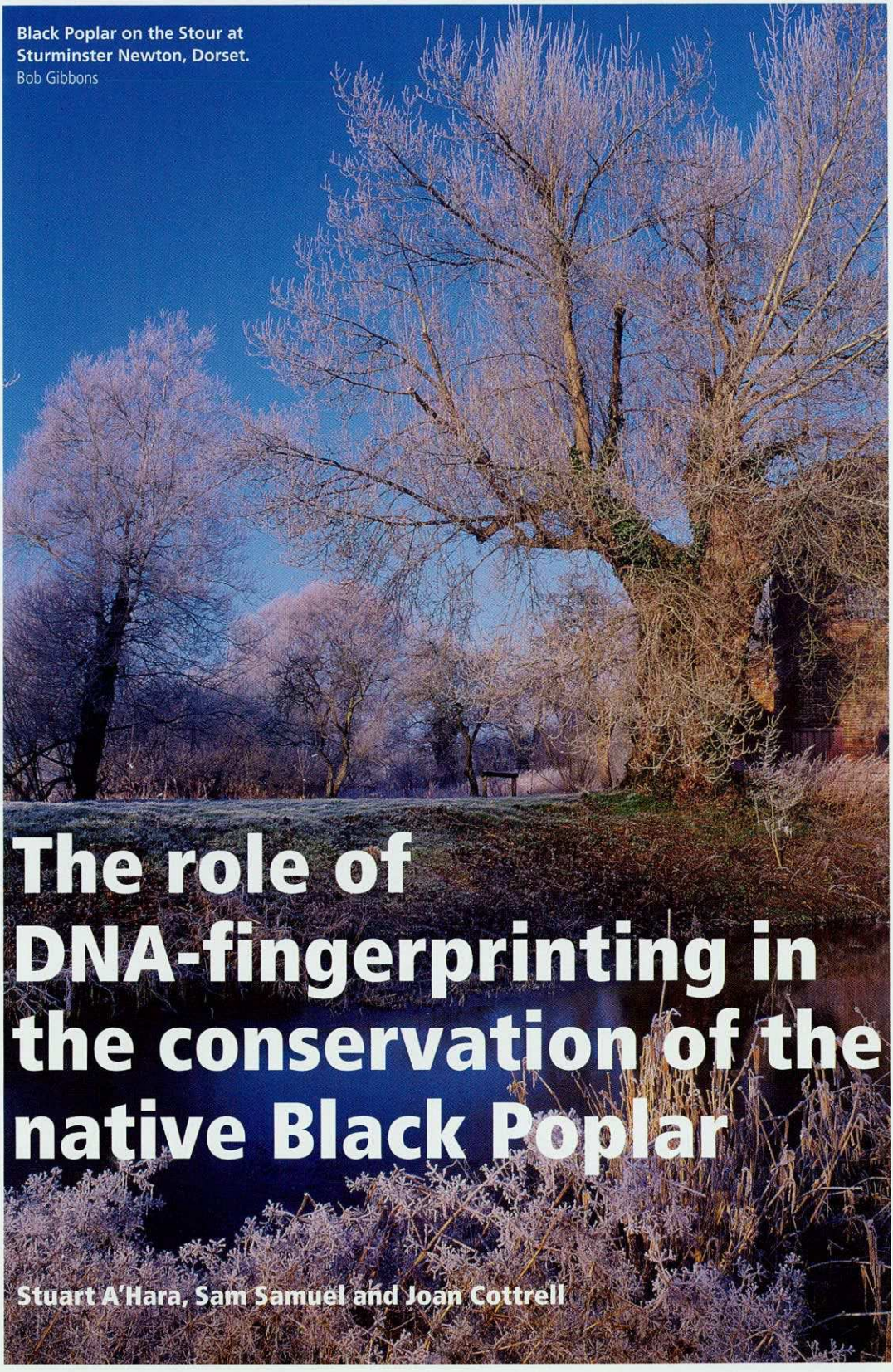


Black Poplar on the Stour at Sturminster Newton, Dorset.
Bob Gibbons

The role of DNA-fingerprinting in the conservation of the native Black Poplar

Stuart A'Hara, Sam Samuel and Joan Cottrell

As one of Britain's most endangered native tree species, the native Black Poplar *Populus nigra* subsp. *betulifolia* is currently the subject of considerable interest, with a range of conservation bodies and local authorities actively working to support the species. There is no national Species Action Plan for Black Poplar (Lawrence & Molteno 2009), but the number of local Biodiversity and Species Action Plans (BAP/SAPs) that have been written for the species in recent years reflects the high level of interest in its conservation. An internet search revealed 23 local BAP/SAPs for Black Poplar written by local authorities in 18 English administrative regions (Berkshire [Reading only], Cheshire, Durham, Essex, Greater London, Greater Manchester, Hertfordshire, Lancashire, Leicestershire, Norfolk, North Yorkshire [Hambleton only], Northamptonshire, Shropshire, Staffordshire, Suffolk, Sussex, Warwickshire, Worcestershire) and five Welsh administrative regions (Conwy, Wrexham, Carmarthenshire, Denbighshire, Flintshire).

These BAP/SAPs list a range of recommended actions directed at conservation of the species. All of these recognise the importance of surveying and creating inventories of the locations of existing Black Poplar trees. Every plan also lists the need to carry out additional planting of locally sourced rooted cuttings. A large majority (83%) include an action aimed at publicising the plight of the Black Poplar in order to raise public awareness of the need to conserve the local resource of this species. Also, most plans (60%) state the need to use planning policy and legislation, such as Tree Preservation Orders, to protect existing trees that might be threatened. Other actions listed by a minority of the plans include: ensuring a mixture of sexes and age classes, removal of flood banks, and the creation of conditions suitable for floodplain woodlands.

Clonal identification

Notably, only a third of the action plans list genetic-fingerprinting as a method to reveal how many clones are present in the area covered by the plan. The low priority given to this action may reflect a lack of awareness among conservationists that DNA-fingerprinting is now a routine, relatively inexpensive technique that falls within the budget of most of the conservation bodies involved.

The benefit of such fingerprinting to Black Poplar conservation activities first came to light in the late 1990s, when two studies using molecular methods demonstrated that many of the trees in Britain had been established by vegetative propagation, which had produced considerable clonal duplication. One of the studies (Cottrell *et al.* 1997) used the RAPD (Random Amplification of Polymorphic DNA) method to fingerprint native Black Poplars held in Forestry Commission clone banks. The original trees on which the clone banks were based came from across the distributional range of the Black Poplar in Britain. This study revealed only 17 clones in the 36 fingerprinted trees. Some of the clones were common and had a very widespread distribution. For example, one clone occurred in locations as far apart as Wales, Dorset and Suffolk. In the other study (Winfield *et al.* 1998), 146 trees from the Upper Severn were fingerprinted by means of AFLP (Amplified Fragment Length Polymorphism) markers. The data were not used directly to determine the number of clones but, using 95% similarity as the threshold for clonality, the results indicate that their sample consisted of 54 clones. Some of the most common clones in the Upper Severn were represented by as many as 18 trees in the sample.

Since these early studies, a collaborative European Union-funded project known as EUROPOP has examined the diversity of the Black Poplar on a European scale. In the course of this project, microsatellite DNA markers were developed (van der Schoot *et al.* 2000; Smulders *et al.* 2001). These markers are superior to RAPDs and AFLPs in their reliability in fingerprinting individuals. Seven of these microsatellite markers were used by Smulders *et al.* (2008) to fingerprint Black Poplars growing along river systems in nine European countries. In the highly dynamic river systems of Spain, France, Czech Republic and Ukraine, no duplication of clones was detected, indicating that all sampled trees were derived from seed rather than from vegetative propagation. Similarly, less than 15% clonal duplication was found in rivers in Italy, Austria and Germany, and this low incidence of vegetative propagation was considered to result naturally from the rooting of broken branches which had been swept downstream from the original trees to new locations. In contrast, there was much higher clonal duplication along the Rhine in the Netherlands (41%) and the Usk in Wales (97%). In the Usk Valley, only two clones consisting of 68 and 4 trees, respectively, were found to

be present (Cottrell *et al.* 2002). These high levels of clonality in the Netherlands and Britain were considered to reflect much higher levels of human-mediated vegetative propagation.

Reasons for human-mediated vegetative propagation in Britain

The documented history of the Black Poplar in Britain supports molecular data which indicate that in this country the species has experienced considerable human intervention. A recent analysis of the locations of trees in Norfolk found that the majority of Black Poplars were associated with farms, kilns, mills, smithies and malthouses (Barnes *et al.* 2009). This suggests that the trees have mostly been planted, rather than being the product of natural seed dispersal. Barnes *et al.* (2009) suggest that the many uses to which its wood can be put in an agricultural context explain the proximity of the Black Poplar to farms. It is shock-absorbent and therefore useful for carts, and its fire-resistant qualities make it the timber of choice for barns and hop kilns. This property, along with its light colour and durability, meant that it was made into floorboards and used for a variety of domestic ware in farmhouses. The aesthetic beauty of the tree might also have influenced the choice of Black Poplar around farmsteads. Its distinctive shape may also have encouraged its planting to mark boundaries of commons and parishes.

The proximity of trees to brickworks, limekilns and smithies may also be a result of the fire-resistant qualities of its timber, which was used in the manufacture of bellows and pumps.

Other uses that are mentioned in the literature include clogs and brake-blocks. The wood can absorb paraffin and was used to make matches. It is also very resistant to rot, and the branches often grow in a curve, which made it excellent for the erection of timber-framed buildings. Some First World War rifle butts were made of Black Poplar wood, and the wood was used in the arrows that were found in the Tudor warship, the *Mary Rose*.

Molecular approaches to hybrid identification

The non-expert can find it difficult to distinguish native Black Poplar from the introduced hybrid *Populus* × *canadensis*, which is a cross between the American and the European Black Poplar, i.e. *P. deltoides* × *P. nigra*. The hybrid arose spontaneously in France in the early 1700s and was introduced around 1750 in Britain, where its faster growth rate meant that it was subsequently planted in preference to native Black Poplar (Jobling 1990). Misidentification of the hybrid as native Black Poplar resulted in the number of Black Poplars in Britain being overestimated, so that its rarity was overlooked until relatively recently. This rarity was first appreciated by Edgar Milne-Redhead following his national survey during 1973-88, and this was later confirmed when Peter Roe instigated the *Daily Telegraph* 'Black Poplar Hunt' in 1994. Currently, around 7,000 trees are recorded, of which only about 600 are females (Cooper 2006).

The implications of the difficulties in identification are highlighted by Barnes *et al.* (2009), who comment that in Norfolk many of the trees supplied by nurseries as native have latterly been identified as hybrids. Fortunately, several molecular tests are now available to distinguish the European Black Poplar from the introduced *P.* × *canadensis* hybrid, and these tests are also useful to Black Poplar conservationists (Holderegger *et al.* 2005). Forest Research, an agency of the Forestry Commission, has recently fingerprinted and hybrid-tested much of the material that is being marketed by nurseries for forest planting and hybrids have been eliminated, so that mislabelling of nursery stock should be reduced in the future in this sector.

Recent molecular work by Forest Research

Forest Research has recently employed the set of microsatellite markers used by Smulders *et al.* (2008) to perform a detailed fingerprinting exercise on Black Poplars. The sampling consisted of trees that are being used by local councils and Wildlife Trusts in England and Wales to provide locally sourced rooted cuttings for the planting schemes recommended by the local action plans. Considerable effort is being made by local authorities to establish cuttings from all the Black Poplars in particular localities in order to capture the maximum amount of genetic diversity that is present in the district. However, because of the high level of duplication of clones in the adult population, the new plantings based on rooted cuttings may consist of very few clones and, consequently, contain very little genetic diversity. The Forestry Commission therefore invited local authorities and Wildlife

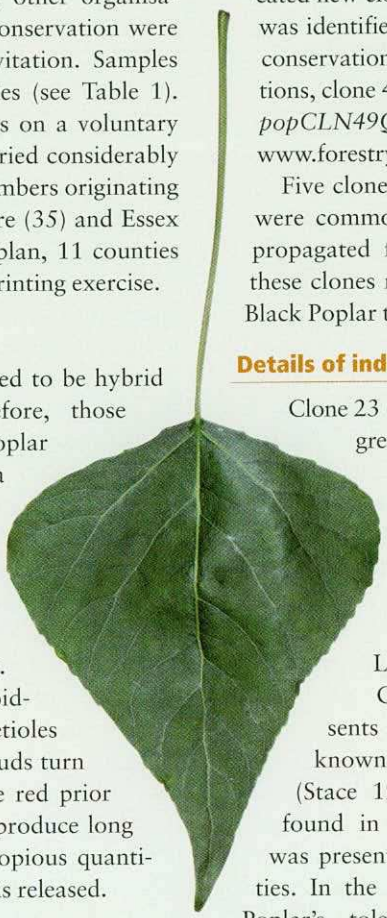
Trusts to submit material from source trees that were being used to provide cutting material in order that they could be genetically fingerprinted and hybrid-tested. The results of this exercise will enable a rational choice of source trees for the supply of rooted cuttings, so that conservation plantings can be designed that represent as much of the local genetic diversity as possible.

The genetic-fingerprinting survey

In total, samples of 250 trees from 12 local authorities, Wildlife Trusts and other organisations involved in Black Poplar conservation were received in response to the invitation. Samples were submitted from 12 counties (see Table 1). As the provision of samples was on a voluntary basis, the number of samples varied considerably between counties, the highest numbers originating from Suffolk (102), Hertfordshire (35) and Essex (30). Despite having an action plan, 11 counties did not participate in the fingerprinting exercise.

Identifying Black Poplar

Only seven of the samples proved to be hybrid *Populus* × *canadensis*. Therefore, those charged with identifying Black Poplar in the field generally achieve a high level of accuracy. The most important morphological features of native Black Poplar include the down-sweeping lower branches and the dark, deeply furrowed bark. The vivid green leaves are deltoid-ovoid in shape, with hairy petioles and serrated margins, and the buds turn outwards. The male catkins are red prior to elongation, and the females produce long pendulous seed capsules with copious quantities of white fluff when the seed is released.



Black Poplar leaf. Paul Sterry/Nature Photographers

Number of clones present

The marketing of Black Poplar for forest planting is covered by the Forest Reproductive Material (FRM) (Great Britain) Regulations 2002, which require the registration of all clones of the genus *Populus* used in propagation. As the FRM regulations operate at the genus rather than the species level, clone numbers 1-21 in the FRM register have previously been allocated to commercial Western Balsam-poplar *P. trichocarpa* clones and hybrids of other poplar species. The numbers for native Black Poplar clones identified in recent studies therefore begin at 22.

In fingerprinting work on material held in the Forest Research clone bank and by the commercial forest nurseries sector several unique clones were identified. In the current survey for the conservation sector, many of these clones were encountered again and therefore an existing clone number could be allocated to them. The new clones that emerged in the current fingerprinting exercise were allocated new clone numbers. A total of 15 clones was identified in the samples provided by the conservation sector. (Under the FRM Regulations, clone 49, for example, takes the identity *popCLN49QU*. For further information, see www.forestry.gov.uk/frm.)

Five clones (clones 23, 25, 28, 32 and 35) were common in the material that is being propagated for conservation plantings, and these clones represented 84% of the sampled Black Poplar trees (Table 1).

Details of individual clones

Clone 23 (male) was frequent in Suffolk and grew also in Essex and was found among a small number of samples from Wiltshire. Clone 25 (male) was the most frequent clone in Suffolk and was present also in Essex. It accounted for two of the six samples received from Leicestershire.

Clone 28 (male) probably represents the clone that is commonly known as 'the Manchester poplar' (Stace 1971). This was the only clone found in samples from Manchester but was present also in six other English counties. In the area around Manchester, Black Poplar's tolerance to industrial pollution resulted in its being a popular species for planting in parks, roadsides and cemeteries in the late 19th and early 20th centuries. This male clone would have been preferred for amenity plantings, as it would not have produced the unsightly fluffy seeds characteristic of female trees. The majority of the trees occur in the eastern and northern parts of the city. These areas used to be slums, where the level of airborne pollution would have been at its worst. As a result, it was estimated that there

Table 1 The number of trees of each clone from the 12 English counties which provided samples for the fingerprinting exercise. Where known, the sex of each clone is indicated.

County	Clone number														
	23	25	28	29	32	34	35	37	43	44	45	46	47	48	49
Yorkshire			3		5										
Middlesborough					1	1	1								
Cheshire			3		1	5			11					1	
Manchester			8												
Leicestershire		2			4										
Avon			4												
Hertfordshire							35								
Buckinghamshire							16	3							
Surrey					3										
Suffolk	14	43	8	1	28					1	5	1	1		
Wiltshire	1		1												2
Essex	5	10	3		5	4							3		
Total	**20**	**55**	**30**	**1**	**47**	**10**	**52**	**3**	**11**	**1**	**5**	**1**	**4**	**1**	**2**
Sex	M	M	M	F	F	M	M	?	?	F	M	M	F	?	F

were as many as 7,000 Black Poplars in Greater Manchester in 2000 (www.gmbp.org.uk/site/.../BLACK%20POPLAR%20BAP15.5.08.pdf). Since 2000, about half of the trees have died or been felled owing to a high incidence of poplar scab in the city. It is not known whether this clone is exceptionally susceptible to this disease or whether the conditions in Manchester are particularly conducive to poplar scab. It is thought that the warmer wetter winters, lack of cold periods and earlier onset of spring resulting from climate change may lead to an increase in the incidence of poplar scab.

Clone 32 was the most common female Black Poplar, and was very widespread in that it occurred in seven of the 12 counties that provided samples.

Clone 35 (male) is restricted to Hertfordshire and Buckinghamshire and a single representative in Middlesborough. This clone is particularly common in the Aylesbury district. This is likely to be the clone to which Richard Mabey (1996) refers when he writes 'On late afternoons in March, ... parts of the Vale of Aylesbury in Buckinghamshire are suffused with an autumn glow. All over the flood-plain, ...rows of craggy pollards begin to shine, as if they have been coated in amber. ...This spectacular display is the largest concentration of our grandest native tree, the Black Poplar, in all its spring finery, and there is not another treescape like it in Britain.' Currently, there are thought to be about 5,000 trees in the Vale of Aylesbury, many of which are in the form of maidens and tall or short pollards. The fast-growing Black Poplars were a resource for the local economy. In this district the lopped branches were used for cattle fodder, wattle, matchsticks, bean poles and fruit baskets. Their use as hedging stakes may account for the appearance of mature Black Poplar trees at regular intervals in hedgerows in the district. The wood was also used in sheep hurdles to confine the sheep at night when they were brought down from the Chiltern downland (http://enquire.herts-scc.gov.uk/cms/explore/walk/blackpoplar.htm).

Clones 29, 44 and 46 all have only one representative each in the current survey. Although they were submitted to the survey by Suffolk Wildlife Trust, they were originally obtained as seedlings from Cheshire. This is interesting, because Mabey (1996) states that for a while the only known place where male and female trees grew close together and away from hybrid trees was at Hallwood Farm Marl Pit, in Cheshire. He further records that seedlings from these trees were transported in 1979 to Sturminster Newton, in Dorset, and planted along the banks of the River Stour. Clones 29, 44 and 46 may therefore also have originated from seeds produced by the trees at Hallwood Farm that were later transported by conservationists to Suffolk.

The remaining seven clones were represented by between one and 11 trees in the sample. Clone 34 occurred in Cheshire, Middlesborough and Essex. Clone 37 occurred only as three trees in Buckinghamshire. Clone 43 was restricted to 11 trees in Cheshire and Clone 45 was represented

by five trees in Suffolk. Clone 47 was female and was sampled only in Suffolk and Essex. Clone 48 occurred only as a single tree in Cheshire and clone 49 was found only as two trees in Wiltshire.

The sex was recorded for 228 of the 250 trees sampled (173 male and 55 female), which consisted of 7 male and 5 female clones.

Implications of the Forestry Commission regulations for Black Poplar

It should be noted that the regulations apply when marketing of Black Poplar takes place for forestry purposes. Forestry Commission guidance defines marketing as 'displaying with a view to sale, selling or delivering under a contract (other than on an agent and client basis) including a contract for the supply of services'. 'Forestry purposes' is defined as woodland planting (woodland is an area greater than 0.25ha or more than 15m wide, with a minimum of 20% canopy cover at maturity) of any description for any multi-purpose forestry use. All Forestry Commission grant-aided planting is covered by the regulations, including those covering planting as part of urban regeneration work and landscape enhancement. The regulations apply to any planting of more than 1,000 trees. If Black Poplar is used as a component of a scheme of this size, however small in proportion to other species, the regulations apply.

Regulatory control provides registration of individual clones, certification of cuttings production, labelling, and a full audit trail between original source and planting. This can therefore be used to monitor the use of individual clones and to provide information for the development of a more balanced availability of clones in use. Any organisation which feels that its plant production might fall under the control of the regulations, or wants to benefit from the documentation which these provide, should contact: The FRM Officer, Forestry Commission, Silvan House, 231 Corstorphine Road, Edinburgh EH12 7AT; tel: 0131 334 0303.

Recommendations

Conservationists should arrange to have their Black Poplar material hybrid-tested and DNA-fingerprinted prior to establishment of rooted material for planting schemes. They should aim to include as many locally sourced clones as possible. If this is not practicable, they should broaden the geographical scale over which they obtain their cuttings. If possible, they should aim to propagate female as well as male clones. Hybrids should not be propagated.

A knowledge of the rarity of a clone will also help in determining which trees are most in need of conservation and/or vegetative propagation. For example, clones 23, 25, 28, 32 and 35 are represented by many trees, and individual trees belonging to these clones should be given lower priority than those clones for which only a few representatives have been located.

The DNA-fingerprinting service is available at Forest Research and the price of fingerprinting is available on request.

References

Barnes, G, Dallas, P, & Williamson, T 2009 The Black Poplar in Norfolk. *Quarterly Journal of Forestry* 103 (1): 31-37

Cooper, F 2006 *The Black Poplar History, Ecology and Conservation*. Windgather Press, Macclesfield

Cottrell, J E, Forrest, G I, & White, I M S 1997 The use of RAPD analysis to study diversity in British Black Poplar (*Populus nigra* L. subsp. *betulifolia* (Pursch.) W Wettst. (Salicaceae) in Great Britain. *Watsonia* 21: 305-312

Cottrell, J E, Tabbener, H E, & Forrest, G I 2002 Distribution of variation in British Black Poplar: the role of human management. In: B C Van Dam & S Bordács (eds) *Genetic diversity in river populations of European Black Poplar*. Csiszár Nyomda, Budapest

Holderegger, R, Angelone, S, Brodbeck, S, Csencsics, D, Gugerli, F, Hoebee, S E, & Finkeldey, R 2005 Application of genetic markers to the discrimination of European Black poplar (*Populus nigra*) from American Black Poplar (*P. deltoides*) and Hybrid Poplars (*P.* x *canadensis*) in Switzerland. *Trees* 19: 742-747

Jobling, J 1990 Poplars for Wood Production and Amenity. *Forestry Commission Bulletin* 92

Lawrence, A, & Molteno, S 2009 *From rationalism to reflexivity? Reflections on change in the UK Biodiversity Action Plan. REFGOV Working Paper Series GPS-15*. Centre for Philosophy of Law, Université catholique de Louvain (final version submitted to MIT Press)

Mabey, R 1996 *Flora Britannica*. Sinclair-Stevenson, London

Smulders, M J M, van der Schoot, J, Arens, P, & Vosman, B 2001 Trinucleotide repeat microsatellite markers for Black Poplar (*Populus nigra* L.). *Molecular Ecology Notes* 1: 188-190

Smulders, M J M *et al.* 2008 Structure of the genetic diversity in Black poplar (*Populus nigra* L.) populations across European river systems: consequences for conservation and restoration. *Forest Ecology and Management* 255: 1388-1399

Stace, C A 1971 The Manchester Poplar. *Watsonia* 8: 391-393

van der Schoot, J, Pospíšková, M, Vosman, B, & Smulders, M J M 2000 Development and characterization of microsatellite markers in Black Poplar (*Populus nigra* L.). *Theoretical & Appllied Genetics* 101: 317-322

Winfield, M O, Arnold, G M, Cooper, F, Le Ray, M, White, J, Karp, A, & Edwards, K J 1998 A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers. *Molecular Ecology* 7: 3-10

The three authors work for Forest Research, an agency of the Forestry Commission. Stuart A'Hara and Joan Cottrell are molecular scientists involved in various applications of genetic markers for the study of trees and woodland-dwelling species (e-mail: Joan.Cottrell@Forestry.gsi.gov.uk). Sam Samuel is responsible for the scientific aspects of the implementation of the Forest Reproductive Material Regulations.