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E.S. Mace · C.G. Gebhardt · R.N. Lester

AFLP analysis of genetic relationships in the tribe Datureae (Solanaceae)

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Abstract The AFLP technique was evaluated as a tool for assessing species relationships within the tribe Datureae and genetic distances were estimated for 47 accessions of over 12 species. The phenetic trees from various analyses of the AFLP data gave very high co-phenetic correlation values, and were found to be consistent with previous trees based on the analysis of different data types, in particular ITS-1 sequences, isozymes and morphology, carried out on the same accessions. These results indicated that the AFLP technique is both an efficient and effective tool for determining genetic relationships among taxa in the Solanaceae. A new classification is proposed for the tribe Datureae, which maintains the arborescent species as a separate genus, *Brugmansia*, and recognises three sections within the genus *Datura*; *Stramonium*, *Dutra* and *Ceratocaulis*. *D. discolor*, previously placed in section *Dutra*, was found to be intermediate between sections *Dutra* and *Stramonium*.

Key words DNA · AFLP · Solanaceae ·*Datura* · *Brugmansia* · phenetic analysis

Introduction

The tribe Datureae belongs to the family Solanaceae, subfamily Solanoideae. It contains between nine (e.g. Hammer et al. 1983) and 12 (e.g. Bye 1986) herbaceous species, all considered to be in the genus *Datura*, and between three (Bristol 1966) and 14 (Safford 1921) arborescent species of disputed nomenclatural status. These arborescent species are commonly known as the tree daturas or *Brugmansia*, and are considered either as a section or subgenus of the genus *Datura*, or else awarded separate generic status, as *Brugmansia*. There are a number of species-level problems within the tribe; however, the delimitation of the two genera is the most controversial issue of the tribe and, as Satina and Avery (1959) have stated, "the most confusing and unsettled problem in the taxonomy of *Datura* is its relationship to the *Brugmansia* group of species". The main centre of diversity for the tribe is the Americas, even though the present distribution of the genus *Datura* is almost world-wide. The arborescent *Brugmansia* species are South American in origin, whereas the herbaceous *Datura* species originate from Central America and south-western USA. All species of the tribe contain potent tropane alkaloids, in particular hyoscine (scopolamine), which is highly psychoactive (Roddick 1991). This accounts for their widespread medicinal and psychotropic use among cultures throughout the world, but particularly by the American Indians (Lockwood 1979).

Species of the genus *Datura* were first established by Linnaeus in his 'Species Plantarum' (1753), in which he described three species, one of which, *D. arborea* L., was arborescent. Linnaeus' original concept of the genus, therefore, was one that encompassed both herbaceous and arborescent species. Persoon (1805) was the first to challenge this concept, with the valid publication of the generic name *Brugmansia* for the arborescent species, as he considered the differences between the herbaceous and arborescent species great enough to warrant this separate generic status. Bernhardi (1833) opposed Persoon's view and established four sections in the genus *Datura*, *Stramonium*, *Dutra*, *Ceratocaulis* and *Brugmansia*, primarily due to the nature of many characteristics of *D. ceratocaula*, which Bernhardi viewed as being intermediate between the herbaceous and arborescent species. Bernhardi's view of the arborescent species as a section within the genus *Datura* has been followed by numerous botanists, e.g. Dunal (1852), Safford

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E.S. Mace
Plant Improvement, School of Land and Food,
The University of Queensland, Brisbane, QLD 4072, Australia

C.G. Gebhardt
Max-Planck-Institut für Züchtungsforschung,
Carl-Von-Linné-Weg 10, D-50829 Köln, Germany

R.N. Lester (✉)
School of Biological Sciences, University of Birmingham,
Edgbaston, Birmingham, B15 2TT, UK
e-mail: r.n.lester@bham.ac.uk
Fax: +44-121-414-5925

(1921), Danert (1955), DeWolf (1956) and Bristol (1966). However, increased support for Persoon's view has recently arisen, e.g. Lockwood (1973), Haegi (1976), Symon and Haegi (1991) and Persson et al. (1999). Lockwood (1973) provided evidence that the herbaceous and arborescent groups of taxa have evolved independently, based on the adaptation of characters of *Datura* species to a xeric environment, and those of *Brugmansia* species to a mesic environment. Lockwood also demonstrated that several characters retained in the *Brugmansia* species are primitive relative to those in the *Datura* species, such as the bilocular ovary, self-incompatibility and the long-lived perennial condition. Despite Lockwood's evidence, the argument still continues, e.g. Nee (1991) once again proposed the lowering of the genus *Brugmansia* to the level of a section within the genus *Datura* based on the linking species concept of *D. ceratocaula*. Satina and Avery (1959) advised that "a fresh approach with new criteria and data might be of help" to this long-standing problem. Persson et al. (1999) followed this advice with the application of cladistic analysis to pollen morphology. The application of molecular techniques to the problem has so far been limited to protein chemotaxonomy (amino-acid sequencing of ferre-

Table 1 Accessions of the tribe Datureae used for AFLP analysis. The full Nijmegen Botanical Garden's accession numbers are listed in this Table: in the text these are abbreviated by replacing '4750', the code for Solanaceae, by '–'

Taxon	Code	Accession no.	Donor	Origin
Datura stramonium L.	str	944750032	Bergarac	–
D. stramonium var. *stramonium* L.	strS	884750260	Luik	Luik
D. stramonium var. *stramonium* L.	strS	884750067	Marburg	Marbella
D. stramonium var. *stramonium* L.	strS	914750062	Gatersleben	–
D. stramonium var. *tatula* (L.) Torr.	strT	954750094	BIRM	–
D. stramonium var. *tatula* (L.) Torr.	strT	954750098	BIRM	–
D. stramonium var. *tatula* (L.) Torr.	strT	954750104	BIRM	–
D. stramonium var. *tatula* (L.) Torr.	strT	954750157	Paris	–
D. stramonium var. *inermis* Jacq.	strI	884750126	Milan	–
D. stramonium var. *inermis* Jacq.	strI	894750327	–	–
D. stramonium var. *inermis* Jacq.	strI	954750099	BIRM	–
D. ferox L.	fer	954750103	BIRM	–
D. ferox L.	fer	954750091	BIRM	S. Australia
D. metel L.	met	884750130	Milan	–
D. metel L. cv. Golden Queen	met	944750019	Fosdinovo	–
D. metel var. *fastuosa* (Bernh.) Danert	metF	934750013	Univ. Toronto	–
D. wrightii Regel	wrg	954750095	BIRM	S. Australia
D. wrightii Regel	wrg	934750177	Luik	Utah
D. wrightii Regel	wrg	884750177	Milan	–
D. wrightii Regel	wrg	894750303	Friulano Udine	–
D. discolor Bernh.	dis	924750157	Schwerdtfeger	–
D. discolor Bernh.	dis	894750219	Gatersleben	–
D. inoxia Mill.	inx	954750058	Fosdinovo	–
D. inoxia Mill.	inx	924750080	Dresden	–
D. inoxia Mill.	inx	924750047	Brno	–
D. inoxia Mill.	inx	944750096	Cuernavaca	–
D. inoxia Mill.	inx	944750030	Bergarac	–
D. inoxia Mill.	inx	924750094	Guadalajara	Mexico
D. inoxia Mill.	inx	944750079	Weerden	Gelderland
D. inoxia Mill.	inx	884750149	Milan	–
D. inoxia Mill.	inx	924750024	Meise	France
D. ceratocaula Ortega	cer	954750090	BIRM	California
D. eckensis Ledeb.	eck	904750225	BIRM	–
Datura sp.	spp	954750080	Bergen	Honduras
Datura sp.	spp	914750139	Los Angeles	–
Brugmansia sanguinea (Ruiz & Pav.) D. Don.	san	944750003	Chiltern Seeds	–
B. aurea Lagerh.	aur	924750208	–	–
B. × *candida* Pers.	can	814750020	Weerden	–
B. × *candida* Pers.	can	944750004	Chiltern Seeds	–
B. × *candida* Pers. cv. Klerx Variegatum	can	934750212	Monaco	–
B. × *candida* Pers. cv. Grand Marnier	can	934750216	Weerden	–
B. suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & Presl.	suv	934750205	Genova	–
B. suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & Presl.	suv	904750061	Monaco	–
B. suaveolens (Humb. & Bonpl. ex Willd.) Bercht. & Presl.	suv	894750239	Genova	–
B. × *insignis* (Barb. Rodr.) Lockwood	ins	934750211	Monaco	–
B. versicolor Lagerh.	ver	904750059	Weerden	–
Atropa spp.	Atr	944750014	J-L. Gatard	–

doxin: Mino et al. 1993; Mino 1994a, b), cytology (Palomino et al. 1988) and isozymes (cf. Lester 1979). The direct study of nuclear DNA diversity, through the application of the AFLP technique, therefore constitutes a fresh approach bringing new data to this problem of the generic recognition of the arborescent and herbaceous species of the tribe Datureae.

The objectives of the present study were: (1) to detect AFLP variation in the tribe Datureae; (2) to determine systematic relationships within the tribe Datureae; and (3) to evaluate the usefulness of AFLPs as systematic characters, with regards to the Solanaceae family. For comparative purposes, the same accessions have been used here as in other previous studies, utilising isozymes, ITS (the internal transcribed spacer of nuclear ribosomal DNA) sequences and morphological analyses (Mace et al. 2000).

Materials and methods

Plant material

A total of 47 accessions of the tribe Datureae, plus one outgroup *Atropa* accession, were used in this study. Table 1 lists the sources of the accessions. The plant material was grown at the Botanical Garden of the University of Nijmegen, The Netherlands.

DNA isolation and AFLP analysis

DNA was extracted from 0.4 g of freeze-dried leaf samples of the accessions using the QIAGEN Genomic DNA Purification from Plant Leaves protocol (QIAGEN GmbH, Max-Volmer-Strasse 4, 40724 Hilden, Germany). The DNA was then purified using the QIAGEN Genomic-tip Protocol, using midi prep-volumes. The AFLP procedure was carried out essentially as described by Zabeau and Vos (1993) and Vos et al. (1995). Modifications are as in Mace et al. (1999).

Data analysis

For each accession, a binary matrix reflecting specific AFLP-band presence (1) or absence (0) was generated. Only heavy bands were scored, faint bands were discarded. These data were analysed using the NTSYS version 1.80 (Rohlf 1993) as detailed in Mace et al. (1999). Three different similarity coefficients and four clustering methods were used to produce dendrograms with the TREE program. The goodness of fit of the clustering to the data matrix was calculated by the COPH and MYXCOMP programs. Principal coordinate analysis (PCO) employed the DCENTER and EIGEN procedures. Cut-off points were assigned to group the accessions into clusters on all dendrograms produced by selecting an appropriate similarity measure. The cut-off points, and consequently the number of clusters, varied between the taxa and depended on the number of accessions and the level of diversity within each taxon. Therefore, the cut-off point had to be flexible in order to take account of the variations between taxa.

Results

The AFLP primer combinations *Hind*III+ACC and *Mse*I+ACC, *Hind*III+ACC and *Mse*I+AGC, *Hind*III+ACC and *Mse*I+ACA, *Hind*III+ACC and *Mse*I+AAG, *Hind*III+AAT and *Mse*I+ACA, *Eco*RI+ACT and *Mse*I+CAG, *Eco*RI+AAC and *Mse*I+CAG, and *Eco*RI+ACA and *Mse*I+CCA were used to analyse 50 accessions. They yielded 45, 42, 41, 21, 9, 36, 30 and 49 polymorphic AFLPs respectively. The sizes of the AFLP fragments were determined by comparing sequencing ladders of control template DNA to AFLP patterns. AFLP fragment sizes ranged from approximately 50 to 700 base pairs (bp). Polymorphic fragments were distributed across the entire size range with the major proportion being between 150 and 300 bp.

Table 2 Comparison of co-phenetic correlation values obtained from the three similarity coefficients and four clustering methods employed for analysing the present AFLP data

Clustering Method	Similarity Coefficients		
	DICE	Jaccard's	SM
UPGMA	0.981	0.987	0.965
WPGMA	0.978	0.984	0.959
Complete linkage	0.970	0.973	0.960
Single linkage	0.964	0.971	0.927

The dendrograms constructed using the three different similarity coefficients (Dice, Jaccard's and SM) and various different clustering methods (UPGMA, WPGMA, complete linkage and single linkage) were examined and the co-phenetic correlation values produced by each coefficient compared (Table 2). The UPGMA method gave consistently higher co-phenetic correlation scores, and Jaccard's (1908) coefficient also gave consistently higher co-phenetic correlation values than either the Dice or SM coefficients. Four main "ball-clusters" (*sensu* Rohlf 1993) are present in all the dendrograms produced when a cut-off point of approximately 50% was selected, which largely correspond to the sections within the genus *Datura* and the genus *Brugmansia*. Figure 1 shows the dendrogram produced by Jaccard's coefficient and the UPGMA clustering method, with the four main clusters identified. Cluster analysis was also performed with each primer combination individually and the four ball-clusters were still observed, although the internal structure did differ slightly between the different primer combinations (data not shown). In all cases, the outgroup *Atropa* accession was separated from the four main clusters by a large genetic distance.

Cluster 1 contains taxa only from *Datura* section *Stramonium*, namely *D. stramonium*, *D. stramonium* var. *stramonium*, *D. stramonium* var. *tatula*, *D. stramonium* var. *inermis*, and *D. ferox*. There is also an unidentified *Datura* species, 95-080, and an accession called *D. eckensis*, 90-225, which is a very rare name that is not mentioned, even in synonymy, by any of the three major treatments of the tribe Datureae this century (Safford 1921; Satina and Avery 1959; Hammer et al. 1983). Cluster 2 contains taxa from section *Dutra* of the genus *Datura*. There are three subclusters within this cluster that can be identified at the 75% similarity level and which correspond to the three species, *D. inoxia*, *D. wrightii* and *D. metel*. Most variation is exhibited within

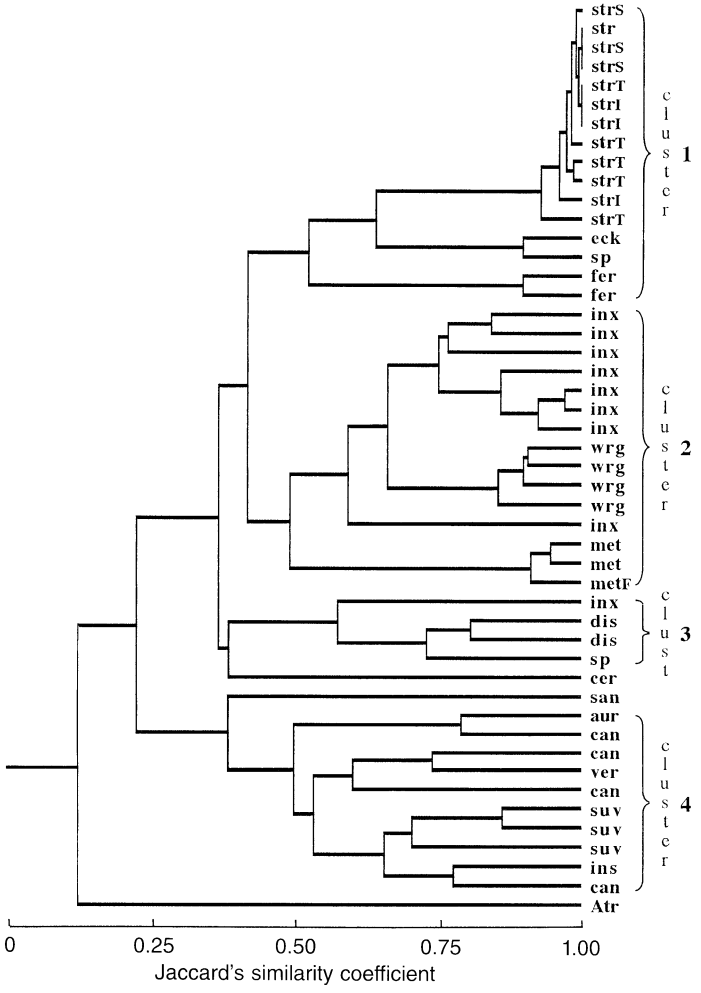

Fig. 1 A dendrogram constructed for 48 Datureae accessions, using Jaccard's coefficient of similarity and UPGMA clustering. The samples are labelled with the codes listed in Table 1

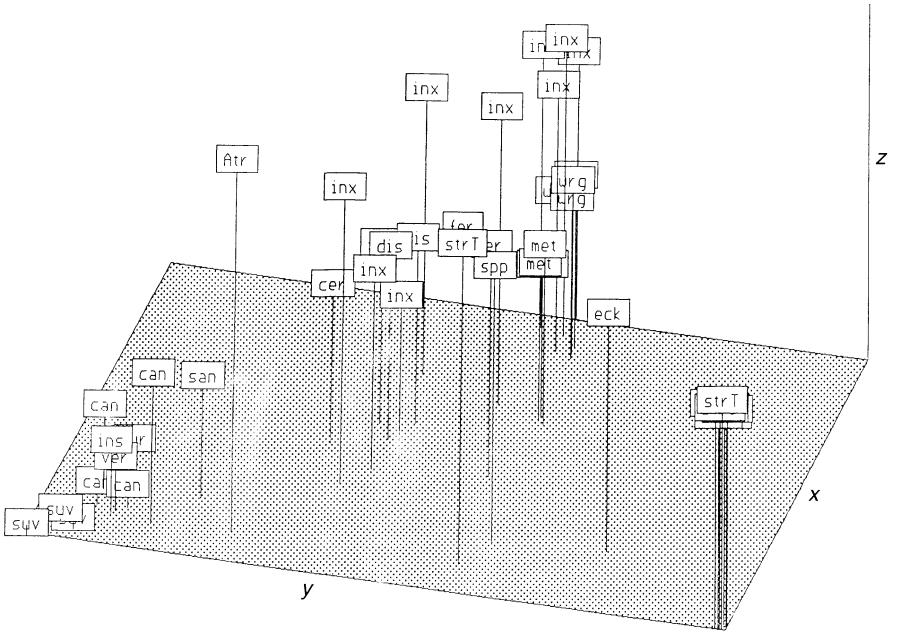

Fig. 2 A 3-D plot of the Principal Coordinates Analysis of the AFLP data. The samples are labelled with the codes listed in Table 1

D. inoxia, as indicated by the loose clustering between the accessions of this species. Cluster 3 contains two accessions of *D. discolor* (92-157, 89-219), an accession of *D. inoxia* (92-094, perhaps misidentified), and a previously unidentified *Datura* accession, 91-139. The tight clustering of this accession to *D. discolor* suggests that, based on the available AFLP data, it can now be confidently identified as *D. discolor.* Linked to cluster 3, but only at about 40% similarity, is *D. ceratocaula* of section *Ceratocaulis*. Cluster 4 contains arborescent *Brugmansia* species, with *B. sanguinea* (94-003) only grouping with the rest at about 35% similarity.

Figure 2 shows a 3-dimensional PCO plot of the Datureae AFLP data set. Of the total variation, 48.1% is represented on the *x* axis, the next 22.7% is represented on the *y* axis, with the next 11.7% on the *z* axis. The accessions plotted are labelled according to the abbreviated species names as listed in Table 2. The *Brugmansia* accessions form a tight distinct group, separate from the herbaceous *Datura* accessions. Within the genus *Datura*, the majority of the accessions from section *Stramonium* form a distinct group, with the accessions from section *Dutra* being more dispersed.

Discussion

AFLP analysis has been shown, from the present study, to provide an independent estimate of genetic relationships in the Solanaceae which is reliable and quite consistent with other molecular and morphological studies, thus supporting the conclusions of Kardolus et al. (1998). The repeatability of the AFLP banding patterns was very high, providing credibility to the conclusions derived from the analyses. As a new molecular technique with few guidelines on the infinite number of ways in which unexpected results can arise, reproducibility is the only easy way of assessing the quality of the data (Karp et al. 1996). Likewise, the general consensus among the dendrograms produced by the different similarity coefficients and clustering methods shows that the data are robust and strengthens confidence in the resulting groups of taxa. AFLP analysis of the taxa in the tribe Datureae supports the following systematic conclusions.

The arborescent species deserve separate generic recognition as *Brugmansia*. The *Brugmansia* species formed a distinct cluster (Fig. 1, cluster 4) that only linked with the herbaceous species at a level of 20% similarity. Within the *Brugmansia* cluster, a number of species-level issues were given support, in particular the hybrid nature of *B.* × *insignis* and *B.* × *candida*, as suggested by Lockwood (1973), which has not yet been fully addressed in recent literature. Two accessions of *B.* × *candida* (94-004 and 81-020) cluster with one accession of *B. versicolor* (90-059), lending weight to Lockwood's claim that *B.* × *candida* is a natural hybrid of *B. aurea* and *B. versicolor*. Furthermore, another accession of *B.* × *candida* (93–212) groups with an accession of *B. aurea* (92-208) at a level of approximately 80% similarity. Support is also given for the hybrid status of *B.* × *insignis*, which Lockwood suggested is a natural hybrid between *B. suaveolens* and *B. versicolor*, as the *B.* × *insignis* accession (93-211) is grouped at a fairly high level of similarity (65%) with the three accessions of *B. suaveolens* (93-215, 90-061 and 89-239), which are shown to represent a distinct species. Furthermore, the accession of *B.* × *insignis* (93-211) and another accession of *B.* × *candida* (93-216) group together at a level of approximately 80% similarity, which could be due to the influence of *B. versicolor* in both natural hybrids, lending further support to Lockwood's claims. *B. sanguinea* was found to be distinct from other species in the genus. This has also been noticed by Beath (1987), who observed that, as well as being morphologically quite distinct, the pollen of *B. sanguinea* was more similar to *D. stramonium* than any of the other *Brugmansia* species. While these results do not support a link between *B. sanguinea* and *D. stramonium*, they do suggest that a real distinction exists between *B. sanguinea* and the other *Brugmansia* species.

Within the genus *Datura*, section *Stramonium* formed another distinct cluster (Fig. 1, cluster 1), in which *D. ferox* was shown to be genetically quite distant from the taxa of *D. stramonium*. Within *D. stramonium*, the distinction between the different varieties was less well-supported, in particular the recognition of the two varieties *inermis* (with spineless fruits and white flowers) and *tatula* (with spiny fruits and purple flowers). Breeding experiments, initially carried out by Safford (1921) and later by Satina and Avery (1959), proved that flower colour was under the control of a single gene, and likewise a single gene for spininess and smoothness was identified. Recent work by Mino et al. (1993) on the amino-acid sequences of ferredoxin again supported the conspecificity of the white and purple-coloured varieties. The AFLPs support the recognition of *D. stramonium* var. *stramonium*; however, the distinction between the spiny and smooth forms of the fruit is not supported. The taxon labelled *D. eckensis* might represent *D. quercifolia*, the third species of the section *Stramonium*. It is unlikely to represent a variety of *D. stramonium*, as the accessions of *D. stramonium* are grouped at such a high level of similarity. It is also unlikely to represent *D. ferox*, for the same reason that the accessions of this taxon are grouped together at a very high level of similarity. The AFLP analysis revealed quite a high level of variation between the taxa of section *Stramonium*, even though there is very little variation within taxa. It has been suggested (e.g. Beath 1987) that taxa of the section *Stramonium* are the most ancient of the tribe, and represent the ancestral species. The lack of variation within this section and the large amount present between it and other sections lends support to this theory.

Section *Dutra* was also distinguished (Fig. 1, cluster 2) and the constituent species, *D. inoxia*, *D. wrightii* and *D. metel*, were clearly delimited, despite previous confusion. In particular, *D. wrightii*, a species morphologi-

cally very similar to *D. inoxia*, has often been referred to as *D. meteloides* (Safford 1921; Satina and Avery 1959); however, the majority have placed *D. meteloides* in synonymy under *D. wrightii* (e.g. Ewan 1944; Hammer et al. 1983; Symon and Haegi 1991). *D. metel* has also been confused with *D. inoxia* (e.g. Sims 1812; Dunal 1852), although the results presented here indicate that *D. metel* is very distinct from the other taxa of section *Dutra*, only grouping with the other *Dutra* accessions at a level of 50% similarity. Beath (1987), on the basis of analysis of pollen morphology, observed that *D. metel* showed greater affinity to members of section *Stramonium* than to members of section *Dutra*. However, the results presented here contradict this view, and suggest that the continued inclusion of *D. metel* in section *Dutra* is valid.

The continued inclusion of *D. discolor* (Fig. 1, cluster 3) in section *Dutra* is not supported, as this species is found to be no closer to taxa of section *Dutra* than it is to taxa of section *Stramonium*. This has also been observed by Barclay (1959), who noted that *D. discolor* had morphological characteristics that were intermediate between sections *Stramonium* and *Dutra*, as it has nodding capsules (characteristic of section *Dutra*) which dehisce regularly (characteristic of section *Stramonium*), and more recently by Mace et al. (2000) who noted that *D. discolor* was intermediate between the two sections at the molecular level also, as observed through isozyme and ITS-1 sequence analysis. The results presented here, and by Mace et al. (2000), suggest that *D. discolor* could have evolved through a hybridisation event between taxa of section *Stramonium* and section *Dutra*, or else that it is ancestral to both sections. Due to the small size of the data set (only two well-authenticated accessions of *D. discolor* were available for the analysis) and the variable nature of the species, a definite proposal to establish a new section for *D. discolor* is not yet appropriate.

D. ceratocaula was shown to be distinct from sections *Dutra* and *Stramonium*. It is the status of this taxon that has caused the controversy between those authors who believe the arborescent species should be given separate generic status (e.g. Persoon 1805; Lagerheim 1895; Lockwood 1973) and those that believe they should be relegated to a section within the genus *Datura* (e.g. Bernhardi 1833; Safford 1921; Bristol 1966). Lockwood (1973) postulated that rather than being a primitive connecting link between the *Datura* and *Brugmansia* species, *D. ceratocaula* represented a highly specialised *Datura*. This view was given further support recently by Beath (1987) who found evidence, from pollen morphology, that *D. ceratocaula* was a specialised member of section *Dutra*. However, the present AFLP data support the maintenance of the separate section *Ceratocaulis* for *D. ceratocaula*, as it appears to be no closer to members of section *Dutra* than it is to members of section *Stramonium*.

In comparison to a recent study which used the same accessions and undertook isozyme, morphological and ITS-1 sequence-variation analyses to determine species relationships (Mace et al. 2000), the results derived from the AFLP analysis are found to be largely congruent. The previous study supported the recognition of distinct groups within the tribe Datureae, as recognised here; however, the delimitations between the groups were less precise than from the present study. The previous study provided support for the generic recognition of *Brugmansia*, based on the isozyme and morphology data sets, but the results from the ITS-1 data set did not confidently support this generic delimitation, as the accessions of *Brugmansia* included in the ITS-1 study were found to be more closely related to accessions of *Datura* section *Dutra* than were accessions of *Datura* section *Stramonium*. The delimitations of the sections *Stramonium* and *Dutra* within the genus *Datura*, from both the isozyme and morphological analysis, were less well-defined than from the AFLP analysis. In particular, the species-level resolution within section *Dutra* was found to be far more highly resolved from the AFLP analysis than from either the isozyme or morphological analysis. Consequently, the genome-wide comparison of the AFLP technique, considering many loci, was able to distinguish and delimit the natural groups within the tribe, namely sections *Stramonium*, *Dutra*, *Discolor* and *Ceratocaulis* of the genus *Datura* and the genus *Brugmansia*, more clearly than the isozyme, morphology and ITS-1 data sets.

AFLP analysis has several advantages over other molecular techniques for the analysis of genetic diversity and the determination of genetic relationships. One of the major advantages is the large number of DNA loci that can be assayed in a relatively short period of time, which is referred to as a high multiplex ratio. The amount of information obtained from AFLP analysis appears to be approximately proportional to the number of primers employed; however, until very recently, e.g. Ellis et al. (1997), there have been very few reports in the literature that attempt to quantify the effects of the amount of primers used. Ellis et al. (1997) concluded that by selecting the six best combinations of primers it is possible to explain more than 80% of the expected relatedness. The present project selected the eight best primer combinations from a total of 32, so it is possible to conclude, with a high level of confidence, that the majority of the expected relatedness has been found in this study. Nevertheless, it is more likely that it is the total number of polymorphic bands scored, and not the total number of primers used, that is more important. AFLPs have also been shown to be as reproducible as RFLPs, but have the advantage of the added power of the PCR technique. A recent study by Jones et al. (1997) demonstrated that the AFLP technique was highly reproducible between seven European laboratories, with only a single-band difference observed in one track, and was comparable with the reproducibility of SSR markers. Like RAPDs, AFLPs are dominant markers, and therefore heterozygotes cannot easily be detected. However, the development of appropriate computer software (Breyne et al. 1997) will further

Table3 Proposed new classification for the tribe Datureae, based on AFLP and other data

***Datura* L.**

Section I. *Stramonium* Bernh.
D. stramonium L.
D. ferox L
D. quercifolia Humb., Bonpl. and Kunth

Section II. *Dutra* Bernh.
D. metel L.
D. wrightii Regel
D. inoxia Mill.
D. leichhardtii F. Muell. ex Benth.
D. lanosa Barclay ex Bye

Section III. *Ceratocaulis* Bernh.
D. ceratocaula Ortega

Section IV. *Discolor*
D. discolor Bernh.

***Brugmansia* Pers.**

B. arborea (L.) Lagerh.
B. aurea Lagerh.
B. sanguinea (Ruíz and Pav.) D. Don
B. suaveolens (Humb. and Bonpl. ex Willd.) Bercht. and C. Presl.
B. versicolor Lagerh.
B. × *candida* Pers.
B. × *dolichocarpa* Lagerh.
B. × *insignis* (Barb. Rodrigues) Schultes
B. × *rubella* (Saff.) Moldenke

increase the value of the AFLP technique by allowing for the efficient scoring of both homozygotes and heterozygotes for a specific fragment. A wider issue in the consideration of the use of AFLPs in assessing species relationships is that of band homology. The presence of a similar band of apparently identical molecular weight in different individuals cannot necessarily be taken as evidence that the two individuals share the same homologous fragment (Karp et al. 1996); however, the mutual coincidence of several bands strengthens the likelihood of the pairwise homology of all of them. The more sensitive separation range of AFLPs over RAPDs makes this issue less serious for AFLP data (Powell et al. 1996), though ideally additional genetic analysis and hybridization studies should be carried out in tandem. A recent study by Rouppe van der Voort et al. (1997) indicated that AFLP markers of the same molecular weight, from different potato genotypes, were homologous.

In conclusion, this research represents one of the most comprehensive studies of DNA diversity for the Datureae, and is among the first to report on the effectiveness of the AFLP technique for determining genetic relationships in the Solanaceae, at both specific and generic levels. The results presented here support the classification for the tribe Datureae shown in Table 3. AFLP analysis has been demonstrated to be quick, robust and effective, and it requires only minimal preliminary work to detect a large number of genetic loci, which far exceeds that possible, in the same amount of time and at the same cost, by using other techniques.

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