Origin of the invasive Arundo donax (Poaceae): a trans-Asian expedition in herbaria

Laurent Hardion1,*, Régine Verlaque1, Kristin Saltonstall2, Agathe Leriche1 and Bruno Vila1

1Institut Méditerranéen de Biodiversité et d’Écologie, UMR CNRS IRD, Aix Marseille Université, 13331 Marseille, France and 2Smithsonian Tropical Research Institute, Apartado 0843-03092, Panama

* For correspondence. E-mail laurent.hardion@imbe.fr

INTRODUCTION

Human activities have disrupted the distribution ranges and dispersal barriers of many organisms, introducing them both deliberately (e.g. for food, gardening, construction or erosion control) and accidentally around the globe (Elton, 1958; Pysek and Richardson, 2006). Some species have become invasive and constitute a major threat to global biodiversity by competing with native species for ecosystem resources (Shea and Chesson, 2002). These alien taxa can also cause significant damage and economic losses to human activities (Pimentel et al., 2005). To control and reduce their impacts, specific knowledge is needed regarding not only their biology and ecology (Dandelot et al., 2005; Blight et al., 2012), but also their genetic and taxonomic delimitation (Saltonstall, 2002; Verlaque et al., 2011), and their geographical origins (Lafuma et al., 2003; Verlaque et al., 2003). Indeed, the designation of weed origin can be extremely useful from a management perspective as it can assist with limiting introduction vectors, studying the adaptive potential of invasive species in their native environments and informing biological control efforts (Clay, 2003).

Among alien species, the literature distinguishes archaeophytes, i.e. species introduced between the beginning of Neolithic agriculture and the discovery of the Americas (approx. 1500 AD), from neophytes, i.e. taxa spread after 1500 AD (Pysek et al., 2004a). In addition, several archaeophytes have become neophytes, following secondary introductions from Eurasia and Africa to the Americas (La Sorte and Pysek, 2009). However, many assumptions of ancient introductions remain hypotheses needing validation by further investigation (Preston et al., 2004). For many archaeophytes, the literature cannot designate a ‘native’ or ‘introduced’ status, a position termed ‘cryptogenic species’ (Carlton, 1996). Thus, the origins of archaeophytes are often difficult to identify, even using phylogenetic investigations. Nevertheless, several distinctive generalizations can be used to help identify ancient introductions: (1) many archaeophytes were introduced with crop translocations, and their affinities for agricultural or human-disturbed habitats are always noticeable despite millennia of occurrence (Pysek et al., 2005); (2) due to their ancient presence, archaeophytes are generally more widespread than neophytes (Preston et al., 2004; Pysek et al., 2004b); and (3) in their introduced ranges, alien taxa often contain lower genetic diversity than in their native area due to founder effects and genetic drift following their introduction (Dlugosch and Parker, 2008). However, this genetic pattern may be disrupted by recurrent introductions and admixture of invasive populations or by hybridization and introgression with native taxa (Baumel et al., 2002; Suehs et al., 2004).

Among invasive species, Arundo donax (Poaceae) represents one of the most aggressive plant taxa in sub-tropical and temperate...
wetlands. This giant cane is a tall (up to 6 m), rhizomatous grass considered invasive in many warm regions, including Oceania, Africa and the Americas (Herrera and Dudley, 2003) where it has invaded mainly riparian areas via clonal growth and dispersal (Wijte et al., 2005). Described from Spain and south-eastern France by Linnaeus in 1753, Arundo donax has generally been considered as native to sub-tropical Eurasia (including the Mediterranean Basin, Middle East, northern India and East Asia), but the origin of invasive populations remains unknown. As an emblematic species of Mediterranean landscapes, this robust reed mainly occurs in human-disturbed habitats, including agrosystems, wastelands, and riparian and coastal zones. Since antiquity, giant cane has had multiple human uses, such as agriculture, fodder, construction, weaponry, fishing, hunting, music, erosion control, medicine and fuel (Perdue, 1958; Tackolm and Drar, 1973; Postgate, 1980). In addition, this species is one of the most promising biomass crops for biofuel production, leading to an increasing interest on its genetic characterization (Pilu et al., 2014; Sablok et al., 2014).

Previous work on A. donax has revealed low genetic diversity across broad geographical areas. Ahmad et al. (2008) tested 185 putative clones of A. donax from the southern USA using SSR-based molecular markers, transposable element (TE)-based molecular markers, and found only one widespread genotype that was similar to four populations from southern France. Further, Hardion et al. (2012) used AFLP (amplified fragment length polymorphism) fingerprints to show the occurrence of only one clone among 16 localities around the Mediterranean Basin. This lack of genetic diversity calls into question the native status of A. donax in the Mediterranean, and redirects research of native populations toward Asia. Using AFLP and inter-simple sequence repeat (ISSR) markers on 67 Mediterranean and ten Asian herbarium specimens, Mariani et al. (2010) showed support for a monophyletic origin of A. donax in Asia. This eastern origin is also supported by the botanical literature, which reports lower cytotypes (2n = 12x approx. 72) in Thailand (Larsen, 1963), India (Christopher and Abraham, 1971; Mehra and Kalia, 1975) and Uzbekistan (Bochantseva, 1972) than in the Mediterranean region (2n = 18x approx. 108; Pizzolongo, 1962; Gorenflo et al., 1972; Hardion et al., 2011, 2013; Bucci et al., 2013). Further, although seed production has not been detected in the Mediterranean or the USA (Johnson et al., 2006; Hardion et al., 2012), caryopses have been found in Iran and Afghanistan (Bor, 1970), China and Pakistan (Brach and Song, 2006). The non-fruited character of Mediterranean populations has been attributed to their high ploidy level which may disrupt meiotic processes (Balagh et al., 2012).

The widespread ruderal distribution of a single non-fruiting clone leads us to suspect an ancient introduction of A. donax to the Mediterranean Basin. Here we test the hypothesis of a founder effect linked to this introduction from Asia to the Mediterranean using plastid DNA genetic markers. Secondly, we characterize and localize the invasive lineage among the Eurasian distribution of A. donax using genetic, morphometric and seed production data. Finally, we develop a bioclimatic species distribution model calibrated on Mediterranean occurrence data to localize potential Asian origins for the worldwide invasive genotype. Due to difficulties in obtaining fresh specimens across the Asian range of A. donax, our sampling is based on herbarium specimens from European collections. In order to extract the most information from these specimens, we set up a three-tiered integrative approach: (1) phylogenetics, using sequencing of plastid DNA micro-/minisatellites and substitutions; (2) morphometry, including stomatal length as an estimation of ploidy; and (3) ecology, using ecological niche modelling.

**MATERIALS AND METHODS**

### Plant collections

The Eurasian sampling of Arundo donax was divided into three inclusive datasets, according to the markers used: (1) 57 well-preserved herbarium specimens suitable for molecular analyses; (2) a broader collection of 127 herbarium specimens with leaves and panicle used for morphometric measurements (Supplementary Data Table S1); and (3) a large dataset of 3429 occurrences for A. donax in the Mediterranean region. Provided by B, BM, E, G, K, MARS, P and W Herbaria, these valuable specimens were collected from the 18th century to the present, in particular by some pioneering botanists in Asia such as V. Jacquemont (1757–1836), J. G. König (1728–1785), T. Thomson (1817–1878) and J. S. Gamble (1847–1925). We also included three samples of invasive A. donax collected from New Caledonia (Oceania), Peru (South America) and Arizona, USA (North America), and seven samples of the Taiwanese endemic Arundo formosana (2n = 12x approx. 72; Hardion et al., 2013) which is a close relative of A. donax (Hardion et al., 2012).

### DNA extraction, sequencing and phylogeographic analysis

DNA extractions were performed on samples collected after 1930 to avoid unnecessary destruction of older specimens and sequencing of highly degraded DNA (Telle and Thines, 2008). About 50 mg of leaves were mechanically ground after treatment with liquid nitrogen. Total DNA was extracted following Doyle and Doyle (1987). DNA concentrations were estimated using a Biophotometer (Eppendorf, Germany) and diluted to 50 ng μL⁻¹.

Plastid DNA diversity was screened on five intergenic spacers: trnT–trnL (Taberlet et al., 1991), trnCF–rpoB, psaA-ORF170, rbcL-psaI and trnS(GCU)–psbD (Salontall, 2001). Polymerase chain reactions (PCRs) were performed in 50 μL volumes containing 1 × PCR buffer [10 mM Tris–HCl, 50 mM KCl, 0.001 % (w/v) gelatin], 1.5 mM MgCl₂, 2.5 mM each dNTP, 40 pmol each primer, 0.1 μg μL⁻¹ bovine serum albumin (BSA) and 2.5 U of Taq polymerase (Qiagen, Illkirch, France). The thermal cycling profile was programmed on a PTC-200 Gradient Thermal Cycler (MJ Research, Watertown, MA, USA) as follows: 2 min at 94 °C followed by 35 cycles of 94 °C for 1 min, 56 °C annealing for 1 min, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. Purification and sequencing of PCR products were carried out by Eurofins MWG Operon (Ebersberg, Germany).

The five plastid DNA regions were manually aligned on five intergenic spacers: trnT–trnL (Taberlet et al., 1991), trnCF–rpoB, psaA-ORF170, rbcL-psaI and trnS(GCU)–psbD (Salontall, 2001). Polymerase chain reactions (PCRs) were performed in 50 μL volumes containing 1 × PCR buffer [10 mM Tris–HCl, 50 mM KCl, 0.001 % (w/v) gelatin], 1.5 mM MgCl₂, 2.5 mM each dNTP, 40 pmol each primer, 0.1 μg μL⁻¹ bovine serum albumin (BSA) and 2.5 U of Taq polymerase (Qiagen, Illkirch, France). The thermal cycling profile was programmed on a PTC-200 Gradient Thermal Cycler (MJ Research, Watertown, MA, USA) as follows: 2 min at 94 °C followed by 35 cycles of 94 °C for 1 min, 56 °C annealing for 1 min, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. Purification and sequencing of PCR products were carried out by Eurofins MWG Operon (Ebersberg, Germany).

The five plastid DNA regions were manually aligned in MEGA 5.05 (Tamura et al., 2011). Haplotype relationships were inferred using the median-joining network algorithm implemented in Network 4-6 (Bandelt et al., 1999). Insertion/deletion (indel) sites (including repetitive regions as mini- and microsatellites) were reduced, considered as a fifth state and weighted as one-tenth of a substitution. This down-weighting is classically adopted for hypervariable sites with high probabilities
of homoplasy (Saltonstall and Lambertini, 2012). In order to distinguish haplotypes based on substitution patterns from those supported by hypervariable sites, the same analysis was also generated without considering indels.

Morphometry

Seven morphometric variables previously used in resolving the taxonomy of Mediterranean Arundo were measured on spikelets collected in the middle part of the panicle: number of flowers per spikelet, and lengths of lower and upper glumes, lemma, palea, lemma awn and hairs (Hardion et al., 2012). As leaf epidermal structures are highly resolving in grass systematics (Prat, 1932; Grass Phylogeny Working Group, 2001) and can also provide information on polyploid events (e.g. in Phragmites; Hansen et al., 2007), we also measured three characters under light microscopy (Dialux 20, Leitz, Welterz, Germany): length of stomatal guard cells; stoma density (per 10^4 μm²); and the number of prickles per millimetre of rib line. To avoid damaging herbarium specimens, leaf epidermis peels were prepared with clear nail polish, following Hiltu and Randall (1984), and mounted on slides. All ten variables were measured ten times and averaged for each sample. The occurrence of caryopses was also noted as a qualitative feature. Morphological data were explored using UPGMA hierarchical clustering on Euclidian distances using the ade4 package in R v.2.15 (Thioulouse et al., 1997; R Development Core Team, 2013), after replacement of the few missing values by variable means. For each morphological variable, we used a Kruskal–Wallis rank sum to test for significant differences between the three groups identified by UPGMA clustering, and the coefficient of determination R² illustrated the proportion of variability explained by this clustering.

Ecological niche modelling

Using ArcGIS 10 (Environmental Sciences Research Institute, Redlands, CA, USA), we created a dataset of A. donax occurrences collected from the literature, herbaria and databases of Mediterranean countries, including Croatia (http://hirc.botanic.hr/fcd/), France (www.silene.eu), Greece (Flora Hellenica database; Strid, 2000), Palestine (http://flora.huji.ac.il) and Spain (www.anthos.es). To reduce spatial bias due to differential search intensities across regions (Segurado et al., 2006), we subsampled this dataset to a grid of 5 arc-min cells (i.e. 0.5 x 0.1°) with random origin, reducing the dataset from 3429 to 1221 occurrences. Based on our biological knowledge of A. donax, we chose five bioclimatic variables from the BioClim database as predictors of its distribution (www.worldclim.org; Hijmans et al., 2005): minimum temperature of the coldest month (BIO6) reflecting potentially lethal frost events; mean temperature of the warmest (BIO10) and coldest (BIO11) quarters, delimiting phenological stage for biomass production; precipitation of the driest month (BIO14) indicating potentially lethal drought events; and precipitation of the warmest quarter (BIO18) providing information on water availability during the main period of biomass production.

To model the potential area of origin for Mediterranean A. donax, we used a machine learning algorithm based on presence-only data and implemented in MaxEnt software v. 3.3.3 (Phillips and Dudík, 2008). This method uses the principle of maximum entropy to estimate the most uniform distribution within the study area given the constraint that the expected value of each environmental predictor variable under this estimated distribution matches its empirical average (Phillips et al., 2006). Model outputs correspond to posterior probabilities of habitat suitability. All combinations of type features (linear, quadratic, threshold and product) were investigated to find the best model with 50 replicates (cross-validation, training gain threshold of 0.0001). The best model was chosen as the simplest model with the highest area under the curve (AUC) value and the lowest standard deviation among the 50 replicates. After calibration on the 1221 Mediterranean occurrences, this model was then used on 5 arc-min cells located from 15 to 50°N in latitude to detect suitable bioclimatic conditions across Eurasia.

RESULTS

Phylogeographic structure

After indel reduction, the plastid DNA alignment of 4518 bp contained 77 variable sites, i.e. 44 substitutions and 33 indels including ten microsatellites, eight minisatellites and three inversions (2, 3 and 5 bp). This dataset distinguishes 13 haplotypes within A. donax (Fig. 1A), which reduces to nine after the removal of hypervariable sites (Fig. 1B). Based on the branching position of other Arundo spp., A. donax appears to be polyphyletic and divided into eastern and western lineages (Figs 1 and 2A). Haplotype diversity is divided into four biogeographic clusters which differentiate along the Himalayas, i.e. Middle-East (four haplotypes M), Western Himalaya (two haplotypes W), Central Himalaya (three haplotypes C) and Eastern Himalaya–China (four haplotypes E) (Fig. 1A). These patterns are also supported when considering only substitutions, although the number of haplotypes in the Middle East cluster is reduced to one which groups with haplotypes in the Western Himalaya cluster (Fig. 1B). Haplotype M1 was found in the 28 samples from the Mediterranean and Irano-Touranian regions (Fig. 2A), and its nearest relatives (M2, M3 and M4), which are distinguished only by hypervariable sites, are located in Afghanistan and Pakistan (Indus valley). The haplotype M1 was also found in invasive samples from New Caledonia, Peru and the USA.

Morphometric differentiation

The UPGMA hierarchical clustering of morphological characters also divided A. donax into two clusters along a longitudinal gradient (Fig 2A, B): (1) the Mediterranean and Persian morphotype T1 corresponding to A. donax sensu stricto, with the largest morphometric sizes and exclusively associated with plastid DNA haplotype M1; and (2) the smaller Sino-Himalayan morphotype T2 (Fig. 3). The larger stomatal guard cells of the morphotype T1 (mean 38.4 ± 4.3 μm) significantly differ from those of the morphotype T2 (mean 29.5 ± 3.8 μm) and A. formosana (mean 25.5 ± 2.5 μm; Figs 3 and 4). In addition, the leaf epidermis of Sino-Himalayan morphotype T2 is characterized by numerous prickles and long hairs, whereas those of morphotype T1 show few prickles and not any long hairs (Fig. 4). Although the majority of samples were collected during the autumn months (i.e. flowering period), seed-set was found in only 12 samples of the morphotype T2 from the Middle-East
Based on 1221 occurrences, the ecological niche model for *Arundo donax* in the Mediterranean projected high importance of bioclimatic variables BIO10 (mean temperature of the warmest quarter), BIO14 (precipitation in the driest month) and BIO6 (minimum temperature of the coldest month). The most parsimonious model was obtained using only linear features (test AUC value = 0.90, AUC standard deviation = 0.0065, gain threshold reached after 180 iterations). Projected on Asian bioclimatic data, this model suggests the highest occurrence probabilities reached after 180 iterations). Projected on Asian bioclimatic value

**DISCUSSION**

**Genetic uniformity in the Mediterranean**

Previous work using AFLPs has questioned the native status of *Arundo donax* in the Mediterranean, due to its genetic uniformity and lack of seed production (Hardion et al., 2012). Similarly, this study finds no genetic variation in Mediterranean *A. donax* across several plastid DNA loci and, further, we document the expanded range of this clone to the Middle East and other recently invaded regions worldwide. To date, such an absence of genetic diversity in plastid and nuclear markers has rarely been shown for such a widespread plant species. Based on equivalent markers and sampling, the nearest plant model is represented by *Pinus pinea*, which has a single Mediterranean-wide haplotype and three other locally restricted haplotypes (Vendramin et al., 2008). However, those results were explained not only by plant clonality but also by demographic bottlenecks linked to human-mediated dispersal. Such patterns of genetic uniformity are more common in invasive species, such as *Spartina anglica* and *Pennisetum setaceum* (Poaceae), which also display clonal reproduction, polyploid genomes and low genetic diversity (Baumel et al., 2001; Le Roux et al., 2007). Despite low mutation rates and preponderant clonality in *Arundo* (Hardion et al., 2012), our results highlight haplotype variation and phylogeographic structure across the Asian range of *A. donax*. Consequently, human-mediated dispersal and its invasive ability remain prevailing explanations for the widespread Mediterranean distribution of this non-fruiting clone.

**Restricted origin of a worldwide invasive clone**

The nearest relatives of the invasive haplotype M1 are found in Afghanistan and Pakistan, along the Indus Valley (haplotypes M2, M3 and M4). These phylogenetic affinities are reinforced by sequence differences seen only in hypervariable sites (i.e. mini- and microsatellites), with mutation rates approx. 10^5-fold higher than plastid DNA substitutions (Cozzolino et al., 2003). Further, species distribution modelling predicts suitable bioclimatic conditions for the Mediterranean clone along the Indus Valley and in south-western Iran, where seeds were found, but also to the south of the Caspian Sea. Consequently, this study clearly supports a Middle East origin for the Mediterranean clone of *A. donax*. However, the robust morphotype T1 contrasts with the morphotype T2 which is also found in this region. This mismatch between morphometric and phylogenetic clustering could be a consequence of gigantism associated with differences in ploidy level from fruiting lower cytotypes, as seen between *A. plinii* and *A. donaciformis* in the Mediterranean (Hardion et al., 2012). This polyploid differentiation is supported by stomatal sizes, which clearly distinguish the two morphotypes within *A. donax*, probably corresponding to its two ploidy levels: 2n = 18x approx. 108 in the Mediterranean (Gorenflot et al., 1972; Hardion et al., 2011, 2013) and 2n = 12x approx. 72 in Asia (Larsen, 1963; Christopher and Abraham, 1971; Bochantseva, 1972; Mehra and Kalia, 1975). Following this hypothesis, the seed production of the morphotype

![Fig. 1. (A) Combined plastid DNA network of the genus *Arundo* based on substitutions and mini-/microsatellites, and (B) on substitutions only. Haplotypes are colour coded; their character code corresponds to Supplementary Data Table S1; their size correspond to the number of individuals detected in the dataset. Small black circles indicate haplotypes not detected in the dataset.](image-url)
**Fig. 2.** (A) Geographical distribution of plastid DNA haplotypes and morphotypes. White circles, *A. donax* morphotype T1; grey circles, *A. donax* morphotype T2; black circles, *A. formosana* morphotype; *, seed occurrence. Coloured rings correspond to plastid DNA haplotypes (Fig. 1). (B) UPGMA tree based on morphological data. (C) Ecological niche modelling of *A. donax* calibrated on 1221 Mediterranean occurrences (black dots) and projected on sub-tropical Eurasia using MaxEnt.

**Fig. 3.** Boxplots of morphological variables between *A. donax* T1 (*A.d. T1*), *A. donax* T2 (*A.d. T2*) and *A. formosana* (*A.f.*), i.e. number of flowers per spikelet (nbF), and length (mm) of lower glume (G1), upper glume (G2), lemma (L), palea (pa), lemma hair (pL) and awn (A), length of stomatal guard cells (X; µm), stoma density (dX; per 10^4 µm^2), number of rib prickles per µm (dP, per mm of rib line). Grey numbers, numbers of measures per variable per morphotype; bold lines, median values; dashed lines, entire variable range; R^2, coefficient of determination; ***Kruskal–Wallis P-value < 0.0001.
T2 could be linked to its lower polyploidy level of $2n = 12x$ approx. 72. Further studies including extensive sampling of living material across the Middle East are needed to distinguish closer fruiting relatives from the invasive clone using chromosome counts and highly variable molecular markers.

**One of the oldest invasive species?**

The use and trade of reeds by human civilizations since the Neolithic has been documented across scholarly fields (Täckholm and Drar, 1973; Postgate, 1980; Faiella, 2005) and in numerous Greek and Roman texts summarized by Pliny the Elder. For example, several Sumerian tablets mentioned thousands of bundles of reed culms being imported across Mesopotamia since 2500 BC (Joannes et al., 2001). Among the reeds, *A. donax* is an easily transplantable and highly productive species offering an exceptional combination of culm robustness, lightness and flexibility (Perdue, 1958). Numerous plant species have been dispersed by humans from south-western Asia to the Mediterranean since the early Neolithic (Zohary et al., 2012). As a consequence, phylogeographic studies on European domesticated species often exhibit phylogetic origin or diversity in the East, mainly from the Levant, e.g. olive trees (north Levant, Besnard et al., 2013) and wheat (Kilian et al., 2007), but also from the Middle East, e.g. grapevine (Caspian region, Arroyo-García et al., 2006) or the wheat D-genome (*Aegilops tauschii*, north-eastern Iran, Saeidi et al., 2008), and Central Asia, e.g. domesticated apple (Cornille et al., 2012). Joining the list of ancient introductions from south-western Asia to the Mediterranean, the invasive clone of *A. donax* could even represent one of the oldest plant invasions, in accordance with its broad naturalized distribution.

**Conclusions**

The present study confirms the genetic uniformity of *A. donax* from the Mediterranean to Iran, supporting the hypothesis of its ancient introduction from eastern to western Eurasia. The three datasets used here suggest that this archaeophyte, i.e. alien species introduced before 1500 AD, originates from the Middle East and was probably introduced to the Mediterranean Basin in antiquity. In recent times, it has also become a neophyte as it has subsequently been introduced around the world to other locations with similar bioclimatic conditions. Due to its high level of invasiveness and continued spread worldwide, this clone may represent one of the oldest and most persistent biological invasions. Because human selection and polyploid differentiation could have played a crucial role in the current range of *A. donax*, further studies dealing with phylogeographic, cytogenetic and archaeological aspects of its distribution should resolve the human-mediated and evolutionary history of this clonal reed species across Eurasia.

**SUPPLEMENTARY DATA**

Supplementary data are available online at [www.aob.oxfordjournals.org](http://www.aob.oxfordjournals.org) and consist of Table S1: plant sampling list, geographical origin, herbarium barcode, plastid DNA haplotype and morphotype assignments. Table S2: GenBank accession numbers.

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**LITERATURE CITED**


