Strategies for a successful plant invasion: the reproduction of *Phragmites australis* in north-eastern North America

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Summary

1. Knowing the relative contribution of vegetative propagation and sexual reproduction to the dispersal and establishment of exotic plants is crucial for devising efficient control strategies. This is particularly true for the common reed (*Phragmites australis*), one of the most invasive species in North America.

2. For the first time, we combined *in situ* field observations and genetic evidence, based on two genotyping techniques, that is microsatellite markers (SSR) and genotyping by sequencing (GBS), to determine the propagation strategies of this invader at its northern distribution limit in North America and especially in roadside ditches.

3. Field observations revealed that, in a region where the common reed is already abundant, both seeds and plant fragments contributed to the establishment of new populations. Newly established individuals originated mostly (84%) from seeds rather than fragments, but a larger proportion of individuals originating from fragments survived the second year compared to seedlings.

4. High genetic diversity among marsh and roadside common reed stands indicated the prime role of sexual reproduction for dispersal. The vast majority of genotypes were found in only one stand; such high genetic variability can only be explained by sexual reproduction. Half the surveyed stands comprised a single clone, suggesting that local expansion mainly occurred vegetatively. As the small proportion of SSR genotypes initially thought to be common between distant stands proved to be distinct (as revealed by GBS data), it is likely that all the stands examined were initially founded by genetically distinct individuals.

5. *Synthesis.* Our study suggests that long-distance dispersal by seeds is important for the common reed, in marshes and roadsides, while both seeds and plant fragments contribute to short-distance dispersal along roads, at least in regions where the species is already abundant. The success of this invader in North America seems to be attributable to a reproduction strategy combining the advantages of sexuality with those of vegetative propagation. Moreover, this study shows that the GBS approach strongly reduces uncertainties associated with the use of a limited number of markers. This approach is especially valuable for ecologists dealing with an ever-increasing number of invaders, of which few have identified microsatellite markers.

Key-words: biological invasion, common reed, dispersal, freshwater wetlands, genotyping by sequencing, invasion ecology, road ecology, sexual reproduction, vegetative propagation

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Introduction

Invasive plants can use different reproductive strategies to colonize new areas. Ramets from vegetative propagation have higher growth and survival rates than seedlings from sexual reproduction, but their dispersal is generally restricted to short distances (Holsinger 2000; Barrett, Colautti & Eckert 2008). Seeds may be produced in great numbers and are usually more effective for rapidly initiating new populations, especially far from the parental plants (Barrett 2011a). However, invasive plants using mainly sexual reproduction for establishing new populations are subject to several constraints, such as the absence of pollination or dispersal vectors, adverse climatic conditions preventing seed maturation, or a scarcity of suitable sites for seed germination; all these constraints can slow the spread of the plants (Barrett 2011b). Consequently, exotic species using both strategies (vegetative propagation and sexual reproduction) may have a higher invasion success than others (Winkler & Fischer 2002). Knowing the relative contribution of each reproduction mode to the propagation of an invasive species is crucial for devising efficient control strategies. Control methods based on a misunderstanding of dispersal mechanisms risk failure because such efforts do not target plant parts from which new populations arise.

The common reed *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) is an invasive plant for which understanding dispersal mechanisms is important for a better control. The haplotype M of this species was accidentally introduced in North America from Eurasia at one or more coastal ports along the Atlantic coast, potentially as early as the first half of 19th century (Saltonstall 2002). This haplotype M has become very abundant in coastal tidal marshes (Rice, Rooth & Stevenson 2000; Warren *et al.* 2001; Philipp & Field 2005; Chambers *et al.* 2008) and, more recently, in freshwater wetlands of the Great Lakes – St. Lawrence River system (Wilcox *et al.* 2003; Hudon, Gagnon & Jean 2005; Trebitz & Taylor 2007; Tulbure, Johnston & Auger 2007). The plant has used road drainage ditches for spreading inland (Jodoin *et al.* 2008; Lelong, Lavoie & Thériault 2009; Brisson, de Blois & Lavoie 2010). The haplotype M represents 95% of all common reed populations of the province of Québec (Canada) and is consequently much more common than the native haplotypes (Lelong *et al.* 2007).

Since the common reed is abundant in roadside ditches, and these ditches are frequently disturbed through maintenance, it was previously hypothesized that stem or rhizome fragments, accidentally carried over short or long distances by mechanical diggers, were responsible for the spread of the plant (Bart & Hartman 2003; Mal & Narine 2004; Catling & Mitrow 2011). Moreover, at the northern distribution limit of the species, low seed viability rates resulting from short growing seasons were assumed to preclude seedling establishment (Gervais *et al.* 1993; Maheu-Giroux & de Blois 2007). However, the recent rapid spread of the common reed over large areas (Lelong *et al.* 2007), and the

development of vast populations over very short periods (Hudon, Gagnon & Jean 2005; Maheu-Giroux & de Blois 2007; LeBlanc, de Blois & Lavoie 2010), challenged the assumption that plant fragments were the only effective diaspore. Common reed seedlings found in freshly dug roadside ditches in Québec indicated that sexual reproduction also contributed, at least locally, to the spread of the plant (Brisson, Paradis & Bellavance 2008). Moreover, high levels of genetic diversity in common reed populations from eastern Canada (Belzile *et al.* 2010; Kirk *et al.* 2011) and the eastern coast of the United States (McCormick *et al.* 2010a, b) strongly supported the role of seeds in the establishment of new populations.

Direct evidence of successful sexual reproduction for the invasive common reed, such as seedlings, is nevertheless scarce, and the relative contribution of sexual reproduction and vegetative propagation to the short and long-distance dispersal of the plant remains unknown. It is likely that the common reed uses seeds for establishing populations over large distances and vegetative structures (rhizomes, stolons) for locally (within a few metres) expanding populations, but this hypothesis remained to be substantiated with large-scale field data. Here, we combine for the first time *in situ* field observations and genetic evidence to show that the common reed uses this double strategy to successfully invade new sites and establish populations at its north-eastern distribution limit in North America.

Materials and methods

Two approaches were used to assess the relative importance of vegetative and sexual reproduction for the common reed. First, we used field observations to determine the relative frequency and survival rate of new individuals (physically distinct, physiologically independent units) originating from seeds or plant fragments. Secondly, we examined small- and large-scale spatial patterns of genetic diversity and structure within and between common reed stands. Vegetative reproduction should result in low genetic diversity, and we expected clonal genotypes to be found only in close proximity within stands or in nearby stands. Sexual reproduction should result in high genetic diversity within and between stands, and natural limits to the dispersal and establishment of seeds should lead to greater genetic similarity in spatially proximate stands.

STUDY AREA

The study area is located in the St. Lawrence River valley, southern Québec, eastern Canada (Fig. 1). These fertile lowlands are mostly occupied by large agricultural fields (corn, soya bean) and crossed by roads and several rivers. The two main urban centres are Montréal and Québec, which are linked by a four-lane highway (H20) on the south shore of the St. Lawrence River. The climate is humid continental, with short, warm and humid summers and long, cold and dry winters. Average mean temperatures in July and January range from 19 (Québec) to 21 °C (Montréal), and from −10 (Montréal) to −13 °C (Québec), respectively. Mean annual precipitation totals 1000 (Montréal) and 1190 mm (Québec; Environment Canada 2014).
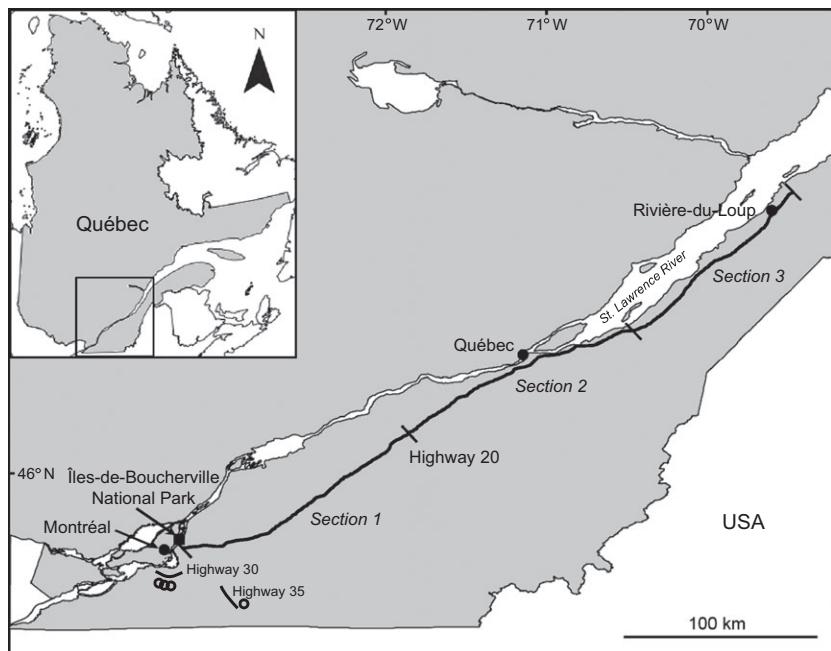
Fig. 1. Location of the sites sampled in southern Québec (Canada) for the evaluation of the genetic diversity of common reed (*Phragmites australis*) stands: Highway 20 (black line) and Îles-de-Boucherville National Park (black square). The locations of Highway 30, Highway 35 and of the national roads (grouped into four sectors, each corresponding to an open circle) sampled for field observation of new common reed individuals are also indicated, along with the other place names cited in the text.

ESTABLISHMENT AND SURVIVAL OF COMMON REED INDIVIDUALS

Newly created roadside ditches represent *a priori* excellent sites to test the relative success of vegetative versus sexual reproduction for establishing new common reed stands. There is a high probability that plant fragments found on such sites were inadvertently transported by machinery or contained in fill. Roadside ditches also offer the bare, wet and unshaded grounds propitious for seed germination and seedling establishment. An exhaustive search for appropriate study sites was undertaken by visiting all roadwork projects underway in the summers of 2010 and 2011 on highways and national roads in the Montérégie region in southern Québec, a region where the common reed invasion is well advanced (Jodoin *et al.* 2008; Lelong, Lavoie & Thériault 2009). We located four sections along two nearly completed highways (H30 and H35) with newly excavated ditches that were not yet colonized by the common reed, but were bordered by common reed stands, and 14 additional sections along national roads (Fig. 1), for a total of 9650 m of ditches.

Marshes near the shores of the Îles-de-Boucherville National Park (hereafter IBNP), a protected group of islands in the middle of the St. Lawrence River close to Montréal (Fig. 1), were also scouted for new common reed individuals. The largest (86 ha) complex of common reed stands in the St. Lawrence River is located in the marshes and along the shores of this park; the area covered by the stands has increased by 20% each year since the beginning of the 1990s (Tougas-Tellier 2013). The water level of the river was exceptionally low in 2010, exposing mud-flats and thus creating potential establishment sites for new individuals of the common reed (Weisner & Ekstam 1993; Coops & van der Velde 1995; Tulbure & Johnston 2010). We found two small marshes that were easily accessible and presumably suitable for the establishment of common reed stands. Their total area was about 800 m^2. They were also in close proximity to existing common reed stands.

Study sites were scouted for new common reed individuals at the end of the summer (August) in 2010 (roads, marshes) and 2011 (roads). New individuals were georeferenced with a global positioning system. Those emerging from a seed (very thin) or from a stem or rhizome fragment (very robust) were easily distinguished *in situ* (see Brisson, Paradis & Bellavance 2008 for details).

While the survival rate of new individuals established from plant fragments is assumed to be high beyond the first growing season (Juneau & Tarasoff 2013), seedlings require a sufficiently long growing season to reach a critical size and accumulate enough resources in their storage organs to survive their first winter (Haslam 1975). To investigate the respective survival rates of individuals emerging from seeds versus plant fragments, we resurveyed in 2011 the central section of H30 (4.2 km long) where the largest number of new individuals had been detected in 2010 (over 3700), and relocated all surviving individuals using the global positioning system.

POPULATION GENETICS OF COMMON REED

Sampling scheme

Leaves of common reed were sampled in summer 2009 along a 416-km stretch of H20, from Montréal to Rivière-du-Loup (Fig. 1). H20 is a split highway, with two parallel lanes for each travel direction (east and west). For sampling purposes, H20 was divided into three sections which differed in size and density of common reed stands, according to a survey carried out in 2003 (Jodoin *et al.* 2008). In the first and southernmost section (130 km), constructed between 1964 and 1966, the common reed forms nearly continuous hedges in the drainage ditches. In the second section (150 km), constructed between 1964 and 1970, stands are less abundant, and about 5–20% of the length of the ditches are occupied by the common reed. In the third and northernmost section (136 km), constructed in 1972, common reed stands are scattered, and less than 5% of the length of ditches are invaded by the plant.

A total of 99 official kilometre markers (33 in each of the three sections of H20) were randomly selected, and the common reed stand located in front of a selected marker was used for sampling leaf tissues. If no stand was located in front of the marker, the next closest stand was used. Leaf tissues were collected at three different spatial levels. A sample was collected from a shoot located at the centre of each stand, or in front of the kilometre marker in the case of very large stands, in the middle of the drainage ditch (level 1). Eight additional samples were collected from shoots at 3-m intervals (four

towards the west and four towards the east) along the drainage ditch (level 2, $N = 9$). Some stands were very short (< 12 m, especially in the northeasternmost section of H20), and they were sampled only if at least five shoots could be collected at level 2; otherwise, another stand (the next one) was selected. Following a first round of genetic characterization with microsatellites, the five most diverse stands within each highway section were revisited for a more detailed characterization. Four additional samples were collected 1 m from each of the nine shoots sampled at level 2, one in each cardinal direction (level 3, $N = 45$).

The marshes located in the IBNP were also sampled. Several circular stands of common reed were clearly visible on aerial photographs of the marshes, suggesting clonal (vegetative) expansion. Ten distinct circular common reed stands were randomly selected for sampling. As for H20, three sampling levels were used. One shoot was sampled at the centre of the stand (level 1). Eight other shoots were collected in each cardinal direction, four positioned at the margin of the stand and four others located at the mid-point between the margin and the central point (level 2, $N = 9$). Finally, four additional samples were collected in the immediate vicinity (1 m) of each of the nine previously sampled shoots in each cardinal direction (level 3, $N = 45$).

Genetic characterization

Fresh leaf samples (1 cm²) were collected in tubes with desiccant, kept at ambient temperature and protected from light. DNA was extracted from dried leaves using the procedure of Wenzl *et al.* (2004). Two different genotyping techniques were used and compared. First, genotyping was performed with a set of six polymorphic microsatellite (SSR) markers (Saltonstall 2003) that were suitable for analysis in multiplex: PaGT-4, PaGT-8, PaGT-9, PaGT-11, PaGT-13 and PaGT-16. Amplifications and electrophoresis were performed (see Belzile *et al.* 2010 for details). Results were visualized and analysed with SAGA^GT microsatellite analysis software (LI-COR Biosciences 2013). Given the uncertainty about ploidy levels and di- or tetrasomic inheritance, no attempt was made to estimate the copy number of each allele. Following the approach used in previous studies (McCormick *et al.* 2010b; Kirk *et al.* 2011), alleles at each SSR locus were scored as present (1) or absent (0) and used to define allelic genotypes (hereafter 'SSR genotypes').

Secondly, single nucleotide polymorphisms (SNPs), developed by genotyping by sequencing (GBS), were used to evaluate whether identical SSR genotypes were truly identical and whether a finer evaluation of clonal identity affected genetic diversity estimates. As the resolution of the SSR analysis was likely limited by the small number of loci, it was hypothesized that individuals sharing the same multilocus SSR genotype were not truly identical genomewide. A single shoot from 79 stands along H20 (18 in section 1, 31 in section 2 and 30 in section 3, respectively), and from nine stands of the IBNP, was analysed. An 88-plex *Pst*I/*Msp*I GBS library was produced using the protocol of Poland *et al.* (2012) at the Plate-forme d'analyses génomiques (Université Laval, Québec, Canada) and sequenced on a single lane of an Illumina HiSeq2000 (McGill University – GénomeQuébec Innovation Center, Montréal, Canada). After sequencing, the UNEAK pipeline (Universal Network Enabled Analysis Kit; Lu *et al.* 2013) was used to call SNPs. Initial filtering was performed using a minimum minor allele frequency (mnMAF) of 0.05 and a minimum call rate (mnC) of 0.8. An in-house Python script was used to more stringently filter genotype calls in the context of a tetraploid. A minimum of 11 reads supporting a genotype call was required; genotypes supported by fewer reads were replaced with an N (missing

data). If all reads for an individual were identical, it was deemed homozygous. If two alleles were detected, the frequency of reads corresponding to the minor allele (i.e. the less frequent of the two) had to be ≥0.15; if lower than this, the individual was called homozygous. This was implemented to correct the propensity of UNEAK to falsely call heterozygotes based on highly unbalanced read counts supporting two alleles (e.g. 1A:60G). Following this correction, SNPs were again filtered for mnMAF (0.05) and mnC (0.8) in TASSEL (Bradbury *et al.* 2007). In addition, we wished to verify that shoots from stands considered monoclonal based on SSR markers truly shared an identical genotype. We thus compared pairs of samples taken in seven monoclonal stands (three along H20 and four from IBNP). A 14-plex *Pst*I/*Msp*I GBS library was sequenced on one-sixth of a sequencing lane, and SNP calling and filtering was performed as described above.

As a complement, RFLP analysis of chloroplast DNA (see Lelong *et al.* 2007 for details) was conducted on all samples to confirm that they corresponded to the exotic common reed haplotype M. In addition, the recent discovery of a new exotic common reed haplotype (L1) in eastern Québec (Meyerson & Cronin 2013) led us to verify whether some of the sampled stands were colonized by this haplotype, especially in the northeasternmost section of H20 that is very close to the two L1 populations that have been found. The six H20 stands closest to these populations and six other stands (randomly selected) from the two other H20 sections were tested for the presence of haplotype L1 using the method described in Meyerson & Cronin (2013).

Genetic diversity

Genetic diversity within common reed stands was described using the number of different multilocus SSR genotypes (G) and an index of genotypic richness ($R = [G − 1] / [n − 1]$; see Dorken & Eckert 2001 for details). Identical genotypes were initially assumed to represent clones resulting from vegetative reproduction, and unique genotypes were interpreted as probable evidence of sexual reproduction. All comparisons between stands were performed with a reduced data set including only one representative of each genotype per stand.

Pairwise genetic distances between genotypes were estimated for both SSRs and SNPs. For SSRs, a binary (0/1) score was used for each allele at each locus, and the resulting matrix was used to calculate genetic distances with TASSEL (Bradbury *et al.* 2007). For SNPs, the DNADIST application in PHYLIP (Felsenstein 1989) was used to produce a distance matrix. Mantel's test was conducted with the XLSTAT package (Addinsoft, 2014) to evaluate the correlation between the two resulting genetic distance matrices. The genetic similarity between stands was analysed with SNP data (more accurate than SSR data for this purpose) and using a model-free principal coordinate analysis (PCOA; Gower 1966). The analysis was implemented in GEN-ALEX (Peakall & Smouse 2006, 2012) using Euclidean genetic distance matrix converted to standardized covariance.

Results

ESTABLISHMENT AND SURVIVAL OF COMMON REED INDIVIDUALS

New common reed individuals were observed at all roadside sites visited in 2010 and 2011. A total of 5340 new individuals were found over a distance of about 9.7 km of drainage ditches (Table 1). Most (84%, all roadside ditches) had

Table 1. Number of new common reed (*Phragmites australis*) individuals discovered in two marshes of the St. Lawrence River (Îles-de-Boucherville National Park (IBNP)) and along the ditches of several highways (H) and national roads located in southern Québec, and percentage of the individuals originating from seeds

Site	Year	Area (marsh) or length (ditch) covered	*N* shoots	Shoot density	Seedlings (%)
IBNP	2010	800 m^2	16	200 ha^{-1}	100
H30, eastern section	2011	950 m	69	73 km^{-1}	64
H30, central section	2010	4220 m	3727	883 km^{-1}	94
H30, western section	2011	545 m	753	1382 km^{-1}	50
H35	2011	2535 m	346	137 km^{-1}	81
National roads	2011	1400 m	445	318 km^{-1}	66
All (road ditches only)		9650 m	5340	553 km^{-1}	84

emerged from seeds; this proportion differed between sites, but was never below 50%. The other individuals were sprouts from rhizome fragments. Most seedlings were observed on soils that had been left bare during the winter and not disturbed the following spring and summer. The two marshes of the IBNP had a few seedlings; no individuals from plant fragments were discovered (Table 1).

In the follow-up survey performed in the central section of H30, only 50 of the 3727 new individuals found in 2010 were still present in 2011. Among the surviving individuals, those originating from plant fragments survived in greater proportion (31 out of 215; 14.4%) than those that emerged from seeds (19 out of 3512; 0.5%).

POPULATION GENETICS OF COMMON REED

The analysis of chloroplast DNA (level 1) indicated that all but two samples corresponded to the exotic common reed haplotype M; the two distinct samples corresponded to the native common reed. In addition, the haplotype L1, documented by Meyerson & Cronin (2013), was not found in the 12 shoots analysed. Summed over all six microsatellite loci, 27 different alleles were identified (PaGT-4: 5 alleles; PaGT-8 and PaGT-13: 2 alleles each; PaGT-9, PaGT-11 and PaGT-16: 6 alleles each). The number of genotypes detected (H20 and IBNP combined) with SSR markers was high at all sampling levels, with 91 ($N = 106$ samples), 184 ($N = 818$) and 251 ($N = 1451$) unique genotypes identified at levels 1, 2 and 3, respectively. At every level, about 85% of the documented genotypes were found at a single reed stand.

Genotypic richness within stands was variable but generally low with SSR markers. At level 2, nearly half (49%) the stands comprised a single genotype, and only 10% comprised five or more; however, up to 28 distinct genotypes were found at one H20 site (level 3, $N = 37$, $R = 0.75$; Table S1 in Supporting Information). Genotypic richness also varied spatially. It was very low at IBNP, where nine out of the ten sampled stands were monoclonal, while the other stand comprised only two genotypes. Along H20, monoclonal stands were interspersed with genetically diverse stands, but there were no marked differences in genetic diversity among sections or any suggestion of a gradient along the highway (Fig. 2). Sampling more shoots per stand (level 3 versus 2) resulted in apparent lower genotypic richness as measured by the index used (one-sided paired t-test on 15 values of R: $P < 0.0001$; Table S1).

The distribution of samples with identical SSR genotypes was spatially clustered within and between stands. Within stands, identical genotypes found in genetically diverse stands tended to be located near each other, as expected from clonal growth (Fig. 3). Between stands, identical genotypes also exhibited some spatial clustering (data not shown). Along H20, 31 identical multilocus SSR genotypes were found at more than one site (50 sites in total).
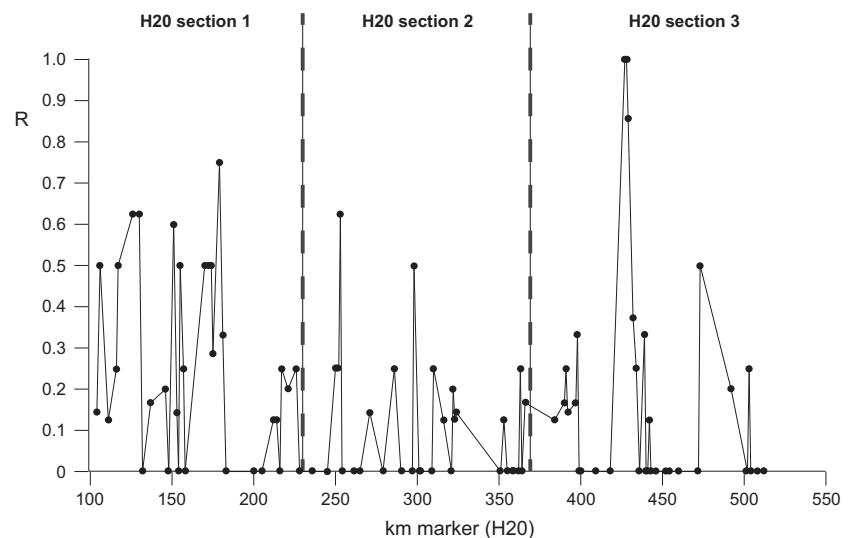

Fig. 2. Spatial distribution of the genotypic richness (R; SSR markers) of the common reed (*Phragmites australis*) stands sampled along Highway 20 (H20) in southern Québec. R values were calculated for sampling level 2, that is with a maximum of nine samples (shoots) per stand (see text for details). The borders between the different sections of H20 are indicated with dotted lines.
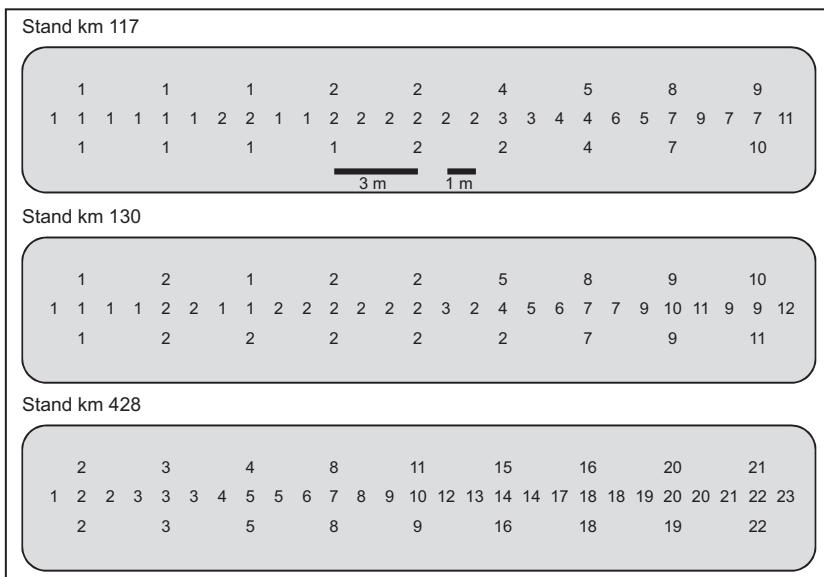
Fig. 3. Representative distribution of genotypes (SSR markers) in three of the most diverse stands of common reed (*Phragmites australis*) sampled along Highway 20 in southern Québec. Within each stand, identical Arabic numerals represent plant shoots belonging to the same genotype (numbered arbitrarily).
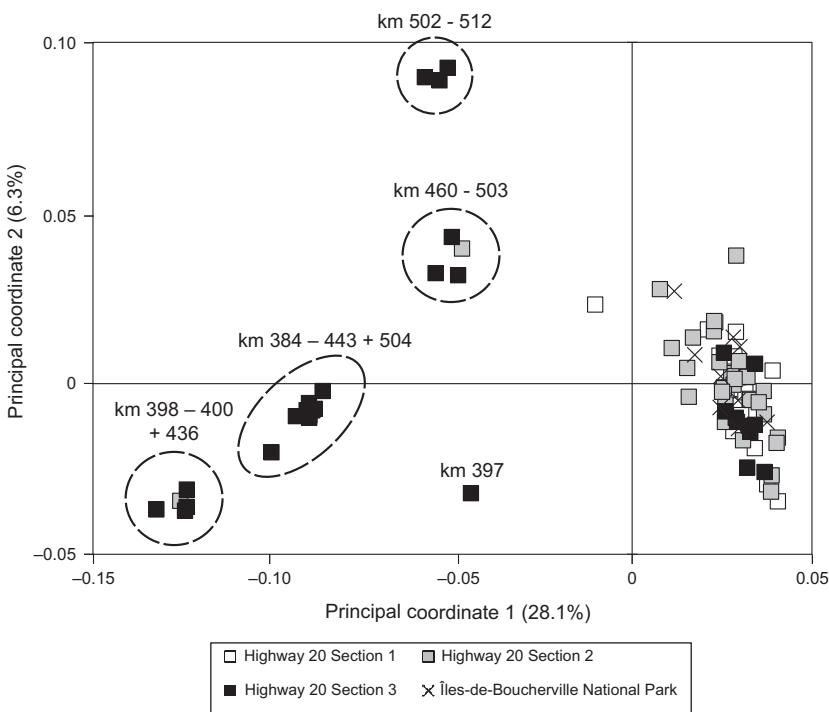

Fig. 4. Principal coordinate analysis (PCoA) showing the two-dimensional genetic (SNP markers) relationships between individuals (shoots) of common reed (*Phragmites australis*) sampled in southern Québec along Highway 20 and at the Îles-de-Boucherville National Park. Subgroups of Highway 20 section 3 in the negative values of axis 1 are indicated by circles, with their associated kilometre markers (minimum – maximum + outlier).

Genotyping by sequencing clearly showed that shoots with the same SSR genotype were genetically different when located in different stands, but truly identical when sampled in the same stand. Among the 88 samples from different stands, a total of 75 different genotypes were distinguished using SSRs (65 singletons, 7 duos and 3 trios). In contrast, based on the catalogue of 5538 biallelic SNPs, all 88 shoots proved to have distinct SNP genotypes. However, duplicate stand samples with identical SSR genotypes also had identical SNP genotypes at all loci for which a genotype could be called in both pair members (90.5% of all loci on average).

Although GBS provided a better resolution for identifying clones, it appears that genetic similarity between genotypes was nevertheless correctly assessed with SSRs. Distance matrices based on SSRs and SNPs were significantly correlated ($r = 0.62$, $P < 0.0001$), indicating that both sets of markers yielded similar estimates of genetic distances. Samples that shared identical SSR genotypes (i.e. with a genetic distance of 0) exhibited small but measurable genetic distances based on SNPs (mean = 0.106). Overall, genetic distances ranged between 0 and 0.630 with SSRs and between 0.003 and 0.430 with SNPs.

PCoA calculated with SNP data indicated that common reed shoots sampled from H20 section 3 formed a distinct genetic group (Fig. 4). The first multivariate axis explained 28% of the genetic variation, and clearly separated genotypes

found in H20 section 3 (negative values on axis 1) from all other genotypes (positive values on axis 1). Five H20 section 3 subgroups, geographically clustered, were also grouped by the PCoA along the second multivariate axis.

Discussion

In situ field observations revealed that both sexual and vegetative reproduction modes contribute to the establishment of new stands of common reed along roads in southern Québec. The relative contribution of each was quite variable between sites, but overall, new common reed individuals originated mostly (84%) from seeds rather than plant fragments. The environmental characteristics suitable for the germination of seeds – a bare and wet soil free from plant competition (Mal & Narine 2004; Saltonstall, Lambert & Meyerson 2010) – were present in the bottom of newly excavated ditches.

Mortality following the first winter may reduce the proportion of new common reed individuals arising from seed. Along H30, a low proportion (0.5%) of the seedlings found in year one (2010) were still alive in year two (2011), whereas a greater proportion of sprouts (14.4%) from fragments survived the winter and roadside management. In a previous survey also conducted in southern Québec, the 2-year survival rate for 38 seedlings established in an undisturbed roadside ditch was 29% (Brisson, Paradis & Bellavance 2008). This suggests that along H30, most of the common reed mortality was caused by human activities rather than by natural factors. It also indicates that sprouts from plant fragments may be more resistant to disturbances than seedlings.

Despite the high mortality rate of common reed along H30, 19 individuals from seedlings and 31 from plant fragments were recovered over the 4.2 km of roadside ditches surveyed in year two, and all of them initiated local vegetative propagation with rhizomes and stolons: they thus also contributed to the spread of the plant. This could be particularly true in a region where common reed stands are already abundant (which is the case for H30): established stands generate a dense seed rain but are also potential sources of vegetative fragments that may be accidentally transported through soil fill or machinery used for roadworks.

While short-term surveys indicate the relative contribution of each diaspore type, genetic diversity of established populations provides a long-term account of establishment modes. The very high genetic diversity observed among highway stands indicates the prime role of sexual reproduction in the dispersal of common reed, at least in a context where reed stands were initially not very abundant in the landscape (H20 was built from 1964 to 1972, when common reed density was much lower). SSR markers strongly suggested, and SNPs further confirmed, that separate stands rarely, if ever, share genotypes. Such a high level of genetic variability can only be explained by the dispersal of sexually produced seeds over extensive areas (Holsinger 2000; McCormick *et al.* 2010b; Kirk *et al.* 2011). It is likely that a large majority, if not all stands examined in this work, were initially founded by genetically distinct individuals. This finding is in agreement

with other recent studies on this species in eastern Canada (Belzile *et al.* 2010; Kirk *et al.* 2011) and the United States (McCormick *et al.* 2010a,b). Our results also suggest that vegetative reproduction is very important for the local spread of common reed (see also Douhovnikoff & Hazelton 2014). Half the surveyed stands comprised a single clone, suggesting that local expansion occurs by means of stolons or rhizomes forming new shoots. This expansion often extends over at least 26 m (sampled length of H20 stands) or 100 m (IBNP).

Our sampling along more than 400 km of highway corridor confirmed that long-distance dispersal by seeds is very important, but spatial genetic clustering along the highway also suggests that gene flow by seeds is constrained. In the northeasternmost section of H20 (section 3), many stands comprised a distinct group of genotypes compared to the other sections of H20 and IBNP. This suggests that each group of genotypes has great difficulty to establish in the territory occupied by the other group. Given the apparent continuity in ecological conditions along H20 sections, this genetic discontinuity may be the result of the colonization of H20 section 3 by a distinct lineage. Dense stands already established in the other H20 sections may have prevented the establishment of rare long-distance seeds dispersed westward. Physical limits to the dispersal and establishment of seeds have also apparently played a role at a smaller spatial scale. Within H20 section 3, distinct subgroups of genotypes spanned over separate stretches of the highway but did not mix. This suggests that patches, once established, are not easily invaded by other genotypes.

In the studied marshes (IBNP), the unique genotype found in each common reed (with one exception) stand suggests that dispersal and establishment happen by seeds. Lateral expansion is wholly attributable to vegetative reproduction resulting in monoclonal stands. Although the number of sampled stands is small, our results contrast with those found in brackish wetlands of Maryland by McCormick *et al.* (2010b). These authors also found that seeds likely initiated stands, but virtually no stands were monoclonal. Possible explanations include that seed production and/or seedling survival were much higher in their wetland area, which is probable considering its more southern location (warmer climate). Also, samples were taken from shoots at the periphery of very large stands that may have arisen from the aggregation of several smaller patches established by distinct genotypes.

Conclusion

Field and genetic data suggest that the invasive haplotype M of the common reed disperses predominantly by seed, in all environments studied to date (lakes: Belzile *et al.* 2010; brackish wetlands: McCormick *et al.* 2010a,b; freshwater wetlands: this study, Kirk *et al.* 2011; highways: this study, Kirk *et al.* 2011). However, in a region already highly invaded by the plant, accidental transport of fragments over short distances during road construction or maintenance can also be a source of new individuals. Once initiated, either from seedling or fragments, local expansion occurs through vegetative

growth. Our study focused on roadside ditches, and additional data are needed for marshes, but since the common reed essentially spreads along roads in north-eastern North America for expanding its range (Lelong *et al.* 2007; Jodoin *et al.* 2008; Lelong, Lavoie & Thériault 2009), the phenomenon described here is important for a better understanding of the dispersal of the invader.

Microsatellite (SSR) markers provided useful information on genetic diversity within and among stands, but SNPs resolved clone identity. With only SSR markers, we would have erroneously concluded that a substantial number of common reed stands resulted from the dispersal of plant fragments over distances as long as 250 km along highways. The GBS approach strongly reduces uncertainties associated with the use of a limited number of markers. This approach is especially valuable for ecologists dealing with an ever-increasing number of invaders, of which few have identified microsatellite markers.

Some practical management considerations and preventive strategies can be drawn from these findings, at least for roads. We showed the high occurrence of common reed establishment through seeds. Environmental managers should reduce the size and number of suitable germination beds by promoting the rapid establishment of a dense plant cover on bare disturbed roadsides. Species seeded should include fast-growing highly competitive native grasses: they will suppress common reed seedlings, which are poor competitors (Byun, de Blois & Brisson 2013). Planting shrub hedges on roadside banks also generate shade that would be unfavourable to this invader (Albert *et al.* 2013). Our study also confirmed the occurrence of accidental rhizome transport during road construction or maintenance over short distances. In regions widely invaded, no soil suspected of containing common reed fragments should be used as fill, and road machinery should be inspected and washed if they carry plant fragments. The dual capacity (seeds, fragments) of the haplotype M of the common reed for establishment and spread is certainly one of the reasons for its remarkable invasion success in North America.

Acknowledgements

Data accessibility

Albert, A., Brisson, J., Belzile, F., Turgeon, J. & Lavoie, C. Data from: Strategies for a successful plant invasion: the reproduction of *Phragmites australis* in north-eastern North America. *Dryad Digital Repository*, doi:10.5061/dryad.rp77j.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Genetic diversity (SSR markers) within stands of common reed *(Phragmites australis)* sampled in southern Québec at the Îles-de-Boucherville National Park (IBNP) and in the ditches of Highway 20 (H20). For each stand sampled, the number of different multilocus genotypes (G) and the index of genotypic richness (R) are indicated for two levels of sampling intensity (see text for details).