Plant Ecol (2013) 214:317–327
DOI 10.1007/s11258-013-0170-5

Human-induced changes on fine-scale genetic structure in *Ulmus laevis* Pallas wetland forests at its SW distribution limit

Martin Venturas · Pablo Fuentes-Utrilla · Richard Ennos · Carmen Collada · Luis Gil

Received: 22 October 2012 / Accepted: 16 January 2013 / Published online: 24 January 2013

Abstract Human activities have deeply transformed the Mediterranean landscape for millennia. Wetland and riverbed vegetation are among the most affected ecosystems because of the value of these areas for agriculture, due to their rich soil and water availability. This has caused the fragmentation, population reduction, and extinction of many species. We focus our study on *Ulmus laevis* Pallas, an endangered tree species in the Iberian Peninsula, as an extreme example of these formations. We study the diversity and fine-scale spatial genetic structure of two human disturbed wetland populations with chloroplast markers and nuclear microsatellites. We evaluate their recovery possibilities, and how they will be affected by future aridification and water table depletion. Our results show that although these populations have suffered bottlenecks and have low genetic diversity, they maintain the same diversity levels as the European populations. Despite the low genetic variation that could contribute to inbreeding problems in the future, we discuss that the main threat of the species is habitat destruction. Finally, we propose some management and conservation policies to ameliorate these effects.

Keywords *Ulmus laevis* · Spatial genetic structure · Endangered species · Conservation · Wetland forests · Management guidelines

Carmen Collada and Luis Gil contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s11258-013-0170-5) contains supplementary material, which is available to authorized users.

M. Venturas · C. Collada · L. Gil (✉)
GENFOR Grupo de Investigación en Genética y Fisiología Forestal, Departamento de Silvopascicultura, E.T.S.I. de Montes, Universidad Politécnica de Madrid, Ciudad Universitaria S/N, 28040 Madrid, Spain
e-mail: luis.gil@upm.es

P. Fuentes-Utrilla · R. Ennos
Institute of Evolutionary Biology, The University of Edinburgh, West Mains Road, Edinburgh EH9 9JT, UK

Introduction

The Iberian Peninsula has a rich cultural heritage; hence human activities have been one of the main driving forces in landscape transformation (Antrop 2005). These human-induced changes started with the Neolithic cultures over 4,000 years ago and have intensified during the last centuries, deeply altering forest ecosystems (Iriarte 2009; Valbuena-Carabaña et al. 2010). Phreatophyte trees (which are deep-rooted and obtain a significant part of the water they need from water table) and mesophyte trees (which are neither adapted to very wet nor particularly dry environments) are among the most affected species as they live in wetlands, riverbeds and fluvial plains, water and nutrient rich areas soon converted to agricultural or pasture land. In addition, many

wetlands were drained to prevent human health risks from mosquito-borne diseases or poor water quality. Nowadays, these riparian forests are also subject to human disruption by poplar plantations, sand and gravel mining, hydrological control of rivers, urbanization, recreation, and construction of golf courses (Hooke 2006). Lowered water tables due to groundwater pumping for agricultural and municipal activity have also caused loss of wetland vegetation and its substitution by climatic types. Although waterlogged areas were very common in the past, most of them have disappeared. In many places, local toponymy is the only evidence that they once existed. Consequently, many species have suffered fragmentation, population reduction, and regional, or local extinction. For example, over the last 60 years the extent of Las Tablas de Daimiel National Park wetlands has been reduced to less than a seventh of its original extent, causing vegetation shift and the loss of three quarters of hydrophyte species (plants adapted to grow in water) (Álvarez-Cobelas et al. 2001).

In the last 30 years seasonal precipitation patterns have changed in the Iberian Peninsula: winter to summer rainfall has decreased and there has been an increase in autumn rainfall percentage (De Luis et al. 2010). If this trend continues it will affect many tree species, crops, and pastures as the water availability will be lower at the beginning of the growing season. For example, stone pine (*Pinus pinea* L.) cone yield has decreased 55 % in the last 40 years due to rainfall reduction (Mutke et al. 2005). Current climate change scenarios estimate an increase in aridity and irrigation needs for the Iberian Peninsula (Moreno 2005; Rodríguez et al. 2007); this will enhance water demand and deplete water tables, putting more pressure on existing riparian woods. Landscape fragmentation increases vulnerability to disturbances which, in combination with reductions in propagule dispersal, can compromise the survival of remnant populations (Thuiller et al. 2005). The effects of genetic drift, inbreeding, and low gene flow on genetic diversity and fitness can put rare plants and small populations at genetic risk (Ellstrand and Elam 1993). Therefore, the knowledge of spatial genetic structure (SGS) of populations is important for establishing sound conservation strategies (Newton et al. 1999; Moritz 2002) and for understanding the response to perturbations. Adults from plants species do not move and their propagules, i.e., pollen, seeds, and cuttings, often show moderate to strong spatial restriction in their dispersal, frequently causing SGS in plant populations (Vekemans and Hardy 2004). Human-induced changes in wetlands and fluvial systems have caused disturbances in large-scale and fine-scale SGS of riparian species (Kikuchi et al. 2011).

The European white elm (*Ulmus laevis* Pallas) is a temperate deciduous tree that grows in riparian forests and damp soils in Central and Eastern Europe (Collin 2003). It is an anemophilous tree and its seeds are dispersed by wind and water. In Southwest Europe its populations are extremely rare, very small, fragmented and, due to their geographic location more sensitive to increasing aridification due to climate change. One of *U. laevis* glacial refuges was the Iberian Peninsula (Fuentes-Utrilla 2008), where the species is native and critically endangered. In the Mediterranean regions of Spain *U. laevis* mainly grows in seasonal waterlogged soils linked to aquifer discharge areas, river floodplains and small closed basins. Habitat destruction and Dutch elm disease (DED) have affected this species all over Europe, resulting in a need for specific conservation strategies (Collin et al. 2004; Goodall-Copestake et al. 2005). At its northern range, due to the low genetic diversity, the strong genetic structure and the outcrossing nature of the species, it may be especially vulnerable to size reduction (Nielsen and Kjær 2010). However, these northern populations are relatively large and continuous compared to the southern ones. In Southwest Europe, based on the genetic structure and diversity of its populations, some general conservation guidelines have been proposed for *U. laevis* (e.g., inclusion of the species in the Red List; establishment of evolutionary significant units and management units; and increasing population sizes within management units by reforestations) (Fuentes-Utrilla 2008). However, more detailed information on the fine-scale SGS and gene dispersal in the region are needed to implement sound conservation measures. To investigate the effect of anthropogenic habitat disruption on local SGS, we have studied two of the largest Spanish *U. laevis* populations (Fig. 1; Online Resource 1) with known human disruptions. The first one, Quitapesares, was first transformed to agricultural land and has recently become a golf course. The second, Valdelatas, suffered water table depletion and deforestation, and seems to have undergone a much higher population size reduction.

The aims of this study are (i) to determine the fine-scale genetic structure of *U. laevis* in two disturbed populations, (ii) to determine the demographic processes they have undergone, and (iii) to evaluate the recovery capabilities of these populations.

Materials and methods

Elm populations

The two populations are located in central Spain (Fig. 1) and belong to two different contiguous hydrological systems basins, that of the river Duero (Quitapesares) and that of the river Tagus (Valdelatas). The distance between them is 52 km and they are separated by the Sistema Central mountain range. They are isolated populations with no other known white elms in their proximity.

Quitapesares (40°54′N, 4°3′W) is located in Palazuelos de Eresma (Segovia), in a 115 ha estate at 1,100 m above sea level (a.s.l.). This farm was traditionally used for livestock grazing, and a document from 1,573 already mentions the presence of elms in this area (Archivo de la Real Chancillería de Valladolid, Registro de Ejecutorias, Caja 1255, 50). The terrain is flat and the water table is very high; thus many parts of the farm get waterlogged for several months. The elms grow in these areas and around a water reservoir, while fodder was cultivated in other parts of the farm (Fig. 2). In 2004, the population consisted of 130 mature elms (diameter at breast height [DBH] >10 cm) (Online Resource 2) and more than 200 saplings. However, in 2007 the farm was transformed into a golf course, and only 102 mature elms were still alive in April 2010, when samples were collected.

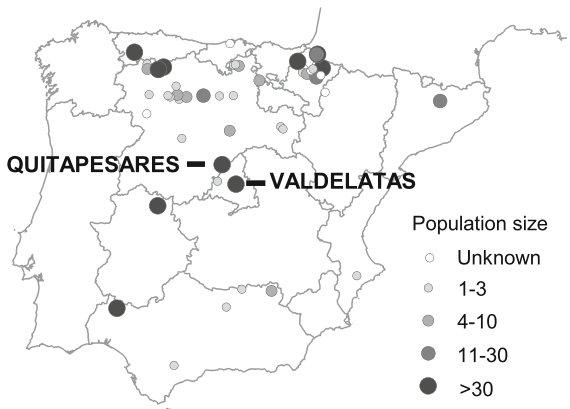

Fig. 1 Location of *Ulmus laevis* Pall. populations in Spain

Valdelatas is a 330 ha forest located close to Madrid (40°32′N, 3°40′W) at 700 m a.s.l. The first written evidence from this population is a 1,287 document where this forest is called *Monte-Negriello* (Colmenares 1640), which means "elm forest". This public domain forest was used as a Royal hunting-ground and for collecting firewood. During the Spanish Civil War (1936–1939) this woodland was nearly destroyed. In the early 1940s holm oak (*Quercus ilex* L.) resprouts were favored, and the clearings were reforested with this oak and with pines (*P. pinea* L. and *Pinus pinaster* Ait.). At that time, a nursery was established in the lower part of the stream where the elms are located. Very few large trees can be seen where the elm grove is nowadays in a 1946 aerial photograph of Valdelatas (Instituto de Estadística de la Comunidad de Madrid, www.madrid.org/nomecalles).

Valdelatas used to be an aquifer discharge area, and therefore seasonal waterlogging occurred in many areas, and water flowed in the small creeks despite their small catchment basins. However, nowadays the water table has been depleted and the population survives because the stream has a constant water flow from two sewage treatment plants installed in 1926 and 1968. The current elm population consists of 53 mature trees (DBH > 10 cm) and 104 saplings that grow along 325 m of a small stream (Fig. 2). Among the mature trees, one individual has a DBH of more than 100 cm, while DBH for the rest are less than 60 cm (Online Resource 2). The history of the population and the distribution of diameters among existing elms suggest that there has been a severe population size reduction in Valdelatas.

Sample collection

In Quitapesares leaves were collected from 57 randomly selected trees of the 102 remaining trees. In Valdelatas, they were collected from all 53 mature elms. Leaves were preserved in tagged tubes with silica-gel till DNA extraction.

Laboratory methods

DNA was extracted with Invisorb Spin Plant Mini Kit from Invitek (Germany). Total DNA was diluted to

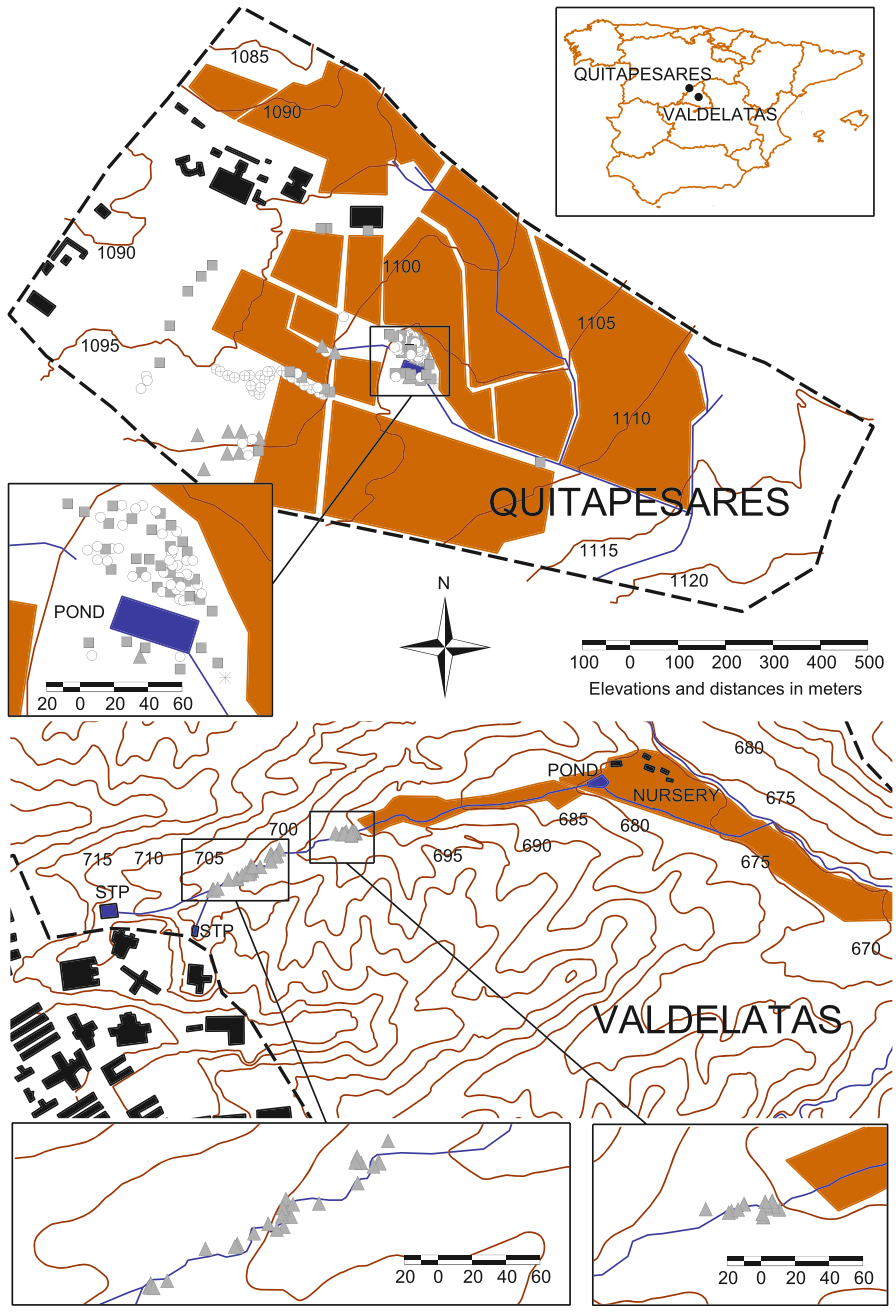

Fig. 2 Quitapesares and Valdelatas detailed map. *Triangles* chlorotype A elms, *squares* chlorotype B elms, *white circles* elms not sampled, *crossed circles* elms felled during the golf course construction before sample collection, *Asterisk* non-amplified haplotype, *STP* sewage treatment plant, *Shaded areas* transformed and plowed

2.5 ng/μL for the polymerase chain reactions (PCRs). Nineteen microsatellites were tested: Ulm2, Ulm3, Ulm9, Ulm 12, and Ulm19 (Whiteley et al. 2003), Ulmi1-11, Ulmi1-21, Ulmi1-98, Ulmi1-165, Ulmi2-20, and Ulm1-181 (Collada et al. 2004), and UR101, UR138, UR141, UR153, UR158, UR173, UR175, and UR188a (Zalapa et al. 2008). PCRs were carried out in 10 μL. Amplifications were done on Perkin-Elmer GenAmp 9700 thermal cycler using PCR protocol described in the respective articles. Amplified products IRDye labeled were denatured and visualized in a Li-Cor 4200 sequencer.

To distinguish the two chloroplast DNA (cpDNA) haplotypes, named A (widespread over Western Europe) and B (unique to Southern France and Spain), differentiated by a deletion (Fuentes-Utrilla 2008) in

the fragment SfM, a pair of specific primers flanking the variable cpDNA region were designed (SfM_UlaevisF CTCCCCAAGTTTTTCAGGTTGT and SfM_UlaevisR AGTTTGACCCCTCCCGCTAC). PCR amplifications were performed in 10 μL that contained: 1 μL 10× PCR buffer, 0.8 μL of 25 mM $MgCl_2$, 0.8 μL dNTPs (2.5 mM each), 1 μL of 2 μM, IRD-800 labeled, forward primer (SfM_UlaevisF), 1 μL of 2 μM reverse primer (SfM_UlaevisR), 1 μL of 5 ng/μL DNA, 025U *Taq* DNA polymerase, and 4.15 μL H_2O. Thermocycling conditions were as follows: 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s, and final extension at 72 °C for 5 min. Amplified products IRDye labeled were denatured and visualized in a Li-Cor 4200 sequencer, 108 pb haplotype A and 115 pb haplotype B were obtained.

Data analysis

Genetic characterization of the populations and Nei's *F* statistics (Nei 1987) calculation were performed with FSTAT 2.9.3.2 (Goudet 2001). The parameters evaluated per locus, population and overall were number of alleles (*A*), allelic richness corrected for sample size 53 (*Ar*), observed heterozygosity (*Ho*), within sample gene diversity (*Hs*), total gene diversity (*Ht*), expected gene diversity (*He*), gene diversity among populations corrected for number of samples (*Dst'*), an estimator of the fixation index (*Fst*), and an estimator of the inbreeding coefficient (*Fis*). Significance of *Fst* and *Fis* was tested randomizing, respectively, individuals among populations and genes among individuals within a population, in 20,000 permutations with SPAGEDI 1.3 (Hardy and Vekemans 2002).

To analyze the level of admixture between the populations, we evaluated the genetic clustering of individuals with STRUCTURE 2.3.3 (Pritchard et al. 2000). We tested the admixture model, giving prior population assignment information and without doing so. This program uses a Bayesian approach to identify the number of genetic clusters (*K*) that better explains the observed genotypes. The program was run at least three times with each *K* value (from 1 to 4) and model to make sure results were consistent. Burning-in period used was 10,000, with 100,000 post-burning simulations used to estimate model parameters.

The SGS within each one of the populations was studied with SPAGEDI 1.3 (Hardy and Vekemans 2002). Loiselle et al. (1995) pairwise kinship coefficient was calculated within each population for seven distance classes (0–20, 20–70, 70–170, 170–230, 230–325, 325–500, and 500–850 m; the last two distance classes do not apply to Valdelatas as there are no trees separated more than 325 m). Significance of the deviation from the estimated average kinship coefficient was tested randomizing locations among all individuals in 20,000 permutations. We also tested the significance of the regression slope of the kinship coefficient with logarithm of distance. Neighborhood size (*Nb*) and half the mean square parent-offspring distance (σ_g) were also calculated with SPAGEDI.

The demographic evolution of both populations was studied with several methods. Population bottlenecks were first tested with Wilcoxon sign rank test under infinite alleles model (IAM), stepwise mutation model (SMM), and two-phased model (TPM). This method is based on the premise that populations experiencing recent reductions in size develop an excess of heterozygosity at selectively neutral loci relative to the heterozygosity expected at mutation-drift equilibrium (Cornuet and Luikart 1996). These tests were run on BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) with 10,000 iterations. The *M* statistic (Garza and Williamson 2001), which is the mean ratio of the number of alleles to the range in allele size, was also calculated. *M* values below 0.68 identify long lasting bottlenecks under any mutation model. Finally, we also inferred the past demographic change of both populations with MSVAR 1.3 (Storz and Beaumont 2002), a full-likelihood Bayesian method. This method gives an estimate of the current (N_{POP}) and ancestral (N_{ANC}) population sizes, and the time since the population started changing in size. This model is based on a SMM, but it is robust to moderate departures from this model (Girod et al. 2011). We used an exponential population size change model. Six Monte Carlo simulations of 50,000 thinned update steps (every line was registered after 10,000 outputs) per population were analyzed with R-CODA package (Plummer et al. 2006).

The effective population size (N_e) was calculated with two single-sample methods. LDNe software (Waples and Do 2008) was used to calculate N_e by linkage-disequilibrium method, assuming random mating. N_e was also calculated by Bayesian inference using ONeSAMP (Tallmon et al. 2008). We tested the sensitivity of ONeSAMP results by running several simulations per population and per prior sizes (2–500; 2–1,000; 2–2,000).

Results

Of the nineteen nuclear microsatellites tested, nine were polymorphic (Table 1), five monomorphic (Um1-165, Um1-181, UR138, UR153, UR173), and five gave either unspecific amplifications or did not amplify at all (Ulm 12, Ulmi1-11, Ulmi1-21, Ulmi2-20, UR101). For the nine polymorphic loci the overall number of alleles per locus varied from 2 to 10 and the mean was 4.1. Within each population the number of alleles varied from 2 to 7 and the average was 3.4. Each population had three private alleles at locus UR158. The overall nuclear diversity (*Ht*) was 0.48. Quitapesares gene diversity ($He = 0.45$) was slightly higher than Valdelatas (0.43). These populations are differentiated as the observed fixation index ($Fst = 0.17$) clearly differs ($P < 0.001$) from the one obtained randomizing individuals (Fst_{random} 95 % confidence interval = [−0.006, 0.004]). *Fis* was not significantly different from 0 ($P > 0.05$) for Valdelatas nor for the entire dataset, and was slightly negative in Quitapesares ($Fis = -0.07$, $P < 0.05$) indicating an excess of heterozygotes in this population (Table 1). Therefore, these populations do not present inbreeding.

The cpDNA analysis revealed that in Quitapesares 10 trees presented haplotype A, common in Western Europe, and 46 had haplotype B, which is only present in Southern France and the Iberian Peninsula (Whiteley 2004; Fuentes-Utrilla 2008). All trees from Valdelatas showed haplotype A. Spatial grouping was observed in Quitapesares when haplotypes were mapped (Fig. 2). Seven out of the ten chlorotype A trees were grouped together in the Southwest part of the farm. However, Loiselle et al. (1995) kinship coefficient calculated for nuclear markers presented no correlation with logarithm of distance in Quitapesares ($P = 0.14$; Fig. 3). Therefore, in this population *Nb* and σ_g could not be calculated. Meanwhile, Valdelatas had a clear negative correlation between kinship coefficient (Loiselle et al. 1995) and the logarithm of distance (regression slope = −0.022; two-sided test $P < 0.0001$; Fig. 3). *Nb* for this population ranged between 44.3 and 32.1 individuals, and σ_g between 18.8 and 41.3 m, depending on the effective density established for the estimation (first values correspond to 100 trees/ha and second values to 15 trees/ha; densities within those figures gave intermediate values).

The number of clusters (*K*) that better fitted the data under the admixture model without prior population information was $K = 2$ (estimated logarithm for the probability of the data for each number of clusters: $K_1 = -1{,}770.0$; $K_2 = -1{,}558.6$; $K_3 = -1{,}610.0$; $K_4 = -1{,}667.4$). Cluster 1 was formed by 52 elms from Valdelatas and 4 from Quitapesares, while cluster 2 was formed by 53 trees from Quitapesares and 1 from Valdelatas (Fig. 4). The results with prior population information were very similar. These results show almost no overlap in nine-locus genetic composition. No subgroups were found within each

Table 1 Genetic characterization of the populations with Nei's *F* statistics (Nei 1987)

Loci	Overall ($n = 110$)								Quitapesares ($n = 57$)					Valdelatas ($n = 53$)				
	A	*Ar*	*Ho*	*Hs*	*Ht*	*Dst'*	*Fst*	*Fis*	*A*	*Ar*	*Ho*	*He*	*Fis*	*A*	*Ar*	*Ho*	*He*	*Fis*
Ulm19	4	4.0	0.54	0.58	0.69	0.21	0.26	0.07	3	3.0	0.46	0.49	0.06	4	4.0	0.62	0.68	0.08
Ulm9	6	6.0	0.67	0.68	0.79	0.23	0.25	0.01	6	6.0	0.77	0.76	−0.02	5	5.0	0.57	0.59	0.04
Ulm1-98	3	3.0	0.41	0.37	0.39	0.04	0.09	−0.10	3	2.9	0.25	0.22	−0.12	3	3.0	0.57	0.52	−0.08
Ulm2	2	2.0	0.49	0.42	0.43	0.01	0.03	−0.16	2	2.0	0.56	0.47	−0.20	2	2.0	0.42	0.37	−0.11
Ulm3	3	3.0	0.42	0.39	0.43	0.09	0.18	−0.10	3	3.0	0.58	0.52	−0.12	3	3.0	0.26	0.25	−0.04
UR175	2	2.0	0.23	0.26	0.26	0.00	−0.01	0.11	2	2.0	0.30	0.28	−0.07	2	2.0	0.17	0.25	0.31
UR141	4	4.0	0.53	0.44	0.46	0.04	0.08	−0.20	3	3.0	0.42	0.37	−0.15	3	3.0	0.64	0.52	−0.24
UR158	10	7.8	0.47	0.53	0.54	0.02	0.04	0.11	7	6.8	0.49	0.43	−0.15	7	7.0	0.45	0.63	0.29
UR188	3	2.5	0.25	0.26	0.35	0.19	0.42	0.03	2	2.0	0.48	0.50	0.03	2	2.0	0.02	0.02	0.00
Overall	4.1	3.8	0.45	0.44	0.48	0.09	0.17	−0.02	3.4	3.4	0.48	0.45	−0.07	3.4	3.4	0.41	0.43	0.03

n sample size, *A* number of alleles, *Ar* allelic richness corrected for sample size 53, *Ho* observed heterozygosity, *Hs* within sample gene diversity, *Ht* total gene diversity, *He* gene diversity, *Dst'* gene diversity among samples, *Fst* estimator of the fixation index, *Fis* estimator of the inbreeding coefficient

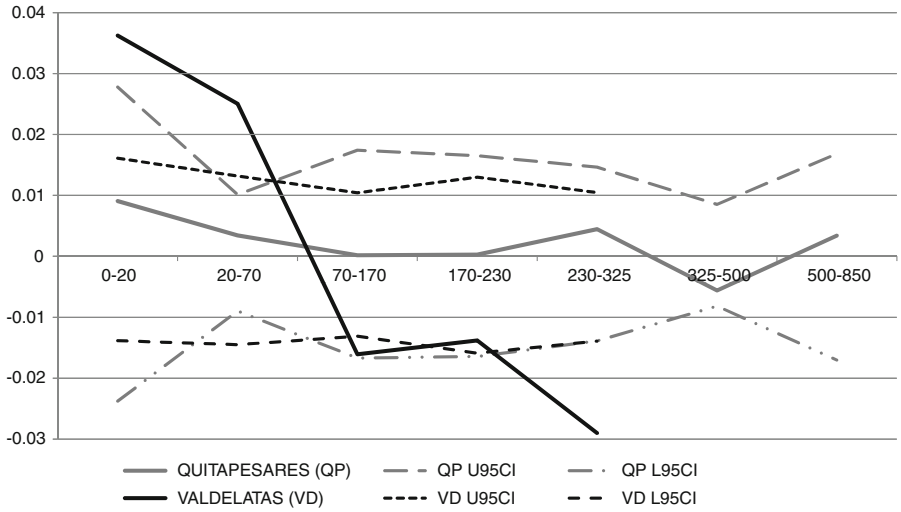

Fig. 3 Quitapesares and Valdelatas spatial genetic structure. The kinship coefficient (Loiselle et al. 1995) is represented for different distance classes (m). *Solid lines* represent the observed values and *broken lines* mark the 95 % confidence intervals for random distribution obtained with 20,000 permutations

population. We also ran nuclear DNA (nDNA) analysis in Quitapesares considering trees with chlorotypes A and B as different populations, and no differences were found among them (data not shown).

The *M* statistic was 0.56 for Quitapesares and 0.48 for Valdelatas, indicative of population bottleneck in both populations. However, with the Wilcoxon sign rank test (Luikart et al. 1998) bottleneck signal was only detected for Valdelatas and only under IAM ($P = 0.024$; Table 2). Under a relaxed probability ($P < 0.10$) excess in heterozygosity was also detected for Quitapesares under the IAM. Despite MSVAR wide confidence intervals, its estimations show that both population sizes have declined, and that this decline is not recent (Table 2).

N_e estimated by ONeSAMP did not depend on prior population size and was convergent for different runs. N_e was similar for Quitapesares (27) and Valdelatas (30) (Table 2; data shown for prior size 2–1,000). N_e obtained for Valdelatas by LDNe was very similar. However, LDNe estimated a higher N_e (91) for Quitapesares with an enormous confidence interval (Table 2).

Discussion

The low genetic diversity observed in Quitapesares and Valdelatas is in accordance with other *U. laevis* populations studied in Europe (e.g., The Netherlands $He = 0.50$, Denmark $He = 0.50$, Central Europe $He = 0.59$) (Whiteley 2004; Fuentes-Utrilla 2008; Nielsen and Kjær 2010). Nevertheless, Quitapesares, with two haplotypes, is more diverse at cpDNA level than most European populations (Whiteley 2004; Fuentes-Utrilla 2008).

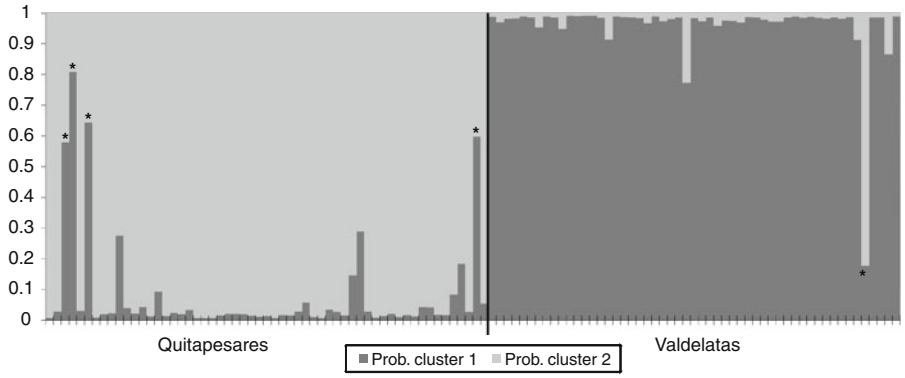

Fig. 4 Clusters inferred with structure. *Bar* plot representing the probabilities assigned to each tree of belonging to cluster 1 or 2 under admixture model. The *black line* separates samples from both populations. Samples clustered with the other population (*asterisk*)

Table 2 Demographic analyzes

Population	N	M	Wilcoxon test: # locus with heterozygosity excess (P value)		
			IAM	TPM	SMM
Quitapesares	57	0.56	7 (0.064)	6 (0.248)	6 (0.500)
Valdelatas	53	0.48	7 (0.024)	5 (0.500)	5 (0.820)

Population	N_e [95 % CI]		MSVAR: mean [95 % CI]		
	ONeSAMP	LDNe	N_{POP}	N_{ANC}	D_Y
Quitapesares	27 [21–50]	91 [34–∞]	326 [6–16,436]	157,435 [3,701–6,697,304]	171,002 [3,305–8,847,081]
Valdelatas	30 [22–66]	30 [16–89]	210 [4–11,290]	372,396 [6,321–11,738,159]	87,862 [1,768–4,365,447]

N is the number of sampled trees, M is the statistic described by Garza and Williamson (2001). Wilcoxon test was run over the 9 locus. *IAM* infinite allele model, *TPM* two phase model (20 % variance and 80 % assigned to SMM), *SMM* single mutation model. N_e effective population size calculated with ONeSAMP (Tallmon et al. 2008) and with LDNe (Waples and Do 2008), and their 95 % confidence intervals. MSVar demographic analyzes (Storz and Beaumont 2002): estimated current population size (N_{POP}), estimated ancestral population size (N_{ANC}), and years since the populations started to decline (D_Y)

Pollen records show that the Iberian Peninsula has been a glacial refuge for *Ulmus* genus during the Pleistocene (Gil and García-Nieto 1990; López 2000), and that the maximum representation of the genus is reached in the Holocene during the Atlantic (8,000–5,000 years BP) and Subboreal (5,000–2,500 years BP) periods (López 2000). Afterwards, the proportion of elm and other tree pollen is considerably reduced, mainly due to human habitat transformation, as pasture and agricultural species pollen increased significantly (López 2000; Valbuena-Carabaña et al. 2010). Unfortunately, the three Iberian elm species (*U. laevis*, *U. glabra* Hudson, and *U. minor* Mill.) pollen is not distinguishable (Stafford 1995), and therefore we cannot infer *U. laevis* populations evolution from pollen records. However, the M values and MSVAR results (Table 2) are indicative of long lasting bottlenecks in both populations (Garza and Williamson 2001; Storz and Beaumont 2002). Our MSVAR time estimations have very wide confidence intervals (Table 2), which happens when there is no prior mutation rate knowledge (Girod et al. 2011). Yet, the estimation of time since the decline started locates the bottleneck in the glacial periods of the Pleistocene (2,500,000–11,000 years BP). Nevertheless, taking into consideration the lower confidence interval it is also possible that the bottlenecks occurred in the last 3,500 years, when pollen records show a decline in elm populations due to human landscape transformation (López 2000; Valbuena-Carabaña et al. 2010).

We know that both populations have suffered recent demographic size reductions due to anthropogenic habitat transformation: Quitapesares, initially because it was partially cultivated, and afterwards due to the golf course construction; and Valdelatas, due to the water table depletion, Spanish Civil War and the nursery and hospital construction (Fig. 2). The Wilcoxon sign rank test, which is good for detecting recent bottlenecks (Cornuet and Luikart 1996; Luikart et al. 1998; Williamson-Natesan 2005), did not show bottleneck signal under TPM nor SMM for neither population (Table 2). However, if it is extremely recent (i.e., this generation) it would not be detectible. Only under IAM Valdelatas presents bottleneck signal. However, the TPM appears to provide a better fit to empirical evidence about the mutation process (Di Rienzo et al. 1994; Garza and Williamson 2001). It is important to recognize that populations suffering a reduction in census size may not suffer a severe reduction of N_e (a genetic bottleneck), if historical N_e has always been low, due to fluctuations in population size, mating system dynamics, or metapopulation structure involving local extinctions and recolonizations (Pimm et al. 1998). The small effective population sizes observed in Valdelatas and Quitapesares, and the low genetic diversity of the species all over its range (Whiteley 2004; Fuentes-Utrilla 2008; Nielsen and Kjær 2010), also support this idea. Nevertheless, population sizes nowadays are similar to N_e and therefore, further size reductions could cause a genetic bottleneck in future generations.

No clones were found in Quitapesares or in Valdelatas, and no root-suckers were detected. However, stool–shoots were observed in the stumps of felled

elms in both populations. Thus, although *U. laevis* stool–shoots and root-suckers may play an important role in the regeneration and colonization of riparian formations after flooding disturbance (Collin 2003; Deiller et al. 2003), in wetland formations regeneration seems to be accomplished by seedling recruitment. However, when trees were felled for wood in Valdelatas, before and during the Civil War, stool–shoots could have been crucial for the maintenance of genetic diversity and recovery of the *U. laevis* grove. Clonal reproduction by sprouting has been described as a mechanism in rare tree species to buffer the genetic impacts of fragmentation by delaying the time between generations, and thereby, moderating the loss of alleles through genetic drift (Wei and Jiang 2012).

No nDNA spatial structuring was detected in Quitapesares (Fig. 3). However, spatial grouping of cpDNA haplotypes distribution can be observed in Quitapesares (Fig. 2). Chloroplast is maternally inherited in elms, so this grouping indicates that the seeds, although they are winged, are not usually dispersed long distances by wind. The lack of differences at the nuclear level among the trees with different cpDNA haplotypes within the population, and the lack of fine-scale SGS, indicates that local pollen flow is not limited. Nielsen and Kjær (2010) established that the average pollination and seed dispersal distance in a Danish forest were relatively low (under 40 m), although some pollen came from far away, and in open areas pollination distance was 1,100–1,200 m. These distances are greater than the two most separated trees in Quitapesares, therefore all the trees within this population can potentially mate among each other. In fluvial systems, long distance dispersal of propagules (hydrochory) can reduce spatial aggregation of related individuals (Kikuchi et al. 2011), but in bogs (area of soft muddy ground) and seasonal wetlands this means of transport might not be that effective. The flatness of seasonal wetland areas and their open forest structure favors pollen and seed dispersal in all directions, and not only along the river axis. Low population density, resulting from historical degradation of forests, also favored greater effective dispersal distances in other tree species (López de Heredia et al. 2010; Kikuchi et al. 2011). Therefore, we consider that these factors contribute to eliminate the nDNA SGS within Quitapesares.

Currently, the only *U. laevis* trees that are left in Valdelatas grow along the stream that has a constant water flow from two sewage treatment plants. The lowered water table, mainly due to the overexploitation of the aquifer, have caused the disappearance of the seasonal waterlogged areas in this forest, and ultimately, of suitable areas for the elms. Moreover, the most suitable area for this species in the area corresponds to the debris cone located at the bottom of the stream, where the tree nursery was established (Fig. 2), which caused the disappearance of pre-existing vegetation. Therefore, the strong genetic structure of Valdelatas (Fig. 3) could be partly due to the human-induced constrictions in suitable habitat, and not only to natural gene dispersal limitations as in Denmark (Nielsen and Kjær 2010). The elm population in Valdelatas, with average gene dispersal distance ranging between 19 and 41 m, is an example of the limitations for pollen and seed dispersal in Mediterranean climates, due to the canopy barrier effect (Milleron et al. 2012) of sclerophyllous vegetation (evergreen oaks and pines) and the increasing rarity of humid areas suitable for the establishment of seedlings.

No inbreeding is observed in Quitapesares or in Valdelatas. This result supports previous evidence of *U. laevis* being highly outcrossing and self-incompatible (Mittempergher and Porta 1991; Nielsen and Kjær 2010). Both populations produce great quantities of sound seeds in mast years, that have high germination (55.9 ± 22.0 %, mean $\pm$ standard deviation) and establishment rates (over 90 % in ex situ conservation plots) (unpublished data), indicating no fitness problems. However, seedlings cannot establish themselves in Quitapesares since it has been transformed into a golf course, nor in Valdelatas as there is no suitable area. Therefore, we consider that the main risk for these populations is lack of regeneration possibilities due to habitat transformation.

More than 60 % of the wetlands have disappeared in Spain in the last 50 years mainly due to land-use changes and mechanization of agriculture (Casado and Montes 1995; Gallego-Fernández et al. 1999). Moreover, under the scenario of increasing aridity in the Iberian Peninsula and higher water demand (Moreno 2005; Rodríguez et al. 2007), remaining wetland ecosystems will be compromised. This will put more pressure on *U. laevis* and will jeopardize the recovery and persistence of its remaining populations. Hence, managers might have to consider the necessity of recharging aquifers or pumping water into wetland

formations for their conservation. Quitapesares and Valdelatas are not at genetic risk, nonetheless, for long-term conservation they do require in situ conservation measures that permit the establishment of seedlings, in order to maximize the genetic variation preserved and to facilitate dynamic conservation strategies (Collin et al. 2004).

Conclusions

Vegetative propagation does not occur in *U. laevis* wetland populations, and stool–shoots help to maintain genetic diversity after felling and fragmentation. This contrasts with riparian formations, where clonal reproduction also permits colonization of new sites and population expansion.

Quitapesares and Valdelatas maintain the same levels of genetic diversity as other European populations and are not at genetic risk, regardless of the long lasting bottleneck and recent size reductions they have undergone. Seed production and viability do not limit the regeneration of these stands; it is the lack of suitable areas for establishment due to human habitat transformation that avoid population expansion and recruitment. This also affects their fine-scale SGS. Therefore, habitat destruction, fragmentation, aridification, and water table depletion present higher risks to the conservation of these populations.

Acknowledgments We would like to thank César Cardo, Alberto Pérez, and Julio Orellana for having shown us Valdelatas elm grove. We thank Segovia XXI for having allowed us collect samples in Quitapesares and helping us preserve this population. We thank Eudaldo González for the elm surveys, he has carried out all over Spain. We are also grateful to Salustiano Iglesias, Pablo Sanjuanbenito, and Cuenca Alta del Manzanares park managers for their support. We also show gratitude to Zaida Lorenzo, Elena Zafra, Eva Miranda, Jorge Domínguez, David Macaya, Gerrie Seket, Guillermo González, Miriam Fajardo, and Ana Moreno for their technical assistance and help. This study was financed by the Comunidad de Madrid (Project S2009AMB-1668). M.V. is sponsored by a PIF Grant from the Technical University of Madrid.

References

Álvarez-Cobelas M, Cirujano S, Sánchez-Carrillo S (2001) Hydrological and botanical man-made changes in the Spanish wetland of Las Tablas de Daimiel. Biol Conserv 97:89–98

Antrop M (2005) Why landscapes of the past are important for the future. Lands Urban Plan 70:21–34

Casado S, Montes C (1995) Guía de los lagos y humedales de España. Ed. J.M. Reyero, Madrid

Collada C, Fuentes-Utrilla P, Gil L, Cervera MT (2004) Characterization of microsatellite loci in *Ulmus minor* Miller and cross-amplification in *U. glabra* Hudson and *U. laevis* Pall. Mol Ecol Notes 4(4):731–732

Collin E (2003) EUFORGEN Technical guidelines for genetic conservation and use for European white elm (*Ulmus laevis*). Forestry. International Plant Genetic Resources Institute, Rome

Collin E, Rusanen M, Ackzell L et al (2004) Methods and progress in the conservation of elm genetic resources in Europe. Invest Agrar: Sist Recur For 13(1):261–272

Colmenares D (1640) Historia de la insigne ciudad de Segovia y compendio de las historias de Castilla. Segovia, Madrid

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

De Luis M, Brunetti M, Gonzalez-Hidalgo J, Longares LA, Martin-Vide J (2010) Changes in seasonal precipitation in the Iberian Peninsula during 1946–2005. Glob Planet Change 74:27–33

Deiller AF, Walter JMN, Trémolières M (2003) Regeneration strategies in a temperate hardwood floodplain forest of the Upper Rhine: sexual versus vegetative reproduction of woody species. For Ecol Manag 180:215–225

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer MB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA 91:3166–3170

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Evol Syst 24:217–242

Fuentes-Utrilla P (2008) Estudio de la variabilidad genética del género *Ulmus* L. en España mediante marcadores moleculares. Dissertation, Universidad Politécnica de Madrid

Gallego-Fernández JB, García-Mora MR, García-Novo F (1999) Small wetlands lost: a biological conservation hazard in Mediterranean landscapes. Environ Conserv 26(3):190–199

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10(2):305–318

Gil L, García-Nieto ME (1990) Paleobotánica e historia de los olmos de la Península Ibérica. In: Gil L (ed) Los olmos y la grafiosis en España. ICONA, Madrid, pp 29–65

Girod C, Vitalis R, Leblois R, Fréville H (2011) Inferring population decline and expansion from microsatellite data: a simulation-based evaluation of the Msvar method. Genetics 188:165–179

Goodall-Copestake WP, Hollingsworth ML, Hollingsworth PM, Jenkins GI, Collin E (2005) Molecular markers and ex situ conservation of the European elms (*Ulmus* spp.). Biol Conserv 122(4):537–546

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from: goudet J (1995) FSTAT (vers. 1.2): a computer program to calculate *F*-statistics. J Hered 86:485–486

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Hooke JM (2006) Human impacts on fluvial systems in the Mediterranean region. Geomorphology 79:311–355

Iriarte MJ (2009) Vegetation landscape and the anthropization of the environment in the central sector of the Northern Iberian Peninsula: current status. Quat Int 200(1–2):66–76

Kikuchi S, Suzuki W, Sashimura N (2011) Gene flow in an endangered willow *Salix hukaoana* (Salicaceae) in natural and fragmented riparian landscapes. Conserv Genet 12(1): 79–89

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understorey shrub, Psychotria officinalis (Rubiaceae). Am J Bot 82:1420–1425

López RA (2000) Paleobotánica de los olmos. In: Gil L, Solla A, Iglesias S (eds) Los olmos ibéricos. Conservación y mejora frente a la grafiosis. Organismo Autónomo Parques Nacionales, Madrid, pp 49–68

López de Heredia U, Venturas M, López RA, Gil L (2010) High biogeographical and evolutionary value of Canary Island pine populations out of the elevational pine belt: the case of a relict coastal population. J Biogeogr 37:2371–2383

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89(3):238–247

Milleron M, López de Heredia U, Lorenzo Z et al (2012) Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus sylvatica* L.). Plant Ecol. doi:10.1007/s11258-012-0125-2

Mittempergher L, Porta N (1991) Hybridization studies in the Eurasian species of elm (*Ulmus* spp.). Silvae Genet 40(5/6):237–243

Moreno JM (2005) Evaluación preliminar de los impactos en España por efecto del cambio climático. Ministerio de Medio Ambiente, Madrid

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 51: 238–254

Mutke S, Gordo J, Gil L (2005) Variability of Mediterranean stone pine cone production: yield loss as response to climate change. Agric For Meteorol 132:263–272

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Newton AC, Allnutt TR, Gillies ACM, Lowe AJ, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol Evol 14:140–145

Nielsen LR, Kjær ED (2010) Fine-scale gene flow and genetic structure in a relic *Ulmus laevis* population at its northern range. Tree Genet Genomes 6(5):643–649

Pimm SL, Gittleman JL, McCracken GF (1998) Plausible alternatives to bottlenecks to explain reduced genetic diversity. Trends Ecol Evol 4(6):176–178

Plummer M, Best N, Cowles K, Vines K (2006) CODA: convergence diagnosis and output analysis for MCMC. R News 6:7–11

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rodríguez JA, Weatherhead EK, Knox JW, Camacho E (2007) Climate change impacts on irrigation water requirements in the Guadalquivir river basin in Spain. Reg Environ Chang 7:149–159

Stafford PJ (1995) The Northwest European Pollen Flora. Ulmaceae. Rev Palaeobot Palyno 88:25–46

Storz JF, Beaumont MA (2002) Testing for genetic evidence of population expansion and contraction: an empirical analysis of microsatellite DNA variation using a hierarchical Bayesian model. Evolution 56:154–166

Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Mol Ecol Resour 8(2):299–301

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. Proc Natl Acad Sci USA 102(23):8245–8250

Valbuena-Carabaña M, López de Heredia U, Fuentes-Utrilla P, González-Doncel I, Gil L (2010) Historical and recent changes in the Spanish forests: a socio-economic process. Rev Palaeobot Palynol 162(3):492–506

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol 13:921–935

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8(4):753–756

Wei X, Jiang M (2012) Limited genetic impacts of habitat fragmentation in an "old rare" relict tree, *Euptelea pleiospermum* (Eupteleaceae). Plant Ecol 213:909–917

Whiteley RE (2004) Quantitative and molecular genetic variation in *Ulmus laevis* Pall. Dissertation, Swedish University of Agricultural Sciences

Whiteley RE, Black-Samuelsson S, Clapham D (2003) Development of microsatellite markers for the European white elm (*Ulmus laevis* Pall.) and cross-species amplification within the genus *Ulmus*. Mol Ecol Notes 3:598–600

Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. Conserv Genet 6:551–562

Zalapa JE, Brunet J, Guries RP (2008) Isolation and characterization of microsatellite markers for red elm (*Ulmus rubra* Muhl.) and cross-species amplification with Siberian elm (*Ulmus pumila* L.). Mol Ecol Resour 8(1):109–112