown) is from east to west.

Comparisons between *P. australis* patches among the subestuaries were used to address the questions listed above.

SAMPLING STRATEGY

We divided each subestuary into five segments with approximately equal amounts of shoreline using aerial photographs. For each subestuary, leaf samples for DNA were collected in each segment from four distinct patches, two on each side of the river (20 patches per subestuary) with the exception of the Battle Creek subestuary where all seven patches were sampled. We defined the perimeter of each *P. australis* patch as being a robust stand of plants separated from other plants by a distance of at least 5 m. Patches we sampled were separated by a minimum of 60 m, as they were chosen to cover the entire segment. In each sampled patch we collected leaves from four plants approximately equally spaced around the perimeter of the patch. In a few cases, where the patch was very small (i.e. < 5 m diameter) only a single plant sample was collected. Leaves were kept in plastic storage bags at 4 °C until DNA was extracted.

ASSESSMENT OF GENETIC VARIATION

We extracted DNA from approximately 20 mg of fresh tissue using a BioSprint 96 (QIAGEN, Inc., Valencia, CA, USA) following the supplied protocol. We used primers developed by Saltonstall (2003a) to amplify via polymerase chain reaction (PCR) eight microsatellite DNA loci. Optimal annealing temperatures for each primer pair were determined during trials before analysing samples to ensure maximum yield of amplified fragments (Table 1). We performed PCR amplification using a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Waltham, MA, USA) programmed using the following conditions: an initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 50–58 °C for 30 s and 72 °C for 10 s, with a final polymerization step at 72 °C for 2 min. The PCR was run as 12.5-μL reactions with concentrations as follows: 1.25 μL template DNA (diluted 1:5–1:100 depending on fluorophor and primer pair, see Table 1), 3.2 μL distilled water, 0.75 μL of each primer (10 μM), 0.3 μL 25 mM $MgCl_2$, 6.25 μL RedMix Plus (Gene Choice, Inc., Frederick, MD, USA).

Table 1. Microsatellite primers and polymerase chain reaction conditions used in the current study. Primer names reference Saltonstall (2003a). The range of fragment sizes (in base pairs) and numbers of alleles obtained with each primer pair are given

Primer pair	Annealing temperature (°C)	Fluorophor	DNA dilution	Fragment sizes (bp)	Numbers of alleles
PaGT4	50	FAM	1:100	254–278	1–2
PaGT9	50	HEX	1:50	182–208	1–2
PaGT12	56	FAM	1:100	148–194	1–5
PaGT13	50	HEX	1:50	159–210	1–6
PaGT14	58	FAM	1:100	168–194	1–4
PaGT16	56	NED	1:10	185–290	1–3
PaGT21	58	HEX	1:50	173–195	1–4
PaGT22	50	NED	1:10	177–197	1–3

After amplification, PCR products with different fluorophores and different expected fragment sizes were combined before sequencer analysis as follows: primers *Pa*GT4, *Pa*GT9 and *Pa*GT16 were combined, primers *Pa*GT12, *Pa*GT13 and *Pa*GT22 were combined and primers *Pa*GT14 and *Pa*GT21 were combined. Amplicons were subjected to analysis on an ABI 3100 Automated Capillary DNA

Sequencer (Applied Biosystems, Inc.; Foster City, CA, USA) using a custom ROX500 size standard to determine fragment sizes. Fragment sizes were determined using GeneMapper v4.0 (Applied Biosystems, Inc.).

We aligned fragments for all samples using a TRFLP peak sorting function for Excel (Rees *et al.* 2004, http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls) and removed shadow peaks manually. The ranges of fragment sizes for each locus are presented in Table 1.

DATA ANALYSIS

Phragmites australis is an allopolyploid (Raicu *et al.* 1972) and displays disomic inheritance in some loci (i.e. alleles behave as diploid, segregating with the DNA from a particular parent during meiosis; Saltonstall 2003a). It is difficult to determine how many copies of each allele are present and with which copy of parental DNA each allele segregates. As a result we used genetic phenotypes instead of genotypes. With genetic phenotypes, two individuals with the same alleles but different numbers of copies of each allele would appear identical. This may underestimate the genetic diversity present but avoids overestimating genetic diversity as a result of incorrectly calculating the number of alleles present. Plant samples with distinct multilocus phenotypes were assumed to result from sexual reproduction. We considered all multilocus phenotypes repeated among patches to indicate establishment of new patches by rhizome fragments and repeated phenotypes within patches to be ramets of a single genet. *Phragmites australis* is known to have a range of ploidy levels and both 4× and 6× have been reported from the mid-Atlantic area, with 4× being the dominant type (Clevering & Lissner 1999). Because *P. australis* is an allopolyploid and populations may have different ploidy levels (Saltonstall 2003a), many calculations of genetic similarity were not appropriate (Obbard, Harris & Pannell 2006). Because plants with different ploidy levels may not be cross-compatible, mixed ploidy levels could also influence the amount of sexual vs. clonal reproduction. We inferred plant ploidy level based on the maximum number of distinct alleles present for a microsatellite locus (Table 1). All analyses used data coded as tetraploid. If fewer than four alleles were apparent, additional alleles were coded as missing as per Saltonstall (2003a). The few samples that had more than four alleles were given a separate code.

The similarity of genetic phenotypes was compared within patches relative to other patches in each subestuary and also in each subestuary relative to all subestuaries using two general approaches. First, we examined the distribution of genetic variation within subpopulations (here patches) compared to the total population (here each subestuary) and within vs. among subestuaries using Wright's F_{ST} (Wright 1951) and the analogous R_{ST} (Slatkin 1995) jack-knifed across all eight loci. F_{ST} and R_{ST} are widely used measures of population genetic subdivision and so facilitate comparisons among studies. Because repeated sampling of a single clonal individual can unduly influence estimates of the distribution of genetic variation, both sets of statistics were calculated with and without repeated samples removed (Halkett, Simon & Balloux 2005) using the method of Weir & Cockerham (1984) for *F*-statistics and using the method of Slatkin (1995) for *R*-statistics, both of which are appropriate for polyploid samples. *F*- and *R*-statistics differ in their model for mutation accumulation. *F*-statistics assume an infinite allele model and *R*-statistics assume a stepwise mutation model. The two represent opposing extremes, neither of which is likely to strictly match the mutation of microsatellite loci; rather, each is more appropriate in some conditions, so both are reported here as suggested by Balloux & Lugon-Moulin (2002). We also calculated G_{ST}, which is analogous to F_{ST} except specifically designed for haplotype DNA (Pons & Petit 1996), for each case but it differed little from F_{ST} so it is not reported here.

Although the methods used for calculating *F*- and *R*-statistics were appropriate for mixed ploidy levels, they were more appropriate for auto- as opposed to allopolyploids so we also calculated this distribution of genetic (phenotypic) diversity using mean Jaccard similarity (as per Lo, Stefanović & Dickinson 2009) and mean Bruvo diversity (Bruvo *et al.* 2004) between all pairs of sampled plants within and among patches in each subestuary. Bruvo diversity (*D*), which was specifically designed to address diversity using microsatellite loci, can accommodate mixed ploidy levels and uses a stepwise mutation model similar to that used in *R*-statistics, but scales less steeply with increasing numbers of mutations separating numbers of repeats. Jaccard similarity (*J*) uses an infinite allele model. We were explicitly interested in examining the likelihood of multiple genetic phenotypes occurring within patches, so we included repeated genetic phenotypes in these calculations. We used GENODIVE (Meirmans & van Tienderen 2004) to calculate both *J* and Bruvo's *D*. We then calculated the mean similarity and diversity within patches for each subestuary. For each diversity statistic (F_{ST}, R_{ST}, *D* and *J*), we calculated a regression of diversity or similarity on percent forest in the surrounding watershed using SYSTAT 11 for Windows (Systat Software Inc.; San Jose, CA, USA) to test for a significant relationship between development and diversity.

To understand the distance component of spread, we calculated genetic similarity as a function of distance using Moran's *I* statistic (SPAGeDi v1.2 g; Hardy & Vekemans 1999, 2002) at distances of 10, 50, 100, 500, 1000 and 50 000 m. Distance classes were chosen to incorporate a range of within and among-patch Euclidean distances while maintaining adequate numbers of sample pairs (> 100) within each distance class.

Results

All but one patch that we sampled appeared morphologically and genetically to belong to the non-native haplotype M (Saltonstall 2003a). All plants in one *P. australis* patch in Parkers Creek appeared morphologically native and the alleles present in plants from this patch did not appear in any other plants we sampled. These alleles were reported to be common among native types in the mid-Atlantic but very rare in non-native plants (Saltonstall 2003a). This one apparently native patch was removed from all genetic analyses and is currently the subject of additional study by our group. There was no morphological or genetic evidence of hybrids in nearby patches.

POPULATION GENETIC DIVERSITY

All pairs of plants with identical genetic phenotypes occurred within patches and likely resulted from clonal growth. Repeated genetic phenotypes were never found among patches, so establishment of all new patches was considered to be a result of sexual reproduction. Overall, 92% (81 of 88 patches with multiple samples) of patches sampled contained multiple genetic phenotypes. In 55% (48 of 88) of patches all four leaf samples had distinct genetic phenotypes. Subestuaries had similar numbers of alleles and genetic phenotypes,

regardless of development in the watershed (regression for alleles $P = 0.206$, $R^2 = 0.105$; for genetic phenotypes $P = 0.585$, $R^2 < 0.001$; Table 2) but the number of genotypes per patch decreased with the percentage of forest in the watershed surrounding the subestuary. This relationship was marginally significant with all watersheds included in the analysis ($P = 0.061$, $R^2 = 0.332$) but Battle Creek was a significant outlier (studentized residual −7.314). When Battle Creek, which had very low diversity, was removed from this analysis the significance of the relationship increased ($P = 0.013$, $R^2 = 0.617$).

F-statistics calculated among subestuaries (Table 3) indicated that there was significant genetic variation among subestuaries ($F_{ST} = 0.07 \pm 0.01$) but most variation was within subestuaries. However, when patches within subestuaries were considered relative to samples from each subestuary as a whole, the amount of genetic variation found within as opposed to among patches varied. Removing repeated genetic phenotypes produced only slight decreases in the proportion of genetic variation found among patches (data not shown). R_{ST} calculated across subestuaries was very similar to F_{ST} but with a higher standard error ($R_{ST} = 0.08 \pm 0.03$) and R_{ST} values calculated within each subestuary are not shown. F_{ST} of patches within each subestuary was not significantly related to amount of forest in the surrounding watershed ($P = 0.123$, $R^2 = 0.197$).

Within-patch Jaccard similarity and Bruvo diversity were both significantly related to the percent of forest in the watershed surrounding a subestuary (Jaccard, $R^2 = 0.430$, $P = 0.023$; Bruvo, $R^2 = 0.386$, $P = 0.044$; Fig. 2).

GENETIC SIMILARITY WITH DISTANCE

With all subestuaries combined, plants that were separated by distances less than 500 m were genetically more similar than those farther apart, indicated by positive Moran's *I* values (Fig. 3).

Discussion

IMPLICATIONS OF ALLEE EFFECTS

Despite the potential for clonal reproduction and partial self-compatibility, which are noted factors that promote invasion specifically because they allow establishment and persistence despite small population size, it appears that the lag in *P. australis* spread in the Chesapeake Bay may be largely explained by Allee effects. These Allee effects, acting on viable seed production through the effects of partial self-compatibility and limited mate availability, have generated different genetic signatures in subestuaries with different amounts of development. The patterns of genetic variation point to the effects of nutrients and disturbance on recruitment from seed as the initial mechanisms promoting *P. australis* spread, but the rate and extent of the invasion eventually become primarily driven by seed production and dispersal, especially in eutrophic subestuaries.

Clonal reproduction removes the impact of strong Allee effects (those effects that result in a population size threshold below which populations inevitably decline), but it does not alleviate weak Allee effects (those effects that affect rate of population spread as an effect of population size without a lower threshold), as has also been found in another invasive wetland grass, *Spartina alterniflora* (Davis *et al.* 2004; Taylor *et al.* 2004). *Spartina alterniflora* is also clonal, partially self-compatible, wind-pollinated and it experienced a similar lag with recent expansive spread. Both pollen limitation and availability of outcross pollen appeared to contribute to Allee effects in *S. alterniflora* and clonal reproduction was limited (Daehler & Strong 1994; Stiller & Denton 1995; Daehler 1998).

If *P. australis* reproduction were dominated by clonal processes, then Allee effects could not explain the observed lag in spread. We found that the patches of the invasive haplotype that we sampled in nine subestuaries of the Chesapeake Bay were most likely all established by sexual reproduction.

Table 2. Number of alleles (A) and genetic phenotypes (G) for each microsatellite locus (*Pa*GT4-*Pa*GT22) in each subestuary and with all subestuaries combined (All). Microsatellite names (*Pa*GT4 through *Pa*GT22) follow Saltonstall (2003a). The mean and standard error (SE) across all loci are also given for the number of alleles and number of genetic phenotypes in each subestuary

	*Pa*G-T4		*Pa*G-T9		*Pa*G-T12		*Pa*G-T13		*Pa*G-T14		*Pa*G-T16		*Pa*G-T21		*Pa*G-T22		Mean	SE	Mean	SE
Subestuary	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	A	G	G
PC	2	3	6	8	6	10	10	21	6	7	5	5	7	14	5	7	5.87	0.79	9.38	2.03
BC	3	3	4	4	4	4	3	3	3	3	4	4	5	5	4	6	3.75	0.25	4.00	0.38
MC	2	3	5	7	9	16	8	18	7	10	3	4	6	10	5	7	5.63	0.84	9.38	1.89
SMR	3	4	5	6	10	14	9	29	5	9	3	4	6	11	4	7	5.63	0.93	10.50	2.91
SOR	6	6	6	8	5	5	4	6	6	7	3	7	8	17	4	7	5.25	0.56	7.88	1.34
SVR	5	6	6	9	5	8	4	6	6	14	4	7	8	14	6	7	5.50	0.46	8.88	1.17
CC	2	3	8	9	11	13	12	25	5	12	5	7	6	10	4	9	6.63	1.22	11.00	2.28
BR	2	2	4	5	4	6	4	7	7	15	3	5	7	15	4	9	4.38	0.63	8.00	1.68
ER	2	3	5	12	8	20	10	21	4	7	4	8	7	16	7	12	5.88	0.92	12.38	2.24
All	6	7	9	22	12	49	12	76	9	27	7	19	9	42	8	18	9	0.756	32.50	7.84

Subestuary abbreviations are as follows: BC: Battle Creek; PC: Parkers Creek; SMR: Saint Mary's River; MC: Mill Creek; SOR: South River; SVR: Severn River; BR: Back River; CC: Curtis Creek; ER: Elizabeth River.

Table 3. F_{ST}, a measure of how much genetic variation is found among patches compared to the whole subestuary, Jaccard's index of similarity (J) and Bruvo's index of diversity (D) calculated for each subestuary and for all nine combined with all samples included. Statistics were calculated using all samples, including ramets, within patches using the technique of Weir & Cockerham (1984) and jack-knifed across all eight microsatellite loci

Subest.	%Forest	%Dev	F_{ST}	Jaccard (J)	Bruvo (D)
PC	80.13	6.28	0.352 ± 0.090	0.511 ± 0.076	0.038 ± 0.015
BC	75.64	4.41	0.457 ± 0.043	0.753 ± 0.068	0.002 ± 0.002
MC	72.20	11.32	0.328 ± 0.067	0.638 ± 0.085	0.029 ± 0.009
SMR	66.15	6.81	0.370 ± 0.091	0.593 ± 0.061	0.027 ± 0.009
SOR	60.48	14.95	0.244 ± 0.075	0.446 ± 0.056	0.062 ± 0.013
SVR	53.62	25.44	0.403 ± 0.085	0.539 ± 0.069	0.065 ± 0.016
CC	21.83	56.21	0.296 ± 0.078	0.351 ± 0.043	0.065 ± 0.012
BR	18.17	66.53	0.307 ± 0.036	0.456 ± 0.066	0.065 ± 0.016
ER	19.64	56.85	0.263 ± 0.039	0.426 ± 0.076	0.060 ± 0.012
All 9			0.065 ± 0.013	0.503 ± 0.024	0.048 ± 0.005

Subestuary (Subest.) abbreviations are as follows BC: Battle Creek; PC: Parkers Creek; SMR: Saint Mary's River; MC: Mill Creek; SOR: South River; SVR: Severn River; BR: Back River; CC: Curtis Creek; ER: Elizabeth River. %Forest and %Dev refer to the percent of the surrounding watershed that is forested or developed, respectively. Within each subestuary and for the combined means of J and D each patch is treated as a subpopulation, while, for all nine combined, each subestuary is a subpopulation.

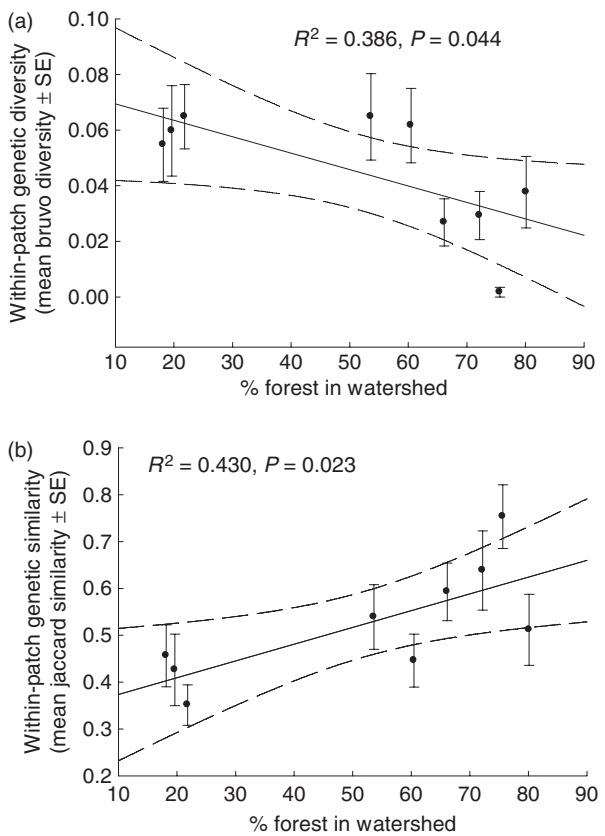

Fig. 2. The relationship between within-patch genetic diversity and percent forest in the watershed surrounding nine subestuaries studied in the Chesapeake Bay, USA. (a) Bruvo diversity (D) and (b) Jaccard similarity (J).

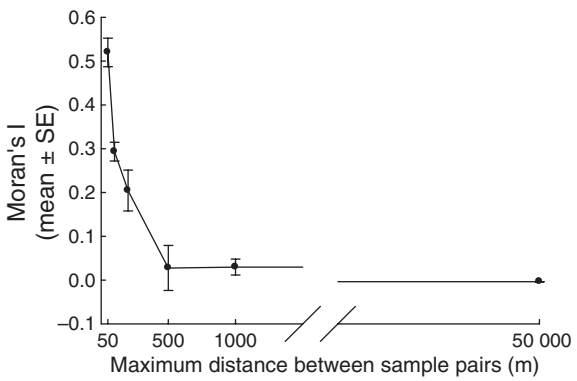

Fig. 3. Genetic similarity (Moran's I) jack-knifed across all eight microsatellite loci as a function of distance between pairs of *Phragmites australis* samples across all sampled subestuaries in the Chesapeake Bay. An axis break between 1500–49 500 m is included to make it easier to see changes in genetic similarity at small distances.

Multilocus genetic phenotypes were not repeated among patches and expansion of individual patches also apparently involved substantial recruitment from seed, as 92% of patches were composed of multiple genetic phenotypes. The preponderance of poly-clonal patches and the lack of repeated genetic phenotypes among patches suggest that the production of viable seeds and subsequent seedling recruitment have been responsible for much of the recent spread of the invasive haplotype. Further, they suggest that recent spread may have resulted from the elimination of the Allee effects that may have limited the rate of spread of the non-native haplotype in the past. These results support findings by Belzile *et al.* (2010), Guo *et al.* (2003), Alvorez, Tron & Mauchamp (2005) and Lambertini *et al.* (2008) but are in contrast to other studies in Europe and North America that found that *P. australis* population dynamics were largely driven by rhizome growth (e.g. Haslam 1972; Gervais *et al.* 1993; Pellegrin & Hauber 1999; Keller 2000; Hudon & Gagnon 2005).

This study provides strong support for spread of the non-native invasive haplotype of *P. australis* by sexually produced seeds. Genetic signatures within and among differently developed subestuaries may be interpreted to explain the greater

spread of the invasive haplotype with increasing development, the substantial time lag between the introduction of the invasive haplotype in the mid-Atlantic region and its recent explosive spread. These data suggest that sexual reproduction is initially very low when only self pollen is locally available, but is followed by a dramatic increase in seed production when one or more genetically distinct seedlings are able to establish in or next to an existing patch or when adjacent genetically distinct patches grow together, making outcross pollen locally available.

DIFFERENCES IN ALLEE EFFECTS AMONG ENVIRONMENTS

If the processes bringing genetic diversity to subestuaries differed, then subestuaries should have different amounts of genetic variation. Subestuaries with different amounts of development in surrounding watersheds had similar numbers of alleles and genetic phenotypes indicating that they received genetic material from other subestuaries at similar rates. The exception was the Battle Creek subestuary, which had the lowest amount of development (4.41%) and no major historical disturbances within or adjacent to the subestuary. The few *P. australis* patches that were growing in Battle Creek were largely monoclonal and were genetically very similar. The other subestuaries had evidence of large-scale development activities such as a large transmission line that was constructed across a part of the Parkers Creek subestuary and bridges that were constructed across parts of other subestuaries (e.g. Mill Creek). The presence of invasive patches immediately adjacent to every large-scale development suggests that they provided historical colonization opportunities.

Similar genetic diversity at the subestuary level suggests that differences in *P. australis* spread with development are attributable to processes within, rather than among, subestuaries. Patches within subestuaries were more likely be to polyclonal and have more genets as subestuary development increased. Genetic diversity (D) and the number of genets within patches increased, while genetic similarity (J) decreased as development in the surrounding watershed increased. The distribution of genetic variation in differently developed subestuaries is especially important when combined with the high values of Moran's I for 10, 50 and 100 m distance classes (i.e. within-patches). These high Moran's I values at distances within patches suggested that most gene flow was substantially local and within patches. In contrast, the genetic similarity among subestuaries, indicated by low Moran's I at >5000 m and low F_{ST} among subestuaries, suggested that there has been a low level of ongoing gene flow among patches, which was not greatly increased within, compared to among, subestuaries. Thus, the low level of gene flow among subestuaries and among patches within subestuaries, while sufficient to maintain genetic diversity, is not sufficient to maintain high levels of seed production and seedling recruitment. This implies that most mates come from within patches and the limited availability of non-inbred mates within patches produces the observed Allee effect and that limitation would need to be overcome with the establishment of each new patch.

A link between genetic variation within patches and invasive spread is supported by studies demonstrating that diverse patches of the invasive haplotype produced more viable seeds than monoclonal patches and that availability of outcross pollen, rather than pollen limitation or nutrient levels, was the major factor limiting production of viable seeds (Kettenring & Whigham 2009; Kettenring *et al.* 2010). This suggests that if development affects patch genetic diversity, it may also impact the Allee threshold and the rate of invasive haplotype spread in subestuaries.

Our findings result in a new interpretation of how patches of multiple genetic phenotypes form and subsequently lead to further invasion of the non-native haplotype M through seed production and dispersal (Fig. 4). A combination of seedling establishment and subsequent clonal growth results in patches with multiple genetic phenotypes. In the early stages of colonization most small individual patches probably consist of a single genetic phenotype and few viable seeds are produced because pollen exchange is mostly between inflorescences on ramets that are genetically identical. Once cross-pollination begins, more and more viable seeds are produced and dispersed within and outside the patches of origin. The establishment and expansion of new patches occurs most rapidly in subestuaries where there are high levels of human activities that are associated with increased establishment of seedlings (i.e. disturbances; Silliman & Bertness 2004) and clonal (Hudon & Gagnon 2005) and seedling (Chambers, Meyerson & Saltonstall 1999; Saltonstall & Stevens 2007) growth. Eventually the spread of the non-native haplotype depends less on disturbance for initial establishment of seedlings and more on the production and dispersal of large numbers of seeds, resulting in a larger number of seeds being dispersed to safe sites that lead to successful seedling establishment.

The role of within-patch genetic variation in determining the duration of the lag in invasive spread, combined with an apparently long tail of dispersal among subestuaries, suggests that patches within all invaded subestuaries might eventually accumulate enough within-patch genetic variation to overcome their Allee threshold and spread explosively, even in those subestuaries that are not yet nutrient enriched and have experienced little direct human-induced disturbance. Indeed, in Europe, where haplotype M is thought to have originated (Saltonstall 2002), Lambertini *et al.* (2008) compared genetic variation between *P. australis* in differently developed subestuaries and found that all had similar, high amounts of genetic variation.

ALTERNATIVE EXPLANATIONS FOR THE GENETIC PATTERNS

While the Allee threshold scenario we propose helps to explain why the invasive haplotype is more abundant in wetlands with increasing development of the adjacent watershed, we also considered four possible alternative explanations for this pattern. First, developed subestuaries might have been colonized

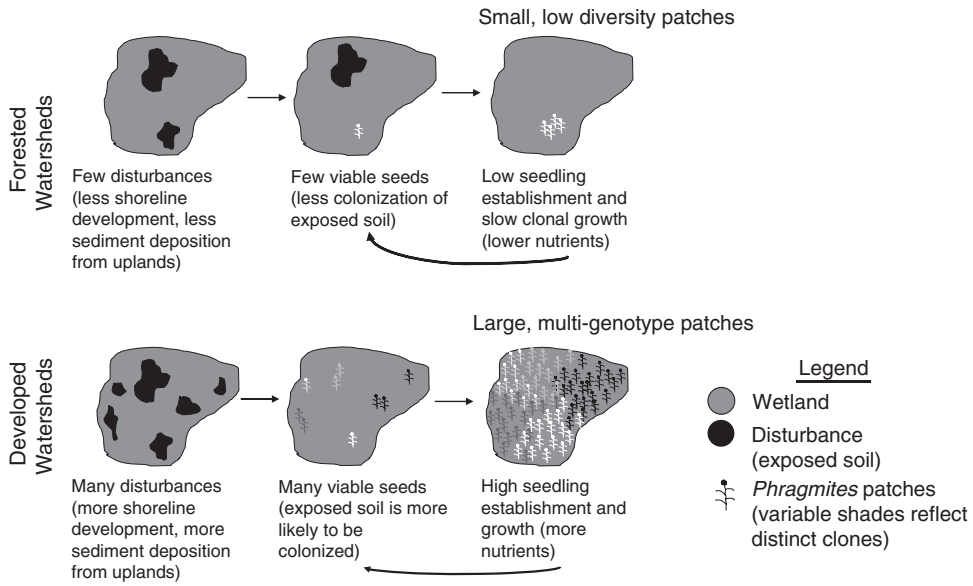

Fig. 4. A feedback model for *Phragmites australis* spread. Intertidal brackish wetlands (grey areas) in forested subestuaries have low levels of disturbance. This produces few, low-diversity *P. australis* patches, which produce few viable seeds to colonize new disturbed areas. When seeds do arrive in disturbed areas, establishment rates and seedling growth are low in low-nutrient environments. In contrast, developed subestuaries have relatively high levels of disturbance and seeds that reach disturbed areas have higher establishment and high growth rates allow patches to spread quickly by both clonal propagation and further seedling establishment in the high nutrient waters, producing multi-genotype patches when independent patches grow together. These patches then produce substantial viable seeds to colonize new disturbances, forming more genetically diverse patches.

earlier than forested subestuaries and have simply had more time to accumulate genetic variation. Comparison of present-day *P. australis* cover with that mapped in the early 1970s (McCormick & Somes 1982) showed that Curtis Creek (a developed subestuary) had only five patches of the invasive haplotype while the South River (a mixed-developed watershed) had 11 patches and Parker's Creek and Battle Creek (both largely forested subestuaries) had also been colonized by 1971–1972. Thus, there does not seem to be any evidence that there were historical differences in the timing of invasion by the non-native haplotype.

Second, some of the differences in spread among subestuaries could have resulted from differences in ploidy level, which have been reported from haplotype M plants (Saltonstall 2003b). Hexaploid plants are reported to produce very few fertile seeds (Clevering & Lissner 1999) and crosses between plants of mixed ploidy may also yield few viable seeds. Logically, this suggests hexaploid populations would spread primarily clonally (e.g. Pellegrin & Hauber 1999) and be less diverse, while tetraploid plants would produce more viable seeds and more spread would be attributable to sexual reproduction. Although flow cytometry data were not available, the maximum number of alleles identified was four or fewer in nearly all plants sampled. In our study, a few plants in three subestuaries had five or six alleles at a locus but the distribution of genetic variation in these subestuaries was not substantially different from other subestuaries with similar forest cover in the surrounding watersheds. These results suggest that a few hexaploid genets when most plants were likely tetraploid did not substantially affect population dynamics.

Third, mixtures of native and non-native haplotypes could also affect genetic diversity if the two types spread differently (e.g. if natives were hexaploid and invasives were tetraploid as suggested, but not definitively demonstrated, in Saltonstall *et al.* 2007). If the different haplotypes had distinct alleles (Saltonstall 2003a; Meyerson, Viola & Brown 2010), then the presence of both types would increase diversity in the subestuary, which we did not see and could also affect how diversity was distributed within vs. among patches if the two types did not interbreed. We only encountered one potentially native patch, which we excluded from all analyses (see 'Results').

Fourth, increased development might promote somatic mutation, resulting in increasing diversity with development. However, it is unlikely that the multiple clones we detected within patches could have arisen from somatic mutations (e.g. Keller 2000) because even closely related genets differed at several loci. In nearly all polyclonal patches, one or more of the genets differed from all others in the patch by as much as a genet sampled at random from the subestuary, as McCormick *et al.* (2009) found in a single subestuary in the Chesapeake Bay.

Conclusion

These findings have substantial implications for prioritizing which subestuaries will be most successfully targeted for management and may allow Davis *et al.*'s (2004) models for *S. alterniflora* to be applied to management of the invasive haplotype of *P. australis* and perhaps even more widely. Tobin *et al.* (2007) found that geographic variation in Allee thresh-

olds, which they attributed to differences in habitat quality, could explain differences in rates of spread in gypsy moth outbreaks in the eastern United States. This suggests that variation in Allee thresholds as a function of environmental differences, in the present case, development, could be broadly important for predicting and managing spread of invasive species.

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References

Allee, W.C. (1931) *Animal Aggregations, a Study in General Sociology*. University of Chicago Press, Chicago, IL.

Alvarez, M.G., Tron, F. & Mauchamp, A. (2005) Sexual versus asexual colonization by *Phragmites australis*: 25-year reed dynamics in a Mediterranean marsh, southern France. *Wetlands*, **25**, 639–647.

Balloux, F. & Lugon-Moulin, N. (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155–165.

Barney, J.N. (2006) North American history of two invasive plant species: phytogeographic distribution, dispersal vectors, and multiple introductions. *Biological Invasions*, **8**, 703–717.

Barrett, S.C.H., Eckhert, C.G. & Husband, B.C. (1993) Evolutionary processes in aquatic plant populations. *Aquatic Botany*, **44**, 105–145.

Belzile, F., Labbé, J., LeBlanc, M.-C. & Lavoie, C. (2010) Seeds contribute strongly to the spread of the invasive genotype of the common reed (*Phragmites australis*). *Biological Invasions*, **12**, 2243–2250.

Berec, L., Angulo, E. & Courchamp, F. (2007) Multiple Allee effects and population management. *Trends in Ecology and Evolution*, **22**, 185–191.

Bertness, M.D., Ewanchuk, P. & Silliman, B.R. (2002) Anthropogenic modification of New England salt marsh landscapes. *Proceedings of the National Academy of Sciences of the USA*, **99**, 1395–1398.

Bruvo, R., Michiels, N.K., D'Souza, T.G. & Schulenburg, H. (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology*, **13**, 2101–2106.

Cappuccino, N. (2004) Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). *Oikos*, **106**, 3–8.

Chambers, R.M., Meyerson, L.A. & Saltonstall, K. (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany*, **64**, 261–273.

Clevering, O.A. & Lissner, J. (1999) Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. *Aquatic Botany*, **64**, 185–208.

Courchamp, F., Luděk, B. & Gascoigne, J. (2008) *Allee Effects in Ecology and Conservation*. Oxford University Press, New York.

Cronk, J.K. & Fennessy, M.S. (2001) *Wetland Plants – Biology and Ecology*. Lewis Publishers, Boca Raton.

Crooks, J.A. (2005) Lag times and exotic species: the ecology and management of biological invasions in slow motion. *Ecoscience*, **12**, 316–329.

Crooks, J.A. & Soulé, M.E. (1999) Lag times in population explosions of invasive species: Causes and implications. *Invasive Species and Biodiversity Management* (eds O.T. Sandlund, P.J. Schei & A. Viken), pp. 103–125. Kluwer Academic Press, Dordrecht.

Daehler, C. (1998) Variation in self-fertility and the reproductive advantage of self-fertility for an invading plant (*Spartina alterniflora*). *Evolutionary Ecology*, **12**, 553–568.

Daehler, C.C. & Strong, D.R. (1994) Variable reproductive output among clones of *Spartina alterniflora* (Poaceae) invading San Francisco Bay, California: the influence of herbivory, pollination, and establishment site. *American Journal of Botany*, **81**, 307–313.

Davis, H.G., Taylor, C.M., Civille, J.C. & Strong, D.R. (2004) An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. *Journal of Ecology*, **92**, 321–327.

Dennis, B. (2002) Allee effects in stochastic populations. *Oikos*, **96**, 389–401.

Elam, D.R., Ridley, C.E., Goodell, K. & Ellstrand, N.C. (2007) Population size and relatedness affect fitness of a self-incompatible invasive plant. *Proceedings of the National Academy of Sciences of the USA*, **104**, 549–552.

Gervais, C., Trahan, R., Moreno, D. & Drolet, A.-M. (1993) Le *Phragmites australis* au Québec: distribution, géographique, nombres chromosomiques et reproduction. *Canadian Journal of Botany*, **71**, 1386–1393.

Guo, W., Wang, R., Zhou, S., Zhang, S. & Zhang, Z. (2003) Genetic diversity and clonal structure of *Phragmites australis* in the Yellow River delta of China. *Biochemical Systematics and Ecology*, **31**, 1093–1109.

Halkett, F., Simon, J.-C. & Balloux, F. (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology and Evolution*, **20**, 194–201.

Hamrick, J.L. & Godt, M.J.W. (1990) Allozyme diversity in plant species. *Plant Population Genetics, Breeding and Genetic Resources* (eds A.H.D. Brown, M.T. Clegg, A.L. Kahler & B.S. Weir), pp. 43–63. Sinauer Associates, Sunderland, MA.

Hardy, O.J. & Vekemans, X. (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, **83**, 145–154.

Hardy, O.J. & Vekemans, X. (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Haslam, S.M. (1972) Biological flora of the British Isles. *Journal of Ecology*, **60**, 585–610.

Hastings, A., Hall, R.J. & Taylor, C.M. (2006) A simple approach to optimal control of invasive species. *Theoretical Population Biology*, **70**, 431–435.

Hudon, C.P. & Gagnon, M.J. (2005) Hydrological factors controlling the spread of common reed (*Phragmites australis*) in the St. Lawrence River (Québec, Canada). *Ecoscience*, **12**, 347–357.

Ishii, J. & Kadono, Y. (2002) Factors influencing seed production of *Phragmites australis*. *Aquatic Botany*, **72**, 129–141.

Keller, B.E.M. (2000) Genetic variation among and within populations of *Phragmites australis* in the Charles River watershed. *Aquatic Botany*, **66**, 195–208.

Kettenring, K.M. & Whigham, D.F. (2009) Seed viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. *Aquatic Botany*, **91**, 199–204.

Kettenring, K.M., McCormick, M.K., Baron, H.M. & Whigham, D.F. (2010) *Phragmites australis* (common reed) invasion in the Rhode River subestuary of the Chesapeake Bay: disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. *Estuaries and Coasts*, **33**, 118–126.

King, R.S., Hines, A.H., Craige, F.D. & Grap, S. (2005) Regional, watershed and local correlates of blue crab and bivalve abundances in subestuaries of Chesapeake Bay, USA. *Journal of Experimental Marine Biology and Ecology*, **319**, 101–116.

King, R.S., Deluca, W.V., Whigham, D.F. & Marra, P.P. (2007) Threshold effects of coastal urbanization on *Phragmites australis* (Common Reed) abundance and foliar nitrogen in Chesapeake Bay. *Estuaries and Coasts*, **30**, 1–13.

Lambert, A.M. & Casagrande, R.A. (2007) Characteristics of a successful estuarine invader: evidence of self-compatibility in native and non-native lineages of *Phragmites australis*. *Marine Ecology Progress Series*, **337**, 299–301.

Lambertini, C., Gustafsson, M.H.G., Frydenberg, J., Speranza, M. & Brix, H. (2008) Genetic diversity patterns in *Phragmites australis* at the population, regional, and continental scales. *Aquatic Botany*, **88**, 160–170.

Le Roux, J. & Wieczorek, A.M. (2009) Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Annals of Applied Biology*, **154**, 1–17.

Lelong, B., Lavoie, C., Jodoin, Y. & Belzile, F. (2007) Expansion pathways of the exotic common reed (*Phragmites australis*): a historical and genetic analysis. *Diversity and Distributions*, **13**, 430–437.

Lo, E.Y.Y., Stefanović, S. & Dickinson, T.A. (2009) Population genetic structure of diploid sexual and polyploidy apomictic hawthorns (*Crataegus*; Rosaceae) in the Pacific Northwest. *Molecular Ecology*, **18**, 1145–1160.

Marks, M., Lapin, V. & Randall, J. (1994) *Phragmites australis (P. communis)*: threats, management, and monitoring. *Natural Areas Journal*, **14**, 285–294.

McCormick, J. & Somes Jr, H.A. (1982) *The Coastal Wetlands of Maryland*. Maryland Department of Natural Resources, Coastal Zone Management, Jack McCormick and Associates, Inc., Chevy Chase, MD.

McCormick, M.K., Kettenring, K.M., Baron, H.M. & Whigham, D.F. (2009) Extent and reproductive mechanisms of *Phragmites australis* spread in brackish wetlands in Chesapeake Bay, Maryland (USA). *Wetlands*, **30**, 67–74.

Meirmans, P.G. & van Tienderen, P.H. (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, **4**, 792–794.

Meyerson, L.A., Viola, D.V. & Brown, R.N. (2010) Hybridization of invasive *Phragmites australis* with a native subspecies in North America. *Biological Invasions*, **12**, 103–111.

Minchinton, T.E. (2002) Disturbance by wrack facilitates spread of *Phragmites australis* in a coastal marsh. *Journal of Experimental Marine Biology and Ecology*, **281**, 89–107.

Minchinton, T.E. & Bertness, M.D. (2003) Disturbance–mediated competition and the spread of *Phragmites australis* in a coastal marsh. *Ecological Applications*, **13**, 1400–1416.

Obbard, D.J., Harris, S.A. & Pannell, J.R. (2006) Simple allelic-phenotype diversity and differentiation statistics for allopolyploids. *Heredity*, **97**, 296–303.

Ouborg, N.J., Piquot, Y. & van Groenendael, J.M. (1999) Population genetics, molecular markers, and the study of dispersal in plants. *Journal of Ecology*, **8**, 1667–1681.

Pellegrin, D. & Hauber, D.P. (1999) Isozyme variation among populations of the clonal species, *Phragmites australis* (Cav.) Trin. Ex Steudel. *Aquatic Botany*, **63**, 241–259.

Pons, O. & Petit, R.J. (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144**, 1237–1245.

Raicu, P., Staicu, S., Stoian, V. & Roman, T. (1972) The *Phragmites communis* Trin. chromosome complement in the Danube delta. *Hydrobiologia*, **39**, 249–252.

Saltonstall, K. (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the USA*, **99**, 2445–2449.

Saltonstall, K. (2003a) Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology*, **12**, 1689–1702.

Saltonstall, K. (2003b) Genetic variation among North American populations of *Phragmites australis*: implications for management. *Estuaries*, **26**, 444–451.

Saltonstall, K. & Stevenson, J.C. (2007) The effect of nutrients on seedling growth of native and introduced *Phragmites australis*. *Aquatic Botany*, **86**, 331–336.

Saltonstall, K., Glennon, K., Burnett, A., Hunter, R.B. & Hunter, K. (2007) Comparison of morphological variation indicative of ploidy level in *Phragmites australis* (Poaceae) from eastern North America. *Rhodora*, **109**, 415–429.

Silliman, B.R. & Bertness, M.D. (2004) Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology*, **18**, 1424–1434.

Slatkin, M. (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Stephens, P.A., Sutherland, W.J. & Freckleton, R.P. (1999) What is the Allee effect? *Oikos*, **87**, 185–190.

Stiller, J.W. & Denton, A.L. (1995) One hundred years of *Spartina alterniflora* (Poaceae) in Willapa Bay, Washington: random amplified polymorphic DNA analysis of an invasive population. *Molecular Ecology*, **4**, 355–363.

Taylor, C.M., Davis, H.G., Civille, J.C., Grevstad, F.S. & Hastings, A. (2004) Consequences of an Allee effect in the invasion of a Pacific estuary by *Spartina alterniflora*. *Ecology*, **85**, 3254–3266.

Tobin, P.C., Whitmire, S.L., Johnson, D.M., Bjørnstad, O.N. & Leibhold, A.M. (2007) Invasion speed is affected by geographical variation in the strength of Allee effects. *Ecology Letters*, **10**, 36–43.

Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright, S. (1978) *Evolution and the Genetics of Populations. VI. Variability Within and Among Natural Populations*. University of Chicago Press, Chicago, IL.

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