Application of RAPDs to the critical taxonomy of the English endemic elm *Ulmus plotii* Druce

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Received June 1999; accepted for publication November 1999

The taxonomy of the British elms is notoriously complicated and a satisfactory consensus classification remains elusive. This taxonomic complexity appears to be attributable to the reproductive biology of the species. *Ulmus glabra* Huds. reproduces sexually and its taxonomic status is widely (albeit not universally) accepted. In contrast, the suckering elms of the *U. minor* complex (*U. minor* Mill. emend. Richens) rarely reproduce by seed in Britain. Instead they perpetuate predominantly by vegetative reproduction; arguments regarding their taxonomy are legion. We have used molecular markers (RAPDs) to investigate the amounts and partitioning of clonal diversity and taxon inter-relationships in the British elms, focusing on a particularly enigmatic suckering elm, *U. plotii* Druce. Our molecular data suggest that all samples of *U. plotii* that precisely match the type description are ramets of a single genet, the distribution of which is attributable to human planting. Morphologically similar samples, which have many but not all of the *U. plotii* diagnostic characters, do not cluster with *U. plotii* when the RAPD data are analysed using principal coordinates analysis (PCO). Instead, they are scattered on the PCO plots throughout the broader range of variability of the *U. minor* complex. The implications of these results for the taxonomy of the British elms are discussed, and the need to combine knowledge of population structure with taxonomic pragmatism is emphasized.

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ADDITIONAL KEY WORDS:—Ulmaceae—elm—clonal reproduction—molecular systematics—multivariate analysis—plant dispersal—plant conservation.

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INTRODUCTION

The British elms

The classification of the genus Ulmus L. (Ulmaceae) in Britain has been notoriously controversial and there is a history of taxonomic confusion and disagreement dating back 400 years to the early English herbalists (for reviews see Moss, 1912; Richens, 1983; Armstrong & Sell, 1996). As with much early taxonomic work, poor species descriptions and a general absence of type specimens resulted in nomenclatural confusion. However, the adoption of more rigorous taxonomic practices, starting in the 18th century, has shown that the failure to reach any form of consensus is due to fundamental biosystematic disagreements.

Interestingly, a general consensus has been reached as to the recognition of Ulmus glabra Huds. (Rackham, 1980), although the status of geographical variants has been an issue in both Britain and Europe. For instance, Lindquist (1930) described northern and southern European varieties of U. glabra primarily based upon variation in leaf shape, a distinction that Stace (1991) regarded as ill-marked in Britain. Generally speaking, however, U. glabra has no more disagreements over its taxonomic integrity than many other plant species. Ulmus glabra is unique among the British elms in two respects. Firstly, it is exclusively reliant upon sexual reproduction, and sets abundant viable seed in most years (Christy, 1922; Grime, Hodgson & Hunt, 1988). Secondly, it has a ubiquitous distribution in Britain, although it occurs more frequently in the north and west on calcareous soils. Consequently, this species is generally accepted as a British native (Hill & Preston, 1997).

The remaining British species constitute a closely related complex that is often treated collectively under the name U. minor Mill. sensu latissimo, and which we refer to in this paper as the U. minor complex. In contrast to U. glabra, reproduction in the U. minor complex is primarily vegetative, via root suckers, and viable seed is rarely set in Britain (Henry, 1910; Christy, 1922). Whether or not the U. minor complex is native to Britain is unclear. It has been suggested that the poor seed set is indicative of introduction from warmer parts of Europe (Richens, 1955, 1980), where viable seed is normally set (E. Collin, pers. comm. 1999). Alternatively, the apparently natural distributions of some taxa within the complex have been proposed as evidence of their native status (Armstrong & Sell, 1996). Unfortunately, pollen
deposits cannot clarify the situation as no taxonomically reliable differences in pollen morphology between the *U. minor* complex and native *U. glabra* have been found (Godwin, 1975). As a whole, the *U. minor* complex is restricted to central and southern England.

It is the *U. minor* complex that has been the source of the taxonomic conflict, and the problem is perhaps best illustrated by the widely divergent treatments of R.H. Richens and R. Melville.

**Richens’ two species treatment**

Richens regarded the *Ulmus minor* complex to be introduced to northern Europe, and represented by “a series of clonal populations whose distribution is explicable in terms of human migration and trading contacts” (Richens, 1980: 307; for a fuller account see Richens, 1983: 15–31). This view was based upon the discovery of a large number of distinguishable entities, as recognized using eight morphometric measurements recorded from the subdistal leaves of British and European elms. Multivariate analysis of Richens’ data has provided further support for the recognition of numerically classifiable entities with discrete geographical distributions (Jeffers & Richens, 1970; Richens & Jeffers, 1975, 1978; Jeffers, 1996). The distribution of these putative clones has been used to infer introduction routes and source populations on mainland Europe (Richens & Jeffers, 1978; Richens, 1983).

Although Richens was able to define morphometric entities, he did not consider them worthy of recognition at the species level due to the absence of structural or physiological discontinuities. He did, however, recognize four taxa at the rank of variety: *U. minor* Mill. var. *vulgaris* (Aiton) Richens; *U. minor* Mill. var. *cornubiensis* (Weston) Richens; *U. minor* Mill. var. *sarniensis* (C.K. Schneid.) Richens; and *U. minor* Mill. var. *lockii* (Druce) Richens (Richens, 1977). Consequently, in Britain, Richens’ taxonomic treatment consists of two species: the broadly defined *Ulmus minor* Mill. emend. Richens sensu latissimo (Richens, 1968, 1976), and *U. glabra*.

**Melville’s six species treatment**

Melville (1975) also recognized *U. glabra*, but split *U. minor* into five more narrowly defined species: *U. angustifolia* (Weston) Weston; *U. carpinifolia* Gled; *U. coriacea* Melville; *U. plojii* Druce; and *U. procera* Salisb. In addition, he recognized a total of 11 hybrid combinations involving two to four parents, including hybrids within the divided *U. minor* (Melville, 1975). These hybrid derivatives were determined by a method that used rectangular coordinates to define critically the shapes of distal and subdistal leaves of adult short shoots (Melville, 1937a, 1939). In Melville’s (1955, 1978) opinion hybrid elms rarely exhibited intermediate leaf shapes. Instead, a pattern of dominance in which the leaf base strongly resembled one species while the apex strongly resembled another was common, thus allowing parentage to be inferred. Melville (1978 and references therein) regarded hybridization and the development of complex hybrid swarms to be responsible for the taxonomic recalcitrance of the genus. Richens, with his broader species concept, also acknowledged that hybridization occurred where *U. minor* had been introduced into
the range of *U. glabra*, resulting in the formation of the morphologically variable *U. × hollandica* Mill (Richens, 1967,1980).

**Current treatments**

Recent work on *Ulmus* has failed to clarify the situation. Two further taxonomic treatments have been proposed. Stace (1991) has followed a compromise between Richens and Melville by recognizing *U. glabra* along with the two most distinctive members of the *U. minor* complex, *U. plotii* and *U. procera*, and referring the remainder to one of three subspecies of *U. minor*. In contrast, Armstrong & Sell (1996) advocate a microspecies treatment. These authors argue that the factors responsible for the morphological variation are to some extent irrelevant, and are more concerned with partitioning the variation observed. They believe that "all past treatments have lumped the species together to a greater or lesser extent and that because of this the species are difficult to define" (Armstrong & Sell, 1996: 47). The recognition of a large number of species is intended, but the details of this treatment remain unpublished and are presently only available in a PhD thesis (Armstrong, 1992). At the opposite extreme Machon et al. (1995) have advocated a single species approach. This view is based upon investigation of isozymes that has demonstrated a genetic continuum between French populations of *U. minor* and *U. glabra*. Consequently, elm taxonomy appears to be further from reaching a consensus than at any time in the last 400 years, and taxonomic clarification is still needed in what has been regarded as one of the most critical genera in the British flora (Stace, 1991; Armstrong & Sell, 1996).

**The present study**

The key question in elm taxonomy in Britain appears to be whether or not there are morphologically and genetically discrete entities within the *Ulmus minor* complex larger than a genet (clone)? The presence of at least some degree of morphological subdivision is generally accepted and, assuming morphology is reliable for recognising subdivisions within the complex, two possible explanations of this exist. Firstly, sexual reproduction has created a series of related genotypes that share some morphological characters, leading to their taxonomic recognition. In such a situation the members of a taxon would be genetically closer to each other than to the members of other such taxa. Secondly, the subdivisions may simply represent individual clones that have been propagated and dispersed.

In order to make progress in elm taxonomy, genetic relationships within morphologically defined groups need to be investigated. We have, therefore, used molecular markers in a case study to examine the population genetic basis of the morphological variability in the *U. minor* complex, focusing on the particularly enigmatic *U. plotii*. Like other members of the *U. minor* complex, *U. plotii* shows low to zero seed set in most years in Britain, with reproduction being by vegetative suckering. Although originally described by Druce (1911), Melville (1940) provided a more thorough description. *Ulmus plotii* is distinguished from other elms in the *U. minor* complex by a particularly characteristic habit of growth in which the apex of the crown leans to one side, referred to as the 'unilateral' habit. Further distinguishing
features are equal or subequal leaf bases with a cordate margin, and so called 'proliferating' side shoots that produce more than the normal five leaves in a single flush. Despite these unusual morphological characteristics the species has not been universally accepted (Boulger, 1912; Richens, 1958). Questions over the taxonomic status of U. plotii go beyond academic interest. It is a British endemic restricted to the English Midlands, and qualifies as nationally scarce (recorded from 33 10-km squares since 1970) (Messenger, 1994). This has lead to its inclusion as a conservation priority species in the UK Biodiversity Action Plans (Anon, 1995). If species are given priority rankings for conservation resources, it is clearly important that these rankings should be based on sound taxonomic information.

To test the genetic distinctness of U. plotii and to assess the amounts and distribution of clonal diversity, we have used RAPDs (randomly amplified polymorphic DNA). The technique was chosen as it has been used to estimate clonal diversity in other plant species (e.g. Wolf & Petersvanrjijn, 1993; Adams et al., 1998; Hollingsworth et al., 1998; Tyson, Vaillancourt & Reid, 1998). In addition, RAPDs have proved useful in the study of hybridization and differentiation among closely related species (e.g. Smith, Burke & Wagner, 1996; Hollingsworth et al., 1998), both important issues in elm taxonomy.

MATERIAL AND METHODS

Plant material, collection and identification

Nomenclature

In the remainder of this paper we follow the nomenclature of Stace (1997). Our only deviation from this is when we talk about the U. minor complex. As mentioned in the introduction, when we refer to the U. minor complex, we include all of the suckering elms (i.e. Stace's U. minor, U. procera and U. plotii)

Plant material

A total of 82 British elm samples were collected (Table 1). As well as material of U. plotii, we have sampled material of U. minor and U. glabra, along with putative hybrids. The living collection of elms held by the Royal Botanic Gardens Kew at Wakehurst Place was an important source of material. This collection was established by Melville in the 1970s and consists of 60 wild collected elm accessions. These elms have been maintained as a low hedge in order to minimise the risk of Dutch elm disease.

In addition to the material collected from Wakehurst Place (25 samples), 52 samples were wild collected from the English Midlands and five samples were collected from ornamental plantings and botanical collections in Edinburgh. Locality details, including the original collection sites for the Wakehurst Place material, are given in Table 1.

Plant identification

Our approach to sample identification was split into two stages. Firstly, overall appearance was assessed against published descriptions. Secondly, the samples were scored for characters we regard as diagnostic (see Table 2), defined as those restricted
Table 1. Locality data and determination of British elm samples. Samples organized by taxon using the following abbreviations: PL = *U. plotii*; PS = ‘pseudoplotii’; MI = *U. minor*, GL = *U. glabra*; HO = *U. × hollandica*; PL × MI = *U. plotii × U. minor* (see text for explanation of ‘pseudoplotii’ and *U. plotii × U. minor*). Doubtfully determined juvenile material is indicated by a question mark after the taxon code. Samples from Wakehurst Place are indicated by Melville’s collection number in parentheses after the collection locality.

| Sample No. | Determination | Collection locality | Grid reference | County         |
|------------|--------------|---------------------|----------------|----------------|
| 1          | PL           | Ryhall              | TF038117       | Leicestershire |
| 8          | PL           | Caythorpe           | SK694455       | Nottinghamshire|
| 10         | PL           | Aslockton           | SK728393       | Nottinghamshire|
| 11         | PL           | Muston              | SK832382       | Leicestershire |
| 12         | PL           | Barrowby            | SK878372       | Lincolnshire   |
| 13         | PL           | Foston              | SK849429       | Lincolnshire   |
| 16         | PL           | Westborough         | SK850443       | Lincolnshire   |
| 17         | PL           | Westborough         | SK851448       | Lincolnshire   |
| 48         | PL           | Saxby (7432)        | SK900750       | Lincolnshire   |
| 53         | PL           | Ashby Folville (7440)| SK711118       | Leicestershire |
| 67         | PL           | Bulwick             | SP959852       | Northamptonshire|
| 68         | PL           | Bulwick             | SP959850       | Northamptonshire|
| 72         | PL           | Laxton              | SP949861       | Northamptonshire|
| 74         | PL           | Laxton              | SP949862       | Northamptonshire|
| 19         | PL           | Gilmorton           | SP579885       | Leicestershire |
| 22         | PL           | Cold Newton         | SK706079       | Leicestershire |
| 23         | PL           | Cold Newton         | SK706074       | Leicestershire |
| 64         | PL           | Lyddington          | SP876965       | Leicestershire |
| 83         | PL           | Carly               | TF065154       | Lincolnshire   |
| 4          | PS           | Graftam             | TL160697       | Cambridgeshire |
| 27         | PS           | South Luffenham     | SK938017       | Leicestershire |
| 42         | PS           | Tedstone de la Mere (7676)| SO6923| Leicestershire |
| 47         | PS           | Ashby Folville (7439)| SK697123       | Leicestershire |
| 57         | PS           | Barholm             | TF081107       | Lincolnshire   |
| 58         | PS           | Barholm             | TF081107       | Lincolnshire   |
| 59         | PS           | Barholm             | TF081107       | Lincolnshire   |
| 61         | PS           | Hambleton           | SK927073       | Leicestershire |
| 69         | PS           | Harrington          | SP917972       | Northamptonshire|
| 95         | PS           | Edinburgh           | NT260726       | Midlothian     |
| 6          | MI           | Aisworth            | TL119991       | Cambridgeshire |
| 7          | MI           | Ufford              | TF118039       | Cambridgeshire |
| 18         | MI           | Barrowden           | SK946009       | Leicestershire |
| 20         | MI           | Peating Magna       | SP939917       | Leicestershire |
| 40         | MI           | Bloxham (7668)      | SP425352       | Oxfordshire     |
| 55         | MI           | Bramfield (7410A)   | TL289144       | Hertfordshire   |
| 75         | MI           | Blatherwycke        | SP978968       | Northamptonshire|
| 76         | MI           | Duddington          | TL012999       | Northamptonshire|
| 77         | MI           | Callyweston         | TF006016       | Northamptonshire|
| 78         | MI           | Ufford              | TF092049       | Cambridgeshire |
| 79         | MI           | Elton               | TL090939       | Cambridgeshire |
| 80         | MI           | Peterborough        | TL149975       | Cambridgeshire |
| 81         | MI           | Peterborough        | TL149974       | Cambridgeshire |
| 82         | MI           | Carly               | TF063154       | Lincolnshire   |
| 2          | GL           | Ryhall              | TF038117       | Nottinghamshire|
| 9          | GL           | Caythorpe           | SK694436       | Nottinghamshire|
| 14         | GL           | Foston              | SK849430       | Lincolnshire   |
| 21         | GL           | Peating Magna       | SP959917       | Leicestershire |
| 25         | GL           | Cold Newton         | SK707074       | Leicestershire |
| 26         | GL           | Glaston             | SK887012       | Leicestershire |
| 41         | GL           | Tedstone de la Mere (7674)| SO669591 | Hereford   |
| 63         | GL           | Egleton             | SK868072       | Leicestershire |
| 65         | GL           | Harringworth        | SP920969       | Northamptonshire|

Continued
### TABLE 1—continued

| Sample No. | Determination | Collection locality | Grid reference | County       |
|------------|---------------|---------------------|----------------|--------------|
| 66         | GL            | Harringworth        | SP920969       | Northamptonshire |
| 71         | GL            | Laxton              | SP951962       | Northamptonshire |
| 73         | GL            | Harringworth        | SP922967       | Northamptonshire |
| 84         | GL            | Ullesthorpe         | SP909906       | Leicestershire |
| 85         | GL            | Ullesthorpe         | SP908905       | Leicestershire |
| 96         | GL            | Edinburgh           | NT260726       | Midlothian   |
| 99         | GL            | Edinburgh           | NT247753       | Midlothian   |
| 3          | HO            | Ryhall              | TF038117       | Leicestershire |
| 28         | HO            | Levels Green (7649) | TL480242       | Essex        |
| 29         | HO            | Ware (7645)         | TL354152       | Hertfordshire |
| 35         | HO            | Broxted (7651)      | TL585282       | Essex        |
| 38         | HO            | Pury End (7661)     | SP718463       | Northamptonshire |
| 43         | HO            | Syde (7677)         | SO944118       | Gloucestershire |
| 49         | HO            | Wold Newton (7428)  | TF235993       | Lincolnshire |
| 54         | HO            | Farnham Green (7411)| TL476243      | Essex        |
| 60         | HO            | Barholm             | TF076120       | Lincolnshire |
| 30         | HO?           | Ware (7643)         | TL354152       | Hertfordshire |
| 31         | HO?           | Holme (7636)        | TQ966540       | Kent         |
| 56         | HO?           | Duntisbourne Rouse (7616)| SO985060 | Gloucestershire |
| 5          | PL × MI       | Easton              | TL142721       | Cambridgeshire |
| 37         | PL × MI       | Maryland (7658)     | TL178463       | Essex        |
| 44         | PL × MI       | Little Hadham (7410)| TL443242      | Hertfordshire |
| 45         | PL × MI       | Lovesby (7441)      | SK276079       | Leicestershire |
| 46         | PL × MI       | Hungarton (7445)    | SK06074        | Leicestershire |
| 50         | PL × MI       | Bramfield (7420)    | TL292159       | Hertfordshire |
| 51         | PL × MI       | Barrow (7456)       | SK97181        | Leicestershire |
| 86         | PL × MI       | Edinburgh           | NT247753       | Midlothian   |
| 87         | PL × MI       | Edinburgh           | NT247753       | Midlothian   |
| 34         | PL × MI?      | Lenham (7634)       | TQ980512       | Kent         |
| 36         | PL × MI?      | Great Dunmow (7654) | TL638238       | Essex        |

### TABLE 2. Diagnostic characters used in the determination of British elm samples. Taxa are indicated using the following abbreviations: PL = U. plotii; MI = U. minor; GL = U. glabra; HO = U. × hollandica

| Diagnostic character                        | PL | MI | GL | HO |
|--------------------------------------------|----|----|----|----|
| Dense cover of simple hairs on leaf upper surface | -  | -  | +  | ±  |
| Red club-shaped glandular hairs on leaf surface | +  | +  | -  | +  |
| Mature crown of unilateral habit            | +  | -  | -  | -  |
| Subequal cordate leaf base                  | +  | -  | -  | -  |
| Short shoots produce more than five leaves in a flush | +  | -  | -  | -  |

Key to character states: - = absence; + = presence; ± = intermediate state

to a single taxon and present in nearly (see below) all individuals. Unlike overall appearance, this has the advantage of easy communication and application by others. In only one case, sample 43, was strict adherence to the diagnostic characters overturned by overall appearance. This sample, overall, combined the characteristics of *U. glabra* and *U. minor*, yet did not exhibit the combination of simple and glandular hair types on the upper leaf surface that characterized all of our other putative *U. × hollandica* samples. We believe determination of sample 43 as *U. × hollandica* is reasonable as one of the most widely planted clones resulting from the same taxonomic cross
(Huntingdon elm) displays the same absence of simple hairs on the upper leaf surface. Thus evidence from hair types should not be relied upon in isolation.

We stress that identification of material in a taxonomically critical group such as the British elms is not always straightforward, a problem compounded by Dutch elm disease which has removed the vast majority of mature elms from the landscape. Part of the difficulty stems from the fact that elm leaves undergo a gradual change in size, shape and density of simple and glandular surface hairs from the seedling through to the adult stage (Melville, 1937b). Suckers and coppice growth revert to an essentially juvenile leaf form and it is therefore important that leaf comparisons are from growth at an equivalent stage in the gerontic spectrum. Most elm researchers have used adult foliage for identification purposes. Melville (1978) used distal and subdistal leaves of adult short shoots, and Richens (1983) used subdistal leaves of adult shoots. As the elms at Wakehurst Place (an important component of our sample) have been kept in a low hedge, and are therefore juvenile in character, it was necessary to examine the voucher specimens deposited by Melville at K (Herbarium, Royal Botanic Gardens Kew) in order to score adult foliage characters. From these vouchers we were able to obtain information on adult characters for all but five of the 25 Wakehurst Place samples included in the study (the remaining five herbarium specimens could not be relocated). These five samples (along with five other wild collected samples) were available only as juvenile material and are highlighted in Table 1. Their identifications are tentative.

A second problem we encountered related to *U. plotii*. Some of our samples exactly matched the type description and had all of the diagnostic characters such as narrow unilateral habit, equal or subequal cordate leaf bases, and proliferating side shoots. Other cases were more complicated. We encountered some samples very similar to *U. plotii*, but which differed in one character or another from the type. Some of these plants had been identified previously by elm experts such as Ronald Melville and Guy Messenger (in addition to the Wakehurst Place samples identified by Melville, we have also sampled wild trees recorded and identified by Melville and Messenger, traced using the Biological Record Centre’s database at Monks Wood). In some cases these samples had been identified as *U. plotii*, in other cases they had been identified as *U. plotii* × *U. minor* (of course Melville did not use the name *U. minor*, rather one or other of his subdivisions of it, such as *U. coritana* or *U. carpinifolia*).

It is difficult to know how best to treat these samples. What we have done is to be pragmatic. Where Melville or Messenger effectively referred them to *U. plotii* × *U. minor* we have used that name. Alternatively, where Melville or Messenger identified them as *U. plotii*, or, if they were trees discovered during the course of this study, we have accepted that they look very like *U. plotii*, but are not quite the same, and have therefore given them the informal name ‘pseudoplotii’. We should stress, that we can see no difference between ‘pseudoplotii’ and Melville and Messenger’s *U. plotii* × *U. minor* (and indeed we are not clear why they differentiated them). This is kept firmly in mind in all subsequent discussion. Consequently, *U. plotii* has been interpreted *sensu stricto* and has only been used to refer to samples that exactly match the type description.

As some of our other samples used in this study were given different determinations by Melville or Messenger we have presented a table in the Appendix listing their determinations, and why we have differed. Many of the differences are simply due to different breadths of species concepts and do not impinge on the general
conclusions drawn in this paper. Others are due to our disagreeing with the identity of a specimen, because we felt it had been misidentified.

A voucher specimen of each sample has been deposited at E (Herbarium, Royal Botanic Garden Edinburgh). While the amount of uncertainty over material identification initially seems disturbing, this is inevitable in studies on the British elms due to the complexity that arises from the combination of them being a critical taxonomic group, and the scarcity of mature trees due to Dutch elm disease. In this respect we would make two points to reassure readers alarmed by the above identification problems.

Firstly, the method of data analysis has been explicitly chosen so that it is completely insensitive to a priori determinations (see data analysis section below).

Secondly, we have deposited an annotated version of the results figures at E and K with the specimens used in this study. Researchers with different concepts of these taxa can reinterpret the plot with their own determinations if they so wish.

**DNA extraction and electrophoresis**

For each sample approximately 0.5 g of fresh leaf material was placed in 20 g of 28–200 mesh non-indicating silica gel, with a small amount of indicating silica gel. Samples were stored in plastic bags with a fully airtight zip seal at room temperature. DNA was then extracted from approximately 2 cm² of dry leaf material using a protocol modified from Doyle & Doyle (1990). Briefly, 400 µl of 2 × CTAB buffer (preheated to 65°C) was added to each sample followed by a pinch of PVPP (polyvinylpolypyrrolidone) and acid washed sand. Samples were homogenized using a ground glass rod attached to a domestic power drill. A further 800 µl of preheated buffer was added, followed by inversion to ensure thorough mixing. Samples were incubated for 1 hour at 65°C. They were then allowed to cool to room temperature and centrifuged at 13 000 rpm for 10 min. The supernatant was removed and proteins were extracted by adding 500 µl of dichloromethane and shaking the samples gently for 20 min. Samples were again centrifuged at 13 000 rpm for 10 min, the supernatant removed, and the dichloromethane step was repeated. DNA was precipitated by adding 0.33 volume freezer-cold isopropanol followed by gentle mixing. The DNA was pelleted by centrifugation at 13 000 rpm for 10 min, and the isopropanol poured off. Samples were allowed to air-dry for 30 min before being dissolved in 0.5 ml of TE buffer. RNA was removed by adding 10 µl of 1 mg/ml RNase followed by incubation at 37°C for 1 hour. To precipitate the DNA 50 µl of 3 M sodium acetate was added followed by 2.5 volumes freezer-cold 95% ethanol. The DNA was pelleted by centrifugation at 13 000 rpm for 10 min, and the ethanol poured off. The sample was allowed to air-dry for 30 min before being dissolved in 200 µl TE buffer. DNA concentration was assessed against standards by running 5 µl of each sample on 1% agarose gels, with visualization by ethidium bromide and ultra-violet light. All samples were diluted to 2 ng DNA/µl.

**RAPDs**

Arbitrary DNA fingerprinting was carried out using standard 10-base RAPD primers (Operon Technologies). To select primers for the RAPD analysis a subset of eight samples (two *Ulmus plotii*, three *U. minor*, and three *U. glabra*) was screened.
using 35 primers from the Operon primer kits P, A and F (OPP2-4, 7-19; OPA2-6, 9-12, 14-20; OPF1-2). From this initial screen, 10 primers were selected which gave clear and reproducible banding patterns: OPA10 (5'-GTGATCGCAG-3'); OPA11 (5'-CAATCGCCGT-3'); OPA12 (5'-TCGGCGATAG-3'); OPA14 (5'-TCTGTGCTGG-3'); OPA15 (5'-TTCCGAACCC-3'); OPA17 (5'-GACCAGTTGT-3'); OPA19 (5'-CAACCGTCCG-3'); OPA20 (5'-GTTGCCATGC-3'); OPP-03 (5'-CTGATACGCC-3'); OPP04 (5'-GTGTTCAGG-3'). The reaction mixture per sample (total volume 25 μl) was: 2.5 μl dNTPs (2 mM); 2.5 μl ammonium sulphate buffer (160 mM); 2.5 μl primer (5 μM); 1.25 μl magnesium chloride (50 mM); 0.5 μl formamide (100%); 5 μl template DNA (2 ng/μl); 1 unit of Taq polymerase (not included in volume calculation); 10.75 μl water. Samples were covered with one drop of mineral oil to prevent evaporation. The thermal cycle was: 2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min at 37°C, 2 min at 72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, 2 min at 72°C; 41 cycles of 30 s at 94°C, 1 min at 35°C, 2 min at 72°C; followed by a final 5 min extension at 72°C.

Negative controls, lacking template DNA, were included in each PCR. Amplification products were separated by electrophoresis on 1.6% agarose gels (1 x TBE) and visualized by staining with ethidium bromide. A 1 kb ladder (Gibco) was used as a molecular weight marker. In addition to the use of a molecular weight marker, eight samples were run on all gels to facilitate cross gel comparisons. Scoring of RAPD bands was carried out on the basis of presence/absence. Co-migrating bands with very marked variation in intensity were excluded from the analysis, along with groups of bands that varied slightly in molecular weight.

Data analysis

The presence/absence matrix generated using the 10 RAPD primers was converted to a similarity matrix using Jaccard's Coefficient: $D_{ij} = 2n_{xy}/n_x + n_y$, where $n_x$ is the number of bands present within accession $x$, $n_y$ is the number of bands present within accession $y$, and $n_{xy}$ is the number of bands shared by accessions $x$ and $y$ (Jaccard, 1908). In calculating this measure of similarity between pairs of samples, only shared presence of RAPD bands is taken into account. The absence of a RAPD fragment may be caused by a number of factors, thus using shared absences to represent similarity may misrepresent relationships (Weising et al., 1995). The relationships of inter-individual similarity in multi-dimensional space were examined by principal coordinates (PCO) analysis on the Jaccard's similarity matrix using the computer program PCO3D, provided by Roger Adams (Baylor University, U.S.A.). Given the problems encountered with sample identification, it is clearly vital that the method of data analysis does not depend on a priori taxon definition. PCO simply measures the inter-relationships of individual samples based on the inter-individual similarities. When graphical representations of the analysis are produced, it is then possible to see whether the inter-relationships of samples coincides with the names and affinities that have been given to them.

RESULTS

A total of 77 reproducible and polymorphic bands was resolved with 10 RAPD primers (mean 7.7 bands per primer, range 4–14). A total of 61 genotypes was
TABLE 3. Sample size and distribution of five shared RAPD genotypes (A, B, C, D & E) between British elm samples

| Taxon                  | Sample size | Number of each shared genotype |
|------------------------|-------------|-------------------------------|
| *U. glabra*            | 16          |                               |
| *U. minor*             | 14          | A(3)                          |
| *U. plotii*            | 14          | B(14)                         |
| *U. plotii?*           | 5           | B(3), D(2)                    |
| 'pseudoplotii'         | 10          | C(2)                          |
| *U. x hollandica*      | 9           |                               |
| *U. x hollandica?*     | 3           |                               |
| *U. plotii x U. minor* | 9           | E(2)                          |
| *U. plotii x U. minor?*| 2           |                               |

Samples for which only juvenile material was available are indicated by question marks.

TABLE 4. Summary of variance extracted in three separate principal coordinate (PCO) analyses (see text) of RAPD data from British elm samples

| PCO analysis                     | PCO 1 to 10 | PCO 1, 2 and 3 |
|----------------------------------|-------------|----------------|
| All data                         | 53.92%      | 18.91%; 5.89%; 5.44% |
| *U. minor, U. plotii & 'pseudoplotii' | 84.05%      | 18.41%; 13.98%; 12.83% |
| *U. glabra & U. minor*           | 78.83%      | 27.52%; 10.73%; 7.68% |

detected from the 82 samples (mean 42.5 genotypes detected per primer, range 31–52). All 61 genotypes could be detected by using only three primers: OPA11 in combination with OPP03 and either OPA17 or OPA19.

Five genotypes, A, B, C, D and E, were detected from multiple samples (see Table 3 and Appendix). All 16 samples of *Ulmus glabra* and nine samples of *U. x hollandica* possessed unique genotypes. Of the 14 samples identified as *U. minor* three shared the A genotype. Of the 14 samples identified as *U. plotii* all shared the B genotype, whilst of the ten samples of 'pseudoplotii', two shared the C genotype. Of the nine samples identified as *U. plotii x U. minor* two shared the E genotype. Finally, of the ten samples available only as juveniles, and thus of doubtful determination, three shared the B genotype (present in all *U. plotii* samples) and two shared the D genotype.

Three separate PCO analyses were run on the RAPD data: (a) all data, (b) *U. minor, U. plotii* and 'pseudoplotii', and (c) *U. glabra* and *U. minor*. The PCO analyses generated eigenroot values for the first ten principal coordinates. These were examined to establish the point at which the increase of total variance extracted by the addition of each successive coordinate began to asymptote, at which point much of the signal is considered to equate to random noise. In each case the extracted variance began to asymptote after the third principal coordinate (Table 4). As all
Figure 1. Principal coordinates plot showing the inter-relationships of RAPD genotypes of British elm samples. The figure shows the relationships among 82 samples representing 61 genotypes. The analysis is based on the presence or absence of 77 polymorphic bands amplified using 10 decamer RAPD primers. The filled circle represents 14 samples of *U. plotii* and three juvenile samples tentatively identified as *U. plotii*. Four other shared genotypes (not highlighted) were recovered, see text.

Three analyses suggested the same relationship among the samples only the 'all data' analysis has been presented here (Fig. 1).

A clear separation of our samples of *U. glabra* (filled triangles) from a cluster composed of samples of *U. plotii* (filled circle), 'pseudoplotii' (open circles), *U. minor*
(filled squares) and *U. plotii* × *U. minor* (open inverted triangles) was found (Fig. 1). Samples identified as *U. × hollandica* (open diamonds) occupied an intermediate position between these two clusters (Fig. 1). Within the cloud of points representing *U. plotii*, 'pseudoplotii', *U. minor* and *U. plotii* × *U. minor* no further taxonomic groupings were evident (Fig. 1). The *U. plotii* and 'pseudoplotii' samples did not form a discrete cluster in the PCO analysis, and instead were scattered within the broader range of variability of the *U. minor* complex (Fig. 1).

**DISCUSSION**

Our RAPD data were clearly able to separate a cluster of samples corresponding to *Ulmus glabra* from a cluster of samples corresponding to the *U. minor* complex (including *U. plotii*, 'pseudoplotii' and *U. plotii* × *U. minor*). In contrast, the lack of clustering of samples of *U. plotii* with 'pseudoplotii' and *U. plotii* × *U. minor*, combined with the existence of a single genotype (B) in all 14 samples of *U. plotii*, is considered an important result that may be generally applicable to the taxonomic problems of the *U. minor* complex. Before dealing with the taxonomic implications of this, however, we first address the issues of clone identification using RAPDs and evidence for elm cultivation based upon clone distribution.

**Evidence for clonal reproduction**

Five RAPD genotypes (A, B, C, D and E) were recovered from multiple samples. The most frequently recovered genotype (B) occurred in all 14 samples of *Ulmus plotii* and three samples of juvenile material tentatively determined as *U. plotii*. We have interpreted samples with identical RAPD banding patterns as multiple ramets of the same genet (i.e. clones). Clearly, this relies on the assumption that identical RAPD profiles for 10 primers can be equated to genet identity. That the RAPD primers used were sensitive and able to detect genetic variation is demonstrated by their ability to uniquely genotype 56 of the 82 samples, including all samples of *U. glabra* and *U. × hollandica*. The other explanation for RAPD profile uniformity is that the loci amplified are homozygous and uniform and that sexual reproduction within and among identical genotypes is perpetuating the same RAPD genotypes. We feel this is unlikely as the European elms are wind pollinated and protogynous, which should promote outcrossing. Higher levels of seed set have been documented in cross versus self-pollinated trees (Mittempergher & La Porta, 1991; J.C. López-Alamansa pers. comm. 1999). In addition, high levels of genetic (allozymic) diversity have been found (Machon *et al.*, 1995, 1997). Based on this one might expect that the probability of preferential mating within and among samples with completely homozygous and uniform RAPD profiles is low. In addition, field observations show that in Britain, seed set is rare (Henry, 1910; Christy, 1922) and that vigorous suckering occurs in the *U. minor* complex. Furthermore, there is a general absence of *U. minor* complex elms from semi-natural woodland, and where such elms are present they have been regarded as invaders of semi-natural communities originating from hedgerows or human habitation (Rodwell, 1991). The typical habitat of all *U. minor* complex elms (including *U. plotii*) in Britain is hedgerows, a setting in which
planting is a likely origin. If planting is accepted as the probable origin of the majority of these elms, then vegetative propagation would have been favoured due to the rarity of seed set and the ease with which cuttings may be rooted (C. Clennet, pers. comm. 1999), or suckers transplanted.

All things considered, it seems that the simplest explanation of the data is that uniform genotypes do indeed equate with clones. The same conclusion has been reached in studies using RAPDs to investigate clonal growth in other plant species (e.g. Wolf & Petersvarnrijn, 1993; Adams et al., 1998; Hollingsworth et al., 1998; Tyson et al., 1998).

Evidence for elm cultivation

The hypothesis that the geographical pattern of variation in the Ulmus minor complex is a product of human cultivation of individual clones was originally devised by Richens (1955). Alternatively, it has been suggested that many taxa have such natural distributions that introduction seems unlikely (Armstrong & Sell, 1996). Using morphometric methods Richens distinguished minute taxonomic categories that he regarded as individual clones. However, such methods may not provide conclusive proof of clonal identity. As we believe RAPDs provide a more reliable method for the identification of elm clones it is possible to reinvestigate this issue.

The age of a clone can be calculated if the rate of vegetative spread is known. Such calculations have been made for several species (Pteridium aquilimum (L.) Kuhn, Oinonen, 1967; Eucalyptus ridsoni Hook. f. × E. amygdalina Labill., Tyson et al., 1998). In a similar manner the maximum theoretical extent of a clone can be calculated for a given time period. Using estimates of both the rate of natural vegetative spread and the length of time that elms of the U. minor complex have been a part of the British flora, such a calculation can be made for elm clones. If the observed spread of individual clones greatly exceeds the expected maximum resulting from natural suckering alone, human propagation would seem a likely cause. Estimating the time that U. minor complex elms have been present in Britain is complicated by the fact that it is not possible to distinguish individual elm species solely upon pollen samples (Godwin, 1975). As U. glabra is better adapted to cooler and wetter climates than the U. minor complex elms, it is generally assumed that this was the first species to colonize at the end of the last glaciation. When, or indeed if, U. minor complex elms naturally colonized Britain remains unclear. Nevertheless, to overcome this difficulty we have followed a conservative approach and have taken the first appearance of elm pollen as marking the upper limit on the time period. The first appearance of elm pollen following the last glaciation occurs in south-west England 8829 ± 100 BP (Godwin, 1975). Rates of vegetative spread in suckering elms have been estimated under various woodland management regimes and it has been concluded that around 0.6 m per year is the maximum (Rackham, 1980). These figures give a theoretical maximum spread per clone of 5.3 km. In marked contrast, the B genotype, found in all 14 samples of U. plotii and three juvenile samples, was recovered from an area of 50 km² in the English Midlands, with the greatest distance between two samples being 80 km. The large disparity between the expected and the observed spread in this case indicates that dispersal has been achieved by means other than normal suckering alone. As elms are not agamosperms, and the rooting of detached twigs has not been observed, the most obvious explanation is human agency. This
result provides strong support for the argument that past human dispersal of clones has been a significant factor in the present day distribution of variation in the *U. minor* complex.

**Taxonomic relationships within the Ulmus minor complex**

If clonal spread and human propagation is responsible for *Ulmus plotii sensu stricto*, this immediately raises the question as to the relationship of this clone to the morphologically similar samples called here ‘pseudoplotii’ or *U. plotii × U. minor*. It is clear from the PCO plots that these samples do not represent a discrete entity, but are instead scattered throughout the broader range of *U. minor*. In the past some of these samples have been identified as *U. plotii* (e.g. samples 4, 27, 42, 61), or as *U. plotii × U. minor* (e.g. 5, 34, 36, 37, 44, 45, 46, 50, 51, 86, 87) (Appendix). Certainly it seems that their designation as *U. plotii* is erroneous and it suggests that ‘very similar but not exactly the same’ is not an appropriate indicator of taxonomic affinities in this group. An interesting example of how we think this can produce biologically misleading information is the isoperoxidase isozymes study of Richens & Pearce (1984). In contrast to the present study, Richens & Pearce (1984) found a different banding pattern in each of four samples of *U. plotii* (cited as *U. minor var. lockii*) investigated. This discrepancy with our single clone result could be explained by the lumping together of a number of different ‘pseudoplotii’ clones, possibly with the single *U. plotii* clone, thus giving a polymorphic (and polyphyletic) species. It is not known which characters Richens & Pearce (1984) used in their determinations of *U. plotii*. Evidence that Richens may not have been using all of the characters (and was actually aware that this may be a problem), comes from Richens himself. Richens (1958: 139–140) stated “There are good reasons for supposing the unilateral habit may have arisen independently in a number of different localities”, and “If the unilateral habit is polyphyletic, it hardly seems proper to employ it as a specific criterion, in which case *U. plotii* would have to be rejected as a species”. Clearly Richens was aware of the pitfalls in identifying *U. plotii*. In this respect we stress that using the *U. plotii* diagnostic characters strictly, all individuals examined by us that match the type shared an identical RAPD profile. In conclusion, in order to avoid inferring misleading affinities, it is necessary to employ rigorous identification procedures based on the full set of diagnostic characters.

It is clear that the samples that are close to *U. plotii*, but differ in one or other character from the type, should not be called *U. plotii*. However, the designation of samples like these as *U. minor × U. plotii* does not seem a satisfactory approach either. These putative hybrid samples are so genetically disparate in the PCO analysis there seems little value in naming them under a collective descriptor (a predictable outcome from hybridization between species that differ greatly in their genetic breadth, especially with one of the parental species apparently genetically nested within the other). It may be that all British elm clones that look similar to *U. plotii* have this taxon in their parentage in one way or another. However, it is also possible that such similarities are due to chance, relating to common origins from the same ancestral gene pool. We view the *U. minor* complex as an open gene pool, which is prevented from forming a complete continuum of morphological variation due to the relative rarity of sexual reproduction in Britain. When sexual reproduction occurs in the *U. minor* complex it does not seem unreasonable to expect
that by chance certain individuals will become established, which although unrelated, share some distinctive morphological features (analogous to the sometimes unnerving similarities between unrelated humans). The key point here is that the sharing of two or three morphological features does not necessarily equate to fine scale genetic relatedness.

**Taxonomic and conservation implications**

It has been suggested that *Ulmus glabra* and *U. minor sensu latissimo* (i.e. the *U. minor* complex) and their hybrids should be “regarded as belonging to a common species” based on their open gene pool and isozyme continuum (Machon et al., 1995: 46). Our local sampling of these widespread taxa prevents us from providing firm data for this debate, although we do note that our samples of *U. glabra* and *U. minor* were separated in our PCO analyses (Fig. 1). However, were we to target intermediate forms, then a genetic continuum could probably be demonstrated, as was observed with isozymes. The translation of all these results to a formal taxonomic treatment is difficult, and finding a hard-line where one could draw a distinction between the two species is probably not possible. However, the creation of a single inclusive species would obscure valuable biological information. Communication is an important function of taxonomy and where there is a general understanding of the morphological, reproductive and ecological distinction between species, as with *U. glabra* and the *U. minor* complex, such changes need to be very carefully considered.

In our opinion the information content of these names warrants their continued application, albeit with caution. A great deal of research has been carried out on plant hybridization and many morphologically defined plant species have the potential to reproduce with other taxa. This does not fit in well with a biological species concept; but practising plant taxonomists seldom use this concept anyway. In practice there is a trade-off between lumping taxa together to produce a tidy taxonomy on the one hand, and the information content of the names on the other. In the case of *U. glabra* and *U. minor*, intermediate individuals are not evenly distributed through the range of these species in Britain or Europe. In northern Britain or Scandinavia a botanist would simply not encounter intermediates, as all plants fit the description of *U. glabra* (other than rare ornamentals). In other areas, particularly where planting has obscured ecological boundaries and nursery crosses are abundant the situation is not so clearcut. On balance, our sympathies lie with those who recognize two species in spite of the intermediates, to avoid information loss. This is taxonomic pragmatism, and similar decisions have been reached in a huge array of other genera. In the British flora *Gentianella* Moench, *Geum* L., *Dactylorhiza* Necker ex Nevski, *Primula* L. and *Silene* L. include examples (Stace, 1997). As long as the users of a classification are aware of the difficulties presented by hybridization, this need not be a barrier to a workable taxonomy.

Clonal propagation of the *U. minor* complex elms seems fundamental to their taxonomic complexity. It seems likely that certain genotypes in the *U. minor* complex will remain restricted and effectively unnoticed. Others, however, due to either serendipity or having some characteristic useful or attractive to man, will become widely distributed. The widespread occurrence of a single genetic individual can lead to its recognition on a fine scale. This, we believe, is what has happened in the case of *U. plotii*. A single clone (genotype B) has been propagated and distributed
around the English Midlands. Its particular combination of morphological characteristics and geographically discrete distribution has resulted in it attracting the attention of taxonomists. In effect it represents a frozen snapshot of a delayed sexual process. In a fully sexual species, combinations of many morphological characters come and go as recurrent gamete fusions produce a myriad array of different combinations and assortments of genes. In the *U. minor* complex in Britain, however, sexual events are rare, and the success of individual clones appears to be determined by human selection. This process produces a punctuated pattern of variation and discrete distributions similar to those found in agamospermous species.

Given that we believe *U. plotii* sensu stricto consists of a single clone, it is worth asking whether it should be given specific rank. Opinions on how to treat morphologically distinct entities of an extremely limited genetic base vary among taxonomists. On the one hand, ‘microspecies’ treatments have lead to some rather derisory comments (“rather pointless and a wasting of print and paper”, Winge, 1938); however, in the case of elms, we believe there is some value in the recognition of units for communication. For instance, at the time of writing, there is a large Europe-wide collaborative project assessing the genetic resources of the European elms. One goal of this project is testing the susceptibility of different elm clones to Dutch elm disease. Clearly, if morphology can be reliably used for identifying clones, then the results from this could be extrapolated to include additional field based observations. However, aside from the very real practical difficulties of accurate identification (e.g. the ‘pseudoplotii’ and putative *U. minor × U. plotii* discussed above), and while we fully appreciate the value of names for communication, we have three reasons for not recommending the allocation of specific rank to segregates of the *U. minor* complex.

Firstly, we have reservations about giving individual genotypes full specific rank, although we do, however, accept Stace’s (1998) argument that even among sexual species there is no ‘standard’ amount of diversity, thus invalidating arguments based upon the idea that species should be somehow genetically equivalent.

Secondly, we agree with Stace (1998) that any taxonomic classification of the *U. minor* complex elms will struggle to cope with (a) “periodic promiscuous sexual interludes” and (b) being applicable to the rest of Europe where seed set is more common.

Thirdly, and perhaps most importantly (at least based on our data from *U. plotii*), we question whether the distributions of segregates of the *U. minor* complex are natural, and instead feel the evidence strongly supports human planting. The apparently natural distributions of segregates of the *U. minor* complex could equally well have arisen from a history of farmers propagating elms from their neighbours’ hedges. In this respect, work on another named segregate of the *U. minor* complex is of interest. In a molecular study of Cornish elm (*U. minor* Mill. subsp. *angustifolia* (Weston) Stace), 65 samples collected from widespread localities in Cornwall, showed complete band uniformity from eight RAPD primers (66 fragments) indicative of a single clone (P.M. Hollingsworth & J.V. Armstrong, unpublished data).

In light of this we raise the question as to whether individual clones, if they are to be named, may not better be considered as cultivars. A cultivar is defined as a taxon selected for a particular attribute or combination of attributes, and that is clearly distinct, uniform and stable in its characteristics and that, when propagated by appropriate means, retains those characteristics (Trehane & Brickell, 1995). This may prove to be a useful method for recognizing clones of the *U. minor* complex in
Britain. However, we strongly believe that stability is vital to taxonomic treatments and do not suggest burdening an already complicated literature with further name changes until there are more data to support these observations. If individual taxa placed in the _U. minor_ complex can be shown to be genetically and morphologically discrete (i.e. form a cohesive genetic group larger than a genet, thus giving some natural biological rather than horticultural meaning to the taxon), then our suggested application of the rank of cultivar would need to be reconsidered.

In terms of biodiversity conservation, a cultivated clone that falls within the range of variation of a widespread and common species would seem less important than a rare taxon that exhibits genetic variability and is not reliant upon man for dispersal. As our data suggest that _U. plotii_ is an example of the former situation we feel it would be inappropriate to regard it as a conservation priority.

For the future, an exploration of the levels and relationships of clonal diversity in other commonly recognized segregates of the _U. minor_ complex, such as _U. procera_ and _U. minor_ Mill. subsp. _sarniensis_ (C.K. Schneid.) Stace, would be informative. If the same pattern of data is obtained from these taxa as has been found in _U. plotii_ and the Cornish elm, we will perhaps be closer to understanding the population genetic processes responsible for the morphological complexity of the _U. minor_ complex in Britain.

**ACKNOWLEDGEMENTS**

We acknowledge the helpful comments of Susan Wiegrefe and another anonymous reviewer. We are also grateful to Rolf Holderegger and Quentin Cronk for commenting on previous drafts of this manuscript, Chris Clennett for providing access to the living collection at Wakehurst Place, Chris Preston for historical biological records that enabled the relocation of many _Ulmus plotii_ sites, Clive Stace for the collection of samples, loan of herbarium material and helpful discussion in the early stages of this research, Richard Griffiths for technical advice, Andy Lowe for help with principal coordinates analysis, Stephen Droop for assistance with sigma plot and to Petra Hoffman for providing access to herbarium material. The work was supported in part by NERC grant GST/02/833 and European Union grant 'Coordination for conservation, characterisation, collection and utilisation of genetic resources of European elms' (DGVI).

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RAPD genotypes and determinations of British elm samples organized numerically by sample number. Taxon abbreviations: PL = *U. plotii*; PS = 'pseudoplotii'; MI = *U. minor*; GL = *U. glabra*; HO = *U. x hollandica*; PL × MI = *U. plotii* × *U. minor*, CA = *U. carpinifolia*; CO = *U. coristana*. Doubtfully determined juvenile material is indicated by a question mark after the taxon code. Previous determinations include source of identification in parentheses: GM = Guy Messenger; RM = Ronald Melville; RBGE = Royal Botanic Garden Edinburgh. Where our determination differs from a previous worker reasons are given: 1 = Proliferating short shoots absent; 2 = Equal to subequal and cordate leaf bases absent; 3 = Typical *U. plotii* unilateral habit absent; 4 = Red glandular hairs absent; 5 = Red glandular hairs present; 6 = Upper surface of adult leaves with dense covering of simple hairs; 7 = Adult material not seen. RAPD genotypes: A, B, C, D and E = shared; + = unique.

| Sample number | Previous determination | Present determination | RAPD genotype | Reason for different determination |
|---------------|------------------------|----------------------|---------------|-----------------------------------|
| 1             | PL (GM)                | PL                   | B             |                                   |
| 2             | —                      | GL                   | +             |                                   |
| 3             | —                      | HO                   | +             |                                   |
| 4             | PL (GM)                | PS                   | 1             |                                   |
| 5             | PL × MI (GM)           | PL × MI              | +             |                                   |
| 6             | —                      | MI                   | +             |                                   |
| 7             | —                      | MI                   | A             |                                   |
| 8             | PL (GM)                | PL                   | B             |                                   |
| 9             | —                      | GL                   | +             |                                   |
| 10            | PL (GM)                | PL                   | B             |                                   |
| 11            | PL (GM)                | PL                   | B             |                                   |
| 12            | —                      | PL                   | B             |                                   |
| 13            | —                      | PL                   | B             |                                   |
| 14            | —                      | GL                   | +             |                                   |
| 15            | —                      | PL                   | B             |                                   |
| 16            | —                      | PL                   | B             |                                   |
| 17            | —                      | PL                   | B             |                                   |
| 18            | —                      | MI                   | +             |                                   |
| 19            | PL (GM)                | PL?                  | 7             | D                                 |
| 20            | —                      | MI                   | +             |                                   |
| 21            | —                      | GL                   | +             |                                   |
| 22            | PL (GM)                | PL?                  | 7             | B                                 |
| 23            | PL (GM)                | PL?                  | 7             | B                                 |
| 24            | —                      | GL                   | +             |                                   |
| 25            | —                      | GL                   | +             |                                   |
| 26            | —                      | GL                   | +             |                                   |
| 27            | PL (GM)                | PS                   | 1             | C                                 |
| 28            | GL × PL × CA (RM)      | HO                   | 5, 6          | +                                 |
| 29            | GL × CA × PL (RM)      | HO                   | 5, 6          | +                                 |
| 30            | GL × PL (RM)           | HO?                  | 7             | +                                 |
| 31            | CO × PL × GL (RM)      | HO?                  | 7             | +                                 |
| 32            | CO × CA × PL (RM)      | PL × MI?             | 7             | +                                 |
| 33            | GL × PL (RM)           | HO                   | 5, 6          | +                                 |
| 34            | PL × CA (RM)           | PL × MI?             | 7             | +                                 |
| 35            | CO × PL (RM)           | PL × MI              | +             |                                   |
| 36            | CA × GL × PL (RM)      | HO                   | 5, 6          | +                                 |
| 37            | GL × PL (RM)           | MI                   | 1, 2, 3       | +                                 |
| 38            | GL × PL (RM)           | GL                   | 4, 6          | +                                 |
| 39            | PL (RM)                | PS                   | 3             | +                                 |
| 40            | GL × PL × CA (RM)      | HO                   | 5             | +                                 |
| 41            | CA × PL × CO (RM)      | PL × MI              | +             |                                   |
| 42            | CO × PL (RM)           | PL × MI              | +             |                                   |
| 43            | PL × CO (RM)           | PL × MI              | +             |                                   |
| 44            | GL × PL (RM)           | PS                   | 1, 6          | +                                 |
| 45            | PL (RM)                | PL                   | B             |                                   |
| 46            | CO × PL × GL (RM)      | HO                   | 5, 6          | +                                 |

continued
## APPENDIX—continued

| Sample number | Previous determination | Present determination | Reason for different determination | RAPD genotype |
|---------------|------------------------|-----------------------|-----------------------------------|---------------|
| 50            | PL x CO (RM)           | PL x MI               |                                   | +             |
| 51            | PL x CA (RM)           | PL x MI               |                                   | +             |
| 53            | PL (RM)                | PL                    |                                   | B             |
| 54            | PL x GL x CO (RM)      | HO                    | 5,6                               | +             |
| 55            | CO x PL (RM)           | MI                    | 1,2,3                             | +             |
| 56            | GL x PL x CA (RM)      | HO?                   | 7                                 | +             |
| 57            |                        | PS                    |                                   |               |
| 58            |                        | PS                    |                                   |               |
| 59            |                        | PS                    |                                   |               |
| 60            |                        | HO                    |                                   |               |
| 61            | PL (GM)                | PS                    | 1                                 | C             |
| 63            |                        | GL                    |                                   |               |
| 64            |                        | PL2                   |                                   | D             |
| 65            |                        | GL                    |                                   |               |
| 66            |                        | GL                    |                                   |               |
| 67            | PL (GM)                | PL                    |                                   | B             |
| 68            | PL (GM)                | PL                    |                                   | B             |
| 69            |                        | PS                    |                                   |               |
| 71            | GL x PL (GM)           | GL                    | 4,6                               | +             |
| 72            |                        | PL                    |                                   | B             |
| 73            |                        | GL                    |                                   |               |
| 74            |                        | PL                    |                                   | B             |
| 75            |                        | MI                    |                                   | A             |
| 76            |                        | MI                    |                                   | A             |
| 77            |                        | MI                    |                                   |               |
| 78            |                        | MI                    |                                   |               |
| 79            |                        | MI                    |                                   |               |
| 80            |                        | MI                    |                                   |               |
| 81            |                        | MI                    |                                   |               |
| 82            |                        | MI                    |                                   |               |
| 83            |                        | PL?                   |                                   |               |
| 84            | GL x PL (RM)           | GL                    | 4,6                               | +             |
| 85            | GL x PL (RM)           | GL                    | 4,6                               | +             |
| 86            | MI x PL (RBGE)         | PL x MI               |                                   | E             |
| 87            | CA x PL (RBGE)         | PL x MI               |                                   | E             |
| 88            |                        | PS                    |                                   |               |
| 89            |                        | GL                    |                                   |               |
| 90            |                        | GL                    |                                   |               |
| 91            |                        | GL                    |                                   |               |
| 92            |                        | GL                    |                                   |               |
| 93            |                        | GL                    |                                   |               |
| 94            |                        | GL                    |                                   |               |
| 95            |                        | GL                    |                                   |               |
| 96            |                        | GL                    |                                   |               |
| 97            |                        | GL                    |                                   |               |
| 98            |                        | GL                    |                                   |               |
| 99            | GL (RBGE)              | GL                    |                                   |               |