Genetic and Morphological Analysis of the Origin and Identity of Perennial *Lavatera* (Malvaceae) Cultivars

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ABSTRACT. Knowledge of the origin of *Lavatera* L. (tree mallows) cultivars helps to predict their cultural requirements. Eighteen accessions representing 15 cultivars, 14 accessions of 7 species, and 5 accessions of an F₁ hybrid between the putative parents of the cultivars were sampled for morphological variation and for randomly amplified polymorphic DNA (RAPD) fingerprint variation. Species-specific molecular markers were identified from the RAPD profiles. Chimeral elements were not distinguishable by RAPD analysis. Principal component analysis identified the majority of the cultivars to be selections of hybrid origin, probably from a narrow genetic base. Two cultivars were derived directly from individual species. The resolving power of RAPD markers and morphology was similar although RAPD data offered greater ability to ascribe parentage while morphology offered optimal discrimination of cultivar selections.

Shrubby *Lavatera* L. (tree mallows) are grown widely and have become popular garden plants (Hardy, 1997; Miller, 1999), especially since the introduction of 'Barnsley' in 1986 (Lancaster, 1987). Several species occur in cultivation although the majority of *Lavatera* grown are cultivars whose origin in relation to wild species is undocumented and their affinities are therefore uncertain. At least 15 cultivars are currently offered in the United States (Hill and Narizny, 2000) and over 20 in the United Kingdom (Lord et al., 2000). The species may be recognized quite easily using traditional morphological characters (Fernandes, 1968) although the generic circumscription is not so clear (Ray, 1995).

Uncertainty over the garden habit and performance of cultivars which nurseries list under one or other of the species, or simply under *Lavatera* is a result of arbitrary classification. This situation arose initially from lack of awareness of the differences between *L. olbia* L. and *L. thuringiaca* L. and the uncertain placement of the first cultivar, 'Rosea', which dates from before 1920 (Bean, 1970–80). Only one other cultivar, later named 'Kew Rose' (Cheek, 1989), was grown for the next 60 years or so until the arrival of 'Barnsley' stimulated selection and introduction of further cultivars in the late 1980s. As *L. olbia* is essentially shrubby in cultivation and *L. thuringiaca* is herbaceous, it is important to horticulturists that the cultivars are not assigned randomly to either species. It is also confusing if the same cultivar name is assigned to two different species, or none at all, in different publications.

Although a few cultivars have arisen as sports, many have been selected from open-pollinated seedlings which arise readily in a garden or nursery situation. These cultivars have been selected primarily on the basis of flower color (white to dark pink), flower size and petal spacing (Miller, 1999). They have

been attributed variously to *L. olbia* or *L. thuringiaca* (Cheek, 1989; Huxley, 1992; Lancaster, 1987, 1989) with little consensus to their true identity. Many do not match either species alone (Miller, 1997), implying that hybridization may have had a role in their origin (van de Laar, 1991). One of the cultivars, 'Barnsley', is thought to be a periclinal chimera (Cheek, 1989) and it regularly produces shoots from the base bearing flowers of the "core" color. The possibility of naturalized plants in the United Kingdom being of hybrid origin has also been suggested (Clement, 1998; Stace, 1997) and would be worth considering for those reported naturalized in Australia (Barker, 1977).

Reported chromosome counts of $2n = 40$ (Davie, 1935) or $2n = 42$ (Luque and Devesa, 1986) for *L. olbia* and $2n = 40$ (Bolkhovskikh, 1969), $2n = 42$ (Markova and Goranova, 1993), or $2n = 44$ (Markova and Goranova, 1993) for *L. thuringiaca* are too close to offer the possibility of determining hybrids karyotypically. Other species grown occasionally in gardens can be discounted as possible parents on morphological grounds, but were included in our study for molecular comparison.

Randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990) have been used widely to assess relatedness and distinguish genotypes in plants. The technique has been used with increasing success in the field of ornamental cultivars (Culham and Grant, 1999; Grant and Culham, 1997a, 1997b; Kumar et al., 1998; Ling et al., 1997; Marquard et al., 1997; Walker and Werner, 1997) but may not be sensitive enough to distinguish somatic mutants (Wolff and Peters-Van Rijn, 1993).

The following research was undertaken to investigate the parental origin of cultivars and the role of hybridization in generation of *Lavatera* cultivars using artificial hybridization, RAPD markers, and morphological characters. In addition, the ability of RAPD markers to distinguish cultivars with a narrow genetic base was also evaluated.

Materials and Methods

PLANT MATERIAL. Material of *L. olbia* and *L. thuringiaca* was of documented wild origin, other species grown occasionally in gardens (*L. arborea* L., *L. cachemiriana* Cambess., *L. maritima*

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Gouan, and *L. oblongifolia* Boiss.) were from nursery sources (Table 1). Cultivars were, where possible, obtained from their originators (Table 1). Artificial F₁ hybrids were produced between *L. olbia* and *L. thuringiaca*. All accessions were field-grown in triplicate at the Royal Horticultural Society (RHS) Garden, Wisley, for 3 years. Voucher specimens were deposited in the Herbarium of RHS Garden, Wisley (WSY) or The University of Reading (RNG).

DNA EXTRACTION. About 1g of leaf material was collected in July 1995 from each accession and DNA isolations were performed using the CTAB method, a DNA extraction protocol derived from Sambrook et al. (1989) and Doyle and Doyle (1987).

DNA extractions were quantified and dilutions to 3 ng·μL⁻¹ made with 'Nanopure' (Barnstead/Thermolyne, Dubuque, Iowa) water which were allowed a working life of 6 weeks. New stocks were prepared at the end of this period or following exhaustion of stock (which ever was first).

RAPD POLYMERASE CHAIN REACTION. Arbitrary primers 10 bases in length were obtained from the University of British Columbia, Vancouver, British Colombia, Canada, (no. 701-800) and used for polymerase chain reaction (PCR) amplification according to the protocol described by Williams et al. (1990). Each PCR reaction mix (25 mL) contained the following components/concentrations: 16 mM (NH₄)₂ SO₄, 67 mM Tris-HCl, 0.1% Tween-20, 2.5 mM MgCl₂, 0.2 mM each dNTP, with 0.1 mM of a single primer, 0.5 Unit of *Taq* Polymerase and 1.5 ng genomic DNA. Reactions were performed on a Hybaid Omnigene Thermocycler (Hybaid, Ashford, United Kingdom) in an air-conditioned laboratory (19 to 20 °C) using the following program: 2 min at 94 °C, 39 cycles each of 30 s at 93 °C, 1 min at 42 °C, 2 min at 72 °C, then cooled to 20 °C. Bands were separated on 1% agarose-TAE pH 8.0 gels at 5V·cm⁻¹ for 2 h and visualized with ethidium bromide under ultraviolet light. One hundred base pair (bp) ladders (Promega, Southampton, United Kingdom) were used to estimate the size of the fragments.

Table 1. Details of plant material used in the study.

Species/cultivar	Accession	Raiser/originator or collection location	Source
<i>L. olbia</i>	21	Italy (collected by D.M.M.)	Liss Forest Nursery, Ltd.
<i>L. olbia</i>	22	Italy (collected by D.M.M.)	Liss Forest Nursery, Ltd.
<i>L. olbia</i>	24	Nursery stock acquired from Writtle Agr. College	Monksilver Nursery
<i>L. olbia</i>	26	Nursery stock	Arne Herbs
<i>L. olbia</i>	40	Morocco, Beni-Snassen (SLJ 15553)	University of Reading
<i>L. thuringiaca</i>	17	S. Yugoslavia, Moraca Gorge (AL&JS 90100JU)	Monksilver Nursery
<i>L. thuringiaca</i>	18	S. Yugoslavia, Moraca Gorge (AL&JS 90100JU)	D.M. Miller
<i>L. thuringiaca</i>	19	Italy (collected by D.M.M.)	Liss Forest Nursery, Ltd.
<i>L. thuringiaca</i>	20	Italy (collected by D.M.M.)	D.M. Miller
<i>L. olbia</i> x <i>L. thuringiaca</i>	33	D.M. Miller (D.M.M. H1)	D.M. Miller
<i>L. olbia</i> x <i>L. thuringiaca</i>	34	A.C. Leslie (ACL H1)	A.C. Leslie
<i>L. olbia</i> x <i>L. thuringiaca</i>	35	A.C. Leslie (ACL H2)	A.C. Leslie
<i>L. olbia</i> x <i>L. thuringiaca</i>	36	A.C. Leslie (ACL H3)	A.C. Leslie
<i>L. olbia</i> x <i>L. thuringiaca</i>	37	A.C. Leslie (ACL H4)	A.C. Leslie
<i>L. arborea</i>	27	South France	Perry's Plants
<i>L. arborea</i> 'Variegata'	25	pre 1882	Perry's Plants
<i>L. cachemiriana</i>	30	Nursery stock	Perry's Plants
<i>L. maritima</i>	28	Nursery stock	Burncoose and South Down Nurseries
<i>L. oblongifolia</i>	29	Nursery stock	Madrona Nursery
'Barnsley'	5	Sport from 'Rosea' early 1970s, discovered by R. Verey	Hopleys Plants, Ltd.
'Barnsley'	6	Sport from 'Rosea' early 1970s, discovered by R. Verey	R. Verey
'Barnsley'	7	Sport from 'Rosea' early 1970s, discovered by R. Verey	Monksilver Nursery
'Barnsley Perry's Dwarf'	4	Seedling from 'Barnsley', raised by P. Perry, Perry's Plants	Perry's Plants
'Blushing Bride'	3	Seedling from 'Barnsley', raised by G. Hellman	Acacia Nurseries
'Bredon Springs'	12	R Sidwell, Bredon Springs	Notcutts Nurseries
'Bressingham Pink'	11	Seedling raised by Blooms	Blooms of Bressingham, Ltd.
'Burgundy Wine'	16	Seedling selected by P. Catt, Liss Forest Nursery, Ltd.	Liss Forest Nursery, Ltd.
'Candy Floss'	10	Seedling selected by P. Catt, Liss Forest Nursery, Ltd.	Liss Forest Nursery, Ltd.
'Ice Cool'	1	Seedling selected by P. Catt, Liss Forest Nursery, Ltd.	Liss Forest Nursery, Ltd.
'Kew Rose'	15	First recorded from Ramparts Nursery	Royal Botanic Gardens, Kew
'Lara Rose'	32	Raised by G.J. Allen	G.J. Allen
'Pink Frills'	8	Discovered by Botanic Nursery in a public garden	Hopleys Plants, Ltd.
'Pink Frills'	9	Discovered by Botanic Nursery in a public garden	Liss Forest Nursery
'Rosea'	14	B. Ladham's, Ltd., The Shirley Nurseries, Southampton	Carlile's Hardy Plants
'Saxtead'	31	Introduced by Notcutts	Notcutts Nurseries
'Shorty'	2	Sport from 'Rosea', raised by Langthorns Plantery	Burncoose and South Down Nurseries
'Wemdbon Variegated'	13	Sport from 'Rosea', raised by M. Cheek	R. Cheek
'Barnsley P.D.' (Accn.4) rootsucker	38	Arose during present investigation	---
'Barnsley' (Accn. 5) rootsucker	39	Arose during present investigation	---

Table 2. RAPD data matrix coded as presence (1) or absence (0) of bands for species and cultivars of *Lavatera*.

[illegible]

In total, 100 RAPD primers were screened to identify primers for taxon specific markers for *Lavatera* and to assess cultivar variation, and 17 were selected for use on a limited number of accessions (University of British Columbia 703, 708, 711, 721, 731, 732, 733, 736, 743, 744, 745, 747, 751, 774, 790, 792, and 796). It was found that five were sufficient to distinguish the cultivars (University of British Columbia 703, 721, 733, 790, and 792) (Table 2). All reactions were triplicated and only reproducible bands were considered for this study.

GEL SCORING AND PRINCIPAL COMPONENT ANALYSIS. Gels were scored for the presence (1) or absence (0) of fragments. Principal component analysis was performed using the NTSYS-pc software package (Rohlf, 1992). In addition the data matrix was screened for species specific markers for *L. olbia* and *L. thuringiaca* using the wild origin material.

MORPHOLOGICAL ASSESSMENT. Winter habit was recorded as being herbaceous (plant dying back to ground level in winter) or shrubby (woody framework persisting). Where possible, five organs were measured from each of the accessions which were grown in triplicate ($n = 15$) and means were calculated. Calyx and epicalyx areas (mm^2) were measured in flowers and in fruit using the Delta-T Image Analysis System version 1.12 software (Delta-T Devices, Ltd, 1993). Degree of expansion for both was calculated (mean area in fruit divided by mean area in flower). Pedicel length was measured in fruit. Hairiness of mature mericarps was assessed on the following scale: 0 = glabrous, 1 = few hairs at apex, 2 = scattered hairs, 3 = sparsely hairy, and 4 = densely hairy.

MORPHOLOGICAL DATA ANALYSIS. The morphological data matrix was subjected to principal component analysis using the NTSYS-pc software package (Rohlf, 1992).

Results and Discussion

Typical DNA yields were in the region of 10 to 40 $\text{mg} \cdot \text{g}^{-1}$ of leaf tissue. Under the stated PCR conditions, 1.5 ng of DNA template was found to yield the best results for reliable and repeatable amplification and to maximize the scorable bands.

Of the 100 primers screened, 17 revealed polymorphisms between at least two of the taxa. The number of variable bands per primer was found to range from 1 to 11 with an average of 4.5 (e.g., Table 2). Band frequencies varied from 3% to 95% with an average of 53%.

The morphological characters recorded showed variation within the wild species but little variation within cultivars. The cultivars were generally intermediate in morphology between the species (Table 3).

HYBRIDITY DETERMINED FROM GENOTYPIC CHARACTERS. The artificial F_1 hybrids showed a high proportion of band sharing (92%). The parents, *L. olbia* and *L. thuringiaca*, share an average of 81% and 93%, respectively, within the individuals screened, and 76% and 68%, respectively, with the hybrids. The cultivars demonstrate a high percentage of band sharing with the postulated parent species. Within the major group of hybrid cultivars, band sharing is 92%. This group shows a 66% similarity to *L. olbia*, a 73% similarity to *L. thuringiaca*, and a 77% similarity to the artificial F_1 hybrids.

MOLECULAR MARKERS. Nine RAPD markers for *L. olbia* and *L. thuringiaca* were identified. Two bands (UBC 721 700 bp, UBC 792 900 bp) were unique to *L. thuringiaca* and two (UBC 790 1150 bp, UBC 790 650 bp) shared with only *L. cachemeriana*, which is considered the most closely related species. One further

Table 3. Morphological data matrix. For explanation of characters measured see Morphological Assessment paragraph.

Accession	Cultivar/ species	Calyx expansion (mm^2)	Epicalyx expansion (mm^2)	Merica p hairiness ^z	Fruit pedicel length (mm)	Winter habit ^y
1	'Ice Cool'	2.2	1.7	0	23.2	H
2	'Shorty'	1.8	1.7	1	17.0	S
3	'Blushing Bride'	1.5	1.4	1	12.4	S
4	'Barnsley Perry's Dwarf'	1.3	1.4	1	12.5	S
5	'Barnsley'	1.3	1.3	1	11.8	S
6	'Barnsley'	1.3	1.5	1	11.1	S
7	'Barnsley'	1.3	1.4	1	13.7	S
8	'Pink Frills'	1.2	1.3	4	3.2	S
9	'Pink Frills'	1.3	1.4	4	3.1	S
10	'Candy Floss'	1.3	2.8	1	19.6	H
11	'Bressingham Pink'	1.3	1.5	1	12.1	S
12	'Bredon Springs'	1.8	1.7	3	8.3	S
13	'Wembdon Variegated'	1.4	1.5	2	16.7	S
14	'Rosea'	1.4	1.5	1	12.9	S
15	'Kew Rose'	1.3	1.3	3	12.2	S
16	'Burgundy Wine'	1.4	1.2	2	8.2	S
17	<i>L. thuringiaca</i>	3.7	2.6	1	14.2	H
18	<i>L. thuringiaca</i>	3.9	2.5	1	22.7	H
19	<i>L. thuringiaca</i>	3.1	2.9	0	16.3	H
20	<i>L. thuringiaca</i>	2.8	2.9	0	11.2	H
21	<i>L. olbia</i>	1.3	1.2	4	6.2	S
24	<i>L. olbia</i>	1.6	1.4	4	4.6	S
34	<i>L. thuringiaca</i> x <i>L. olbia</i>	1.3	1.4	1	7.8	S
35	<i>L. thuringiaca</i> x <i>L. olbia</i>	1.5	1.6	2	9.2	S

^zAssessed on a scale of 0 to 4 where 0 = glabrous, 1 = few hairs at apex, 2 = scattered hairs, 3 = sparsely hairy, and 4 = densely hairy.

^yH = herbaceous (plant dying back to ground level in winter) and S = shrubby (woody framework persisting).

Fig. 1. Principal component (PC) analysis of combined RAPD data for all 36 bands and 37 accessions listed in Table 2. ♦ = *L. olbia*, ■ = *L. thuringiaca*, hexagon = *L. olbia* × *L. thuringiaca*, pentagon = other species, and ● = cultivars.

marker (UBC 721 1050 bp) is present in *L. thuringiaca* and *L. arborea* only and serves to distinguish further the former species from *L. olbia*. In contrast, *L. olbia* has no unique bands but does possess a unique absence of band UBC 733 510 bp. *Lavatera olbia* also shares markers (UBC 703 920 bp, UBC 721 800 bp, and UBC 733 460 bp) with other congeners that distinguish it from *L. thuringiaca*.

PHENOTYPIC CHARACTERS. Morphological observations on the taxa relevant to the issue of hybridity showed the majority of cultivars to be intermediate between *L. olbia* and *L. thuringiaca*. *Lavatera olbia* is typically shrubby in habit whereas *L. thuringiaca* is typically herbaceous. ‘Candy Floss’ and ‘Ice Cool’ were also found to be herbaceous whereas all the deliberately raised hybrids and the rest of the cultivars were shrubby.

Lavatera thuringiaca showed the greatest increase in both calyx and epicalyx area from flower to fruit - an average increase of 3.4 times for calyx and 2.7 times for epicalyx. For *L. olbia*, the increases averaged 1.5 times and 1.3 times, respectively. The majority of cultivars and the deliberate hybrids remained closer to *L. olbia* for both these characters although ‘Bredon Springs’, ‘Candy Floss’, ‘Ice Cool’, and ‘Shorty’ all showed increases closer to *L. thuringiaca* in magnitude.

Fruit pedicel length in fruit averaged 16.1 mm for *L. thuringiaca* and 5.4 mm for *L. olbia*. The deliberate hybrids averaged 8.5 mm and the majority of the cultivars fell into the range of 8.2 to 16.7 mm. The exceptions were ‘Pink Frills’ with an average of 3.1 mm and ‘Shorty’, ‘Candy Floss’, and ‘Ice Cool’ with averages of 17.0 mm, 19.6 mm, and 23.2 mm, respectively.

Mericarp hairiness was found to be densest (densely hairy = 4) in *L. olbia* and absent (glabrous = 0), or nearly so, in *L. thuringiaca*. Of the cultivars, only ‘Pink Frills’ (densely hairy = 4) matched the hairiness of *L. olbia* and only ‘Ice Cool’ (glabrous = 0) was as glabrous as *L. thuringiaca*. The deliberate hybrids and the rest of the cultivars fell between 1 and 3 on the scale.

CULTIVAR DISTINCTION DETERMINED FROM GENOTYPIC CHARACTERS. ‘Pink Frills’ and ‘Ice Cool’ are genetically distinct from the other cultivars, and can be attributed to *L. olbia* and *L. thuringiaca*, respectively (Fig. 1). The remainder fall somewhere between the two species with a group of cultivars forming a

Fig. 2. Principal component (PC) analysis of morphological data for four quantitative and one qualitative character and 24 accessions listed in Table 3. ♦ = *L. olbia*, ■ = *L. thuringiaca*, hexagon = *L. olbia* × *L. thuringiaca*, and ● = cultivars.

closely related core. This core group consists of ‘Barnsley’, ‘Barnsley Perry’s Dwarf’, ‘Blushing Bride’, ‘Bressingham Pink’, ‘Lara Rose’,

'Rosea' and 'Wemdbon Variegated'. Some are indistinguishable using the RAPD markers employed in this study.

CULTIVAR DISTINCTION DETERMINED FROM PHENOTYPIC CHARACTERS. 'Pink Frills', 'Candy Floss', and 'Ice Cool' are distinct from the other cultivars in terms of morphological characters used for distinguishing the species (Fig. 2). 'Pink Frills' groups with *L. olbia* while the other two group with *L. thuringiaca*. The remainder form a more homogenous group that can be distinguished by a combination of flower color, and petal size and arrangement. These additional features are a result of artificial selection of garden material and many of the characteristics have originated from somaclonal mutation that is not predictive of parentage. These morphological differences are explored more fully in Miller (1999).

DISTINCTION OF CHIMERAL ELEMENTS. No genotypic polymorphisms in 'Barnsley' or 'Barnsley Perry's Dwarf' were discernible between the normal shoots (bearing white flowers with a pink eye) and the root-origin shoots (bearing pink flowers). This suggests that RAPD markers are not sensitive enough to detect what may be only a single gene mutation affecting flower color.

Conclusions

Results indicate that 'Ice Cool' is derived from *L. thuringiaca* and 'Pink Frills' from *L. olbia*. The other cultivars are hybrids falling between these two species for which a name, *L. x clementii* Cheek, has recently been published (Cheek, 2000). A distinct clustering of closely related cultivars suggests that they have arisen from a relatively closed gene pool probably derived from the two commonest and oldest cultivars, 'Rosea' and 'Kew Rose'. The genetic position of the hybrids raised deliberately for this study from wild-origin parents is intermediate, but they cluster in a different area of the graph to the cultivars indicating their possession of a novel origin. Morphological data were congruent with the molecular results but the characters identifying parentage were not good at distinguishing the cultivars. Novel morphological elements selected in cultivation such as flower color, flower size and petal spacing are necessary for cultivar distinction (Miller, 1999). Molecular markers to the putative parents were successfully identified and could be used in determination of the parentage of the cultivars.

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