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ORIGINAL PAPER

Seeds contribute strongly to the spread of the invasive genotype of the common reed (*Phragmites australis*)

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Abstract The introduced subspecies of the common reed (*Phragmites australis* (Cav.) Trin. ex Steud. subsp. *australis*; Poaceae) is considered one of the most invasive plants in North American wetlands. Given its relatively low seed set and its tremendous capacity to spread via stolons or rhizomes, it has generally been thought that the spread of vegetative diaspores was responsible for the establishment of new populations. To test this hypothesis, we sampled a single plant from each of 345 visually-distinct common reed stands located along the shores of Lake St. François (southern Quebec, Canada). With a set of six nuclear microsatellite markers, we distinguished 134 different genotypes. The number of individuals sharing the same genotype ranged from one to 16, and averaged 2.1. Most genotypes were encountered only once. We examined the spatial distribution of the most frequent genotypes and found little evidence of clusters along the lakeshore. These data contradict the hypothesis that a common reed invasion is initiated by the introduction of vegetative diaspores from a few clones. Rather, they clearly support the alternative hypothesis that seeds were the primary diaspores responsible for the establishment of common reed populations.

Keywords Common reed · Haplotype M · Lake · *Phragmites australis* · Seed · Wetland

Introduction

Although most aquatic exotic plant species have been established in North America for several decades, ecologists have only just begun to identify specific genotypes that are particularly invasive, and to study their characteristics (Moody and Les 2002; Saltonstall 2002; Lavergne and Molofsky 2007). The relative contribution of vegetative and sexual reproduction to the spread of many species is largely unknown; this lack of knowledge compromises the effective management and control of invaders. This is particularly true for the common reed (*Phragmites australis* (Cav.) Trin. ex Steud.; Poaceae). This grass species is probably the most aggressive aquatic plant invading eastern North American wetlands (excluding Florida). Large populations covering hundreds of hectares are widespread along the Atlantic coast of the United States (from Connecticut to Virginia) and in the Mississippi River delta (Rice et al. 2000; Warren et al. 2001; Lathrop et al. 2003; White et al. 2004; Philipp and Field 2005). Common reed populations

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have also recently expanded on the shores of the Great Lakes and along the St. Lawrence River (Wilcox et al. 2003; Hudon et al. 2005; Trebitz and Taylor 2007; Tulbure et al. 2007). The spread and expansion of common reed populations in marshes are probably associated with the nineteenth century introduction of a Eurasian subspecies (*P. australis* subsp. *australis*), also known as 'haplotype M' (Saltonstall 2002). This subspecies (or haplotype) is particularly productive in wetlands that have been enriched with nitrogen from nearby agricultural or residential sources (Bertness et al. 2002; League et al. 2006). Highway systems have strongly contributed to the inland spread of the haplotype, with roadsides acting as corridors and habitats (Lelong et al. 2007, 2009; Jodoin et al. 2008). The haplotype M of the common reed has serious negative impacts on wetland hydrology (Windham and Lathrop 1999; Hanson et al. 2002; Osgood et al. 2003) and biodiversity (Benoit and Askins 1999; Keller 2000; Lavoie et al. 2003; Silliman and Bertness 2004; Robertson and Weis 2005; Hunter et al. 2006).

The common reed is an abundant seed producer, although seed production is highly variable. Seed weight may range from 0.01 to 0.19 mg (McKee and Richards 1996). A single inflorescence may produce 350–800 seeds (Maheu-Giroux and de Blois 2007) that can be dispersed by water or wind up to 10 km (Fér and Hroudová 2009). There are few studies about the sexual reproductive potential of the common reed in North America. The plant apparently produces very few viable seeds (3–7% of the seeds), at least in the populations located at the northern distribution limit of the species (Gervais et al. 1993; Maheu-Giroux and de Blois 2007). This supports observations from other parts of the world (McKee and Richards 1996; Ishii and Kadono 2002). Moreover, until very recently (Brisson et al. 2008), no common reed seedlings had ever been reported in the field in Canada. Consequently, most ecologists presumed that the establishment of new populations resulted from the natural (water) or human-assisted (road construction) spread of stem and rhizome fragments (Gervais et al. 1993; Chambers et al. 1999; Mal and Narine 2004; Minchinton 2006). However, the swiftness of the spread of the haplotype M in North America (Saltonstall 2002; Lelong et al. 2007), and the rapid rise in the number of common reed stands in sites that have recently been invaded (Hudon et al. 2005; Maheu-Giroux and de Blois 2007), raised doubts about the ineffectiveness of sexual reproduction as a contributor to the spread of the plant.

In Europe, both sexual and asexual reproduction has been found to contribute to the spread of the common reed. For instance, Koppitz and Kühl (2000) proposed that there are three stages in the establishment and growth of a common reed stand. At the settlement stage, colonization is initiated via seed germination, presumably in large numbers, in an area that is free of vegetation. Soon thereafter, each seedling rapidly propagates vegetatively to occupy the available niche. As a result, considerable genetic diversity exists at this stage because a large number of distinct genotypes have succeeded in establishing themselves. At the propagation stage, the different genotypes continue to expand, via rhizomes and stolons, and start to invade and compete each other. During this second stage, genetic diversity remains high. Finally, during the stationary stage, intense competition between the various genotypes occurs and the best-adapted clone(s) dominate(s) the stands. This results in a decrease in genetic diversity such that long-established common reed stands tend to be composed of one or a few clones (Koppitz et al. 1997).

Determining the relative contribution of vegetative and sexual reproduction for the common reed is of major environmental importance. If the species primarily spreads by vegetative fragments, wetland managers must strive first and foremost to prevent the introduction of contaminated vectors (boats, excavators). If new populations mainly emerge from seeds, managers should concentrate on management practices that reduce the availability of favourable germination conditions, thereby preventing the initiation of new invasions. Completely different management strategies are required based on the primary reproductive mechanism. Ideally, to understand the process by which colonization occurs, one needs to observe the very first stages of colonization. Such an opportunity was provided by recently-established (~10 years) common reed stands that have rapidly expanded on the shores of a large lake in southern Canada (Lake St. François, Quebec). We hypothesized that the degree of genetic diversity between distinct stands would allow us to determine the predominant dispersal mechanism in this environment.

Methods

The Lake Saint-François is the third largest (51 km^2) lake in Quebec south of the St. Lawrence River (Fig. 1). It drains a watershed of 1,204 km^2. The mean annual temperature of the Lake St. François watershed varies from 2.5 to 5.0°C. The mean annual precipitation is about 1,000–1,100 mm, and the length of the growing season extends over 170–180 days. The region surrounding the lake is essentially (75%) forested with maple (*Acer saccharum* Marsh.), birch (*Betula alleghaniensis* Britt.) or fir (*Abies balsamea* (L.) Mill.). The remainder of the watershed is occupied by agricultural fields and small villages (Robitaille and Saucier 1998).

Lake St. François was dammed in 1917 for the production of hydroelectricity and to prevent spring floods. The water level is consequently highly variable (±8 m a year), and reaches its minimum level during the winter season. The mean water depth is 16 m, but may reach 40 m during the summer season (Ministère du Loisir, de la Chasse et de la Pêche du Québec 1986). Approximately 63 of the 117 km of lakeshore are protected within Frontenac National Park (Fig. 1), and have not recently been disturbed. The remaining 54 km are occupied by 883 residences. The forest vegetation (trees, shrubs) near these residences has in most cases been eliminated, and nearly all of their shores are now covered with rocks, concrete or lawn.

Preliminary surveys conducted along the shores protected by the Frontenac National Park suggested that the common reed stands, almost absent in 1995, were rapidly increasing in number and area (Société des établissements de plein air du Québec 2005). The shores of the lake (inside and outside the park) were visited with a small boat from July 24th to August 27th, 2006, and all visually-distinct common reed stands (i.e., a group of stems clearly isolated from its neighbours) were mapped and positioned with a geographic information system. A small leaf fragment was taken from a stem located at the centre of each population for genetic analysis. All leaf fragments were kept in a freezer (−20°C) prior to analysis.

Total DNA was extracted as per Edwards et al. (1991). To discriminate between the native and the Eurasian (haplotype M) genotypes, two non-coding regions of the chloroplast genome (*trnT* (UGU)–*trnL* (UAA) and *rbcL–psaI*) were amplified by polymerase chain reaction using the primers described by Saltonstall (2003), and restriction site polymorphism was detected by digesting the amplicons with *Rsa*I or *Hha*I, respectively. To determine the genetic diversity (nuclear DNA) within the Lake St. François population, we used a set of six microsatellite loci (PaGT-4, -8, -9, -11, -13 and -16). The primers followed Saltonstall (2002), except that the forward primer was extended with a M13 tail to allow labelling. Amplification reactions were set up in a 10 μl container with ~50 ng of genomic DNA, 1.5 μl of 10× polymerase chain reaction buffer, 1 μl of 2 mM dNTPs, 0.5 μl of 2.5 μM of each forward and reverse primer, 0.2 μl (0.1 pmol/μl) of M13 primer labeled with either IRD700 or IRD800, and 0.15 μl of Taq DNA polymerase. Amplifications were performed on a gradient thermocycler (T Professional Thermocycler, Biometra, Goettingen) as follows: 95°C, 2 min; 30 cycles of 92°C, 45 s; 54°C, 2 min; 68°C, 1 min. The amplicons were separated on 6.5% polyacrylamide gels with a DNA analyzer (1,500 V, 50°C; Global Edition IR^2, LI-COR Biosciences, Lincoln).

A point pattern analysis was conducted to evaluate whether there was spatial clustering for the most common genotypes that have been identified, i.e., those represented by at least five distinct common reed stands. The degree of clustering was tested by calculating a nearest neighbour index which compares the mean distance separating the stands to a theoretical distance that would result from a random spatial distribution of common reed patches (Clark and Evans 1954; Cressie 1993).

Results

A total of 345 common reed stands were mapped along the shores of Lake St. François (Fig. 1). They were especially abundant along the northern part of the lake and in Sauvage Bay. The analysis of two chloroplastic non-coding regions indicated that all leaf fragments analyzed belonged to the Eurasian subspecies of the common reed (haplotype M). Among the 345 individuals sampled, the number of samples in which the alleles could be scored unambiguously ranged from 298 (PaGT11) to 339 (PaGT8). At three of the six loci (PaGT4, PaGT8,

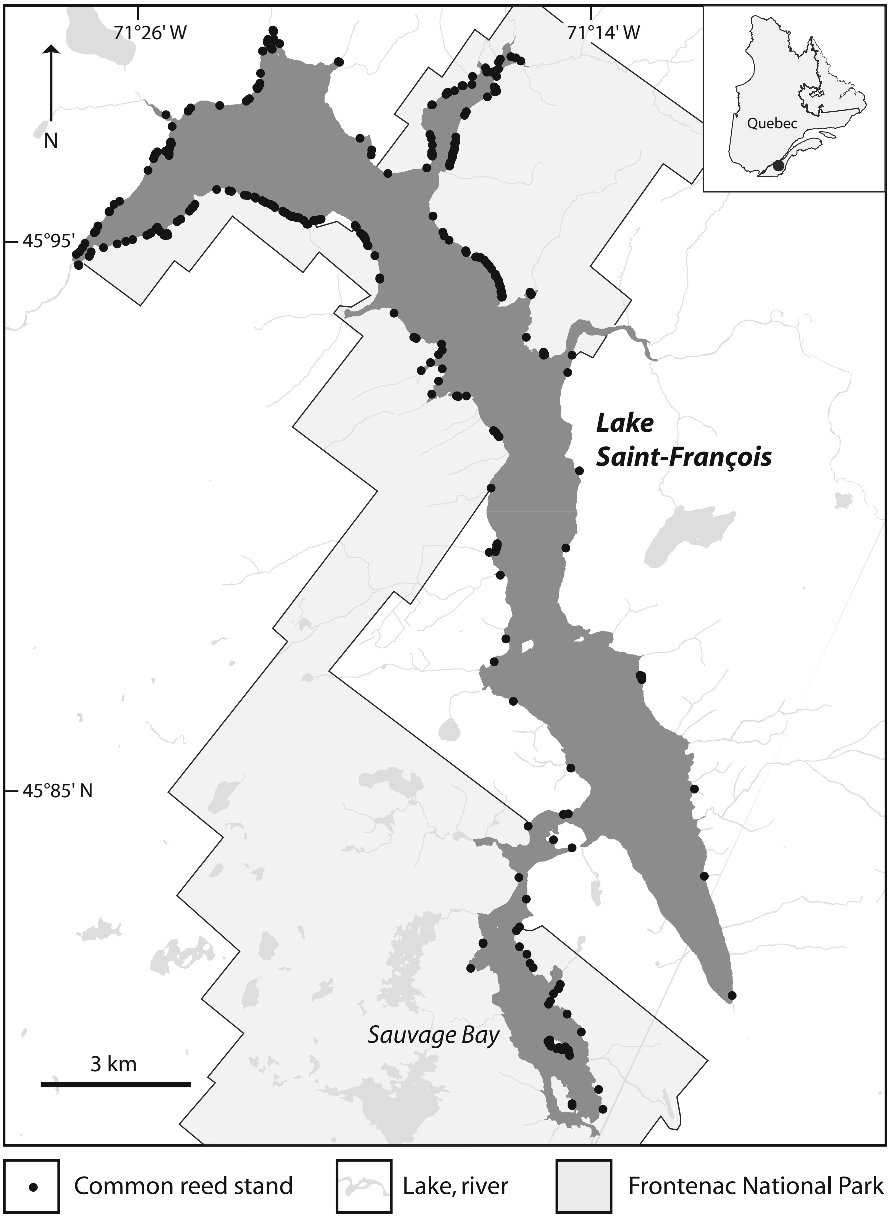

Fig. 1 Spatial distribution of common reed (*Phragmites australis*) stands on the shores of Lake St. François (southern Quebec, Canada) in summer 2006

and PaGT13), only two alleles were observed in the collection of samples whereas three, four and five alleles were detected at the three remaining loci (PaGT16, PaGT9 and PaGT11, respectively). On an individual plant basis, between one and four different alleles were observed, suggesting that polyploids were present in the material examined.

When the marker loci were considered jointly, data on all six markers were available for 280 of the 345 individuals analyzed. A total of 134 different combinations of alleles, or genotypes, were observed, and the number of individuals with the same genotype ranged from one to 16 and averaged 2.1. The number of genotypes increased rapidly with the number of markers considered (Fig. 2). Based on the trend observed, it is conceivable that the use of a few more markers could have produced a unique genotype for each of the 345 sampled individuals.

To examine if the distribution of these genotypes followed a pattern, we mapped the spatial distribution of the common reed stands for each of the 13 most frequent genotypes found around the lake (Fig. 3).

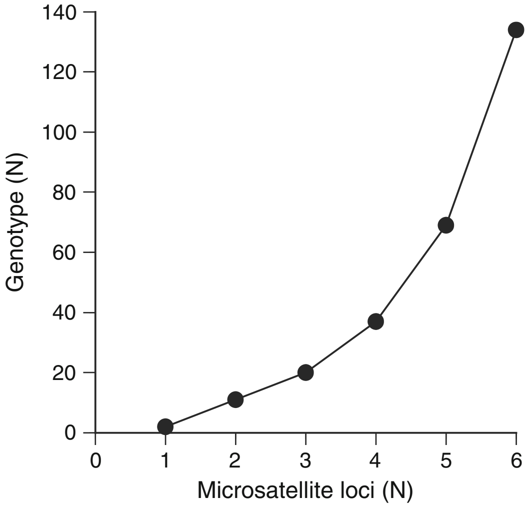

Fig. 2 Number of distinct genotypes among 280 common reed (*Phragmites australis*) stands located on the shores of Lake St. François (southern Quebec, Canada) as a function of the number of microsatellite loci considered

Only three genotypes (79, 111, and 126) showed significant ($P < 0.001$) clustering relative to a random distribution (point pattern analysis). It should be noted that in each of these cases, pairs or trios of common reed stands sharing the same genotype were in such proximity that they are confounded on Fig. 3. Overall, the data suggest that most of these genotypes are dispersed in a random fashion.

Discussion

To date, the spread of the common reed in North America has been presumed to result largely, if not exclusively, from the unintentional introduction of plant parts (rhizomes, stems) following anthropogenic disturbances to the environment (Gervais et al. 1993; Chambers et al. 1999; Mal and Narine 2004; Minchinton 2006). However, the genetic data collected from the specimens along Lake St. François contradicted the hypothesis that asexual spread was the predominant mechanism behind the initial invasion of the lakeshore. Indeed, with as few as six microsatellite loci, we identified 134 different genotypes. Such high genotypic diversity cannot result from vegetative spread and can only be achieved via sexual reproduction. Therefore, the most likely origin of the invasion of the shores of Lake St. François is the spread of seeds. Although the proportion of viable seeds has been reported to be low for this species (3–7%; Gervais et al. 1993; Maheu-Giroux and de Blois 2007), viability can vary considerably from year to year and from one stand to another (McKee and Richards 1996). For example, Ishii and Kadono (2002) observed that although the seed set in the majority of common reed stands studied in southwestern Japan was well below 10%, it could reach as high as 60% in one site in a given year. This suggests that, under favourable climatic circumstances, the production of viable seeds can occasionally reach high levels (McKee and Richards 1996; Brisson et al. 2008). However, for colonization to occur, common reed seeds need to come in contact with a seedbed suitable for the germination and initial establishment of the plants. A disturbed soil surface where the vegetation cover has been removed may represent an excellent seedbed for the common reed, especially if the surface is located close to a source of water (Mal and Narine 2004). More than 44% of the cottages located along Lake St. François were built between 1990 and 2006 (LeBlanc 2008); this intense construction period coincided with the rapid expansion of the number of common reed stands around the lake. There may be a link between the soil disturbance associated with house construction and the establishment of several common reed stands, but this assertion remains to be substantiated.

Have the tremendous asexual reproduction mechanisms of the common reed also played a part in the lakeshore invasion? It is possible that each individual stand was the result of a relatively rare successful establishment of a single, unique genotype derived from a seed, which was followed by the rapid vegetative expansion of the stand. Alternatively, several common reed stands could be composed of a mixture of different genotypes, each resulting from the germination of a seed. To resolve this issue, several individuals from the same stand should be sampled to determine the level of genetic uniformity within stands.

How did the seeds arrive on the lake or its shores in the first place? The seeds have probably first spread from the 2,914 common reed stands located along the highway system surrounding the lake (615 km of roads over an area of 952 km^2; LeBlanc 2008). In southern Québec, road ditches located on surface deposits with low drainage capacity (organic

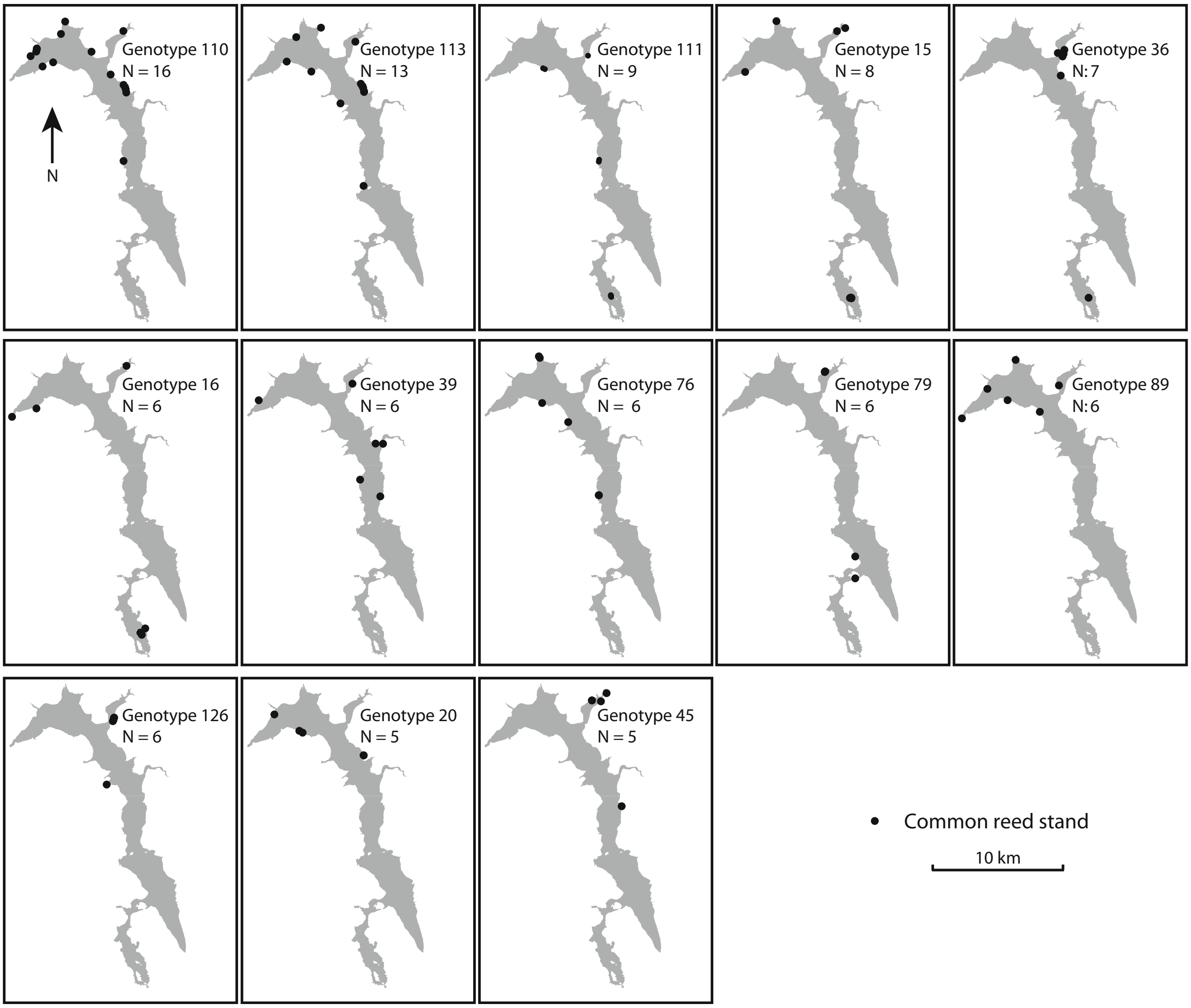

Fig. 3 Spatial distribution of the most common genotypes identified in common reed (*Phragmites australis*) stands located on the shores of Lake St. François (southern Quebec, Canada). Each map shows the location of individuals bearing the same genotype. For a given genotype, the number of individuals ranged between 5 and 16

or clay) are heavily invaded by the common reed (Lelong et al. 2009). The Lake St. François is almost completely surrounded by major (national) roads; the distance separating the roads to the lakeshores is well below the maximum dispersal distance (10 km) that has been measured for common reed seeds (Fér and Hroudová 2009). The seeds could also have been produced by plants introduced accidentally or voluntarily collected in the fall for decorative purposes in the backyards of lakeshore cottages (LeBlanc 2008). Whatever the origin of the seeds, wetland managers in North America should not only focus their attention on dispersal vectors likely to spread common reed plant parts from one place to another. They should also prevent the creation of suitable seedbeds (disturbed soils) in wetlands, or at least revegetate disturbed soils as soon as possible. Management practices leading to a dense plant cover that can compete for space and light with common reed seedlings may represent a good strategy for preventing the establishment of a common reed stand (Brisson et al. 2008).

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