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RESEARCH

# Ghosts of Giants: high levels of clonality may represent massive remnant clones in European aspen (*Populus tremula*)

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**Abstract**
Clonal reproduction is an important strategy for the establishment, expansion and persistence of many plant species. It is well-documented in *Populus* spp. *Populus tremula* (European Aspen) is a native species in Ireland, a pioneer which has persisted from the early Holocene to the present. Our main aim was to determine whether clonal and genetic diversity across Irish populations of *P. tremula*, are lower compared to populations from Britain and continental Europe. Twenty geographically discrete populations of *P. tremula* from Ireland, and 14 populations from mainland Britain, Sweden and Italy, were genotyped for eight nuclear microsatellites and five chloroplast microsatellites. Levels of genetic diversity were extremely low in Ireland compared to those from Britain, Sweden and Italy. While such low levels of variation can be linked to founder effects during postglacial colonisation, all but two of the Irish sites were monoclonal with extensive fragmentation of the Irish clones across large areas. Collectively, these results suggest that the present-day distribution of *P. tremula* in Ireland comprises remnants of once massive clones that have persisted by asexual reproduction. This is consistent with Baker's Law that self-compatible organisms are more likely to colonize the extremities of a species range than self-incompatible organisms. Clonality in *P. tremula* across the Irish landscape may be an adaptation enabling establishment and persistence of isolated populations during climate change associated with glacial advances and retreat. Extreme clonality, however, is also associated with loss of genetic diversity which may increase vulnerability to recent, more rapid, anthropogenic climate change, novel plant pathogens and hybridisation.

**Keywords** Asexual reproduction · Genetic diversity · Clonal diversity and area · European aspen · Baker's Law

## Introduction

Clonal reproduction giving rise to many genetically identical individuals has been studied extensively in *Populus* spp. (poplars, aspens and cottonwoods; Farmer 1962; Barnes 1966). The existence and size of clones before the advent of molecular methods, was estimated using aerial photography and morphological characteristics of bark, leaves and stems (Barnes 1969; Tharakan et al. 2005; Robinson et al. 2012; Reategui-Betancourt et al. 2020). These studies revealed some extremely large clones, the most celebrated of which, in *P. tremeloides*, "Pando", in Utah, USA, covers ca. 43 ha (Kemperman and Barnes 1976). Unequivocal characterization of the relationship between individual trees or ramets (genetically identical but separate members of a clonal colony) and genets (a genetic individual that arises from a single fertilisation event that subsequently may produce multiple, genetically identical individuals through vegetative reproduction), requires genetic analysis (Beatty et al. 2008) e.g.seven microsatellite markers were used to confirm Pando comprised a single genet (DeWoody et al. 2008). Other work on *P. tremuloides*, however, suggests more frequent sexual reproduction and smaller clone sizes (Namroud et al. 2005; Mock et al. 2008). Nevertheless, large clones may have established in and persisted from the

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end of the last glaciation ca. 10–12 KYA (Kemperman and Barnes 1976; Ally et al. 2008).

European aspen (*Populus tremula*) ranges from Ireland to Japan, and northern Norway to northern Africa (Worrell 1995). It is a rapidly growing, broadleaf species with an altitudinal range up to 1900 m (Savill 2013). It is tolerant and adaptable across diverse climatic conditions and habitats (Rogers et al. 2020) and grows in a wide range of soils with a preference for moist, organic-rich soils (Caudullo and de Rigo 2016). It is frequently regarded as a keystone species, especially within its central boreal range (Hardenbol et al. 2020). In contrast to other *Populus* spp., *P. tremula*, has little commercial value and planted only occasionally for horticultural purposes (Rogers et al. 2020).

In continental Europe, *P. tremula* is characteristically part of continuous forest (Caudullo and Rigo 2016). In the British Isles, in contrast, its distribution is wide-ranging but fragmented and in low abundance (Stroh et al. 2023). In Ireland, where forest and woodland amount to around 10% of total land cover, 90% of which is commercial forestry (Devaney et al. 2015), P. *tremula* is particularly sparse and, in many counties, restricted to isolated clumps in hedgerows (WIM/SSJM pers. obs.). There is evidence of a decline in the occurrence of *P. tremula* in Ireland from 2000 to 2019 (Stroh et al. 2023).

Phylogeographical analyses suggest that northern European populations of *P. tremula* such as in Sweden, lack genetic structure and were probably recolonised from multiple connected refugia close to the former ice cap (Fussi et al. 2010). This was supported by Wilson (2019) who found significant but slight genetic population variation within Scottish populations of *P. tremula* and a gradual increase in genetic distance between Scotland and Norway (least), Sweden, Latvia, Russia and China (greatest). There is no phylogeographical analysis involving populations from throughout the British Isles although it seems likely that they are more closely allied to Swedish populations than to those from southern European peninsulas such as Italy.

Loss of genetic diversity within species is recognised as a global issue and must be addressed by identifying vulnerable populations even in common species (Booy et al. 2000; Shaw et al. 2025). Small, isolated populations, especially those which originate from a single founding individual, a genet, and are dependent on asexual reproduction producing many identical ramets, may run a high risk of decreasing genetic diversity due to absence of recombination, founder effects or genetic drift, or as argued by Silvertown (2008), absence of sexual reproduction. However, clonal plants have a range of adaptive mechanisms for surviving environmental change (Douhovnikoff and Dodd 2015; Yang et al. 2023; Yu et al. 2024). Thus, ability to withstand genetic loss may vary between species and between larger continental and smaller, insular populations of the same species. Isolated populations of *P. tremula* tend towards monoclonality whilst larger, continental populations comprise multiple, spatially-aggregated clones (Suvanto and Latva-Karjanmaa 2005; Cristóbal et al. 2014; Politov et al. 2016). Phylogeographical analyses suggest that north European populations of *P. tremula* lack genetic structure and were probably recolonised from multiple, connected refugia close to the former ice cap (Fussi et al. 2010). However, there are few data on genetic population structure in natural populations of *P. tremula* in the British Isles, its most westerly and isolated part of its range, despite recognition of this important knowledge gap (Worrell 1995).

Research on *P. tremula* centres on prolific clonal (vegetative) reproduction via root suckering, which allows it to establish, maintain and expand its populations (Bärring 1988). There is particular interest in the commercial potential of clones of *P. tremula x tremuloides* hybrids (Rytter and Rytter 2017; Rytter and Jacobson 2018; Stener et al. 2018). Clonal reproduction could lead to establishment and persistence under adverse conditions, supporting the pioneering nature of the species and facilitating colonisation of recently deglaciated areas (Klimeš et al. 1997; Honnay and Bossuyt 2005). *P. tremula* can also reproduce sexually but it is not self-fertile and viable seed production with seedling establishment occurs only under optimal conditions (Worrell 1995; Worrell et al. 1999; Latva-Karjanmaa et al. 2003, 2006). *P. tremula* is dioecious with wind-dispersal of pollen and seeds (Easton 1997). Significant levels of sexual reproduction and regeneration via seed have been documented in various population genetic studies across the species range (Lopez-de-Heredia et al. 2004; Suvanto and Latva-Karjanmaa 2005; Hall et al. 2007; de Carvalho et al. 2010).

The link between dispersal and reproduction is the basis of Baker's Law that populations of plants that are self-compatible or can utilise vegetative reproduction, may originate from a single seed facilitating colonisation in geographical and ecophysiological extremities ("Baker's Law"; Baker 1955, 1967; Pannell et al. 2015). Thus, clonal reproduction, which is common in plants (Klimeš et al. 1997), could aid persistence under adverse conditions, such as in recently deglaciated areas (Honnay and Bossuyt 2005). Dering et al. (2015) showed that clonal tree species have a more apparent spatial genetic structure than non-clonal species, concluding that clonality impacts significantly on population processes. Clonality was critical to survival in northern regions amd asexual reproduction also influenced population genetic structure more widely in grey alder *Alnus incana* in southern and northern refugia in Europe (Dering et al. 2017). In *Betula humilis*, 76% of ramets share the genotype of their nearest neighbour at the southwestern limit of its range but in the presence of unrelated individuals, limited pollen and

**Table 1** Aspen sites studied, location, number of trees sampled, and summary of results of nuclear and chloroplast analyses

| Population | | Lat (N) | Long (W) | Nuclear | | | | Chloroplast | | | | | | | | | | | | | | | *H* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *N* | *G* | *R* | *D** | *N* | h1 | h2 | h3 | h4 | h5 | h6 | h7 | h8 | h9 | h10 | h11 | h12 | h13 | hS | |
| Northern Ireland | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | Clonvaraghan | 54.287 | 5.923 | 24 | 2 | 0.043 | 0.083 | 24 | 24 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | Hilltown | 54.225 | 6.076 | 32 | 2 | 0.032 | 0.075 | 32 | - | - | - | 32 | - | - | - | - | - | - | - | - | - | - | - |
| 3 | Rathfriland | 54.244 | 6.176 | 23 | 3 | 0.091 | 0.170 | 23 | - | - | - | 23 | - | - | - | - | - | - | - | - | - | - | - |
| 4 | Poyntzpass | 54.304 | 6.358 | 14 | 2 | 0.077 | 0.440 | 14 | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | Duffmount | 54.635 | 6.594 | 23 | 1 | - | - | 23 | - | - | - | - | - | - | - | - | - | - | - | - | - | 23(1) | - |
| 6 | Peatlands Park | 54.485 | 6.617 | 24 | 1 | - | - | 24 | 24 | - | - | - | - | - | - | - | - | - | - | - | - | | - |
| 7 | Drumrot | 54.669 | 6.686 | 55 | 2 | 0.019 | 0.071 | 55 | - | 55 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | Tullyboy | 54.661 | 6.702 | 22 | 1 | - | - | 22 | - | 22 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | Poplar Hill | 54.618 | 6.725 | 22 | 1 | - | - | 22 | - | 22 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | South Fermanagh | 54.140 | 7.474 | 22 | 1 | - | - | 22 | - | - | 22 | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | Lisnaskea | 54.294 | 7.540 | 31 | 1 | - | - | 31 | - | - | 31 | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | Derrygonnelly | 54.419 | 7.822 | 29 | 1 | - | - | 29 | 29 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | Lough Melvin | 54.433 | 8.113 | 24 | 2 | 0.043 | 0.301 | 24 | - | - | - | 24 | - | - | - | - | - | - | - | - | - | - | - |
| | | | | | | | | | | | | | | | | | | | | | | - | |
| Republic of Ireland | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | Tullycana | 52.273 | 6.682 | 15 | 2 | 0.071 | 0.176 | 15 | - | - | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | Corekeela Lodge | 52.278 | 6.689 | 16 | 1 | - | - | 16 | - | - | 16 | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | Cullenstown | 52.226 | 6.717 | 16 | 1 | - | - | 16 | - | - | - | - | - | - | - | 16 | - | - | - | - | - | - | - |
| 17 | Kiltraw Bridge | 52.248 | 6.753 | 12 | 1 | - | - | 12 | 12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 18 | Vicarstown | 53.049 | 7.083 | 31 | 1 | - | - | 31 | - | 31 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 19 | Montrath | 53.011 | 7.499 | 21 | 2 | 0.050 | 0.092 | 21 | 21 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | Lissarda | 51.854 | 8.874 | 9 | 1 | - | - | 9 | 9 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Scotland | | | | | | | | | | | | | | | | | | | | | | | |
| 21 | Edinvillie | 57.444 | 3.224 | 32 | 1 | - | - | 32 | 32 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | Cardhu | 57.438 | 3.368 | 32 | 1 | - | - | 32 | - | - | - | - | - | - | - | - | - | - | - | - | - | 32(1) | - |
| 23 | Lettoch | 57.364 | 3.524 | 29 | 6 | 0.179 | 0.766 | 29 | 10 | - | - | - | - | 9 | - | - | 7 | - | - | - | - | | 0.741 |
| 24 | Speybridge | 57.320 | 3.606 | 29 | 15 | 0.500 | 0.919 | 29 | 12 | - | - | - | - | - | - | 4 | - | - | - | - | - | 3(1) | 0.766 |
| | | | | | | | | | | | | | | | | | | | | | | 13(5) | |
| England | | | | | | | | | | | | | | | | | | | | | | | |
| 25 | Wistow | 52.414 | 0.115 (E) | 26 | 1 | - | - | 26 | | - | - | - | - | - | - | - | - | - | - | - | - | 26(1) | - |
| 26 | Loxwood | 51.062 | 0.515 | 21 | 1 | - | - | 21 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 27 | Haslemere | 51.085 | 0.692 | 23 | 3 | 0.091 | 0.514 | 23 | | 16 | - | - | - | - | - | - | - | - | - | - | - | 7(1) | 0.443 |
| Sweden | | | | | | | | | | | | | | | | | | | | | | | |
| 28 | Tandsjoborg | 61.709 | 14.721 (E) | 16 | 11 | 0.667 | 0.933 | 15 | - | - | - | - | 7 | - | - | - | - | - | - | 1 | - | 7(3) | 0.733 |
| 29 | Dorotea | 64.262 | | 27 | 15 | 0.538 | 0.915 | 18 | - | - | - | - | 1 | - | 3 | - | - | - | 3 | - | - | 11(2) | 0.784 |
| 30 | Sorsele | 65.535 | 16.415 (E) | 30 | 8 | 0.241 | 0.756 | 30 | - | - | 7 | - | 3 | - | - | - | - | - | 4 | - | 1 | 15(1) | 0.690 |
| 31 | Skuleskogen | 63.111 | | 29 | 9 | 0.286 | 0.817 | 27 | - | - | - | - | 6 | - | - | - | - | 9 | - | 4 | - | 8(1) | 0.758 |
| 32 | Harads | 66.083 | 17.542 (E) | 27 | 10 | 0.346 | 0.863 | 26 | - | - | - | - | 10 | - | 9 | - | 3 | - | - | 1 | - | 3(1) | 0.760 |

**Table 1** (continued)

| Population | | Lat (N) | Long (W) | Nuclear | | | | Chloroplast | | | | | | | | | | | | | | *H* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *N* | *G* | *R* | *D** | *N* | h1 | h2 | h3 | h4 | h5 | h6 | h7 | h8 | h9 | h10 | h11 | h12 | h13 | hS | |
| 33 | Hakkas | 66.915 | 18.483 (E)<br>20.963(E)<br>21.565 (E) | 27 | 7 | 0.231 | 0.752 | 24 | - | - | - | - | 9 | - | 10 | - | - | 1 | - | - | 4 | - | 0.685 |
| Italy | | | | | | | | | | | | | | | | | | | | | | | |
| 34 | Monte Bondone | 46.005 | 11.035 (E) | 27 | 8 | 0.269 | 0.630 | 27 | 6 | - | - | - | - | 17 | - | - | - | - | - | - | - | 4(4) | 0.567 |

seed gene flow, leads to biparental pairing without loss of genetic and clonal diversity (Bona et al. 2019).

Clonal dominance in newly colonised areas could simply result from the advantage of reproduction without a mate. Genome-wide sequencing of population of *Fucus vesiculosus* in postglacial populations of the Baltic Sea suggested that clones are favoured in recently invaded areas because of 'uniparental reproductive assurance' (Pereyra et al. 2023). Conversely, sexual reproduction is favoured in core areas so that alternative waves of asexual and sexual reproduction, create a mosaic of clones and sexually reproducing populations which may be maintained over thousands of generations by repeated bottlenecks in marginal areas. Rafajlovic et al. (2017) further supports Baker's Law in explaining clonal reproduction in marginal populations of a fucoid seaweed which is not dependent on selection, but by neutral processes such as random dispersal and population stochasticity. Large, widely distributed clones are predicted in aquatic species dispersing as adults or fragments. Smaller, local clones, in contrast, are predicted in aquatic and terrestrial plants dispersing as sexually produced seeds. Peripheral populations, thus, are dominated by asexual reproduction, leading to few genets and many ramets whilst core or continental populations are dominated by sexual reproduction with many genets and few ramets.

There is some doubt concerning the arrival of aspen in Ireland due to the infrequent occurrence of *P. tremula* pollen in peat and lake sediments both between sites and chronologically. This cannot be explained by a low production of pollen (Caudullo and Rigo 2016). However, preservation of pollen of *Populus* spp. compared to other species may be poor (Campbell 1999). *P. tremula* is uncommon in Irish pollen records but believed to date from Dunshaughlin, Co Meath in the midlands during the Early Flandrian ca. 9,600 years ago (Watts 1977). Two other early records are: 11,565 years ago from the midlands at Cornagher Lough, West Meath, (unpublished, Professor Gill Plunkett, Queen's University Belfast); and, 11,184 years ago, from Sluggan Moss, Co Antrim in the north (Smith and Goddard 1991). These records suggest that *P. tremula* was present and widespread in Ireland from the start of the Mesolithic ca. 10,000 years ago. The first human settlement in postglacial Ireland dates to 9,000 years ago (Woodman 2015) up to 1,500 years after the first dates for pollen or *P. tremula* (Watts 1977; Smith and Goddard 1991). Furthermore, because Ireland was cut off from Britain and Europe ca. 15,000 years ago (McCabe et al. 2005; Montgomery et al. 2014), recolonization would have to have occurred rapidly over long distances across the sea (Edwards and Brooks 2008). Hence, colonisation of Ireland likely involved few genets with an emphasis on asexual reproduction of ramets (Baker 1955, 1967; Beatty et al. 2015b). Such a scenario could result in low levels of genetic

variation which could compromise the capacity of *P. tremula* in Ireland to adapt to a range of global threats (reviewed by Mackenzie, 2010; Caudullo and de Rigo 2016).

Our principal aim was to describe genetic and clonal diversity in populations of *P. tremula* in Ireland and make comparisons with *P. tremula* from Britain and representative areas of continental Europe. The latter were opportunistic samples from Sweden towards the northern limit of the species' continental range, and Italy, towards its southern limit. The status of *P. tremula* in Ireland is poorly understood (Stroh et al. 2023) whereas it is regarded as increasing in Britain and Sweden (Caudullo and de Rigo 2016). We aimed to determine the relative importance of sexual and clonal reproduction in island and continental populations of *P. tremula* (Suvanto and Latva-Karjanmaa 2005) and to assess whether European *P. tremula* populations are consistent with Baker's Law. More specifically, we test the hypothesis that insular populations (from Ireland and Britain) of *P. tremula* have fewer genets with more ramets than continental populations (from Sweden and Italy). The present research should assist the evaluation of the vulnerability of scattered, small populations of *P. tremula* in a wider context.

## Materials and methods

### Sampling

The National Biodiversity Data Centre for Ireland (https://biodiversityireland.ie/) holds 731 records (all years) of *P. tremula* from 322 10 km squares with two main foci: mid-Ulster in the north and Wexford in the south east. Samples (465 individual trees) were collected from 20 sites across Ireland (Table 1; Fig. 1). Woodlands previously identified as ancient or semi-natural were selected on the basis of data collected for the Woodland Trust Inventory of ancient and long-established woodland in Northern Ireland (www.backonthemap.org.uk) and the National Survey of Native Woodlands 2003–2008 in the Republic of Ireland (www.npws.ie). Other woodlands were selected based on information from the Department of the Environment such as ASSIs (Areas of Special Scientific Interest), as well as the landscape character areas listing the woodlands and species present in each region (https://www.doeni.gov.uk). Although *P. tremula* is widespread, it is sparsely distributed, so records from the Centre for the Northern Ireland Environmental Data and Recording (CEDaR) (http://www2.habitas.org.uk/records/home) and the Botanical Society of Britain and Ireland (BSBI) (http://bsbi.org/maps) were also used to pinpoint locations where the species might be present. Further sites were found fortuitously due to the 'quaking' movement of aspen in leaf. Given the overall pattern of deforestation, low woodland cover and predominance of farmland with hedgerows in Ireland (Mitchell and Ryan 1992), it is likely that these fortuitous sites have a similar history to sites with a documented presence of *P. tremula*.

At each location, 3–5 leaves were taken from each, physically separate individual tree or ramet and stored on silica gel. The number of individual trees or ramets sampled varied (9 to 55) from site to site (Table 1). Mature trees (maximum height 20 m) and saplings (minimum height 1.5 m), where present, were sampled as the aim was to collect leaves from all putative ramets in a clump of genetically identical individuals or genet, to give a full picture of each population and its level of clonality. The spatial limits of a putative genet or clump were determined by surveying beyond its outer ramets. GPS coordinates were recorded for each ramet sampled. Maximum length and total area sampled are presented in Table 2. Sampling was restricted to July and August when trees were in full leaf before undergoing senescence.

Samples (375 individual trees) were also obtained from four sites in Scotland, three sites in England, seven sites in Sweden and one in Italy for comparison with northern and southern British and continental populations (Table 1; Fig. 1). GPS coordinates were not available for the Swedish and Italian samples, but they were collected from areas of woodland comparable in size to those from the islands of Great Britain and Ireland. Genetic diversity may increase with range area and habitat availability. The continental samples were also collected from more continuous forest and woodland than the British and Irish samples which were confined to and easily delineated in scattered fragments of woodland often associated with field boundaries. Range areas of the four semi-isolated countries were within a single order of magnitude. Samples of *P. tremula* from larger continental areas may be expected to have greater genetic variety than those from smaller areas, but Ireland and Italy, and Great Britain and Sweden are roughly similar in area.

### DNA extraction and genotyping

DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). Clonal analysis using genetic markers e.g. microsatellites is an effective method of establishing levels of asexual reproduction in natural populations (Mori et al. 2018). Following precedence, samples were genotyped for 13 loci comprising 8 nuclear microsatellite loci and 5 chloroplast microsatellite loci. Four of the nuclear loci (PTR4, PTR5, PTR6 and PTR14) were originally developed for *P. tremuloides* and described in Dayanandan et al. (1998) and Rahman et al.

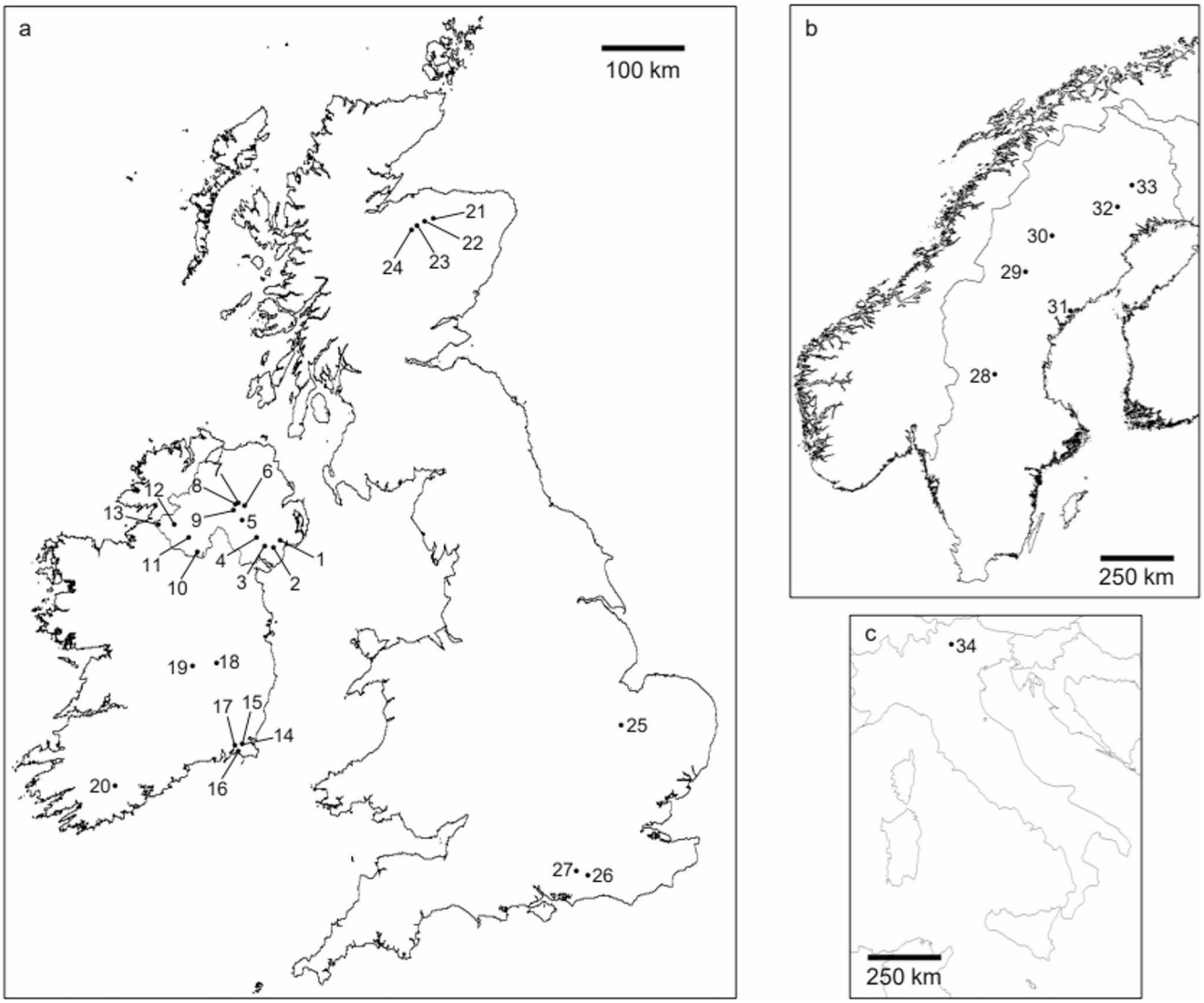



**Fig. 1** Map showing the locations of populations sampled in (**a**) Great Britain and Ireland, (**b**) Sweden. Numbers refer to locations in Table 1

(2000). Rajora and Rahman (2001) showed that SSR primer pairs developed in *P. tremuloides* were successful in resolving microsatellites in all 24 *Populus* species. Nevertheless, to avoid aberrant results from using only non-specific primer pairs, we developed specific primers to amplify the remaining loci (BU819241, BU821046, BU889575 and BU891546) from the corresponding GenBank entries using the Primer3 program (Untergasser et al. 2012; Table S1). Forward primers included a 19 bp M13 tail (CACGACG TTGTAAAACGAC) and reverse primers included a 7 bp tail (GTGTCTT). PCR was carried out in a total volume of 10 µl containing 100 ng genomic DNA, 5 pmol of 6-FAM labelled M13 primer, 0.05 pmol of each M13-tailed forward primer, 5 pmol each reverse primer, 1× PCR reaction buffer, 200 µM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR used the following conditions: initial denaturation at 94 °C for 3 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored and inconsistencies suggesting null alleles identified, using the GeneMarker software (V1.8, SoftGenetics).

Chloroplast microsatellites were designed from the complete chloroplast genome sequence of *P. tremula* (GenBank accession KP861984). Primers were designed using the Primer3 program to amplify five loci in two multiplex reactions (Popcp51246/Popcp76696/Popcp80893 and Popcp186/Popcp114331), which were combined for a single genotyping run (Table S2). PCR and genotyping were carried out as described above, except that 30 cycles were performed.

**Table 2** Maximum distance (m) between individual trees and minimum area (ha) of multi-ramet clones found in Irish and British populations. Minimum area was estimated using a convex hull measurement in the QGIS software package (V2.18.4)

| Clone | Length (m) | Area (ha) | Population(s) |
|---|---|---|---|
| N001 | 107 | 0.093 | Clonvaraghan |
| N003 | 11,790 | 43.852 | Hilltown, Rathfriland |
| N007 | 28 | 0.003 | Poyntzpass |
| N008 | 36 | 0.004 | Poyntzpass |
| N009 | 60 | 0.033 | Duffmount |
| N010 | 183 | 0.016 | Peatlands Park |
| N011 | 11,187 | 311.122 | Drumrot, Tullyboy, Poplar Hill |
| N012 | 12 | N/A | Drumrot |
| N013 | 4,602 | 32.296 | South Fermanagh |
| N014 | 5,250 | 321.779 | Lisnaskea |
| N015 | 3,934 | 25.234 | Derrygonnelly |
| N016 | 1,728 | 16.166 | Lough Melvin |
| N017 | 34 | 0.003 | Lough Melvin |
| N018 | 92 | 0.017 | Tullycana |
| N019 | 30 | N/A | Tullycana |
| N020 | 48 | 0.016 | Corakeela Lodge |
| N021 | 67 | 0.020 | Cullenstown |
| N022 | 60 | 0.023 | Kiltraw Bridge |
| N023 | 1,725 | 41.086 | Vicarstown |
| N024 | 1,101 | 2.018 | Mountrath |
| N026 | 27 | 0.007 | Lissarda |
| N027 | 87 | 0.122 | Edenvillie |
| N028 | 63 | 0.298 | Cardhu |
| N029 | 194 | 0.112 | Lettoch |
| N030 | 73 | 0.028 | Lettoch |
| N032 | 113 | 0.010 | Lettoch |
| N034 | 6 | N/A | Lettoch |
| N037 | 100 | 0.146 | Speybridge |
| N038 | 52 | N/A | Speybridge |
| N040 | 4 | N/A | Speybridge |
| N042 | 25 | 0.003 | Speybridge |
| N043 | 135 | 0.030 | Speybridge |
| N050 | 144 | 0.158 | Wistow |
| N051 | 61 | 0.031 | Loxwood |
| N052 | 31 | 0.015 | Haslemere |
| N054 | 50 | 0.041 | Haslemere |

## Data analysis

Clonal individuals belonging to the same genet were identified using the method of Parks and Werth (1993), which calculates the probability $P_{GEN}$ of each multi-locus genotype (MLG) arising through sexual as opposed to clonal reproduction:

$$P_{GEN} = \left[2^h \pi \left(X_{1i} X_{2i}\right)\right]^{n-1}$$

where $h$ is the number of loci at which the genotype is heterozygous, $x_{1i}$ is the allele frequency of the first allele in the genotype at locus $i$, $x_{2i}$ is the allele frequency of the second allele in the genotype at locus $i$. $P_{GEN}$ values were calculated using the GENCLONE software package (V2.0; Arnaud-Haond and Belkhir 2007). GENCLONE was also used to calculate genotypic richness ($R$) and clonal diversity ($D$*) for each population. Levels of chloroplast haplotype diversity ($H$) were calculated using the ARLEQUIN software package (V3.5.1.2; Excoffier and Lischer 2010). The minimum area of each MLG (or clone comprising genetically identical individuals) was estimated using a convex hull measurement in the QGIS software package (V2.18.4).

To characterise the relationships among nuclear MLG's the proportion of shared alleles (Dps) (Bowcock et al. 1994) between each pair of MLGs was calculated using MICROSATELLITE ANALYSER (Dieringer & Schlötterer, 2003) and then viewed using a Principal Coordinates Analysis (PCoA) performed in GenAlex (Peakall and Smouse 2006). Similarly, Dps and PCoA was used to assess the genetic relationships among population samples. Genetic differences among samples were further quantified using $F_{ST}$ (Weir and Cockerham 1984). Mean pairwise relatedness within samples was calculated using the relatedness estimator, rqg, of Queller and Goodnight (1989) in GENALEX with associated 95% confidence intervals determined by 1000 bootstraps. Permutation of genotypes among all samples (999 times) was used to calculate the upper and lower 95% confidence intervals for the expected range of rqg under a panmictic model.

## Results

Between six (PTR-4, BU821046 and BU891546) and 17 (PTR-6) alleles were detected per nuclear microsatellite locus (mean = 9.125). Three alleles were detected in all individuals from the Clonvaraghan population at locus PTR-5, and in all individuals in the Lisnaskea population at locus BU889575 (Fig. S1). This might have resulted from cross-species amplification of non-homologous products from non-orthologous loci (Yue et al. 2010). A fixed null allele was detected at locus BU889575 in the Corakeela Lodge, Kiltraw Bridge and Wistow populations. The analysis revealed a total of 121 separate genets from 841 individual trees (ramets) studied, with levels of genotypic richness ($R$) and clonal diversity ($D$*) ranging from zero (16 populations) to 0.667 (Tandsjoborg, Sweden), and from zero (16 populations) to 0.933 (Tandsjoborg) respectively (Table 2). Zero indicates absence of genotypic richness and clonal diversity. The probability of multiplied genotypes emerging due to independent generative reproduction events ranged from 0.14 to < 0.0001 (average = 0.008) (Table S3).

Box and whisker plots and Wilcoxon rank sum tests indicate genetic diversity for both measures was significantly

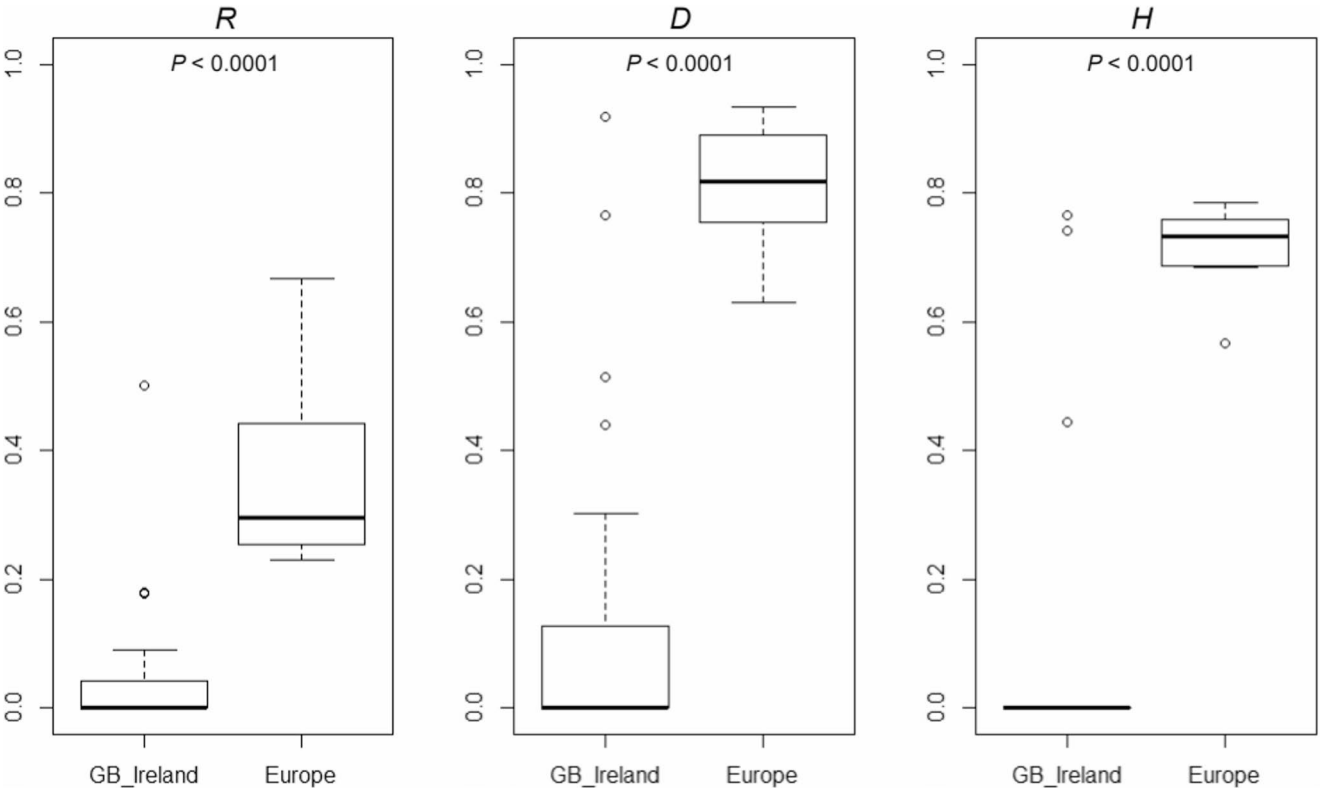



**Fig. 2** Box and whisker plots showing comparisons of genetic diversity between Great Britain / Ireland and Europe (Sweden and Italy): R – genetic richness; D* – clonal diversity; H – haplotype diversity. P-values from Wilcoxon rank sum tests

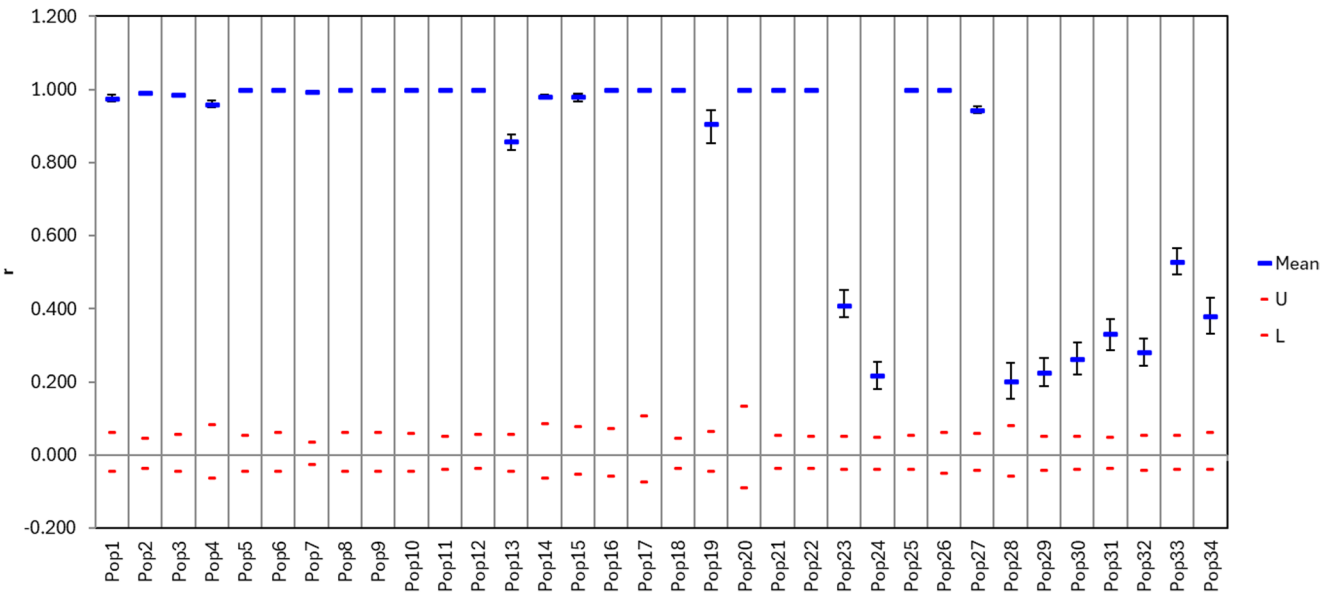



**Fig. 3** Mean within-sample pairwise relatedness, rqg, and 95% confidence intervals determined by bootstrap resampling. Red bars are the 95% upper and lower expected values for a null distribution generated from 999 permutations of data among all samples, and enclose the values expected within a panmictic system. Population numbers correspond to Table 1.

lower in Britain and Ireland compared to the samples from Italy and Sweden (Fig. 2). This was also evident at the population level with British and Irish samples except for Lettoch and Speybridge, showing much higher levels of mean relatedness than Swedish and Italian samples (Fig. 3). Levels of diversity were particularly low in Ireland, with only eight of the 20 populations exhibiting more than a single clone (Fig. 4). When potential somatic mutation (i.e. clones that

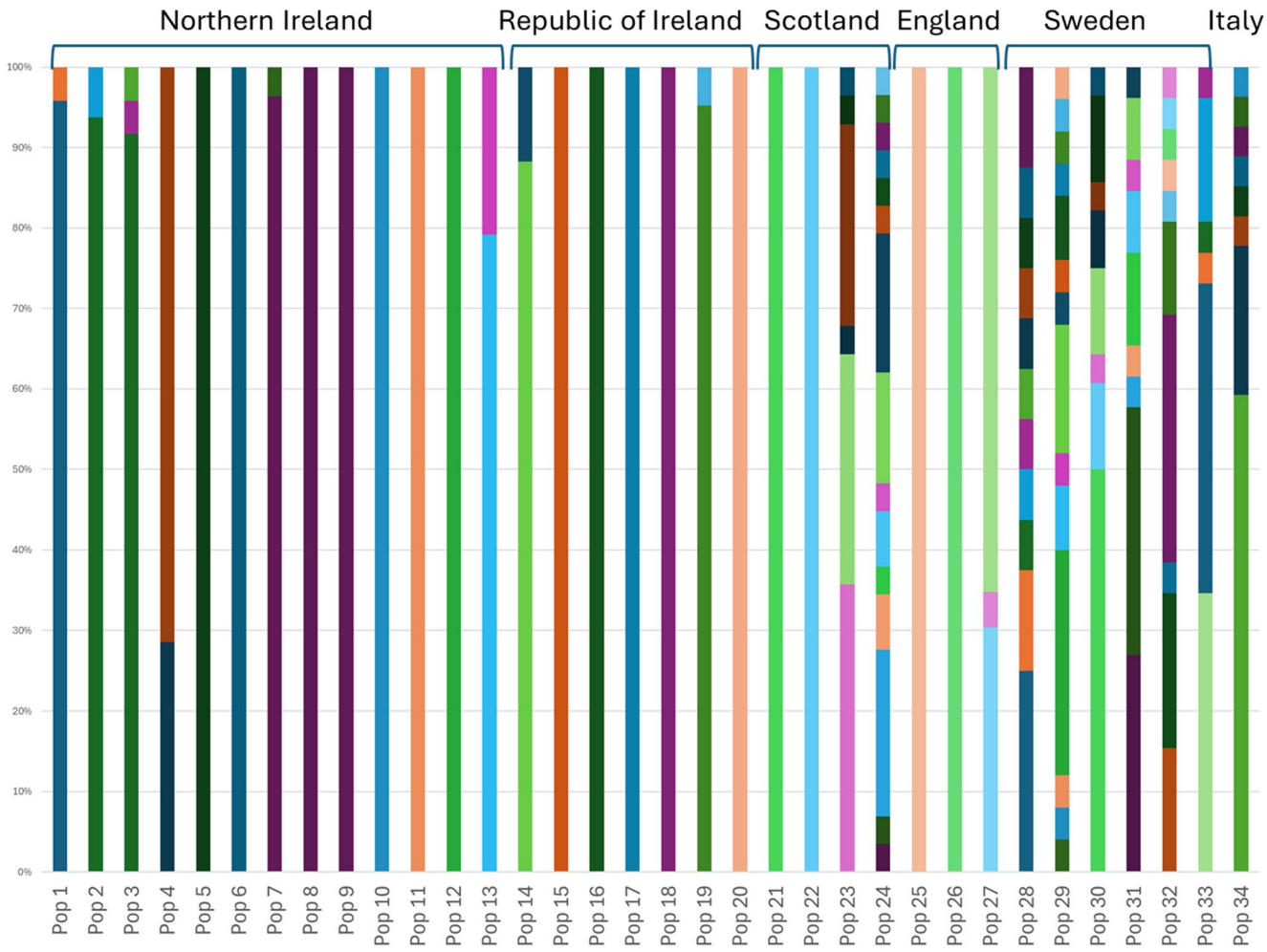



**Fig. 4** Frequency of the different multilocus genotypes (N = 121), defined by 8 nuclear microsatellite loci in each sample. Population numbers correspond to Table1.

differ by a single allele) was considered, all but two (Lough Melvin and Montrath) could be considered monoclonal. The majority of multi-ramet clones were relatively small (< 1 ha) and confined to a single area sampled or population (Table 2), but several very large clones were detected, two of which (N003 and N011) were found across different populations. One of these, clone N003, was the second largest by area (311 ha) and was found in the Drumrot, Tullyboy and Poplar Hill populations, whilst the largest, clone N014 in the Lisnaskea population, covered 322 ha (Fig. 5).

As might be expected from such clonal structure the overall $F_{ST}$ for the Irish and British samples was very high ($F_{ST}$ = 0.503) and much higher than the $F_{ST}$ across the Swedish and Italian samples ($F_{ST}$ = 0.167). The average $F_{ST}$ in comparison between Irish/British samples vs. Swedish/Italian samples was 0.22. These patterns were evident from the PCoA of population samples which revealed a greater spread of Irish/British samples compared to the mainland European samples (Fig. 6). Across all samples there was a total of 121 MLG's, with MLG's 1–54 found among Irish and British, and 55–121 found among the Swedish/Italian sites. Despite the lack of genotype sharing between these regions phylogenetic analysis revealed considerable overlap in the MLG's (Fig. 7). Higher resolution genetic analyses of these potentially important genetic components of biodiversity are required to confirm the ramets comprising each genet.

Between five (Popcp51246) and ten (Popcp80893) alleles were found in the five chloroplast microsatellites analysed (mean = 7.2; Table S2). Combining these gave a total of 34 unique haplotypes, 13 of which were found in multiple populations, with the remaining 21 being unique to a single population. Only three haplotypes were shared between Ireland/Britain and continental Europe (Table 2). Levels of haplotype diversity ranged from zero (all British and Irish populations except Lettoch, Speybridge and Haslemere) to 0.784 (Dorotea, Sweden; Table 2) and were significantly




**Fig. 5** Maps showing distributions of clonal individuals in (**a**) Lisnaskea and (**b**) Drumrot, Tullyboy and Poplar Hill.

lower in Britain and Ireland compared to samples from Italy and Sweden (Fig. 2).

## Discussion

Our study suggests extremely low levels of genetic diversity and high levels of clonality in Irish *P. tremula* compatible with a predominance of asexual reproduction in most of the populations sampled. These results contrast with similar studies on *P. tremula*. Suvanto and Latva-Karjanmaa (2005) found that clones in old-growth and managed forests in Finland were genetically distinct and that sexual reproduction was common with 70% of the clones identified consisting of a single ramet. Variability in reproductive method was also apparent in stands of *P. tremula* in European Russia (Politov et al. 2016). Three stands consisted of 40.6 to 83.3% of discrete genotypes whilst the fourth was a single distinct genotype (ramet). Even under uniform 'garden' environmental conditions mating systems in *P. tremula* vary giving rise to genetic diversity in different demes (Zhang et al. 2016).

The patterns for Irish populations are consistent with both postglacial recolonization and Baker's Law which predicts extreme founder effects are followed by extensive vegetative reproduction. Baker's Law holds that self-compatible organisms are more likely to colonize successfully from a single propagule than self-incompatible organisms (Baker 1955, 1967; Pannell et al. 2015). In *P. tremula*, the former condition, self-compatibility, is satisfied by asexual reproduction and the latter, self-incompatibility, by sexual reproduction.

Fussi et al. (2010) reported absence of any clear phylogeographic structuring in *P. tremula* across mainland Europe. This was attributed to high ancestral gene flow among connected refugia and rapid recolonisation of previously glaciated regions, resulting in similar levels of genetic variation among mainland sites. This contrasts with the present study which suggests lower levels of genetic diversity observed in Ireland and, to a lesser degree, Britain. The lower level of genetic variation among Irish sites, however, is consistent with similar patterns reported for the Lusitanian flora of Ireland (Mitchell and Ryan 1992) which are

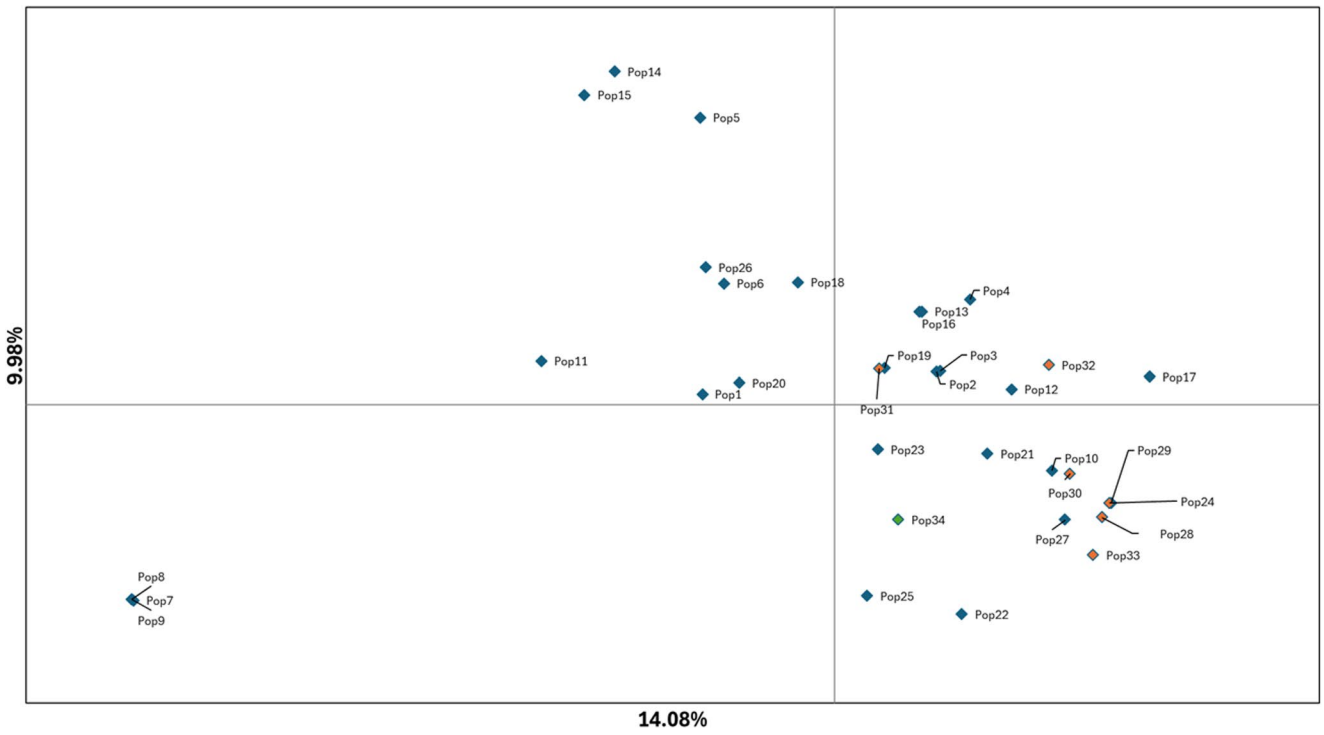



**Fig. 6** Relationships among population samples inferred by Principal Coordinates Analysis of genetic distance (Dps) between samples. Numbers correspond to Table 1. Swedish populations are highlighted in amber and the Italian population is in green.

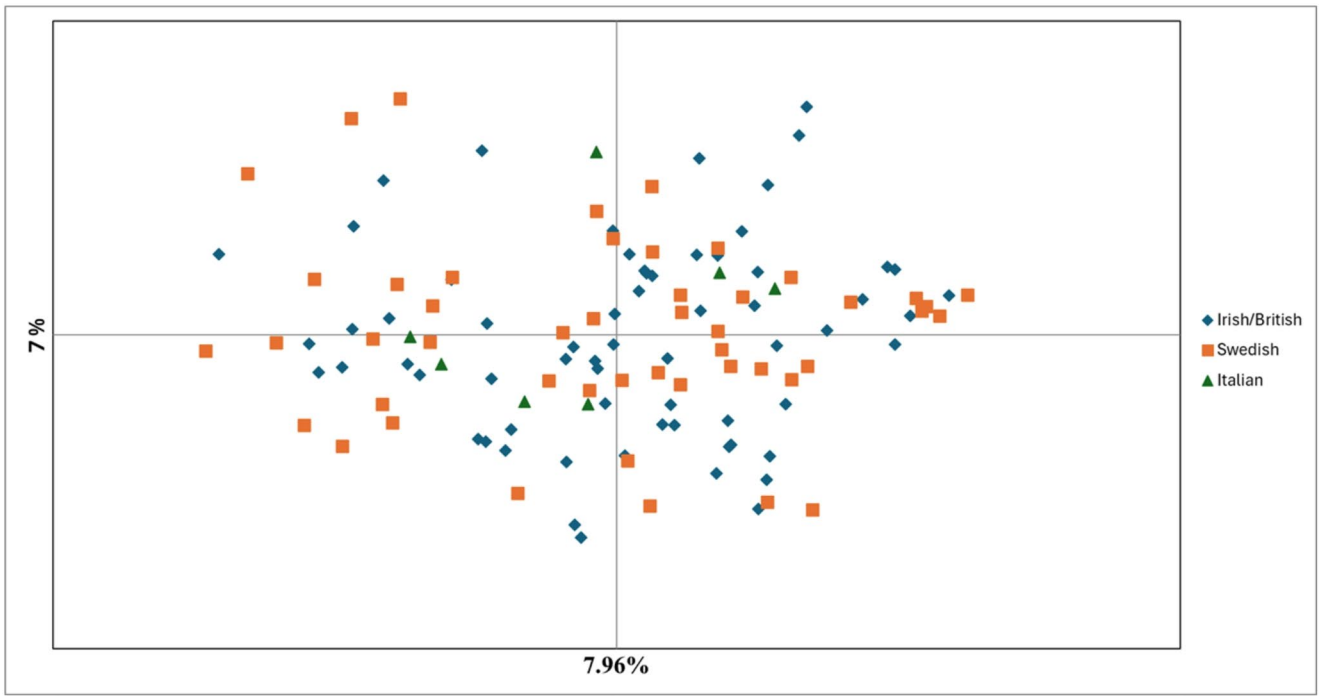



**Fig. 7** Relationships among the distinct nuclear multilocus genotypes (MLG) (n = 121) inferred by PCoA of Dps. MLGs are colour coded according to area of sampling.

restricted to western Ireland and the northern Iberian Peninsula. Irish populations exhibit far lower diversity than their Iberian counterparts (Beatty & Provan 2013, 2014; Beatty et al. 2015b). While phylogenetic cohesion among Irish, British and mainland Europe MLGs is compatible with ancestral gene flow, a reduced level of diversity can be attributed to the restricted access to Britain and particularly Ireland during postglacial colonisation as part of the "Littletonian steeplechase" (Mitchell and Ryan 1992), and postglacial genetic loss (discussed below).

The Italian sample had less variation than most Swedish samples. While more extensive sampling of southern sites is needed to confirm this result, this digresses from the common pattern of Southern richness – Northern purity (e.g. Dapporto et al. 2024). This may reflect a combination of (i) lower genetic variation in Italian sites as these are towards the southern edge of the species range and (ii) increased variation among Swedish sites due to admixture among refugial groups (de Carvalho et al. 2010). Refugial richness may also explain similar levels of nuclear genetic variation for island (Sardinia) and mainland Italian populations of *P. alba* (Brundu et al. 2008).

An alternative to natural colonisation followed by deforestation, that might explain low genetic richness and diversity of *P. tremula* in Ireland, is that early people brought *P. tremula* with them and moved genets around the island. This hypothesis seems tenable given that 987 non-native plant species have been recorded in Ireland, including 97 ancient (pre-1500) introductions, but none of these is a tree species (Stroh et al. 2023). After the last glaciation, juniper *Juniperus communis*, and birch *Betula pubescens*, arrived in Ireland ca. 13,500 years ago. These gave way to hazel *Corylus avellanus* and primary forest tree species, mainly oak *Quercus* spp., and elm *Ulmus* spp. so that dense forest covered Ireland 9,000 years ago (Pilcher and Hall 2001). The earliest date for *P. tremula* in Ireland is ca. 11, 500 years ago (see Introduction). Ireland did not have Palaeolithic people; the earliest hunter-gatherers date from 9,000 years ago and earliest farmers, from 6,000 years ago (Woodman 2015). Whilst it is possible that the former imported animal species (Montgomery et al. 2014), Neolithic farmers were more likely to introduce useful plants (McClatchie et al. 2014). Celtic people made limited use of *P. tremula* to make shields and was also regarded as a link to the spiritual world (treesforlife.org.uk/into-the-forest/trees-plants-animals/trees/aspen/aspen-mythology-and-folklore/). Its general lack of utility (Jobling 1990) is reflected in just one Irish placename associated with An Crann Creathach, the Irish name for aspen (McLoughlin 2016). Thus, the hypothesis of anthropogenic introduction is inconsistent with the known chronology of the arrival of trees in Ireland and their subsequent deforestation with the introduction of agriculture, the history of exotic trees in Ireland and the relative lack of utility of wood from *P. tremula*. Anthropogenic introduction and random dispersal is also unlikely to preserve local genetic homogeneity at the level of the ramet and island wide heterogeneity at the level of the genet, without access to 21 st century genetics. Without identifying discrete ramets, isolated genets could not be maintained preventing mixing of ramets from different sources.

Gradual recolonisation from isolated propagules and multiple sources is more likely to produce genetic diversity unique to Ireland and distinct from Britain and parts of the continent, as well as populations comprising one or two clones. Present distribution of ramets within discrete genets suggests only local anthropogenic translocation occurred and did not disrupt the genetic population structure established in Ireland early in the Holocene. While we consider anthropogenic introduction of *P. tremula* into Ireland less likely than natural colonisation. Direct testing of the former could be facilitated by increased genomic coverage allowing more accurate aging of clones (Macaya-Sanz et al. 2016). However, we cannot yet dismiss an anthropogenic basis for isolated clones with low genetic diversity of *P. tremula* in Ireland. The natural dispersion of recolonising *P. tremula* and human mediated decline with deforestation seem a more likely and generally applicable model for marginal areas of Europe. For example, *P. tremula* is similarly widespread and sparse in the Outer Hebrides and is regarded as an important pioneer surviving from the start of the Holocene with deforestation from the Neolithic onwards (Spence 1960).

Hybridisation zones between contiguous *Populus* spp. occur in continental Europe (e.g. Castiglione et al. 2010). Introgression with *P. tremula* adds to genetic diversity of *P. alba* in northern Italy but the main driver of population genetic structure of *P. alba* is a physical barrier to gene flow and riparian habit. A hybridisation zone between *P. alba* and *P. tremula* exists along the river Duoro of the Northern Iberian Plateau (Santos-del-Blanco et al. 2013). Maximum individual clonal 'extension' of *P. alba* was 158 km and large clones had two genomic regions derived from *P. tremula* (Macaya-Sanz et al. 2016). Both male and female *P. alba* form spatially discrete genetic aggregations consisting of similar numbers of ramets which in females were positioned to avoid competition. Males sought to exploit new areas and had 2.7 times the number of genets of females (Dering et al. 2016). *P. nigra* is also a riparian species with asexual and sexual reproduction that varies with age of tree stand along 30 km of the Garonne in southwest France (Barsoun et al. 2004). Young stands had fewer replicated genotypes than middle-aged or old stands, while middle-aged stands had the greatest number of replicated genotypes. Dispersion of spatially organised clones was strongly influenced by

river flow and generally formed small compact units (up to 4 trees) but translocated woody fragments could occur up to 19 km downstream. Most (95%) of clonal MLG, consisted of fewer than 5 ramets (maximum 18) in a single large stand (7 ha) of *P. nigra* beside the Loire (Chenault et al. 2011). There was also evidence of introgression involving *P. nigra* var *italica.*

Clones of *P. alba*, *P. nigra* and *P. tremula* and their hybrids along the rivers of southern Europe have a different basis from those of *P. tremula* in northern Europe and, in particular, Britain and Ireland, which do not involve hybrids and are not restricted to riparian habitats. *P. tremula* is not regarded as a riparian species. There is also strong evidence for asexual reproduction involving root suckers but not woody fragments in *P. tremula* with most populations comprising quite compact groups of ramets (Bärring 1988). Hence, there would be limited chance for long distance translocation of woody fragments as in populations of *P. nigra* on the Garonne (Barsoun et al. 2004).

Although the overall genetic diversity of *P. tremula* in Great Britain and Ireland in the present study was very low, two Scottish populations exhibited patterns of variation more akin to those found by Suvanto and Latva-Karjanmaa (2005). Lettoch contained four multi-ramet clones as well as two unique individuals, whilst Speybridge had five multi-ramet clones and ten unique individuals. Although clone areas were not calculated in the earlier study, mean clone length (13.5 m) was much smaller than those from Lettoch (mean 94.5 m) and Speybridge (mean 64.8 m). As in the Finnish study, the Scottish populations exhibited a "phalanx" structure, where clones are tightly packed together due to connections between ramets having few or short internodes with no mixing of clones, as opposed to the "guerrilla" form where clones are more spaced out due to their many and/or long connections between ramets, with subsequent mixing of clones (Lovett Doust 1981). This was not always the case in the few multi-clonal populations examined in the present study. The Lough Melvin population in Ireland exhibited a phalanx distribution, but those from Poyntzpass and Tullycana (Ireland), and Haslemere (England) had a guerrilla structure, with groups of trees containing a mix of clones.

The only other study on genetics and conservation of *P. tremula* in Scotland (Easton 1997) mentioned "Strathspey" which probably includes the Speybridge and Lettoch populations, but was carried out over a much larger area, without specifically addressing clonal structure. It has been suggested that some of these "Speyside" sites may contain more than one clone (Worrell 1995). *P. tremula* is dioecious and thus the sex of a clone should be recorded in future studiesusing a PCR based marker (Pakull et al. 2015).

Suvanto and Latva-Karjanmaa (2005) proposed that *P. tremula* clones are not as large as those found in American aspen, *P. tremuloides* (but our findings suggest that this may not be the case. Even allowing for the convex hull measurements of fragmented stands of the same clone most likely underestimating the size of these clones, several were larger than reported for contiguous clones of *P. tremuloides* clones in Utah. The clones in Lisnaskea (321.78 ha) and Drumrot, Tullyboy and Poplar Hill (311.12 ha), were almost an order of magnitude larger than the *P. tremuloides* "Pando" clone (Kemperman and Barnes 1976; De Woody et al. 2008). Large clones fragment over time due to small scale disturbances, as well as competition from other clones and vegetation types (Mock et al. 2008). This occurs in *P. tremuloides*, where genetically identical ramets were up to 500 m apart (Hipkins and Kitzmiller 2004; Mock et al. 2008). Although trees may die, suckers of *P. tremula* can remain alive and active (Bärring 1988). These suckers have been observed up to 40 m away from trees (Jobling 1990), and, given that they can potentially spread at a rate of 1 m per year (Myking et al. 2011), it seems possible that the clones found in Ireland are very old. Based on the present study with clones over 11 km in length, it is conceivable that some of these are remnants of the original clones from the initial recolonization around 10–11,000 years ago. Rafajlović et al. (2017) suggests that postglacial recolonization, particularly in long-lived species such as trees, is characterized by the asexual spread of large, dominant clones. Thus, the combination of rare but important long-distance dispersal (Trakhtenbot et al. 2005) and limited suitable habitat for germination and establishment of seedlings (Latva-Karjanmaa et al. 2003) probably led to the expansion of a small number of very large clones, which subsequently fragmented into the sparse, dispersed stands observed in the present-day. In Ireland, deforestation started in Neolithic times and led to almost total loss of tree cover followed by field enclosure by hedgerows (Mitchell and Ryan 1992) such that remnants of clones of *P. tremula* today, are generally isolated along field boundaries in a matrix of farmland.

No flowering was observed in *P. tremula* during the sampling period of the present study (JB, SSJM, WIM pers. obs.). The high levels of clonality observed in *P. tremula* contrast with other population genetic studies of native trees in Ireland, including juniper *Juniperus communis* (Provan et al. 2008), ash *Fraxinus excelsior* (Beatty et al. 2015a), alder *Alnus glutinosa* (Beatty et al. 2015c), both pedunculate oak *Quercus robur* and sessile oak *Quercus petraea* (Beatty et al. 2016), hawthorn *Crataegus monogyna* (Brown et al. 2016a) and hazel *Corylus avellana* (Brown et al. 2016b). Vegetative reproduction having been suggested in juniper and hazel (Ward 1982; Persson et al. 2004), but there is no indication of asexual reproduction in either in Ireland. An extreme level of clonal reproduction was also found in the herbaceous plant *Orthillia secunda* in Ireland (Beatty et al.

2008). Like *P. tremula*, *O. secunda* has a highly fragmented distribution in Ireland. The cost of survival of both these species in Ireland may be loss of genetic diversity. There is structural spacing of clones in blackthorn *Prunus spinosa* (Brown et al. 2022).

Suvanto & Latvia-Karanjanmaa (2005) described fine scale structuring (average $F_{ST}$ across loci = 0.117) among *P. tremula* in Finland similar to that reported among our Swedish samples (average $F_{ST}$ = 0.091). Based on the species seemingly high capacity for pollen and seed dispersal by wind (Altman & Dittmer, 1964) Suvanto & Latvia-Karjanmaa (2005) suggested such fine scale structure was due to patchy establishment of clones possibly in combination with local selection. Comparisons between the Italian and Swedish samples here yielded a slightly larger average $F_{ST}$ value of 0.129. Given the large geographical distance between this samples this suggests that restricted gene flow due to geographical distance is not the primary driver of genetic structure in this species. Further studies employing rapidly evolving nuclear markers may reveal structure beyond the resolution of traditional chloroplast markers and elucidate the various roles of selection, drift, and restricted dispersal in this species. Such studies are vital as loss of genetic diversity in any fragmented, small population is a matter of conservation concern (Ellstrand and Elam 1993). Genetic diversity is critical in combating threats such as emerging plant diseases (Panzavolta et al. 2021;Gibson 2022) and climate change (Begna and Begna 2021; Muluneh 2021), and hybridisation with introduced *Populus* spp. and commercial varieties (Zhang et al. 2023). The results here confirm that compared to the more continuous, larger and more genetically diverse populations of continental Europe, the smaller more fragmented and genetically homogeneous populations of Britain and particularly Ireland, are clearly highly vulnerable to these threats.

Asexual reproduction in *Populus* is associated with the occurrence of triploidy, where failure in meiotic pairing results in sterility (Gustafsson 1935). Mock et al. (2008) observed three alleles at one or more loci in most larger genets in their study areas including the "Pando" clone. They also noted that triple alleles were especially common among spatially fragmented clones. In the present study, three alleles were found at a locus in two of the clones, including the largest clone at Lisnaskea. Whilst this might be an artefact related to use of markers developed for *P. tremuloides*, the occurrence of triploidy in *P. tremula* was originally noted over 80 years ago (Müntzing 1936) in a particularly vigorous form which had especially large leaves. It is possible that the increased vigour of such triploids aided the initial establishment and subsequent clonal expansion of aspen in Ireland.

## Conclusion

The present study suggests that British and Irish populations of *P. tremula* complement those of continental Europe, raising overall species genetic diversity. However, there are extremely high levels of asexual reproduction and low levels of genetic diversity in Irish and to a lesser degree British populations of *P. tremula* compared to Swedish and Italian populations. These data are consistent with Baker's Law suggesting that asexual reproduction was advantageous compared to sexual reproduction in newly accessible, marginal habitat during postglacial afforestation. The formation of clones in *P. tremula* in northern Europe and in particular Britain and Ireland, however, may have a very different basis than in *P. alba*, *P. nigra* and *P. tremula* and their hybrids in riparian habitats of southern Europe. The early history of *P. tremula* in Ireland and Britain was probably not influenced by people although this cannot be completely discounted. Subsequently, populations of *P. tremula* were lost or became fragmented due to deforestation related to agriculture. The scattered remnants of clones of *P. tremula* across Ireland may represent the "ghosts" of ancient, giant clones. These clones survive as fragments characterised by low genetic diversity, which are potentially vulnerable to climate change, habitat loss, novel plant diseases and pathogens, invasive species and hybridisation.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10592-026-01767-w.

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**Author contributions** JP and WIM conceived the study. JAB, WIM and SSJM obtained samples. JAB carried out the laboratory work. JAB, GEB, NJMcK and JP analysed the data. All authors participated in preparation of manuscript.

**Data availability** The dataset underpinning this study, including microsatellite genotypes and derived clone membership data for 34 populations of Populus tremula, is available from the Aberystwyth University PURE repository: https://doi.org/10.20391/2c118cef-b724-4029-9f90-e78e1dc78232.

## Declarations

**Competing interests** The authors declare no competing interests.

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