Clonality and genetic structure of an endangered aquatic plant, *Typha minima*, in the French Alps. Consequences for conservation.

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Abstract

The dwarf bulrush (*Typha minima* Hoppe) is a perennial aquatic plant that has been rapidly disappearing in Northern Europe following flood control methods (dikes, dams, embankments). Floods, by erasing and creating new banks, maintain a metapopulation system (extinction/recolonization of populations).

The largest and most diverse populations are located in France. In order to identify the size of the metapopulations, we studied clonality, genetic diversity and genetic structure of an extensive sample of the French populations using AFLP markers.

Clonality was high (on average, each genotype was found in three copies) but variable across sites: some genotypes had a high number of copies (> 20) and were distributed over several river catchments while 239 genotypes were unique. Genetic diversity was high but did not accumulate downstream indicating both up- and down-stream long distance gene flow through pollen and seeds.

Genetic diversity is structured in three major clusters. One (cluster N) is restricted to sites north of 44°4N. The other two (clusters S and E), coexist in river catchments or even in the same site. However, the highest F_{st} were found between cluster E and clusters N or S, indicating a recolonization from different refugia, one possibly located east of the Alps (cluster E) and one or two on the Western side.

Therefore conservation actions should take into account these three major conservation units (CU) in France. These CU cover large areas. It is thus important to maintain a natural river dynamics with frequent extinction/recolonization events over whole drainage basins.

Key-words (4-6): metapopulation, dwarf bulrush, natural river systems, genetic diversity, river restoration

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Introduction

The dwarf bulrush (*Typha minima* Hoppe) is a perennial aquatic plant that was widely distributed along pioneer habitats in the braided reaches of alpine European rivers until the middle of the 19th century (Prunier et al. 2010a; Csencsic and Müller 2015). It has since been rapidly disappearing from landscapes and is now considered as an endangered species throughout Europe (IUCN 1982). It is restricted to highly fragmented landscapes of open sand banks and islands along rivers and their secondary channels or dead arms (Armand et al. 2008). In Europe, the species is now present from Eastern Romania to Italy and South-Eastern France (Austria, Czech Republic, France, Germany, Switzerland, Hungary, Italia, Yugoslavia and Romania). Most of the existing populations are in France, in more or less continuous areas along rivers in the Isère, Durance, Var and Arve drainages, and with some sites in Alsace. Some large populations are still found in Italy and Austria while Germany and Switzerland harbor only small and isolated populations (Prunier et al. 2010; Csencsic and Müller 2015).

As a pioneer species, the eco-hydrological conditions for regeneration include freshly disturbed, competitor-free sediments. Prime seedling recruitment habitat in the active alluvial zone occurs at the immediate margins of the main or secondary channels and is highly dependent on water table level fluctuations. *T. minima* populations have an effect on hydrogeomorphological processes, fluvial landforms and related habitat conditions by trapping fine sediment, nutrients and organic matter. This ecosystem engineering effect (sensu Jones et al. 1994) creates favorable conditions for the establishment of the next succession stages with *Alnus* and *Salix* shrubby and woody communities (Prunier et al. 2010b) and the elimination of the species through competition. On the other hand, the largest floods create new fluvial bars built with fluvial sediments reworked through banks and bed erosion and more generally rejuvenates the river landscape, leading to the disappearance of populations, together with the creation of new colonisation sites. This natural river dynamics leads to a metapopulation system, with fragmented populations submitted to frequent extinctions and recolonisations (Hanski et al. 2017). This means that it is crucial for the survival of populations that floodplains have near-natural sediment and flow dynamics and do not fall below a minimum size (Csencsic and Müller 2015).

During the past two centuries, dikes were constructed along river beds to reduce river dynamics, control flood and gain cultivable land. This thereby drastically reduced *T. minima* population sizes and increased population isolation. The lower regression in France can be attributed to the strategy implemented by 19th century hydraulic engineers. Girel (2010) suggests that, even if the channelization of French alpine braided rivers initially contracted more drastically the fluvial bandwidth compared to the *golène* embankment practiced in Italy or Switzeland in which a narrow channel is associated to a larger floodplain, this kind of hydraulic engineering turned out, in the long term, to be more favourable to biodiversity. The construction of relatively wide (100 – 150m) channels allowed the centennial flood to run its course and permitted, within the channel, the lateral development of islands or sandbars on which typical alluvial taxa such as*Calamagrostis pseudophragmites* or *Myricaria germanica* could be maintained in addition to *T. minima* (Greulich 2017). Several conservation or reintroduction programs are underway in Switzerland (Werner 1998, Csencsic et al. 2008, Lambelet-Haueter et al. 2009), Austria (Csencsic and Müller 2015) and France (Greulich 2017, Popoff et al. 2021). To produce viable and autonomous conservation units, the major challenge of these reintroduction programs is to maintain or restore the metapopulation dynamics. For this, it is necessary to determine the size or range of existing effective

metapopulations. This can be answered by studying the genetic diversity and structure of natural populations in areas where metapopulations are still functional. In these areas, the genetic structure inferred from exhaustive (or extensive fine-grained) sampling should allow to identify the number and range of functional metapopulations (Pritchard et al. 2000).

The genetic diversity of *T. minima* was studied in the remaining Swiss populations using genetic markers (isozymes). It showed a low level of genetic diversity (Galeuchet et al. 2002), interpreted as the consequence of the drastic reduction in population size and number in the last century. Another study was conducted using microsatellite markers on 12 natural and 9 reintroduced populations from Switzerland, Austria, Germany France and Italy (Csencsic and Müller 2015). They found (i) a higher diversity in the French and Italian populations compared to Swiss and Austrian ones, confirming the low diversity found by Galeuchet et al. (2002) in Switzerland and (ii) a strong genetic structure with populations clearly separated into seven geographic groups that could represent different metapopulations. This study, however, does not provide information on the actual range of these metapopulations because sampling was very patchy and, for the French populations, very limited compared to the actual number of sites where *T. minima* can still be found (only three samples from France). One study performed on 30 sites collected along a 60 km section of the Isère River using AFLP markers (Till-Bottraud et al. 2010) showed a much higher diversity compared to the Swiss populations of Galeuchet et al. (2002), which could be a consequence of using a more variable set of markers, and no major genetic discontinuity indicating that the whole section belongs to one single metapopulation. Although this study used a comprehensive sample along a river segment, sampling was uneven across sites due to very variable population sizes, which may have affected inferences.
All three studies indicated a high level of clonality and long-distance dispersal of genets (over 30 km; Till-Bottraud et al. 2010). In successional species such as *T. minima*, the level of clonality may provide indications on the regeneration and age of the populations (young populations with a high regeneration by seeds will have a low level of clonality, while older populations exhibit mostly clonal reproduction; Galeuchet et al. 2002). Clonality is also important to identify as it can have a significant impact on spatial genetic structure (SGS) because some multilocus genotypes can be overrepresented over small distances (Chenault et al. 2011). Finally, over longer distances (among sites) clonality provide information on rhizome fragment dispersal.

In this context, we decided to extend the sampling of Till-Bottraud et al. (2010) to the whole distribution range of *T. minima* in Southeastern France, using an extensive sampling scheme and to analyze the whole dataset in order to answer the following questions:
1-What is the genetic diversity and clonality of the French *T. minima* populations?
2-How is this diversity structured? Is it possible to identify large groups / metapopulations?
3-Is it possible to infer the historical sources (glacial refugia) of these groups?
4-What is the level of gene flow within and between these groups?
5-What are the consequences for conservation?

Material and Methods

Study species

The dwarf bulrush (*Typha minima* Hoppe) is wind pollinated. Seeds are embedded in a hairy pericarp. They are disseminated both by wind and water and can remain on the water surface for a long time without sinking (Baur et al 2015). It is rhizomatous with a strong potential for vegetative growth and colonisation when the habitat is favourable. Rhizome fragments can be transported along the river to colonise new sites (Csencsics et al. 2008). In natural conditions, seeds are short-lived (they cannot survive more than a few weeks, Galeuchet et al. 2002), and germination only occurs immediately after arrival on suitable bare moist alluvial bars (Baur et al. 2015).

Sampling

The samples collected in 2008 along the Isère River (Till-Bottraud et al. 2010) were complemented in the fall of 2010 by 108 sites belonging to five river catchments (Fig.1) in order to cover the whole distribution range in southeasternFrance and resulting in a quasi-exhaustive sampling of this area, except for some sites that were inaccessible. One site consisted in one patch where *T. minima* was present and separated by at least 20 m from other individuals. Sampling effort was adjusted to site size, *i.e.* the larger the site, the more samples were collected, and therefore, some sites were represented by very few samples. Consequently, for population-level analyses (diversity estimates), we merged sites that were geographically close to each other (within a radius of ~500m) and had few samples into a single population, resulting in 23 merged populations and 20 isolated sites (Table 1). The number of samples per population/site varied from 1 to 127 with an average of 29 samples and a total 1069 samples. For the genetic structure analyses no merging was performed. One sample consisted of a 5 cm long fragment of green leaf stored in silicagel.

Genetic analyses

DNA extraction and AFLP genotyping

Total DNA was extracted using the DNeasy 96 plant extraction kit (QIAGEN) according to the manufacturer instructions. The AFLP procedure followed Vos et al. (1995) with minor modifications. Digestion of genomic DNA was performed together with ligation of double stranded adaptors for 2 h in a 11 µL mix using 1 U of *Mse*I, 5 U of *Eco*RI (New England Biolabs) and 1 U of T4 DNA Ligase (Roche). Products were diluted 1:10 and preselective polymerase chain reaction (PCR; 120 s at 72°C, 30 cycles 30 s at 94°C, 30 s at 56°C, 120 s at 72°C with a final elongation of 10 min at 72°C) was carried out in a 25 µL volume containing Tris–HCl and MgCl2 both at 1.5 mM, dNTP mix at 0.2 mM each, each primer at 0.2 µM, and 0.5 U of Ampli*Taq* DNA polymerase (Applied Biosystems). After a 1:20 dilution of pre-selective PCR products, the selective amplification (10 min at 95°C, 13 cycles 30 s at 94°C, 60 s at 65–55°C, and 60 s at 72°C, 23 cycles 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C with a final elongation of 10 min at 72°C) was carried out in a 12.5 µL volume containing Tris–HCl and MgCl2 both at 2.5 mM, dNTP mix at 0.2 mM each, each primer at 0,2 µM (*Eco*RI + 3 / *Mse*I + 3), and 1 U of Ampli*Taq* Gold DNA polymerase (Applied Biosystems). Three primer combinations were chosen which resulted in clear bands of sufficient variability (EAAT– MCAC, EAGC–MCTG and EAGA–MCTA). PCR products were purified using columns of half to half Sephadex G50 at 5% and Sephacryl S200. Finally 1.5 µL of the purified products was mixed with 10 µL of HiDi formamide and 0.1 µL Genescan ROX 500 size standard (Applied Biosystems), and electrophoresed on an ABI PRISM 3130 XL capillary sequencer (Applied Biosystems). PCR products from each primer combination were run separately.

The results from the 2008 sample were published in Till-Bottraud et al. (2010). In order to analyze simultaneously both samples (2008 and 2010), 16 DNA extracts from the 2008 sample were randomly distributed in the 96-well plates with the 2010 samples before the AFLP procedure and electrophoresis. In addition, 13 samples were duplicated in 2008 and 18 in 2011 to check the reliability of AFLP genotyping. Raw data from the 2008 and 2010 AFLP genotyping were then collected and sized together using GENEMAPPER 3.7 (Applied Biosystems). The AFLP profiles were scored using a semi-automated procedure to code presence (1) or absence (0) of markers. Markers were defined manually with GENEMAPPER for presence or absence of each marker in each individual. The quality of markers were then automatically checked with an R script described in Herrmann et al. (2010), similar to that of Whitlock et al. (2008). This script removes non-reproducible markers and screens phenotype identification (presence/absence of markers). AFLP fragments shorter than 50 bp and monomorphic fragments were discarded. Only fully repeatable and polymorphic markers (lowest allele frequency above 0.01) were kept, resulting in 80 markers and a total genotyping error of 0. Eight samples did not amplify correctly and were discarded, resulting in 1061 samples.

Data analysis

1- Clonality

The analysis of clonality was performed using the Genalex version 6.5 (Peakall and Smouse 2006) software using the "Multilocus match" procedure. From this data, we estimated the number of genets and the proportion of distinguishable genotypes (G-1/N-1; Arnaud-Haond et al. 2007) for the whole sample and for each population.

To test the discrimination power of the dataset, we computed the probability of occurring multiple times through random mating (probability of identity, *p(id)*) for each of the replicated genotypes and for 60 randomly chosen unique genotypes following Till-Bottraud et al. (2012). To avoid biased estimates due to the high number of ramets of several replicated genotypes, we used the band frequencies estimated from the genets to compute these probabilities. Moreover, as our dataset is strongly structured in 3 major clusters (see below), we computed *p(id)* on 20 unique genotypes from each cluster.

2- Diversity

The subsequent analyses were performed on the genets: within each population, we kept one ramet for each replicated genotype in order to obtain unbiased diversity estimates (Chenault et al. 2011). Eight of the 43 populations/sites had less than five genets. In the 35 remaining populations we estimated general parameters of genetic diversity [percentage of polymorphic loci (%P), effective number of alleles (Ne) and expected unbiaised heterozygosity (uHe)] using the Genalex software. We tested whether the three diversity parameters were correlated, and whether diversity was correlated with population size, using sample size (N) as an estimate of population size, or to the number of genets (G) using a linear regression (*lm* function in R). To see if there was an accumulation of diversity downstream, we tested whether there was a linear variation of uHe along the two major rivers (Isère and Durance) by computing as above the linear regression between uHe and the distance from most upstream population (Table 1).

We tested isolation by distance by performing Mantel tests between the genetic ($F_{st}/1-F_{st}$) and geographic distance between populations. We computed two types of geographic distances: the euclidian distance (Datm) corresponding to a straight line between each pair of

populations and a "river" (Dval) distance corresponding to the distance along the river corridors using classical GIS tools for hydrographic network analysis (*ArcGIS 10.5 Network Analyst Tools*). We computed the genetic distances using Spagedi 1.5a (Hardy and Vekemans 2002) and performed the Mantel tests using Genalex. We performed Mantels for the whole sample and for the Isère and Durance rivers separately.

3- Structure

We first analyzed our data using the R package MEMGENE (Galpern et al. 2014). This software was designed to detect spatial patterns of genetic structure using Moran's eigenvector maps and a regression framework to eliminate the issues related to the Mantel test (mantel tests suffer from inflated type I error rate; Legendre and Fortin 2010; Guillot and Rousset 2013). It is moreover supposed to be efficient at detecting weak spatial genetic structure (Galpern et al. 2014). MEMGENE requires the geographic coordinates of each sample, thus only a subset of 306 samples could be used.

We then used the bayesian structuring method STRUCTURE (v 2.3.4; Pritchard et al. 2000). All analyses were done on a dataset containing only one ramet for each replicated genotype, with 20 replicate runs for each K value, a Burnin period of 100 000 iterations followed by 200 000 additional MCMC iterations, with an admixture model, uncorrelated allele frequencies and recessive alleles. The data was visualized using CLUMPAK (Kopelman et al. 2015). In all cases, all runs provided the same solution for the best K. We used two methods to infer the genetic structure of our sample. First, we followed the method of Evanno et al. (2005) to detect major clusters and sub-clusters. The best K was estimated using ΔK, visually verifying that the highest values of LnP were not obtained with K=1. A first run was done on the whole sample with K=1 to 6 (preliminary shorter runs showed that the best number of clusters was small). A second run was then performed on each cluster identified by the first run with K = 1 to 6. A third run was performed on the sub-clusters identified by the second run with K = 1 to 6. This method has, however, been criticized in case of uneven sample sizes (Kalinowski 2011; Puechmaille 2016). We therefore used the "corrected PP" of Puechmaille (2016) to estimate the number of clusters. The "corrected PP" performs reasonably well even in the case of uneven sample sizes. Given the very uneven distribution of the species along river corridors, and the structure of our sample, which was meant to capture this distribution we could not *a priori* delineate populations or sub-populations, precluding the use of Puechmaille's other parameters. We then computed pairwise F_{st} between the clusters using Genalex.

Results

1- Clonality

We found clonality in most of the river catchments. We identified 82 replicated and 239 unique genotypes, giving a total of 321 different genets (table 1). Global G-1/N-1 was 0.3, and ranged from 0.12 to 1 across the 35 populations. The number of copies of the replicated genotypes ranged from two to 190 (one genotype had 190 ramets, 17 genotypes had 10 ramets or more; Fig.2). Probabilities of identity (*p(id)*) ranged from $4.8\ 10^{-9}$ to $5.2\ 10^{-2}$ for the replicated genotypes and from $8.1\ 10^{-17}$ to $1.9\ 10^{-3}$ for the unique genotypes. We found a significant correlation between *p(id)* and the number of copies (R^2= 0.611, r = 0.78, df = 141, p< 0.001) and the replicated genotype with the highest number of ramets (190) had the highest *p(id)*. Within six populations, some patches (sampling sites) harbored only one

replicated genotype (one site on Arve with 20 samples, four on Isère with 12, 11, 4 and 3 samples and one on Durance with 7 samples). Large replicated genotypes were distributed over wide areas and often in more than one river catchment (see the distribution of four of the largest replicated genotypes on the map Fig.3).

2- Diversity

Global uHe was 0.144 and ranged between 0.011 (pop AY4) and 0.240 (pop D6) for the 35 populations (table 1). The global number of effective alleles (Ne) was 1.21 and ranged from 1.02 to 1.40. The percentage of polymorphic loci (%P) ranged from 2.5 to 85%. All three estimates were highly correlated with each other (all $p<0.001$). Diversity (uHe) was correlated to the number of genets per population (G; $p = 0.027$), but not with sample size (N, a proxy for population size; $p = 0.797$). In Isère and Durance, diversity did not increase from upstream to downstream ($p = 0.927$ and 0.811, respectively).

Mantel tests of genetic vs. euclidian (Datm) and river (Dval) distances were significant for the whole sample ($p = 0.001$ with Datm, $p=0.005$ with log(Datm), and $p=0.001$ with Dval), but not for the Isère and Durance rivers separately (with Datm $p = 0.382$ and 0.101, respectively).

3- Structure

MEMGENE results show that almost half of the genetic variation is explained by the spatial pattern of distribution ($R^2 = 0.44$). The first two eignefunctions represent, respectively, 85 (MEMGENE1) and 13 (MEMGENE2) percent of this explained variation (Fig.4). MEMGENE1 separates the Eastern and Western-most sites together with some sites on the Durance (white circles) from the rest (black circles). MEMGENE2 separates the Northern and Eastern sites (white circles) from the Southern sites (black circles).

For the first STRUCTURE run, ΔK identified two clusters (Red and Blue clusters, Fig.5). Most genets were assigned to either cluster with membership coefficients ranging from 0.6 to 1 (71 and 242 genets to the Orange and Blue clusters, respectively). This separation corresponds to the first eigenvector identified by the MEMGENE analysis (Fig.4). The Orange cluster could be further divided into 2 sub-clusters (Fig.5). One sub-cluster (E, 64 genets) was widely distributed in the Durance, Aygues and Var catchments, the other sub-cluster (Z, 7 genets) was restricted to two populations on the Aygues River (AY2 and AY3). The Blue cluster could be further divided into 3 sub-clusters (Fig.5). One sub-cluster (N, 148 genets) was restricted to the northern part of the sampling range. The second was restricted to the southern part of the sampling range (S, 83 genets). The third (X) included only eight genets distributed over the whole range. This separation corresponds to the second eigenvector identified by the MEMGENE analysis (Fig.4). Sub-clusters E, N and S did not show further substructure. We also identified several individuals with mixed ancestry (average membership coefficient over the 20 runs of 0.5 ± 0.1 to two clusters or sub-clusters). Eight genets were of mixed ancestry between the Orange and Blue clusters and came all from the Durance or Aygues Rivers in areas where Clusters E and S coexist (see Fig.1). One genet from the Orange cluster on the Durance River was of mixed ancestry between sub-clusters E and Z. Three genets from the Blue cluster (two on the Durance River and one on the Isère River) were of mixed ancestry between sub-clusters N, S or X. Moreover, 11 individuals belonged unequally to two sub-clusters in the second run (membership coefficient to each sub-cluster ranging from 0.2 to 0.4), and sub-clusters X and Z correspond to genets that showed partial admixture between the Orange and Blue clusters in the first STRUCTURE run (membership coefficient to each

cluster ranging from 0.2 to 0.4). Fig.1 summarizes the distribution of samples assigned to the different Sub-clusters and mixed ancestry individuals (hybrids) in the different sites.

Using the "corrected PP" parameter on the results from the first STRUCTURE run, we identified four clusters. Three of them correspond exactly to the N, S and E Sub-clusters using ΔK and the fourth one corresponds to Sub-cluster X and individuals with mixed ancestry between the Orange and Blue clusters. Genets assigned to Sub-cluster Z were not assigned to a specific cluster, but showed mixed ancestry between two clusters with a membership coefficient to one cluster ranging between 0.2 and 0.4 with this method.

The two methods ("ΔK" of Evanno et al. 2005 and "corrected PP" of Puechmaille 2016) thus converged to identify three major clusters, one or two minor clusters and several genets with various levels of mixed ancestry. This structure is congruent with the results of the MEMGENE analysis. The northern cluster (Cluster N) groups all populations north of 44.4°N. South of this line, Cluster E occurs in all river catchments (Durance, Aygues and Var) while Cluster S is predominant in the Durance and is present in only two sites in the Aygues River. The minor clusters X and Z are probably "spurious clusters" as defined by Puechmaille (2016), with no real biological meaning. Pairwise F_{st} was 0.85 and 0.86 between Sub-cluster E and Sub-clusters N or S, respectively, and 0.54 between Sub-clusters N and S.

Clonality was distributed differently in the three major clusters (Fig.2, Table 2). Sub-cluster E had no replicated genotypes and *p(id)* of the unique genotypes from E ranged from $2\ 10^{-14}$ to $2\ 10^{-4}$. Sub-cluster S had the largest replicated genotype (190 ramets) but other replicated genotypes had no more than 21 ramets. In this cluster, *p(id)* ranged from $4.8\ 10^{-9}$ to $5.2\ 10^{-2}$ for the replicated genotypes and from $8.3\ 10^{-10}$ to $3.2\ 10^{-3}$ for the unique genotypes. Sub-cluster N had seven highly replicated genotypes (between 20 and 60 ramets) and 6 genets had between 10 and 20 ramets. In Sub-cluster N, *p(id)* ranged from $4.2\ 10^{-6}$ to $1.9\ 10^{-2}$ for the replicated genotypes and from $8.1\ 10^{-17}$ to $1.9\ 10^{-3}$ for the unique genotypes. Cluster E had the highest diversity followed by S and N (uHe estimated on the genets = 0.103, 0.069 and 0.053, respectively; Table 2).

Discussion

1- Clonality and genetic diversity

Clonality was very important in our *Typha minima* populations, with a global level of clonality (G/N value) of 0.3, meaning that each genotype was found in more than three copies on average. This value is higher than what was found in 2008 on the Isère sample (G/N value of 0.58, Till-Bottraud et al. 2010). These global values however, hide an extremely large variation among populations (levels of clonality ranging from 0.12 to 1, with some monogenotypic patches within six large populations) and among genotypes (one genotype had 190 ramets, 17 genotypes had 10 ramets or more, and 239 genotypes were unique).

The discrimination power of our dataset was globally high, except for some replicated genotypes. The genotypes with the highest number of ramets also had the highest probability of occurring multiple times through random mating indicating that some individuals that we identified as ramets of a clone could be in fact different genets. However, several clones had a significantly low probability of occurring multiple times. Moreover, probabilities in the range of 10^{-2} to 10^{-4}, although arguing for a possible origin of identical genotypes through sexual reproduction, cannot explain the very high number of copies of some genets. Thus, although it might be slightly overestimated, clonality is very important in most sites.

The global variability of our extensive sample of French populations was similar to the values obtained on the Isère sample alone in 2008 (Till-Bottraud et al. 2010). Genetic diversity, estimated on the genets was of 0.144 compared to 0.139 in 2008. However diversity per population had a broader range than in 2008 (uHe ranged from 0.011 to 0.240 compared to 0.033 to 0.141 in 2008). Galeuchet et al. (2002) studied the clonality and the genetic diversity of six Swiss natural populations using isozymes. They found a high clonality level and a low genetic diversity (1 to 17 genotypes per population with a sample size of 30; number of alleles per locus ranging from 1.3 to 1.8), which could be due to a low discrimination power of isozyme markers (Till-Bottraud et al. 2010). Csencsics and Müller (2015) studied populations from France, Italy, Switzerland, Germany and Austria using microsatellite markers and found a higher diversity in the French and Italian populations compared to Swiss and Austrian ones, confirming the low diversity found by Galeuchet et al. (2002) in Switzerland.

The genetic diversity values we obtained fall in the medium range of diversity obtained with AFLPs on other species (Gaudeul et al. 2000; Jump et al. 2006; Tsumura et al. 2007) and is quite similar that of other clonal species (Widen et al. 1994). Genetic diversity was very variable across other *Typha* species. Almost no variability was found for *T. latifolia* with isozymes (Mashburn et al. 1978; Sharitz et al. 1980). Using AFLPs, Lamote et al. (2005) found a high diversity in Flanders, and greater in *T. angustifolia* compared to *T. latifolia* (% polymorphic bands = 81 vs. 46%). The same result was found using microsatellite markers in Ukraine (He= 0.66 and 0.37, respectively; Tsyusko et al. 2005), China (He = 0.205 and 0.101, respectively; Zhou et al. 2016) and northeastern United States (He = 0.339, and 0.271, respectively; Pieper et al. 2020). Clonal diversity is similarly quite variable, ranging from 0.32 to 0.86 with AFLPs (Lamote et al. 2005) and microsatellites (Tsyusko et al. 2005; Pieper et al. 2020). As with *T. minima*, clones were observed within populations as well as among populations (Lamote et al. 2005). A lower clonality was found in *T. angustifolia* than in *T. latifolia* (Lamote et al. 2005; Tsyusko et al. 2005; Pieper et al. 2020).

2- Genetic structure

The genetic diversity of the French *Typha minima* populations shows a very strong structure (three major groups) over large distances, but not among drainage basins. This was unexpected given the strong geographical discontinuity of the distribution as shown on the map in Fig.1 (we sampled *T. minima* from almost all know sites). Significant IBD was identified over the whole range but not along the two major rivers harboring *T. minima* populations (Durance and Isère) and diversity did not accumulate downstream along these river systems. The strongest genetic differentiation does not correspond to a geographic structuration: although the North/South divide is spatially very clear and individuals from Clusters E and S occur in the same sites or in adjacent sites, the predominant genetic structure was between Cluster E and Clusters S or N. The genetic differentiation follows this pattern (largest F_{st} - greatest genetic differenciation- between Cluster E and Clusters N and S). An additional difference was that we found no replicated genotypes in Cluster E.

In a study of populations from France, Italy, Switzerland, Germany and Austria Csencsics and Müller (2015) identified seven different clusters. One of their French population was collected on the same site as our Giffre population (Arve catchment, cluster N), another one on the same site as our D34 population (Durance catchment, Cluster S). Cluster N and S are thus probably equivalent to the “Giffre/Rhône” and “Durance” clusters, respectively, of Csencsics and Müller (2015). The third population was collected in the “Haute Durance” reach but we

did not find plants in this site in 2010. The Italian populations that were sampled on the Dora Riparia are geographically close to our southeastern populations of Cluster E (Var River) and could belong to the same cluster.

Over more restricted distances and using isozymes, Galeuchet et al. (2002) found low genetic differentiation among populations (mean genetic distance of 0.03) and no isolation by distance. Using microsatellite markers, isolation by distance was found in *T. latifolia*, but not in *T. angustifolia* in Ukraine (Tsyusko et al. 2005) and China (Zhou et al 2016), while the reverse was found in the eastern United States (Pieper et al 2020). Genetic structure was also different between the two species across continents, with 3 distinct groups in *T. latifolia* and one in *T. angustifolia* in Flanders (Lamote et al. 2005), 3 groups in *T. angustifolia* in the eastern United States with genotypes of two of them scattered across the sampling area (similarly to our E and S clusters) and one in *T. latifolia* (Pieper et al 2020) and no apparent structure for either species in Ukraine (Tsyusko et al. 2005). In general aquatic macrophytes tend to show little geographic structure (*Sisymbrium austriacum* in Belgium, Jacquemyn et al. 2006; *Sparganium emersum* in Flanders, Pollux et al. 2007; *Nuphar Lutea* in the Czeck Republic; Fer & Hroudova 2008; Phragmites australis in the northeastern USA, Kirk et al 2011; *Phragmites australis* and *Triglochin procera* in Australia, James et al. 2013; *Ranunculs subrigidus* in the Qinghai-Tibet plateau, Wu et al. 2019) although Garcia-Giron el al. (2019) identified a strong structure in *Myriophyllum alterniflorum* over short distances (~30 km) in Spain. The results we obtained on *T. minima* also show a striking similarity to what was found for the Black Poplar, *Populus nigra*, a protected species that requires a similar habitat for its natural regeneration and which shows little natural regeneration in northern Europe: Faivre-Rampant et al (2016) found a large genetic diversity and only a restricted number genetic clusters over Europe, indicating large scale dispersal and populations or metapopulations distributed over large areas.

3- Historical sources (refugia)

Although Kalinowski (2011) shows that the clusters created by STRUCTURE are not systematically consistent with the evolutionary history of the populations, the convergent results provided by our different analyses and the F_{st} values suggest that Cluster E was separated from Clusters N and S for a longer time, or by stronger barriers.

During the late glacial maximum, many European species survived in isolated southern refugia (Iberian Peninsula, Italy, Balkans; e.g. *Sedum album*, Listl et al. 2017) or at the southern edge of the cold and dry steppe-tundra area in eastern, central, and southwestern Europe, leading to strong divergence among different lineages (Petit et al. 2003). Recolonisation from these refugia was constrained by geographical barriers such as the Alps (Taberlet et al. 1998). On the other hand, several Alpine species were shown to have survived in local ice-free peripheral areas or on nunatacks (Schönswetter et al. 2005). In their review of the phylogeographic patterns of Alpine species, Schönswetter et al. (2005) identified a well-supported southwestern-Alpine peripheral refugium between Nice and the Aoste valley and several potential peripheral refugia on the western border of the French Alps, the latter confirmed by a study on *Arabis alpina* with a clear divide between southern and northern populations (Rogivue et al. 2018). *T. minima*, while not a strictly alpine species, is restricted to the braided reaches of alpine European rivers and has probably a similar history than some alpine species. Indeed, the distribution of native North American hydrophytes can be related to historical processes such as the retreat of the Wisconsin glacier while climatic factors seem to have limited importance (Santamaria 2002). Moreover, the biogeography of *Ranunculs subrigidus*, an aquatic macrophyte of the Qinghai-Tibet plateau, is similarly suspected of being influenced

by mid-to-late Pleistocene events (Wu et al. 2019). Cluster E could thus originate from an eastern (Italian) refugium or from the "southwestern-Alpine peripheral refugium" of Schönswetter et al. (2005), while clusters N and S originate from one or several western refugia.

The North/South divide in the French external Alps is interesting: in the Durance catchment, the most upstream population of the Durance River (HD) is mostly assigned to Cluster N. Symmetrically, the southernmost population of the Isère catchment, the Drac population, is assigned to Cluster S. These two populations lie, respectively, north and south of 44.4°N, suggesting a phylogeographic or a climatic limit at this latitude. Ozenda (1985) describes a climatic limit in the French Alps at 45°N separating the "Alpes nord-occidentales" from the "Alpes sud-occidentales". This limit also corresponds to the divide between southern and northern populations of *A. alpina* (Rogivue et al. 2018). This suggests that Clusters N and S either originated from two separate refugia in the French external Alps, or that local climatic conditions have recently separated these two groups, or as a combination of both causes, *i.e.* that recent climatic conditions have maintained or even reinforced a divergence due to post-glacial recolonisation.

An alternative explanation for cluster E is that, similarly to what was suggested by Pieper et al (2020) for *T. angustifolia* in the United States, it is an escaped cultivated lineage. Garden centers sell *T. minima* by internet over all of Europe. One garden center calls it the "dwarf Japanese bull rush" indicating a possible foreign origin. However, no plants belonging to cluster E were found in the northern part of our study area, which makes this a less plausible explanation than for *T. angustifolia* in the US.

4- Gene flow within and between groups

Although the global large-scale structure is very robust, evidence of current gene flow is pervasive in our data. First, several clones are distributed along river reaches and even across river catchments, indicating long-distance transport of rhizome fragments. The distribution of clones over long river reaches can be explained by downstream transport of rhizome fragments by water, most probably during bankfull flood events for which sediment transport, erosion and deposition are the most efficient in the long term (Wolman and Miller, 1960). The occurrence of the same clone in different river catchments (e.g. clone II in Durance, Aygues and Drac) suggests other means of transport, such as human accidental transport with river sediments or active transplantation, or possibly aquatic birds (Santamaria 2002; James et al. 2014). Alternatively, different genets that could not be distinguished due to the medium discrimination power of our dataset could occupy the different catchments.

Second, diversity did not accumulate downstream along these river systems. This indicates that gene flow is bidirectional (upstream and downstream). The upstream movement is probably due to wind transport of pollen and seeds, while the downstream movement could be due to both water and wind transport of seeds, and water transport of rhizome fragments.

Third, several genets showed various levels of admixture between clusters or sub clusters, suggesting gene flow and hybridization over a large range of distances (see Fig.1). Eleven individuals that were found to have roughly equal probability of belonging to two clusters are probably F1 hybrids. Some occur in sites where individuals from two different clusters coexist, but three were found in a river catchment where only one parent cluster is present. Additionally, 26 individuals belonging unequally to two clusters are possibly the result of various levels of backcrosses. Some could thus be the result of local hybridization with no

further dispersal, but others suggest long distance pollination events across river catchments or long distance seed dispersal.
On the other hand, we identified a strong genetic discontinuity in the upper reaches of the Drac and Durance Rivers. Important dam systems occur downstream of both the Haute Durance and the Drac sites (see Fig.1), suggesting that they could represent a barrier to gene flow. However, as these are the only river reaches inhabited by *T. minima* where dams are present, generalization is not possible.
High levels of gene flow are usually estimated in aquatic plants. In his review, Santamaria (2002) describes compelling evidence of high dispersal rates due to potential long distance dispersal by waterfowl and describes a dispersal function that peaks locally but has a thick tail. This has been confirmed by more recent studies (e.g. Jacquemyn et al. 2006; Pollux et al. 2007; Fer & Hroudova 2008; Kirk et al 2011; James et al. 2013; Wu et al. 2019; Pieper et al. 2020; but see also Garcia-Giron et al. 2019 for an example of strong IBD and gene flow restricted mostly between neighboring populations).

5- Consequences for conservation

France is the European country with the largest number of natural populations of *T. minima* (Prunier et al. 2010a). It also harbors a very high genetic diversity, together with Italy (Csencsics and Müller 2015). Our exhaustive sampling showed that French populations belong to three different genetic clusters. One of these clusters (N) corresponds to the Western Switzerland cluster identified by (Csencsics and Müller 2015), another (E) is probably the same as the one found in Italy, and the third one (S) is unique to France. All this highlights the responsibility of France for the protection of this species. Consequently, a regional action plan ("plan régional d'action Auvergne-Rhône-Alpes et Provence-Alpes-Côte d'Azur") is being developed by a network of stakeholders (réseau Flore sentinelle https://floresentinelle.fr/).

In most species, existing populations inhabit easily delimitated areas that can be set aside for protection. For pioneer species inhabiting very dynamic habitats and functioning as metapopulations, as is the case for *T. minima*, protecting sites where the species is present at a certain point in time is not sufficient. We need to identify the range at which a metapopulation can be viable, *i.e.* what is the size of a functional conservation unit? For *T. minima*, we identified three major genetic groups that are distributed over very large areas (each approx. 2.10^4 km²). We moreover found significant gene flow between sites and even river catchments, through transport of rhizome fragments, but also pollen and seeds that are transported both down- and up-stream. This gene flow confirms the large geographical reach and the strong dynamics of *T. minima* metapopulations.
The structure could, however, be the signature of an ancient favorable situation (before the major flood control actions started in European Alpine rivers ~100-200 years ago) in which the time elapsed since fragmentation was not sufficient to develop a local genetic structure and reduce genetic diversity. Such a situation was suspected in two wetland macrophytes in Australia (*Phragmites australis* and *Triglochin procera*; James et al. 2013), the long-lived Alpine perennial *Eryngium alpinum* L. (Gaudeul and Till-Bottraud 2008), and in the short-lived perennial *Laserpitium prutenicum* L., where it was called "silent goodbye" (Reichel et al. 2016). To detect effective actual gene flow would require testing seeds or very young seedlings.
In this context, it is important to maintain natural river processes and dynamics with frequent extinction/recolonization events to maintain functional connectivity along whole drainage basins or whole river systems. Climate change impacts on Alpine rivers may also drive,

through contrasted effects on the upstream and the downstream parts, the development of a much more contrasted, or even fragmented, riverine landscape restricting the connectivity between populations (Bard et al., 2015; Giuntoli et al., 2013). On the other hand, there is a strong incitation in France and the EU to restore fluvial longitudinal and lateral connectivity of sediment and water in order to thwart the general alpine rivers incision resulting from both natural and anthropic controlling factors (Peiry et al, 1994). This should result in a much more natural fluvial dynamics promoting transient fluvial landforms such as braiding islands, which are a favourable pioneer habitat for *T. minima*.

Reinforcement/Reintroduction programs require using genotypes originating from the same gene pool to avoid maladaptation (Derry et al. 2019) or outbreeding depression (Frankham et al. 2011). We suspect a climatic limit at Lat 44.4°N between individuals from Cluster N and Cluster S. This local adaptation should therefore be tested using e.g. reciprocal transplant experiments. However, as a precautionary approach we suggest the reintroduction of individuals from Cluster N in the northern part of the range. In the southern part, Clusters E and W, although exhibiting the strongest genetic differentiation, coexist, indicating similar climatic requirements, and thus a reduced risk of maladaptation. The risk of outbreeding depression might however be greater as the low level of clonality of the inter-cluster hybrids (only three of the 37 admixed genotypes were replicated and had only two copies) indicate a low vegetative propagation capacity or a reduced lifespan. This interpretation must however be put into perspective because we found no clonality in Cluster E where we have no indication of reduced fitness. In the South, we thus suggest to reinforce populations with individuals from the same cluster or to reintroduce individuals from only one cluster to prevent inter-cluster hybridization.

Conclusions

In this study, we showed that *Typha minima,* an endangered pioneer species, is structured in three genetic clusters over its distribution range in southeastern France. Similarly to what was found in several aquatic macrophytes, these clusters span very large areas (2. 10^4 km²), exhibit high levels of diversity and high levels of gene flow via both rhizome fragments and reproductive propagules (pollen and seeds). This indicates that in this region, three metapopulations are present and are still potentially viable if the river dynamics is maintained. The absence of fine-scale genetic structure despite the large geographic discontinuities of the distribution was unexpected and could be due to a faster rate of habitat loss compared to the loss of genetic diversity. Evidence of actual (present) gene flow should be investigated by studying e.g. in situ germinations. The origin of the strong structure is, on the other hand, still unclear and could be the result of post-glacial recolonization or local adaptation. The latter could be tested using e.g. reciprocal transplant experiments.

T. minima is restricted to the braided reaches of alpine European rivers. These reaches were severely impacted by the flood control methods implemented during the 19th century, strongly restricting the natural flow dynamics to reduce flood impacts and increase available farming areas. The consequence was globally detrimental to rare habitats now protected by the EU Habitats Directive such as water courses of plain to montane levels, alluvial forests, calcareous fens but also floodplain grasslands and was more globally detrimental to biodiversity. However, the impact of the channeling system chosen for the Isère River was not as dramatic as that of the Italian “golena system” used in other Alpine rivers. The awareness

that these measures, together with climate change, also impact water availability and sediment transport along the river continuum, opens avenues for restauration of better river continuity and of the habitats and species that depend on dynamic riverine systems.

The regeneration habitats created by dynamic rivers are also important for other endangered plant species such as *Populus nigra* or *Centaurium favargeri, Corispermum gallicum, Polygala exilis* which are notably difficult to detect, but also protected animal species such as the Eurasian beaver (*Castor fiber*) or the Eurasian otter (*Lutra lutra*). For all these species, natural / unhampered river dynamics is a crucial factor for their maintenance. *Typha minima*, one of the finest indicator species for the health or naturality of a river (Prunier et al 2010a), is a true umbrella species for these habitats. The rapid metapopulation dynamics of the species requires now to move away from a classical approach of site protection and to take into account river dynamics, functionality and the presence of endangered species over large river sections in all future river development projects such as dams, gravel extraction etc. both upstream and downstream of the sites where the species is present. Although *T. minima* still occupies large and continuous river sections, France has a major stake in the protection of the species in Europe.

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824 **Table 1:** Characteristics, diversity estimates and cluster assignment of the sites and merged populations studied.

Pop	Isolated/ Merged	River	Year	N	G	D(km)	G-1 /N-1	%P	Ne	SE(Ne)	uHe	SE(uHe)	Main cluster	2nd clusters
Arve	M	Arve	2010	38	12		0,30	37,50	1,149	0,032	0,095	0,019	N	X
Giffre	I	Arve	2010	20	3								N	
Isère1	I	Isere	2010	1	1								N	
Isère3	M	Isere	2010	35	11	0,00	0,29	12,50	1,048	0,019	0,031	0,011	N	
Isère11	I	Isere	2008	22	11	3,23	0,48	12,50	1,060	0,022	0,038	0,013	N	
Isère12	M	Isere	2010	59	24	4,26	0,40	17,50	1,048	0,016	0,034	0,011	N	
Isère15	M	Isere	2010	70	32	14,37	0,45	28,75	1,062	0,020	0,042	0,012	N	
Arc1	I	Isere	2010	7	3								N	
Arc2	I	Isere	2010	22	9		0,38	13,75	1,058	0,022	0,037	0,013	N	
Arc3	M	Isere	2010	29	9	18,67	0,29	12,50	1,041	0,016	0,030	0,010	N	
Isère37	I	Isere	2010	10	7	19,77	0,67	10,00	1,036	0,014	0,027	0,010	N	
Isère42	I	Isere	2008	23	16	35,31	0,68	12,50	1,052	0,020	0,033	0,012	N	
Isère43	M	Isere	2008	19	15	35,69	0,78	21,25	1,083	0,025	0,053	0,015	N	
Isère45	I	Isere	2008	5	4								N	
Isère47	M	Isere	2008	16	7	40,69	0,40	6,25	1,039	0,019	0,024	0,011	N	
Isère49	M	Isere	2008	21	12	42,57	0,55	28,75	1,113	0,027	0,075	0,017	N	X
Isère55	M	Isere	2008	33	18	44,15	0,53	22,50	1,073	0,021	0,050	0,013	N	H
Isère59	I	Isere	2008	23	13	48,68	0,55	10,00	1,048	0,021	0,030	0,012	N	
Isère61	I	Isere	2008	28	9	52,25	0,30	7,50	1,038	0,019	0,024	0,011	N	
Isère62	I	Isere	2008	16	8	55,89	0,47	8,75	1,043	0,019	0,028	0,011	N	
Isère64	M	Isere	2008	25	17	59,37	0,67	17,50	1,050	0,017	0,035	0,011	N	
G72	I	Isere	2010	21	11	60,32	0,50	7,50	1,061	0,024	0,035	0,014	N	
Isère67	I	Isere	2008	10	4								N	
Isère69	M	Isere	2008	16	5	71,77	0,27	7,50	1,044	0,020	0,029	0,012	N	
Drac	I	Isere	2010	3	2								S	
DuranceH	M	Durance	2010	13	10	0,00	0,75	17,50	1,069	0,023	0,045	0,014	N	S

Durance34	M	Durance	2010	51	23	53,82	0,44	45,00	1,117	0,026	0,079	0,016	S	N/H/X
Durance6	M	Durance	2010	29	28	75,69	0,96	81,25	1,401	0,043	0,240	0,022	E	S/H
Durance9	M	Durance	2010	17	12	102,52	0,69	13,75	1,082	0,026	0,050	0,015	S	
Durance43	M	Durance	2010	20	7	125,64	0,32	56,25	1,312	0,037	0,206	0,023	S	H/Z
Asse	M	Durance	2010	77	10	145,64	0,12	18,75	1,071	0,023	0,047	0,014	S	
Durance12	M	Durance	2010	46	25	190,67	0,53	85,00	1,215	0,035	0,140	0,018	S	E/H/X
Durance23	M	Durance	2010	127	27	191,22	0,21	56,25	1,104	0,026	0,069	0,015	S	X/H
Durance15	M	Durance	2010	11	8	193,39	0,70	28,75	1,134	0,031	0,086	0,018	S	X
Durance3	M	Durance	2010	10	8	194,51	0,78	10,00	1,040	0,017	0,028	0,010	S	
Durance2	I	Durance	2010	2	2								E	X
Durance1	I	Durance	2010	6	3								S	
Aygues3	I	Aygues	2010	5	5		1,00	20,00	1,143	0,036	0,088	0,021	Z	E
Aygues4	I	Aygues	2010	15	5		0,29	2,50	1,018	0,013	0,011	0,008	S	
Aygues5	M	Aygues	2010	12	12		1,00	23,75	1,120	0,031	0,073	0,018	E	
Aygues1	I	Aygues	2010	15	6		0,36	6,25	1,030	0,017	0,020	0,010	S	
Aygues2	I	Aygues	2010	20	20		1,00	61,25	1,262	0,036	0,167	0,021	E	Z/H
Var	M	Var	2010	12	12		1,00	20,00	1,108	0,031	0,065	0,017	E	
Grand Mean and SE over Loci and Pops (for pops with more than 5 Genets)				28,7	13,3		0,54	24,04	1,096	0,005	0,062	0,003		
Global				1061	321		0,30	100	1,211	0,030	0,144	0,016		

Pop: site or population name (listed from North to South). Isolated/ Merged; I: sites with a high number of samples or isolated; M: the populations result from merged sites that were geographically close to each other and had few samples. Year: sampling year. N: sample size. G: number of genets. D: distance from the uppermost populations (for Isère and Durance Rivers only). G-1/N-1: level of clonality (proportion of distinguishable genotypes). %P: percentage of polymorphic loci. Ne: effective number of alleles. uHe: unbiaised heterozygosity. SE(Ne) and SE(uHe) are the respective standard errors. Main cluster: cluster to which most of the genets were assigned. 2nd clusters: clusters to which the remaining genets were assigned.

Table 2: Genetic diversity of the different clusters identified by the STRUCTURE analysis.

Cluster	N	G	G-1 /N-1	%P	Ne	SE(Ne)	uHe	SE(uHe)
E	63	63	1,00	41,25	1,171	0,035	0,103	0,019
N	582	141	0,24	60,00	1,075	0,020	0,053	0,012
S	388	82	0,21	45,00	1,101	0,023	0,069	0,014
X	8	8	1,00	72,50	1,248	0,029	0,180	0,018
Z	7	7	1,00	15,00	1,110	0,033	0,064	0,018

N: sample size. G: number of genets. D: distance from the uppermost populations (for Isère and Durance only). G-1/N-1: level of clonality (proportion of distinguishable genotypes). P: percentage of polymorphic loci. Ne: effective number of alleles. uHe: unbiaised heterozygosity. SE(Ne) and SE(uHe) are the respective standard errors.

Fig.legends

Fig.1: Map of all investigated sites of *T. minima*. For each site, the size of the circle is proportional to the number of individuals sampled, and the colors show the proportion of individuals assigned to each of the five clusters (N, X, S, E and Z; see fig. 5 and text) or to admixed individuals (H), as determined by the STRUCTURE analysis. To prevent overlapping of symbols, close populations were slightly moved apart.

Fig.2: Distribution of copy number per replicated genotype; number of genotypes with 1, 2, .. 190 copies from Clusters E(blue), S (orange) and N (green), as determined by the STRUCTURE analysis. "15-": 15 to 19; "20-": 20 to 29; "30-": 30 to 39; "40-": 40 to 49; "50-": 50 to 189".

Fig.3: Distribution of four of the largest replicated genotypes. Clone D and Clone E belong to Cluster N; Clone HH and Clone II belong to Cluster S. Clusters determined by STRUCURE and clones determined by Genalex.

Fig.4: MEMGENE results for eigenvector 1 (left; 85% of the variance explained by the model) and eigenvector 2 (right; 13% of the variance explained by the model). Dashed lines represent watershed boundaries (as in Fig.1). Values next to the circles in the legend box represent the scores: large black and white circles describe opposite extremes on the MEMGENE axis (eigenvector) and the size of the circle diminishes towards the center of the axis. In the graph, circles of a similar size and color indicate individuals with similar scores and groups of circles with similar scores close to each other indicate a spatial pattern.

Fig.5: Inference of population structure obtained from Bayesian analysis in STRUCTURE. The first run (First analysis) indicated an optimum of two clusters (Orange and Blue clusters). Each cluster was further subdivided into two or three sub-clusters (Second analysis). For each analysis, the population structure is shown on the left (sites arranged from north to south) and the ΔK values are on the right.

Supplementary material:

Fig.S1: Map of all investigated sites of *T. minima* showing the grouping of sites into populations (each population is represented by a different color).

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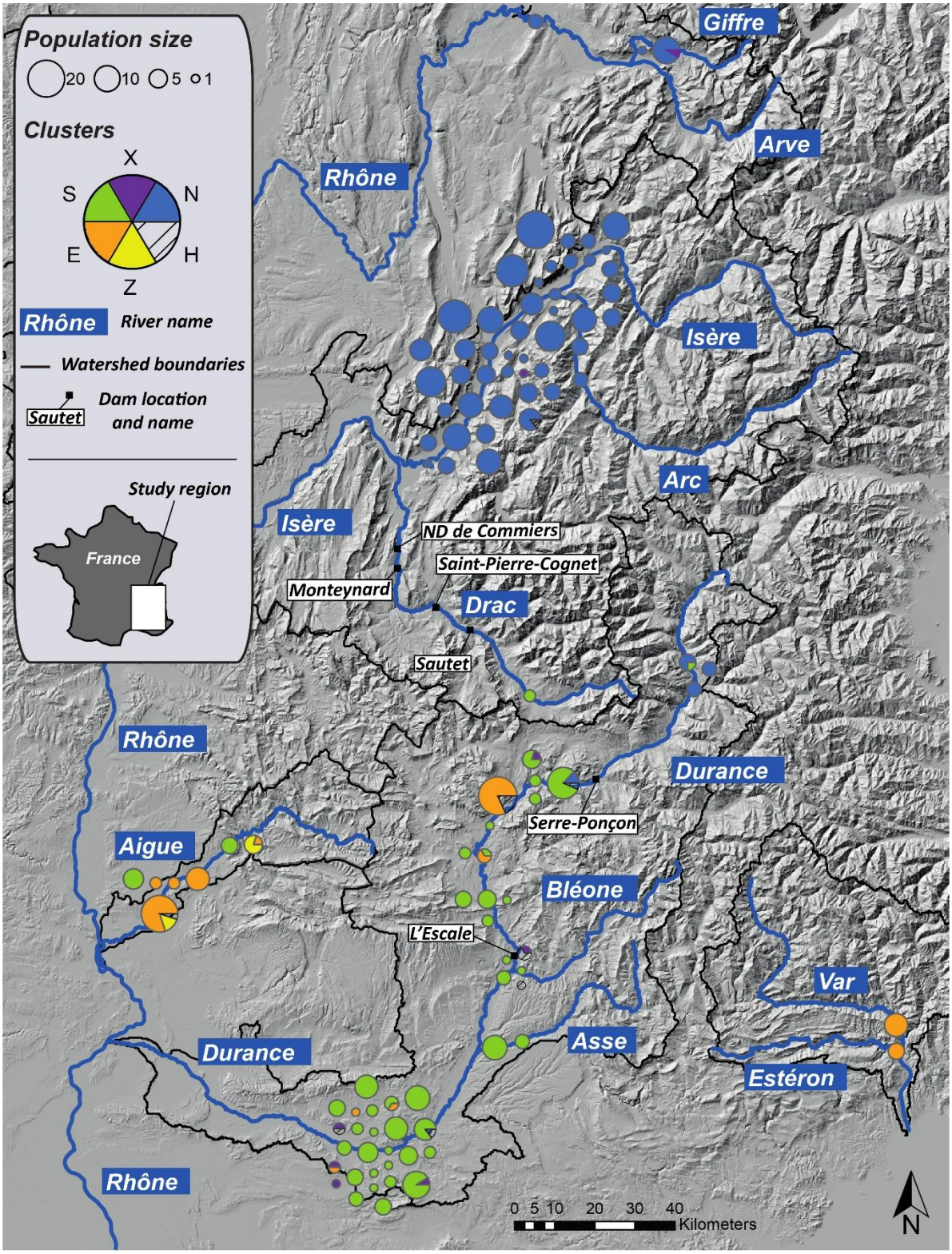
883



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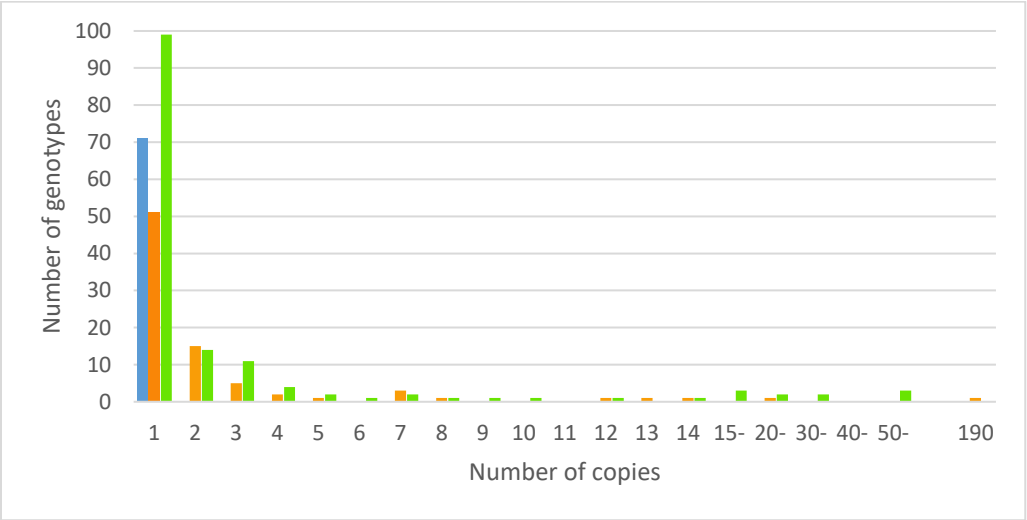
Excel histogram



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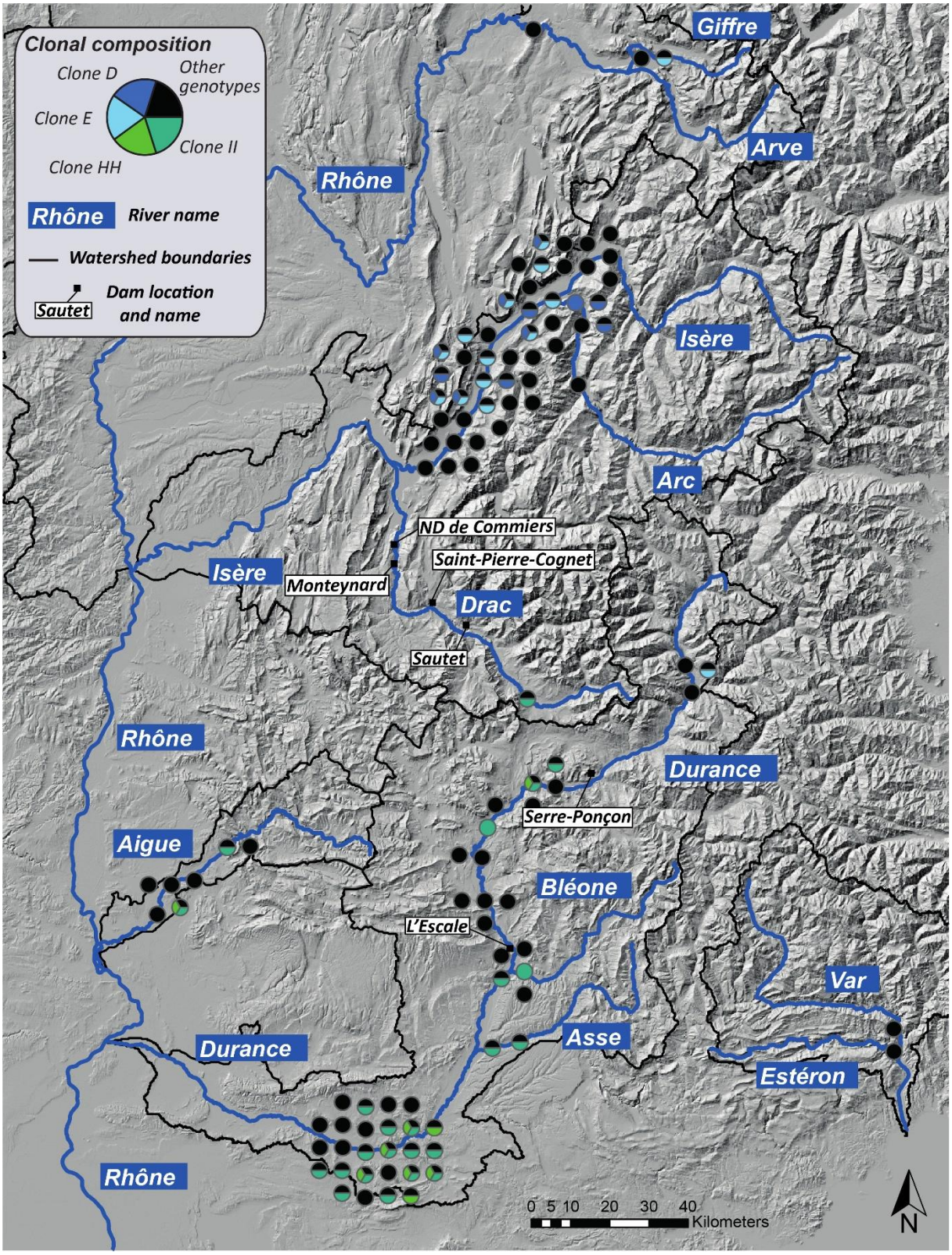

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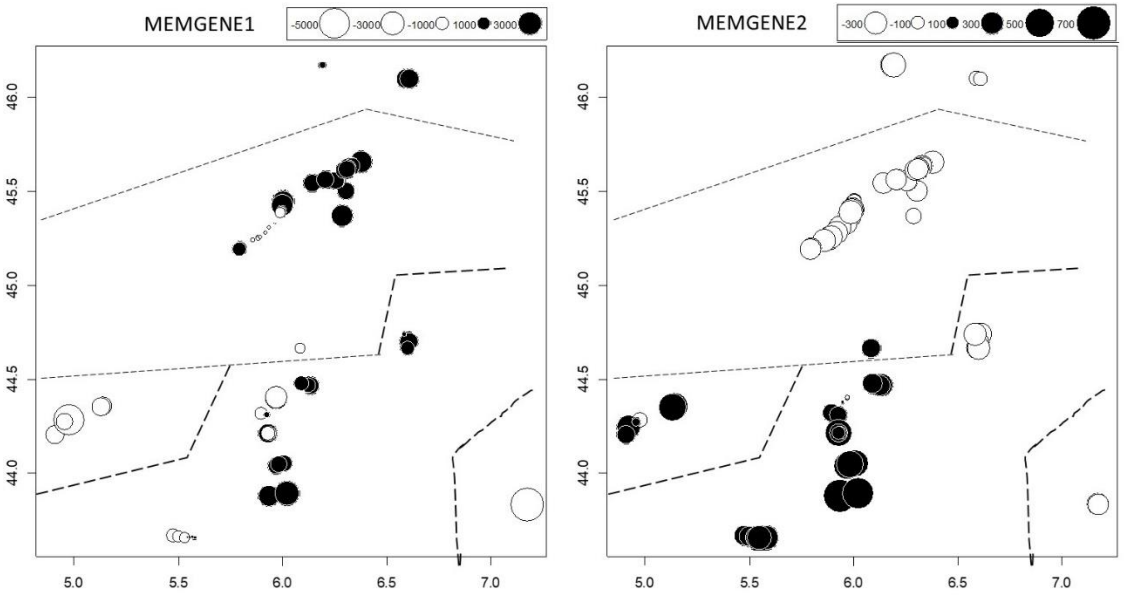
MEMGENE output



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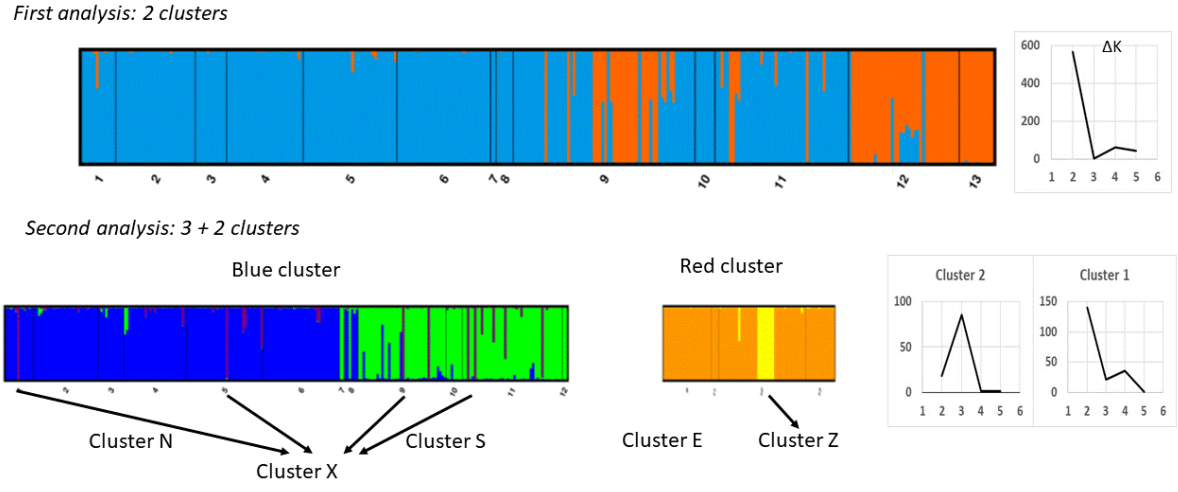
CLUMPAK outputs assembled using Powerpoint



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