Increased genetic variation and evolutionary potential drive the success of an invasive grass

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Despite the increasing biological and economic impacts of invasive species, little is known about the evolutionary mechanisms that favor geographic range expansion and evolution of invasiveness in introduced species. Here, we focus on the invasive wetland grass *Phalaris arundinacea* L. and document the evolutionary consequences that resulted from multiple and uncontrolled introductions into North America of genetic material native to different European regions. Continental-scale genetic variation occurring in reed canarygrass' European range has been reshuffled and recombined within North American introduced populations, giving rise to a number of novel genotypes. This process alleviated genetic bottlenecks throughout reed canarygrass' introduced range, including in peripheral populations, where depletion of genetic diversity is expected and is observed in the native range. Moreover, reed canarygrass had higher genetic diversity and heritable phenotypic variation in its invasive range relative to its native range. The resulting high evolutionary potential of invasive populations allowed for rapid selection of genotypes with higher vegetative colonization ability and phenotypic plasticity. Our results show that repeated introductions of a single species may inadvertently create harmful invaders with high adaptive potential. Such invasive species may be able to evolve in response to changing climate, allowing them to have increasing impact on native communities and ecosystems in the future. More generally, multiple immigration events may thus trigger future adaptation and geographic spread of a species population by preventing genetic bottlenecks and generating genetic novelties through recombination.

biological invasion | genotypic diversity | multiple immigration | range expansion | phenotypic plasticity

Invasive species are increasingly threatening biodiversity worldwide (1) and causing substantial economic damage (2, 3). Despite these detrimental effects, little is known about the evolutionary mechanisms that permit geographic range expansion and evolution of invasiveness in introduced species (4, 5). Theoretical models of species geographic range predict that species boundaries will be static when peripheral populations lack heritable phenotypic variation (6) or cannot adapt to local environmental conditions because of continuous gene flow from central populations (7, 8). Yet, many introduced species have expanding range margins and thus can provide insights into important evolutionary processes that drive adaptation and range expansion (9, 10). Over a relatively short time interval, some invasive plants have evolved higher growth and reduced anti-herbivore defenses (11, 12), higher sexual and/or clonal reproductive rates (13, 14), and have adapted to local climatic conditions (15, 16). Thus far, the influence of species immigration history on such high adaptive potential of newly founded populations has been little investigated. Multiple colonization events, whether natural or human induced, may overcome bottlenecks in recently founded populations (17–19) and permit new genetic combinations (20–22), especially when propagules have long residence time (23) or retain high genetic diversity as do seeds of out-crossed species (24). Consequently, immigration history can influence how bottlenecks, recombination, and natural selection interact to contribute to phenotypic evolution and increased invasiveness in introduced species.

Grasses constitute a major group of invasive plants that can dramatically alter native plant community structure and ecosystem processes such as fire frequency, nutrient cycling, and water circulation (25). Here, we show that the invasive potential of the perennial grass *Phalaris arundinacea* L. in North American wetlands is an evolved feature that resulted from multiple and uncontrolled introductions of genetic material native to different European regions. The continental-scale genetic variation occurring in the species' European range has thus been reshuffled and recombined within introduced populations in North America. This alleviated any potential genetic bottlenecks, including at the leading invasion front, and increased the species' genetic diversity and heritable phenotypic variation relative to its native range. The resulting higher evolutionary potential of invasive populations finally allowed for rapid selection of novel genotypes with higher vegetative colonization ability and phenotypic plasticity. Our results suggest that multiply introduced invasive species are particularly predisposed to exhibit high rates of phenotypic evolution after their introduction, and may be very successful in adapting to predicted climate change in future decades.

Results

We used a hierarchical sampling design in which we collected 350 rhizome pieces of reed canarygrass from populations located at the center and the margin of its native range in Europe (Czech Republic and southern France, respectively) and its invasive range in North America (Vermont and North Carolina, respectively). Sampling the center versus the margin in the native and invasive range of reed canarygrass allowed us to compare the evolutionary mechanisms that confine its currently stable range in Europe to those that allow its aggressive range expansion in North America.

Genotypic Diversity and Composition of Invasive and Native Populations. Reed canarygrass exhibited high overall genetic diversity (based on 12 neutral allozyme markers exhibiting 34 distinct alleles, Fig. 1 *a–c*). More importantly, geographic distribution of different alleles provided convincing evidence for a history of several introductions of strains originating from disparate European regions. Alleles unique to French and Czech populations
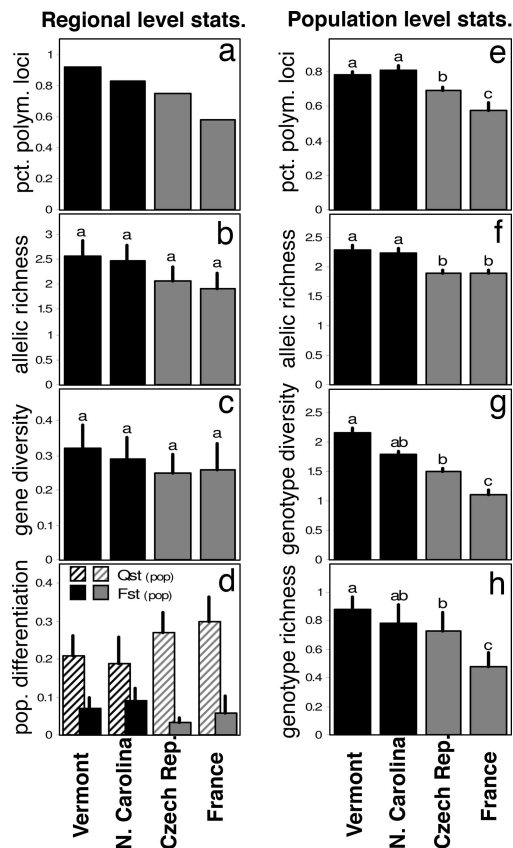
Fig. 1. Regional-level (*Left*, *a–d*) and population-level (*Right*, *e–h*) statistics of genetic diversity for invasive regions (black/hatched black histograms) and native regions (gray/hatched gray histograms) of reed canarygrass. Regional-level statistics are: overall percentage of polymorphic loci (*a*), overall allelic richness (*b*), weighted overall gene diversity (*c*), and genetic differentiation between populations (*d*) for both neutral markers ($Fst_{[pop]}$) and phenotypic traits ($Qst_{[pop]}$). Population-level statistics are: mean percentage of polymorphic loci (*e*), mean allelic richness (*f*), corrected Shannon–Wiener index for genotypic diversity (*g*), and mean richness in multilocus genotypes (*h*). Error bars represent standard errors. Letters indicate means that were not significantly different at the 5% level after a 10,000-permutation test.

(two and three alleles, respectively) cooccurred within invasive North American populations (see Fig. 2), supporting a scenario of at least two separate introductions of strains native to southern and central European regions. In fact, given the large number of rare alleles detected in North American populations (Fig. 2), it seems likely that European strains have been introduced into North America several times. These multiple introductions of European strains resulted in the continental-wide genetic diversity of reed canarygrass in its European range being redistributed into North American introduced populations.

After introduction, European strains of reed canarygrass have extensively recombined, generating new genotypes in North American populations. Among the 210 multilocus genotypes identified overall, we found that only 1.5% of North American genotypes also occurred in European populations, although North American and European regions shared 85% of the total allelic diversity. Multilocus genotypic structure was significantly different between invasive and native ranges of reed canarygrass [see supporting information (SI) Table 1], indicating that invasive populations display a number of novel interlocus allelic combinations. Thus, the vast majority of invasive genotypes have arisen by recombination between introduced European genotypes. Because introduced genotypes were drawn from across

Europe, their mixing in North America created the opportunity for novel genetic recombinations to be formed that had little chance of occurring in Europe.

The history of repeated introductions of reed canarygrass into North America has also resulted in substantially higher within-population genetic diversity (Fig. 1 *e* and *f*) and genotypic diversity (Fig. 1 *g* and *h*) and in much lower genetic depletion at the southern periphery of the invasive range relative to the native range (Fig. 1 *e–h*). Moreover, a resampling procedure demonstrated that this pattern is robust to unevenness in sampling design: Vermont and North Carolina populations displayed a higher genotypic diversity than the Czech Republic, followed by France (Fig. 3). Thus, introduced North American populations of reed canarygrass did not experience the strong genetic depletion at the current range limits that was found in their native European range. Reed canarygrass' higher genotypic diversity in the invasive range, including within peripheral populations, may promote geographic expansion in North America.

Phenotypic Consequences of Genetic Identity. The new genotypic composition of introduced populations was found to translate into phenotypic differences, based on a common garden greenhouse experiment that used replicated clones of 41 native and 49 invasive, randomly selected, genotypes. The best models to explain the data, based on Akaike's Information Criterion (AIC) (see SI Table 2 for model selection), indicated that invasive genotypes emerged faster and with a slightly higher asymptotic emergence probability than native ones (Fig. 4*a*), with no differences between regions within ranges (SI Table 2). Invasive genotypes also exhibited significantly higher tillering rates (SI Table 2 and Fig. 4*b*) and leaf production (SI Table 2 and Fig. 4*c*) than native genotypes, indicating a higher potential for clonal spread and leaf canopy expansion. Finally, invasive genotypes had significantly higher biomass production than native ones, mainly because of a higher above-ground biomass (SI Table 2 and Fig. 4*d*). This higher potential for vegetative establishment, biomass production, and clonal spread of invasive genotypes relative to native ones explains the greater aggressiveness of reed canarygrass in North America than in Europe (26).

A genotype's phenotypic performance in the greenhouse was correlated with its phenotypic performance under field conditions. Using clones of 36 genotypes (9 genotypes from each study region) transplanted into a Vermont wetland, we found that the ranking of genotypes with respect to their phenotypic traits measured in the greenhouse translated into natural conditions. Tiller height and tiller number measured in the greenhouse significantly predicted a genotype's tiller height and tiller number in natural conditions (tiller height $F_{(1, 32)} = 9.99$ $P < 0.01$; tiller number $F_{(1, 32)} = 5.09$ $P < 0.05$). Early emergence, rapid vegetative spread, and high relative growth rate generally confer a competitive advantage for light and space (27, 28); thus, the novel genotypes of reed canarygrass may have a selective advantage in North American natural conditions because of their higher potential for vegetative colonization. The consistent differences among European and North American genotypes under natural conditions demonstrates that rapid phenotypic evolution is likely responsible for reed canarygrass invasiveness in North America.

Evolutionary Potential and Phenotypic Plasticity. The extensive recombination that occurred in introduced populations may have stimulated the evolution of invasive ability through several nonexclusive mechanisms: (*i*) a change in the mean phenotype after recombination (hybrid vigor), where the newly created genotypes would be immediately more invasive than the parent ones, (*ii*) an increase in genetic variance and subsequent natural selection of invasive ability, and (*iii*) an increase in phenotypic
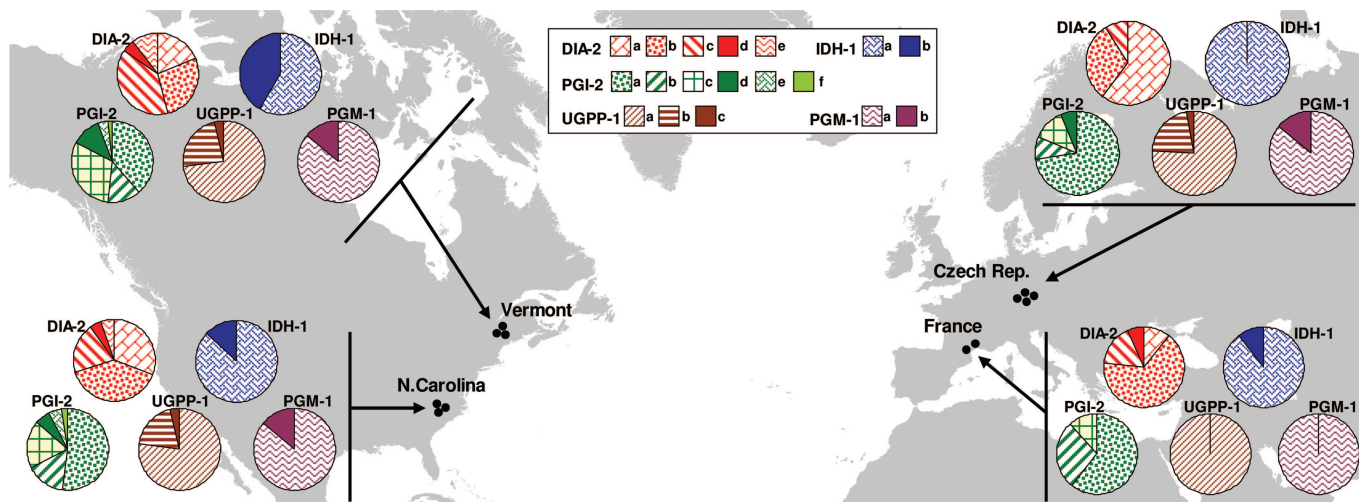
Fig. 2. Geographic distribution of neutral genetic diversity of reed canarygrass for five highly variable allozyme loci (among 12 studied). Pie charts display allele frequencies within central vs. southern regions of occurrence in the native range (Czech Republic vs. France) and invasive range (Vermont vs. North Carolina) of reed canarygrass. Note that alleles unique to southern France (DIA-2d and IDH-1b) and the Czech Republic (PGI-2d, UGPP-1c, and PGM-1b) cooccur within invasive regions of reed canarygrass.

plasticity, which may promote invasion by allowing growth in a variety of environmental conditions.

We found no evidence for hybrid vigor in introduced populations. Observed heterozygosity did not significantly differ between invasive and native populations (10,000-permutation test, $P = 0.188$); and, for both invasive and native genotypes, there was no relationship between the heterozygosity of individual genotypes and either their tillering rate or biomass production (Pearson correlations $r_{(invasive)} = 0.092\ P = 0.388$, and $r_{(native)} = -0.104\ P = 0.523$). However, we found that invasive populations exhibited greater broad-sense heritability and, thus, greater potential response to natural selection, for a number of phenotypic traits such as emergence time, tillering rate, and root biomass (Fig. 5a). Because experimental plants were at least second-generation transplants, this pattern cannot be attributed to differential maternal effects. The observed higher heritable variation thus provides the substrate for further evolutionary change in invasive populations relative to native ones.

Moreover, within each region, we observed significant phenotypic differentiation between populations (nonnull Q_{st}) for a number of phenotypic traits; and population differentiation was generally higher for phenotypic traits than for selectively neutral markers ($Q_{st} > F_{st}$, Fig. 1d). This higher population differentiation in phenotypic traits indicates that invasive populations experience divergent selection regimes within each region (29), despite the observed high gene flow (indicated by low F_{st}) that should homogenize phenotypes between adjacent populations. Thus, it appears that invasive populations of reed canarygrass have a higher potential response to natural selection than native ones for ecologically important phenotypic traits (i.e., emergence time, tillering rate) and that these same traits are currently under selection. The observed vegetative advantage of invasive genotypes over native ones in greenhouse and North American natural conditions likely results from a response to natural selection after their introduction.

Phenotypic plasticity may also enhance invasion success and could have been selected for in the introduced range of reed canarygrass. In a field experiment where clones of 36 genotypes were transplanted along a moisture gradient, invasive genotypes exhibited higher phenotypic plasticity than European native genotypes for stem height, leaf number, and morphological principal component (Fig. 5b). Phenotypic plasticity allows reed canarygrass to compete for resources and space under a wide

range of moisture conditions, as observed in invaded habitats (26). Our data thus conform to a scenario where both increased phenotypic plasticity and response to selection contributed to the invasive ability of reed canarygrass after its introduction in North America.

Discussion

Multiple introductions of a species into a new region may thus inadvertently increase its evolutionary potential and allow for rapid evolution and geographic spread in the region of introduction. In reed canarygrass, multiple introductions of European strains have occurred in North America since the mid-19th century. This has resulted in the continental-wide genetic diversity of reed canarygrass in its European range being redistributed and recombined into introduced North American populations, yielding a number of novel genotypes. This not only alleviated the consequences of genetic bottlenecks, including in peripheral invasive populations, but also increased reed canarygrass's genetic diversity and heritable phenotypic variation for ecologically important traits. The resulting high evolutionary potential of introduced populations has stimulated rapid selection for vegetative establishment, clonal growth, and phenotypic plasticity, allowing for rapid range expansion of reed canarygrass in North American wetlands. Reciprocal transplant experienced between North American and European sites will test whether "novel" North American genotypes have higher fitness irrespective of geographic location and whether a lack of natural enemies has selected for vegetative vigor in North American populations.

Because reed canarygrass was introduced to North America for a variety of agronomic and soil-management purposes (26), the increased aggressiveness of reed canarygrass in North America could also have resulted from the introduction and subsequent escape of previously bred agronomic strains. Under this scenario, we would expect to see low genotypic diversity in the introduced range because only a few strains would be represented in North American populations. In contrast, we find very high genotypic diversity in the introduced range. Moreover, previous results showed that invasive populations of reed canarygrass in Vermont are genetically unrelated to widely cultivated varieties of reed canarygrass (30). Finally, the higher vegetative success of invasive genotypes in North American natural conditions and the high phenotypic differentiation occurring between adjacent populations suggest that North Amer-
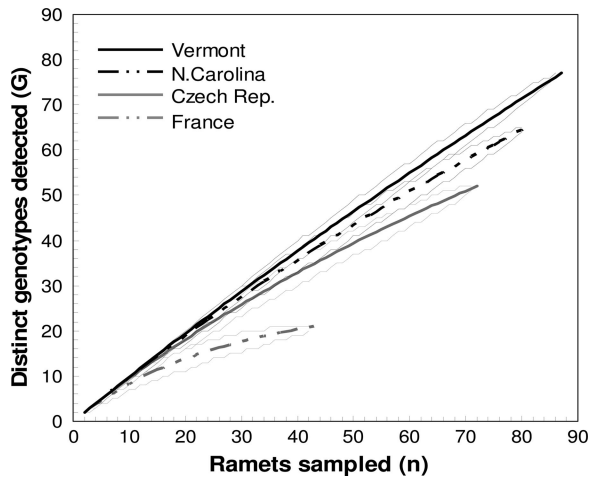
Fig. 3. The number of multilocus genotypes (G) of reed canarygrass detected in central and peripheral regions of its invasive range (Vermont and North Carolina, respectively) and native range (Czech Republic and France, respectively), as a function of sample size. Dot–dash lines represent 95% confidence limits obtained by randomly generating 50,000 replicates of G for every sample size. This permutation procedure was used to assess the robustness of regional patterns of genotypic diversity to unevenness in sample size.

ican populations of reed canarygrass currently experience selection pressures. The fact that spatially variable selection pressures are coupled with high gene flow (as indicated by low F_{st}) has likely maintained high genetic variance and high evolutionary potential within invasive populations (31). Thus, the most likely scenario is multiple introductions and extensive recombination of reed canarygrass' European strains promoting the rapid evolution of invasive ability.

Presettlement populations of reed canarygrass in North America may also have contributed to the invasive populations. Although we cannot completely rule out the mixing of presettlement reed canarygrass and more recent agronomic strains, we have found only anecdotal evidence for the presence of these presettlement populations of reed canarygrass in North America and no evidence at all concerning the eastern United States (26, 32, 33). Moreover, our data demonstrate that at least 85% of the genetic diversity present in North American populations has a European origin and not ≈50% as expected under a scenario of a random mixing between native and introduced genotypes. Thus, North American invasive populations of reed canarygrass may primarily result from the multiple introductions of European strains that started with early European settlers and were repeated several times throughout the mid-19th century (32, 33). Our data also show that introduced strains were drawn from disparate European regions, which created the template for original genetic recombination after introduction.

Conclusion

The major implication of our work is that a species' immigration history can have profound consequences for fundamental evolutionary processes regulating its geographic range. First, depletion of genetic diversity at the periphery of a species range is generally thought to slow down its geographic spread; but, large scale and repeated introductions may overcome genetic bottlenecks and create genetically diverse peripheral populations that have the potential for continued range expansion. Second, repeated introduction may allow the emergence of genetic novelties that are necessary to exploit new environments by increasing the probability of recombination between introduced conspecific genotypes (22, 34) or between interfertile introduced species (35). Finally, our results shed light on the relative roles of drift and natural selection after colonization events and suggest that natural selection can be stronger than drift in
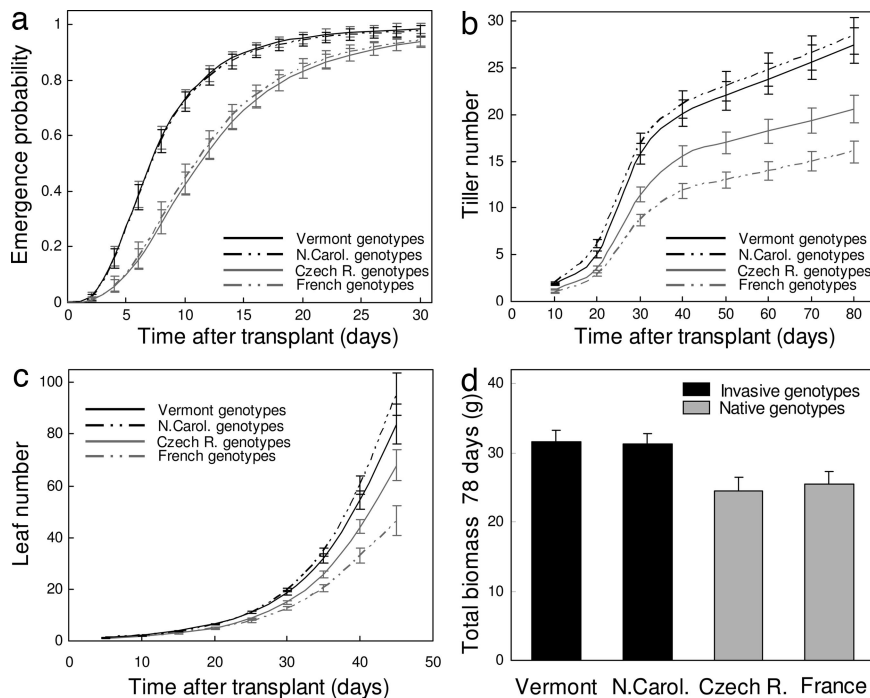

Fig. 4. Dynamics of emergence (*a*), vegetative spread (*b*), leaf production (*c*), and final biomass production (*d*) of 90 genotypes sampled in the four study regions: Vermont, North Carolina, the Czech Republic, and France. Means and standard errors (error bars) were back-transformed fitted values from generalized linear models (see *Materials and Methods* and SI Table 2 for model structure). Best models explaining the data were determined by AIC-based model selection (see SI Table 2).
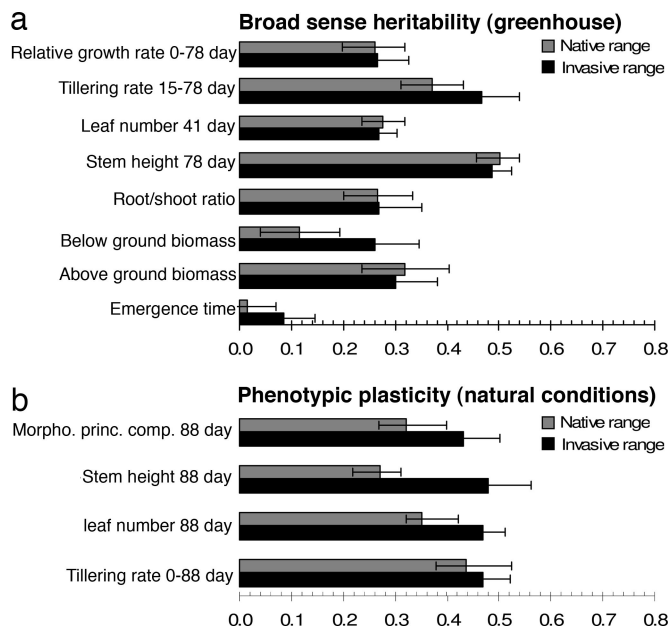
a

Broad sense heritability (greenhouse)

- Relative growth rate 0–78 day
- Tillering rate 15–78 day
- Leaf number 41 day
- Stem height 78 day
- Root/shoot ratio
- Below ground biomass
- Above ground biomass
- Emergence time

Native range
Invasive range

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8

b

Phenotypic plasticity (natural conditions)

- Morpho. princ. comp. 88 day
- Stem height 88 day
- leaf number 88 day
- Tillering rate 0–88 day

Native range
Invasive range

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8

Fig. 5. Broad-sense heritability (*a*) and phenotypic plasticity (*b*) of phenotypic traits within populations of reed canarygrass sampled from its invasive and native range. (*a*) Broad-sense heritability was computed from a greenhouse experiment using clones of 90 genetically distinct genotypes, as the ratio $H^2 = V_G/(V_G + V_E)$, where V_G is the within-population genetic variance and V_E is the environmental variance. Error bars represent 95% confidence intervals obtained by bootstrapping 1,000 draws of genotypes. (*b*) Phenotypic plasticity was computed from a field experiment, where clones of 36 genotypes were transplanted along a moisture gradient, as the ratio $PV = (V_{GxE} + V_E)/(V_{GxE} + V_E + V_G)$, where V_{GxE} is the variance associated with genotype by environment interactions, V_E is the environmental variance, and V_G is the genotypic variance. Error bars represent 95% confidence intervals obtained by jackknifing over genotypes. Morphological principal component (Morpho Princ Comp) is the score on the first axis of a PCA performed on all morphological traits.

recently founded populations, which contrasts with the classic assumption that Fisherian evolution may be negligible during colonization processes (36, 37).

From the perspective of invasive species management, our results caution against repeated introductions of nonnative invasive species. Although a single introduction may not necessarily result in a harmful invader, repeated introductions of a single species can result in unplanned recombination events and inadvertently create novel genotypes that have higher aggressiveness than their native counterparts. This may be particularly problematic (*i*) for agronomically or horticulturally important species that have been previously inbred for various desirable traits and (*ii*) for species that can reproduce both sexually and clonally because sexual reproduction promotes recombination, and clonal reproduction preserves successful combinations. Finally, the enhanced genetic variation of multiply introduced invasive species may enable them to evolve in response to changing climate, allowing them to have increasing impact on native communities and ecosystems in the future.

Materials and Methods

Study Species and Sampling Locations. Reed canarygrass (*P. arundinacea* L., Poaceae) is a 1- to 2-m-tall, cool-season perennial grass, with a C3 photosynthetic pathway and an out-crossing mating system. Reed canarygrass is native to circumboreal regions of Eurasia and, probably, to a small fraction of western North America (for an exhaustive review, see ref. 26). The species was repeatedly introduced into North America after the

mid-19th century for agronomic purposes, soil management, and wastewater treatment (26).

In 2002, we sampled 20–30 rhizome pieces per population in the Czech Republic (four populations near Trebon, centered on 49°00′ N, 14°46′ E) and France (three populations near Montpellier, centered on 43°37′ N, 3°52′ E), thus located at the center and the southern margin of the European native range, respectively. In 2003, we sampled in Vermont (three populations near Burlington, centered on 44°28′ N, 73° 9′ W) and in North Carolina (three populations near Franklin, centered on 35°19′ N, 83°38′ W), regions that are located at the center of the invasive range of reed canarygrass and at the southern migration front. The sampled 350 rhizome pieces were transplanted in a greenhouse and vegetatively propagated twice before experiments to alleviate maternal effects.

Eurasian reed canarygrass is mostly represented by a diploidized tetraploid cytotype ($2n = 42$) distributed throughout temperate regions and by a hexaploid cytotype ($2n = 64$) restricted to the Iberian Peninsula. Using flow cytometry, we found that populations of Vermont, North Carolina, and the Czech Republic are composed of tetraploid cytotypes (mean DNA 2C values ± SE: 9.14 ± 0.06 pg, 9.25 ± 0.05 pg, and 9.35 ± 0.05 pg, respectively). Among French populations, two were made up of tetraploid cytotypes (mean DNA 2C value ± SE: 9.31 ± 0.08), whereas the third one was hexaploid (mean DNA 2C value ± SE: 13.49 ± 0.12). For at least the eastern part of its invasive range, reed canarygrass is thus composed of tetraploid cytotypes, implying that invasive populations must be compared with European tetraploid ones. The finding of a hexaploid population in southern France suggests that French populations occur at the southern margin of the native range of the reed canarygrass tetraploid cytotype. Thus, we discarded the European hexaploid populations for the rest of the study to compare central and southern margin range populations in both the invasive and native range of the tetraploid cytotype. Because the tetraploid cytotype of reed canarygrass is an allotetraploid cytotype with diploid segregation, i.e., forming 14 bivalents in meiosis (26), codominant markers such as allozymes can be used to infer population genetics processes without any problems with interpretation. Multiple banding or expression disequilibrium, typical of gene duplication and nondiploid segregation, was never observed in this species (30).

Genetic Analyses. We conducted starch gel enzyme electrophoresis on leaf tissue from each plant, using 12 allozyme loci (see protocols in *SI Methods*). We computed the percentage of polymorphic loci and the allelic richness for each population and each region. Unbiased Nei's estimate of genetic diversity (Ht) and between-population genetic diversity $F_{st\ (pop)}$ were computed separately for every region [Goudet J (2001) available from www2.unil.ch/popgen/softwares/fstat.htm]. In each population, we computed the number of multilocus genotypes G, the genotype richness $R = (G - 1)/(N - 1)$ and an unbiased Shannon–Wiener index of genotypic diversity (38). To account for potential biases resulting from unequal sample sizes, we assessed the relationship between sample size and the number of genotypes G detected within each study region. This indicates whether sample sizes are big enough to detect differences of genotypic diversity between study regions. This was done by jackknifing the data set with increasing sample size and generating 50,000 replicates of G for each region. Multilocus genotypic structure was compared between invasive and native populations by using multinomial models to depict frequencies of interlocus allelic associations occurring in each range.

Greenhouse Experiment. Among the 210 genotypes identified overall, we randomly selected a subsample of 90 genotypes (49 invasive and 41 native genotypes) and grew four identical clones

of each genotype in a common greenhouse environment for 78 days (see *SI Methods*). Emergence and morphological variables (leaf and tiller number, maximum tiller height) were measured periodically until final harvest, when above-ground, below-ground, and total dry biomass were determined. Maternal effects are likely very low because every clone used is at least a second-generation transplant from the plants that were collected in natural conditions. Broad-sense heritability of early traits (time to emergence, tiller height at 15 days, leaf and tiller number at 15 days) was consistently very close to zero ($0.016 <$ $H^2 < 0.10$), indicating very low, if any, maternal effects.

We determined whether phenotypic traits of reed canarygrass genotypes varied consistently between ranges or between regions by fitting hierarchical mixed-effects models (39), with genotype nested within populations as a random effect, and with three possible fixed effects to account for the three alternative models: null model, range effect, or region effect. Null models were an intercept model for the traits measured at final harvest and an intercept function of time for measurements repeated over time (SI Table 2). Models were fitted by maximum-likelihood, and the best model explaining the data was determined by AIC-based model selection (40). Models were fitted with a distinct variance parameter for every population, with a normal error for all variables with the exception of emergence probability (binomial error). An autoregressive correlation structure was added in the model for measurements repeated over time.

Within the invasive and native range, we partitioned a phenotypic trait's variance among regions, populations, and genotypes, using a procedure of hierarchical variance partitioning. We calculated the broad-sense heritability (H^2) of phenotypic traits by extracting between-genotype and environmental (residual) variance within all populations (see Fig. 5 legend). We also computed the proportion of traits variance explained between populations within each region ($Q_{st(pop)}$). Variance components were computed by restricted maximum likelihood, and confidence intervals of H^2 and $Q_{st(pop)}$ were determined by drawing 1,000 bootstraps over genotypes (41, 42).

Field Experiment. From the 90 study genotypes, we randomly chose a subset of 36 genotypes (18 invasives and 18 natives) and transplanted nine clones of each genotype into nine randomized blocks, along a wetland–wet meadow ecotone in Vermont (see *SI Methods*). Morphological variables (leaf number, tiller number, and maximum tiller height) were measured periodically for 88 days. They were analyzed separately for every date by fitting linear mixed-effects models (39) with block as a random effect, and the corresponding phenotypic trait was measured for the same genotype in the greenhouse as a fixed effect. We also compared the phenotypic plasticity along the moisture gradient of invasive and native populations for the three morphological traits measured 88 days after transplantation and for a principal morphological component (score on the first axis of a PCA performed on all morphological traits). Phenotypic plasticity was estimated as the proportion of phenotypic variance explained by soil moisture (see *SI Methods*) and genotype by soil moisture interactions (43), by using a general linear model with soil moisture, genotype, and soil moisture by genotype interaction as main effects. The plasticity confidence interval was computed by jackknifing over genotypes.

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