Forestry Commission

Research Note

Conservation of black poplar: insights from a DNA fingerprinting approach

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Black poplar is Great Britain's rarest native hardwood and there is considerable interest in conserving the genetic diversity present in the remaining population. However, multiplication by vegetative propagation has led to issues in identifying and selecting genetically diverse native planting material. The ability to use DNA markers to identify poplars at the level of the individual enables conservation efforts to be directed to deploy and maintain the current genetic diversity. This Research Note summarises the results from the DNA fingerprinting of 811 non-hybrid black poplars which identified a total of 87 clones. The results split the British black poplar clones into two groups, a small group which contains individuals with a large number of rare alleles (rare alleles are DNA variants occurring at a low frequency in a population) and a larger group containing less diversity and the more common alleles. In terms of their geographical distribution, some clones had a restricted distribution whereas others were widespread. The results highlight that the British native black poplar population has clearly been influenced by human intervention and, due to a number of historical factors it rarely acts as a naturally sexually regenerating species. Black poplar needs to regenerate sexually if it is to respond to environmental changes and management should aim to provide the conditions required for seed germination. DNA-based clonal identification can be utilised to ensure the current genetic diversity in the British population is protected into the future.

Introduction

This Research Note provides an update to the Forestry Commission Information Note concerning the conservation of black poplar (Cottrell, 2004), which reported on some of the early genetic diversity studies of black poplar in Great Britain and Europe, and highlighted the utility of developing a robust DNA fingerprinting system that would provide an overview of the degree of clonal duplication present in Great Britain. Such a system, using microsatellite DNA markers, has now been used for over a decade to study the diversity in British black poplars, adding considerably to the body of knowledge regarding the distribution, gender and genetic diversity of the black poplar population in Great Britain. This Note also considers some of the factors, both natural and human-mediated, that may have influenced the current situation.

Taxonomy, ecology and threats

The European black poplar (*Populus nigra*) is a pioneer species of riparian ecosystems whose geographic range extends from south and central Europe to central Asia and northern Africa (Zsuffa, 1974). Black poplar is a dioecious species, meaning there are separate male and female trees. It is wind-pollinated and its seeds are similarly wind-dispersed. Seed viability is short-lived and successful germination requires an availability of winter-flooded meadows.

Black poplars in Great Britain belong to the subspecies *betulifolia*, which is known as the Atlantic race of European black poplar, due to its distribution being confined to Great Britain and northern France (Bean, 1976). The main distinguishing feature of the *P. nigra* subspecies *betulifolia* is the presence of hairy petioles on the young expanding leaves (Figure 1). The species is considered to be the rarest native hardwood in Britain with approximately 7000 surviving trees (Milne-Redhead, 1990). A high proportion of the remaining British black poplars occur in Lancashire, Manchester, the Welsh borders, East Anglia and Buckinghamshire – especially the Vale of Aylesbury (Cooper, 2006). There are also many trees along the Thames Valley with individuals recorded in 21 of the London boroughs (www.gigl.org.uk/london-bap-priority-species).

The species was once a common sight across southern Britain. Naturally fire-resistant and resilient, the timber was used in buildings, carts and wagons, scaffolding, farm equipment and matches. There are several factors which have led to the decline of this iconic species. The drainage and management of waterways since the 17th century means that the specific substrate requirements for seed germination have, in the main, been lost in Great Britain. However, it is important to note that black poplar also reproduces readily by vegetative means, both naturally, for instance when detached branches are carried downstream in flooded rivers, and also through human-mediated planting of rooted cuttings.

Figure 1 Hairy petioles in young leaves characteristic of native black poplar.

In addition to the wide-scale loss of suitable germination sites, a further threat to British black poplar arrived in the 19th century with the introduction of *Populus* x *euramericana*, the product of the hybrid cross between the American black poplar (*Populus deltoides*) and the European black poplar. Since its introduction, *P.* x *euramericana* has often been planted in preference to native black poplar because of its superior growth rate. The presence of these hybrids in the landscape potentially confounds conservation efforts for native poplars because distinguishing hybrid trees by their physical characteristics (the phenotype) is not always straightforward. Another potentially confounding issue is the crossing of native black poplars with the non-native Lombardy black poplar, *Populus nigra* var. *italica*, which has also been widely planted in Great Britain due to its visually striking upright habit. There are also instances of other non-native black poplar clones being introduced into Great Britain from the continent (Jobling, 1990). A third reason for the decline of the black poplar in Great Britain is that male clones were preferred because female trees produced large amounts of white seed fluff, which was considered unsightly and undesirable. Consequently, this has led to a situation where the sex ratio of the surviving trees in the landscape is heavily male-biased.

Faced with this triple threat of habitat loss, potential contaminating pollen sources and gender imbalance, a cohesive strategy to categorise and manage the genetic diversity in the remaining native British black poplar population is required.

Molecular approaches

In 2007, an initiative was launched in which the Forestry Commission invited local authorities and Wildlife Trusts to

submit material from black poplar trees in their local areas to Forest Research, first to be screened for hybrid status using a molecular test (Heinze, 1997) which identifies first-generation hybrids, then genotyped with molecular markers (often referred to as DNA fingerprinting) allowing the identification of different clones in a population (the term clone is used to indicate a genetically unique individual). This was with the objective of listing the different clones on the Forest Reproductive Material (FRM) National Register of Basic Material (https://www.forestry.gov.uk/frm), which is the source of information on approved planting stock in Great Britain. By having the clones categorised and listed on the National Register, foresters and conservation groups would then have a shared frame of reference when discussing future black poplar plantings. Once different clones had been identified by the DNA fingerprinting work, this would provide a platform to facilitate setting up clone banks, which could be managed to contain as much genetic diversity as possible. This was with the ultimate aim of having material available for vegetative propagation of clones that were not only local to any particular planting site, but also captured as high a proportion of available genetic diversity as possible (Figure 2). A degree of balance between known male and female trees could also be addressed. The list of different clones identified by Forest Research continues to be available on the FRM National Register (www.forestry.gov.uk/frm).

A'Hara, Samuel and Cottrell (2009) reported the findings of this initiative and highlighted some interesting and stark results. They used seven microsatellite markers to DNA fingerprint 243 British black poplar trees. Microsatellites are highly variable DNA markers that are very suitable for distinguishing individuals in a population. They identified that only 15 different clones were present among this tranche of 243 samples. The limited number of clones recorded highlighted the need to try and conserve the standing diversity that exists in the species, and further explore the existing population with the aim of identifying additional clones. Funding for tree conservation is typically scarce, and with the realisation that some clones are present in relative abundance whereas others have only one known representative, this work highlighted a need to identify and focus conservation efforts on the rarer clones which are in much greater danger of being lost from the gene pool.

Figure 2 Clone bank with trees representing a range of unique black poplar clones.

The publication of this article (A'Hara, Samuel and Cottrell, 2009) proved to be something of a catalyst for poplar enthusiasts, and subsequently a large number of black poplar samples were submitted to Forest Research for DNA fingerprinting through various Wildlife Trusts, conservation bodies and local authority councils, and also by interested members of the public keen to assist with conservation of their local flora.

Methods

A total of 989 trees have now been DNA fingerprinted using the same seven microsatellite markers as previously reported by A'Hara, Samuel and Cottrell (2009). Twenty of these 989 samples were found to contain alleles that indicated they were likely to be offspring of the Lombardy poplar (Figure 3). A further 66 trees, comprising 31 clones, were confirmed as

Figure 3 Row of Lombardy poplars exhibiting upright habit.

P. x *euramericana* hybrids with the Heinze (1997) molecular test. These 86 samples were genotyped but excluded from the analysis. A further 112 trees submitted for testing did not have an exact grid reference location, and so these are not reported here. The analysis is therefore based on 811 genotyped trees, each accompanied by a precise grid reference location, and each regarded as a non-hybrid black poplar and not the product of a cross with a Lombardy poplar. While recognising that this does not represent a structured sampling, this sample set nonetheless allowed exploration in more detail of the nature of the surroundings in which black poplar grows in Great Britain, thus enabling a better understanding of the underlying reasons for the distribution patterns exhibited by particular clones and helping infer why little sexual reproduction occurs in Great Britain. The web-based grid reference look-up facility in Herbaria United (herbariaunited.org) was used to determine the vice-county in which each sample was located. In addition, the website's map was utilised to examine in greater detail the immediate locale of each sample (a 100 m^2 area centred on each tree) and search for landscape features, for instance whether the tree was situated near a visible source of water or a public park, and also whether it was located in an urban or a rural setting. This information might help paint a picture of those landscape features which most influenced the distribution of British black poplar clones. For comparison with the genetic diversity in the British population, a panel of 15 trees from mainland Europe (eight from France, five from Germany and two from Holland); the 31 hybrid clones identified in the course of the study and three *P. deltoides* samples were also fingerprinted.

Results

Clonal composition

A total of 87 clones were identified in the 811 trees analysed; their genetic fingerprints are presented in Table 1. Notably, the contribution of individual clones to the total sample set was imbalanced; of the 87 identified clones, 19 occurred four or more times in the sample set (Table 2). The most common clone, clone 28, occurred in 169 (20%) samples. The five most frequent clones made up over 66% of the trees in the total sample set. The remaining 34% consisted of samples which belonged to 68 clones that were each represented by three or fewer trees in the total sample (Table 3). With the exception of two clones, these rare clones each had a distribution that was restricted to a single vice-county. Only rare clones 29 and 58 occurred in two vice-counties, with clone 29 having samples from Cardiganshire in Wales and Mid-West Yorkshire, and clone 58 occurring in Middlesex and North Essex. A total of 55 clones were only represented by a single tree in the dataset.

Allele frequency and distribution

The allele frequency (the relative frequency of an allele at a particular locus in a population), based on a single representative of each clone, is shown in Table 4, and the genetic similarity of clones is illustrated in Figure 4. The number of alleles per locus ranges from five to 10 and each locus has several alleles present in very low frequencies. These rare (low frequency) alleles are not randomly distributed across all the clones but instead often aggregate across several loci in particular clones. The following clones each have three or more rare alleles in their genetic fingerprint: 25 (85 trees), 27 (two trees), 40 (four trees), 45 (six trees), 106 (one tree), 107 (one tree), 108 (seven trees), 113 (one tree), 114 (one tree) and 115 (one tree). Clone 25 is represented by 85 trees in this sample whereas the other nine clones which are rich in rare alleles are each represented by between one and seven trees. The majority of the trees rich in rare alleles are located in East Anglia, Surrey, Buckinghamshire and Gloucestershire. This aggregation of rare alleles in particular clones suggests that these genotypes may have a different origin from the clones which contain only the common alleles. The observation that some clones are particularly rich in rare alleles suggests that these trees may have arrived relatively recently and have not introgressed (the spread of alleles from one gene pool into the gene pool of another through mating) into the native British population due to the currently low frequency of sexual reproduction. If these clones with rare alleles had arrived at the same time as the rest of the native material, then the rare alleles would be expected to exhibit a much more random distribution across the clones than is seen from the results. The presence of nine clones which have only one or two rare alleles in their fingerprint may indicate that a small amount of introgression between the two origins of British black poplar has begun to occur.

Clone 40 is particularly rich in rare alleles with nine of its 14 alleles falling into this category. It has the same genetic fingerprint as a commercial non-hybrid black poplar clone known as Vereecken, a sample of which was obtained from a British commercial nursery and fingerprinted. This fast-growing, narrow-crowned clone is widely grown in Holland and is thought to have originated in Belgium. It was imported by the Forestry Commission into Great Britain in 1950 (Jobling, 1990). This clone, along with clones 108 and 113, shows the least genetic similarity to the rest of the samples as shown in the dendrogram (Figure 4), which illustrates the relationships between the samples based on the genetic data. The next most different group consists of clones 106, 25, 117, 96, 92, 99, 95, 115 and 114. Of the nine clones in this group, the seven which are each represented by a single tree in the dataset all occur in the Cotswold Water Park in East Gloucestershire. The fact that these cluster closely together in the dendrogram suggests that

Table 1 Genotype of each individual clone in the 811 sampled trees according to seven microsatellite markers containing two alleles per locus. The figures in each column represent the number of base pairs of the amplified fragment for each of the loci. There are two alleles per locus in a diploid organism. Rare alleles are highlighted in dark grey.

FRM Clone number	PMS9		PMS12		PMS14		PMS16		PMS18		PMS20		PMGC14		Total number of trees
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
23	254	248	172	172	241	229	148	148	241	232	234	228	210	204	121
25	260	254	172	166	247	241	148	142	232	226	246	234	204	201	85
27	262	252	164	164	280	247	148	148	241	232	228	222	225	210	2
28	266	248	172	164	247	241	148	148	232	232	234	234	210	210	169
29	266	248	172	164	253	241	148	148	232	229	228	222	210	210	2
32	266	248	172	164	253	241	148	148	232	232	234	222	210	204	89
33	266	254	172	164	247	241	148	148	232	229	0	0	210	210	1
34	266	254	172	164	253	229	148	148	241	229	234	222	210	210	75
35	266	254	172	164	253	241	148	148	232	229	234	228	210	204	41
36	266	254	172	164	253	241	148	148	241	232	234	228	210	210	1
38	296	248	172	164	253	229	148	148	241	229	234	222	210	204	1
40	296	252	166	164	280	253	154	142	241	223	246	234	225	207	4
41	296	254	172	164	247	241	148	148	241	229	234	222	210	204	1
43	296	254	172	164	253	241	148	148	232	232	234	234	210	210	30
44	254	248	172	172	253	229	148	148	232	229	234	234	210	210	2
45	254	254	164	164	280	253	148	139	232	232	234	222	210	207	6
46	266	254	172	164	253	241	148	148	241	232	234	222	210	204	2
47	296	248	172	164	247	241	148	148	232	232	234	222	210	204	11
48	296	254	172	164	253	229	148	148	232	232	234	222	210	210	1
49	296	254	172	164	253	229	148	148	241	229	234	228	210	204	43
54	266	248	172	164	241	229	148	148	232	229	234	222	210	204	2
55	266	254	172	164	253	241	148	148	232	229	228	222	210	204	7
56	266	266	164	164	253	247	148	148	232	232	234	234	210	210	3
57	254	248	172	164	253	253	148	148	232	232	234	228	204	204	5
58	266	254	172	164	247	241	148	148	232	232	234	228	210	210	3
59	266	266	164	164	241	241	148	148	232	232	234	234	210	210	4
60	248	248	172	172	241	241	148	148	232	232	234	222	210	204	2
61	266	254	172	164	256	241	148	148	241	232	234	228	210	210	2
62	296	248	172	164	253	229	148	142	232	229	234	222	210	210	3
63	248	248	172	172	253	247	148	148	232	232	234	222	210	210	1
64	254	248	172	164	241	229	148	148	232	229	234	222	210	210	1
65	254	248	172	164	241	229	148	148	241	241	234	234	210	204	1
66	254	254	172	172	253	241	148	148	232	229	234	234	210	204	1
67	260	248	172	172	253	247	148	148	232	232	234	222	204	204	1
68	266	248	172	164	253	241	148	148	232	229	234	222	210	204	1
69	266	248	172	164	253	241	148	148	241	232	234	222	210	204	1
70	266	248	172	164	253	247	148	148	232	232	234	222	210	204	1
71	266	248	164	164	241	229	148	148	232	229	234	222	210	210	1
72	266	248	164	164	241	241	148	148	232	232	234	222	210	210	2
73	266	248	172	172	253	247	148	148	232	232	234	222	210	210	1
74	266	248	164	164	241	241	148	148	232	232	234	234	210	204	1
75	266	248	164	164	253	241	148	148	232	232	234	234	210	210	1
76	266	248	164	164	253	241	148	148	241	232	234	234	210	210	1

Table 1 (continued)

FRM Clone number	PMS9		PMS12		PMS14		PMS16		PMS18		PMS20		PMGC14		Total number of trees
77	266	254	172	164	241	229	148	148	232	229	222	222	210	210	1
78	266	254	172	164	253	241	148	148	232	232	234	222	210	204	4
79	266	254	172	164	253	253	148	148	241	232	234	222	210	210	1
80	266	254	164	164	241	229	148	148	232	229	234	234	210	210	1
81	266	266	164	164	253	247	148	148	232	226	234	222	207	204	1
82	266	266	164	164	256	241	148	148	232	232	234	222	210	210	1
83	266	266	172	164	253	253	148	148	241	229	234	222	210	210	1
84	266	266	172	164	253	229	148	148	232	232	234	228	210	210	1
85	266	266	164	164	241	229	148	148	241	232	234	234	210	204	2
86	248	248	172	164	241	241	148	148	232	232	234	222	210	204	2
87	266	266	164	164	247	241	148	148	232	232	234	234	210	204	2
88	266	266	164	164	253	241	148	148	232	229	234	234	210	204	1
89	266	266	172	172	253	247	148	148	232	232	234	234	210	204	1
90	266	266	172	164	253	253	148	148	232	229	234	234	210	210	1
91	296	254	172	172	253	253	148	148	232	229	228	222	210	204	1
92	296	254	172	164	229	229	148	148	241	229	234	m	204	204	1
93	296	248	172	164	253	229	148	148	232	229	228	228	210	204	1
94	296	248	172	172	247	229	148	148	241	232	234	228	210	204	1
95	296	254	172	164	253	247	148	148	229	226	228	228	204	204	1
96	296	254	164	164	241	229	148	148	241	229	234	228	204	204	1
97	266	248	172	164	241	229	148	148	232	229	234	222	210	210	1
99	266	254	172	164	241	229	148	148	241	229	228	228	210	204	1
100	266	254	172	164	253	229	148	148	241	232	228	222	210	210	1
101	254	248	172	172	241	229	148	148	241	241	234	228	210	210	1
102	254	254	172	172	229	229	148	148	241	232	234	234	210	204	1
103	248	248	172	164	247	241	148	148	232	232	234	234	210	204	1
104	266	248	172	164	241	241	148	148	232	232	234	234	210	210	3
105	266	248	172	164	253	241	148	148	232	232	234	234	210	210	4
106	254	252	164	164	253	250	148	148	226	226	246	246	207	201	1
107	266	252	180	172	256	241	148	148	244	232	234	228	210	201	1
108	296	296	170	164	265	232	136	136	232	232	234	222	207	204	7
109	266	266	172	164	241	238	148	148	232	232	234	222	210	204	1
110	266	266	164	164	253	253	148	148	232	229	234	234	210	204	2
111	266	254	180	172	253	241	148	148	232	232	234	228	210	201	4
112	266	248	172	164	247	241	148	148	232	232	234	222	210	210	14
113	252	248	172	166	280	241	154	148	241	241	246	228	225	210	1
114	254	254	172	166	253	229	148	136	229	226	252	228	210	201	1
115	254	254	172	164	229	229	148	136	229	226	228	228	204	201	1
116	296	248	172	164	253	241	148	148	241	229	234	234	204	204	1
117	296	254	164	164	241	229	148	148	229	229	228	228	204	204	1
118	296	266	172	164	241	229	148	148	232	229	234	234	210	210	1
119	296	266	164	164	280	253	148	142	241	229	234	222	210	204	1
120	296	248	172	172	253	229	148	148	241	232	228	228	210	210	1
121	254	248	172	172	229	229	148	148	241	229	234	234	210	204	1

Table 2 Number of trees per vice-county for clones which have more than four trees per clone in the sample set.

VC number	VC name	Clone 23	Clone 25	Clone 28	Clone 32	Clone 34	Clone 35	Clone 40	Clone 43	Clone 45	Clone 47	Clone 49	Clone 55	Clone 57	Clone 59	Clone 78	Clone 105	Clone 108	Clone 111	Clone 112
VC5	S. Somerset	6		1																
VC6	N. Somerset	1		15																
VC7	N. Wiltshire	2		1								3								
VC11	S. Hampshire			2	1	1														
VC12	N. Hampshire	1		1									7							
VC13	West Sussex			4													1			
VC16	W. Kent			4	2			1												
VC17	Surrey		6	31	16		2	2						5	4	4	3	4		
VC18	S. Essex	2	2	32	5	1														
VC19	N. Essex	4	12	14	5	4					7							2		
VC20	Hertfordshire			1			30				2									
VC21	Middlesex	1		24	1	2		1										1	4	
VC22	Berkshire																			
VC23	Oxfordshire																			
VC24	Buckinghamshire						9													
VC25	E. Suffolk	8	23	7	32	2				5										
VC26	W. Suffolk	12	36	2	4					1	2									
VC27	E. Norfolk		1																	
VC33	E. Gloucestershire	3		4	1	1			1			40								
VC34	W. Gloucestershire	1																		
VC35	Monmouthshire																			
VC36	Herefordshire																			
VC37	Worcestershire					1														
VC39	Staffordshire					1														
VC40	Shropshire	1				5			4											
VC46	Cardiganshire																			
VC47	Montgomeryshire					1														
VC48	Merionethshire	5																		
VC50	Denbighshire	66				3														14
VC51	Flintshire	6				6			1											
VC53	S. Lincolnshire				3															
VC54	N. Lincolnshire				2															
VC56	Nottinghamshire		2		4															
VC58	Cheshire	1		12	1	38			23											
VC59	South Lancashire	1		5		4			1											
VC62	N.E. Yorkshire			2	5															
VC64	Mid-W. Yorkshire																			
VC65	N.W. Yorkshire			1																
VC66	County Durham			5	2	1														
VC67	S. Northumberland		1		1															
VC69	Westmorland					1														
VC70	Cumberland					3														
VC83	Midlothian		2	1	1															
VCH18	Offaly				3															
Grand total =723	Total	121	85	169	89	75	41	4	30	6	11	43	7	5	4	4	4	7	4	14

VC – Vice-county, VCH – Vice-County Hibernia (vice-county in Ireland)

Table 3 Number of trees per vice-county for clones which have more than four trees per clone in the sample set.

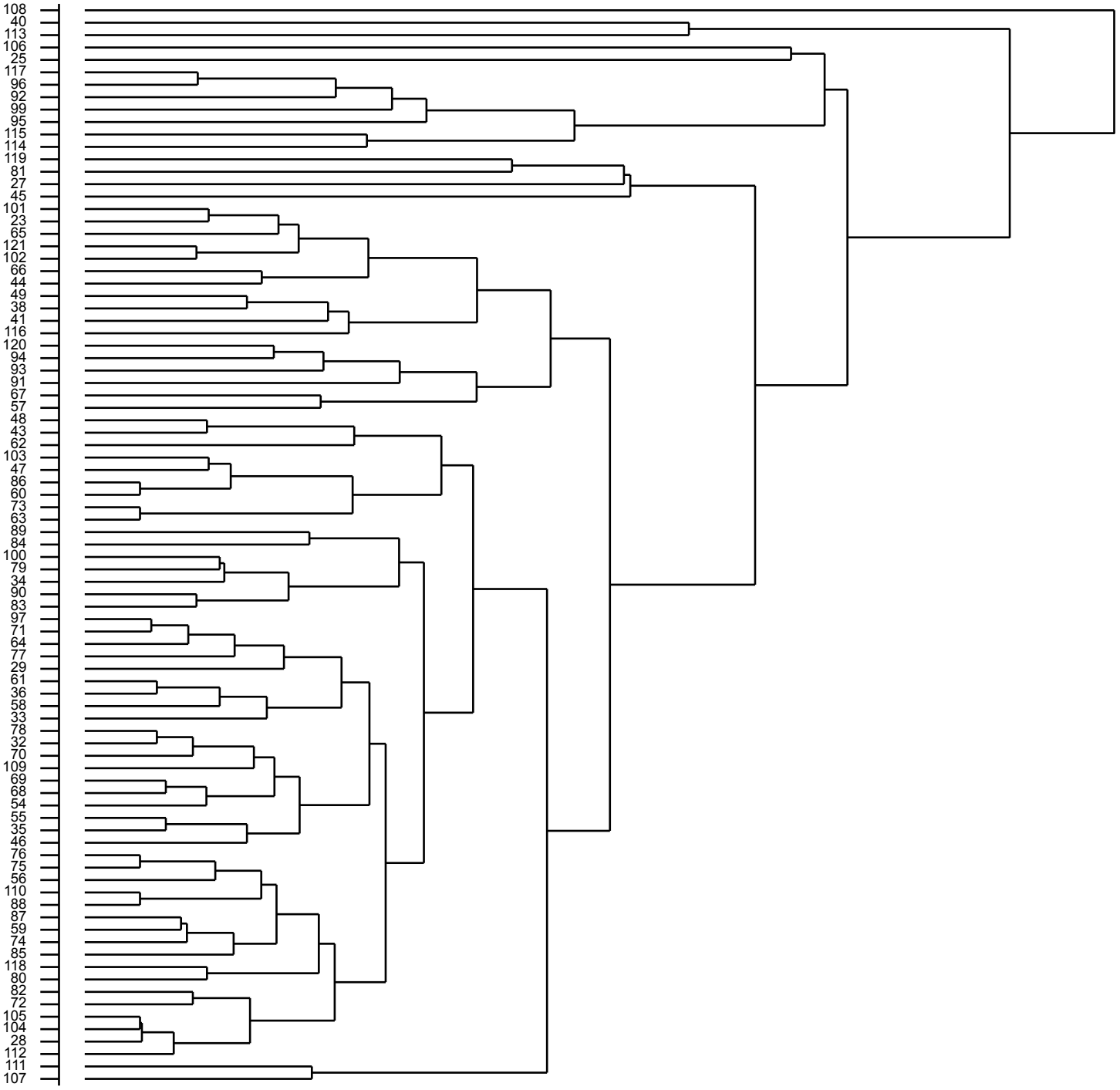
VC	VC name	FRM Clone number (and the number of trees of each clone for those clones present three times or less in the sample set)	Total number of trees	Number of clones
VC5	S. Somerset	88 (1)	1	1
VC6	N. Somerset			
VC7	N. Wiltshire	62 (3)	3	1
VC11	S. Hampshire			
VC12	N. Hampshire			
VC13	W. Sussex			
VC16	W. Kent			
VC17	Surrey	56 (3), 60 (2), 63 (1), 64 (1), 70 (1), 71 (1), 72 (2), 73 (1), 74 (1), 75 (1), 76 (1), 80 (1), 81 (1), 82 (1), 83 (1), 84 (1), 85 (2), 86 (2), 89 (1), 97 (1), 103 (1), 104 (3), 107 (1), 109 (1), 110 (2)	34	25
VC18	S. Essex	69 (1), 106 (1)	2	2
VC19	N. Essex	58 (2)	2	1
VC20	Hertfordshire			
VC21	Middlesex	36 (1), 58 (1), 61 (2), 67 (1) 87 (2)	7	5
VC22	Berkshire	41 (1)	1	1
VC23	Oxfordshire	100 (1)	1	1
VC24	Buckinghamshire	27 (2)	2	1
VC25	E. Suffolk	44 (2), 46 (2), 54 (2), 68 (1)	7	4
VC26	W. Suffolk	77 (1), 79 (1)	2	2
VC27	E. Norfolk	90 (1)	1	1
VC33	E. Gloucestershire	65 (1), 66 (1), 91 (1), 92 (1),93 (1), 94 (1), 95 (1), 96 (1), 99 (1), 101 (1), 102 (1), 113 (1), 114 (1), 115 (1), 116 (1), 117 (1), 118 (1), 119 (1), 120 (1), 121 (1)	20	20
VC34	W. Gloucestershire			
VC35	Monmouthshire	33 (1)	1	1
VC36	Herefordshire	38 (1)	1	1
VC37	Worcestershire			
VC39	Staffordshire			
VC40	Shropshire			
VC46	Cardiganshire	29 (1)	1	1
VC47	Montgomeryshire			
VC48	Merionethshire			
VC50	Denbighshire			
VC51	Flintshire			
VC53	S. Lincolnshire			
VC54	N. Lincolnshire			
VC56	Nottinghamshire			
VC58	Cheshire	48 (1)	1	1
VC59	S. Lancashire			
VC62	N.E. Yorkshire			
VC64	Mid-W. Yorkshire	29 (1)	1	1
VC65	N.W. Yorkshire			
VC66	CountyDurham			
VC67	S. Northumberland			
VC69	Westmorland			
VC70	Cumberland			
VC83	Midlothian			
VCH18	Offaly			
	Total		88	

VC – Vice-county, VCH – Vice-County Hibernia (vice-county in Ireland)

Table 4 Allele frequency for seven microsatellite markers. Alleles are ordered according to their size in terms of base pairs (bp). Rare alleles are highlighted in dark grey for the British sample set.

PMS9		PMS12		PMS14		PMS16		PMS18		PMS20		PMGC14	
Allele size (bp)	Frequency	Allele size (bp)	Frequency	Allele size (bp)	Frequency	Allele size (bp)	Frequency	Allele size (bp)	Frequency	Allele size (bp)	Frequency	Allele size (bp)	Frequency
87 non-hybrid British black poplar clones present in the sample set													
248	0.2356	164	0.5057	229	0.1954	136	0.0230	223	0.0057	m	0.0172	201	0.0345
252	0.0287	166	0.0230	232	0.0057	139	0.0057	226	0.0402	222	0.2184	204	0.2931
254	0.2471	170	0.0057	238	0.0057	142	0.0230	229	0.2069	228	0.1839	207	0.0287
260	0.0115	172	0.4540	241	0.3276	148	0.9368	232	0.5632	234	0.5460	210	0.6264
262	0.0057	180	0.0115	247	0.1092	154	0.0115	241	0.1782	246	0.0287	225	0.0172
266	0.3506			250	0.0057			244	0.0057	252	0.0057		
296	0.1207			253	0.2989								
				256	0.0172								
				265	0.0057								
				280	0.0287								
31 hybrid black poplars present in the sample set													
236	0.0968	156	0.0167	235	0.0161	127	0.0179	217	0.3793	210	0.0161	174	0.0164
242	0.0323	160	0.1833	238	0.0161	133	0.2321	220	0.0172	219	0.0161	182	0.0164
248	0.1613	164	0.2500	241	0.0807	139	0.2857	223	0.0172	222	0.5968	189	0.0328
252	0.0968	168	0.1000	247	0.3710	145	0.0179	229	0.0690	226	0.0161	192	0.1639
254	0.0323	169	0.0333	250	0.0161	148	0.4107	232	0.4655	228	0.0161	195	0.1639
258	0.0807	170	0.0833	253	0.0323	151	0.0179	241	0.0517	234	0.2903	198	0.0656
260	0.3548	172	0.0833	256	0.3387	154	0.0179			236	0.0161	204	0.1312
266	0.1452	174	0.0333	265	0.0161					246	0.0161	207	0.1639
		178	0.1500	271	0.0323					252	0.0161	210	0.1475
		180	0.0333	274	0.0323							213	0.0656
		195	0.0167	280	0.0484							225	0.0328
		204	0.0167										
15 black poplars from mainland Europe													
244	0.033	162	0.036	229	0.16	136	0.033	223	0.033	222	0.23	201	0.100
248	0.166	164	0.500	232	0.05	142	0.330	226	0.23	228	0.130	204	0.023
252	0.330	166	0.071	238	0.05	148	0.430	232	0.36	234	0.400	207	0.206
254	0.160	168	0.071	247	0.22	154	0.200	241	0.23	240	0.100	210	0.260
258	0.033	172	0.071	250	0.05			244	0.10	246	0.066	213	0.200
262	0.066	174	0.036	253	0.05			256	0.03	252	0.066	216	0.033
282	0.033	180	0.210	256	0.05							225	0.130
284	0.066			265	0.05								
294	0.066			280	0.22								
296	0.033			283	0.05								

Figure 4 Dendrogram showing the genetic relatedness of the black poplar clones.



some of the trees in this park are closely related and may be either the products of sexual reproduction, or possibly are from the same source population.

The average number of alleles per microsatellite marker was greatest for the hybrid clones (9.1), followed by the European samples (7.1), and then the British samples with the lowest average (6.1). Thus, genetic diversity in the 15 black poplar samples from mainland Europe is greater than is found in the 87 clones sampled across Great Britain. In the relatively small sample set of 15 European trees, 24 of the rare British alleles were found, with only three of the rare alleles not present. In addition, looking at the allele size and frequencies in the 31 hybrid clones, eight of the rare alleles in the British sample set were not found in the known first generation hybrids. One of the rare British alleles (PMS16 allele size of 139 base pairs) was present in a high proportion in the three *P. deltoides* that were sampled, but this was the only case.

Therefore, the presence of the vast majority of the rare British alleles in this relatively small number of mainland Europe *P. nigra* samples, and the absence of a large number of rare alleles

in the panel of hybrids, makes it unnecessary to consider *P.* x *euramericana* in order to account for their presence.

Gender balance

Perhaps surprisingly, despite molecular-based gender tests now being available for other poplar species, there is not yet such a test available for black poplar. Attempts to apply the sex marker developed for *Populus tremuloides* to identify the sex of *P. nigra* trees have been unsuccessful (Pakull *et al*., 2011). Any information provided on the gender of the trees was recorded when they were submitted for analysis.

Many of the gender-unknown trees were members of frequently occurring clones which meant that although the sex of the tree itself was not known, the gender of the clone to which it belonged was known and considered robust. On the basis of this, it was therefore possible to infer the gender of the majority of trees in the sample set. Of the 811 trees analysed, 554 (68%) were males, 218 (27%) were females, and the gender of 39 (5%) was unknown (Table 5). The ratio of known male-to-female trees was therefore 2.5:1. Four of the five most common clones were male. Of the 44 vice-counties represented in the sample set there were 22 vice-counties which contained only a single sex of tree, thereby making it unlikely that sexually derived seedlings could be produced in these vice-counties. The majority of these single-sexed vice-counties contained only male trees. In an extreme example, the 69 trees of known gender in Denbighshire in Wales were all male. Two vice-counties, East Gloucestershire and Surrey, were particularly rich in clones of both genders, with the former containing 15 male and 10 female clones, and the latter having 16 male and 13 female clones in the sample.

Distribution and association with landscape features

Of the 811 analysed trees, 808 came from 43 vice-counties in England, Scotland and Wales, and three originated from a single vice-county in southern Ireland and belonged to a single clone which was not unique to Ireland. This was clone 32 which was present in 86 other samples from locations in Great Britain.

In total, 490 trees occurred in a rural setting, with a further 41 located in villages away from cities or towns (Table 6). The remainder occurred in cities (167) and towns (113). The frequency of particular clones was notably different in the rural/village compared to the town/city locations. Clones 28 and 32 were much more common in urban settings, making up 41% and 31% of the urban samples, respectively (Figure 5). This compares with only 10% of the sample represented by clone 28 and 10% represented by clone 32 in the rural/village samples. The eight most common clones in the whole sample made up 65.5% of rural and village samples, whereas these made up 90.6% of the town and city samples. Consequently, the rural and village samples contain more trees that belong to the less frequently occurring clones than samples originating from town and cities, with 34.5% of samples consisting of less common clones in rural and village settings compared with only 9.4% of clones in towns and cities. Surprisingly, however, the rural and village samples consisted of 49 clones, which is a total close to the 47 clones present in the town and city samples. Of course, some of the current urban locations may have been classified as rural at the time the sampled trees were initially established.

Winter-flooded meadows are required for seed germination in black poplar. Of the total sample, for 432 (53%) trees there was a source of water such as a drain, stream, river, pond or moat in the 100 m^2 area surrounding each tree, but there was no visible source of water near the other 379 trees. There was the same proportion of trees near water irrespective of whether the location was a city/town or rural/village.

The proportion of trees that was close to water was much greater in the samples that were members of rare clones. Of the 88 trees which belonged to clones that were present in the sample on three or less occasions, 68 (77%) were close to water. Of these, 29 were located along the banks of the river Thames

Figure 5 Example of mature black poplar in an urban environment showing the characteristic downward sweep of branches.

Table 5 Number of trees, sex and number of clones according to vice-county in the sample set.

VC	VC name	Total number of trees	Number of male trees	Number of female trees	Number of gender unknown trees	Total number of clones	Number of male clones	Number of female clones	Number of clones of unknown gender
VC5	S. Somerset	8	7	0	1	3	2	0	1
VC6	N. Somerset	16	16	0	0	2	2	0	0
VC7	N. Wiltshire	9	6	3	0	4	3	1	0
VC11	S. Hampshire	4	3	1	0	3	2	1	0
VC12	N. Hampshire	9	9	0	0	3	3	0	0
VC13	West Sussex	5	4	1	0	2	1	1	0
VC16	W. Kent	7	5	2	0	3	2	1	0
VC17	Surrey	111	64	41	6	34	16	13	5
VC18	S. Essex	44	38	6	0	7	5	2	0
VC19	N. Essex	50	36	14	0	8	5	3	0
VC20	Hertfordshire	33	31	2	0	3	2	1	0
VC21	Middlesex	41	31	2	8	13	7	2	4
VC22	Berkshire	1	0	0	1	1	0	0	1
VC23	Oxfordshire	1	0	1	0	1	0	1	0
VC24	Buckinghamshire	11	9	2	0	2	1	1	0
VC25	E. Suffolk	84	47	36	1	9	5	3	1
VC26	W. Suffolk	59	51	6	2	8	4	2	2
VC27	E. Norfolk	2	1	0	1	2	1	0	1
VC33	E. Gloucestershire	70	20	49	1	26	15	10	1
VC34	W. Gloucestershire	1	1	0	0	1	1	0	0
VC35	Monmouthshire	1	0	0	1	1	0	0	1
VC36	Herefordshire	1	0	0	1	1	0	0	1
VC37	Worcestershire	1	1	0	0	1	1	0	0
VC39	Staffordshire	1	1	0	0	1	1	0	0
VC40	Shropshire	10	6	4	0	3	2	1	0
VC46	Cardiganshire	1	0	0	1	1	0	0	1
VC47	Montgomeryshire	1	1	0	0	1	1	0	0
VC48	Merionethshire	5	5	0	0	1	1	0	0
VC50	Denbighshire	83	69	0	14	3	2	0	1
VC51	Flintshire	13	12	1	0	3	2	1	0
VC53	S. Lincolnshire	3	0	3	0	1	0	1	0
VC54	N. Lincolnshire	2	0	2	0	1	0	1	0
VC56	Nottinghamshire	6	2	4	0	2	1	1	0
VC58	Cheshire	76	51	24	1	6	3	2	1
VC59	South Lancashire	11	10	1	0	4	3	1	0
VC62	N.E. Yorkshire	7	2	5	0	2	1	1	0
VC64	Mid-W. Yorkshire	1	0	0	1	1	0	0	1
VC65	N.W. Yorkshire	1	1	0	0	1	1	0	0
VC66	County Durham	8	6	2	0	3	2	1	0
VC67	S. Northumberland	2	1	1	0	2	1	1	0
VC69	Westmorland	1	1	0	0	1	1	0	0
VC70	Cumberland	3	3	0	0	1	1	0	0
VC83	Midlothian	4	3	1	0	3	2	1	0
VCH18	Offaly	3	0	3	0	1	0	1	0

VC – Vice-county, VCH – Vice-County Hibernia (vice-county in Ireland)

Table 6 Percentage of the total sample in rural/village locations compared to town/city locations..

Type of location	Percentage of the total sample represented by each of the most common clones								
	Clone 23	Clone 25	Clone 28	Clone 32	Clone 34	Clone 35	Clone 43	Clone 49	Other clones
Rural/ village (531 trees)	1.3	13.1	10.0	10.1	10.7	6.8	5.4	8.1	34.5
Town/ city (280 trees)	4.6	5.7	41.4	31.1	6.4	1.4	0.0	0.0	9.4

and 19 were close to ponds at the Cotswold Water Park, both areas which potentially offer opportunities for sexual regeneration. These 68 trees had a more equal distribution of the sexes (47% males, 38% females and 15% unknown) than was the case in the whole sample set, which suggests they were the products of sexual as opposed to vegetative reproduction, or were planted using material propagated from a sexually reproducing population.

Discussion

The Manchester poplar

The high frequency of occurrence of clone 28, particularly in urban locations, suggests that it is the Manchester poplar. Adams (2010) mentions that there are likely to have been 7000 trees of this clone planted historically in the Manchester area alone, with large numbers also planted in London and Essex. In this more extensive fingerprinting exercise large numbers of this clone were also found in south-west England, and one individual as far north as Edinburgh. Cooper (2006) provides an interesting background to the frequency of this particular clone. After Manchester led the way by opening the first three public parks in Great Britain in 1846, it was found over time that pollution killed the oak, ash and elm that had been planted, and so, in 1913, the lost trees were replaced in one of the parks with black poplars at a cost of £2500. The trees for the city were grown by a nursery owned by the Manchester Parks and Cemeteries Committee at Carrington Moss in Cheshire, where the 25 000 black poplars grown in 1915 made up two-thirds of the total trees raised that year, which were destined to be planted in Manchester.

Evidence of two origins

Cottrell *et al*. (2005) carried out a chloroplast DNA study of black poplar samples from across Europe. Chloroplast DNA is maternally inherited in poplars and is informative in revealing post-glacial routes of colonisation. It was discovered that material currently present in Great Britain had two origins, a Spanish lineage and another that had arrived from Italy and south east Europe. In Great Britain, the southeast European lineage which migrated northwest-ward during the period of re-colonisation occurs more frequently than the Spanish lineage, which colonised in a northward direction through France. Some of the samples used in the Cottrell *et al*. (2005) study were also present in the current sample set, and by careful backtracking it was discovered that clone 25 is from the Spanish lineage whereas clones 23, 28, 29, 32, 33, 36 and 41 are from the southeast European lineage. In the current study, clone 25 is rich in rare microsatellite alleles whereas the seven clones identified as having a southeast European lineage have no rare alleles. The fact that the rare alleles are often aggregated in particular clones suggests that their arrival has been relatively recent, possibly as a result of human-mediated activity. It would be informative to carry out a chloroplast DNA analysis of all the clones in the current sample set in order to reconcile nuclear microsatellite fingerprints with chloroplast DNA lineage.

Links with Ireland

Cooper (2006) reported finding a Manchester poplar clone (clone 28) in Ireland, as well as other representatives of this clone in Sussex and Yorkshire. In the current DNA fingerprinting exercise the three samples from southern Ireland belonged to clone 32, which is present in a further 86 trees in this study, which are mostly concentrated in southeast England. These findings confirm the transfer of vegetatively propagated planting stock between England and Ireland. In a previous genotyping study of 80 trees growing in southern Ireland, Keary *et al*. (2005) identified six clones, three of which were present in Offaly, where the samples from Ireland in our study originated. The three Offaly trees in our sample set that belonged to clone 32 were also present in the Keary *et al*. (2005) study (here they were referred to as clone 8 because a different numbering system to the FRM National Register was being used). This clone was represented by 10 trees at Offaly and one near Tipperary in the Keary *et al*. (2005) study. It is not known exactly when planting material was transferred between England and Ireland, but in the mid-16th century the English monarchy parcelled out land in Ireland to English, Scottish and Welsh settlers who cleared large areas to create pasture for livestock and tillage for crops (Forbes, 1932). The interest in forestry that

began to develop during the 1700s quickly spread to England and Ireland, and changes in the aesthetic tastes among wealthy landowners resulted in the establishment of many small woods at this time. These new English landowners may well have been responsible for the transfer of black poplar clones that occur in both England and Ireland today.

Links with Holland

Clone 40 produced an identical fingerprint to Vereecken, the commercial poplar clone introduced from Holland in 1950. Cooper (2006) also tested a black poplar from Holland and found it produced the same fingerprint as trees in Great Britain growing in Suffolk, Caernarvonshire, Leicestershire and Lancashire. However, Cooper's (2006) Amplified Fragment Length Polymorphism (AFLP) molecular marker approach may have been less efficient at differentiating individual clones because an enigmatic result of the same fingerprint for both male and female trees was obtained. This did not occur in the current study when microsatellite markers were used. Two other clones, 108 and 113 which, in common with clone 40, also had a large number of rare alleles and were least similar to the rest of the samples according to the dendrogram which represents genetic relatedness (Figure 4). These two clones may therefore not be native in origin, but instead may represent trees transferred from mainland Europe to Great Britain.

Black poplar in Wales

Wales has large populations of black poplar in Denbighshire and Flintshire in north Wales and along the banks of the river Usk in south Wales. Indeed, black poplars in Wales are listed on the Tree Register's native species database of champion trees, with one at Rosset, Denbighshire, and another at Christ's College, Brecon, displaying impressive girths of 1.0 m and 1.3 m, respectively (www.treeregister.org/champion-trees.shtml).

In a previous study of black poplar along European river systems, Smulders *et al*. (2008) found only two clones in the 72 trees DNA-fingerprinted with microsatellites along the river Usk in Wales. Seventy of these trees represented a single clone. In the current study, a similarly low clonal diversity in the Welsh samples, most of which came from north Wales, was found. Of the 104 samples from Wales, 77 were represented by clone 23, 10 by clone 34, and 14 by clone 14. The remaining three samples were represented by a single tree each of clones 29, 33 and 43. The dominant clone 23 is relatively common in East and West Suffolk, North Essex and South Somerset. One of the samples in the current study which was DNA-fingerprinted as clone 23 was included in an earlier study using RAPD (Random Amplified Polymorphic DNA) markers (Cottrell, Forrest and White, 1997). The RAPD markers also found that this clone was present in Flintshire, Denbighshire and Breconshire, as well as in West Gloucestershire, Dorset and North Essex. The large contribution of clone 23 to the population in Wales may reflect the vegetative propagation carried out by nurseries based in Wales which relied on a limited range of clones for propagation. Linnard (1979) records that there were six nurseries in Cardiganshire and Carmarthenshire at the beginning of the 19th century. The largest occupied 18 acres in Newcastle Emlyn and sold about 400 000 plants per year. It is recorded in one of its annual inventories that black poplar made up 3000 of the 2.5 million plants on that site. Black poplars were recommended for shelter planting in places exposed to sea winds. The presence of large numbers of clone 23 in Breconshire may reflect its use in the making of cartwheels, an industry that operated along the river Usk around 200 years ago (Cooper, 2006).

Black poplar in the British landscape

It has been commented on extensively that although black poplar is a species of riverine ecosystems adapted to grow in winter-flooded meadows, it is frequently seen in Great Britain in several very different environments. Barnes, Dallas and Williamson (2009) discuss the landscape context in which black poplars are found in Norfolk by examining the immediate environment around existing trees on 17th and 18th century estate maps. Their work demonstrates that black poplar is most certainly not a woodland tree, and only 13% of their sample of 75 locations in Norfolk showed black poplar occurring in floodplains; almost one third (29%) were associated with commons and nearly half (46%) were near farms and farmyards. The association with common land, village greens and proximity to ponds has also been remarked upon by Cooper (2006). However, the most striking association was with kilns (13%), mills (12%), smithies (6%) and malt houses (4%). Although many were clearly planted, they were nevertheless established in damp locations with 22% of the samples near ponds or moats.

Natural sexual reproduction

Opportunities for the natural sexual regeneration of black poplar to take place appear to be rare in Great Britain at the present time. The greater frequency of male to female clones, coupled with the finding from this study that several vice-counties only had trees of one sex, means that both sexes may rarely be present in close proximity. Also, over half the trees in the sample set do not grow near an obvious source of water, which suggests that in the rare locations where sexual reproduction could occur, the seeds would most likely lack the appropriate conditions on which to germinate. However, two areas were found where either sexual reproduction may have commonly occurred in the past, or where the current material

was propagated from a sexually reproducing population growing elsewhere. These are Cotswold Water Park in Gloucestershire and the wharves in London docks in the Barnes area. Both of these areas contain trees of both sexes, a high diversity of clones, and several clones that were only present as a single tree in the total sample set, features which indicate sexual reproduction may have taken place.

Cotswold Water Park consists of a wetland landscape supporting extensive biodiversity-rich habitats of standing water together with associated marginal vegetation of woodlands and scrub. It contains many veteran trees, hedgerows and river banks that support large black poplar specimens. The current landscape is the product of excavations of the mineral reserves in the Upper Thames Valley which started in the early 20th century and changed the landscape from one of extensive areas of farmland within the low-lying floodplain area to a mosaic of lakes of varying size and character. Cotswold Water Park now provides both a nationally important area of inland open standing water as well as the most extensive marl lake system in Great Britain. This is a consequence of the proximity to limestone areas and the presence of lime-rich water. In our study, notes provided with the black poplar samples indicate that the samples are mainly taken from mature trees which probably started life when the area comprised farmland in the low-lying floodplain and might have offered suitable conditions for seed germination and the subsequent growth of black poplar. Although significantly modified since the early 20th century, this area continues to offer opportunities for sexual reproduction and seedling establishment for black poplar around the many marl ponds that were formed during the 20th century. Intriguingly, there is evidence that recent sexual reproduction has taken place in Cotswold Water Park, as the DNA fingerprint from a sapling at the site can be assigned as the putative offspring of a DNA-fingerprinted male and female tree in the park.

The London samples, that may be the product of sexual reproduction, are the group of trees which Adams (2010) refers to as the 'enigmatic mixed population of male and females in a line along the southwest bank of the tidal Thames from Hammersmith to Putney' which consists of over 40 trees. These trees were recorded as early as 1852 (Lousley, 1976) and may represent a relict population of seedlings which germinated in the gravel at the side of the river. The Victorian engineers constructed revetments around the bases of these mature trees to protect the river bank, making it probable that these trees are now over 200 years old. Unfortunately, establishment of a new sexually derived generation is unlikely as the stone walls of the revetments and the tarring of the adjacent towpath mean that suitable areas for germination are no longer available (Adams, 2010).

Conclusion and implications

Through the efforts of many different organisations and individuals supplying samples for analysis, a picture emerges of a native black poplar population that has clearly been greatly influenced by human intervention, and which due to a number of historical factors, rarely acts as a naturally sexually regenerating species. The DNA results can be used to split the British black poplars sampled into two groups: a small group which contains individuals with a large number of rare alleles and a larger group containing less diversity and the more common alleles. Registration of the clones on the FRM National Register provides a standardised system of clone numbering so that a shared frame of reference exists for all interested parties. In an era of climate change and increasing threat from introduced pests and diseases, the authors hope that this work raises the awareness of the ongoing plight of the black poplar and helps direct conservation efforts to capture the genetic diversity present in the population thus giving British poplars the best chance of surviving into the future.

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