Forest Ecology and Management 409 (2018) 148–160

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Genetic variation in *Taxus baccata* L.: A case study supporting Poland's protection and restoration program

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ARTICLE INFO

Keywords:
Bottleneck
Conservation
English yew
Genetic differentiation
Genetic resources
Microsatellite markers

ABSTRACT

English yew (*Taxus baccata* L.) is a strictly outcrossing and dioecious species with small and isolated populations, which has led to its endangered species status within its natural range in Europe. In the present study, we determined the level of genetic variation and genetic differentiation of thirty-one natural *T. baccata* populations in Poland (2725 individuals) and focused on the impact of demographic processes on the species' population structure using five nuclear microsatellite loci. The populations demonstrated generally moderate to high levels of genetic diversity (AR = 2.4–12.5; H_o = 0.262–0.593; H_e = 0.441–0.839). The genetic differentiation between populations was geographically structured and occurred at a moderate level (F_{st} = 0.155). A Bayesian analysis identified a significant north-south population structure. Additionally, populations showed significant isolation by distance, suggesting the recent isolation and fragmentation of local *T. baccata* populations. All the examined populations experienced a demographic bottleneck and significant fluctuations in size. The improved understanding of genetic variation has practical implications for developing conservation strategies for this rare and endangered forest species. Our study supplements the limited knowledge regarding the fine-scale genetic structure of *T. baccata*; thus, the Polish populations are now among the most-surveyed stands of this species in Europe. Based on our findings, we suggest that the gene pools of *T. baccata* should be actively preserved and that local populations should be prioritized according to their potential significance for *in situ* or *ex situ* conservation.

1. Introduction

Forest trees cover approximately 30% of the world's terrestrial area, are an important facet of biodiversity and play a pivotal role in the function of forest ecosystems. Forest trees generally maintain high genetic variation within populations and low genetic differentiation between populations compared with other plant species because of their large effective population sizes, longevity, outcrossing, and wind pollination, which allow for extensive gene flow over great geographical distances (Hamrick et al., 1992; Robledo-Arnuncio, 2011). Genetically diverse populations are more likely to survive in unfavourable conditions because they have the potential to transmit many combinations of alleles to future generations (Hampe and Petit, 2005; Savolainen et al., 2007; Gienapp et al., 2008; Alberto et al., 2013). Global environmental change, such as climate change and natural habitat fragmentation, which are intensified by human activities, alters the genetic structure patterns and survival of many plant species (Young et al., 2006; Bacles and Jump, 2011). Limited gene flow among populations resulting from geographical isolation may lead to genetic erosion via increased inbreeding and greater genetic drift in populations with small effective sizes (Jump and Peñuelas, 2006). Consequently, a higher risk of extinction is observed for small isolated populations.

English yew (*Taxus baccata* L.) is a tertiary relict and a long-lived wind-pollinated dioecious tree species located all over Europe. In Scandinavia, it grows up to the 61°N latitude. The eastern border of its natural distribution range in Europe extends from the Gulf of Riga through the Białowieża Forest south towards the Carpathians and farther to the southeast (Thomas and Polwart, 2003). Across the natural range in Europe, English yew grows under a wide range of environmental conditions. This tree was one of the forest-forming species with a wide natural distribution and has high ecological and economic importance. The present distribution and number of natural populations have drastically decreased in recent years in many parts of Europe, and yew is under threat (Thomas and Polwart, 2003). This decline is partially caused by long-term human impacts, including extensive timber harvesting, and progressively drier climatic conditions. Despite its high shade tolerance, other factors have contributed to the decline of the species, such as a lack of natural regeneration because of grazing and a competitive disadvantage with respect to other plant species (Hulme, 1996; Svenning and Magard, 1999; Iszkuło et al., 2009; Linares, 2013).

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https://doi.org/10.1016/j.foreco.2017.11.026
Received 24 August 2017; Received in revised form 6 November 2017; Accepted 10 November 2017
Available online 15 November 2017
0378-1127/

Currently, most populations of *T. baccata* are small and fragmented, which increases inbreeding and the potential for genetic drift. Previous research on the genetic structure of *T. baccata* in Europe using different marker systems demonstrated a high level of overall genetic variation and significant differentiation between populations (Lewandowski et al., 1995; Cao et al., 2004; Dubreuil et al., 2010; Myking et al., 2009; Tröber and Ballian, 2011; Chybicki et al., 2012).

Furthermore, the extinction risk for the dioecious yew is high compared with that of co-sexual species. Dioecy means that individual plants are distinctly male or female, which can result in an insufficient number of individuals of the opposite sex in small and isolated populations (Heilbuth, 2000; Vamosi and Vamosi, 2005). The species has become endangered in many European countries and has priority status in many *in situ* and *ex situ* conservation and restoration programmes. In Poland, a conservation programme for the native populations of the species was launched in 2006, and it resulted in the establishment of 29 forest reserves of approximately 590 hectares, including isolated older trees growing outside the reserves that are protected as natural monuments.

In the present study, we used a set of nuclear microsatellite markers to explore the genetic diversity and structure of many yew populations throughout their range in Poland. We aimed to (1) determine the level of genetic diversity and genetic differentiation within and among populations, (2) estimate the degree of population inbreeding and the effective population size, (3) investigate the demographic history of the species in Poland to test for the signature of a recent bottleneck, and (4) discuss implications of this research on conservation efforts.

2. Materials and methods

2.1. Plant sampling and DNA extraction

This study examined 31 populations of *T. baccata* from the entire species range in Poland, all of which are included in the species' conservation and restoration programme (Fig. 1). The sample from each population ranged from 27 to 100 individuals and a total of 2725 individuals were analysed (Table 1). Genomic DNA was extracted from the needles of each sample using a modified CTAB protocol (Dumolin et al., 1995).

2.2. Microsatellite genotyping

Eight nuclear microsatellite loci originally described for *T. baccata* by Dubreuil et al. (2008) were initially selected for analysis. However, after the initial tests, the final set of loci selected for this study included five nSSRs (Tax23, Tax26, Tax31, Tax36, Tax92) because they can provide repeatable, high-quality amplification with sufficient polymorphisms and unambiguous allele binding products. Three nSSR loci (Tax26, Tax36 and Tax92) were simultaneously amplified in a multiplex reaction using a Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany). The PCR multiplex reactions for each sample were performed in a total volume of 10 µl containing 5 µl of Qiagen Multiplex Master Mix (2×), 0.2 µl of primer mix (20 µM), 1 µl of Q-Solution (5×), 0.8 µl RNase-Free water, and 3 µl DNA template (approximately 10–20 ng). The other two loci (Tax23 and Tax31) were amplified separately in a total volume of 10 µl containing 10–20 ng of DNA template, 1× PCR, pH 8.3 (Novazym, Poznan, Poland), 25 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 µM of each primer, 10 ng/µl BSA, and 1.25 U VivaTaq polymerase (Novazym, Poznan, Poland). The PCR conditions for the single and multiplex reactions included a denaturation step at 95 °C for 15 min; 10 touchdown cycles at 94 °C for 30 s, 60 °C for 30 s (−1 °C/cycle), and 72 °C for 40 s; 30 cycles at 94 °C for 30 s, 50 °C for 50 s, and 72 °C for 40 s; and a final extension at 72 °C for 7 min. The fluorescently labelled PCR products and a size standard (GeneScan 500 LIZ) were separated on an ABI 3130 capillary sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The allele size was determined using GeneMapper software (ver. 4.0; Thermo Fisher Scientific, Waltham, Massachusetts, USA), and all variants were manually verified and approved.

2.3. Data analysis

2.3.1. Genetic diversity

The linkage disequilibria between the pairs of loci were assessed at the single population level and across all populations and its significance was tested with a Bonferroni correction using FSTAT v. 2.9.3 software (Goudet, 2001). The genetic diversity within and among populations was estimated based on the following parameters: number of alleles (A), rarefied allelic richness (AR_{27}) for a minimum sample size of 27 individuals, observed heterozygosity (H_o), and unbiased expected

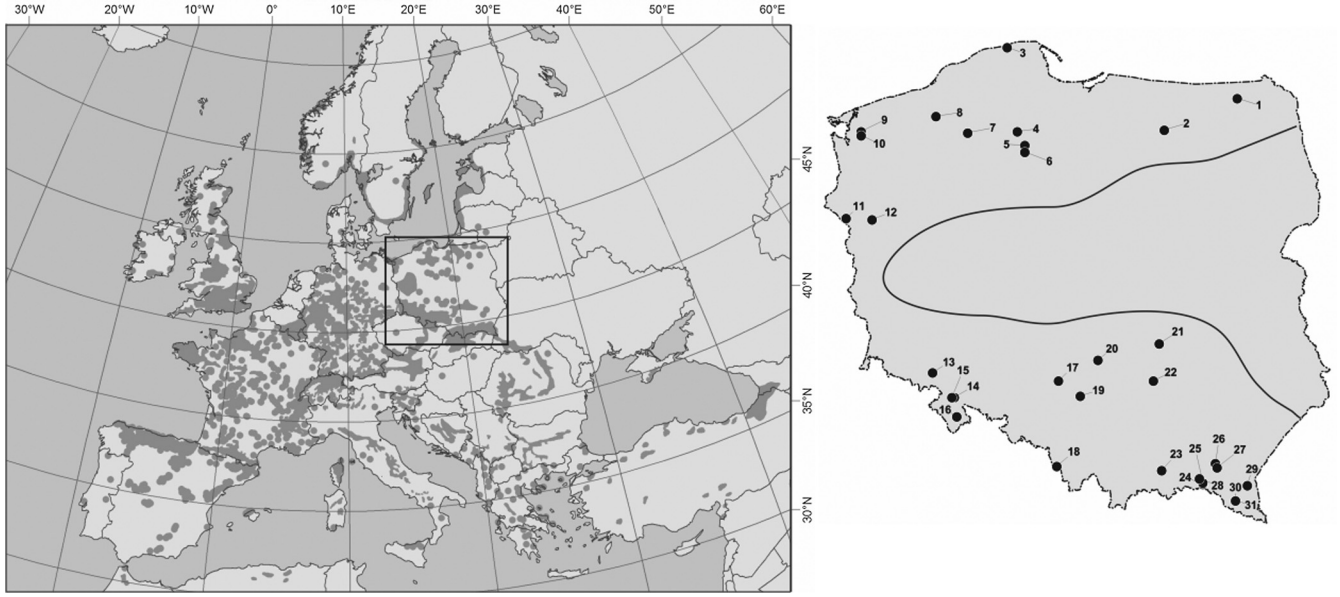

Fig. 1. Map of the current natural range of *Taxus baccata* in Europe (based on EUFORGEN, the European Forest Genetic Resources programme, www.euforgen.org) and the locations of the 31 Polish populations included in this study. The codes representing the populations are listed in Table 1, and the borders of the natural distribution of *T. baccata* in Poland are indicated with a black line.