ABSTRACT

Wild grapevine (Vitis vinifera ssp sylvestris) is considered to be nearly extinct in its western range as a result of the expansion of American diseases combined with drastic hydraulic works in the region. In such a context, the discovery of a population of 120 individuals, offered an excellent opportunity to evaluate the status of the plant at the current edge of its western range. We focussed on the distribution patterns and the reconstruction of the history of the population through a combination of ecological and genetic data.

Fifty-one individual plants present in a forest plot of 9.72 ha were studied. For each individual, geographical, morphological and growth strategy data were collected. Individuals were genotyped at 14 SSR loci.

Most grapevines were clumped and their density varied between forest plots with different management over the past few centuries. Overall, the number of stems possessed by each plant ranged from one to nine. Heights varied from 14 to 24 m, with diameter ranging from 0.9 to 4.6 cm (mean = 2.4 cm).

The analysis of the genetic data showed that (i) no cultivar or rootstocks were present in the population; (ii) out of 57 individuals considered in this study, six were clones; and (iii) all 14 loci were polymorphic. Six groups were shown to be significantly related.

In spite of a relatively high-genetic diversity, the population is currently at a critical state at the local scale because of the low chances of seedling survival for more than 1 year. The clones were also directly destroyed by human management.

The ideal way to improve the status of the wild grapevine in the Basseé Forest would be to re-create zones of erosion as well as to create a strict conservation area or reserve encompassing the entire region. Copyright © 2009 John Wiley & Sons, Ltd.

INTRODUCTION

The historic natural range of the wild grapevine (Vitis vinifera L. ssp sylvestris, Gmelin, Hegi) was relatively vast in Eurasia. The plant was distributed around the Mediterranean basin between the 43rd and 49th northern parallels, from sea level up to an altitude of 1000 m. The wild grapevine is a dioecious woody vine (a liana), using tendrils to climb up to 30 m (Oberdorfer, 1992). It has entire and trilobate deciduous leaves, which are hairy on the lower face. Female plants produce grapes of black berries. These are 1 cm in diameter and contain up to four round seeds (Hegi, 1966; Arnold et al., 1998). Genetically, the haplotype richness is particularly high in Caucasus and in the Levant (Arroyo-Garcia et al., 2006).

A previous large-scale study on the ecology of the wild grapevine in Europe indicated that the main habitats are gaps and river edges of flood-prone areas (Arnold, 2002). Competition between other large lianas typical of large river plains (e.g. Hedera helix L., Clematis vitalba L., Humulus lupulus L. in western Europe) for light and nutrients is avoided by marked differences in a few species traits (e.g. climbing strategy, requirement for germination and...
establishment of juveniles) and habitat range. The wild grapevine is better adapted to temporal anoxia and immersion than \textit{Hedera helix} and \textit{Clematis vitalba}, but more sensitive to shade and competition with grasses during the establishment phase. After erosive flooding, it has a greater capacity to colonize riverbanks and newly formed gaps similarly to \textit{Humulus lupulus} (Schumann, 1974; Schnitzler, 2007). The latter is however limited in calcareous and coarse-textured soils while grapevines can colonize a wide range of soils (Arnold, 2002).

The wild grapevine has suffered particularly from deforestations, forest and river management, genetic pollution and the expansion of exotic pathogens (oïdium: 1845; phylloxera: 1863; mildew: 1878). The decline of the liana was dramatic at the beginning of the 20th century (Issler, 1938; Kirchheimer, 1946; Schumann, 1974, 1975) and was further exacerbated by hydraulic works that occurred after the Second World War (de Waal et al., 1999; Innis et al., 2000) and the intensification of forestry. The loss of large areas that were regularly flooded allowed phylloxera to penetrate into the last refugia zones of the wild grapevine so that most of the grapevines died (Ocete et al., 2004a; Ocete et al., 2006). Increasingly drier soils have favoured \textit{Clematis vitalba}, which is now showing an advantage over many plants (Arnold et al., 2005). Other Vitaceae also interfere with the natural habitats of the wild grapevine: grapevine cultivars (\textit{Vitis vinifera} ssp. \textit{vinifera}), rootstocks of American \textit{Vitis} hybrids grapevine and \textit{Parthenocissus quinquefolia} have all escaped from gardens. Some exotic \textit{Vitis} species are also suspected to hybridize with the native Eurasian \textit{Vitis} (Terpo, 1976; Arrigo and Arnold, 2007).

These events led to the addition of the wild grapevine to the IUCN (International Union for Conservation of Nature) list of endangered European plants in the 1980s. Since 2000, it has been considered a critically endangered species. The plant has been strictly protected in some countries including France, the Czech Republic, Spain and Italy. However, recent prospecting (Arnold et al., 1998, 2002; Arnold et al., 2005; Lacombe et al., 2003; Arroyo-Garcia et al., 2006; de Ruffray et al., 2008) indicates that the decline of the plant continues, especially in the western part of its range where human impacts are most dominant, but there still exist remnant populations in remote sites, such as colluvial zones and the forests of small tributaries.

The results of these global interdisciplinary studies of wild grapevine metapopulations in Europe have indicated that the largest dense alluvial populations currently found in Europe are present below a latitude of 45° N: the Donana National Parc (Ocete 2004a); the Pô Delta (Anzani et al., 1990); in Croatia and Slovenia (personal observations); the Basque region of Spain (Ocete et al., 2004b); and the Danube Delta (Arnold, 2002). Only two large alluvial populations are found above a latitude of 48° North: one in Austria along the Danube, currently well studied (Margl and Fraissl, personal communication; Kirchheimer, 1946; Kirchheimer, 1955), and a second, recently discovered (Arnal and Zanré, 1990) in the Seine valley. In order to complete studies of wild grapevines in Europe we focussed on the distribution pattern of the wild grapevine of the Seine on a small scale by using a combination of ecological, genetic and historical data. Based on the results and our knowledge of the ecology of the species we aim to: (i) reconstruct the history of the wild grapevine in the Seine valley, (ii) evaluate the survival chances of the population and (iii) propose priorities for conservation.

This study will also provide information on the criteria that should be selected to optimize the survival of the plant in other floodplain forests in Europe.

THE STUDY SITE

The Bassée National Natural Reserve (854 ha) is a part of the middle Bassée floodplain in the Seine valley (N 48°27’; E 3°19’) (Figure 1), France, and is integrated within the conservation network of Natura 2000. The mean slope of the Seine valley is 0.03% between Nogent-sur Seine and Montereau-Fault-Yonne. The climate is temperate and oceanic (the mean temperature in winter is 4.5°C and the mean temperature in summer is 17.5°C). The mean annual rainfall is 600–700 mm. Flooding occurs at discharge points of more than 250–400 m³/s, mainly during the winter. Spring and summer floods may occur during exceptional climatic events (Currie et al., 2007). Forest soils range from fluvisol to reductisols (hydromorphic pseudogley at 20–40 cm depth) (USDA, 1975).

The natural flooding regime of the river was relatively well preserved until the 1960s (except for small regulated channels used for navigation) but was greatly altered after this period; the lake of the Orient Forest was dug, and there was intensive drainage in the upstream sector of the Bassée, with canalization in the downstream sector. As a result, the river Seine in the Bassée region has lost most of its natural dynamics, particularly in the downstream.
sector (Hydratec, 2001). For example, from 1998 to 2003 the Bassée has been flooded each year and several times in winter and spring, either by a rise of the groundwater table or by surface water, but without any erosive action. From 2003 to 2008, there were no floods (Parisot, personal observation).

Forests cover very small areas (about 325 ha) because of large-scale deforestation in the past. Most current forest plots were recorded in a map generated by Cassini in the 18th century and can thus be classified as ‘ancient forest’ (Hermy et al., 1999), but new forests have recolonized some meadows or pastures after the Second World War due to changes in land use. Ancient forests are composed of oak (Quercus robur L.), ash (Fraxinus excelsior L.) and elm (Ulmus laevis Pall) and can be included in the alliance of the Alno-Padion (Oberdorfer, 1992). Four lianas reach canopy: Hedera helix L., Vitis vinifera ssp sylvestris (Gmelin, Hegi), Clematis vitalba L. and Humulus lupulus L., which develop within the forest or at the forest edges.

Trees are mainly used for firewood, without any management for productivity. Given the number of private owners, forest practices have varied greatly, from coppices with rotations of 25–50 years to coppices associated with the conservation of a few big trees (mainly oak) or a total absence of management for several decades.

After the discovery of wild grapevines in 1990, forest managers and naturalists prospected the entire region (Parisot, 1999). Wild grapevines were thus recorded in four municipalities of the Bassée region (Grisy-sur-Seine, Nogent-sur-Seine, Le Mériot, Saint-Just–Sauvage). The total was less than 120 individuals with the highest density in the Feuchelle forest (municipality of Nogent-sur-Seine). Some of these samples have been recorded in a general study on wild grapevines in France by the INRA, Montpellier.

**METHODS**

For this study, we selected the forest of La Feuchelle, which contains 57 wild grapevine individuals in an area of 9.72 ha. The floristic components of the vegetation and the soil (fluvisol) are homogenous over the entire forest surface (Parisot, 2005). This forest has been divided into 20 private properties numbered from A to T (Figure 2). Plot B was transformed into an agricultural area and was thus removed from the study. The other 19 plots presented various levels of management. Their size varied from a few square metres to a few hectares, with a mean area of 51 acres. Each wild grapevine individual (including five seedlings) found in the study area was mapped and measured. Fifty-one of the 57 individuals could be genotyped. Six individuals were removed from the analysis because their leaves were out of reach and we did not want to injure the main stem in the collection process. Complete analysis of the following aspects was performed on the remaining 51 individuals.

![Figure 1. The study area. This figure is available in colour online at wileyonlinelibrary.com](image-url)
Genetic data

Fifty-one leaf samples were collected and dried in silicagel for DNA analysis (Adams, 1997). The DNA of these 51 grapevine leaves was extracted with the Qiagen DNEasy Plant Mini Kit, following the manufacturer’s instructions. Fourteen microsatellite loci were analysed, but in some cases 9 additional loci were investigated when clones were suspected (Table I). The PCR products were run on an ABI3100 and scored using Genotyper 3.7. Allele sizes were standardized by comparison with known genotypes of standard cultivars. The raw data were compared with a database containing all cultivars and rootstocks (Vouillamoz, personal Vitis SSR database).

Ecological and life-history trait data

Fifty-one individuals were studied according to the methods proposed in Gerwing et al., (2006) and Schnitzer et al., (2008). The following data were taken for each individual: the latitude and longitude coordinates (quantitative), the presence of grapevines in a given plot (nine plots contained grapevines: A, C, D, F, F, H, I, P, T) (binary), the number of shoots (quantitative), height (quantitative) and the circumference of the stem (quantitative). When the grapevine had several stems, only the largest circumference was measured according to Gerwing et al., (2006).

Statistical analysis

The data linked to the ecology and the life-history traits of wild grapevines were first grouped and then standardized. A correspondence analysis (CA) was performed on the global data set to identify general tendencies. To further investigate the tendencies, the data were separated into two matrices. A matrix called ‘Structure’ regrouped the quantitative data linked to life traits (number of shoots, circumference and height). An Euclidian distance matrix was calculated on the 51 individuals. A similarity matrix called ‘Plot’ was calculated with a Jaccard
coefficient on the asymmetric binary data from the nine forest plots where the wild grapevines were found. This matrix was converted into a distance matrix using the transformation $D = 1 - S$. A Mantel test was performed between the distance matrix ‘Structure’ and the distance matrix ‘Plot’.

The genetic data were regrouped in a matrix with the 51 individuals in the rows and the value of both alleles of the 14 microsatellites in the columns.

The microsatellite data were analysed using the GENALEX program (Peakall and Smouse, 2006).

1. The first step was to compare the genetic profiles of the samples with cultivated grapevines in order to detect the presence of escaped cultivars or rootstock in the population.
2. The genetic diversity of the individuals of the Seine population was evaluated by: the number of alleles per locus, minimum and maximum allele size, and heterozygosity.
3. In order to group the individuals most genetically linked, a genetic distance matrix generated in GENALEX was imported in progiciel R (BIOGEO) (Casgrain and Legendre, 2006). Based on the geographic coordinates (latitude/longitude), 134 geographic links between the 51 individuals were obtained by a triangulation of Delaunay with rectangular constraint (progiciel R).
4. Finally, a spatial autocorrelation based on the genetic and geographic data was calculated in GENALEX in order to confirm or infer the presence of a significant genetic relationship at a short distance.

**RESULTS**

*Life-history traits of individuals and distribution in the forest*

The mean density of the grapevines per ha was 5.3, but most individuals were clumped. Among 51 individuals, the number of stems ranged from one to nine. Individuals with more than two stems (nine individuals) were situated along the edges of plots C and B in the agricultural area, as well as along the edge between plots E and D and within plot A. In all cases, the multiplication of stems was probably a response to damage caused by private owners or
farmers. In more aged and better structured forests that have been unexploited for decades, such as plot E, wild grapevines had single stems.

Heights varied from 0.1 (for seedlings) to 26 m, with diameters ranging from 0.1 to 4.6 cm (mean = 2.4 cm) (Photos 1, 2). Vines were smaller in diameter in plots recently exploited by clearcuts (plots D, A, J to S) and larger in unexploited forests, for example, plot E.

The smallest distance between individuals was 5 m, and the maximum was 240 m. Most of the adults had reached the canopy. Of these, 60% were currently present in closed forests and the others developed along forest edges (30%) or in open areas (10%). Seedlings were only present in plot D, which was recently clearcut.

CA results

The Mantel test between ‘Structure’ and ‘Plot’ was highly significant (p < 0.001).

The first three axes of the correspondence analysis explained 86.3% of the total variance (First axis 60%). In the projection of the first two axes of the CA, longitude and number of shoots were positively correlated to the first and the second axis of the CA, respectively. Larger and higher stems seemed to be present on the left hand side of the forest plots, while smaller and lower plants were regrouped in plots A, D and T. Plots C and D were significantly different from the others. The grape individuals seemed to be regrouped in aggregates.

Genetic identification of the individuals

The analysis of the raw data showed that (i) no cultivar or rootstocks were present in the population and (ii) 6 out of the 51 individuals considered in this study were clones (numbers 4-5, 14-15, 23-24, 41-42, 49-50, 53-54-55) (Figure 3). The clones were separated by a distance of 5–10 m and, in many cases, a physical link could be observed between them. The seedlings were grouped within the same clump together with adult plants from adjacent plots (16, 17, 18, 36, 40).

All 14 loci were polymorphic. The allele size for the 14 primers ranged from 135 to 269. The number of alleles per locus varied from 4 to 17 (Table I). The average expected and observed heterozygosity for all loci were
He = 0.63 and Ho = 0.53. The overall genetic diversity of the 51 samples was relatively high. The population was not at a Hardy-Weinberg equilibrium.

**Spatial distribution and genetic relationship between individuals**

Based on the genetic and the geographical distance, six clumps occurred. Individuals were genetically closer to each other in the same clump. Group 1 links two individuals in plot P; Group 2 links 15 individuals in plot A; group 3 links four individuals in plot A; Group 4 links 10 individuals in plots A, B, C and D; Group 5 links 20 individuals; and Group 6 links seven individuals in plots F, G, H, I. Between individuals within a given clump, the distance ranged from 5 to 70 m. The mean distance between the clumps was 45 m.

Looking at the spatial autocorrelation analysis, five distance classes were determined. At a distance between 0 and 23 m, individuals were significantly genetically close. Outside this range, the genetic relation tended to no longer be significant.

**DISCUSSION**

**Vine population dynamics in the Feuchelle forest**

With the changes in hydrological conditions, the past and the present variations in forest management are responsible for the current pattern seen in the vine population. Indeed, five categories of ecological situations can be found:

1. Closed forest with a lack of management for more than 50 years (plots A, E). In this case, the grapevines have large diameters (either one single stem or many stems). Most of them are clones. There are no grapevine seedlings.

Vines with a single trunk in these plots have reached the greatest size found in the forest with a diameter (Dbh) of 9 cm. The development of a single trunk suggests growth in uniform light conditions during the ascension phase, without temporary gap phases, which favour the reiteration of the main axis (for the definition of the reiteration process, see Oldeman, 1990) and without traumas resulting from tree falls or human cutting (Caballé, 1986). The presence of clones more than 5 m distant from each other indicates a process of vegetative reproduction at a long distance initiated by the falling of a host tree followed by the crawling of the vine on the soil and the ascension on another host, which is a frequent process in a natural alluvial environment (personal observations from Arnold in the Danube forests). This process may have lasted a few years, during which there must not have been human management (cutting of vines or plantations). The presence of several big stems suggests growth within a gap (now closed by tree growth), which corresponds to supra-optimal conditions for the grapevine, i.e. light, availability of supports if associated to lack of human cuttings.

There is a lack of seedlings in the understorey because of low-light availability.

2. Closed forest with a lack of management for more than 50 years (plots F, G, H, I, P). Grapevines have large diameter and one single stem. There are no clones and no seedlings.

The degree of maturity is less important than in the category 1 and gaps are rare, which explains the absence of clones crawling within the forest. Vertical conditions for growth are favourable for the edification of a single large trunk. Seedlings are lacking in understoreys because of very low-light availability.

3. Edge environment (plot C, partly plot A). Grapevines have many stems with small diameters. Some are clonal and there are no seedlings. This environment is favourable for the growth and the sexual reproduction of grapevines, but individuals are regularly cut by forest managers and farmers. Grapevines survive through reiteration at the level of the cutting and small clones. There are no seedlings because of competition with grasses along the edges.
4. Intensive forest management, closed forest (plots J, K, L, M, N, O, Q, R, S, partly South of A). There are no grapevines because of direct destruction.

5. Frequent forest cuttings but no direct destruction of grapevines (plots C, D). Grapevines have been cut at around 50 cm in height (plot D, individuals n° 36, 39, 40) and have developed many small stems. In plot C there were some recent seedlings. These seedlings were closely related to each other and to proximally located adults. This supposes a limited dispersal of the grapevine, which occurs for all species that reproduce by zoogamy and disperse by zoochory or barochory (Briggs and Walters, 1997). Additionally, the pollen and the berries of the wild grapevine are relatively heavy (Mezouar, 1999; Azevedo, 2000). However genetic results indicate that gene fluxes occurred between different clumps of vines.

Since the dispersal conditions of the 1960s have changed with river management, the conjunction of increasing drought in soils during the spring (which corresponds to the period of germination for wild grapevines) and the increasing competition with tall grasses (favoured by the limitation of anoxic periods) have inhibited the survival chances of seedlings. None of them lived more than 1 year (Parisot, personal observations).

Currently, the relatively high number of individuals and the overall high level of heterozygosity still reflect the former suitable situation that occurred in the Seine Valley. The Seine forests probably acted as refugia for this species during the phases of deforestation and conversion into grasslands during the 17th and 18th centuries, as can be seen in the map of Cassini. Moreover, sources of propagation of the homoptere phyloxera from cultivated zones disappeared relatively early in the region compared to other parts of Europe because most of the vineyards located in the surroundings were destroyed immediately after contamination (Boisset, 1991). A final favourable factor for the survival of wild grapevines was the maintenance of river dynamics until recent times, which maintained conditions on which wild grapevines are strongly dependent for reproduction, the establishment of seedlings and successful ascension (Arnold et al., 2005).

In spite of a relatively high genetic diversity, the population is currently in a critical state at the local scale because there is no possibility for seedlings to survive for more than 1 year. At a larger scale, the distances between the populations at La Feuchelle are by far too large to allow for gene flow. Clones are also directly destroyed in many alluvial forests of the region, in spite of strict rules of protection.

**What roles do river dynamics play in the long-term maintenance of the wild grapevine in floodplain forests?**

In recent years, interest in the floodplain forest has stimulated research into the relationships of plants and river dynamics (among many: Siebel et al., 1998; Trémolières et al., 1998; Badre et al., 1998; Carter-Johnson, 2000; Andersson et al., 2000; Deiller et al., 2001). Among this work, some researchers have focussed on liana ecology (Beekman, 1984; Allen, 2007; Schnitzler and Heuzé, 2006; Heuzé et al., 2008). All of these studies conclude that the dispersal and establishment of these plants are facilitated by flood events.

It is clear that much remains to be discovered about the natural dispersal of wild grapevine and its relationship to river dynamics, but we can hypothesize that flood events play a major role in propagation and hence the genetic diversity of the liana, which is naturally limited by short-distance dispersal. Floods can disperse seeds or vegetative parts of the liana throughout the forest floor, increasing the distances between individuals. They also increase the success of the long-term establishment of seedlings through several actions, including the temporal anoxia created by standing water, which kills many herbaceous plants and subsequently decreases competition for seedlings and also accelerates gap dynamics (Walter, 1979; Schnitzler, 1995). Indirectly, floods favour seed dispersal by creating optimal conditions for frugivorous passerines, whose densities are higher at these sites than in upland forests (Dronneau, 2007).

Grapevine seedlings found in these zones are in adequate niches that protect against herbivory because of the accumulation of woody debris, which provides trellises for ascending (Arnold, 2002). Floods also provide enough water during the sensitive phase of development of young wild grapevines in the spring and a high degree of moisture and nutrients throughout the year. The combination of these factors helps the young individuals to rapidly reach the canopy and to reproduce in the light.

As with most tendrillar woody species, a proportion of the grapevine stem is formed by the clonal extension of individuals (Oldeman, 1990; Fisher and Ewers, 1991; personal observations). This strategy, which greatly helps in
the colonization of new supports in shaded areas (Hegarty and Caballé, 1991), was frequently observed by the authors in the well-preserved floodplain forests of Austria, Romania and Turkey. In these forests, adults of wild grapevines develop enormous stems (up to several decimetres, with diameters of 15–45 cm) in the shady parts of the forest, sometimes forming upright giant loops more than 1 m in height.

The steps of the decline

Based on our results, we can reconstruct the main events that led to the current situation of the wild grapevine in the northern part of France:

1. The optimal conditions for the long-term maintenance of wild grapevine metapopulations in their western range in France lasted until 1830, although many potential sites for wild grapevine development had already disappeared with the deforestation of floodplains.

2. In the second part of the 19th century, the decline of the species was dramatic, with the general development of hydraulic works and the expansion of American diseases. Foresters also destroyed lianas, including wild grapevines. At this stage, the remnant-inundated forest sites became the last refugia for wild grapevines by protecting roots from phylloxera. In the Bassée Forest, the high fragmentation of these last surviving individuals demonstrates that reproduction occurred more and more frequently among neighbours, affecting the fitness of the remnant population.

3. After the 1960s, increased flood management of the Seine led to a total failure of sexual reproduction and hence a senescence of the remnant adults of the Bassée population, which are moreover still subjected to illegal destructions. Recent initiatives by conservationists (i.e. integration within the network of Natura 2000), the creation of a natural reserve with softer forestry practices, and better management of water levels via the creation of new floodgates for regulating fluxes of water and the adequate monitoring of water levels are not sufficient for preventing extinction if river dynamics is not restored. Furthermore, the population is likely currently too small and deprived of any connectivity with other refugia zones.

The ideal way to improve the status of the wild grapevine in the Bassée Forest would be to re-create zones of erosion and to create a wide strict reserve, in order to permit the ecosystem to recover a natural state in terms of gap dynamics, natural competition and co-existence between native species, and to allow the expansion of the grapevine populations. This would also permit the re-creation of referent sites for scientific research in the future. In the strictly protected flooded forests of the Congaree National Park in South Carolina, USA., research has been carried out over several decades. The results indicate a great variety of colonization patterns between lianas and, in particular, between Vitaceae (Allen, 2007).

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