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THE ORIGINS OF LEYLAND'S CYPRESSES (X*CUPRESSOCYPARIS LEYANDII*) BASED ON DNA DATA

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ABSTRACT

Leyland's cypress (*xCupressocyparis leylandii*) is a putative, spontaneous hybrid between *Chamaecyparis nootkatensis and Cupressus macrocarpa.* To investigate this putative origin, twenty five Leyland's cypresses were sampled, along with living, putative parents of *Ch. nootkatensis* and *Cup. macrocarpa* from gardens in the UK. The DNA was extracted and analyzed by DNA fingerprinting (RAPDs) and Inter-Simple Sequence Repeats (ISSRs). DNA fingerprinting indicated that Leyland's cypresses were intermediate between the putative parental species. When a total of 77 RAPD bands were examined by principal coordinates analysis, the Leyland's cypresses were ordinated in an intermediate position between *Ch. nootkatensis* and *Cup. macrocarpa,* suggestive of hybrid origin. Several additive bands between 'Aurea' (*Ch. nootkatensis*) and 'Lutea' (*Cup. macrocarpa*) were found in Leyland's cypresses. Neither the sequences of nrDNA (nuclear ribosomal ITS region) nor chalcone synthase were informative due to heterozygosity in both the parents, and the putative hybrids. Examination of Inter-Simple Sequence Repeats (ISSR) by capillary electrophoresis revealed Castlewellan and Galway Gold trees to be intermediate in their bands between Aurea and Lutea, suggesting hybrid origin. A recent nomenclatural moving of *Ch. nootkatensis* to *Xanthocyparis* (*X. nootkatensis*), resulted in a change of the name Leyland's cypress to *xCuprocyparis leylandii*. But, recently, Little et al. proposed restoring *Callitropsis* in place of *Xanthocyparis* and thus, *Callitropsis nootkatensis*. If accepted, this new name, *Callitropsis nootkatensis*, will lead to a new name for Leyland's cypress.

KEY WORDS: Leyland's Cypress, *xCupressocyparis leylandii*, hybrids, *Cupressus macrocarpa*, *Chamaecyparis nootkatensis*, *Xanthocyparis nootkatensis*, *Callitropsis nootkatensis*, RAPDs, ISSR.

Leyland's Cypresses (*xCupressocyparis leylandii*) are some of the most widely cultivated horticultural trees in the UK (Rushforth, 1987). Yet, the origin of such plants is a horticultural mystery. *xCupressocyparis leylandii* is a putative spontaneous hybrid between *Cupressus macrocarpa* Hartw. (Monterey Cypress) and *Chamaecyparis nootkatensis* (D. Don) Spach (Nootka cypress). However, the parentage of *xCupressocyparis leylandii* has been assigned on the basis of seed origin and the similarity of foliage, not on any specific intentional crossings, or other experimental data.

The history of the initial trees of Leyland cypress is reviewed by Owens et al. (1964), and Mitchell (1996) adds details on several more recently discovered trees. Owens et al. (1964), report that in 1888, some seeds obtained from *Ch. nootkatensis* (Nootka cypress) growing at Leighton Hall were sown and six of the plantlets raised differed in foliage from the others. Mr. C. J. Leyland (brother-in-law of Mr. Naylor of Leighton Hall), took the 6 plantlets [Note: these seedlings were unfortunately, called clones 1 to 6, but they are not actually clonal (e.g. asexually derived)] and planted these at his home at Haggerston Castle (Kew Bull. No. 3, 1926). "Clone 2" was later named 'Haggerston Grey' and is very widely planted. These six plants were later described as intergeneric hybrids, *xCupressocyparis leylandii* Dallimore.

In 1911, Mr. Leyland's nephew, Capt. J. M. Naylor, picked a cone from a *Cupressus macrocarpa* (Monterey cypress) at Leighton Hall that grew about 50 yards from a *Ch. nootkatensis* tree (Owens et al., 1964). He planted these *C. macrocarpa* seeds. When the seedlings developed adult foliage, two were unusual (different from *C. macrocarpa*). These two plants were re-planted about one-half mile apart behind Leighton Hall and these plants became known as 'Leighton Green' ("clone 10") and 'Naylor's blue' ("clone 11"). In 1940, seed was collected from a Monterey cypress and two plantlets with

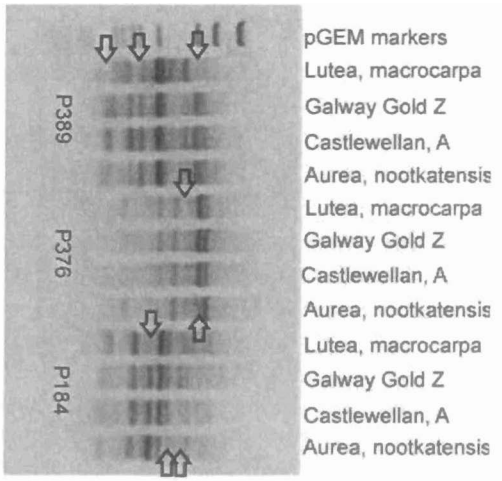

Figure 1. RAPDs gel showing complementary bands between putative parents (Lutea and Aurea) and in putative hybrids.

unusual foliage were raised as "clone 20" and "clone 21". The cultivar 'Castlewellan' was derived from a seed from a cone on a fallen branch of Monterey cypress 'Lutea', growing near a slightly yellow foliaged Nootka cypress, 'Aurea'. The cultivars 'Harlequin', 'Golconda' and 'Silver Dust' are reported to have arisen as sports on older clones (cultivars). Other clones (cultivars) have no recorded history: 'Clun Rectory', 'Galway Gold', 'Hyde Hall' and 'Rostrevor'.

Leyland's cypresses were recognized as hybrids as *Cupressus x leylandii* Jackson & Dallimore (Kew Bull. #3, 1926). Later, when the genus was divided into *Chamaecyparis* and *Cupressus*, the present name was applied (*xCupressocyparis* Dallimore).

The analysis of DNA-genetic variation within and among populations and among species has been developed in our lab for the *Cupressaceae* (Adams and Turuspekov, 1998; Adams, 1999, 2000a, 2000b, 2000c, 2000d; Adams, 2001; Adams et al., 2001a, 2001b). DNA fingerprinting, RAPD (Random Amplified Polymorphic DNAs)

	P398			P376		P184		
Arw	1	2	3	1	2	1	2	3
M	-	-	-	+	-	+	+	-
Z	+	+	+	+	+	+	+	+
A	+	+	+	+	+	+	+	+
N	+	+	+	-	+	-	-	+

Table 1. Bands showing complementary inheritance for three primers in figure 1. Arw = Arrow; M= *Cup. macrocarpa*. 'Lutea'; Z = 'Galway Gold'. A = 'Castlewellan'; N = *Ch. nootkatensis*. 'Aurea'.

and ISSR Inter-Simple-Sequence-Repeats) are ideally suited for the identification of clones and resolving the question of parentage.

The purpose of this paper is to attempt to resolve the historical and genetic relationships among clones of Leyland's Cypresses and their putative parents and to provide identification of the individuals and their parentage by DNA fingerprinting and DNA sequencing.

MATERIALS AND METHODS

In addition to the samples of Leyland's cypress (appendix 1), samples were collected from the living putative parents, *C. macrocarpa* (3) and *Ch. nootkatensis* (2), from gardens in the United Kingdom to use as putative parents in the analysis. Although both species are endemic to the west coast of North America, it seemed appropriate to select from cultivated trees in the United Kingdom as that is the place of origin of Leyland's cypresses.

One (1) gram (fresh weight) of the foliage was placed in 20 grams of activated silica gel and transported to the lab, where it was stored at -20° C until the DNA was extracted by use of the Qiagen DNeasy mini plant kit.

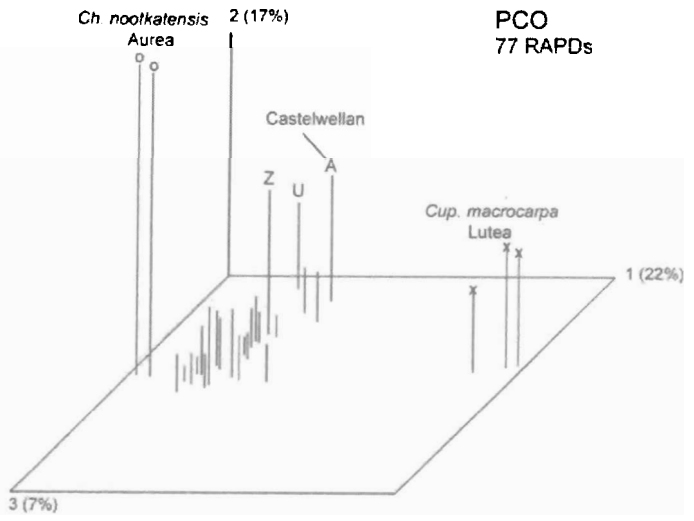

Figure 2. PCO ordination based on 77 RAPD bands. A = Castlewellan A, Z = Galway Gold, U ='Ferndale'.

The RAPD analysis follows that of Adams and Demeke (1993). Ten-mer primers (University of British Colombia and IDT, Inc.): (5'-3') 131: GAA ACA GCG T; 184: CAA ACG GCA C; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 239: CTG AAG CGG A; 244: CAG CCA ACC G; 268: AGG CCG CTT A; 338: CTG TGG CGG T; 376: CAG GAC ATC G; 389: CGC CCG CAG T; 413: GAG GCG GCG A were used.

For RAPDs, PCR was performed in a volume of 15 µl containing 1.5 µl Promega 10X buffer, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). RAPDs were run as: 94°C (1.5 min) for initial strand separation, then 40 cycles of 40°C (2 min), 72°C (2 min),

91°C (1 min). Two additional steps were used: 40°C (2 min) and 72°C (5 min) for final extension. Amplification products were analyzed by electrophoresis on 1.5 % agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667.

Inter-Simple Sequence Repeat (ISSR) analysis follows Adams et al. (2003) and utilized ISSR primer UBC-811: GAGAGAGAGAGAGAGAC and the RAPD protocol (above) except using an annealing temperature of 50°C in the PCR amplification. The resulting reactions were then analyzed by adding 1 µl of product plus 1 µl of 400 bp size standard to the CEQ sample loading solution and running the samples on the Beckman CEQ 8000 capillary instrument at 6 kv for 60 min.

Numerical analysis follows Adams' (1975) minimum spanning networks and Gower's (1966) formulation of principal coordinate ordination.

RESULTS AND DISCUSSION

Figure 1 shows RAPD bands for the putative parents ('Lutea', *Cup. macrocarpa*; 'Aurea', *Ch. nootkatensis*) and the putative hybrids, Castlewellan A and Galway Gold, Z. Notice for primer 389, there are three bands that show inheritance. For primer 376, there are two bands that are complementary and for primer 184 there are three complementary bands. This can be seen more diagrammatically in Table 1.

So in five cases, parental bands came from *Ch. nootkatensis* ('Aurea') and in three cases parental bands came from *Cup. macrocarpa* ('Lutea'). This is perhaps the strongest evidence to date that 'Aurea' and 'Lutea' are the parents of 'Castlewellan' (although this does not exclude other *Ch. nootkatensis* growing in the area). 'Galway Gold' also appears to be a hybrid but it is also a distinct cultivar from 'Castlewellan'.

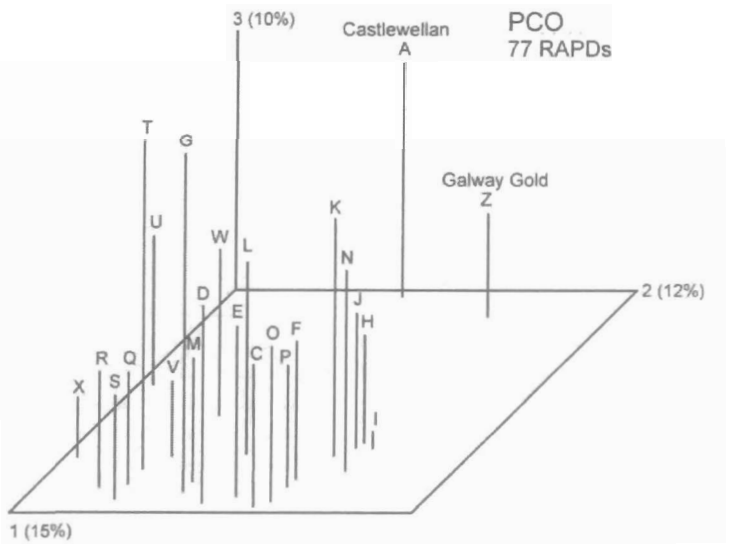

Figure 3. PCO of Leyland's cypresses (with *Ch. nootkatensis* and *Cup. macrocarpa* removed).

Considering all 77 RAPD bands, PCO ordination shows the parental species well resolved (fig. 2) and the Leyland's cypresses somewhat intermediate between *Ch. nootkatensis* and *C. macrocarpa* (fig. 2). Note particularly trees 'Castlewellan' (A) and Galway Gold (Z) are intermediate between the putative parents ('Aurea' and 'Lutea', fig. 2). 'Ferndale' (U) appears to be very similar to 'Castlewellan' and 'Galway Gold' in this PCO. The balance of the Leyland's cypresses show a general clustered that suggests they had a different parentage(s) from 'Castlewellan' and 'Galway Gold' cultivars.

In order to examine the relationships between Leyland's cypresses clones more closely, PCO was performed without the putative parents. Several smaller clusters are visible (fig. 3) suggesting that 'families' of Leyland's cypresses came from cones of a single tree (ex. [X, R, S, Q]; [E, C, O, P, F]; [K, N, J, H]).

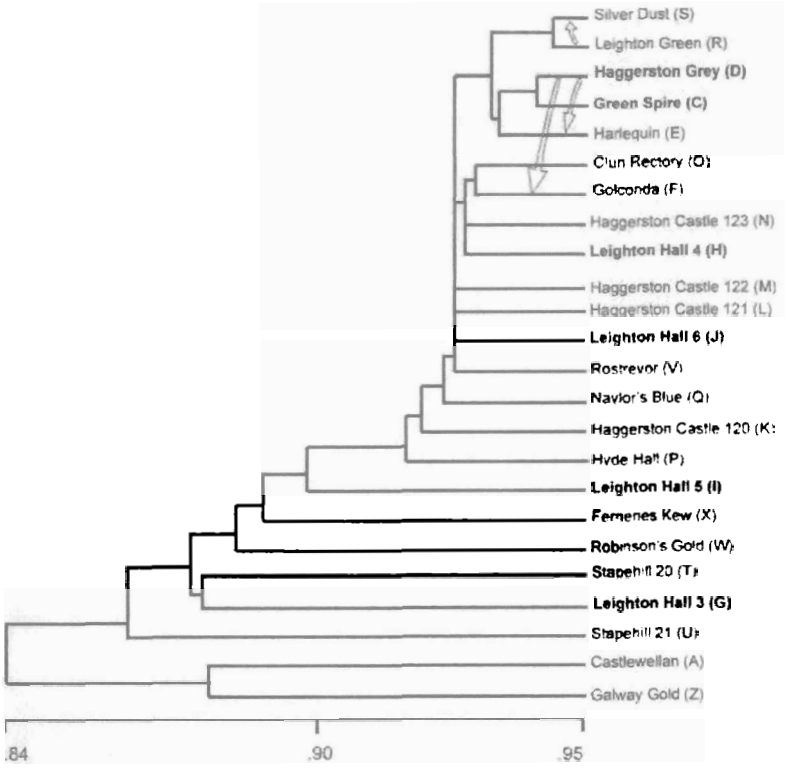

Figure 4. Minimum spanning network based on 77 RAPDs. Arrows show the putative origins of 3 clones.

Some Leyland's cypresses are thought to be clones from previous Leyland's cypresses [e.g., 'Silver Dust' (S) from 'Leighton Green'(R)] and these do cluster together in figure 3. To examine these kinds of differences, a minimum spanning network was produced (figure 4). Notice that 'Silver Dust' (S), a branch sport cloned from 'Leighton Green' (R) at Washington, D.C. is nearly identical to 'Leighton Green'. Likewise, 'Harlequin' (E), having white patches on the foliage, reputedly a branch sport obtained 'Haggerston Grey' (D), is nearly identical to 'Haggerston Grey' (D). However, 'Golconda' (F), with

uniform pale golden green foliage, another reputed branch sport from 'Haggerston Grey' (D), is actually more similar to 'Clun Rectory' (O). This indicates that 'Golconda' is probably not a sport from 'Haggerston Grey', but perhaps from 'Clun Rectory'.

In general, one can see (fig. 4), that from 'Silver Dust' to 'Hyde Hall', these Leyland's cypresses are very genetically similar. The six individuals obtained as seedlings from *Ch. nootkatensis* cones at Leighton Green (1888) are in boldface (Fig. 4). By the diversity of their DNA, it seems unlikely that these seedlings were half sibs (i.e., had the same mother tree). 'Castlewellan' and 'Galway Gold' are rather distinct, as is 'Stapehill' to a lesser degree. The Kyloe Woods clones, numbers 120 (K), 121 (L), 122 (M) and 123 (N), have been recorded as very early cuttings from the original 1888 seedlings, at least as far as three of the clones are concerned (Mitchell, 1996). Figure 4 shows that clones 121 (L) and 122 (M) are similar to the un-named original seedling 'clone' 6 and that clone 123 (N) is very similar to un-named original seedling 'clone' 4. However, the analysis shows that clone 120 (K) (as represented in the stock bed at Alice Holt) is different. This suggests that it is of independent origin.

'Rostrevoer' (V) (as represented in the stock bed at Alice Holt) is shown to be very similar to the un-named seedling 'clone' 6. There are two possible explanations. The stock bed labels could have become switched at some stage, with the result that the plant recorded as 'Rostrevor' and the clone 120 (K) have been switched. This would be consistent with Mitchell's assertion (1996) that three of the Kyloe Wood trees were recorded as cuttings from one original seedling plant. The other possibility is that Rostrevor Garden received one of the early cuttings distributed before the hybrid was suspected (Mitchell, 1996), and thus received a cutting of 'clone' 6.

Adams et al. (2003) found that ISSRs were useful in delimiting closely related *Juniperus* species. So, in addition to RAPDs analyses, a preliminary ISSR analysis was made using 'Aurea', 'Lutea' and 'Castlewellan' (A) and 'Galway Gold' (Z). Figure 5 shows the capillary electrophoresis chromatograms for these samples and one can see several peaks (bands) that are complementary (arising from

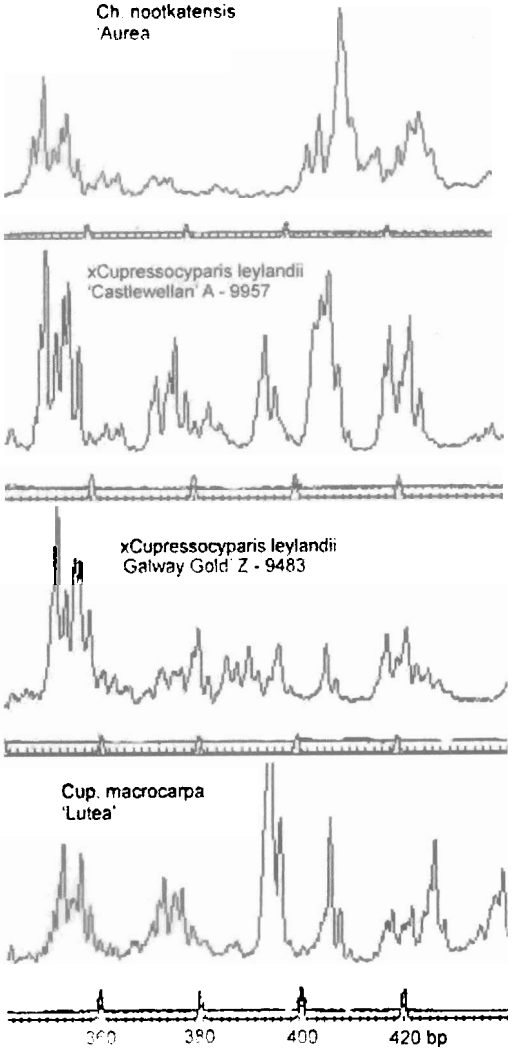

Figure 5. ISSR capillary electrophoresis (partial, ~300 - 440 bp).

either one of the putative parents), just as seen with the RAPDs data (Fig. 1). Note especially the peaks (bands) around 380 bp that are present in 'Lutea', absent in 'Aurea', but present in 'Castlewellan' (A) and 'Galway Gold' (Z). Also peaks around 450 bp in 'Aurea', are absent in 'Lutea', but present in 'Castlewellan' (A) and 'Galway Gold' (Z). The complementary inheritance of RAPD bands is well known (Adams and Demeke, 1993). This is strong evidence that Leyland's cypress is of hybrid origin from the putative parental species.

CONCLUSIONS

The long standing hypothesis that Leyland's cypresses arose by chance crossing of *Chamaecyparis nootkatensis* and *Cupressus macrocarpa*, cultivated in the United Kingdom, seems to be verified by the DNA fingerprinting presented in this study. It is unfortunate that both DNA sequences examined (nrDNA, chalcone synthase) were too polymorphic in the parents to be utilized. However, this study should aid nurserymen and horticulturalists in applying names to Leyland's cypresses of commercial utilization.

The Nootka cypress (*Chamaecyparis nootkatensis*) is of uncertain generic origin at present. Originally named *Cupressus nootkatensis* D. Don, it was transferred by Spach to his new genus *Chamaecyparis*, as *Chamaecyparis nootkatensis* (D. Don) Spach, and more recently by Farjon et al. (2002) to *Xanthocyparis nootkatensis* (D. Don) Farjon & Harder. Gadek et al. (2000) found *Chamaecyparis nootkatensis* to be within *Cupressus* (of the Old World) in their study, but Little et al. (2004), using more extensive DNA sequence data, found it to be congeneric with *Xanthocyparis vietnamensis*; in addition, they presented evidence from DNA sequencing that *Cupressus macrocarpa* (Monterey Cypress) (and the New World cypresses) may not be in the same genus as the Old World cypresses, such as the type species of *Cupressus, C. sempervirens* L.

The recent nomenclatural change (Farjon, et al., 2002) moving *Chamaecyparis nootkatensis* to *Xanthocyparis* (*X. nootkatensis* (D. Don) Farjon), resulted in a change of the name (Leyland's cypress) to *xCuprocyparis leylandii* (A.B. Jackson & Dallimore) Farjon.

However, Little et al. (2004) pointed out that the genus *Callitropsis* and the name *Callitropsis nootkatensis* (D. Don) Orsted has priority and proposed restoring *Callitropsis* in place of *Xanthocyparis* (thus *Callitropsis nootkatensis*). If accepted, this name, *Callitropsis nootkatensis*, will lead to new scientific name for Leyland's cypress.

ACKNOWLEDGMENTS

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x *Cupressocyparis leylandii* :

9463-9468. In 1888, seed was collected from *Ch. nootkatensis* at Leighton Hall and germinated. Six seedlings were unusual and sent to Haggerston Castle in 1892; samples 9463-9468 are from these 6 trees.
9463, C, Clone 1, 'Green Spire'
9464, D, Clone 2, 'Haggerston Grey'
9465, G, Clone 3, un-named
9466, H, Clone 4, un-named
9467, I, Clone 5, un-named
9468, J, Clone 6, un-named
9469-9470. In 1911, two unusual seedlings were obtained from seed from *Cup. macrocarpa* at Leighton Hall, UK. The two samples from these trees are:
9469, Q, Clone 10, 'Naylor's Blue'
9470, R, Clone 11, 'Leighton Green'.
9471-9472. Plants, reputed cuttings from the early seedlings planted at Kyloe Wood, Haggerston Castle (Mitchell (1972) records three as planted in 1897 and one in 1906):
9471, K, Clone 120, un-named
9472, L, Clone 121, un-named
9473, M, Clone 122, un-named
9474, N, Clone 123, un-named
9475-9476. In 1940, seed from *Cup. macrocarpa* at Barthelemy's Nursery at Stapehill were germinated and two unusual individuals were found. No *Ch. nootkatensis* was recorded in the vicinity:
9475, T, Clone 20, un-named
9476, U, Clone 21, 'Stapehill'.
9477, V, 'Rostrevor', reported to have originated from an old tree at Rostrevor, County Down, Ireland, planted circa 1870, which blew down before 1914, but Mitchell (1996, p. 64) indicates that the plants in cultivation are from cuttings made much later.
9478, O, 'Clun Rectory', a tree growing at Clun, Shropshire, UK circa 1900.
9479, S, 'Silver Dust', a branch sport obtained from Clone 11 'Leighton Green' tree (=**9470**, R, above) growing in Washington, D. C. Date uncertain.
9480-9481, branch sports with patches reputedly obtained from clone 2 'Haggerston Grey'(9464, D, above):

9480, E, 'Harlequin', Weston Park, Shropshire. Harlequin has patches of white foliage.
9481, F, 'Golconda', Wyborton, Bedfordshire. Golconda has pale golden, green foliage.
9482, Z, 'Galway Gold', origin unknown but often thought to be a renaming of 'Castlewellan'.
9483 B, 'Castlewellan', Castlewellan, County Down, Northern Ireland, 1962.
9484, W, 'Robinson's Gold', County Down, Ireland, 1964.
9485, X, 'Ferneries Kew' tree found growing in the Fernery at Kew Gardens, UK, date unknown.
9486, P, 'Hyde Hall', tree growing at Hyde Hall, Essex, date unknown.
Material collected by Michael Lear from trees at Castlewellan Arboretum, County Down, Northern Ireland:
9957, A, 'Castlewellan', Castlewellan, UK, from the original tree, reputedly from a cone from *Cup. macrocarpa* 'Lutea' growing near a *Ch. nootkatensis* 'Aurea'. 1962.
Chamaecyparis nootkatensis
9956, N1, 'Aurea', tag 0177, Castlewellan, UK, planted in 1892.
10069, N2, Westernbrit, UK, planting date unknown.
Cupressus macrocarpa
9953, M1, 'Lutea', tag 0045, Castlewellan, UK, planting date unknown
9954, M2, tag 0051, Castlewellan, UK, planting date unknown.
9955, M3, Tregrehan, UK, planted in early 1800s.

Appendix 1. Leyland's cypresses, *Ch. nootkatensis* and *Cup. macrocarpa* collected for analysis, with notes on their origins. Four digit numbers refer to Adams' Lab analyses numbers. The letters (A, B, C..) refer to labels in figures. Clone numbers are those of Owens et al. (1964).