



GENETIC AND ENVIRONMENTAL CHARACTERIZATION OF *ABIES ALBA* MILL. POPULATIONS AT ITS WESTERN REAR EDGE

Caracterización genética y ambiental de poblaciones de Abies alba Mill. en su retaguardia occidental

D. Sancho-Knapik^{1,†}, J.J. Peguero-Pina^{1,†}, E. Cremer², J.J. Camarero³, Á. Fernández-Cancio⁴, N. Ibarra⁵, M. Konnert² & E. Gil-Pelegrín^{1,*}

¹Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria, Gobierno de Aragón, 50059, Zaragoza, Spain

²Bavarian Office for Forest Seeding and Planting (ASP), Forstamtsplatz 1, 83317 Teisendorf, Germany

³ARAIID, Instituto Pirenaico de Ecología (CSIC). Apdo. 13034, 50080 Zaragoza, Spain

⁴Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Departamento de Ecología y Genética Forestal, Carretera de La Coruña km 7.5, 28040, Madrid, Spain

⁵Unidad de la Salud de los Bosques, Gobierno de Aragón, 50059, Zaragoza, Spain

*Author for correspondence. E-mail: egilp@aragon.es

†These authors contributed equally to this study

Recibido: 09-06-2014. **Aceptado:** 02-09-2014. **Fecha de publicación on-line:** 21-10-2014

Citation / Cómo citar este artículo: Sancho-Knapik, D., Peguero-Pina, J. J., Cremer, E., Camarero, J. J., Fernández-Cancio, Á., Ibarra, N., Konnert, M. & Gil-Pelegrín, E. (2014). Genetic and environmental characterization of *Abies alba* Mill. populations at its western rear edge. *Pirineos*, 169, e007. doi: <http://dx.doi.org/10.3989/Pirineos.2014.169007>

ABSTRACT: The genetic structure of ten *Abies alba* populations at the western rear edge in the Spanish Pyrenees was characterized and compared with two German populations, looking for the role of climatic factors in the fir decline. Growth, defoliation, aridity and cumulative summer water deficit summer were also characterized. Spanish populations show a lower genetic diversity and a high genetic differentiation than German ones, mainly established by an East (higher diversity)-West (lower diversity) gradient. The three defoliated populations are the western ones, with higher summer aridity. Contrastingly, the southern population (Guara) shows climatic variables close to western sites but without defoliation and with a higher genetic diversity, indicating a possible adaptation to the sub-Mediterranean conditions. Silver fir in the Spanish Pyrenees constitutes a “stable” rear edge because of their isolation, small sized and small genetic diversity. Western Pyrenean sites subjected to dryer conditions and presenting lower genetic diversity are prone to drought-induced mortality in the context of global warming.

KEYWORDS: Decline; drought; genetic diversity; silver fir; Pyrenees.

RESUMEN: Se ha caracterizado la estructura genética de diez poblaciones de *Abies alba* en la retaguardia occidental de su distribución en el Pirineo español en comparación con dos poblaciones de Alemania, buscando la influencia de factores climáticos en el decaimiento del abeto. También se caracterizó crecimiento, defoliación, aridez y déficit acumulado de agua durante el verano. Las poblaciones españolas mostraron una menor diversidad genética y una mayor diferenciación genética que las poblaciones alemanas, establecida principalmente a través de un eje Este (mayor diversidad)-Oeste (menor diversidad). Las tres poblaciones con defoliación son las del suroeste, con mayor aridez estival. Por el contrario, la población más meridional (Guara) muestra variables climáticas cercanas a estas poblaciones del suroeste pero sin defoliación y con mayor diversidad genética, indicando una posible adaptación a las condiciones sub-Mediterráneas. El abeto blanco en el Pirineo español constituye una retaguardia estable debido a su aislamiento, pequeño tamaño y baja diversidad genética. Las poblaciones más occidentales, bajo mayor aridez y con una diversidad genética más baja, son propensas a una mortalidad inducida por la sequía en un contexto de calentamiento global.

PALABRAS CLAVE: Decaimiento; sequía; diversidad genética; abeto blanco; Pirineos.

1. Introduction

Tree populations are facing new and rapidly changing selective pressures such as more frequent extreme droughts which threaten the conservation of forests and related ecosystem services (Lindner *et al.*, 2010). Drought-induced forest decline is a major emerging threat for forests in drought-prone areas (Allen *et al.*, 2010) but also in mesic temperate forests (van Mantgem & Stephenson, 2007). At broad biogeographical scales, drought-related forest decline has been usually reported near the species southernmost distribution limit (rear edge *sensu* Hampe & Petit, 2005) where drought stress is often presumed to be more limiting for the species persistence than in the core area of distribution (Jump *et al.*, 2009).

One of the major research gaps identified by Allen *et al.* (2010) is a better knowledge on within-species genetic variability as related to forest decline which may allow understanding the differential responses of rear-edge tree populations to drought stress. First, rear-edge populations may be genetically impoverished because of their historical isolation or due to further fragmentation. Second, rear-edge tree populations living near the species xeric distribution limit may have adapted to drought stress and represent genetic reservoirs of drought-resistant genotypes. Genetic impoverishment may lead to high vulnerability against drought-induced decline. On the contrary, selection of drought-resistant genotypes may lead to populations of a high adaptive value in a warmer and dryer climatic scenario.

Silver fir (*Abies alba*) constitutes a suitable species to test these contrasting ideas since (i) its genetic variation and paleogeography across many European populations has been quantitatively evaluated (e.g., Liepelt *et al.*, 2009; Alba-Sánchez *et al.*, 2010), and (ii) it has experienced drought-induced decline in its western distribution limit in the Spanish Pyrenees since the late 1980s (Camarero *et al.*, 2011). The current geographical distribution of silver fir, which dominates wide forested areas in central Europe, is the result of postglacial recolonization, which started about 11000 years ago from glacial refugia located in southern Europe such as the Pyrenees (Liepelt *et al.*, 2009). However, while its distribution is quite continuous in the center of the range, this species shows a fragmented distribution patterns at the periphery (Gömöry *et al.*, 2012). This is the case of the Pyrenean species' rear edge (Camarero *et al.*, 2004).

The reduced population sizes and the habitat fragmentation of Pyrenean silver fir populations may result in reduced genetic variation, increased levels of inbreeding within populations, and also elevation genetic differentiation among populations (Young *et al.*, 1996; Leimu *et al.*, 2010). In fact, previous studies have shown a lower genetic variability for silver fir in the Pyrenees compared to other European fir populations using either isozymes (Konnert & Bergmann, 1995) or chloroplast microsatellites (Vendramin *et al.*, 1999). The performance of these populations may also be

compromised by high levels of genetic drift (Bacles & Jump, 2010), leading to increased homozygosity, which may negatively affect individual fitness (Leimu *et al.*, 2006). As a long-term consequence, these processes could induce a reduction in the adaptive potential of these fragmented populations reducing their resilience ability to cope with changing climatic conditions (Peroldi *et al.*, 2007).

Rear-edge populations respond more to drought stress than less marginal populations in terms of radial growth (Carrer *et al.*, 2010), being the former more prone to extirpation due to stochastic events than the latter (Lesica & Allendorf, 1995). Thus, rear-edge populations may be very susceptible to rising temperatures and associated aridification trends, which may trigger drought-induced forest decline (Macias *et al.*, 2006). Moreover, selective logging has been proposed as an additional contributing factor of decline through the negative selection of slow-growing trees (Camarero *et al.*, 2011), and the opening of abundant gaps thus reducing air humidity which is a main ecophysiological constraint of silver fir (Peguero-Pina *et al.*, 2007).

Thereby, the genetic status in such peripheral rear-edge silver fir populations is a decisive aspect for assessing their adaptive ability and resilience potential against increasing aridification trends. Thus, the genetic structure of Pyrenean silver fir populations may be hypothesized to be a risk factor for predisposing them to drought-induced decline. On that account we characterize the genetic structure of ten silver fir populations located in the Spanish Pyrenees as compared with two Central European populations using nuclear microsatellite markers. We analyze climatic variables and long-term climatic trends and we characterize forest structure in declining and non-declining Pyrenean sites. Finally, a comprehensive assessment of climatic trends, silver-fir decline patterns and genetic data is made by relating them, concluding with a consideration of potential risk of these interactions on local extinction processes of rear-edge tree populations.

2. Materials and Methods

2.1. Study area

Silver fir (*Abies alba* Mill.) presents a quite continuous distribution area in Central Europe and it is also found in several European mountains (Alps, Carpathians, Balkans, Apennines and Pyrenees) (Pawlaczyk *et al.*, 2005). The studied silver fir populations are located in the Spanish Aragón Pyrenees (rear edge populations, southwestern Europe) and in southern Germany (central Europe) (Fig. 1). The Pyrenees is a mountain range which extends from west to east along the border between Spain and France, and it constitutes a transitional area between more humid conditions in their northern margin and drier Mediterranean conditions southwards (Vigo & Ninot, 1987). This latitudinal gradient over-

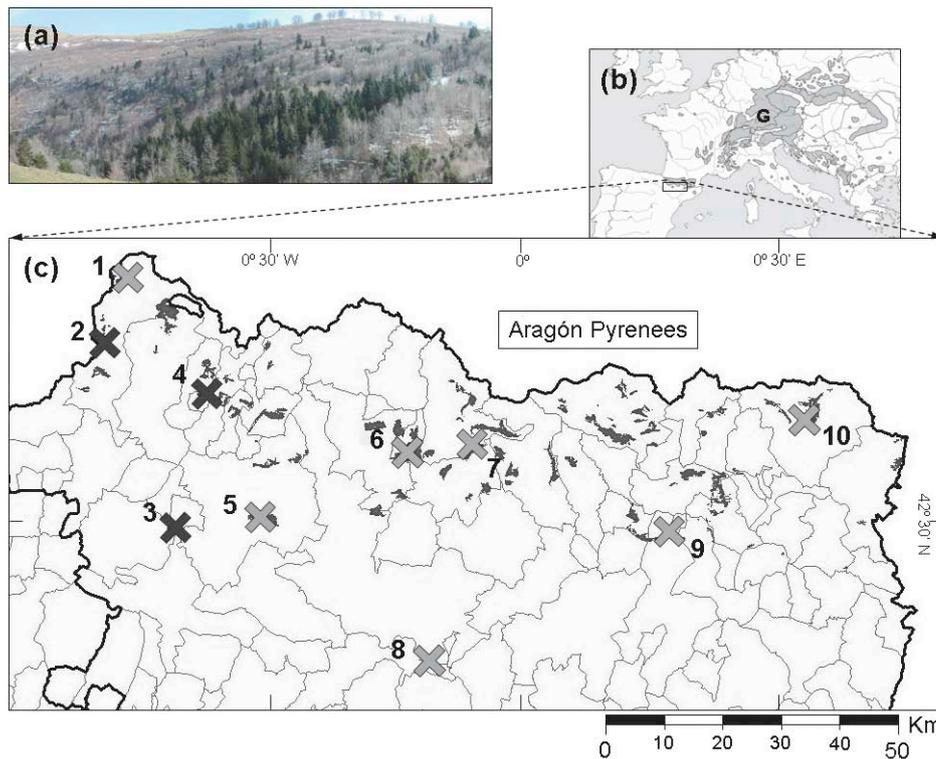


Figure 1. View of a fragmented Pyrenean silver fir population (site Gamueta) (a), distribution maps of silver fir in Europe (b) and the Spanish Aragón Pyrenees (c) showing the location of the study sites forming the species' rear edge (see sites' codes in Table 1). The compared Central European populations are also indicated in the upper map (code G). In the lower map the dark symbols correspond to declining populations.

Figura 1. Fotografía de una población fragmentada de abeto blanco en el Pirineo (Gamueta) (a), mapas de distribución del abeto blanco en Europa (b) y en Aragón (c) mostrando la localización de los lugares de estudio que forman la retaguardia de la especie (ver códigos en Tabla 1). Las poblaciones centroeuropeas comparadas están también indicadas en el mapa superior (código G). En el mapa inferior los símbolos más oscuros corresponden a las poblaciones en decaimiento.

laps with a longitudinal gradient caused by the location of the range between the Atlantic Ocean and the Mediterranean Sea. This location confers to the Pyrenees a continental climate regime influenced by oceanic (westwards) and by Mediterranean (eastwards) cyclonic activity. The westward oceanic influence leads to greater precipitation in winter and a smaller temperature range than eastwards, where the Mediterranean influence is characterized by higher precipitation in summer (Camarero *et al.*, 2011).

In the Aragón Pyrenees, silver fir stands are usually found at humid sites on north-facing slopes, where they form pure or mixed stands with *Fagus sylvatica* L. or *Pinus sylvestris* L. The study sites have an elevation between 1100 and 1700 m, a mean total annual precipitation between 1100 and 1500 mm, and a mean annual temperature ranging between 7.0 and 10.0 °C (Table 1). For comparison purposes two silver fir populations from central Europe (locate in southern Germany) were chosen (Table 1). The latter sites are located in elevations around 770 m, and show means annual precipitations between 1000 and

1300 mm and mean annual temperatures varying between 6.5 and 8.0 °C.

2.2. Climate data

The climate data used in this study (annual and monthly values of mean temperature and total precipitation) corresponded to the period 1930-2010. It was obtained with the GENPT software (Manrique & Fernández-Cancio, 2005), which allows the generation of local climatic data (spatial resolution ca. 15-20 km) using as a reference the dataset collected from meteorological stations managed by the Spanish Meteorological Agency (<http://www.aemet.es/>). The software algorithms used 8000 real meteorological stations all over Spain for local approximations by using combined linear gradient methods based on means, regressions and variance stabilization, with mean square errors of under 0.5 °C in temperature and 5-10 % in precipitation in comparison with the reference stations.

Since silver fir growth is highly sensitive to cumulative water deficit in summer we paid special attention to characterize this deficit. First, we calculated the Gausser aridity index as the number of months in which $P_i < 2T_i$, where P_i is the monthly precipitation (in mm) and T_i is the monthly mean temperature (in °C) for the month i . Moreover, the annual (MAI = $P/(T + 10)$) and the monthly Martonne aridity indices ($12 P_i/(T_i + 10)$) were also calculated. The mean value of the monthly Martonne indices for June, July and August was regarded as the summer Martonne aridity index (sMAI). Second, we obtained the ratio between the relative difference between summer (SP) and winter precipitation (WP) to describe the seasonal rainfall regime of each site as $(SP - WP)/(SP + WP)$. Positive values of the ratio indicate that more precipitation is recorded in summer than in winter and vice versa.

2.3. Stand description and decline characterization

Sampling of Pyrenean silver fir stands was conducted between 1999 and 2001 and defoliation data were updated in 2012. At least one silver fir population (forests with at least 10 ha of area and silver fir cover >50%) in each 10-km² grid covering the Aragón Pyrenees was sampled. More stands in those 10-km² grids with more defoliated trees were sampled because we were mainly interested in stands experiencing forest decline. Among the initially 32 sampled sites, 10 were randomly selected for further analyses. At each site, 10–15 dominant trees were randomly selected for sampling within a 500-m long and 20-m wide transect randomly located within the stand. We measured the diameter at 1.3 m (dbh, diameter at breast height) and stem height of each of the 10–15 selected trees located within the transect and assessed their percent crown defoliation, here regarded as a proxy of decline intensity or tree vigour, using a 6-classes semi-quantitative scale. Overall stand defoliation was expressed as the percentage of trees with more than 50% of crown defoliation. The basal area of each stand was also estimated by measuring the number and dbh of all neighboring trees found within a circular plot of 7.6 m in radius placed around each sampled tree. Finally, we performed dendrochronological analyses of sampled trees to estimate tree age (number of cross-dated rings) at 1.3 m. See more details on sampling procedures in Camarero *et al.*, (2011).

2.4. Genetic analyses

To quantify the genetic diversity within and among silver fir populations total DNA was extracted from needles of 467 fir trees sampled in the 10 Pyrenean silver fir populations adopting an alkyltrimethyl ammonium bromide method described by Dumolin *et al.*, (1995). These populations were compared with data obtained from two German populations (codes G1 and G2) based on seed

lots sampled from 50 trees for G1 and 91 for G2. The DNA was stored at -30 °C until use. All samples were genotyped using the six nuclear microsatellites (SSR markers): SF1, SFb4, SF78 (Cremer *et al.* 2006) and NFF7, NFF3, NFF3 (Hansen *et al.*, 2005). The PCR products were separated by capillary electrophoresis using the GeXP automated sequencer (Beckam & Coulter GmbH, Krefeld, Germany).

Genetic diversity within populations was estimated by calculating the following variables: mean number of alleles per locus (A), number of private alleles defined as those occurring in only one population but not in the others (AP), number of effective alleles per locus (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and the fixation index (F) (Weir & Cockerham, 1984). These variables were calculated using the software GenAlEx 6.0 (Peakall & Smouse, 2006). The genetic distance according to Nei (1972) was calculated between pairs of populations based on a Principal Component Analysis (PCA) using the aforementioned software. Population differentiation occurs when a large proportion of the total genetic variation is found among populations. To detect and test the significance of the among- and within-populations genetic differentiation, we used an Analysis of Molecular Variance (AMOVA) to compare observed vs. expected patterns being the latter obtained from 9,999 random permutations (Excoffier *et al.*, 1992). In addition, by using the software SWAP 0.9, the differentiation measure D (Jost, 2008) over all populations and loci was computed and significance was tested by performing 10,000 jack-knife simulations.

3. Results

3.1. Climatic trends

Pyrenean sites presented similar annual precipitation and MAI values than their Central European counterparts while lower values were observed in the case of summer precipitation (e.g. 208 vs. 393 mm) and sMAI (e.g. 31 vs. 63) (Table 1). The lowest MAI values corresponded to sites Paco Ezpela, San Juan de la Peña, Paco Mayor and Guara, and the lowest values of sMAI, and therefore, the highest summer aridity levels, were observed in the sites Paco Ezpela, San Juan de la Peña and Paco Mayor. Positive values for the seasonal rainfall regime, indicating more precipitation received in summer than in winter, were observed in the most eastern sites in this study (e.g., Collubert and Benasque) and Central European sites while negative values corresponding to lower rainfall in summer than in winter were observed in western Pyrenean sites (e.g., sites Gamueta and Paco Ezpela). A value close to zero of this ratio indicated that the amount of precipitation during summer and winter were similar (e.g., site Yésero).

The Gausser aridity index and the sMAI show an increase in aridity for all Pyrenean sites since the 1940s

Table 1. Geographical, topographical and climatic characteristics of the Pyrenean and Central European (lower three rows) silver fir populations. The rainfall regime is the ratio between the difference and the sum of summer and winter precipitations. MAI and sMAI are the annual and summer Martonne aridity indices, respectively (period 1970-2010). Declining populations are underlined.

Tabla 1. Características geográficas, topográficas y climáticas de las poblaciones de abeto blanco de los Pirineos y Europa Central (últimas tres filas). El régimen de precipitación es el cociente entre la diferencia y la suma de la precipitación estival e invernal. MAI y sMAI son los índices de aridez de Martonne anual y estival, respectivamente (periodo 1970-2010). Las poblaciones en decaimiento aparecen subrayadas.

Site (code)	Latitude	Longitude	Elevation (m)	Mean annual / summer temperatures (°C)	Total annual / summer precipitations (mm)	Rainfall regime	MAI / sMAI
Gamueta (1)	42° 53' N	0° 48' W	1400	7.7 / 14.9	1324 / 219	-0.21	75 / 35
<u>Paco Ezpela (2)</u>	42° 45' N	0° 52' W	1152	9.5 / 17.1	1090 / 193	-0.18	56 / 28
<u>San Juan de la Peña (3)</u>	42° 31' N	0° 41' W	1393	8.1 / 15.7	1082 / 198	-0.14	60 / 31
<u>Paco Mayor (4)</u>	42° 42' N	0° 38' W	1333	9.5 / 17.1	1244 / 208	-0.20	64 / 31
Peña Oroel (5)	42° 31' N	0° 32' W	1595	7.3 / 14.4	1281 / 249	-0.10	74 / 41
Yésero (6)	42° 38' N	0° 17' W	1399	8.4 / 16.0	1296 / 269	-0.02	70 / 41
Diazas (7)	42° 37' N	0° 5' W	1528	7.9 / 15.1	1479 / 304	-0.06	83 / 48
Guara (8)	42° 18' N	0° 12' W	1428	7.7 / 15.0	1163 / 222	-0.04	65 / 36
Collubert (9)	42° 28' N	0° 18' E	1474	7.4 / 14.9	1183 / 302	0.14	68 / 48
Benasque (10)	42° 38' N	0° 35' E	1600	7.3 / 14.7	1187 / 290	0.09	69 / 47
Tuttlingen (G1)	47° 58' N	8° 49' E	669	7.8 / 15.9	1079 / 344	0.19	60 / 53
Plöckenstein (G2)	48° 47' N	13° 40' E	880	6.6 / 15.1	1291 / 393	0.06	78 / 63

Table 2. Characteristics of the studied *A. alba* populations (means \pm SE). Stand defoliation is expressed as the percentage of trees with more than 50% of crown defoliation. Declining populations are underlined.

Tabla 2. Características de las poblaciones de A. alba estudiadas (medias \pm SE). El grado de defoliación de cada población se expresa como el porcentaje de árboles con una defoliación de copa superior al 50%. Las poblaciones en decaimiento aparecen subrayadas.

Site (code)	Stand defoliation (%)	Diameter at 1.3 m (cm)	Height (m)	Basal area (m ² ha ⁻¹)	Age at 1.3 m (yr)
Gamueta (1)	0.0	64.2 \pm 2.2	30.1 \pm 1.1	55.8	129 \pm 8
<u>Paco Ezpela (2)</u>	50.0	38.0 \pm 1.7	19.5 \pm 0.8	17.3	103 \pm 6
<u>San Juan de la Peña (3)</u>	7.7	46.0 \pm 2.3	16.4 \pm 1.3	17.9	95 \pm 9
<u>Paco Mayor (4)</u>	32.0	49.8 \pm 3.3	24.0 \pm 0.5	32.7	99 \pm 3
Peña Oroel (5)	0.0	52.3 \pm 3.3	21.1 \pm 0.6	47.8	86 \pm 5
Yésero (6)	0.0	48.1 \pm 3.1	20.0 \pm 0.5	31.7	64 \pm 4
Diazas (7)	0.0	56.5 \pm 2.2	25.9 \pm 0.6	45.4	98 \pm 6
Guara (8)	0.0	52.5 \pm 2.6	20.0 \pm 0.7	13.5	80 \pm 9
Collubert (9)	0.0	56.0 \pm 4.4	21.7 \pm 0.4	29.9	83 \pm 10
Benasque (10)	0.0	49.6 \pm 2.3	21.3 \pm 0.9	48.6	107 \pm 5

with a maximum water deficit in the 1980s (Fig. 2). During the 1980s and 1990s the Gaussen index reached values higher than 1 in site Paco Ezpela and also in the southernmost site Guara, indicating that at least one month was arid there sensu Gaussen. Moreover, the strong decrease in sMAI during the late 20th century also indicates a shift in the intensity of the aridity.

3.2. Stand characteristics

The structural characteristics of the ten Pyrenean silver fir populations indicate that defoliation existed in Paco Ezpela, San Juan de la Peña and Paco Mayor, and these sites were hereafter considered as the declining ones (Table 2). Mean defoliation was very high in the

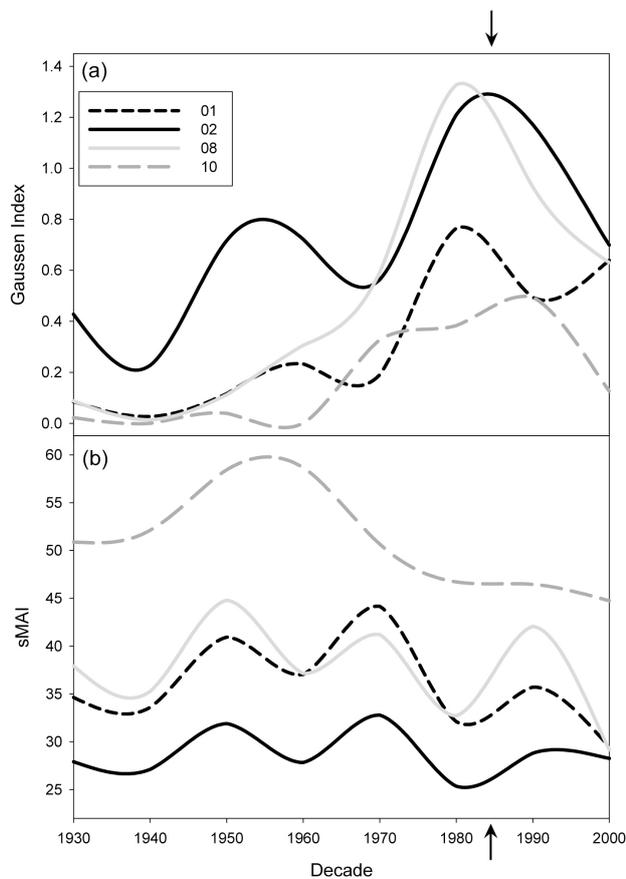


Figure 2. Gausson (a) and summer Martonne aridity (sMAI) indices (b) for several of the studied sites in the Aragón Pyrenees (period 1930-2010) (see sites' codes in Table 1, for clarity purposes only four representative populations are shown). Values are grouped in decades, representing the average value. Arrows indicate the year (1986) when forest decline phenomena started to be observed in the Aragón Pyrenees.

Figura 2. Índices de aridez de Gausson (a) y de Martonne estival (sMAI) (b) para varios de los sitios estudiados en el Pirineo aragonés (periodo 1930-2010) (ver códigos en Tabla 1, para mayor claridad solamente se han representado cuatro poblaciones representativas). Los valores se agrupan por décadas, representándose el valor medio. Las flechas indican el año (1986) donde el decaimiento en el Pirineo aragonés empezó a ser observado.

Paco Ezpela site (50%) where high mortality rates have been also observed until nowadays (J.J. Camarero, pers. observ.) On average, declining sites showed a significantly smaller dbh (44.6 cm) than non-declining sites (54.2 cm) ($F = 6.26$, $P = 0.04$) and tended to have also smaller basal area values ($22.6 \text{ m}^2 \text{ ha}^{-1}$ vs. $39.0 \text{ m}^2 \text{ ha}^{-1}$) despite this last variable did not significantly differ among decline classes ($F = 3.13$, $P = 0.11$). Lastly, declining sites presented the lowest sMAI values, i.e. they were subjected to the highest summer aridity levels (Fig. 3). Lower

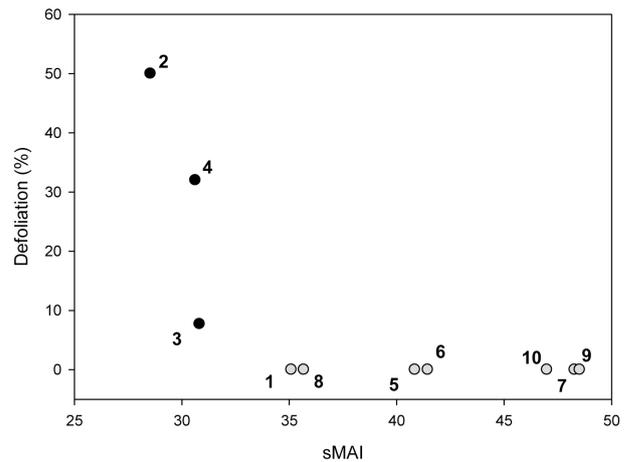


Figure 3. Relationship between the summer Martonne aridity index (sMAI) and the stand defoliation (%) for the ten studied Spanish silver fir populations (sites' codes are as in Table 1).

Dark symbols correspond to declining populations.

Figura 3. Relación entre el índice de aridez de Martonne estival (sMAI) y el grado de defoliación de la población (%) para las diez poblaciones de abeto blanco españolas estudiadas (ver códigos en Tabla 1). Los símbolos más oscuros representan a las poblaciones en decaimiento.

sMAI values were also observed in the westernmost (Gamüeta) and southernmost (Guara) Pyrenean sites.

3.3. Genetic diversity within the silver fir populations

The number of effective alleles (N_e) varies from 2.17 (Gamüeta) to 2.6 (Benasque) (Table 3), i.e. these populations can be considered as the most genetically variable within the Spanish silver fir populations. However, the Spanish populations are also characterized by an extremely low genetic diversity (low values of N_e and A) if compared to the German ones. Overall, the degree of heterozygosity (H_p), which varies from 0.44 to 0.57 in the Pyrenees, is low if compared with values of German populations. The highest number of A_p can be found in the populations Guara and Collubert (0.7) indicating that four alleles each are unique within the respective fir population. Lastly, the fixation index (F) close to zero indicates no deviation from Hardy-Weinberg-equilibrium and, consequently, no inbreeding for eight out of the ten silver fir Pyrenean populations. However, the mean F value of the sites Paco Mayor and Guara are conspicuously high ($F > 0.1$).

3.4. Genetic differentiation among the silver fir populations

With the help of PCA a biplot based on Nei distance was constructed whereas the first and the second axes ex-

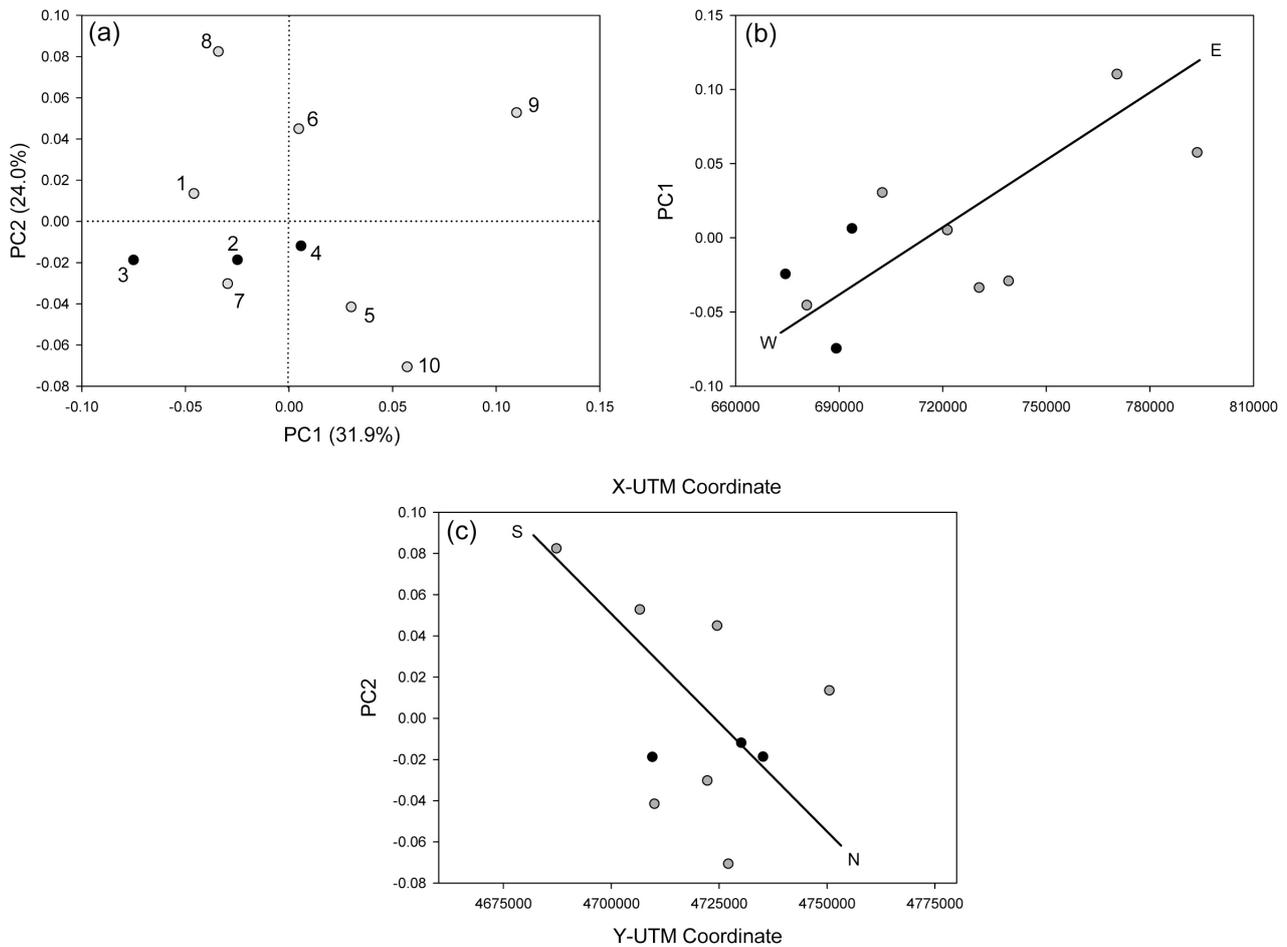


Figure 4. (a) Relationship between the first two principal components (PC1 vs. PC2) of the Principal Component Analysis (PCA) computed on genetic data (Nei) for the ten studied Spanish silver fir populations (see sites' codes in Table 1). Relationships between the first (PC1) and second (PC2) principal components of the PCA as related to site longitude (X-UTM coordinate) (b) and latitude (Y-UTM coordinate) (c), respectively. Dark symbols denote declining populations.

Figura 4. (a) Relación entre los dos primeros componentes (PC1 vs. PC2) del análisis de componentes principales (PCA) basado en datos genéticos (Nei) para las diez poblaciones de abeto blanco españolas estudiadas (ver códigos en Tabla 1). Relaciones entre el primer (PC1) y el segundo (PC2) componente principal del PCA con la longitud (coordenada X-UTM) (b) y latitud del sitio (coordenada Y-UTM) (c), respectivamente. Los símbolos más oscuros representan a las poblaciones en decaimiento.

plain 31.9% and 24.0% of the total genetic variation, respectively (Fig. 4a). The scores of the ten fir Pyrenean populations in the biplot reveal a pattern that resembles their geographical pattern. The first (PC1) and the second (PC2) axes seem to be tightly related to longitude and latitude, respectively (Figs. 4b and 4c). The PCA also shows that Guara, Benasque and Collubert populations differentiate more from the others Pyrenean populations. The AMOVA based on six SSR markers indicated that variance was lower among populations (7%) than within them (93%). However, a differentiation value of 7% can be regarded as comparably high considering the relatively small geographical region studied in the Pyrenees. Accordingly, the differentiation measure D (Jost, 2008) for the ten fir populations is 0.063 which is statistical highly

significant ($p < 0.01$). In contrast, German silver fir population indicated a differentiation among populations of only 1% (Cremer, 2009) on a similar geographic scale.

4. Discussion

Rear edge tree populations inhabiting the southernmost (or lowermost) margins of the species distribution range may act as an important reserve of genetic diversity in changing climates because they have often been long-isolated and lack gene flow, display strong differentiation, and may exhibit local adaptation in response to strong selective pressures such as drought stress (Hampe & Petit, 2005; Parisod & Joost, 2010; Gugger *et al.*, 2011). The

Table 3. Genetic parameters for the ten studied Spanish silver fir populations (codes 1 to 10) as compared with two Central-European populations (codes G1 and G2). Abbreviations of genetic variables: number of analyzed trees (N), mean number of alleles per locus (A), number of private alleles (A_p), number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F). For comparison purpose values of German *A. alba* seed lots are added below (grey part). Declining populations are underlined.

Tabla 3. Parámetros genéticos de las diez poblaciones españolas de abeto blanco estudiadas (códigos del 1 al 10) comparadas con las dos poblaciones centroeuropeas (códigos G1 y G2). Abreviaturas de las variables genéticas: número de árboles analizados (N), número medio de alelos por locus (A), número de alelos privativos (A_p), número de alelos efectivos (N_e), heterocigosidad observada (H_o), heterocigosidad esperada (H_e), índice de fijación (F). Para establecer una comparación se han añadido en la parte inferior los valores de las poblaciones alemanas de *A. alba* (parte gris). Las poblaciones en decaimiento aparecen subrayadas.

Site (code)	N	A	A_p	N_e	H_o	H_e	F
Gamueta (1)	50	5.2	0.2	2.17	0.501	0.453	-0.094
<u>Paco Ezpela (2)</u>	47	3.8	0	2.41	0.487	0.473	-0.006
<u>San Juan de la Peña (3)</u>	48	4	0	2.31	0.456	0.463	-0.011
<u>Paco Mayor (4)</u>	47	5.2	0	2.55	0.429	0.49	0.107
Peña Oroel (5)	19	3.5	0	2.35	0.456	0.441	-0.032
Yésero (6)	49	5	0.2	2.25	0.452	0.458	0.03
Diazas (7)	48	5.5	0	2.35	0.476	0.513	0.05
Guara (8)	45	6.2	0.7	2.24	0.401	0.456	0.127
Collubert (9)	50	7.2	0.7	2.55	0.492	0.538	0.062
Benasque (10)	47	6	0.2	2.6	0.581	0.567	-0.035
Tuttlingen (G1)	50	10.8	1.7	5.2	0.687	0.748	0.113
Plöckenstein (G2)	91	13.8	1.5	7.15	0.648	0.785	–

studied silver fir populations in the Spanish Pyrenees can be considered as the western “stable” rear edge for the species. Hampe & Petit (2005) defined “stable” rear edges as situations where at least some populations have persisted *in situ* at suitable growing sites across Quaternary climatic oscillations, while the species expanded its range into other regions. This is the case of silver fir in the Spanish Pyrenees (Alba-Sánchez *et al.*, 2010). Moreover, the studied populations are isolated and small sized, which also matches the definition of a “stable” edge situation. This, in turn, can be a reason for the comparatively small genetic diversity found within the studied *A. alba* populations in comparison to German ones (Table 3). Bulgarian silver fir populations also show higher diversity than Spanish and German ones with diversity values of up to $N_e = 8.7$ and $A = 18.2$ (data in preparation). A low genetic variation for rear edge populations as a consequence of isolation and the small size has been previously reported in other plant species (Chang *et al.*, 2004).

On the other hand, high levels of genetic differentiation could be found among the ten analyzed fir populations in the Pyrenees (Table 3). The Spanish silver fir populations show a high genetic differentiation in relation to the maximum geographical distance (ca. 100 km). This might be caused by long-term isolation, fragmentation and reduced gene flow among the silver fir populations. Also as local selection processes as discussed in Comps *et al.*, (2001) and Petit *et al.*, (2003) regarding stable rear edges and glacial refugia might have been played a role. Similar

to the fir populations in the Spanish Pyrenees, Gugger *et al.*, (2011) detected a high differentiation among Douglas-fir rear-edge populations in Mexico. Specifically, in our study area, the southernmost Guara population can be considered as the most geographically isolated (Fig. 1). Although the genetic diversity parameters for the silver firs sampled in Guara are comparable to the values of the most eastern populations here studied, inbreeding may be assumed, since there the fixation index F was high (0.127) and deviated from Hardy-Weinberg-equilibrium. An even higher F -value (0.25) was detected in the threatened relict fir species *A. ziyuanensis* in China (Tang *et al.*, 2008). In contrast, a lower level of genetic differentiation was found in the German silver fir populations, i.e. in the core of the distribution range, which also presented similar geographical distances separating them (Cremer, 2009).

In this study we have evidenced that the genetic differentiation among the studied populations in the Spanish Pyrenees is mainly established by an East-West gradient (Fig. 4b). The most eastern populations (Collubert and Benasque) show a higher genetic diversity along this longitudinal gradient, whereas the western populations (Paco Ezpela, San Juan de la Peña and Oroel) are characterized by the lower genetic diversity values among the studied Spanish populations (Fig. 5).

Peripheral rear-edge populations can occupy habitats more stressful from those faced by populations located near the core of the distribution range (Lesica & Allendorf, 1995). In fact, Peguero-Pina *et al.*, (2007) showed that sil-

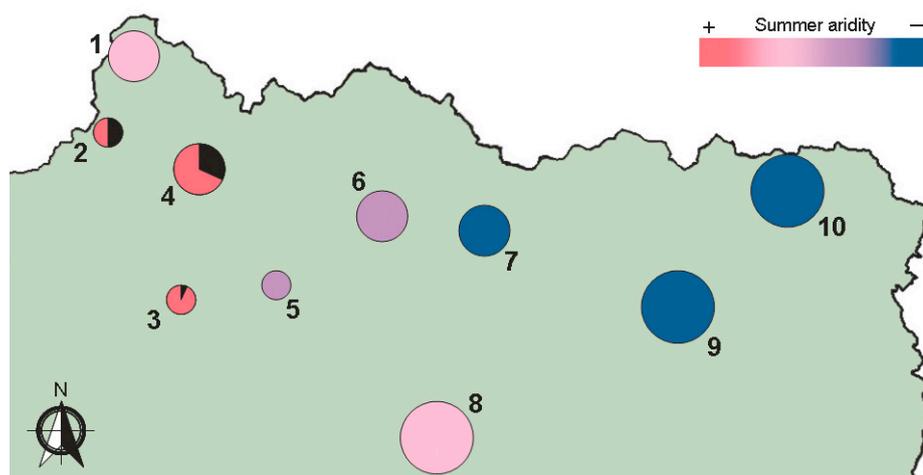


Figure 5. Biogeographical relationships between aridity intensity, genetic diversity and recent decline (assessed as defoliation) in Pyrenean silver fir sites (sites' codes are as in Table 1). Aridity intensity is estimated as the summer Martonne aridity index (colour of the circle and related scale). Genetic diversity is expressed as the mean number of alleles being proportional to the diameter of the circle. The dark portion within the circles denotes the percentage of defoliation of each stand.

Figura 5. Relaciones biogeográficas entre la intensidad de la aridez, la diversidad genética y el decaimiento reciente (a partir de datos de defoliación) para las poblaciones estudiadas de abeto blanco en el Pirineo (ver códigos en Tabla 1). La intensidad de la aridez se estima con el índice de aridez de Martonne estival (color del círculo y escala relacionada). La diversidad genética se expresa a partir del número medio de alelos, siendo proporcional al diámetro del círculo. La porción oscura del círculo indica el porcentaje de defoliación de cada población.

ver fir stands in the southern Pyrenees occupies more xeric habitats than those reported for this species in Central Europe. Our results agree with these assumptions since summer precipitation and sMAI are comparatively low in the Spanish Pyrenees than in Germany. Specifically, the three populations living under the more xeric conditions are located in the western study area and show high defoliation levels (Paco Ezpela, San Juan de la Peña and Paco Mayor) (Fig. 5). In this western group the Gamueta populations does not show defoliation, probably due to the more mesic climatic conditions existing in this site when compared to the declining populations. For this reason, this population may be more prone to experience decline phenomena in the future under the current context of rising temperatures and summer precipitation decrease. The apparent importance of summer aridity, expressed by low sMAI values, on the performance of these western populations implies that a shift in summer temperatures could threaten the survival of these stands, even in the absence of any modification in the precipitation regime. On the other hand, although the climatic conditions experiencing by the central and eastern populations in this study are more similar to those existing in Germany, the Guara site shows climatic variables regarding aridity (sMAI and Gausson index) more similar to those found in the western sites. The absence of defoliation in this southernmost population and a similar genetic diversity with the most eastern populations indicates the possibility that silver fir in Guara could be well adapted to the sub-Mediterranean conditions of this site. Additional studies should be done to assess this possibility.

An evident warming trend has been detected since the 1940s in western European mountains, showing that the latest decades were much warmer than any other period of the instrumental records (Díaz & Bradley, 1997). In the Central Pyrenees, mean temperature increased by +0.8 °C between 1882 and 1970 at a high-elevation site (Bücher & Dessens, 1991). A progressive increase in the length and intensity of the summer aridity period has been detected during the last decades in the studied sites, especially in the westernmost Pyrenean populations (Camarero *et al.*, 2011). This warming trend during the late 20th century involves increasing aridity and should affect the long-term performance of these rear-edge silver fir stands (Macias *et al.*, 2006). If these predictions are confirmed, the present rear edge for *A. alba* in the Spanish Pyrenees will have to be redrawn in a near future. For this reason, conservation of declining Pyrenean silver fir populations may be an urgent need for maintaining their ecosystem services including their regional genetic diversity. Western Pyrenean sites subjected to dryer conditions in summer, high defoliations levels and low genetic diversity are prone to drought-induced decline and mortality. Since these rear-edge populations are small, isolated and genetically impoverished, regional population dynamics cannot easily compensate local extinction events. Based on these considerations, a conservation of the regional genetic diversity, i.e. of the whole gene pool, of Pyrenean silver fir populations must be associated with the preservation of these single populations.

Acknowledgments

This study was partially supported by CAIXA project 2012/GA-LC-002 (Departamento de Ciencia, Tecnología y Universidad, Gobierno de Aragón) and by projects 012/2008 and 387/2011 (Organismo Autónomo de Parques Nacionales, Spanish Ministry of Agriculture and Environment). Financial support from Gobierno de Aragón (A54 research group) is also acknowledged. Jesús Julio Camarero acknowledges the support of ARAID. We also thank the technical assistants of the Bavarian Institute for Forest Seeding and Planting for support in lab.

References

- Alba-Sánchez, F., López-Sáez, J.A., Benito-de Pardo, B., Linares, J.C., Nieto-Lugilde, D. & López-Merino, L. 2010. Past and present potential distribution of *Abies* species: a phytogeographic approach using fossil pollen data and species distribution models. *Diversity and Distributions*, 16: 214-228. doi: <http://dx.doi.org/10.1111/j.1472-4642.2010.00636.x>.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kizberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A. & Cobb, N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, 259: 660-684. doi: <http://dx.doi.org/10.1016/j.foreco.2009.09.001>.
- Bacles, C.F. & Jump, A.S. 2010. Taking a tree's perspective on forest fragmentation genetics. *Trends in Plant Science*, 16: 13-18. doi: <http://dx.doi.org/10.1016/j.tplants.2010.10.002>.
- Bücher, A. & Dessens, J. 1991. Secular trend of surface temperature at an elevated observatory in the Pyrenees. *Journal of Climate*, 4: 859-868. doi: [http://dx.doi.org/10.1175/1520-0442\(1991\)004<0859:STOSTA>2.0.CO;2](http://dx.doi.org/10.1175/1520-0442(1991)004<0859:STOSTA>2.0.CO;2).
- Camarero, J.J., Bigler, C., Linares, J.C. & Gil-Pelegrín, E. 2011. Synergistic effects of past historical logging and drought on the decline of Pyrenean silver fir forests. *Forest Ecology and Management*, 262: 759-769. doi: <http://dx.doi.org/10.1016/j.foreco.2011.05.009>.
- Camarero, J.J., Lloret, F., Corcuera, L., Peñuelas, J. & Gil-Pelegrín, E. 2004. Cambio global y decaimiento del bosque. En: Valladares, F. (ed.) *Ecología del bosque mediterráneo en un mundo cambiante*, pp. 397-423, Organismo Autónomo de Parques Nacionales, Ministerio de Medio Ambiente, Madrid.
- Carrer, M., Nola, P., Motta, R., & Urbinati, C. 2010. Contrasting tree-ring growth to climate responses of *Abies alba* toward the southern limit of its distribution area. *Oikos*, 119: 1515-1525. doi: <http://dx.doi.org/10.1111/j.1600-0706.2010.18293.x>.
- Chang, C.S., Kim, H., Park, T.Y. & Maunder, M. 2004. Low levels of genetic variation among southern peripheral populations of the threatened herb, *Leontice microhyncha* (Berberidaceae) in Korea. *Biological Conservation*, 119: 387-396. doi: <http://dx.doi.org/10.1016/j.biocon.2003.12.003>.
- Comps, B., Gömöry, D., Letouzey, J., Thiébaud, B. & Petit, R.J. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics*, 157: 389-397.
- Cremer, E. 2009. Population genetics of silver fir (*Abies alba* Mill.) in the Northern Black Forest - preconditions for the recolonization of windthrow areas and associated ectomycorrhizal communities. PhD Thesis, Philipps-Universität Marburg, Marburg-Lahn, Germany.
- Cremer, E., Liepelt, S., Sebastiani, F., Buonamici, A., Michalczyk, I.M., Ziegenhagen, B. & Vendramin, G.G. 2006. Identification and characterization of nuclear microsatellite loci in *Abies alba* Mill. *Molecular Ecology Notes*, 6: 374-376. doi: <http://dx.doi.org/10.1111/j.1471-8286.2005.01238.x>.
- Díaz, H.F. & Bradley, R.S. 1997. Temperature variations during the last century at high elevation. *Climatic Change*, 36: 254-279. doi: <http://dx.doi.org/10.1023/A:1005335731187>.
- Dumolin, S., Demesure, B. & Petit, R.J. 1995. Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theoretical Applied Genetics*, 91: 1253-1256. doi: <http://dx.doi.org/10.1007/BF00220937>.
- Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. *Genetics*, 131: 479-491.
- Gömöry, D., Paule, L., Krajmerova, D., Romsakova, I. & Longauer, R. 2012. Admixture of genetic lineages of different glacial origin: a case study of *Abies alba* Mill. in the Carpathians. *Plant Systematics and Evolution*, 298: 703-712. doi: <http://dx.doi.org/10.1007/s00606-011-0580-6>.
- Gugger, P.F., González-Rodríguez, A., Rodríguez-Correa, H., Sugita, S. & Cavender-Bares, J. 2011. Southward Pleistocene migration of Douglas-fir into Mexico: phylogeography, ecological niche modeling, and conservation of "rear edge" populations. *New Phytologist*, 189: 1185-1199. doi: <http://dx.doi.org/10.1111/j.1469-8137.2010.03559.x>.
- Hampe, A. & Petit, R.J. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, 8: 461-467. doi: <http://dx.doi.org/10.1111/j.1461-0248.2005.00739.x>.
- Hansen, O.K., Vendramin, G.G., Sebastiani, F. & Edwards, K.J. 2005. Development of microsatellite markers in *Abies nordmanniana* (Stev.) Spach and cross-species amplification in the *Abies* genus. *Molecular Ecology Notes*, 5: 784-787. doi: <http://dx.doi.org/10.1111/j.1471-8286.2005.01062.x>.
- Jost, L. 2008. GST and its relatives do not measure differentiation. *Molecular Ecology*, 17: 4015-4026. doi: <http://dx.doi.org/10.1111/j.1365-294X.2008.03887.x>.
- Jump, A.S., Mátyás, C. & Peñuelas, J. 2009. The altitude-for-latitude disparity in the range retractions of woody species. *Trends in Ecology and Evolution*, 24: 694-701. doi: <http://dx.doi.org/10.1016/j.tree.2009.06.007>.
- Konnert, M. & Bergmann, F. 1995. The geographical distribution of genetic variation of silver fir (*Abies alba*, Pinaceae) in relation to its migration history. *Plant Systematics and Evolution*, 196: 9-30. doi: <http://dx.doi.org/10.1007/BF00985333>.
- Leimu, R., Mutikainen, P., Koricheva, J. & Fischer, M. 2006. How general are positive relationship between plant population size, fitness and genetic variation? *Journal of Ecology*, 94: 942-952. doi: <http://dx.doi.org/10.1111/j.1365-2745.2006.01150.x>.
- Leimu, R., Vergeer, P., Angeloni, F. & Ouborg, NP. 2010. Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences*, 1195: 84-98. doi: <http://dx.doi.org/10.1111/j.1749-6632.2010.05450.x>.
- Lesica, P. & Allendorf, F.W. 1995. When are peripheral populations valuable for conservation? *Conservation Biology*, 9: 753-760. doi: <http://dx.doi.org/10.1046/j.1523-1739.1995.09040753.x>.
- Liepelt, S., Cheddadi, R., de Beaulieu, J.L., Fady, B., Gömöry, D., Hussendörfer, E., Konnert, M., Litt, T., Longauer, R., Terhürne-Berson, R. & Ziegenhagen, B. 2009. Postglacial expansion and its genetic imprints in *Abies alba* (Mill.) - A synthesis from palaeobotanic and genetic data. *Review of Palaeobotany and Palynology*, 153: 139-149. doi: <http://dx.doi.org/10.1016/j.revpalbo.2008.07.007>.
- Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M.J. & Marchetti, M. 2010. Climate change

- impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management*, 259: 698-709. doi: <http://dx.doi.org/10.1016/j.foreco.2009.09.023>.
- Macías, M., Andreu, L., Bosch, O., Camarero, J.J. & Gutiérrez, E. 2006. Increasing aridity is enhancing silver fir (*Abies alba* Mill.) water stress in its south-western distribution limit. *Climatic Change*, 79: 289-313. doi: <http://dx.doi.org/10.1007/s10584-006-9071-0>.
- Manrique, E. & Fernández-Cancio, A. 2005. Sistema informático para la generación de datos climáticos y fitoclimáticos. *Actas IV Congreso Forestal Español*, pp. 161, Zaragoza, Spain.
- Nei, M. 1972. Genetic distance between populations. *The American Naturalist*, 106: 283-292. doi: <http://dx.doi.org/10.1086/282771>
- Parisod, C. & Joost, S. 2010. Divergent selection in trailing-versus leading-edge populations of *Biscutella laevigata*. *Annals of Botany*, 105: 655-660. doi: <http://dx.doi.org/10.1093/aob/mcq014>.
- Pawlaczyk, E.M., Grzebyta, J., Bobowicz, M.A. & Korczyk, A.F. 2005. Individual differentiation of *Abies alba* Mill. population from the Tisovik Reserve. Variability expressed in morphology and anatomy of needles. *Acta Biologica Cracoviensia Series Botanica*, 47: 137-144.
- Peakall, R. & Smouse, P.E. 2006. GenA1Ex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6: 288-295. doi: <http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x>.
- Peguero-Pina, J.J., Camarero, J.J., Abadía, A., Martín, E., González-Cascón, R., Morales, F., & Gil-Pelegrín, E. 2007. Physiological performance of silver-fir (*Abies alba* Mill.) populations under contrasting climates near the south-western distribution limit of the species. *Flora*, 202: 226-236. doi: <http://dx.doi.org/10.1016/j.flora.2006.06.004>.
- Peguero-Pina, J.J., Sancho-Knapik, D., Cochard, H., Barredo, G., Villarroya, D. & Gil-Pelegrín, E. 2011. Hydraulic traits are associated with the distribution range of two closely related Mediterranean firs, *Abies alba* Mill. and *Abies pinsapo* Boiss. *Tree Physiology*, 31: 1-9. doi: <http://dx.doi.org/10.1093/treephys/tpq092>.
- Pertoldi, C., Bijlsma, R. & Loeschcke, V. 2007. Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. *Biodiversity and Conservation*, 116: 4147-4163. doi: <http://dx.doi.org/10.1007/s10531-007-9212-4>.
- Petit, R.J., Aguinalalde, I., de Beaulieu, J.L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martin, J.P., Rendell, S. & Vendramin, G.G. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, 300: 1563-1565. doi: <http://dx.doi.org/10.1126/science.1083264>.
- Tang, S., Dai, W., Li, M., Zhang, Y., Geng, Y., Wang, L. & Zhong, Y. 2008. Genetic diversity of relictual and endangered plant *Abies ziyenensis* (*Pinaceae*) revealed by ALFP and SSR markers. *Genetica*, 133: 21-30. doi: <http://dx.doi.org/10.1007/s10709-007-9178-x>.
- van Mantgem, P.J. & Stephenson, N.L. 2007. Apparent climatically induced increase of tree mortality rates in a temperate forest. *Ecology Letters*, 10: 909-916. doi: <http://dx.doi.org/10.1111/j.1461-0248.2007.01080.x>.
- Vendramin, G.G., Degen, B., Petit, R.J., Anzidei, M., Madaghiale, A. & Ziegenhagen, B. 1999. High level of variation at *Abies alba* chloroplast microsatellite loci in Europe. *Molecular Ecology*, 8: 1117-1126. doi: <http://dx.doi.org/10.1046/j.1365-294X.1999.00666.x>.
- Vigo, J. & Ninot, J.M. 1987. Los Pirineos. En: Peinado-Lorca, M. & Rivas-Martínez, S. (eds.) *La Vegetación de España*, pp. 351-384, Universidad de Alcalá de Henares, Alcalá de Henares.
- Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358-1370. doi: <http://dx.doi.org/10.2307/2408641>
- Young, A., Boyle, T. & Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, 11: 413-418. doi: [http://dx.doi.org/10.1016/0169-5347\(96\)10045-8](http://dx.doi.org/10.1016/0169-5347(96)10045-8).