CHAPTER ONE - INTRODUCTION

1.1 Aims and objectives

The aims of the study were to establish the level of genetic diversity within the endangered black poplar *(Populus nigra* subsp *betulifolia)* in Britain and Ireland, using molecular genetic methods, and to update the national survey of the species, first undertaken by Edgar Milne-Redhead between 1973 and 1988 (Milne-Redhead, 1990), in order to provide a complete national inventory. Edgar Milne-Redhead first highlighted the plight of the tree, when, upon his retirement from the Royal Botanic Gardens at Kew (White 1993); Rogers 1995), he undertook a national survey of the tree for the Botanical Society of the British Isles (BSBI) and World Wildlife Fund (WWF) between 1973 and 1988 (Milne-Redhead, 1990). By 1975, he had found some 1,000 standard (fig. 1.1) trees (pollarded specimens (fig. 1.2) were excluded from the survey (White 1993), as was the Manchester poplar, although no explanation was given for these omissions) (Milne-Redhead, 1990).

1.2 Why black poplar?

Black poplar was chosen for this study because it has been identified as one of Britain's rarest and most endangered native trees (White, 1993), and considerable interest has been expressed in its conservation by various groups and individuals throughout the country, such as Wildlife Trusts and County Councils, together with local *ad hoc* conservation groups. Britain is a key site as it is on the western fringe of the species range and a great deal of geographical information is known. As the population was thought to be only 2,500 at the commencement of the project in October 1997, it was possible to include a significant proportion of the population in the study. Its notoriety as a difficult tree to identify in morphological terms, coupled with the fact that it is a dioecious (with male and female flowers on separate trees) species, make it an interesting tree to study genetically.

In Britain, the tree appears to have three distinct leaf morphologies, with a clear geographical distribution. Such differentiation has resulted in an unofficial sub-taxonomy being devised by John White, formerly dendrologist for the Forestry Commission at Westonbirt Arboretum. He suggests the following forms; 'large-leafed St Asaph type', found in the west of the range; 'diamond-leafed Rossendale type', found in the north; and the 'Cambridge type', found in the east, and thought by Edgar Milne-Redhead to represent the best leaf form of black poplar (J White, pers comm). There are several explanations of the variations. First, it is possible that either sub-sub-species exist, or that more than one sub-species is present. Second, the variation may reflect the existence of several ecotypes (the adaptation of a species to environmental conditions). Third, the variation may be due to environmental influences, such as micro-climate or altitude, although a tree grown from a cutting in a different locality retained the characteristics of the parent tree (J White, pers comm). Personal observation during fieldwork has indicated that, in addition to variation in leaf morphology, great variation in bark also occurs. Most trees in Britain have bark of a dark brown colour. However, trees in Somerset frequently have pale grey bark, whilst trees in the Vale of Aylesbury and Oxfordshire have a lighter brown bark. In addition to this variation, sexual dimorphism exists between male and female trees, with female trees displaying more slender twigs than male trees particularly in the north of the range. Fissuring and bossing can also vary, with some trees in the Republic of Ireland, Cheshire and Gloucestershire having no bosses at all.

Fig. 1.1 Standard black poplar

Fig. 1.2 Pollard black poplar

1.2.1 Decline of black poplar in Britain

The black poplar population in Britain is thought to have declined for the following six reasons, although other factors may have been influential in its decline.

- Clearance of floodplain forest.
- Land drainage and river engineering operations.
- An unequal number of male and female trees.
- Unsuitable ground conditions for seed set.
- Unpopularity of female trees.
- The introduction of a faster-growing, more commercially viable hybrid species *P. x canadensis.*

Land drainage has been operative in Europe since around 2000BC, although the practice did not become widespread until the 17th and 18th centuries (Haslam, 1991). The practice removed wet river margins and the bare wet soil conditions necessary for black poplar seed to set. Land drainage continued until the late 1980s, when concern arose about over-production in agriculture and the destruction of wetland areas (Purseglove, 1988; Haslam, 1991). River engineering carried out in order to alleviate flooding, involved straightening and deepening of many river channels, in order to carry excess water away as quickly as possible (Peterken and Hughes, 1995). River engineering activities also involved removal of instream and riparian vegetation, as well as riverside trees (Purseglove, 1988), and it is likely that many black poplars fell victim to this type of clearance.

The paucity of females, coupled with unsuitable ground conditions for seed set, even where males and females grow together has resulted in virtually a total lack of natural regeneration, other than at a site in Cheshire (D Hobson, pers comm). Females were deemed to be unpopular with farmers as the fluffy seed was considered to ‘adversely affect the appearance of market produce’ particularly lettuce (Bean, 1976). It also caused a nuisance to farmers at haymaking time and to soft-fruit growers (J White, pers comm). Such unpopularity has resulted in centuries of males being favoured for planting in Britain. A recent survey in Italy found a similar male : female ratio to that in Britain (S Bisoffi, pers comm) and it is possible that fluff was unpopular in parts of Europe, possibly for similar reasons. However, of 70 randomly sampled trees from six natural stands in France, 33 were male and 37 were female (Legionnet *et al*, 1997), suggesting that the male : female ratio is more balanced in France. In assessing such ratios, it must not be forgotten that the species is not as widely planted in hedgerows in Europe as it is in Britain.

A study on seed viability of *P. deltoides* by Braatne *et al*, (1996) found that seed was only viable for one to two weeks. However, Van Splunder *et al*, (1995) found that 10% of *P. nigra* seed germinated after 30 days storage; whilst Turrill (1962) states that black poplar seed must germinate ‘very soon after maturity’. In an *ex situ* study, it was found that no mortality occurred in a greenhouse experiment with seedlings (Van Splunder *et al*, 1996), suggesting that environmental conditions are contributory factors in establishing seed set. An additional problem is that the fluff surrounding the seed prevents it from embedding into the ground.

Finally, a potential major factor in the decline of black poplar was the introduction, thought by Loudon (1842) to have been in 1769, of a faster-growing, more commercially-viable hybrid, then known as *P. serotina,* (Elwes and Henry, 1913) (a name now used to describe a male cultivar), or *P. x canadensis* Moench. This name was abandoned in 1950 by the International Poplar Commission and replaced by *P. x euramericana* (Dode) Guinier, as it was felt that the former name implied a specific geographical origin (Jobling, 1990). The species is a cross between American *P. deltoides* Marshall and European *P. nigra* L, the subspecies of the latter parent is unknown. This hybrid was favoured in view of its economic properties, and led to a decline in planting of the native species, which was thereafter largely forgotten, other than for occasional hedgerow and urban planting (J White, pers comm). Thus, absence of regeneration requirements for seed set and lack of planting have resulted in an even-aged population, with many trees nearing the end of their natural lifespan. However, despite its decline in popularity, it is probable that the tree was never common in Britain, even in the 17th century. Evelyn (1664) stated how the 'black poplar grows rarely with us', but he remarked that 'divers stately ones of these I remember about the banks of Po in Italy' (Evelyn, 1664).

The reputed ease of hybridisation with certain non-native poplar species (J White, pers comm), whose flowering time coincides with black poplar, combined with the ease of wind pollination, has meant that a number of trees have suffered genetic pollution. These are morphologically similar, but are in fact backcrosses (J White, pers comm) and many have been incorrectly recorded as the native sub-species.

Despite these problems, the tree is able to propagate vegetatively, and many trees that have fallen, if left, will produce roots from trunks and survive for many more years. It is thought that many trees may be several hundred years of age, having fallen and regenerated in this manner (Milne-Redhead, 1990; S Falk, pers comm).

1.3 Historical distribution and ecology of black poplar

Black poplar *(P. nigra* L) has a broad distribution from Britain in the west, central China in the north and east, north Africa and Iran in the south, north-west India and Afghanistan in the south east (Zsuffa, 1974). This distribution range applies to black poplar 'in the aggregate sense' (Meikle, 1984), as opposed to the many hybrids and subspecies, which are found in more closely defined areas.

Milne-Redhead (1990) suggested that black poplar's native range in Britain was south of the Mersey and Humber estuaries, excluding Cornwall and West Wales. However, historical data obtained from county floras suggest that the distribution range of black poplar is restricted to England and Wales, south of the Lune and Humber estuaries, with isolated examples to be found in Cumbria and Northumbria, west Wales and Cornwall. It was believed by many flora authors to be an alien, perhaps because it has been extensively planted, coupled with the fact that little or no natural regeneration has taken place.

The Biological Records Centre (BRC) at the Centre for Ecology and Hydrology (CEH) (formerly Institute of Terrestrial Ecology (ITE)) has held records, in conjunction with Edgar Milne-Redhead, of the black poplar population in Britain and Ireland since 1973. An article by Peter Roe in the *Daily Telegraph* (Roe, 1994) launched The Black Poplar Hunt, and generated much public interest in the species. Following the article, the record expanded by some 250 trees, although it was found that many previously recorded trees had been lost (Rogers, 1995). As a result of the foregoing work by Edgar Milne-Redhead and the *Daily Telegraph*, at the commencement of this study in 1997, there were records of approximately 2,500 trees (of which around 150 were known to be female).

Conflict of opinion exists as to the original habitat of the tree. It is believed by some workers to be a component of floodplain forest (Peterken and Hughes, 1995; Tabbush, 1995). Ellenberg (1988) regarded black poplar as a pioneer species of floodplain forest in Europe, which matured into mixed broad-leaved forests, consisting of *Ulmus, Quercus, Fraxinus, Salix* and *Alnus*. In Britain, *Betula* could be added to this group (Peterken and Hughes, 1995). However, Rackham (1990) and White (unpublished) do not regard it as a woodland tree. An argument in favour of black poplar's place in floodplain forest is the presence of its European counterpart in the fragment floodplain forest on the River Drome (N Barsoum, pers comm) and the River Loire in France (personal observation). However, its appearance would have been very different from the isolated individuals seen today. As a heliophilous species, it requires light and space otherwise competition from other species prevents it from developing its characteristic silhouette.

Tansley (1949) mentions black poplar as being rare in oakwood, but occasionally found in marsh wood. Peterken (1996) believes that 'hedgerows and riverside fringes containing black poplar'…'can be regarded as fragments of the floodplain forest of major lowland rivers of Britain'. Bean (1976), suggests that 'it is a mistaken belief that poplar thrives on wet soil', and a study by Van Splunder *et al,* (1996) suggests that black poplar is drought tolerant. The diarist, Evelyn, regarded poplar as an 'aquatical' species (Evelyn, 1664). Cook (1724) refers to the tree as the 'water poplar' and states that it 'loves to grow by riversides, or in ground that is wet, or such as holds water much'. Gilbert-Carter (1932) regarded it as 'not uncommon on rich wet soils in southern and eastern England'.

1.4 Black poplar propagation

1.4.1 Traditional methods of propagation

Black poplar can be propagated via seed or cuttings, although in most cases propagation has been via the latter vegetative route. Cook (1724) recommended planting in wet ground, using 'truncheons around 2-8ft long'. This mode of propagation was supported by Evelyn (1664), who recommended the planting of truncheons, seven or eight feet long, in the ground. Loudon, cited in Elwes and Henry (1913) also describes how the tree grows easily from cuttings, but does not elaborate on cutting size. Contemporary nurserymen recommend taking cuttings approximately 25cm long, in February or March and placing them in damp ground, from which a 50% success rate can be expected (M Le Ray, pers comm). Successful planting of trees by volunteers has taken place in the Vale of Aylesbury, Buckinghamshire, using branches around 1.5m long, which have grown to some 3m in two to three years (A Holmes, pers comm). Natural vegetative reproduction frequently occurs if a fallen tree or branch is left undisturbed on damp ground, where it can take root and continue to grow (White, 1993).

1.4.2 Propagation using tissue culture

A more modern method of plant propagation is that of tissue culture (*in vitro* culture, (IVC)), where plants are regenerated from explants (small cuttings) of leaves and other plant organs (Bowes, 1999). IVC is a useful method of propagation for the conservation of endangered species, where the use of more traditional methods would be detrimental to a species, for example, where very low numbers of individuals exist. Propagation can be from either sterile or non-sterile mother plants, however, propagation from non-sterile plants is quicker and simpler (Bowes, 1999). The explant (approximately 2 x 2 cm^2 of plant tissue) is placed in a Petri dish containing agar (a gel made from seaweed) the dish is sealed with plastic film and incubated at 25^oC with continuous white fluorescent light for up to ten weeks, dependent upon the development of the explant. Once adventitious buds and roots are developed, the explants can be transferred to seed trays with ventilated plastic hoods containing compost for around ten weeks before transferring to larger pots in a greenhouse (Bowes, 1999).

The tissue culture method of propagation has been applied to *P. alba* and *P. canescens* species, with a greater level of success that with the conventional method of growing cuttings in a greenhouse. However, it is perhaps unnecessary to employ tissue culture propagation methods to black poplar, as it readily regenerates vegetatively.

1.5 History of black poplar

1.5.1 Black poplar in historical texts

This section utilises historical texts and flora descriptions to establish historical records for black poplar. The earliest detailed historical record of black poplar is by John Evelyn, in his classic work, *Sylva* (Evelyn, 1664), in which he discussed propagation, distribution and timber use. However, earlier passing references to poplar can be found. In 1310, a John Petye cut down one poplar in Nowton, Suffolk without permission and was fined two shillings; in 1422 an ancient, decayed poplar tree was condemned for growing too far over the King's highway at Great Canfield in Essex (Rackham, 1986). Unfortunately, no mention is made in either case of the exact species involved in these references. However, the trees in question may have been black poplar in view of the fact that hybrid poplars had not been introduced to Britain at that time. Furthermore, it is unlikely that a white poplar (thought to have been introduced from Holland) would have been mature enough to be decayed at that time.

Linnard (1982) mentions Hindes' forest nursery at Felindre near Newcastle Emlyn as holding 3,000 black poplars in their 1815 inventory. He describes the planting between 1804 and 1810 of 75,000 poplars in Denbighshire, but states these were hybrid species (Linnard, 1982). He also mentions a small plantation of black poplar in Tre-ffin in 1774, grown to analyse stem growth. It was found that those trees with the greatest growing space that were unpruned produced most stem volume (Linnard, 1982).

It can be seen from more recent literature that considerable confusion surrounds the correct identification of the species. Threlkeld (1727) briefly mentions that 'there is a sort of poplar called black', but does not elaborate. Edlin (1945) appears to be uncertain about the native status of the tree, stating that it 'is indigenous throughout Europe, and occurs in a wild state in Britain', and illustrates a hybrid poplar. By 1956, he writes that three or four poplars are probably native to Britain, of which black poplar is one (Edlin, 1956). In 1985, he suggests that the tree is one of Britain's 'few native kinds' and regards it as an uncommon species (Edlin, 1985). Step (1940) regards the tree as a 'common introduced species' and the accompanying illustration is in fact a hybrid species, suggesting that he was unaware of the identity of black poplar. Edwards (1962) lists *P. nigra* subsp *betulifolia* as the Manchester poplar, and says it is widely planted but does not confer native or non-native status upon it. Further confusion arose with the publication of *Atlas of the British Flora* in 1962, which included many records of hybrid poplars, giving a completely misleading distribution (Milne-Redhead, 1990). The well-known dendrologist, Alan Mitchell, regarded the tree as native to central and south-east England, and acknowledged its scarcity (Mitchell and Wilkinson, 1988).

1.5.2 Historical nomenclature of black poplar

The earliest reference to the name 'black poplar' occurred in the 17th century by Evelyn (1664). However, poplar was previously recorded in medieval documents as popel, popelar or popular, as distinct from aspe (aspen) or abele (white poplar), and therefore possibly denotes black poplar (Rackham, 1986).

The area in east London known as Poplar was first heard of in 1327 (Rackham, 1986). It is thought that the borough name, Poplar, is derived from the large number of trees growing in moist soil by the river, many of which were still standing in 1720 (Anon, 1927). As hybrid poplars were not introduced until the mid-eighteenth century, and white poplars were known as abele at the time that Poplar was named, it is possible that the trees in question were black poplar. A later article suggests that the singular name Poplar indicates that only one, distinctive specimen existed, which would have been 'held in view by those who passed along the great bend of the river between Limehouse and the Lea mouth' (Anon, 1933).

Cook (1724) refers to the tree as 'in most places called the water poplar', and supposes that it is the 'same which some call the Black-poplar'. Grigson (1958) describes local names for black poplar thus; in Lancashire it was know as catfoot poplar, due to dark knots in the wood. In Suffolk its name was the cotton-tree, as a result of the fluffy female seed, while in Somerset it was known as the water poplar, and in Cambridgeshire, the willow poplar. Gutch and Peacock (1908) mention that the black poplar, not the aspen, is known as the Shivver-tree in Marshland areas, such as Lincolnshire. Edlin (1985) suggested that it was called 'black' to distinguish it from white and grey poplars.

1.5.3 Historical timber uses

Prior to the decline of black poplar in popularity in the mid-18th century, the tree was regarded as an important timber species. Its fire resistant and shock absorbent properties made it suitable for such diverse items as bowls, sabot, small rafters, railings, basket making, cart floors, fence poles, spar-gadds, aircraft ribs, clogs, clothes pegs, railway brake blocks, cruck frames and flooring in agricultural buildings (Selby, 1842; Milne-Redhead, 1985; Milne-Redhead, 1990; White, 1993; Rackham, 1986; Rogers, 1995; Harris, 1974). A submerged, hollow trunk used as a well lining was found in Viking York excavations (Hall, 1984), and arrows found on the *Mary Rose* ship were discovered to be constructed from black poplar, alleged to be from a tree still growing in the town of Portsmouth (McKee, 1982), although it is unlikely that this connection can ever be proven. Trimmer (1866) stated that many trees in Norfolk were shredded for fuelwood, but Selby (1842) regarded the timber as almost useless for fuel. It was thought that early summer shoots were dried and stored for winter animal fodder (Rogers, 1995). A wooden chest in the church at East Bergholt in Suffolk was made from a black poplar half log (Rackham, 1986). Bark from the tree was used for tanning and fishing net floats (Selby, 1842). Within the counties of Herefordshire and Worcestershire, four barns have been found with cruck frames constructed from poplar wood, although the species is unknown (Harris, 1974). However, it is possible that black poplar was utilised as the characteristic curved shape of the species readily lends itself to cruck frame construction. The commercial importance of the tree is highlighted by Cook (1724) who suggests that ‘if you set one of these worth a half penny, if they grow they will bring you that yearly for twenty years or more’.

In Herefordshire folklore, poplar appears to be favoured over other timber species, as evidenced in this anonymous rhyme:

‘Cut me green and keep me dry
And I will oak or elm defy’ (Palmer, 1992).

As the distribution of pollards and standards throughout the country is unequal, it appears that its use as a timber tree was not universal. For example, whilst Herefordshire and Worcestershire have mainly pollards, Shropshire has only one site where five pollards and two coppiced trees exist. Pollards are abundant in hedgerows in Derbyshire, Herefordshire, East Anglia, the Vale of Aylesbury, Gloucestershire and Somerset, despite Rackham’s statement that ‘pollards are now uncommon except in Eastern England’ (Rackham, 1995). Approximately 90 pollarded individuals are to be found on Castlemorton Common, Worcestershire, and are regarded by some as ‘probably the best collection left’ (Snookes, 1986). However, all trees are male and probably planted examples, as they are located in small groups near houses. The trees were pollarded at differing times in order to provide poles of varying sizes (Snookes, 1986). Commoners used young branches for rough basketry, older poles for fencing, wattle and daub walls and ladders, together with fuel wood (Snookes, 1986). The Malvern Hills Conservators have undertaken a propagation programme of these trees to ensure their continued survival (Snookes, 1986). Rackham (1995) states that the Bishop of Ely utilised timber from black and white poplar growing at Brandon in the Breckland area, but does not suggest how the timber was used.

Black poplar was possibly widely used as a parish and county boundary marker, particularly in Cambridgeshire (G Easy, pers comm), although this theory is difficult to prove. In Shropshire, 200 black poplars were thought to have been used to mark the boundaries between Lordstone and Tankerville estates (R J Cook, pers comm), although only five trees remain today (personal observation). It is thought that the trees in Aylesbury were planted as boundary markers the time of the Enclosure Acts in the 18th century, and that black poplar was chosen because of its ability to survive in wet areas, which were prevalent around Aylesbury (M Davies, pers comm). Evelyn (1664) lists many timber uses, such as wooden vessels, trays, bowls and other 'turner's ware', carts, wine and hop-props and 'divers viminious works' and says loppings in January are for firewood and twigs, (supported by Trimmer (1866)) and those with leaves provide useful brooms. The Roman scientist and scholar Pliny, recommends the use of black poplars as support for vines (Newsome, 1964) and this practice continued in Italy until the last century (M Agnoletti, pers comm). The owner of two female trees and one male tree in Herefordshire thought the wood was utilised in hop kilns in view of its resistance to fumes (Watkins *et al*, 1997). The owner of 18 black poplars near Crickhowell in Brecon believes that the trees planted in his woodland were utilised for the cart industry that operated in the River Usk valley some 200 years ago (C Gardner, pers comm). The tree was depicted by John Constable in his famous painting *The Hay Wain* in 1821, and it is possible that the cart in the painting was constructed with black poplar timber (Tabbush, 1998).

1.6 Medicinal applications of black poplar

Balsam from the buds of black poplar was thought to be beneficial in treating bruises, inflammation and gout (Paterson, 1996). An ointment, known as *Unguentum Populeon*, made from black poplar buds, was used by the herbalist, Gerard, in the 15th century for inflammation and bruises (Grigson, 1958). Culpeper (1995) states that 'The water that drops from the hollows of this tree takes away warts, wheals and breakings out of the body'. Culpeper listed black poplar as a treatment for 'falling sickness', and 'warts and wens' (Culpeper, 1995). Friend (1884) also mentions the washing of warts with water collected from black poplar to remove them, as advocated by Culpeper. Gutch & Peacock (1908) discuss the practice of tying a lock of hair to a black poplar branch as a cure for ague, a shaking fever. Modern herbalists believe that the species can be used for the treatment of arthritis, bronchitis, haemorrhoids and rheumatic diseases (Potterton, 1983). In order to ascertain the medicinal value of the tree, it would be useful if research were to be undertaken on the chemical content of the bud exudate.

CHAPTER TWO – GENETIC VARIABILITY OF WOODY SPECIES

2.1 International studies on *Populus* sp

Liu and Furnier (1993) compared allozymes, RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers to determine genetic variation within and between trembling and bigtooth aspen *(P. tremuloides* and *P. grandidentata).* They found levels of similarity of 65% and 81% respectively for RFLP analysis; 68% and 72% similarity for allozyme analysis, and 70% and 65% for RAPD analysis. The authors concluded that trembling aspen has a greater level of diversity than bigtooth aspen and that RAPDs are useful markers but feel that caution should be exercised in their use for studies of genetic variation (Liu and Furnier, 1993).

Reports of levels of genetic diversity within trembling aspen *(P. tremuloides)* appear to be variable, with different studies finding genetic variation levels ranging from 23.5% to 42%. Cheliak and Dancik (1982) used isozyme analysis to study genetic diversity in 142 trembling aspen individuals and found 42% overall variation. A study by Jelinski and Cheliak (1992) of six populations of trembling aspen from a geographically restricted area in Alberta, Canada, found 32% variation. Hyun *et al*,, (1987) also used isozyme analysis to study genetic diversity in trembling aspen individuals from Ontario, and found 23.5% variation. Such wide-ranging results suggest that the methods of analysis may not be reproducible or that a high level of genetic variability does exist within this species, which is perhaps unexpected in view of its vegetative reproduction through suckering.

2.2 European studies on *Populus* genus

Legionnet and Lefevre (1996) studied allozyme variation in 60 black poplar trees from Bulgaria, Romanian, Slovakia, Hungary, Belgium and Italy, together with 111 from throughout France. They found that average diversity was greater in the non-French samples (74.9% similarity), whereas similarity levels in French samples 80.1% similar. The authors found that intra-stand differentiation was 3.5% and inter-stand differentiation was 1.7%, lower than averages previously found for long-lived, woody, outcrossing, wind-pollinated species, which are 7.6% and 9.9% respectively (Legionnet and Lefevre, 1996).

A study by Arens *et al*, (1998) found an average of 82.7% similarity between 143 *P. nigra* individuals on the banks of the Dutch Rhine system, using AFLP (amplified fragment length polymorphism) analysis. Their results suggested that vegetative reproduction tended to be a localised strategy, whereas recolonisation of new areas occurs generatively. The sub-species in the study was unknown (B Vosman, pers comm), but was likely to have been *P. nigra* subsp *betulifolia* in view of the fact that the study was conducted in western Europe, where subsp *betulifolia* is found. Although the level of diversity (82.7%) found was greater than that found within black poplar in the British

Isles (Cottrell *et al,* 1997; Winfield *et al,* 1998), it was felt by the authors to be low (Arens *et al*, 1998).

A study by van der Schoot *et al,* (2000) used a small number (8) of microsatellite markers to study genetic diversity within 23 trees from the EUFORGEN Core Collection of black poplar individuals from throughout Europe. These trees have been previously selected for morphological diversity observed within the species, as it was felt that selection would conserve maximum genetic diversity (S Bisoffi, pers comm). They found 71% similarity, and felt that the total genetic diversity within the species may be higher than previously found. Two trees from Yugoslavia and Slovakia had 25% and 38% similarity respectively, and it is possible, therefore, that these trees may not be pure *P. nigra* L. The collection includes two trees from Britain (Huntingdon female and Cambridge male), and these were found to be the most genetically similar trees in the study, supporting the findings of Cottrell *et al,* (1997) and Winfield *et al,* (1998). Further work is now being undertaken on a greater number of trees from throughout Europe (van der Schoot *et al,* 2000).

Hughes *et al,* (2000) studied the effect of differing water table depths and sediment types on male and female black poplars, using an experimental site on an alluvial island in the River Great Ouse in Cambridgeshire. They found that females tended to prefer wetter and more nutrient-rich sites than males, which is thought by the authors to be due to the fact that they have to invest greater effort in reproductive strategies than males. However, they found that, although females showed higher growth rates than males, it was not statistically significant, suggesting that there was considerable overlap in their requirements. A complementary genetic study of the individuals used in the study indicated that individuals shared around 75-80% of AFLP markers, indicating that genetic variation is very low, and supporting the findings of Cottrell *et al,* (1997), Winfield *et al,* (1998) and (Hughes *et al,* 2000).

2.3 British studies on *Populus nigra* subsp *betulifolia*

A RAPD study by Cottrell *et al,* (1997) on 36 individuals held in a clone bank, taken from trees throughout England and Wales, found an average of 94% similarity, with 17 distinct genotypes, implying that 50% of the population may be genetically identical. However, they feel that 50% is probably an over-estimate in view of the geographical range, as localised populations throughout the distribution are more likely to be clonal. Of six female trees in the sample, only two genotypes were identified, indicating that 33% of females are distinct genotypes. This level of diversity appears to be lower in the subspecies than that found at species level, as the same authors found 63% similarity in a RAPD study of *P. nigra* L using the Jaccard Coefficient, equivalent to 77% using the Nei and Li (1979) method of analysing similarity (Cottrell *et al,* 1997).

Winfield *et al,* (1998) reported on the Environment Agency AFLP study of 146 accessions of black poplar in the Upper Severn Area, covering Shropshire, Montgomeryshire, north Worcestershire and south-west Staffordshire. Most trees recorded on the national record, held by the Biological Records Centre of the Institute of

Terrestrial Ecology, were sampled to evaluate the level of diversity in an area that had suffered comparatively little perturbation. Results from this study indicated that little genetic diversity exists in the area studied, with individual trees on average 97% similar. One tree was identical to an individual 200 km away and most populations were found to be clonal, indicating that the species has been maintained by cuttings (Winfield *et al*, 1998).

CHAPTER THREE – FIELD SURVEY

3.1 Field survey and sample collection

The national record for the species, which consisted of some 2,500 trees, of which around 150 trees were known to be female, was obtained from the Biological Records Centre (BRC) at the Centre for Ecology and Hydrology (CEH) at Abbots Ripton, near Huntingdon in Cambridgeshire (formerly Institute of Terrestrial Ecology (ITE)) in order for individual trees to be selected.

The sampling strategy was based upon randomly selected individuals from each Vice County, together with important individuals or populations, where these are known to exist, such as the reputed relict populations at Welford-on-Avon, Warwickshire (S Falk, pers comm), Widmerpool, Nottinghamshire and Exeter, Devon (Watkins *et al*, 1997). Female trees, in view of their comparative scarcity, and very old trees that were thought to pre-date hybrid introductions, were also targeted. For example, the original Arbor Tree in Shropshire has fallen, but has been replaced by a sapling from the original tree, which is thought to date back to 1705 (P Hand, pers comm), prior to the introduction of alien species. Sites that are believed to have produced seedlings, such as a disused marlpit in Cheshire (Milne-Redhead, 1990) were also chosen for their importance. Eleven saplings from the trees at this site were planted by the River Stour at Sturminster Newton in Dorset (D Hobson, pers comm) and a tree from this site was specifically sampled. Twigs from individuals held in the European clone bank were kindly provided by Stefano Bisoffi of Institute at Casale Monferrato in order to grow leaves hydroponically. In addition, samples were collected from the Republic of Ireland for inclusion in the study.

Where little was known about a county's population (as a result of under-recording, for example), trees were selected by personal observation whilst in the field, based on factors such as location, number of trees and sex. For example, a lone tree in a riverside position would be regarded as an important tree, as it is a tree from a possible remnant population. In contrast, a row of sixty young trees around a playing field in Manchester would not be regarded as ecologically important as they were planted examples, although they have undoubted amenity value.

In view of the survey in the Republic of Ireland (Hobson, 1991), it was felt that west Wales and Cornwall may be part of the native range and these areas were therefore visited to assess their respective populations. In addition, as trees are recorded in the counties of Cumberland, Westmorland, Northumberland and County Durham, field visits to these counties were therefore considered necessary, although these areas were considered by Milne-Redhead, perhaps inaccurately, to be outside what he believed to be the native range, i.e. south of an imaginary line between the Mersey and Humber estuaries (Milne-Redhead, 1990).

Sample selection could not be made from written documentation alone. It was essential that each county was visited and visually assessed, as perceptions of naturalness and site descriptions can vary considerably. For example, a tree at Wadebridge, Cornwall is described in the national record as situated on the north bank of River Camel, but it is in fact a roadside tree some 400m from the riverbank. In addition, as approximately 80% of trees were unsexed, no true estimation could be made at the start of this project as to how many females existed in Britain.

3.2 Field methods and sample collection

Each tree selected for sampling was visited and surveyed. Approximately 2.0g of newly opened leaves were collected for DNA isolation. Young leaves, as opposed to more mature leaves, were selected in order to maximise the number of cells per unit area and thus obtain optimum DNA yields. In addition, young, healthy, actively growing leaves were less likely to have cross-contaminating infections and pests. Leaves from black poplar trees of all ages produce a viscous, yellow bud exudate, therefore, where possible, leaves were selected which appeared to have produced less exudate, as the presence of these secondary compounds may have impacted upon the quality of isolated DNA. The leaves were wrapped in aluminium foil, labelled and snap frozen in liquid nitrogen, and transported to the University of Nottingham for storage at -80^{o}C. The samples that underwent genetic analysis are listed in Appendix 1, and illustrated in fig. 2.1.

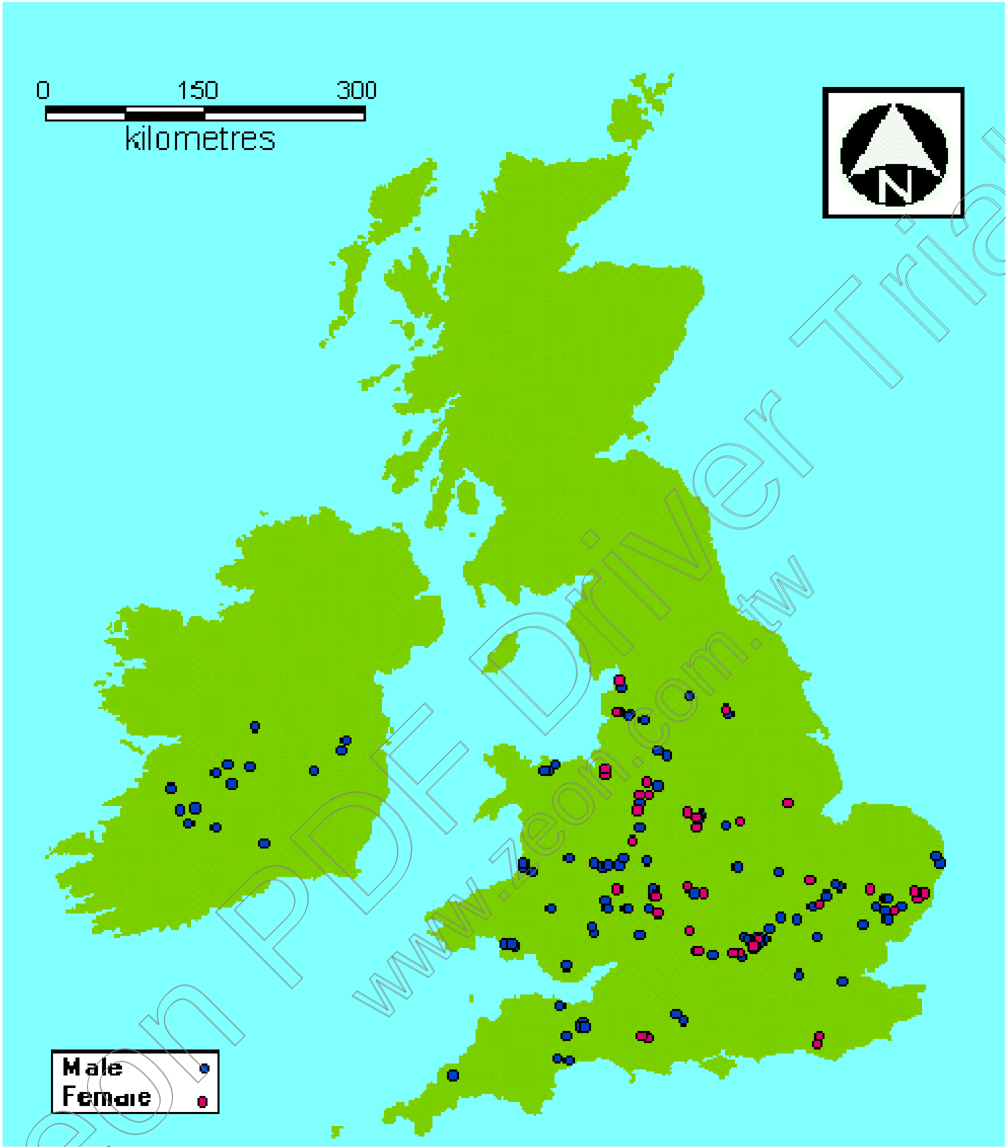

Fig. 3.1: Map of all samples analysed for genetic variation

3.3 Problems encountered in sample collection

Although the national record is generally accurate, there are instances where errors have occurred. The record contains a grid reference and a brief indication of location. In a number of cases (approximately 15%), the grid reference was found to be incorrect. Errors can occur for three reasons: first, human error in transposing figures in grid references; second, when a tree has been observed and a map consulted at a later date; third, hybrid poplars incorrectly identified as black poplar. However, where local records were used, such as those compiled by wildlife groups or County Councils, few inaccuracies were found. Some trees were no longer standing or had been removed for safety reasons. A number of trees at Corse Lawn had been recently pollarded, and no leaf material was present, whilst one tree in Gloucestershire was standing dead. Where landowner permission was essential for access to a tree, for example when a tree was in a private garden, and the landowner was not available, it was not possible to sample the tree. A further problem experienced during lone fieldwork was that of the limited time available for sample collection (due to leaf flush occurring in late May, June and early July).

CHAPTER FOUR – SURVEY RESULTS

4. Results
4.1 Field survey
4.1.1 Pollard and standard distribution

The distribution of pollarded black poplars as opposed to standard trees varies greatly, with pollarded examples clustering in Buckinghamshire, Derbyshire, Herefordshire, Worcestershire and East Anglia, where it is almost certain that cropped branches were utilised in some manner. It is believed that standard trees also had an important timber function as timber for construction of cruck frames. All confirmed pollarded specimens at the conclusion of this study are shown on the dot map in fig 4.1.

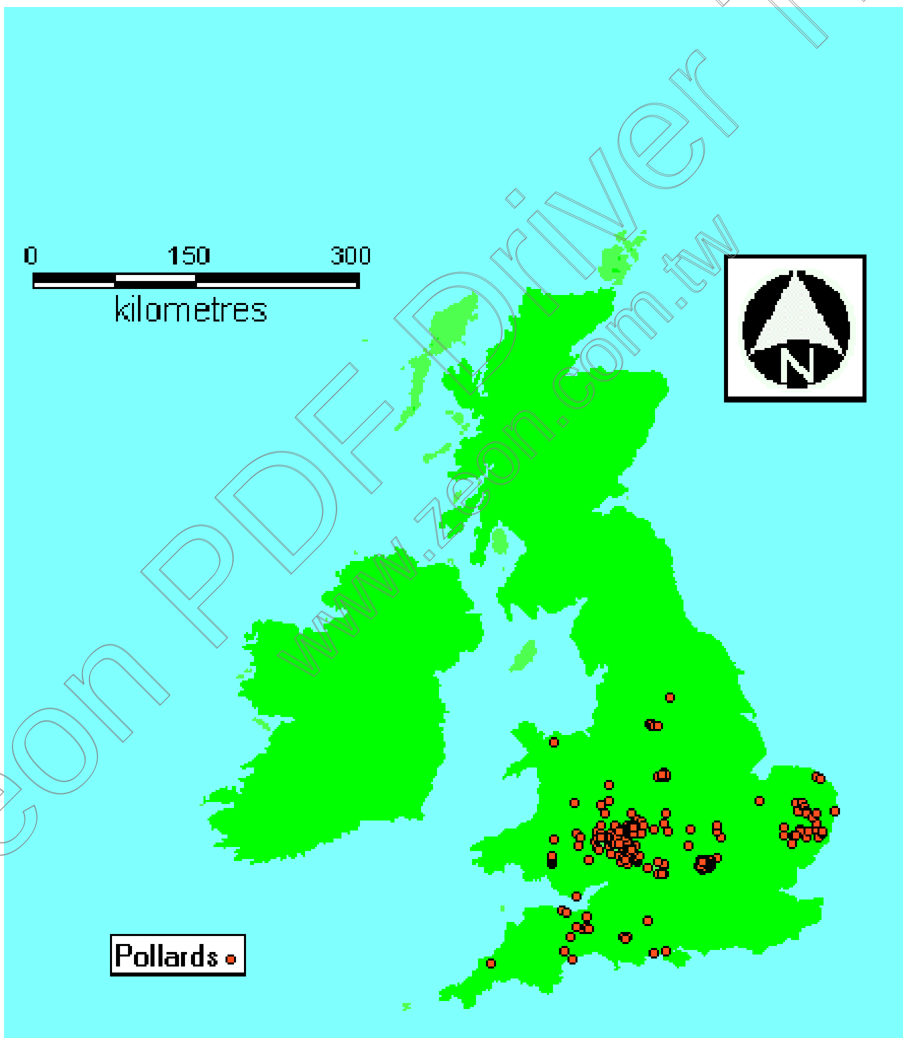

Fig. 4.1: All confirmed pollarded black poplars
at conclusion of this study

Rackham (1995) suggested that black poplar pollards are only common in East Anglia, and that few are found in the Welsh Border area and in the county of Devon. However, whilst his statement is correct about Devon, in Worcestershire, Herefordshire and Gloucestershire, many pollarded examples of black poplar can be found. It is unclear why Shropshire has only six pollarded black poplars, as a number of houses around Acton Scott, near Church Stretton in Shropshire, are reputed to have been constructed of black poplar timber (S Robson, pers comm), and these houses were not constructed with cruck frames. No pollarded trees have been recorded in Cheshire, Lancashire or Cumbria. Preliminary research into estate records held at the County Records Office in Aylesbury has not yielded any information regarding possible uses for the timber. Quite the reverse, as a carpenter living in Haddenham wrote (date unknown) there was 'no use for poplar, this went to Chesham for bush and broom making' (Rose, 1937).

4.1.2 Male and female distribution

Until recently, the female black poplar has been regarded as extremely rare. Prior to the mid-1990s, it was believed that only 150 existed in Britain (Rogers, 1995), probably because of under-recording. Rackham (1995) states that the 'female black poplar is very rare indeed'. It can be seen that the distribution of females is uneven (fig. 4.2), with strong concentrations in Dorset, Gloucestershire, Oxfordshire, Cheshire, Sussex and East Anglia. Interestingly, the most northerly female is in County Durham, an area not regarded as within the tree's native range (Milne-Redhead, 1990). Taking into consideration the unpopularity of females, it is unlikely that a female would have been transported over a large distance for planting purposes, when a more local male tree could have been used. In addition, it is unlikely that the sex of the tree was not known at the time of planting, as applying the same principle to planted female trees throughout Britain would probably have lead to the presence of many more females. All confirmed females at completion of this survey are illustrated in fig. 4.2.

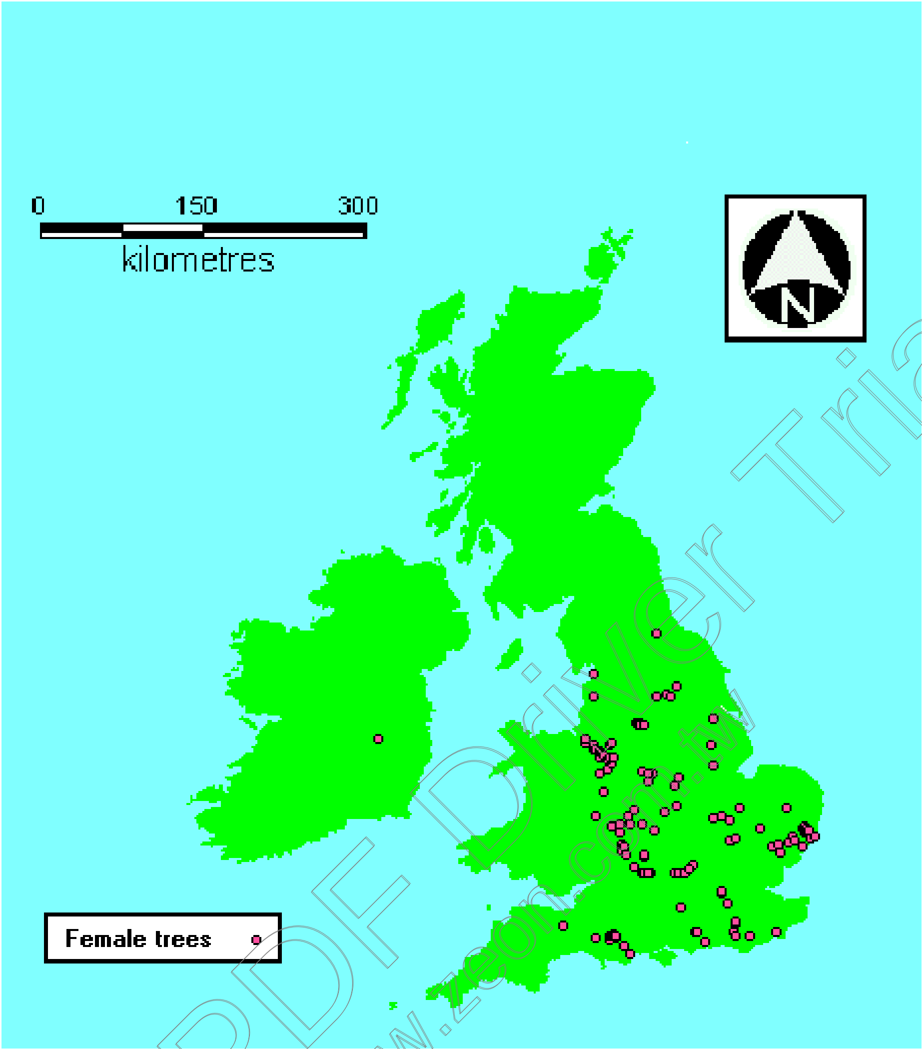

Fig. 4.2: All confirmed female black poplars
at conclusion of this study

4.1.3 Population and distribution of trees at conclusion of study

The distribution map (fig. 4.3) illustrates all trees recorded as a result of information collated for this study, together with those previously held by the Biological Records Centre (BRC). The total recorded population now stands at around 7,000 trees, with 600 confirmed female trees, with the possibility of further undiscovered trees (K Pyne, pers comm; L Weekes, pers comm; D Green, pers comm; M Anderson, pers comm; L Davies, pers comm; J Guest, pers comm; M Le Ray, pers comm; K Adams, pers comm; P Ennis, pers comm, P Jepson, pers comm; S Falk, pers comm). This figure of 7,000 does not include the large population of trees in and around Manchester, which is thought to number around 4,000 individuals, all of which have so far been found to be male. Major increases in recorded trees are in the Vale of Aylesbury, Clwyd, Cheshire, Lancashire, Shropshire, Somerset and Oxfordshire. The probable reasons for the increase in recorded trees as a result of this study are twofold:

- The result of under-recording by Milne-Redhead (1990), who excluded pollards and Manchester poplars from his 1973-1988 survey.
- The result of extensive recording of trees throughout Britain by various conservation groups and by the author for this study.

The current population of trees is illustrated in fig. 4.3.

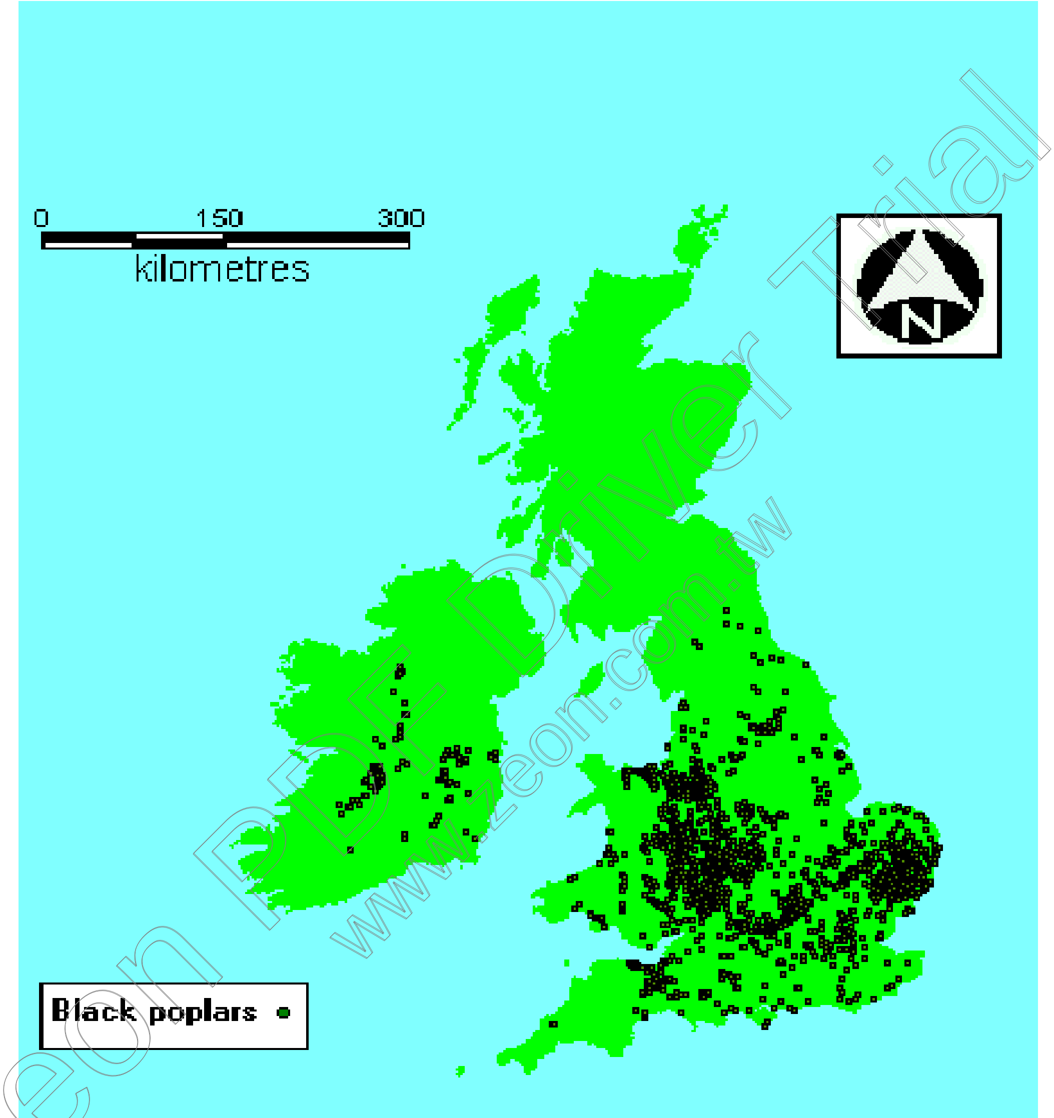

Fig. 4.3: All confirmed black poplars
at conclusion of this study

4.2 Summary

Accounts of black poplar in county floras suggest that confusion surrounding the distribution and origin of the tree has existed from the time of the first mention of black poplar by Evelyn (1664), and perhaps arises from the difficulties encountered with correct identification. Such confusion has therefore possibly given false impressions of the status of the tree at the time that the respective accounts were written. When *Atlas of the British Flora* was written in 1962, many hybrids were recorded as native trees, resulting in an inaccurate record of distribution and numbers.

When considering the national record for the tree, it should be emphasised that Edgar Milne-Redhead only included those trees that he felt to be important, such as those that were natural, or deemed to have been planted from what he regarded as natural trees, found in floodplain habitat, or hedgerows adjacent thereto. He did not include what is known as the Manchester poplar, and should this tree be included in the record, numbers of the tree would increase the national record to around 10-12,000 individuals. However, this potential increase does not imply either that the tree is not rare or that it is not endangered.

Despite the confusion regarding distribution and identification, recent survey work, both locally and for this study, together with improved identification skills, have resulted in a much clearer knowledge of the distribution, and established that northern Britain appears to be part of the native range. It is hoped that genetic analysis will further this understanding, by highlighting geographic relatedness of trees.

The results of the survey work which enabled production of the new map and increased the number of recorded trees, numbers of females and pollarded individuals, has given a complete global picture of the status of black poplar in Britain and will provide information to enable adequate conservation strategies to be implemented.

CHAPTER FIVE – GENETIC ANALYSIS RESULTS

5.1 Genetic analysis

AFLP analysis was chosen as being a suitable method of analysing genetic diversity in black poplar. Three fluorescent primer combinations (blue, green and yellow) were utilized which were analysed using a fluorescent analyzer.

5.2 Percentage similarity and clonal groups

Data from successful samples were used to produce similarity matrices (figs. 5.1 to 5.7) and dendrograms (figs. 5.8 to 5.15) in order to assess percentage similarity and derive clonal groupings (tables 5.9 to 5.16).

5.3 Similarity matrices and dendrograms

Resultant percentage similarities are shown below for each primer and primer combination (table 5.1). Ten similarity matrices were produced for the samples, including each primer individually and the various primer combinations. It is probable that the blue and green primer combinations were more reliable than the yellow primer combination, as results obtained from blue and green primer sets more closely reflect the findings of other studies (Cottrell *et al*, 1997; Winfield *et al*, 1998), who found 94% and 97% similarity in their respective studies. In addition, data were more reproducible with blue and green combinations. Traces produced by these primer combinations were clearer, suggesting that data derived from yellow traces should be viewed with caution.

Numbers of successful samples and the percentage similarity results from each of the primer combinations are as follows:

- **Mse1.1/Pst1.1 (yellow primer):** One hundred and eleven (111) samples successfully amplified using this primer combination, giving an average similarity of 70.10%.

- **Mse1.2/Pst.1.1 (green primer):** One hundred and twelve (112) samples successfully amplified using this primer combination, giving an average similarity of 87.65%.

- **Mse1.3/Pst1.1 (blue primer):** One hundred and forty (140) samples successfully amplified using this primer combination, giving an average similarity of 88.01%.

- **Combined data:** Sixty-seven (67) samples successfully amplified using all primer combinations, giving an average overall similarity of 81.34%. Eighty-nine (89) samples successfully amplified using the blue and green primer combinations, giving an average similarity of 88.44%; eighty-four (84) using green and yellow combinations, giving an average similarity of 74.40%; and eighty-six (86) using blue and yellow combinations, giving an average similarity of 79.70%.

Table 5.1: Overall percentage similarities calculated using Microsoft Excel v7.0 data analysis tools

Primer/primer combination	% similarity
Blue	88.01
Green	87.65
Yellow	70.1
BG	88.4
BY	79.7
GY	76.4
BGY	81.3

Male and female data: No specific differences were detected between male and female trees, and many were in fact identical, for example, tree numbers 12 and 13. In addition, a natural seedling (sample no. 21) produced in Cheshire was in fact identical to a number of both male and female trees from across the range tested.

Clonal group data: Clonal groups of black poplar were identified using computer-generated dendrograms where groupings of trees can be seen, the patterns displayed in the dendrograms reflect those displayed in the similarity matrices. Clonal groups are listed for each primer and primer combination in tables 5.9 to 5.16, and the number of groups identified for each primer and primer combination are illustrated in table 5.17. When a greater number of primers used to analyse clonal groupings, a greater number of clonal groupings are identified. For example, when analysing groups with all primer sets, thirteen clonal groups are identified, whereas the blue primer combination only identified three clonal groups. The presence of the yellow primer combination impacted strongly upon the number of clones, as it did upon the percentage diversity, reflecting the fact that it is likely to have given an unrealistically high clone differentiation. The six clones that were identified by the blue and green primer combination are perhaps a more realistic estimate, considering the wide geographical location. Winfield *et al*, (1998) identified four clonal groups from a geographically restricted area.

Table 5.2: Number of clones identified by individual primers and primer combinations

Primer or primer combination	No. of clonal groups
Blue	3
Green	6
Yellow	7
Blue/green	6
Blue/yellow	7
Green/yellow	8
Blue/green/yellow	13

5.4 Discussion

Although there appears to be a greater level of diversity in the species from the results of this study, it can be seen from the similarity matrices that there are a large number of trees that are identical (figs. 5.1 to 5.7), including the small group of trees that produced clear traces with few, but distinct, bands.

No specific differences were found between male and female trees, and a natural seedling was identical to other trees; trees from geographically isolated areas were often identical, for example, the tree from Holland (165) was identical to male and female trees from Suffolk, Caernarvonshire, Leicestershire and Lancashire. Sample numbers 213 (Portumna, RoI) and 215 (A2) appear to be genetically distinct from other trees when analysed by the blue and yellow primer combinations. However, the green primer combination does not separate these trees into different groups. Sample 213 is from the Republic of Ireland, whilst 215 is from the Vale of Aylesbury. There are three possible reasons why no specific variation between the sexes was detected. First, the fact that the markers used in the analysis may not reflect differences controlling sex determination in black poplar as only a small proportion of the genome was analysed. Second, it may reflect the fact that uncertainty exists regarding sex determination in Salicaceae. Stace (1975) states that, ‘as far as is known, *Salix* does not have an X-Y determining mechanism’, and therefore sex differences within black poplar may not be chromosomal. It is also possible that genetic differences may be present between the sexes, but only as a result of a single gene mutation, which is unlikely to be picked up by the use of three primer combinations and would require sequence analysis. Alstrom-Rapaport *et al*, (1998) successfully located a DNA marker associated with sex determination in *Salix viminalis* and suggest that sex determination is a complex process and several loci may be involved. It is possible that their methods may be appropriate in analysis of sex differentiation in male and female black poplar.

It can be seen from the similarity matrices (figs. 5.1 to 5.7) that one group of trees appears to be significantly different to other clones, displaying only 70%, 60% and 43% similarity respectively for blue, green and yellow primers. This group of samples display levels of similarity ranging from 51% to 68%, when analysed by all primer combination; had these samples been excluded from the study, overall similarity would have been much greater. However, these trees appear similar to each other (between 91% and 100% similarity), but are different from the remainder of samples, and it is therefore probable that these trees are a distinct clone. Given the high degree of similarity between the traces of this group it seems likely that the bands are genuine and not the consequence of artifacts due to PCR based errors. Based on morphological characteristics, all trees in this group were genuine *P. nigra* subsp *betulifolia* and did not have any characteristics associated with hybrid poplar.

The majority of the population of black poplars throughout Britain appears to have been planted (Tabbush, 1998) and the study by Winfield *et al*, (1998) concluded that the population has been maintained by cuttings. This view is supported by estate records which confirm that trees from Ireland have been planted in Somerset (M Anderson, pers comm), and it is probable that further research would highlight other instances of movement of trees by human agency.

5.5 Summary

Whilst these findings differ slightly from previous studies of genetic diversity in *P. nigra* subsp *betulifolia* (Cottrell *et al*, 1997; Arens *et al,* 1998; Winfield *et al*, 1998), and it is probable that the employment of an extra primer could account for the lower level of similarity displayed in the results of this study, particularly as the yellow primer produced 70% similarity, which would have impacted upon levels detected by all combinations where the yellow primer combination was included. This level of similarity is perhaps unlikely to reflect the true level of diversity within the species, and is more likely to occur as a result of the primer favouring non-specific products, which were non reliably amplified and thus incorrectly scored.

AFLP analysis was successfully conducted on 170 trees using three separate primers. A total of 78 bands were analysed with an average of 26 bands generated from each primer. The samples were taken from trees from throughout the range, including trees from the Republic of Ireland and a tree from Holland. Percentage similarities were 88.01% (blue); 87.65% (green) and 70.10% (yellow), suggesting that blue and green primers provided the most reliable results, which were repeated in reproducibility studies. When blue and green data were combined, the overall similarity was 88.44%, and this level of similarity was perhaps a more accurate estimation. No specific differences were found between male and female trees, and a natural seedling was identical to other trees; the tree from Holland was identical to trees from Suffolk, Caernarvonshire, Leicestershire and Lancashire. The level of diversity highlighted in this study is greater than that found by Cottrell *et al,* (1997) and Winfield *et al*, (1998), who found levels 94% and 97% similarity respectively, but less than Arens *et al*, (1998) who found 82.7% similarity on trees from the Dutch Rhine river system.

Comparisons with other studies of genetic diversity within the *Populus* genus indicate that greater levels of genetic diversity exist in other *Populus* species. *P. tremuloides* and *P. grandidentata* are approximately 68% and 72% similar (Liu and Furnier, 1993); other studies on *P. tremuloides* have yielded variable results ranging from 23.5% to 42% diversity (Cheliak and Dancik, 1982; Hyun *et al*, 1987; Jelinski and Cheliak, 1992; Chong *et al*, 1994).

Comparisons with studies on other woody species (*Eucalyptus nitens;* 78.2% similarity (Byrne and Moran, 1994); *Grevillea scapigera;* 75% similarity (Rossetto *et al*, 1995); *Camellia sinensis;* 69.5% similarity (Wachira *et al*, 1995); *Cocus nucifera* L; average 81% similarity (Perera *et al*, 1998); *Castanea* sp.; average 83.5% (Yamamoto *et al*, 1998); *Olea* sp.; average 63.33% similarity (Angiolillo *et al*, 1999); *Moringa oleifera;* average 70.51% similarity (Muluvi *et al*, 1999), indicate that average genetic diversity is lower in black poplar than other woody species.

CHAPTER SIX – CONCLUSIONS AND STRATEGIES

6.1 Origin of black poplar in Britain

The findings from this study suggest that a great deal of human intervention has taken place with black poplar propagation, which may have occurred over some 1,000 years. Possible origins of black poplar include:

- Native, recolonising Britain unaided by humans, after the last ice age.
- Introduced from western Europe by human agency.

This study has provided new information, which helps to bring forward debates regarding the true native status of black poplar in Britain. The absence of a reliable pollen record makes it impossible to confer native or non-native status upon black poplar on this basis alone. Most trees in the British population are planted examples, suggesting that survival of the species has occurred as a result of human intervention. The species occupies an important place in the British flora, folklore, landscape, popular imagination and to a lesser degree, literature, and for the reasons previously stated it is important to implement a conservation programme. Any conservation programme should ensure provision is made for a long-term planting strategy.

6.2 Suggestions for conservation strategies

The popularity of black poplar has resulted in a number of ad hoc planting schemes throughout Britain (A Holmes, pers comm). However, since the publication of Jonathon Spencer's action plan (1994), no updated document has been produced. Since his action plan, knowledge of black poplar ecology and genetic diversity has greatly increased, and it is now important to disseminate this information into a national action plan.

The results of this study, and previous studies on British black poplar (Cottrell *et al,* 1997; Winfield *et al,* 1998), indicate that there does not appear to be any biological reason why one individual tree should be favoured over another in any planting or conservation strategy. However, the historical aspect of the study has highlighted the fact that there are historical and cultural reasons why it is desirable to use local trees when planning clone banks or planting programmes. In addition to historical and cultural reasons for using local trees, there is the morphological variation of trees between east, west and north and it is desirable to maintain this localised variation, although the reasons for such variation are unclear, and morphological variation was not studied. It is possible that future research using different methods of genetic analysis may address this question.

An *ex situ* conservation strategy already exists in the form of a collection of black poplars from throughout Britain that is held in a national clone bank in Norfolk (J White, pers comm). This bank should now perhaps be extended to include a tree from each clonal grouping identified by this study, together trees of each sex from the differing three leaf morphologies. It is desirable to utilise *in situ* methods to ensure the continued survival of black poplar in its natural habitat. Any planting programme should make provision for long-term planting; the population is currently even-aged, with many trees reaching the end of their natural lifespan. In the event of black poplar only remaining a fashionable topic for several years, this situation could then be repeated in the future, which is an undesirable outcome.

The reasons for the conservation of black poplar in Britain are as follows:

- Britain's commitment to the national biodiversity agreement signed in 1992 at the Rio summit.
- The species' commercial importance as one parent of the hybrid *P. euramericana.*
- The lack of natural regeneration and the fact that the population is even-aged, whereby the species could become extinct within 20-30 years.
- The diverse invertebrate fauna supported by black poplar.
- The species' potential for inclusion in floodplain forest, planting of which is currently being researched, particularly for flood control.
- The tree occupies an important place in Britain, not only from an ecological standpoint, but also from cultural and historical standpoints, and these are further reasons for its conservation.
- The tree's significance as a landscape feature.

6.3 Planting guidelines
6.3.1 Propagation

Taking cuttings is quite simple. it is important to make sure that the poplar has been identified as a true native black poplar before cuttings are taken. It is imperative to gain the approval of the landowner.

When collecting material, thought should also be given to the ratio between male and female trees. Female trees are popular because of their rarity but it is important to remember the problems encountered with the copious amounts of fluffy seeds that are produced.

Very often older trees will be lacking the suitable young growth required for cuttings. Newly cut pollards are an excellent source of material. Older trees often form vigorous side shoots from the bark which are also suitable. Great care should be taken when collecting material from Britain's tallest native tree! In addition, older trees can be extremely unstable and should be treated with respect and caution.

Poplar will grow from hardwood or softwood cuttings; the latter requires more elaborate horticultural technology. Hardwood cuttings are taken in the autumn and winter when the tree is largely dormant, the current year's wood has ripened, but root growth still takes place. Softwood cuttings are best taken between the end of July and end of August. It is possible to continue into October, but success rates are poor. Since very good results can be obtained from the hardwood cuttings, it is likely that this will be the most common method chosen. Softwood cuttings may be considered in an emergency situation if a tree collapses or has to be felled during the summer months.

Whilst hardwood cuttings can be taken any time over the dormant months, John Evelyn(1664) tells us to collect cuttings 'after the first full moon in January'. Cuttings taken before Christmas usually start to root and these may then be broken by frost heave in the soil during the latter part winter. Many have reported that cuttings taken in February and March have been extremely successful.

Cuttings should be taken from new growth made in the previous summer. They should be 150-200mm long with at least half their length below ground. The cuttings can be rooted straight into the open ground. Cuttings must be well labelled during their life in the nursery. Weed control is very important over the first year. Using a black plastic or felt mulch can reduce competition from weeds. Rooted cuttings can usually be planted out after their first year. In order to relieve pressure on native trees, save time in collecting material and produce vigorous cutting material, the establishment of stool beds is recommended.

Whilst there is no biological reason why any particular tree should be favoured over another for propagation purposes, it is important to retain the existing distribution pattern of black poplar in Britain, in view of localised morphological variation, and to ensure that sites of key importance are maintained, both by implementation of planting programmes and protection of existing trees. Areas with relatively large populations of black poplar are as follows:

- Welsh borderland
- Vales of Clwyd and Conway
- Somerset and Dorset
- Vale of Aylesbury
- Suffolk and Essex
- Dove Valley, Derbyshire

Where isolated examples of trees are found, for example, river valleys in Yorkshire, Northumbria, Cumbria and Lancashire, consideration should be given to maintaining the presence of trees in these areas.

In addition, there may be cultural or historical reasons why the planting of local trees might be favoured, for example, the Arbor Tree in Shropshire, which is perhaps Britain's most famous black poplar, is of great cultural significance nationally.

A further consideration when implementing a planting programme is that of a long-term strategy; it is important to ensure that a population of different aged trees is created in order to establish long-term stability. Careful consideration should be given to the choice of planting site, and the following factors should be taken into consideration:

- The root system can spread to a radius equivalent to the height of the tree, and new trees should therefore be planted well away from any structures and underground services.

- The ability of the *Populus* genus to dry out wet ground should be considered when introducing new black poplars to wetland or areas of archaeological importance in order to prevent damage to the existing features of a landscape.

- As with all tree planting, the effects of shade and leaf fall upon the existing habitat should be considered. The choice of site for female trees is particularly important in view of the potential nuisance factor of fluffy seed.

- Any planting site needs to have full light, a good moisture supply, alluvial soil and a lowland climate. Trees should be planted at approximately 20m apart in order for them to develop their characteristic silhouette.

- Consideration should be given to the re-creation of floodplain forest, research into which has been undertaken by the Forestry Commission and the Environment Agency.

- Where large numbers of pollarded trees are found, creation of new pollards should be considered, as pollarding extends the life of a tree, ensures continuation of a valuable landscape feature and provides a timber source.

- All cuttings should be labelled with details of the parent tree.

6.3.2 Management guidelines

Newly planted saplings should be protected from grazing animals by the provision of tree guards, and once established, by wooden tree crates, or suitable fencing. Weed control is very important during the first year, and laying black plastic or felt mulch and making small slits for the sapling to grow through can reduce this.

It is vital to look after mature trees that must be protected from physical damage. If they are under threat, it may be important to fence off a tree. As with any tree, poplar will suffer if there is any compaction to the root zone or physical damage such as from grazing or ploughing. Changes to the water table can also have a detrimental effect upon the tree. However, older trees can become very dangerous and unstable, safety should never be overlooked and the advice of a qualified tree surgeon should be sought.

6.4 Future research

- Implementation of a recording system that makes provision for ecologically and historically important trees and approximate age of trees, which is not currently undertaken by BRC.

- Further research into morphological variation within black poplar, in association with genetic analysis, taking into account the methodology of this study.

- Analysis of chloroplast and mitochondrial DNA may reveal differences between the sexes of black poplar, and may reveal greater levels of genetic diversity to support the morphological variation that is present. Microsatellite analysis may be a more suitable method, now that more sequence information is available for black poplar.

- Recording of currently unlisted trees in Vale of Aylesbury, Manchester and other counties should be conducted, together with sexing of those trees not visited as part of this study. Further surveying of the population in the Republic of Ireland is likely to locate more trees, as the survey by Hobson (1991) took place by car and thus only recorded trees near roads.

- Estate records should be examined, particularly in those areas where black poplar is found in large numbers, such as Aylesbury and south Derbyshire, to establish reasons for planting and timber use.

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Appendix 1

Table illustrating successfully amplified samples (B = blue primer, G = green primer and Y = yellow primer)

No	Sex	Site	East	North	B	G	Y
1	F	Bury St Edmunds	5858	2643	*	*	
2	F	Marlesford	6322	2581	*	*	*
3	F	Friday Street	6375	2605	*	*	*
8	M	Stratford St Mary	6042	2342	*	*	
10	M	Westerfield	6176	2483	*		
12	F	Campsea Ashe	6315	2559	*	*	*
13	M	Hadleigh	6025	2422	*	*	*
17	M	Lowestoft	6526	2907	*		
18	F	Hintlesham	6097	2432			
19	M	Gt Finborough	6016	2555	*	*	*
21	M	D2	3782	1136	*	*	*
22	M	Brent Eleigh	5933	2479		*	
23	M	Combs	6038	2567	*	*	*
24	F	Framlingham	6285	2631	*		
25	F	Widmerpool	4627	3278	*	*	*
27	F	Sleaford Park	5073	3456	*	*	*
28	F	Wootton Wawen	4149	2623	*	*	*
29	F	Powick Hams	3847	2515	*		
30	M	Hampton Loade	3746	2868	*		
31	F	Sturminster Newton	3790	1140	*	*	*
32	F	Ivington B	3477	2568		*	
33	F	Middlewich	3727	3635	*		
34	F	Sealand	3352	3689	*	*	*
35	F	Nantwich	3669	3498	*		
36	F	Newton with Scales	3446	4312	*		
37	F	Bourton on Water C	4177	2189	*		
38	F	Leighton	3611	3056	*	*	*
39	F	Lower Lode	3882	2346	*	*	*
41	M	Little Moreton	3844	3599	*		
43	F	Warpsgrove	4649	1985	*		
45	F	Engelsea	3750	3507	*		
46	F	Moreton Morrell	4304	2564	*		*
48	F	Market Drayton	3658	3343			*
49	M	Colwyn Bay	2862	3784		*	*
50	M	Ivington C	3477	2568	*		
51	M	Brewood	3477	2568	*		
54	M	Pye Brook A	3501	2821	*		

57	M	Westbury on Severn B	3715	2137	*		
58	M	Conder Green	3459	4559	*		
69	M	Newcastle on Clun hill	3260	2831	*		
71	M	Bodnant	2797	3724	*	*	
73	M	Tyn-y-Groes	2773	3719			
74	M	Powick Island	3835	2523	*		*
80	F	Widmerpool A	4627	3278	*		
87	M	Mordiford	3571	2375	*		*
88	F	Branston A38	4208	3192	*		*
89	M	Welland	3778	2400	*	*	
90	M	Cold Hill Farm	4466	4354	*	*	
98	M	Beambridge	3533	2883			*
102	F	Bourton on Water B	4177	2189	*	*	
104	M	Llandinam	3015	2868	*		*
110	M	Ickleford	5185	2316	*		*
116	F	Cold Hill Farm	4466	4354	*		
118	M	Dyfatty	2454	2008	*	*	*
119	M	Llanelli Park	2533	1985	*	*	*
120	M	Llanelli Wet/wild	2518	2004	*		*
125		Watlington	4683	1948	*	*	*
126	M	Darwen	3686	4228	*		*
127	M	Stratford on Avon	4212	2560	*		*
132	M	Long Whatton Mill	4493	3233	*	*	*
135	M	Little Wishford	4065	1369	*	*	*
141	M	Preston	3552	4288	*		*
142	M	Duck Street	3369	2457	*		*
147	M	Llanwenarth	3275	2145			*
148	F	Friday St	6380	2603	*	*	*
151	M	Guinea Farm	4249	3320	*		*
152	M	Audlem	3662	3415	*		
154	M	Shenmore	3394	2384			
160	M	Hallow	3822	2585			*
161	M	Gellirhydd A	3248	2197	*		
162	M	Checkley Common	3599	2384			
164		N251			*		
165		IBN-1238			*	*	*
167		N041			*	*	
168		Vulhm 88045				*	*
200	M	Monaster	1505	2401	*	*	
202	M	Herringfleet	6481	2976	*	*	*
203	M	Clare Castle 1	1304	2705	*	*	*
204	M	Clare Castle 2	1305	2704	*	*	*
205	M	Halstead, Essex	5803	2287			*
206	F	Freeman's Wood	3453	4615	*		*
207	M	Ilkley	4116	4481	*		

208	M	Ferrybridge	1407	2503	*	*	*
209	M	Clonmel	2230	1280	*	*	*
212	M	Birr 1	2006	2004	*		*
213	M	Portumna	1806	2005	*	*	*
215	M	A2	4887	2127	*	*	*
216	M	Ware	5388	2147		*	
217	M	Gorteeny	1706	1906	*	*	*
218	M	Vicarstown	2870	2370	*	*	*
220	F	Newton with Scales 1	3443	4314	*	*	
221	F	Lewes	5411	1110		*	
222	M	Sherburn	4484	4327	*	*	
223	M	Wadebridge	1992	715		*	*
224	M	Athlone dup	2004	2400	*	*	*
225	M	Monaster dup	2860	2250	*	*	*
226	M	Birr 1 dup	1860	1820	*	*	
228	M	Clare Castle 2 dup	1305	2704	*		*
229	F	Isfield	5441	1181	*	*	*
231	M	Bamber Bdg 2 dup	3540	4266	*	*	*
232	M	Portumna dup	1806	2005	*	*	*
233	M	Mungret dup	1560	1550	*	*	*
234	M	Ferrybridge dup	6406	9504	*	*	
235	M	Cambridge Bot Gar	5454	2573	*		
236	M	Aberllolwyn	2587	2772	*	*	
237	M	Burwell	5582	2657	*		
239	M	Cambridge Pk	5454	2568	*		*
240	F	Child Okeford	3826	1129	*	*	*
241	M	Hillfarrance 1	3171	1229	*	*	*
242	M	Aberystwyth Park N	2594	2809	*	*	*
243	F	Shepreth	5399	2471	*	*	
244	F	Grafton 2	4268	1998	*	*	
245	M	Welford Nhants	4613	2811	*	*	*
247	M	Norton Fitzwarren	3195	1255	*		*
248	M	Dunster	2980	1431	*	*	
249	M	A27	2704	4141	*	*	
250	M	Uffculme	3055	1117	*		
252	M	A30	2779	4095		*	
253	M	Bassingbourn	5323	2451	*	*	
254	M	Leighton Buzzard	4924	2224	*	*	
255	M	A10	2852	2107	*	*	
256	M	Leighton male	3611	3053	*	*	
257	M	A14	2845	4136	*	*	
264	F	St Ives	5313	2711	*		*
265	M	A46	2814	4054	*	*	*
266	M	A42	2779	4024	*	*	*
267	F	A53	2791	4042	*		

268	M	Hilton	4231	3309	*	*	
269	M	A19	2839	4144	*		
271	M	Timahoe	2620	2040	*	*	
272	M	A1	2887	4126		*	*
273	M	A51	4791	2043	*	*	*
274	M	Flitwick 3	5031	2340	*		
275	M	Manchester	3834	3936	*		*
277	M	Manchester	3911	3905	*		
278	M	A10	4851	2107		*	*
279	M	Thrapston	4990	2787	*		
280	M	A14	4846	2136	*	*	
281	M	Hopton Castle	3352	2788	*		
282	M	Swanage	3025	1804	*	*	*
283	M	Fiddleford Inn	3805	1131	*	*	*
284	F	Stadhampton	4600	1984	*	*	*
285	M	West Buckland	3177	1210		*	*
286	M	Salisbury 1	4140	1298		*	*
287	M	Kelmscott 2	4251	1992		*	*
288	F	A47	4830	2122		*	*
289	F	Bagber	3765	1146		*	*
290	F	Doveridge 2	4133	3338	*	*	*
292	M	Swaffham Prior	5530	2668	*	*	*
293	F	A49	4791	2043		*	*
294	M	A41	4741	2119	*	*	*
295	M	Kelmscott 1	4251	1992		*	*
296	F	Hilton 2	4235	3308		*	*
297	M	Lyford 2	4392	1951		*	
298	M	Clyst St Mary	2973	914		*	
299	F	Hatton 4	4221	3309		*	*
300	M	Arbor Tree	3392	2818			*
301	M	Newton Poppleford	3080	897		*	*
302	M	Trull	3213	1225		*	
304	M	Hammersmith	5231	1778			*
306	M	A13	4846	2136		*	*
307	M	Marston on Dove 2	4235	3292	*	*	*
308	M	Elton	3459	3743	*	*	*
309	M	Hammersmith	5231	1778		*	*
310	M	Ballyshrule	1770	2380		*	*
311	M	Gatesheath	2473	3604	*	*	*
363	M	Kynnersley	3683	3179		*	
384	M	Trawscoed B	2674	2718		*	
A26	M	Aylesbury	4794	2143	*	*	
GE	U	Gravesend	5635	1728	*		

Appendix 2

Table 5.9: Three clonal groupings identified from dendrograms produced from the blue primer combination dataset

Clone 1			Clone 2	Clone 3
1	231	151	12	209
2	232	164	13	213
3	233	165	17	215
8	234	167	18	
10	235	202	21	
19	236	203	24	
25	237	205	27	
35	239	206	28	
36	240	207	29	
43	241	208	30	
45	242	212	31	
46	243	217	33	
69	244	218	34	
71	247	220	37	
73	248	222	38	
74	249	224	39	
80	252	225	50	
87	253	226	51	
88	254	228	54	
89	255	229	57	
90	256	286	58	
39	257	287	98	
41	264	290		
50	265	292		
51	266	294		
54	267	295		
57	268	297		
58	269	301		
98	271	302		
102	272	303		
104	273	304		
110	274	306		
116	275	307		
118	277	308		
119	278	311		
120	279	A26		
125	280	GE		
127	281			
132	282			
141	283			
142	284			
148	285			

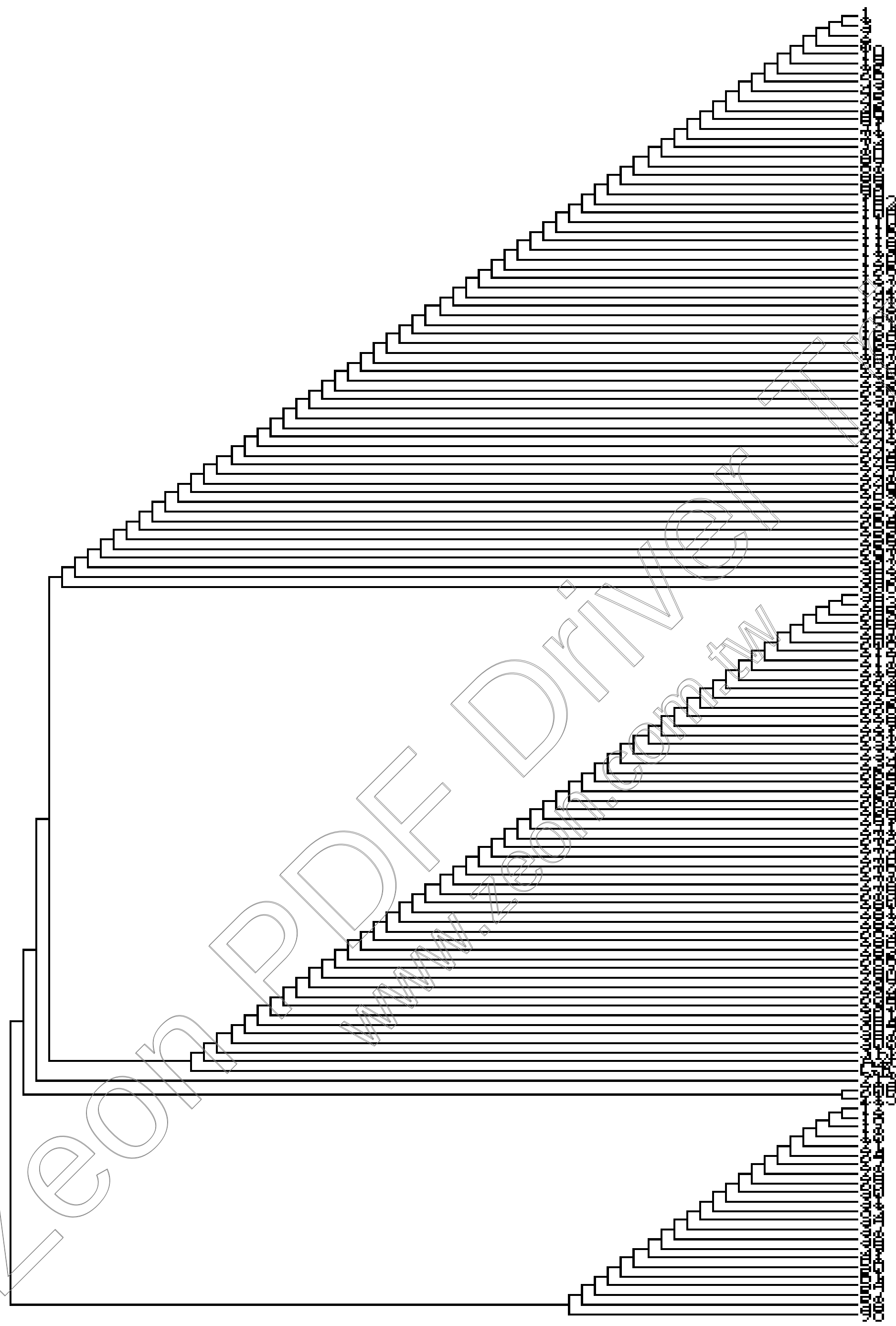

Fig. 5.8: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the blue primer combination

Table 5.10: Six clonal groupings identified from dendrograms produced from the green primer combination dataset

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6
1	3	223	209	236	12
2	208	265		237	13
8	217	266		240	19
22	218	267		241	21
32	221	273		242	23
49	222	275		243	25
71	224	280		245	27
89	225	282		246	28
90	226	283		248	31
102	229	285			34
118	231	286			38
119	232	287			39
122	233	288			
132	234	289			
135	A26	290			
141		292			
142		293			
147		294			
148		295			
151		296			
165		297			
168		300			
200		301			
202		302			
203		303			
204		304			
213		306			
215		307			
216		308			
220		309			
232		310			
254		311			
255		GE			
256					
257					
269					
272					
298					
299					

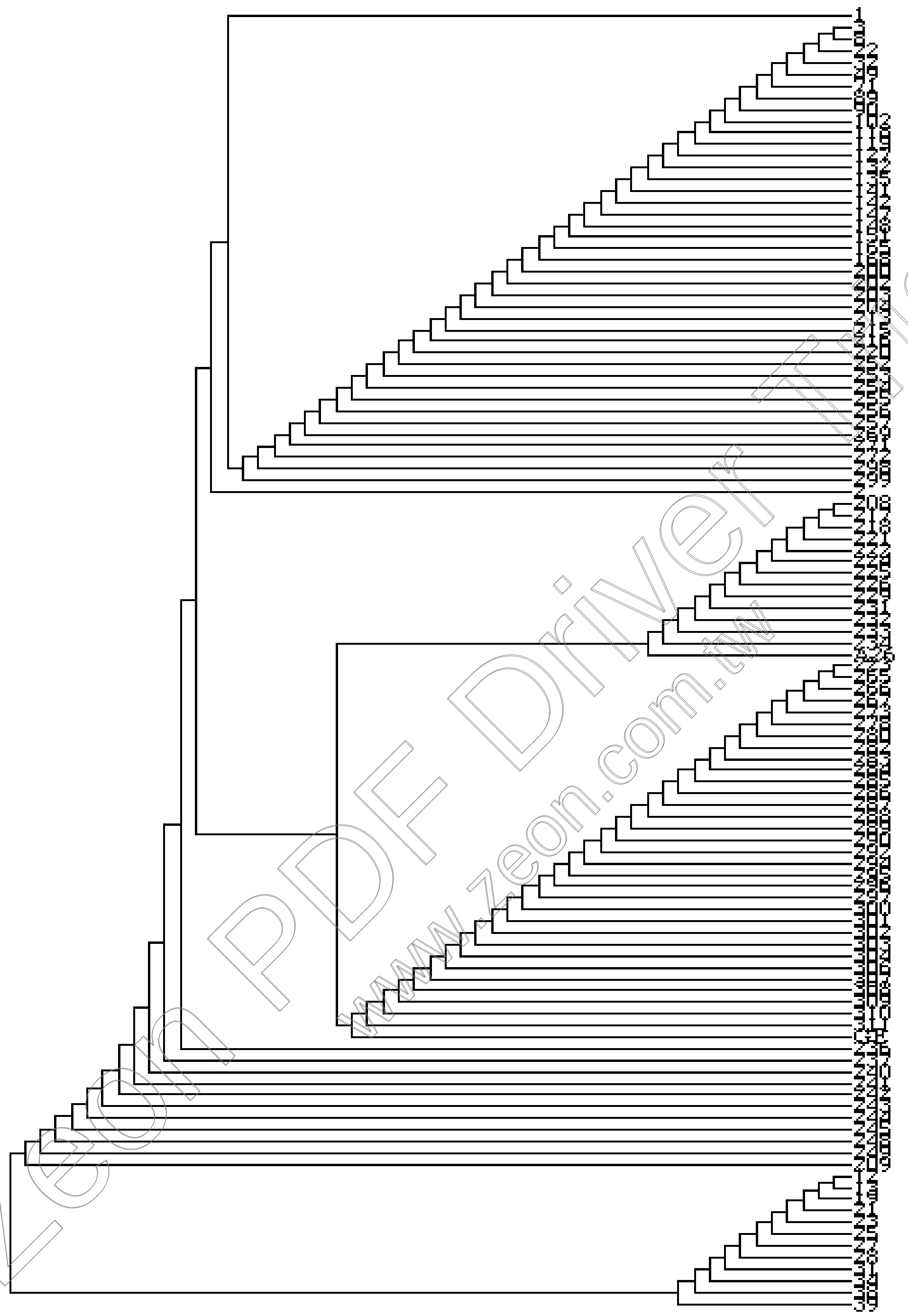

Fig. 5.9: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the green primer combination

Table 5.11: Seven clonal groupings identified from dendrograms produced from the yellow primer combination dataset

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6	Clone 7
2	165	215	208	213	12	13
3	240		237		34	19
90	241		257			21
102	242		265			31
118	245		266			38
119	302		267			39
127	306		269			
132			271			
141			272			
142			273			
148			278			
151			280			
217			282			
218			283			
222			284			
224			285			
225			287			
226			290			
231			292			
232			294			
234			295			
286			301			
307			308			
GE			311			
			A26			

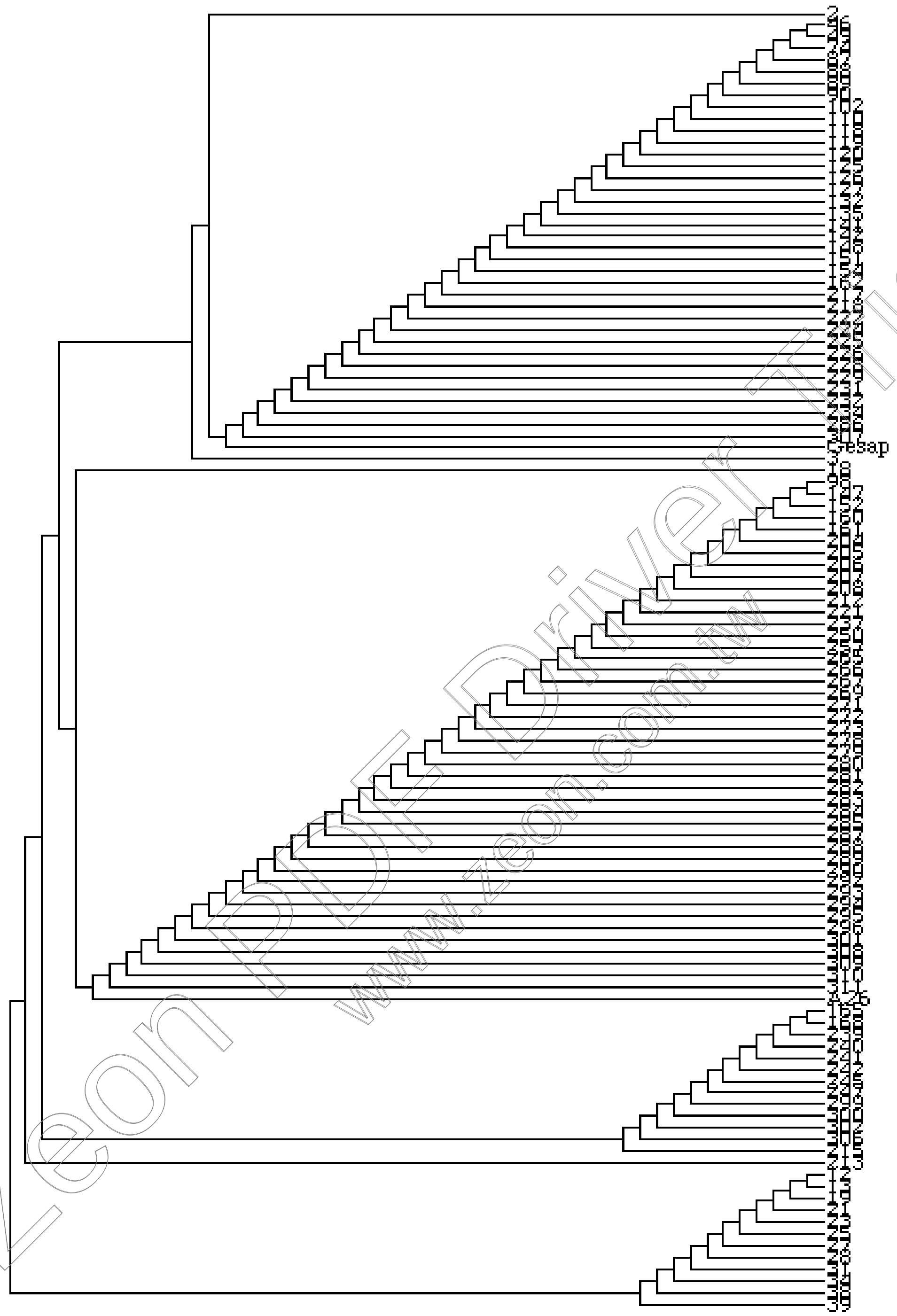

Fig. 5.10: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the yellow primer combinati

Table 5.12: Eight clonal groupings identified from dendrograms produced from the green and yellow primer combinations

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6	Clone 7	Clone 8
2	3	165	215	147	269	237	12
49	217	168		204	271	308	19
89	218	299		257	272	221	13
90	222	300		265		A26	21
102	224	302		266		213	23
118	225	306		267			25
119	226	240		273			27
127	229	241		278			28
132	231	242		280			31
135	234	245		282			34
141				283			38
142				284			39
148				285			
151				287			
286				288			
307				289			
GE				290			
				292			
				294			
				295			
				296			
				301			
				308			
				309			
				310			
				311			

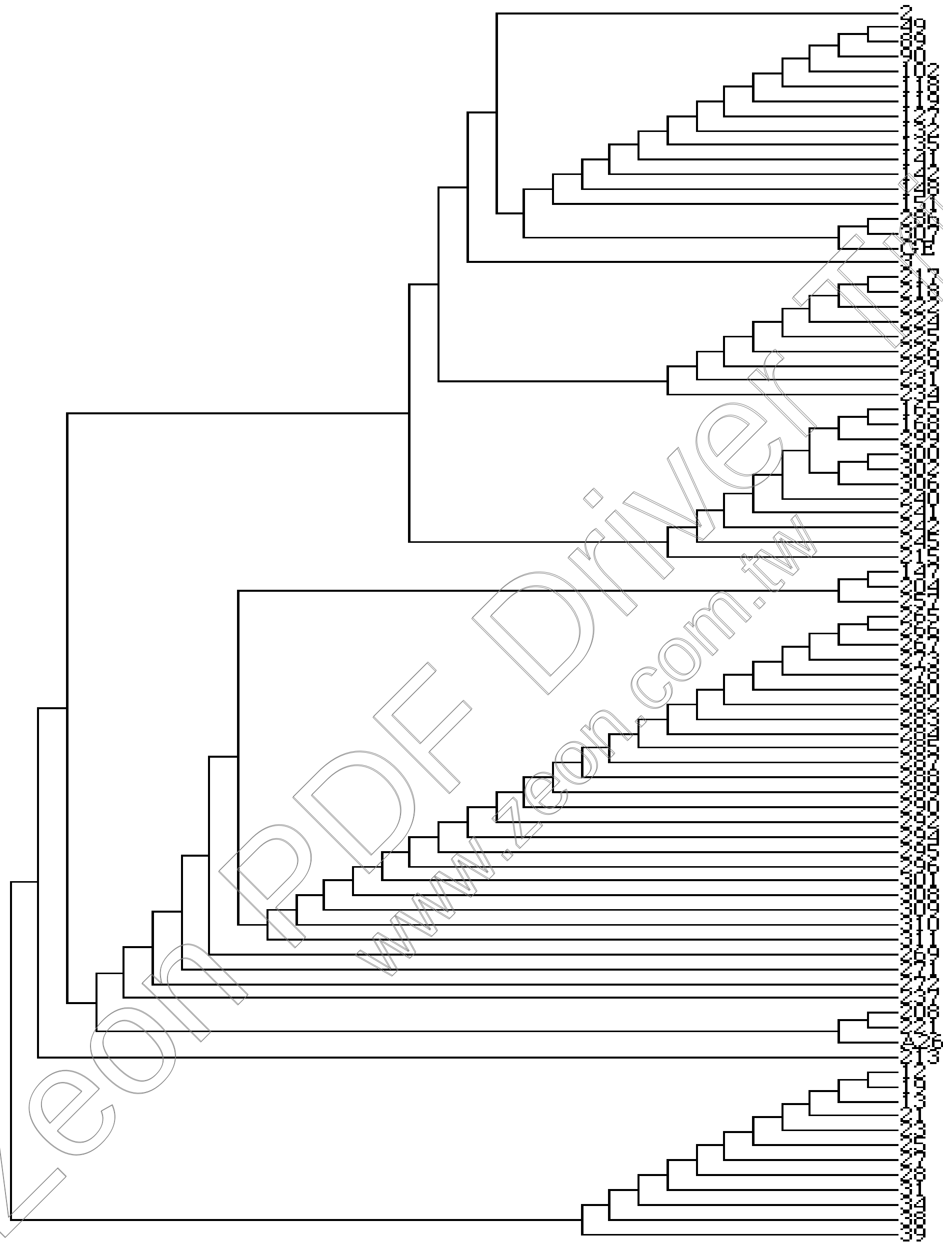

Fig. 5.11: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the green and yellow primer combination

Table 5.13: Six clonal groupings identified from dendrograms produced from the blue and green primer combinations

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6
1	2	236	208	266	12
297	3	237	217	267	28
302	71	240	218	273	31
303	89	241	222	278	34
306	90	242	224	280	38
	102	243	225	282	39
	118	244	226	283	13
	119	245	229	284	21
	127	248	231	285	
	132	249	232	286	
	141		233	287	
	142		234	290	
	148		A26	292	
	151			294	
	165			295	
	202			301	
	220			304	
	252			307	
	253			308	
	254			311	
	255			GE	
	256			213	
	257			209	
				215	
				19	

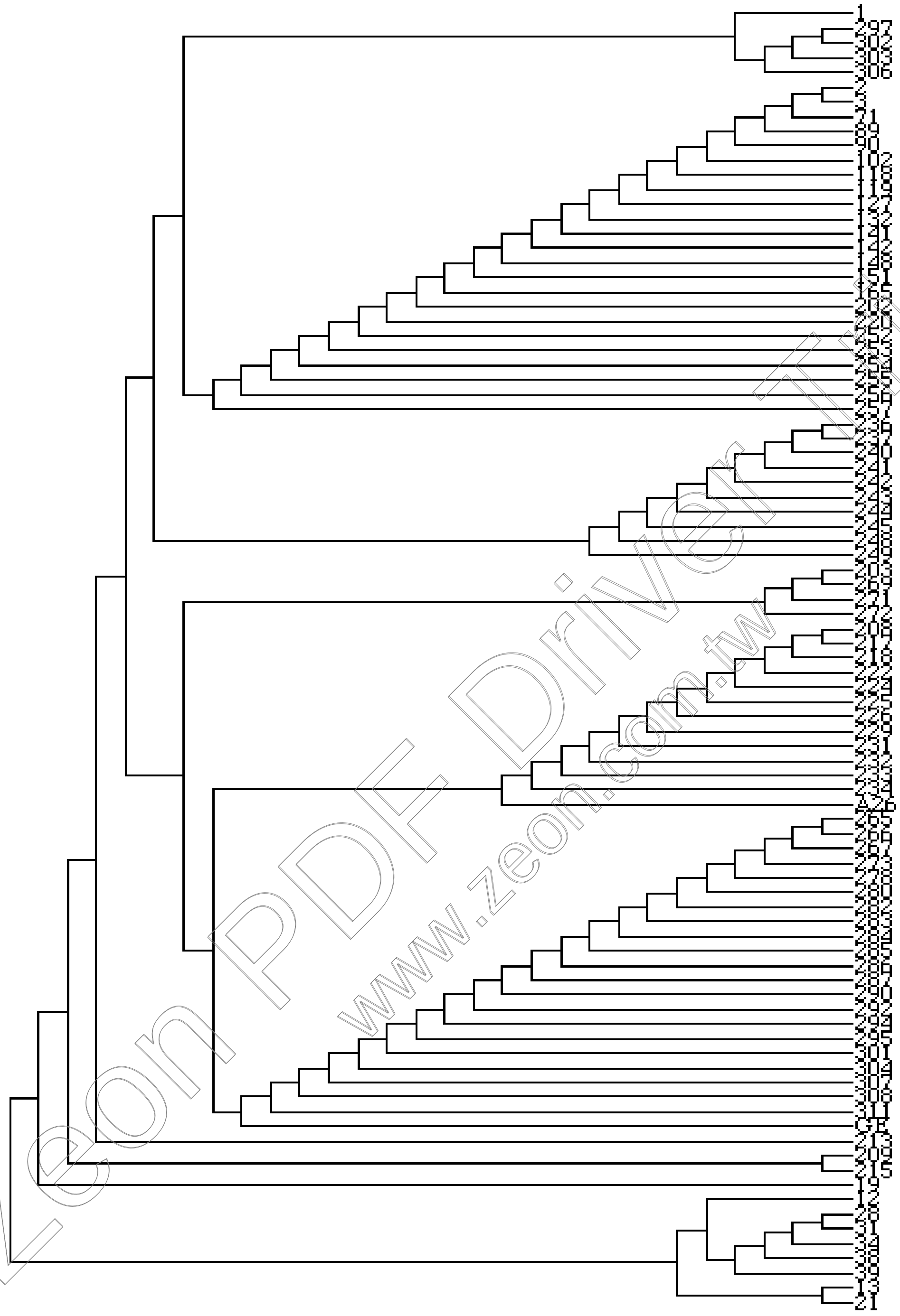

Fig. 5.12: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the blue and green primer combinations

Table 5.14: Seven clonal groupings identified from dendrograms produced from the blue and yellow primer combinations

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6	Clone 7
2	165	215	208	213	12	13
3	240		237		34	19
90	241		257			21
102	242		265			31
118	245		266			38
119	302		267			39
127	306		271			
132			272			
141			273			
142			278			
148			280			
151			282			
217			283			
218			284			
222			285			
224			287			
225			290			
226			294			
229			295			
231			301			
232			308			
234			311			
286			A26			
307						
GE						

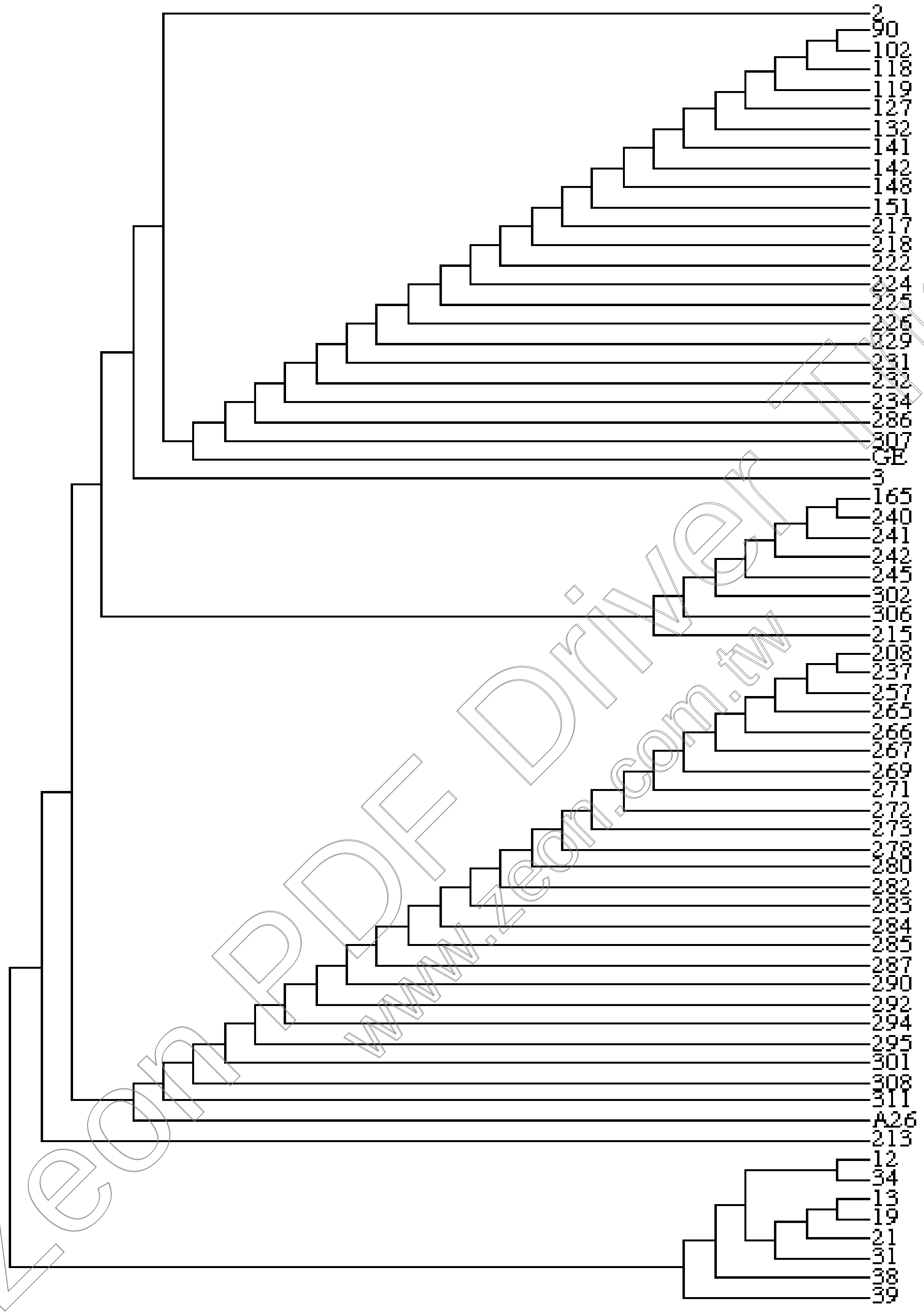

Fig. 5.13: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the blue and yellow primer combinations

Table 5.15: Eight clonal groupings identified from dendrograms produced from green and yellow primer combinations

Clone 1	Clone 2	Clone 3	Clone 4	Clone 5	Clone 6	Clone 7	Clone 8
2	3	165	215	147	269	237	12
49	217	168		204	271	308	19
89	218	299		257	272	221	13
90	222	300		265		A26	21
102	224	302		266		213	23
118	225	306		267			25
119	226	240		273			27
127	229	241		278			28
132	231	242		280			31
135	234	245		282			34
141				283			38
142				284			39
148				285			
151				287			
286				288			
307				289			
GE				290			
				292			
				294			
				295			
				296			
				301			
				308			
				309			
				310			
				311			

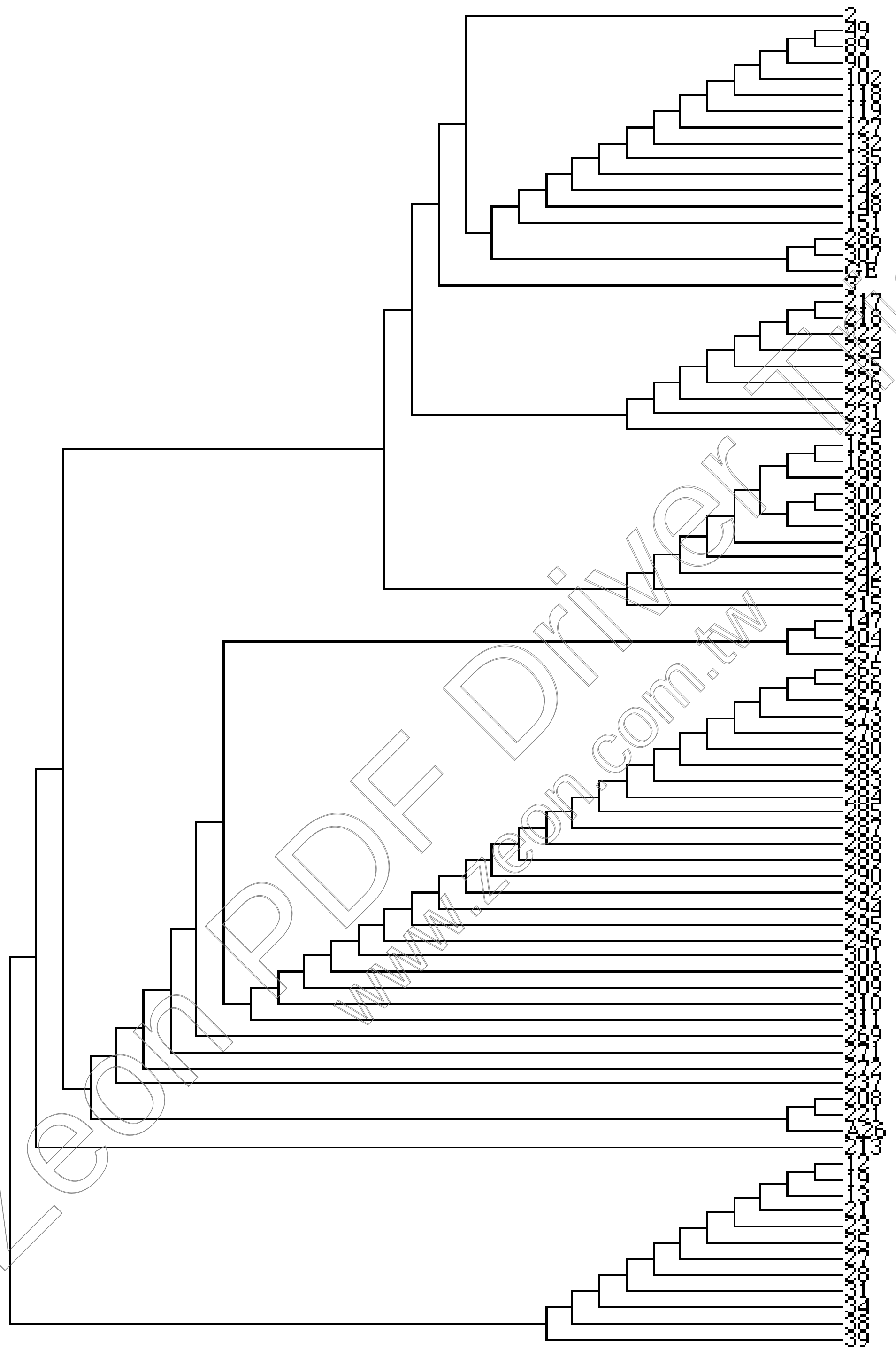

Fig. 5.14: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by the green and yellow primer combinations

Table 5.16: Thirteen clonal groupings identified from dendrograms produced from all primer combinations

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
2	217	286	208	265	269	237	165	240	213	19	12	13
3	218	307	A26	266	271	257	302	241	215		31	21
90	222	GE		267	272		306	242			34	
102	224			273				245			38	
118	225			278							39	
119	226			280								
127	229			282								
132	231			283								
141	232			284								
142	234			285								
148				287								
151				290								
				292								
				294								
				295								
				301								
				308								
				311								

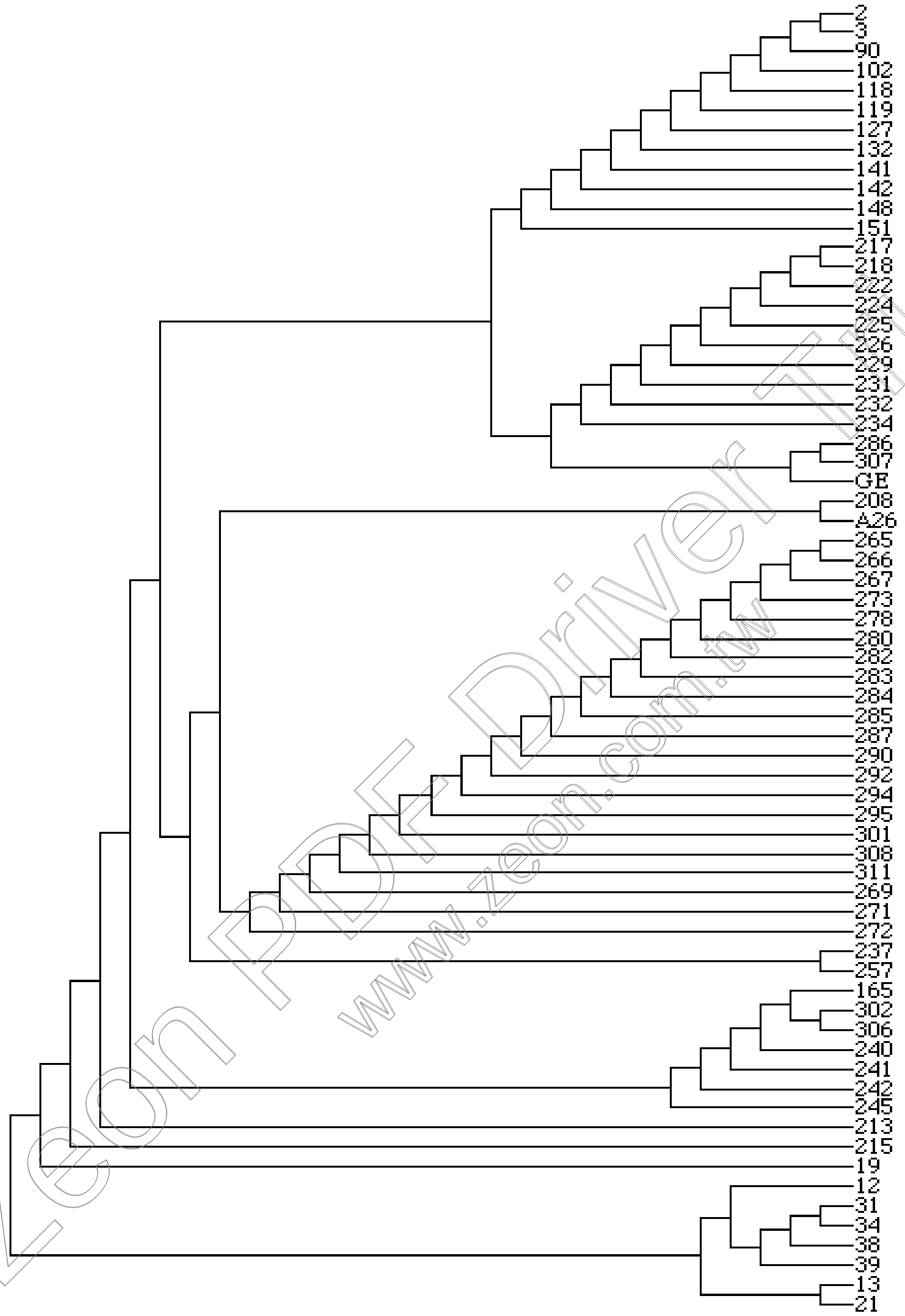

Fig. 5.15: Dendrogram produced using the DOLLOP parsimonious program analysed from data produced by all primer combinations

Appendix 3

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Appendix 4

Glossary

AFLP	Amplified Fragment Length Polymorphism
bp	base pairs
BRC	Biological Records Centre
BSBI	Botanical Society for the British Isles
CBD	Convention for Biological Diversity
CEH	Centre for Ecology and Hydrology
dH_2O	distilled water
DNA	deoxyribonucleic acid
et al	and others
GIS	geographic information system
ITE	Institute of Terrestrial Ecology
IVC	*in vitro* culture
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
STS	sequence tagged satellites
TPO	Tree Preservation Order
VC	Vice County