

INFORMATION NOTE

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SUMMARY

There is considerable interest in the need to conserve the 7000 trees which are all that remain of the population of black poplar growing in Britain. Recent work based on molecular techniques has provided markers which can unambiguously distinguish first generation hybrids of *P. x euramericana* from *P. nigra*. This is an important tool as it enables non-hybrid trees, which should be conserved, to be distinguished from exotic clones of hybrid poplars. Molecular markers have also demonstrated that the British population of black poplar has low diversity, compared with that in other European countries, and that there is a great deal of clonal duplication. Female clones are particularly rare. The ability to identify clones offers the opportunity for conservation effort to be concentrated at the level of the clone rather than that of the individual tree. Most of the British population is derived from ancestral material which colonised Britain after the last glacial period from refugia in southeastern Europe.

Work aimed at understanding the physical processes which drive the ecology of floodplain forests may help to re-establish populations of black poplar which have the capacity to reproduce and adapt to a changing environment.

INTRODUCTION

This Information Note updates the previous version relating to black poplar (*Populus nigra*) written a decade ago (White, 1993). The past ten years have seen great advances in our understanding of the existing populations of the species in Britain and Europe. This has largely occurred through the availability of new molecular markers which have provided the tools to investigate several important aspects of this rare species. They include its origins, genetic diversity, incidence of clonal duplication and the amount of 'introgression' (backcrossing of the hybrid *P. deltoides* x *P. nigra* or the cultivar *P. nigra* 'Italica' with the wild population of *P. nigra*).

TAXONOMY AND ECOLOGY

Black poplar trees in Britain belong to the subspecies *betulifolia*, otherwise known as the Atlantic race of European black poplar. They differ from subspecies *nigra* in that their young expanding leaves have hairy petioles. Black poplar used to grow in the natural floodplain forests which lined the banks of rivers in Europe, however, much of this habitat has been lost since the 17th century through such processes as urbanisation, land drainage and canalisation of rivers. Recently there has

been interest in the re-establishment of floodplain habitats, including the floodplain woodlands which contain black poplar as a key species.

Due to Europe-wide concern over the increased frequency of river flooding, consideration is now being given to the development of natural flood defences. The Natura 2000 network of nature conservation sites across Europe includes many which are located on floodplains. Although there is very little floodplain woodland in the UK today, this may begin to change with the increased recognition that such woodlands:

- have a potentially high amenity value;
- can perform a commercial forestry function;
- have the potential to help in flood management;
- help the control of diffuse pollution of water.

A number of projects and initiatives across Europe are looking at the value of floodplain forests. Of particular interest, in relation to black poplar, are the EU funded FLOBAR 1 & 2 projects that are investigating how to restore the biological function of floodplain forests by restoring the physical processes that drive them.

USES

Black poplar timber was much in demand during the 17th and 18th centuries as it has certain desirable properties. It was used for the flooring near fireplaces where its fire resistance was an advantage. The wood is shock absorbent and was the timber of choice for wagon bottoms, rifle butts, stable partitions and brake blocks. It was used for clogs, furniture and fruit boxes because of its lightweight and white colour. Poles from pollarded trees were used for thatching spars, sheep hurdles, bean poles and in the manufacture of matches. The forked trunks formed the structural support of cruck-framed buildings and branches were used as cattle fodder. It was planted because of its distinctive form to delineate parish and county boundaries. Today, it is largely grown as an amenity tree in parks, where male trees are favoured because they do not produce the seed fluff which is considered to be unsightly (Figure 1).

Figure 1 Female catkins with seed producing capsules.



The popularity of black poplar declined in the 19th century following the introduction of the faster growing hybrid between the American *P. deltoides* and the European *P. nigra* (*P. x euramericana*). Until recently, there had been very little planting of black poplar since this time and, as a result, the trees which remain today are old and are reaching the end of their lives (Figure 2).

Figure 2 A specimen of an old black poplar tree.

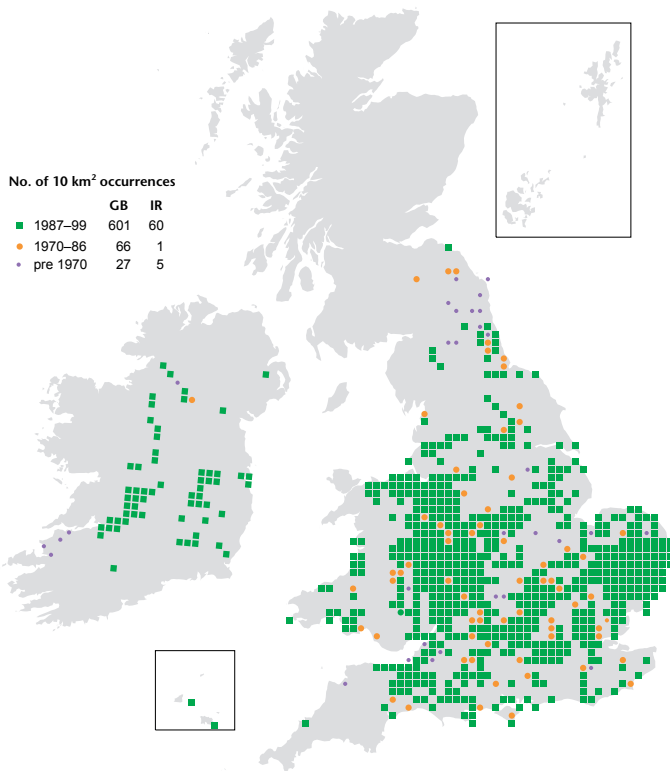


POPULATION SIZE AND DISTRIBUTION

Black poplar is one of Britain and Ireland's rarest trees. The most recent survey (Cooper *et al.*, 2002; Preston *et al.*, 2002) estimates that there are 7000 trees in England, Wales and the Republic of Ireland of which 600 are females. The majority of trees in England exist below the line drawn from the Mersey to the Wash (Figure 3) and there are areas, such as the Vale of Aylesbury, where the tree is particularly frequent with 2400 specimens having been recorded. The majority of pollarded trees are located in the Welsh Borders and in East Anglia (Cooper *et al.*, 2002). Female trees occur throughout the distribution range in England but are rare in Wales (Cooper *et al.*, 2002).

Black poplar seeds have very exacting germination requirements and the drainage of winter flooded meadows has resulted in the loss of suitable land for seedling germination and establishment. Consequently, most of the remaining trees in Britain have probably been artificially propagated from cuttings; there is currently no natural regeneration from seed.

Figure 3 Distribution map of black poplar.



Map produced by the Biological Records Centre, CEH Monks Wood, from records collected by the Botanical Society of the British Isles and others.

MORPHOLOGICAL AND MOLECULAR ANALYSIS

Hybrid identification

It is difficult to distinguish between the indigenous black poplar and the *P. x euramericana* hybrids to the extent that misidentification of hybrids may have led to the endangered status of black poplar being overlooked for a long time. Addressing this difficulty has been one of the tasks of EUFORGEN, a pan-European network developing genetic conservation strategies for forest tree species. EUFORGEN has published an identification sheet to assist with the accurate identification of black poplar but the task continues to be difficult for the inexperienced user. Modern molecular tools are also now available which allow hybrids to be identified unambiguously (Rajora, 1989, Heinze, 1997). Used independently, they have the power to identify *P. nigra*, *P. deltoides* and *P. x euramericana* and, used in combination, they can identify second generation hybrids and their backcrosses with original parents. This is a major step forward in resolving the difficulty of accurate identification. Unfortunately, because these techniques are laboratory-based and require molecular skills for them to be performed, field-based botanists are still faced with difficulties of identification.

The availability of these species-specific markers has allowed the level of backcrossing (introgression) which occurs between black poplar and *P. x euramericana* to be assessed. Both the hybrid *P. x euramericana* and the non-hybrid, fastigate *P. nigra* cv. 'Italica' (Lombardy poplar), are represented by a large number of individual trees which are based on a very restricted number of clones in Britain. The anxiety regarding these sources of introgression is related to the potential introduction of maladapted genes into the native population. More important, however, is the concern that the restricted number of genotypes represented by these exotic clones will, through interbreeding, become over-represented in the genepool of wild *P. nigra*. Such an outcome would represent a reduction in effective population size with an associated loss of diversity.

In situations where female black poplars grow in close proximity to both hybrid and non-hybrid males, no evidence of introgression from *P. x euramericana* has been detected. However, if there are no male *P. nigra* trees within pollinating distance, the female black poplars will cross with the male *P. x euramericana* in the vicinity. The introduced hybrid poplars do not therefore pose a major threat to native black poplars provided there is pollen from black poplar available. Similar studies which have examined whether *P. nigra* cv. 'Italica' pollinates British black poplar found no evidence of crossing. This is thought to be because the Lombardy poplar, being from a more southerly European origin, flowers much earlier than the indigenous black poplars. Furthermore, in the unlikely case that introgressed seeds were produced in Britain, they should not pose a problem because of the absence of any suitable sites for natural seedling germination and establishment to occur.

Glacial origins

Knowledge of the postglacial history of a rare species can greatly assist in the development of appropriate conservation strategies. Molecular markers, based on DNA found in chloroplasts (the parts of green plant cells which carry out most of the processes associated with photosynthesis also contain small quantities of DNA) are the most appropriate tools for this type of study. A recent survey of chloroplast DNA variation in black poplar in Europe showed that samples from eastern Europe differed from those growing in the west and this suggests that at least two postglacial lineages exist for this species (Cottrell *et al.*, in press). The majority of samples from England and Wales had the chloroplast DNA pattern which is typical of the lineage which colonised from southeastern Europe after the last glacial period. However, there was also some material from the southwestern, Spanish lineage present in Britain.

Genetic diversity

Molecular markers indicate that the genetic diversity of black poplar in Europe is largest in southern countries (France, Italy and Spain) and lowest in Britain (Storme *et al.*, 2004). Several factors may account for this, for example the topography of the country and the variety of climate and soil characteristics, the number of different river catchments as well as the (human) population density. In France, Italy and Spain, many rivers remain uncanalised, and this is likely to promote the survival of natural populations of black poplar. The river systems in Belgium, Germany and the Netherlands are partly connected, facilitating gene flow and leading to a higher similarity among populations. Genetic diversity in black poplar is not structured according to absolute geographical distance, since dispersal may be hindered by topography. In France, for example, the Loire and the Rhône are close to each other, but mountain ranges separate the populations which occur along the two rivers. It must, however, be borne in mind that the molecular markers used in this type of study are neutral in effect and do not provide an insight into processes related to local adaptation.

Clonal duplication

Poplar readily propagates from detached shoots and this process occurs both naturally and through human activity. It has been suspected for a long time that much of the British population is likely to be clonal and the research with molecular markers has confirmed this. An analysis of the genetic diversity captured in clone banks (clonal archives) located in several European countries showed that those in France, Austria, Spain and Italy contain the highest number of unique clones (Storme *et al.*, 2004). The British clone banks show the lowest diversity, followed by those in Belgium and the Netherlands. Cottrell *et al.* (1997) showed that 36 accessions taken from a Forestry Commission bank contained many duplicated clones and only 17 separate genotypes. Only two female genotypes were identified. A later study, based on more reliable markers, found 20 clones in a sample size of 88 non-hybrid trees (Cottrell *et al.*, 2002).

Several different marker systems are available for clonal identification and there is a strong interest in studying local populations. This combination tends to generate small projects which aim to identify the number of individual clones present in a small number of trees from restricted areas using one of a range of marker systems. Valuable though such information is at a local level, it would be advantageous for future studies to adopt a more

integrated approach based on a single marker system. Such co-ordination would allow the results of separate, local studies to be united to provide an overview of the degree of clonal duplication present throughout Britain. The system of choice is likely to be microsatellites as this method is the most robust when used across laboratories.

Conservation implications

General guidelines for conservation of black poplar in Europe have recently been published (Lefèvre *et al.* 2001, 2002).

Ex situ conservation

The terms '*ex situ*' and '*in situ*' are used here with the special meaning of places where a species cannot or can reproduce sexually, respectively.

Since the rarity of the species came to light following the Milne-Redhead survey started in the 1970s, (Milne-Redhead, 1990) there have been renewed efforts to conserve Britain's rarest native tree species. Three clone banks were set up by the Forestry Commission at Talybont (South Wales), Downham Market (Norfolk) and Fineshade (Northants). Molecular analysis of the accessions from these clone banks show considerable clonal duplication. Moreover, the original collections were not representative of the whole British population. Some areas such as the Usk valley are over-represented, whereas others, such as Cheshire and Leicestershire, were not sampled. Now that an up-to-date inventory of the species is available, it should be possible to redesign and improve these archives, and base them on a rational and systematic sampling procedure. Molecular markers could be applied to all new archived samples to prevent clonal duplication.

Local authorities are actively encouraging the planting of black poplar as part of their local biodiversity action plans. For example, 2000 black poplars have been planted in the Vale of Aylesbury in the last five years (Davies, 2002). It is hoped that such praiseworthy schemes can make increasing use of better information and improved clone banks to establish diverse clonal mixtures. Molecular studies directed at clone identification show that man has moved genotypes around the country a great deal, with the same clones occurring large distances apart. There is therefore little reason to restrict new plantings to material of local stock as the natural pattern of distribution has already been disrupted. It is much more important to plant a range of genotypes. In view of the fact that trees growing in the same locality often consist of

only one or two clones, it may be necessary to obtain cuttings from a relatively wide geographic range in order to include sufficient genetic diversity.

Many local authorities have established local biodiversity action plans in which they detail their strategy for the conservation of black poplars in their district. This involves such measures as the development of accurate inventories, application of tree preservation orders, raising public awareness, pollarding and tree surgery policies to extend the normal life span of the trees and planting of new woodlands which contain an improved ratio of male to female clones. National Biodiversity Action Plans for black poplar would assist in ensuring a unified approach to the conservation of this species.

***In situ* conservation**

Black poplar can only respond to changing environmental conditions if it is given the opportunity to reproduce sexually. This is not possible at present because of the lack of suitable sites for seed germination and seedling establishment. A determined effort to conserve this species in Britain would therefore benefit from re-establishment of natural floodplain forests which would offer the opportunity to practice *in situ* conservation of black poplar. For successful germination and establishment, black poplar seedlings require sites where there are recent deposits of sand and shingle, free of vegetation and with optimal water/soil conditions. Such conditions do not exist in mature stands and therefore regeneration is restricted to new sites where there are no mature trees. Under such conditions the species could re-establish with its naturally associated ground flora and fauna. The natural habitat needs to be highly dynamic in order to provide a constant supply of new sites for colonisation where conditions would sustain germination and establishment of the next generation. Conservationists need to work together with river managers and flood engineers if *in situ* regeneration of black poplar is to become a reality.

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