A PHYLOGENETIC ASSESSMENT OF *LYCASTE* AND *ANGULOA* (ORCHIDACEAE)

By

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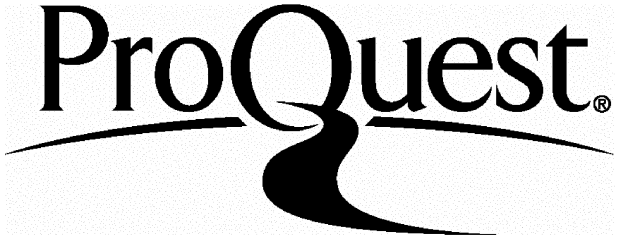

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ABSTRACT

Parsimony analysis has been used to examine the phylogenetic relationships of two genera of Neotropical orchids, *Lycaste* and *Anguloa*. Within these genera, difficulties occur when assigning names to plants using traditional morphological techniques. Many herbarium specimens are in bad condition and some descriptions are incomplete. To date, infrageneric classifications have been based on very few diagnostic characters. Here, three approaches have been evaluated: A systematic analysis of the morphology, an examination of two regions of DNA sequence and an analysis of the chemical composition of the floral fragrances.

Morphological analysis showed that *Lycaste* is not monophyletic. There was a clear division between species currently ascribed to sect. *Fimbriatae* and the other species of the genus. Selection of characters introduced an element of subjectivity into the analysis and it was shown that exclusion of a single character could significantly affect the topology of relationships.

Parsimony analysis of the sequences of both ITS and *matK* placed *Lycaste* sect. *Fimbriatae* closer to *Anguloa* than to the other species of *Lycaste*. *Neomoorea* was identified as nearest neighbour to *Lycaste* and *Anguloa*. A combined analysis of ITS and morphological data gave congruent results.

Morphological and sequence analyses also indicated that the single pendent species, *L. dyeriana*, should remain within sect. *Fimbriatae* and that the taxonomic status of the subspecies of *L. macrophylla* should be revised.

None of the analyses provided sufficient resolution to address the sectional treatment of the remaining *Lycaste* species. To answer this question, comparison of sequence data from a faster evolving region of DNA will be required.

The floral fragrance composition of 28 species and subspecies of *Lycaste* and *Anguloa* was determined. In its current form, the data was found to be unsuitable for addressing phylogenetic relationships at species level and above but may prove useful for population studies.

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GLOSSARY

From Allaby (1992), Dressler (1993) and Pridgeon *et al.* (1999).

Abaxial	The surface of an organ directed away from the main axis.
Anther	That part of the flower that produces pollen.
Anthesis	The time of flowering in a plant or the opening of a flower bud.
Anticlinal	The lateral cell walls that are in contact with each other, as opposed to the inner and outer (periclinal) walls.
Apomorphy	A derived character state.
Autapomorphy	A derived character state that occurs only in the clade under study.
Axis	The main or central stem of a plant.
Bract	Scale or sheath like structure, homologous with leaf, but lacking the blade.
Callus	Crest or fleshy outgrowth of the lip.
Caudicle	Slender, mealy or elastic extension of the pollinium, or a mealy portion at one end of the pollinium. The structure is part of the pollen mass and is produced within the anther.
Column	Central structure of an orchid flower, made up of the style and filaments of one or more anthers.
Column foot	Ventral extension of the base of the column that has the lip attached at its tip.
Distal	Region of an organ furthest from the point at which it is attached to the plant.
Epiphyte	Any plant that grows on another plant.
Fusiform	Spindle shaped.
Genome	The genetic chromosomal complement of a cell, also refers to the circular DNA molecules found in the plastids and mitochondria.
Homoplasy	Similarity that has occurred by independent routes.
Lip	A modified petal.
Monophyletic	Descended from a common ancestor.
Osmophore	A scent producing gland.
Paraphyletic	A group or taxon that does not include all descendants from a common ancestor.
Parsimony	A method of phylogenetic inference based on the simplest or most economic explanation for the observed variation in data.

Pedicel	The stem which supports an individual flower, usually jointed at the base, above the floral bract.
Peduncle	The stem that supports a solitary flower or inflorescence.
Perianth	Collective term for petals and sepals.
Periclinal	Referring to seed coat cells, the walls that are tangential with respect to the seed; the inner and outer cell walls.
Plastid	One of a group of membrane bound plant-cell organelles that vary in their structure and function. Examples are chloroplasts and amyloplasts.
Plesiomorphy	An ancestral character state.
Plicate	Pleated or folded.
Polyphyletic	An artificial group, that has descended from two or more different ancestral groups.
Proximal	Region of an organ nearest to the point at which it is attached to the plant.
Pseudobulb	A thickened stem, usually aerial.
Rostellum	Portion of the stigma which aids in gluing the pollinia to the pollinating agent; the tissue that separates the anther from the stigma.
Scape	The peduncle and rachis of an inflorescence.
Sheath	Leaf-like structure enfolding a stem, pseudobulb or young inflorescence.
Stipe	A pollinium stalk derived from the rostellum.
Style	That part of the pistil that connects the ovary with the stigma. In orchids, part of the column.
Taxon	A taxonomic group of any rank.
Vegetative	Parts of the plant not involved in flowering or fruiting, i.e. the leaves, stems, bulbs and roots.
Velamen	One or more layers of spongy cells on the outside of the root.
Viscidium	Part of the rostellum that is removed with the pollinia and is used to attach them to an insect or other agent.

Chapter 1 INTRODUCTION

This thesis seeks to establish the phylogenetic relationships within and between *Lycaste* and *Anguloa* using three different approaches:

- A systematic examination of the morphology.
- An examination of two regions of DNA sequence: The internal transcribed spacer (ITS) of ribosomal nuclear DNA and the plastid maturase encoding gene *matK*.
- An analysis of the chemical composition of the floral fragrances.

1.1 GENERAL INTRODUCTION

Lycaste and *Anguloa* are two closely related Neotropical orchid genera. *Lycaste* was first described by John Lindley (1843a) and is currently considered to comprise some 50 to 60 species and subspecies. These range from Mexico to Bolivia and Brazil, typically at elevations between 500m and 2500m. *Anguloa,* described by Ruiz and Pavón (1794a), comprises about nine species, found in the Andes from Venezuela to Peru between 1500m and 2500m (Figure 1.1).

Within these two genera, difficulties arise when assigning names to plants using morphological features alone. Some type specimens are in bad condition, others have been destroyed, for example, those of Schlechter in the Berlin Herbarium which was partially destroyed in March 1943; all of the orchid specimens were lost.

Many of the original descriptions lack detail and are often without illustration. At least one (*Lycaste leucantha* Klotzsch) has been illustrated at a later stage with the wrong plant. These problems have been exacerbated by inconsistent and conflicting perceptions of what delimits a species.

Figure 1.1 The geographical range of *Lycaste* and *Anguloa*.

The word "species" derives from the Latin, *speculare*, to look. One of the simplest definitions has been provided by Cronquist (1978): "The smallest groups that are consistently and persistently distinct and distinguishable by ordinary means." Although it is generally accepted that species are discrete groups of organisms (Andersson, 1990), opinion is divided on how discontinuities between groups arise and are maintained. Three popular hypotheses are concerned with reproductive isolation (e.g. Mayr, 1982), geneology (e.g. Wiley, 1978) and differential selection (e.g. Van Valen, 1976), and some definitions encompass one or more of these concepts. Jeffrey (1982), for example, considers a species to be "a series of intergrading and interfertile populations, recognisably distinct from other such series and separated from other such series by genetically controlled barriers".

Infrageneric classifications have been published for both *Lycaste* and *Anguloa*, but these have been based on few diagnostic characteristics. Schlechter (1916) published a revision of *Anguloa*, dividing the genus on the basis of rostellum and lip morphology; Fowlie's later treatment of *Lycaste* (1970) relied upon a combination of geographical range, lip morphology and flower colour. The different techniques adopted for the present investigation aim to clarify these infra- and intergeneric relationships and help resolve some of the confusion that exists at species level.

By way of introduction, what follows is a historical review of the classification of the two genera, a brief description of their gross morphology, some comments on the application of DNA sequence data to botanical problems and finally, a section on the role of floral fragrance in orchid pollination.

Author abbreviations used throughout this thesis follow Brummitt and Powell (1992). Full citations for all recognised *Lycaste* and *Anguloa* species, up to May 2000, are given in Appendix 1. Vouchers for DNA templates and floral fragrance analyses have

been deposited at either the Royal Botanic Gardens, Kew (K) or the Herbario de la Asociación Mexicana de Orquideología A.C (AMO); a full listing is given in Appendix 2.

1.2 THE ORCHIDACEAE AND THEIR CLASSIFICATION

The Orchidaceae are the largest family of flowering plants. Estimates of the number of species range from 19500 to 35000 (Dressler, 1993). Orchids have been known since the earliest botanical literature: Theophrastus, a pupil of Aristotle and Plato, described the testicle-like tubers of some European orchids in *De Historia Plantarum*, written between 370 and 285BC. The use of "Orchidaceae" as a family name came much later and is attributed to de Jussieu (1759).

The family has six major characteristics (Clements, 1995):

1. The sexual parts are at least partially fused into a single structure, the column.
2. The number of anthers is reduced, usually to one or two.
3. The structure of the pollen and the anther that contains it differ from other plant families. Among the Orchidaceae, the pollen is usually clustered into discrete masses called pollinia.
4. One of the inner segments of the perianth is modified into a structure termed a lip or labellum.
5. The seeds are very small, numerous and lack endoplasm.
6. The embryo will only germinate and grow in the presence of an endotrophic mycorrhyzal fungus.

Linnaeus (1753) described 69 orchid species in eight genera in his seminal work, *Species Plantarum*, in which he was the first to adopt a consistent binary naming system, each name comprising the generic name and a specific epiphet. This was the system used by Ruiz and Pavón (1794a,b) when describing their collections from Peru and Chile.

One of Linnaeus' students, Olof Swartz (1800), is generally considered to be the first to attempt to systematically classify the orchids, dividing them into those with one fertile anther, later termed "Monandrae" by Brown (1810), and those with two; the latter represented by a single genus, *Cypripedium* L. (Diandrae). Monandrous taxa were subdivided into three informal groups based on the position of the anther on the column.

Much of the current terminology for describing parts of an orchid plant can be attributed to Richard (1818), who defined terms such as caudicula, gymnostemium and rostellum in a key to the European orchids. In this system, the condition of the pollen masses was the primary diagnostic characteristic, three states being recognised: Sectile, granulose and solid.

The first classifications to include large numbers of tropical orchids were by Lindley (1826, 1830-1840), who divided the family into seven tribes, using the number of anthers and condition of the pollen as the primary characters, as shown in Table 1.1.

The tribe Vandeae was subdivided according to the number of pollinia and caudicles; *Anguloa*, *Maxillaria* Ruiz & Pav. and *Zygopetalum* Hook. all with two pollinia and one caudicle were grouped together, however, no names were given to the subdivisions.

Shortly afterwards, Lindley (1843a) established five new genera based on species formerly ascribed to *Maxillaria*; one of these being *Lycaste*. At the same time, he created the Maxillaridae as a new division of Vandeae, characterised by the arrangement of the lateral sepals, which were oblique at the base so that the flower bud appeared to have a chin, and which lacked a spur on the lip. Twenty eight genera

were placed in this division, including *Lycaste*, *Anguloa*, *Zygopetalum* and *Bifrenaria* Lindl.

Bentham (1881) continued Lindley's work, publishing a revised classification in which he reduced the number of tribes to five: Epidendreae, Vandeae, Neottieae, Ophrydeae and Cypripedieae. He divided the first four tribes into "subtribes". These included Malaxeae Lindl., which was relegated to a subtribe of Epidendreae, and Arethuseae Lindl., which became a subtribe of Neottieae. In his classification, he distinguished between "caudicles", which he defined as extensions of the pollen grains, and "stipes", found on species of the Vandeae tribe, which are formed from the rostellum.

I. Anther 1 only (MONANDRAE)
- A. Pollen masses waxy
  - a. No caudicle or separate stigmatic gland - MALAXEAE or Malaxideae
  - b. Distinct caudicle but no separable stigmatic gland - EPIDENDREAE
  - c. Distinct caudicle united to a deciduous stigmatic gland - VANDEAE
- B. Pollen powdery, granular or sectile
  - a. Anther terminal, erect - OPHREAE or Ophrydeae
  - b. Anther terminal, operculate - ARETHUSEAE
  - c. Anther dorsal (behind the rostellum) - NEOTTIEAE

II. Anthers 2 or 3 (DIANDRAE) - CYPRIPEDIEAE

Table 1.1 Early classification of orchids, after Lindley (1830-1840).

Within Vandeae, Bentham described eight subtribes. Of these, Lindley's Maxillarideae was redefined to include only genera for which buds had a mentum, and which had single flowered scapes, and coriaceous, fleshy or herbaceous leaves. *Lycaste*, *Anguloa*, *Bifrenaria* and *Zygopetalum* were transferred from Maxillarideae to a new and somewhat diverse subtribe, Cyrtopodieae. Bentham described this subtribe as having

"the prominent mentum of Maxillarieae with the foliage and habit of Cymbidieae". A clearer description was provided by Bentham and Hooker (1883), who listed its characteristics as the presence of plicate or veined leaves, a column-foot and a leafless scape. In this later work, 25 *Lycaste* and three *Anguloa* species were recognised, but no details concerning individual species names were given.

In contrast, Pfitzer (1887, 1889) devised a system which relied heavily on vegetative characters, although he did retain Lindley's and Bentham's initial Diandrae/Monandreae separation. His system used several different tiers of heirarchy, but only named one, subfamily, which equates to today's subtribe or alliance. Under this arrangement *Lycaste*, *Anguloa* and *Bifrenaria* were grouped together in the subfamily Lycastinae. He considered three subfamilies, Lycastinae, Gongorinae and Zygopetalinae, to be closely related because their pseudobulbs were morphologically similar, all comprising a single thickened stem internode, a condition he termed "heteroblastic".

Schlechter combined the principles of both the Bentham and Pfitzer systems in a revised classification, published posthumously in 1926. His treatment took the form of a dichotomous key in which he recognised four tribes and 81 subtribes. The Epidendreae and Vandeae were amalgamated into a new tribe, Kerosphaereae; the other tribes approximated those of Bentham. Many of Schlechter's "subtribes" equated to Pfitzer's "subfamilies"; within Kerosphaereae, the grouping of Lycasteae, Gongoreae and Zygopetaleae on account of their pseudobulb morphology was preserved. This classification became the international standard for the next thirty years.

The transition from an orchid classification that emphasised differences based upon a few diagnostic characters, to one based on overall similarity, was initiated by Dressler and Dodson (1960), who also standardised the tribal and subtribal nomenclature. In

their revision, they divided the Orchidaceae into two subfamilies, Cypripedioideae and Orchidoideae, five tribes and 42 subtribes. Cypripedioideae, characterised by the presence of two or three fertile anthers and the absence of a rostellum, had two tribes, Apostasieae and Cypripedieae. Three tribes, Neottieae, Orchideae and Epidendreae were assigned to Orchidoideae. Vandeae were incorporated into Epidendreae on the grounds that there was no clear distinction between them; previous separations had been based on the presence of a stipe, but Dressler and Dodson had found that some genera similar to Vandeae in other respects had no stipe.

As an intermediate between subtribe and genus, they used the concept of "alliances". *Lycaste* and *Anguloa* were placed in the tropical American subtribe Maxillariinae Bentham. Three alliances were defined for this subtribe:

1. Callus usually wide with longitudinal ridges — *Zygopetalum* alliance
2. Callus usually narrow, smooth
   - Leaves plicate — *Lycaste* alliance
   - Leaves conduplicate — *Maxillaria* alliance

The *Lycaste* alliance comprised *Anguloa*, *Bifrenaria*, *Lycaste*, *Rudolfiella* Hoehne, *Teuscheria* Garay, *Xylobium* Lindl. and also tentatively *Eriopsis* Lindl. and *Neomoorea* Rolfe.

Dressler (1979, 1981) presented a more comprehensive and updated classification, which used six subfamilies and 21 tribes. The subfamily Vandoideae was reinstated on account of differences in anther structure; Dressler suggested that although the column structures of the Epidendroideae and Vandoideae were extremely similar, they had developed by different routes. *Lycaste*, *Anguloa* and *Neomoorea* were placed in the subtribe Lycastinae Schltr. *Bifrenaria*, *Horvatia* Garay, *Rudolfiella*, *Teuscheria* and *Xylobium* were transferred to Bifrenariinae.

Dressler (1993) made several changes to his 1981 classification in the light of new information on, for example, seed morphology and chromosome numbers. He also incorporated ideas from cladistic analysis (Hennig, 1966). The new revision was based on five subfamilies: Cypripedioideae, Apostasioideae Spiranthoideae, Orchidoideae and Epidendroideae. Vandoideae were incorporated back into Epidendroideae because, as was noted earlier (Dressler and Dodson, 1960), some species of *Maxillaria* and *Cymbidium* Sw. have no stipe.

He differentiated between primitive and advanced Epidendroideae, listing the following as advanced characteristics: Epiphytism; pseudobulbs or corms; distichous, caducous, fleshy or conduplicate leaves; lateral inflorescences; hard pollinia and caducous anthers. The advanced Epidendroideae were split into two groups, the cymbidioid phylad, which included the tribe Maxillarieae, and the epidendroid phylad. Within Maxillarieae, the subtribe Bifrenariinae, separated in the previous classification (Dressler, 1981), was incorporated back into Lycastinae.

The following year, Chase *et al.* (1994) published the first molecular analysis of the Orchidaceae, using DNA sequences of the plastid gene *rbcL* from 33 genera. Their results indicated that the primary division of the family according to the number of anthers, the basis of most previous classifications, may have been too simplistic. In their sample Apostasioideae, with two or three anthers, were found to be sister to the rest of the Orchidaceae. Cypripedioideae were not placed adjacent to them, leading Chase *et al.* (1994) to speculate that their diandrous state may be derived rather than ancestral.

1.2.1 INFRAGENERIC CLASSIFICATION OF *LYCASTE*

When Lindley published *Genera and Species of Orchidaceous Plants* (1830-1840), *Maxillaria* encompassed 40 species that were quite diverse in appearance and

structure but had two common characteristics. The first was the arrangement of the lateral sepals, which were oblique at the base and fused to a lengthened column-foot to form a chin. The second was the presence of either one or two pairs of pollinia attached to a single caudicle, now termed a stipe, with a distinct viscidium. By 1843, *Maxillaria* had been expanded considerably, causing Lindley (1843a) to narrow the limits of the genus so that it only included those species with two pairs of pollinia resting directly on the viscidium, that is, without a distinct stipe. At the same time, he established five new genera: *Promenaea*, *Scuticaria*, *Warrea*, *Paphinia* and *Lycaste*. Species of *Maxillaria* that had a fleshy callus on the lip, and two pairs of pollinia attached to a long stipe with a small roundish viscidium, seven at that time, were transferred to *Lycaste*. The first reference to the genus, a description of *Lycaste plana* Lindl., had actually been published a year earlier (Lindley, 1842) and it is this species that is considered the type of the genus.

The origin of the name "*Lycaste*" remains a mystery. Lindley (1843b) was somewhat vague: "A fanciful name, Lycaste was a beautiful woman". Oakeley (1977) thought her to be one of Helen of Troy's sisters, a daughter of King Priam. Sheehan and Sheehan (1979) have suggested that the name derives from the Greek word for nymph. More recently, Alcorn and Hallett (1994) proposed that the name came from the *Dionysiaca*, a history of Dionysos, the god of wine, written by the Greek poet, Nonnos; Lycaste was one of his nurses. A more plausible explanation has been provided by two of Lindley's contemporaries, Lemaire (1848) and André (1880), who both claim that she was the wife of Butes, the son of Amycus and was famed for her beauty (Oakeley, 1999 *pers. com.*).

The first attempt at a subgeneric classification was by De Wolfe (1953), who proposed that the genus had its origin somewhere in the Andes of north-western South America and should be split into two sections. These were defined, but left unnamed.

The first section, based on *Lycaste fimbriata* (Poepp. & Endl.) Cogn., was characterised by a fimbriate margin on the mid-lobe of the lip and a bilobed callus on the "plate" of the lip or disc. He assigned nine species to this section, seven of which are found in the Andes of Colombia and Peru, one in the mountains of Venezuela and one in the Greater Antilles; a single taxon, *L. ciliata* subsp. *rossyi* (Hoehne) Fowlie, is found in the mountains of southern Brazil. Within the section, he noted that four species, *L. costata* Lindl., *L. gigantea* Lindl., *L. fimbriata* and *L. fulvescens* Hook., formed a complex of related species which were difficult to distinguish; he thought that this complex was the most primitive of the genus.

The second section was based around the *Lycaste macrophylla* (Poepp. & Endl.) Lindl. alliance, in which he included *L. dowiana* Endres & Rchb.f. from Costa Rica and *L. filomenoi* Schltr. from Peru. This section was characterised by a non-fimbriate margin to the mid-lobe of the lip and an elongate, finger-like callus on the disc. It ranges from the Peruvian Andes to the mountains of Central America and Mexico, where it reaches its greatest diversity.

Fowlie (1970) published a revision of *Lycaste*, dividing the genus into four sections and two subsections, all of which are "*nomina nuda*", that is, they have never been published validly:

Sect. *Fimbriatae*
Sect. *Deciduosae* — Subsect. *Xanthanthae*
Subsect. *Paradeciduosae*
Sect. *Longisepalae*
Sect. *Lycaste* (as sect. *Macrophyllae*)[1]

[1]In accordance with the ICBN, sect. *Macrophyllae* Fowlie, which contains the genus type, *L. plana*, has been renamed "sect. *Lycaste*".

He followed De Wolfe (1953) in his definition of sect. *Fimbriatae* and agreed that it contained the most primitive species of the genus. His rationale was that the two species at its geographical extremes, *Lycaste barringtoniae* (Smith) Lindl. from Jamaica and Cuba and *L. ciliata* subsp. *rossyi* from southern Brazil, are both low elevation species, ranging from sea-level to 700m. As the other species in the section are rarely found below 1600m, he concluded that the origin of this section predated the formation of the Andes.

Fowlie (1970) differed from De Wolfe (1953) in his treatment of the pendulous species *Lycaste dyeriana* Sander. De Wolfe (1953) had treated it as a fimbriate *Lycaste*; however Fowlie (1970) considered it to be more closely allied to *Bifrenaria* and excluded it from the genus.

Sect. *Deciduosae* were characterised by vigorous vegetative growth during the rainy season, followed by a resting period of between two and five months during which they lose their leaves. Flowers are produced simultaneously with new vegetative growth at the end of this resting period. The pseudobulbs of all species except *Lycaste tricolor* Klotzsch have apical spines, formed when the leaves are shed. They are usually epiphytic and found in close proximity to water.

The two subsections of *Deciduosae* reflected differences in geographical range and flower colour. *Xanthanthae,* ranging from Mexico to northern Colombia are primarily yellow flowered; *Paradeciduosae,* found in the highlands of Nicaragua, Costa Rica and Panama, have pink flowers.

A single species, *L. schilleriana* Rchb.f., which resembles a long sepaled *Macrophyllae,* was placed in sect. *Longisepalae*. This species occurs in the highland regions of Colombia, Ecuador and northern Peru.

The geographical range of sect. *Lycaste* is from Guatemala to Bolivia. As Fowlie's original the name suggests, it included species bearing some morphological resemblance to *Lycaste macrophylla*, seven species at that time. He had defined the section in an earlier publication (Fowlie, 1964) as having obscurely spined or unspined pseudobulbs, non-deciduous leaves, spatulate calluses and non-fimbriate lips. He considered some taxa within the section to be subspecies of *L. macrophylla* rather than valid species (Fowlie, 1964,1970), his criterion being their ability to form hybrid swarms in localities where their ranges overlapped.

Rose (1986) recognised only three sections: *Deciduosae*, *Lycaste* (as *Macrophyllae*) and *Fimbriatae*; the position of *L. schilleriana* within this arrangement was not specified.

Oakeley (1991a,b,c; 1993) has adhered to Fowlie's system, but has taken a narrower stance on the delimitation of species. This is particularly evident in the 1993 publication where he has re-instated seven species, almost all from sect. *Fimbriatae*, which Fowlie had reduced to synonomy.

The current status of species within the sections is shown in Figure 1.2.

1.2.2 INFRAGENERIC CLASSIFICATION OF *ANGULOA*

The first *Anguloa*, *A. uniflora*, was collected in Peru by two Spanish botanists, Hipólyto Ruiz and José Pavón, during a 10 year expedition to Peru and Chile. The expedition, which left Spain in 1777, was sponsored by King Carlos III. The primary aim was to prepare a scientific inventory of the natural products of South America with a view to publishing a *Flora Americana*. The expedition yielded almost 3000 different dried plants and over 2000 illustrations. The first volume of their work, containing descriptions and illustrations of all the new genera they had encountered, *Florae*

Peruviae et Chilensis Prodromus, was published in 1794. Ten new orchid genera were described, among them *Anguloa*, named after the Peruvian Director General of Mines, Don Francisco de Angulo, who was a keen botanist and *Maxillaria*, named after the jaw-shaped "nectary" of the flowers.

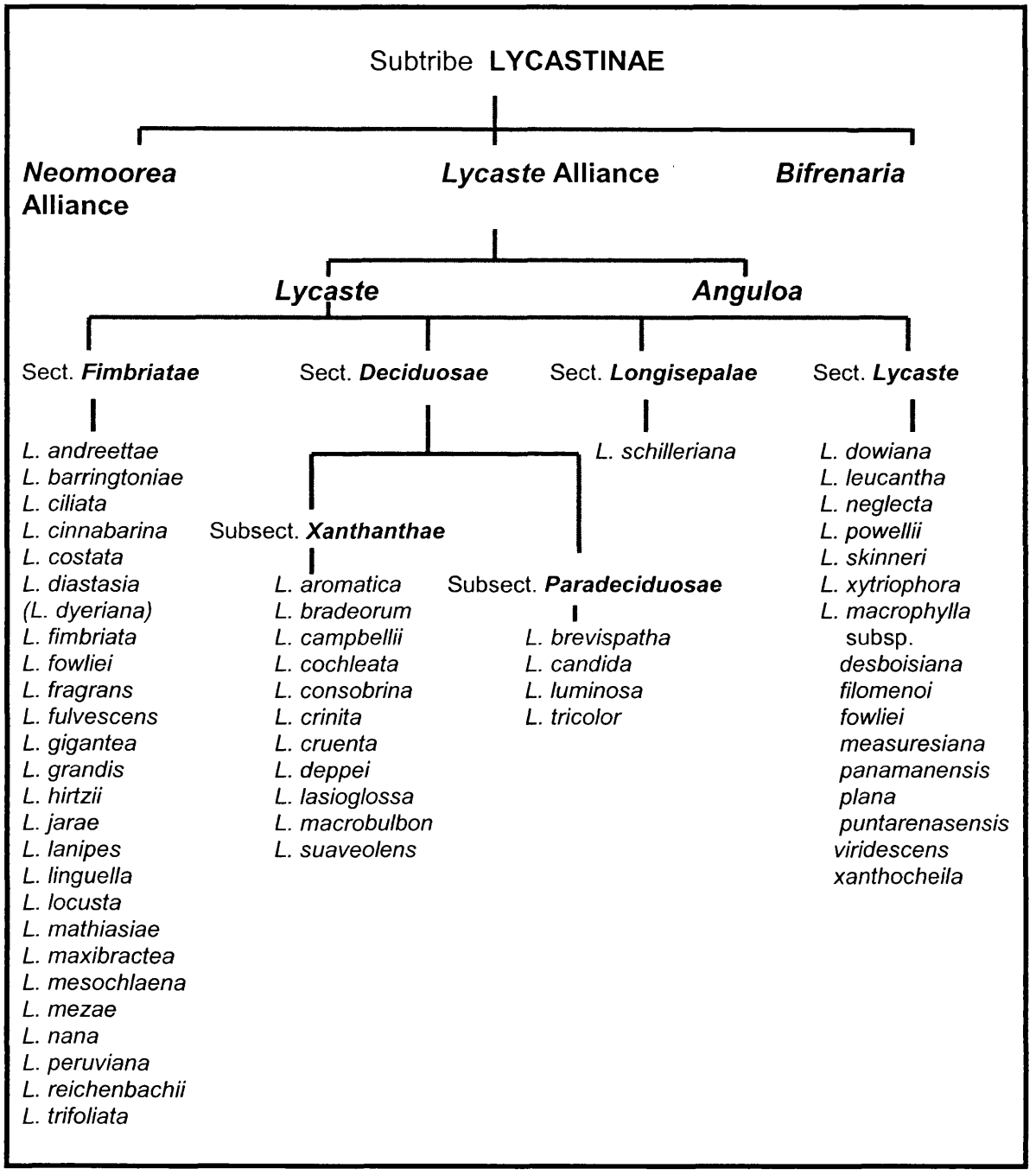


Figure 1.2 Sectional treatment of *Lycaste* species, after Fowlie (1970) and Oakeley (1993).

Production of the main *Flora*, containing descriptions and illustrations of individual species was halted prematurely after the appearance of Volume 3 in 1802. Funds for the project had been donated by the Americas, but these were requisitioned by an almost bankrupt Spanish Treasury in 1797 (Steele, 1964). Although the plates for Volume 4 were finally published, without descriptions, in 1802, the orchid species had been scheduled for Volume 7, which has never materialised. Some compensation was that brief text descriptions of all the new species were included in a smaller publication, *Systema Vegetabilium Florae Peruvianae et Chilensis* (1798), and these included *Anguloa uniflora* (1798a) and *Maxillaria ciliata* (now *Lycaste ciliata*, 1798b).

Berol (1861) noted that the major difference between *Lycaste* and *Anguloa* was in the spatial arrangement of the lateral sepals. These spread apart in *Lycaste* "in the manner of a true *Maxillaria*", but overlap each other in *Anguloa*. He recognised three distinct species of *Anguloa*: *A. uniflora* Ruiz & Pav., *A. clowesii* Lindl. and *A. ruckeri* Rolfe; a fourth species, *A. virginalis* Linden, was considered a synonym of *A. uniflora*. This view was supported by Bentham and Hooker (1883) and also by Veitch (1887-94), who in addition considered *Anguloa eburnea* Williams to be a white form of *A. clowesii*.

Williams (1894) recognised six distinct species, reinstating *Anguloa eburnea* and *A. virginalis* and describing *A. turneri* for the first time. His descriptions of the floral parts of each species placed great emphasis on colour. In addition he listed two hybrids: *Anguloa* X *dubia* Rchb.f. (A. *clowesii* X *A. uniflora*) and *A.* intermedia (A. *clowesii* X *A. ruckeri*).

Schlechter (1916) published the first key to the genus, Table 1.2, recognising nine species, of which two, *Anguloa macroglossa* and *A. goldschmidtiana*, were newly described. He split the genus into two sections: *Euanguloa*, here renamed *Anguloa* in deference to nomenclatural rules, and *Guloanga*. The former, characterised by

additional downward-pointing triangular lobes on either side of the rostellum, contained two species, *A. uniflora* and *A. virginalis*; the remaining seven which lack these lobes were placed in sect. *Guloanga*. Individual species were keyed according to differences in the structure of the lip, particularly the mid-lobe and callus.

Although Schlechter's drawings of lip structures have been reproduced in more recent publications on *Anguloa* (Kennedy, 1976; Tomlinson, 1984; Oakeley, 1994), his sectional names have never been adopted. Whether this was due to oversight or to the fact that no Latin descriptions were published for them is unclear.

Fifty years later, Dodson (1966) was only able to distinguish four species: *Anguloa uniflora*, *A. clowesii*, *A. ruckeri* and *A. cliftonii*.

Kennedy (1976), concerned at the nomenclatural confusion of plants in cultivation, was prompted to reiterate Schlechter's work on differences in lip morphology between the white-flowered species. A similar paper on red-flowered species followed (Kennedy, 1978).

Oakeley (1991d,e) recognised nine species, dividing them into two groups. The first group, containing two species: *Anguloa uniflora* and *A. virginalis*, was characterised by "two fang-like projections at the head of the column on either side of the pollen stalk", corresponding to Schlechter's sect. *Anguloa*. The second group with seven species lacked these projections and corresponded to sect. *Guloanga*. He differed from Schlechter in two respects. First, he included the red-flowered *A. brevilabris* Rolfe, which had been described for the first time in 1915 (Oakeley, 1991d); Schlechter made no reference to this species and may not have been aware of its existence. The second difference was that he failed to assign species status to *A. dubia* (Oakeley,

1991e) and in a later article suggested that its type specimen was identical with *A. clowesii* (Oakeley, 1994).

1. Section *Anguloa* (as sect. *Euanguloa*)

A. Central lobe of labellum egg-shaped, pointed — ***A. uniflora* Ruiz & Pav.**

B. Central lobe of labellum linear, pointed — ***A. virginalis* Linden**

1. Section *Guloanga*

A. Mid lobe of labellum sessile.

I. Entire mid lobe and callus tip densely, velvety papillose. Mid lobe has short lateral lobes.

a. Lateral lobes of the mid-lobe fused to the callus — ***A. hohenlohii* Morr.**

b. Lateral lobes of the mid-lobe free, callus entirely free at apex.

i. Flower exterior greenish, interior covered with brown or red blotches.

+Labellum ~4cm long, pointed lateral lobes — ***A. macroglossa* Schltr.**

++ Labellum < 3cm long, blunt lateral lobes — ***A. ruckeri* Lindl.**

ii. Flower evenly golden yellow or lemon yellow, tip of labellum sometimes orange yellow — ***A. clowesii* Lindl.**

II. Frontal lobes of labellum not separate, densely papillose only at tip. Callus bare.

a. Lateral lobes of labellum pointed, flower yellow finely spotted with red — ***A. dubia* Rchb.f.**

b. Lateral lobes of labellum blunt, flower very densely patterned inside with dark purple blotches — ***A. goldschmidtiana* Schltr.**

B. Frontal lobe of labellum on a distinct claw — ***A. cliftonii* Rolfe**

Table 1.2 A morphological key to *Anguloa* species, from Schlechter (1916).

In a recent revision of the genus, Oakeley (1999) made several modifications to his previous work. Amongst the "white" anguloas, that is, sect. *Anguloa*, he re-established *Anguloa eburnea* as a species, distinguishing it from *A. uniflora* on account of its larger size and less recurved callus. In addition he described a new species, *A. tognettiae*. Changes to sect. *Guloanga* Schltr. have been more dramatic. *A. dubia* has been reinstated, but two other species have been relegated to varietal status: *A. macroglossa* to *A. hohenlohii* var. *macroglossa* and *A. goldschmidtiana* to *A. brevilabris* var. *goldschmidtiana*. The final modification to this group concerns *A. ruckeri*, which he now considers to be a natural hybrid of *A. hohenlohii* and *A. clowesii*.

1.3 MORPHOLOGY

1.3.1 THE MORPHOLOGY OF *LYCASTE*

Lycaste species are epiphytic, lithophytic or terrestrial herbs. Examples from the morphological extremes of the genus, *Lycaste aromatica* (Graham ex Hook.) Lindl. and *L. cinnabarina* Lindl., are shown in Figures 1.3 and 1.4. *L. aromatica* ranges from Mexico to Nicaragua typically at elevations of 700m to 1500m. It is usually epiphytic or lithophytic (Ames and Correll, 1953) and is "deciduous", losing its leaves during the dry season. *L. cinnabarina* is a high elevation terrestrial or lithophytic plant, which is found on mossy rocks at 1600m to 2200m in Ecuador and Peru. This species, like others from sect. *Fimbriatae* Fowlie, keeps its leaves for two to four years.

Lycaste roots are cylindrical, typically 3-5mm in diameter and sheathed in white velamen with only a few millimetres of cortex exposed at the tip. The velamen is a specialised epidermis, which protects the root and probably acts as a sponge, capturing and retaining water and nutrients long enough for them to be absorbed (Pridgeon, 1987).

The rhizomes are typically 5mm in diameter and only a few cm long, causing the pseudobulbs to pack tightly together. In exceptional circumstances they do grow longer; Oakeley (pers. comm., 1999) has observed a rhizome of *Lycaste fragrans* Oakeley that had extended to 30cm after being buried in a landslide. Growth is sympodial, with successive new growths originating from the base of the preceding one at the end of the dry season.

New growth consists of an erect stem bearing two to four leaves surrounded by leafy bracts. Over a period of months, the stem swells to form a pseudobulb, which functions as a water storage organ; the bracts die as the bulb starts to mature. Mature pseudobulbs are dark green, vary in shape from almost circular to elongate-ellipsoid, are almost always laterally compressed and often ribbed. They are frequently armed at the apex with spines, formed when the leaves are shed; these are particularly prominent in two Mexican species, *Lycaste consobrina* Rchb.f. and *L. crinita* Lindl.

The leaves are long, plicate, thinly textured and strongly veined. Their shapes vary from lanceolate to broadly elliptic. Among sect. *Fimbriatae*, the base of the leaf narrows to a short petiole, but this is less pronounced among the other sections. A notable exception is *Lycaste dyeriana* Rchb.f., unique among the genus in that it exhibits pendant growth and has both surfaces of its leaves covered with a bluish bloom.

Inflorescences are lateral, one to several appearing from the base of the pseudobulbs at the onset of new growth. They are generally single flowered; two-flowered scapes occasionally occur in all sections of the genus (Oakeley, pers. comm.). The scapes vary in length from 4cm (*Lycaste campbellii* C.Schweinf.) to about 50cm (*L. costata* Lindl.) and are partially covered by two to four leafy bracts. An additional floral bract sheathes the pedicel and six-ribbed ovary and may extend along the lateral sepal.

Key

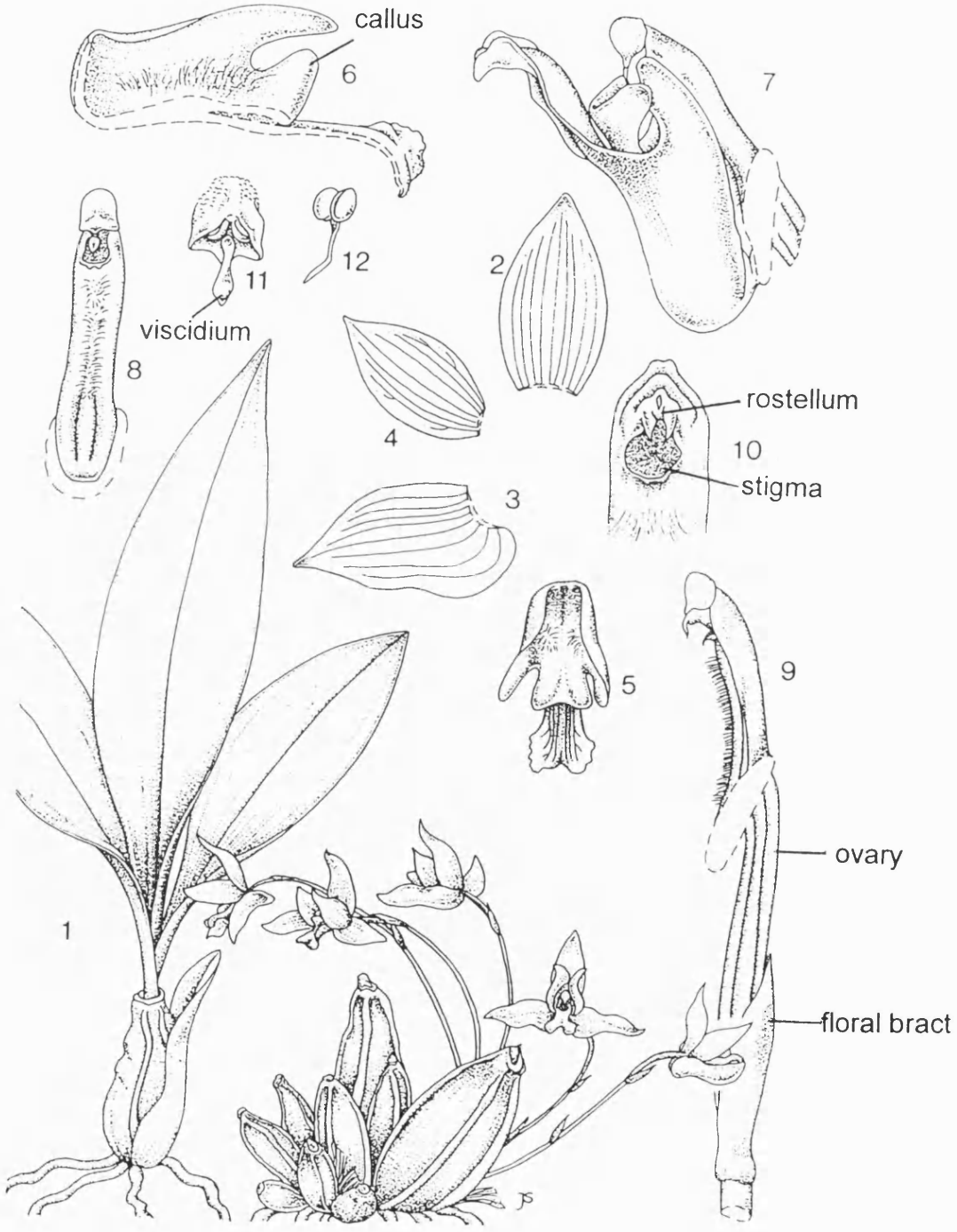
		Magnification
1.	Habit	x 1/3
2.	Dorsal Sepal	x 1
3.	Lateral sepal	x 1
4.	Petal	x 1
5.	Lip, flattened	x 1
6.	Lip, longitudinal cross-section	x 2
7.	Column and lip, side view	x 2
8.	Column, ventral view	x 2
9.	Column with ovary and pedicel, side view	x 2
10.	Column apex with anther removed, ventral view	x 4
11.	Anther, dorsal view	x 4
12.	Pollinia and stipe	x 4

Figure 1.3 *Lycaste aromatica* (Graham ex Hooker) Lindl., drawn by Judi Stone.

Habit drawn after a painting by Patricia Roberts (p.14 in Fowlie, 1970). Flower and dissections drawn from spirit material (*Oakeley* 63859 K).

Lycaste aromatica

Judi Stope
November '96



Key

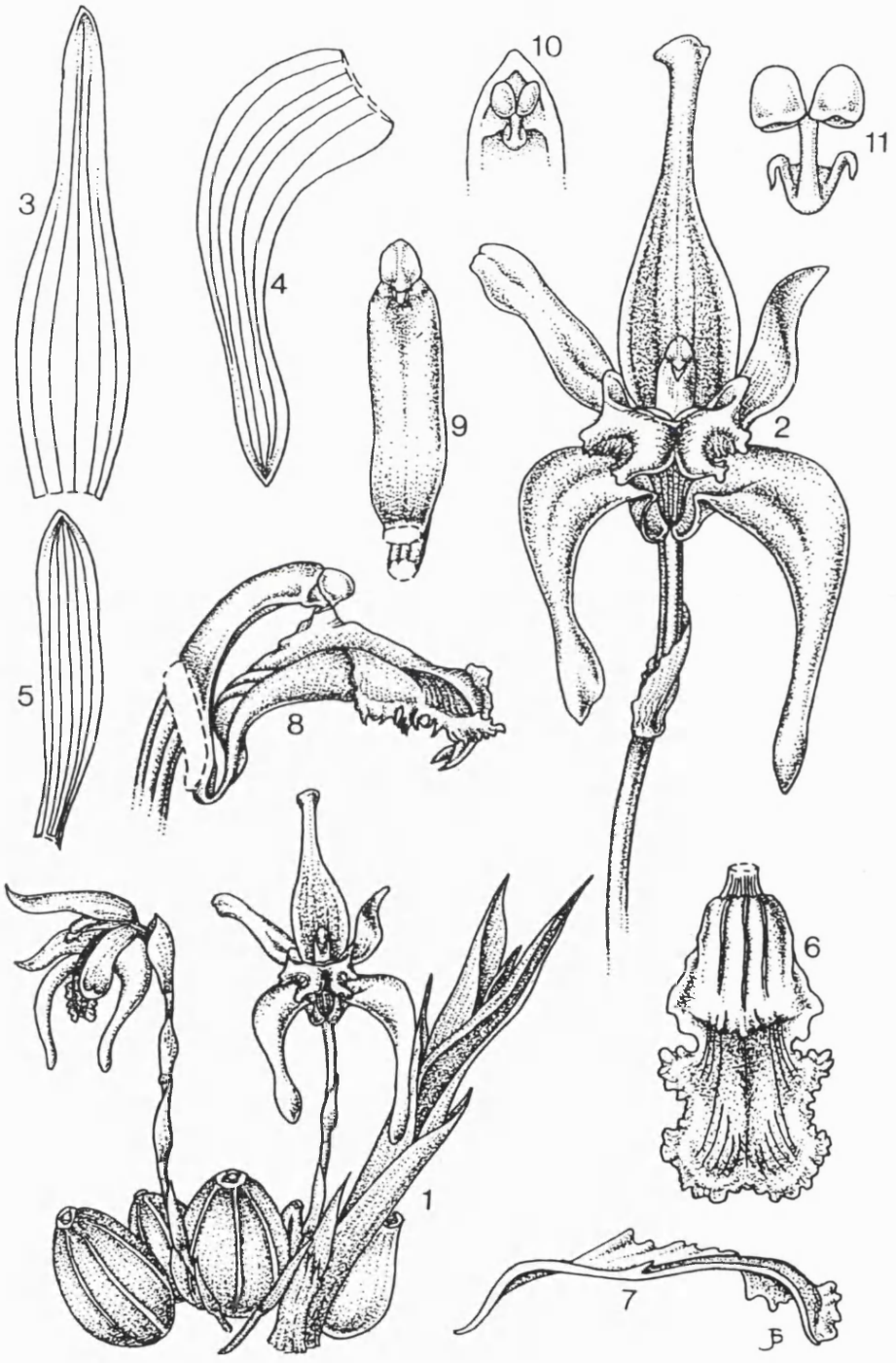
		Magnification
1.	Habit	x 1/3
2.	Flower	x 2/3
3.	Dorsal sepal	x 2/3
4.	Lateral sepal	x 2/3
5.	Petal	x 2/3
6.	Lip, flattened	x 1
7.	Lip, longitudinal cross section	x 1
8.	Column and lip, side view	x 1
9.	Column, ventral view	x 1
10.	Column apex with anther removed, ventral view	
11.	Pollinia and stipe	

Figure 1.4 *Lycaste cinnabarina* Lindl. (synonym *L. denniniana* Rchb.f.), drawn by Judi Stone.

Habit drawn after a painting by Patricia Roberts (p.56 in Fowlie, 1970). Flower and dissections drawn from spirit material (cult. *Oakeley* 64032 K). Pollinia and stipe drawn from slides provided by Henry Oakeley.

Lycaste denningiana

April 1997
Judi Stone



Demarcation between the pedicel and ovary is not always clearly defined, especially in spirit specimens.

The perianth consists of an outer whorl of three sepals, two lateral and one dorsal, and an inner whorl of three petals, one of which, the lip, has been modified to serve as a landing platform for potential pollinators. The entire base of both lateral sepals is joined to the column-foot to form a chin or "mentum".

Sepals are subequal and spreading. The lateral sepals are broader than the dorsal and among "non-fimbriate" *Lycaste* species, such as *L. aromatica,* are usually recurved or reflexed.

The petals are usually similar in shape to the sepals but smaller. They lie parallel to the column, almost enclosing it. Their apices are generally reflexed. Species of sect. *Fimbriatae*, such as *Lycaste cinnabarina*, tend to have petals and sepals of the same colour, usually green. Species from the other sections of the genus often have petals which are either a different colour or have a different pattern of spotting from the sepals.

The lip is three-lobed, shorter than the sepals and may have either a different colour or different markings from both the sepals and petals. The lateral lobes are generally prominent and erect. As with *Lycaste aromatica*, the mid-lobe margin of most species is entire; however those from sect. *Fimbriatae* tend to have a fringed margin. Species of sect. *Fimbriatae* also have a fleshy multi-keeled callus, which runs the length of the lip from the column-foot to the mid-lobe; amongst these species, the front of the callus is always bifid. Species from the other sections of the genus have a callus in which the base is much further forward and the apex entire; for these species, the only keels are those that define the margins.

The column is long, narrow and arched, extending to the front of the lip callus. The tapered section of column below the ovary, the column-foot, tends to be much longer in species from sect. *Fimbriatae* than in species from the other sections. The ventral surface of the column is often hairy. In some species such as *Lycaste aromatica* and *L. cruenta* Lindl., the entire area between the stigmatic surface and ovary is covered with long oily hairs, whereas in others hairs are located only directly under the stigma or around the ovary. The stigma itself is almost hidden under the rostellum.

The anther is terminal, cap-shaped, slightly pointed and contains four laterally compressed ovoid or ellipsoid pollinia which are pressed together in pairs and attached to a single stipe. At the base of the stipe is a sticky pad, the viscidium which attaches the pollinia to an insect visitor. The viscidium is generally ovate; exceptions are several species from sect. *Fimbriatae* in which they are elongate, narrow and bent to form a "V" or "M" shape.

The seed capsule, which is not illustrated here, is dark green and usually erect. Its shape is oblong to fusiform and it has six longitudinal ribs.

1.3.2 THE MORPHOLOGY OF *ANGULOA*

Anguloa species are predominantly lithophytic or terrestrial, growing on damp moss-covered rocks. Other than size, their vegetative features are very similar to those of *Lycaste*. The rhizomes are 5-8mm in diameter and short, causing the pseudobulbs to pack tightly together. Pseudobulbs are dark green, laterally compressed and vary in shape from ovoidal to ellipsoidal. They can grow up to 18cm high and, as in most *Lycaste* species, are spined at the apex. New pseudobulbs have two to three plicate, strongly nerved apical leaves which are shed at the end of the resting period, or more usually when the new growth is about 12cm high and the flower buds have started to

develop. The leaves are broadly lanceolate, up to 1.3m long and narrow at the base to produce a short petiole-like stalk.

There are significant differences in the floral morphology of the two genera, as can be seen from the illustration of *Anguloa clowesii* Lindl., Figure 1.5.

Flower scapes arise from the base of the previous year's pseudobulb after the new growth has started to develop. The scapes are 8 to 17cm long, relatively thick (4-7mm diameter) and loosely covered by two to four overlapping leafy bracts. They almost exclusively bear a single flower. Two-flowered scapes have been observed in cultivated plants by both Schlechter (1916) and Dunsterville (1979), but according to the latter, these are "freak occasions" rather than the norm.

The ovary is cylindrical, hairless and subtended by a floral bract which usually overlaps the dorsal sepal considerably. As with *Lycaste*, separation of the pedicel and ovary is not clearly defined. Mature seed capsules appear six-sectioned.

Superficially, the flowers are similar throughout the genus. The sepals and petals are fleshy and concave. They are held erect and overlap for most of their length to form a cup with a narrow opening, hence one of the popular names, "tulip orchid". Like *Lycaste*, they have a distinct chin, which is formed by the union of the lateral sepals with the base of the column.

Lateral sepals are orbicular-elliptic in shape with an elongated sickle-shaped apex. The dorsal sepal tends to be narrower with a less pronounced apex. With the exception of *Anguloa cliftonii* Rolfe, the petals are smaller than the sepals and resemble the dorsal sepal in shape.

Key

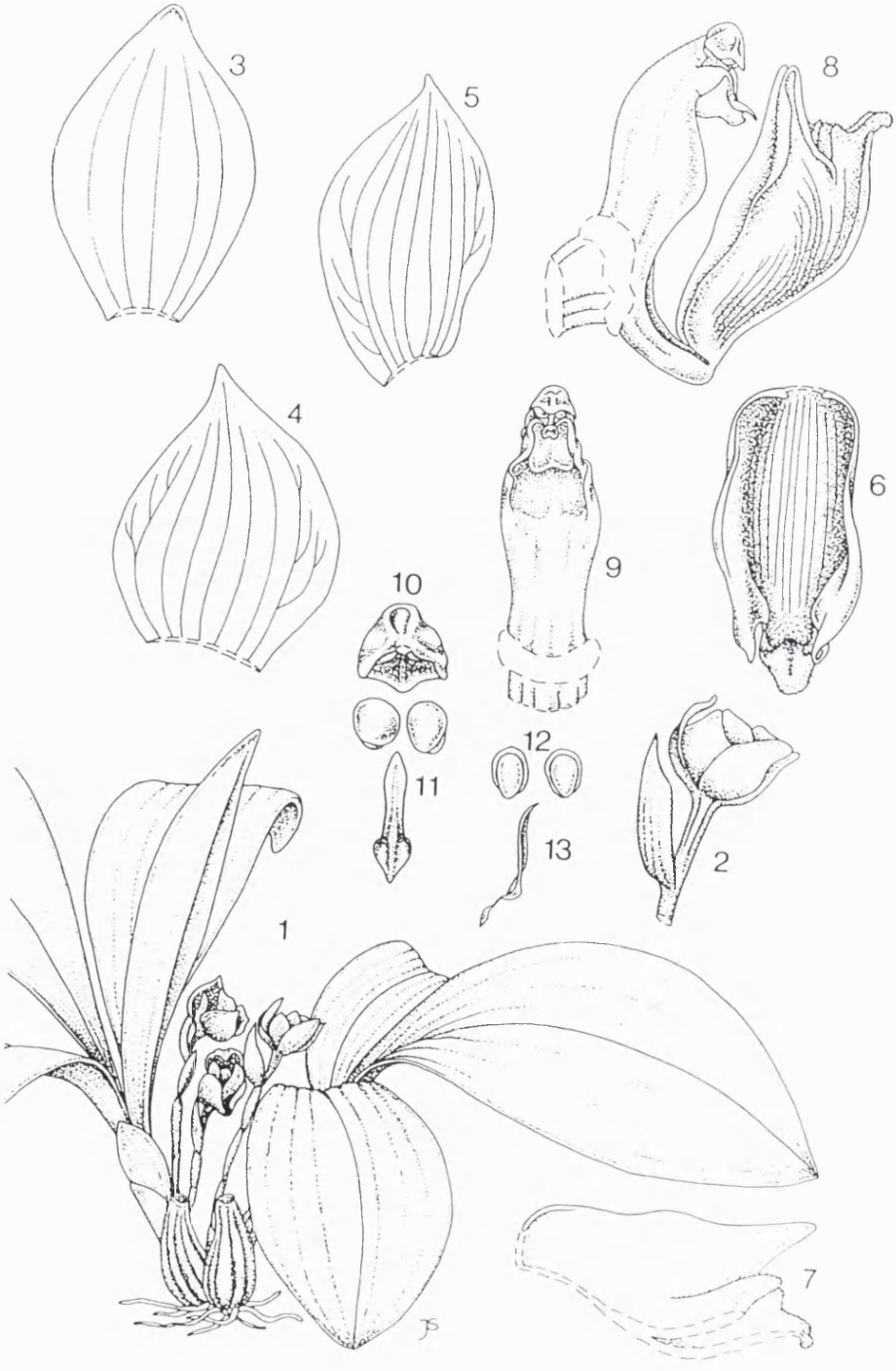
	Magnification
1. Habit	x 1/9
2. Flower	x 1/9
3. Dorsal sepal	x 2/3
4. Lateral sepal	x 2/3
5. Petal	x 2/3
6. Lip	x 1
7. Lip, longitudinal cross section	x 1
8. Column and lip, side view	x 1
9. Column, top section, ventral view	x 1
10. Anther cap	x 2
11. Pollinia and stipe, front view	x 2
12. Pollinia, dorsal view	x 2
13. Stipe, side view	x 2

Figure 1.5 *Anguloa clowesii* Lindl., drawn by Judi Stone.

Habit after a drawing by G.C.K. Dunsterville (1962 K), published in *Venezuelan Orchids Illustrated*, vol. 3 (1965). Flower and dissections from spirit material (*Oakeley 57535* K).

Anguloa clowesii

Jud. Stoyel
November 1996



The lip is smaller than the other perianth parts. It is held upright and hinged to the tip of the column-foot so that it rocks back and forth when touched, the basis for another vernacular name, "cradle orchid". Its base is concave, often described as boat-shaped (e.g. Schlechter, 1916). The side lobes stand upright and unlike *Lycaste*, are usually larger than the mid-lobe. In some species, such as *Anguloa cliftonii* and *A. clowesii*, the mid-lobe may itself be three-lobed or five-lobed. The callus extends from the column-foot to the base of the mid-lobe. The front of the callus is bifid, very fleshy and projects over the base of the mid-lobe.

The column is approximately the same length as the lip. Above the ovary, its ventral surface is concave, with the stigma recessed under an arched rostellum. The side lobes of the rostellum are considerably elongated in species of sect. *Anguloa*.

As in *Lycaste*, the anther is terminal. There are four laterally compressed, oblong-ellipsoid or ovoid pollinia, pressed together in pairs and attached to a single stipe; the viscidium is generally ovate. The anther cap is slightly pointed, drawn out towards the front, and has a prominent ridge running axially between the two pairs of pollinia.

1.4 DNA AND PLANT PHYLOGENY

Plant DNA offers a much larger set of well defined characters than that provided by morphological examination; the plastid genome alone has typically between 130 and 160 kilobase pairs (Palmer, 1985). It offers two other advantages that are pertinent to phylogenetic studies. First, different regions of each genome evolve at different rates, which means that regions can be targeted to address questions at different taxonomic levels. Secondly, it removes the element of bias that is inherent in selecting morphological characters (Pridgeon and Chase, 1998).

There are two different approaches to using DNA for phylogenetic studies: Fingerprinting and direct nucleotide sequencing. The former involves generation of a set of DNA fragments which reflect the genetic constitution of an individual. Within this remit are a range of different techniques, such as "restriction fragment length polymorphism" (RFLP) and "random amplification of polymorphic DNA" (RAPD), which have both been used for plant-population level studies (eg Nybom and Schaal, 1990; Williams *et al.*, 1990).

Direct sequencing has been found to be more suitable for establishing relationships among species, genera and higher levels of classification (e.g. Doyle, 1993) and is the technique chosen for this study. Two regions have been selected, the plastid gene *matK* and the nuclear ribosomal spacers ITS1 and ITS2.

Plant cells have three genomes: In the plastids, in the nucleus and in the mitochondria. Within the animal kingdom, mitochondrial DNA (mtDNA) has been used extensively for evolutionary studies; it is maternally inherited, does not exhibit recombination and evolves rapidly (Avise *et al.*, 1987; Moritz *et al.*, 1987). Plant mtDNA has proved to be less amenable to such work; the genome varies widely in size, is structurally labile and frequently contains plastid DNA sequences, which can be up to 12kilobase pairs long (Palmer, 1992). The mitochondrial genomes of several *Brassica* species have been mapped and found to be highly diverse in terms of both structure and arrangement; however, their nucleotide sequences show little variation (Palmer and Hebron, 1988). At the present time, the potential of plant mtDNA for phylogenetic analysis is still unknown.

To date, the plastid gene *rbcL*, which codes for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), has been the most popular choice for sequencing studies (Chase *et al.*, 1994). It has proved useful for determining

phylogenetic relationships at family and higher levels (Chase *et al.*, 1993) and within the Orchidaceae, has been used to infer relationships at levels above that of genus (Chase *et al.*, 1994).

Of the other protein coding regions within the plastid genome, *matK* is one of the most rapidly evolving (Johnson and Soltis, 1995). It has been used to clarify phylogenetic relationships which were not fully resolved using *rbcL* sequences alone (Johnson and Soltis, 1994). The gene is located within an intron of approximately 2600bp between the 5' and 3' exons of the transfer RNA gene for lysine, *trnK*, as shown in Figure 1. 6.

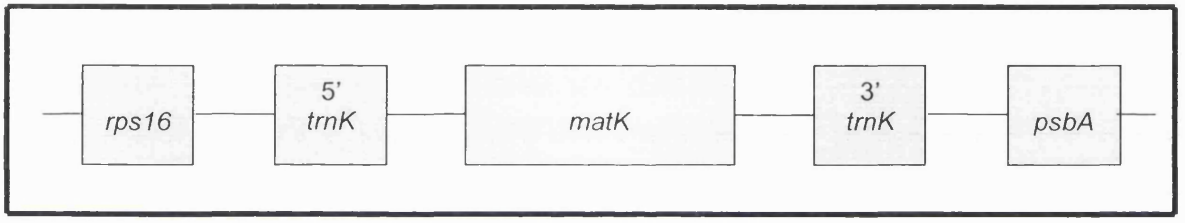


Figure 1.6 Schematic representation of the location of *matK*, from Soltis and Soltis (1998).

Plunkett *et al.* (1997), used a combination of *matK* and *rbcL* sequences to show that Apiaceae and Araliaceae are well-defined families which derive from a common lineage. The two families had not been completely resolved using *rbcL* sequence data, and it had been assumed that Apiaceae had derived from within Araliaceae.

Within the nuclear genome, the 18S-26S ribosomal DNA (rDNA) gene family has been the subject of several studies (eg Hamby and Zimmer, 1992; Nickrent and Franchina, 1990). Ribosomal DNA can represent up to 10% of the total plant DNA (Hemleben *et al.*, 1988). The basic structure is a single repeat unit, illustrated in Figure 1.7, which is reiterated thousands of times (Appels and Honeycutt, 1986). Although the genes within this region, 18S, 5.8S and 26S, are highly conserved, the noncoding spacers between

them, ITS1 and ITS2 evolve much faster (Baldwin *et al.*, 1995). Johnson and Soltis (1995) have found the number of nucleotide differences per site among species of *Gilia* (Polemoniaceae) to be 1.9 times greater in the two ITS regions than in *matK*.

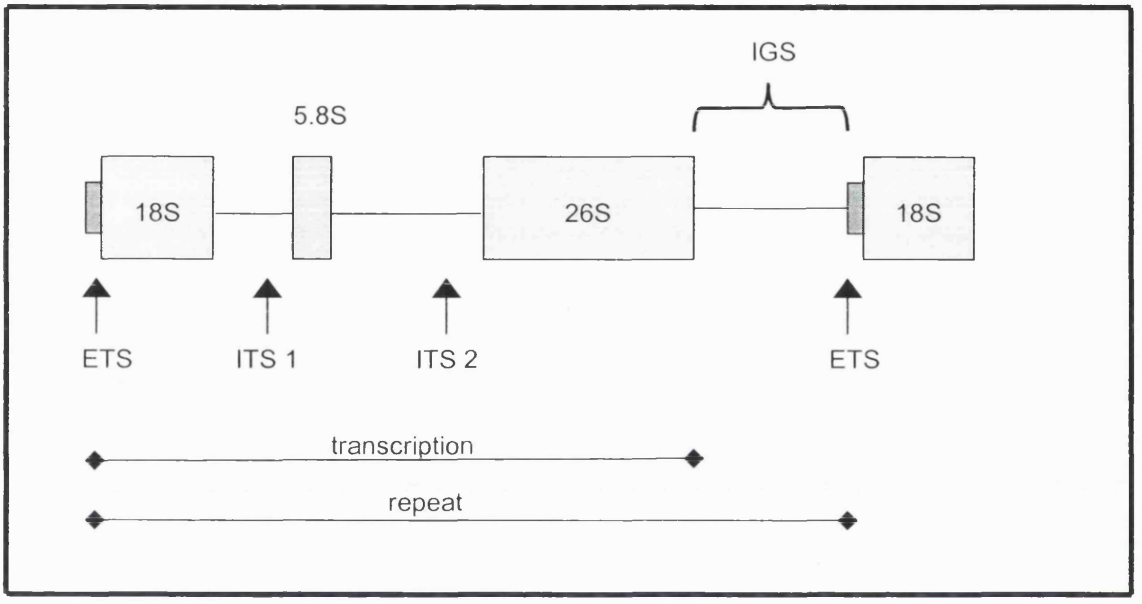


Figure 1.7 Schematic diagram of the rDNA repeat unit in plants, from Soltis and Soltis (1998). ETS=external transcribed spacer, ITS=internal transcribed spacer, IGS=intergenic spacer.

1.5 FLORAL FRAGRANCES AND ORCHID POLLINATION

Orchids are associated with high pollinator specificity because in most cases the entire pollen mass is attached to the first visitor. The first study of their various pollination strategies was by Darwin (1862), based on his observations of European orchids and of tropical cultivated orchids. Although the second edition of this work (Darwin, 1877) was supplemented by information on West Indian orchids, provided by Crüger, serious study of the pollination of Neotropical orchids was started by Dodson and co-workers in the 1960s (e.g. Dodson and Frymire, 1961a,b; van der Pijl and Dodson, 1966).

Many orchids use deception as a pollination strategy, offering little or no reward, instead exploiting some trait in the food foraging, territorial or reproductive behaviour of the pollinators. Moth-pollinated *Epidendrum ciliare* L. falls into the first category, emitting a fragrance similar to that of nectar-containing plants (Knudsen and Tollsten, 1993).

The territorial instinct of bees of the genus *Centris* is thought to be exploited by flowers of *Oncidium hyphaematicum* Rchb.f. and *O. planilabre* Lindl. (Dodson and Frymire, 1961b; van der Pijl and Dodson, 1966). The bees position themselves on twigs from where they not only chase away other insects, but also attack the orchid flowers when they are moved by a breeze. Pollinia are either removed or deposited when the flowers are struck by the bees.

Deception involving the reproductive behaviour of the insect is exhibited by the genus *Ophrys* L.: Male wasps and bees are lured to flowers of different species of *Ophrys* by a combination of shape, colour, texture and fragrance, all of which mimic the female. The deception works so well that the insects attempt to copulate with the flowers, resulting in deposition of pollinia on either their head or abdomen (Kullenberg, 1961).

Lycaste and *Anguloa*, in common with about 600 Neotropical orchid species, mainly epiphytes (Ackerman, 1986), are known to be pollinated by male euglossine bees which collect and store floral fragrances.

A review of the tribe Euglossini (Bombinae: Apidae) was undertaken by Dressler (1982). It comprises five genera, of which two are parasitic. The largest of the free-living genera is *Euglossa* with between 60 and 100 species; the other two are *Eufriesia*, with about 50 species and *Eulaema*, with 13. Of the parasitic genera,

Exaerete has five species and *Aglae* is monotypic. A checklist of euglossine orchid pollinators, compiled by Williams (1982) does not list *Aglae* as a pollinator.

The five tarsal segments of the forelegs of males bear dense tufts of hair, often referred to as "brushes", which absorb oily liquids by capillary action. The male hind tibiae are enlarged to contain a hollow spongy structure, which is used to store the floral fragrances. Access to this structure, known as the tibial organ, is via a "scar" on the external surface, an opening covered with long hairs through which liquids can pass (Dressler, 1982). Tarsal brushes and scars are absent in female euglossines (Dodson and Frymire, 1961a,b) and less well developed in males from the genera *Exaerte* and *Aglae* (Dodson *et al.*, 1969).

The behaviour of these bees was first reported from Trinidad by Crüger (1865), who observed that flowers of *Stanhopea grandiflora* Lindl., *Gloxinia maculata* L. and *Coryanthes macrantha* Hook. had similar perfumes and attracted the same pollinator. He described the pollination of *Coryanthes macrantha* by "humble bees", but thought that although the perfume served as an attractant, the bees had come to eat the lining of the lip. The bees were subsequently identified as euglossines by Smith (in Darwin, 1877). More recently, van der Pijl and Dodson (1966) have listed two of the pollinators of *Coryanthes macrantha* as *Eulaema cingulata* and *Eul. basalis*.

Crüger's suggestion that the flowers were a source of food was finally disproved by Dodson and Frymire (1961b), who observed that although the bees scratched at the surface of the lip, absorbing released substances through the hairs on their front tarsi, they did not attempt to feed on it. They also noted that the flowers were visited exclusively by males.

Detailed descriptions of the behaviour of male euglossines in the flowers have been provided by van der Pijl and Dodson (1966) and by Williams (1982). The bees land on the lip and move to the area of maximum fragrance production where they scrape the surface with their tarsal brushes. They then launch into the air and transfer the collected fragrance to their hind tibiae before returning to the flower. It is while brushing, preparing to launch or during the actual launch that pollinia are removed from the flower by the bee.

The pollination of *Anguloa clowesii* by *Eulaema bolivensis* has been observed by Dodson (1966). In *Anguloa*, the centre of fragrance production is the upper surface of the fold of the callus, at the front of the lip. The only way that bees can access it is by backing into the flower. Once inside, they balance by holding onto the edges of the sepals and petals with their middle pair of legs. As they attempt to leave the flower and release the tepals, their weight is transferred to the lip, which rocks towards the column. The viscidium slips between the thorax and abdomen of the bee, and as it crawls out of the flower, the stipe and pollinia are pulled out of the anther and carried away. On entering another flower, a similar sequence results in the pollinia being forced into the stigma under the rostellum.

By comparison, pollination of *Lycaste* species is straightforward. Dodson and Frymire (1961b) reported the pollination of *Lycaste xytriophora* Linden & Rchb.f. by *Euglossa viridissima*. The bee enters the flower by landing on the outstretched lip. When backing out, after fragrance collection, the viscidium becomes stuck to the underside of the dorsal projection of the metathorax, transferring the stipe and pollinia to the bee. On visiting another flower, the pollinia are deposited on the stigma when the bee backs out.

Why the fragrances are collected remains a matter of speculation. Vogel (1963) suggested that the flowers were mimicking the odour of female bees and that the

males scratched the lip out of frustration when no female was found. Dodson *et al.* (1969) rejected this idea as the brushing appeared to be too systematic, and suggested three other possibilities:

- The compounds are necessary for the synthesis of a vital metabolite which is deficient from their normal diet.

- The compounds are used to attract other males of the same species to a display site.

- The compounds are converted into female sex attractants.

The first of these hypotheses was abandoned when Ackerman and Montalvo (1985) showed that captive male bees which were denied access to floral fragrances did not have a reduced lifespan.

The second was based on the observation that male euglossines establish display sites, typically on small tree trunks, which they mark with fragrance and where they exhibit ritual flying and buzzing behaviour. Other males, usually less than five, are attracted to the display site and either hover or set up adjacent territories; females are attracted by the noise.

Dodson (1975) subsequently expanded this theory, suggesting that these male aggregations or "leks" are only established by "attractor" males who have gathered the requisite blend of fragrance compounds by visiting a number of different species of plant. However, Kimsey (1980) found no evidence to support the idea. She observed that leks were only formed around treefall sites or other large light gaps, which offered a number of territory sites in close proximity and that females would find a mate while foraging in these areas.

The third hypothesis is preferred by Williams (1982), who suggested that the fragrance compounds are modified in the tibia and then transferred to the mandibular glands where they are secreted to mark mating sites.

Whatever the rationale for their behaviour, it has been shown that many of the individual compounds found in the floral fragrances of euglossine pollinated plants attract male bees, and that the bees will brush and transfer these compounds from blotting paper and wicks as well as from flowers (Williams, 1982). Although some substances, such as 1,8-cineole, seem to be universal attractants, others such as limonene attract only a limited number of species. Williams and Dodson (1972) have also demonstrated that blending chemicals modifies their attraction potential.

These findings suggest that analysis of the floral fragrance compositions of euglossine pollinated plants may contribute to our understanding of their phylogeny.

Chapter 2 MORPHOLOGICAL ANALYSIS

The work presented in this chapter details the first systematic examination of the morphology of *Lycaste* and *Anguloa*. It aims to identify relationships that exist within and between the two genera, and to identify the character changes responsible for them.

2.1 INTRODUCTION

The monographs on *Lycaste*, by Fowlie (1970), and on *Anguloa*, by Schlechter (1916), contain the only sectional classifications of these genera and both are based on very few characteristics. Fowlie (1970) concentrated on geographical location, lip morphology and colour, whereas Schlechter (1916) singled out the side-lobes of the rostellum. Both genera are ascribed to subtribe Lycastinae, which has undergone several revisions, as discussed in Chapter 1. The last major change, shown in Figure 2.1, was by Dressler (1993), who expanded the subtribe to include all those genera formerly ascribed to Bifrenariinae Lindl.

Methods for the systematic analysis of morphological data fall into two categories, phenetic and cladistic. The former, the result of a collaboration by Sokal and Sneath (1963), uses standard multivariate techniques such as clustering or principal component analysis to measure overall similarity or difference between species. The analyses are based on measurement of the states of a number of characteristic features (characters), each of which are given equal weight.

Cladistics, pioneered by Hennig (1950, 1966), is concerned with character state changes and is based on the assumption that speciation occurs by dichotomy. Branching patterns are determined by applying the principle of parsimony, i.e. accepting the simplest explanation that accounts for all the facts. Features of a group

are analysed to determine the minimum number and sequence of branchings (speciations) that can have given rise to it. Unlike a phenetic analysis where all characters are given equal weight, cladistics makes a distinction between ancestral (plesiomorphic) and derived (apomorphic) character states; only derived states are held to be of value in determining plant phylogeny. An advantage of this technique over phenetics is that the individual character state changes responsible for the branching patterns can be identified. With traditional multivariate analysis, such information is lost during processing.

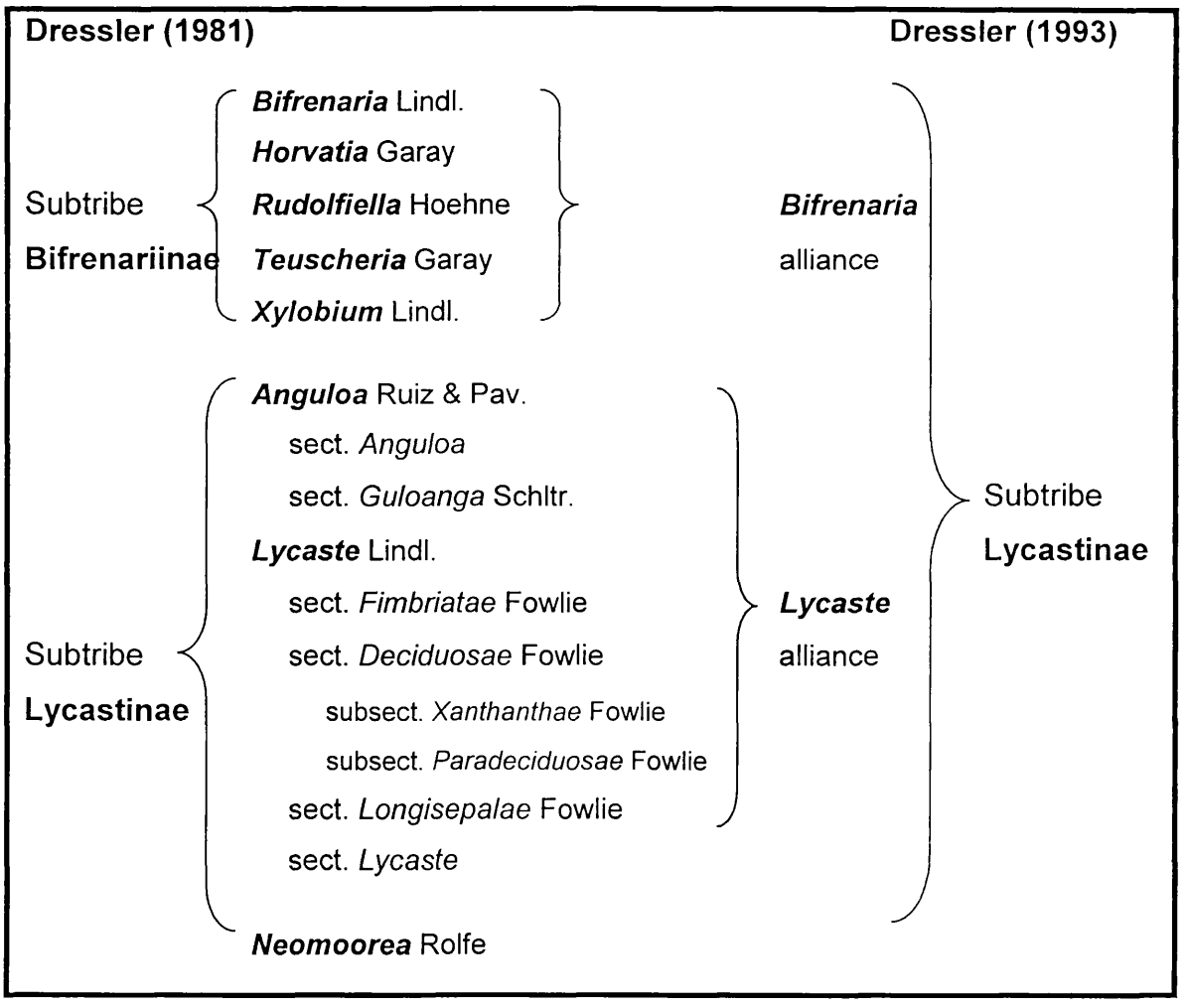


Figure 2.1 Subtribe Lycastinae Schltr., after Dressler (1981,1993).

The relative merits of the two techniques continue to be the subject of heated debate (e.g. Cronquist, 1987; Donoghue and Cantino, 1988; Brummit, 1997; Freudenstein, 1998; Chase 1999). While much of the argument has concerned the degree to which classification should reflect phylogeny, a more contentious issue has been the concept that evolution proceeds parsimoniously. Cladistic analyses have been used throughout this thesis. The author follows the views of Chase (1999), and uses parsimony purely as an estimate of phylogeny. There is no implication that evolution always proceeds by the same mechanism.

The first morphological analysis of the Orchidaceae to use cladistics was that of Burns-Balogh and Funk (1986), who examined the variation of 42 floral characters throughout the family. Their results showed marked differences from the classification of Dressler (1981), such as the separation of *Neuwiedia* from *Apostasia* and also in the circumscripton of sub-families Spiranthoideae and Neottioideae. Although the analysis was criticised for the exclusion of vegetative characters, the quality of the included data and for the method of data processing (e.g. Freudenstein and Rasmussen, 1999), it has served as a starting point for further work.

More recently, Freudenstein and Rasmussen (1999) undertook a cladistic analysis of the family, based on 98 representative genera. Their results showed that most of the traditionally recognised subfamilies, such as Apostasioideae (including *Neuwiedia*), Cypripedioideae, Spiranthoideae and Epidendroideae were monophyletic; the Orchidoideae were not. Their study used 53 vegetative, floral and seed characters, some of which were appropriate to the work presented here.

At a lower taxonomic level, Romero (1990) has used morphological features to examine phylogenetic relationships within subtribe Catasetinae. His analysis identified two monophyletic groups: the first comprising *Clowesia* Lindl., *Dressleria* Dodson,

Cycnoches Lindl. and *Mormodes* Lindl., and the second *Catasetum* Kunth. sections *Catasetum* and *Pseudocatasetum*. Both *Catasetum* and *Cycnoches* have sexually dimorphic flowers and he concluded that this had evolved independently in the two clades.

In this chapter, characteristic features of the species have been defined and their states coded. Plant specimens were examined to determine the status of each of these, and the information used to construct a data matrix. Related genera were included in the matrix (outgroups) to provide a means of polarizing the data (Watrous and Wheeler, 1981).

Some of the floral and vegetative characters that have been used in orchid classification over the years were detailed in sections 1.2 and 1.3. Thus far, little reference has been made to studies on the morphology of orchid seeds; a brief review of these is given below. The final part of this introduction explains the method of data analysis in more detail.

2.1.1 ORCHID SEED MORPHOLOGY

Orchid seeds are small, almost dust-like, typically 0.3-5mm long (Dressler, 1981). They consist of a single embryo without endosperm, which is enclosed in a thin transparent coating, one cell thick, the testa. At one end, a pronounced indentation marks the position of the funicle, which attaches the seed to the carpel. On the seed coat, testa cells usually vary in size, being largest towards the centre. The anticlinal wall of the testa cells, i.e. the wall adjoining other cells, is thickened and raised, giving the seed coat a net-like appearance.

The structure of orchid seeds was first studied by Beer (1863), who examined 166 species distributed among 83 genera. For each species, he published a drawing to

show the size and shape of the seeds, the position of the embryo, the arrangement of testa cells and in the case of three species of *Mormodes*, the presence of spines.

Curtiss (1893) was first to fully recognise the use of seed morphology in orchid classification. He used a light microscope to examine 25 species of North American orchids and concluded that certain features, particularly the shape and form of the testa cells, could be used to distinguish closely related species.

In the first systematic study, by Clifford and Smith (1969), seeds from 49 orchid species were examined, again using a light microscope. The seeds were mainly collected from the Brisbane area of Australia and represented 38 genera from the tribes Epidendreae, Neottieae, Orchideae and Cypripedieae (*sensu* Dressler and Dodson, 1960). Six key characters were identified: seed shape, seed size, testa cell shape, relative wall thickness and secondary thickening on both the anticlinal and periclinal walls.

No difference in gross morphology was observed either between seeds of the same species or between seeds of species belonging to the same genera. At higher taxonomic levels, although there were no obvious attributes that delimited one tribe from another, certain trends were noted: the Neottieae were uniform in seed shape and testa cell shape whereas the Epidendreae showed some diversity in both; none of the Epidendreae showed secondary thickening on the longitudinal walls. The authors concluded that the most useful information was at the sub-tribal level, where they found evidence to support the transfer of *Dipodium* R.Br. from Cymbidiinae to a new subtribe with *Geodorum* Jacks. and the removal of *Anoectochilus* Blume, *Hetaeria* Blume and *Zeuxine* Lindl. from the Spiranthinae. Both sets of transfers have since occurred; *Geodorum* and *Dipodium* are currently ascribed to subtribe Eulophiinae while the other three genera have been placed in Goodyerinae (Dressler 1993).

The use of a scanning electron microscope (SEM) to study seed morphology was first reported by Barthlott (1976). He highlighted the same six characters as Clifford and Smith (1969) and reiterated their view that the information was most useful at sub-tribal level and above.

Seeds of the Oncidiinae and related Neotropical sub-tribes were surveyed by Chase and Pippen (1988). They found no significant differences between seeds of Oncidiinae, Lockhartiinae, Ornithocephalinae, Pachyphyllinae and Telipogonae; Maxillariinae was identified as the nearest relative of the Oncidiinae.

While most studies have been concerned with delimiting tribes and subtribes, variation can occur at and below genus level. For example, within the genus *Cymbidium*, there is a distinct difference between seeds of subgenus *Jensoa* and those of subgenera *Cymbidium* and *Cyperorchis* (Du Puy and Cribb, 1988). Seeds of subgenus *Jensoa* have a filiform shape with lateral striations on the periclinal walls, whilst those of the other subgenera are fusiform with longitudinal striations.

Another example is *Leochilus* Knowles & Westcott (Oncidiinae) where the size and shape of the seeds is uniform across the genus, but there is variation in the degree of ornamentation of the anticlinal walls of the testa cells (Chase, 1986a). Of particular significance are prominent hooks at cell junctions. The hooks are focused around the micropylar pole of the seeds and are only present in species that are twig epiphytes.

In a later study, Chase and Pippen (1990) examined seed from the five genera of the Catasetinae: *Catasetum*, *Clowesia*, *Cycnoches*, *Dressleria* and *Mormodes*, together with *Cyrtopodium* R.Br. and *Cymbidium* from the Cyrtopodiinae. The only useful systematic information came from the nature of the sculpturing on the periclinal walls. This grouped *Clowesia, Dressleria, Mormodes* and some species of *Cycnoches*, but

placed *Catasetum* with *Cyrtopodium* and some *Cymbidium* species. Within Catasetinae, both *Catasetum* and *Cycnoches* have dimorphic unisexual flowers, while *Mormodes* has flowers which have uniform morphology but are functionally either male or female. Traditionally, unisexual flowers had always been considered a more derived character state than "perfect" flowers. However, the groupings inferred from the seed morphology led the authors to suggest that possession of unisexual flowers was in fact a primitive state.

In view of these findings, seeds of *Lycaste* and *Anguloa* species have been examined for morphological variation.

2.1.2 PARSIMONY ANALYSIS

The program used for all the parsimony analyses in this thesis was PAUP* 4.0.2ba (Swofford, 1999). Although PAUP* has the capability of performing exhaustive searches, which guarantee to find the most parsimonious (shortest) trees, the algorithm can only be used for small data sets (less than 20 taxa). For this reason, heuristic searches have been used throughout. Heuristic searching is a two-step process. An initial tree (or set of trees) is obtained by stepwise addition. This is then subjected to trial rearrangements that attempt to find shorter trees.

Stepwise addition operates by connecting taxa sequentially to a developing tree until all have been placed. Three are chosen for the initial tree, followed by one of the unplaced taxa. Each of the trees that would result from addition of that taxon to one of the three branches is examined and the one whose length is optimal saved for the next round. Another unplaced taxon is connected, and the process repeated. It terminates when all taxa have been connected to the tree. For all the parsimony analyses presented here, a pseudorandom number generator (Fishman and Moore, 1982) was used to

determine the order in which taxa were added to the sequence, a process known as "random addition" (Swofford, 1993).

By itself, stepwise addition may not find the shortest trees. As more taxa are added to an existing tree, the position of those placed previously may become sub-optimal. The situation can be improved by performing sets of predefined rearrangements, a process known as branch swapping. Three different algorithms for branch swapping were available to PAUP*: nearest neighbour interchanges (NNI), subtree pruning-regrafting (SPR) and tree bisection-reconnection (TBR). The most rigorous of these, TBR, has been used throughout. With this algorithm, each tree generated by stepwise addition is bisected along a branch, and the two "subtrees" reconnected by joining one branch from each. All possible permutations of bisection and pairwise reconnection are evaluated.

The quality of the analysis can be measured in two ways. The consistency index (Kluge and Farris, 1969), retention index (Farris, 1989) and rescaled consistency index (Farris, 1989) all provide measures of how well the data fits the trees. The level of confidence that can be ascribed to a particular tree topology is measured by the "bootstrap" (Felsenstein, 1985) or "jacknife" (Farris *et al.*, 1996).

For each character, the consistency index (ci) is given by the formula:

$$ci = m/s$$

Where "m" is the minimum number of state changes possible, defined by the coding procedure used, and "s", the observed number of state changes on the tree. The observed number of changes exceeds the minimum number when extra steps, or homoplasy are required to account for the character on the tree. "Homoplasy" refers to perceived similarity, i.e. similarity which has evolved by independent routes or by

character state reversal, as opposed to "homology" or "synapomorphy", which both refer to similarity due to common descent.

The ensemble consistency index of a given tree topology (CI) is given by:

$$CI = \frac{\sum m}{\sum s}$$

Although in general a high CI indicates that the data fits the tree well, the value can be inflated by uninformative (constant) characters, which do not contribute to the tree topology.

The retention index, RI, avoids the problem of uninformative characters. It measures the amount of synapomorphy in a data set and is calculated as the actual amount of homoplasy divided by the maximum possible amount of homoplasy. For each character:

$$ri = \frac{(g - s)}{(g - m)}$$

where "g" is the maximum number of changes possible for the character on the tree. For all characters on a tree:

$$RI = \frac{\sum g - \sum s}{\sum g - \sum m}$$

High RI scores are given to tree configurations in which state changes occur predominantly at internal nodes, lower scores when predominance is at the terminal branches.

The rescaled consistency index, RC, measured as the product of the RI and CI, excludes characters that do not contribute to the "fit" of the tree. It is frequently used for "successive weighting" (Farris, 1969), a method for reducing the effect of highly homoplasious characters on tree topology. This technique successively analyses the

data matrix, calculates the rescaled consistency index for each character, reweights the characters according to the values obtained, and repeats the process until the lengths of trees for successive iterations are constant.

Both the "bootstrap" (Felsenstein, 1985) and "jacknife" (Farris *et al.*, 1996) operate by randomly resampling the data to simulate a new data set for tree construction. During the resampling process, the bootstrap samples, then randomly replaces characters, with a duplicate set of others. The jacknife samples without replacement and the simulated dataset is created by randomly excluding characters or taxa. In general, branches with bootstrap values above 85% are considered to be strongly supported, values between 70% and 85% suggest medium support.

2.2 MATERIALS AND METHODS

Plant material was provided by the Asociación Mexicana de Orquideología, Dr Henry Oakeley and the Royal Botanic Gardens, Kew. The plant material was of four forms: living plants, seeds from mature capsules, pressed herbarium specimens and flowers preserved in either Kew mixture (53%v/v ethyl alcohol, 37% water, 5% glycerol, 5% formaldehyde) or Copenhagen mixture (70%v/v ethyl alcohol, 28% water, 2% glycerol). Details of the vouchers used for this work have been included in Appendices 1 and 2.

Dried flowers were removed from herbarium sheets and rehydrated by boiling in water for 5-10 minutes, prior to dissection and examination using a Wild M8 dissecting microscope. A *camera lucida* extension was used to make detailed drawings of the floral structures. After examination, the flowers were dried with filter paper and returned to their herbarium sheet. To meet Health and Safety regulations, flowers preserved in Kew mixture were transferred to Copenhagen mixture prior to examination.

Dried seeds were mounted onto aluminium stubs using double sided adhesive tape. They were gold coated using an Emscope SC5000 sputter coater, prior to examination under a Cambridge Stereoscan 240 Scanning Electron Microscope. The scanning electron micrographs were taken at an accelerating voltage of 18kV and a working distance of 13-21mm.

Heuristic searches were performed using PAUP* version 4.0b2a (Swofford, 1999). Gaps in the matrix were coded as missing values. Each matrix was subjected to 1000 random stepwise addition replicates, with tree bisection-reconnection (TBR) as the branch swapping algorithm and the Fitch (1971) criterion of unordered states and equal weights. All trees generated by this method were swapped to completion or until memory capacity (10000 trees) was exceeded. Successive approximations weighting, using a base weight of 1000 (Farris, 1969), was applied to the resultant trees and rounds of reweighting and analysis were continued until the tree length remained constant for two successive rounds. Internal support was assessed from 1000 bootstrap replicates (Felsenstein, 1985), with simple stepwise addition, the TBR algorithm and the SW weights applied.

2.3 RESULTS

2.3.1 CHARACTER SELECTION AND CODING

Data was collected from 37 species and subspecies of *Lycaste* and seven species of *Anguloa*, as well as other representatives from subtribe Lycastinae: *Neomoorea wallisii* (Rchb.f.) Schltr., *Bifrenaria harrisoniae* (Hook.) Rchb.f., *Rudolfiella aurantiaca* (Lindl.) Hoehne and *Xylobium latilabium* C.Schweinf. Four species of the subtribe Maxillariinae, *Maxillaria picta* Hook., *M. tenuifolia* Lindl., *M. umbratilis* L.O.Williams and *M. violaceopunctata* Rchb.f. were specified as the outgroup.

In compiling the list of morphological characters used for the analysis, four criteria were considered: Each character had to be unique, easily defined, and both variable and measurable across a wide range of species. Whenever possible, two character states were recognised. Representatives of some of the species used for the analysis are shown in Plates 2.1-2.7. A total of 47 characters were selected.

2.3.1.1 VEGETATIVE CHARACTERS

Owing to their size, few herbarium specimens of *Lycaste*, *Anguloa* or *Neomoorea* include vegetative parts. The following were considered valid:

1. Growth habit (pendent 0, erect 1)

The growth habit is generally erect; the exception is *Lycaste dyeriana* which exhibits pendent growth.

2. Rhizome habit (ascending 0, creeping 1)

All Lycastinae species have a creeping horizontal rhizome, whereas those of the outgroup *Maxillaria* may have an ascending rhizome.

3. Pseudobulb spines (absent 0, vestigial 1, prominent 2)

The pseudobulbs of species from *Lycaste* sect. *Deciduosae* generally bear spines at the apex. Among the other sections of the genus, these are either greatly reduced or absent.

4. Leaf number (single leaf 0, more than one 1)

Lycaste, *Anguloa* and *Neomoorea* have two to four plicate leaves per pseudobulb. The related genera *Bifrenaria*, *Rudolfiella* and *Xylobium* have a single leaf. *Maxillaria* has either one or two leaves.

5. Leaf petiole (absent 0, present 1)

Although none of the species examined have true leaf stalks (petioles), apart from those of *Maxillaria*, all have leaves which narrow towards their base.

6. Leaf vernation (conduplicate 0, plicate 1)

Almost all Lycastinae species have plicate leaves. Those of *Maxillaria* are conduplicate.

7. Leaf texture (papery 0, leathery 1)

Species of *Lycaste*, *Anguloa* and *Neomoorea* have a thin, papery leaf texture. Those of *Maxillaria* and *Xylobium* are more leathery.

8. Leaf persistence (persisting for one year or less 0, more than one year 1)

Species of *Lycaste* sect. *Deciduosae* lose their leaves during the dry season and frequently bear no mature leaves at the onset of flowering and new growth. Among species of the other sections of the genus, the mature leaves are present at the onset of new growth. Those of *Anguloa* are lost at about the same time as the new growth starts to develop.

9. Leaf shape (not-linear 0, linear 1)

Stearn (1983) has defined a wide range of terms to describe two and three dimensional shapes. Leaves of *Maxillaria* are indisputably "linear", i.e. long and narrow with parallel sides. Those of the Lycastinae species included in the study can all be categorised as either "lanceolate" (broadest across the middle, tapering equally towards both ends) or "obovate" (broadest towards the apex). *Bifrenaria harrisoniae*, *Neomoorea wallisii*, and *Lycaste* sect. *Fimbriatae* fall into the former category; the latter includes many species of *Anguloa* and *L.* sect. *Deciduosae*. In practise, it was difficult to delimit obovate and lanceolate, particularly among species of *L.* sect. *Deciduosae* that do not have

persistent leaves; immature leaves of some of these species appeared obovate and mature leaves lanceolate. For this reason the character states were restricted to linear and non-linear.

Excluded vegetative characters:

All the genera examined here have prominent pseudobulbs, sheathed with leafy bracts. The size and shape of the bulbs is variable both within a species and within an individual, and was considered inappropriate for inclusion. In many species, such as *Bifrenaria harrisoniae* and *Lycaste* sects. *Deciduosae* and *Lycaste*, the pseudobulbs show pronounced bilateral compression; in others, such as *L.* sect. *Fimbriatae*, they are more pyriform. However, demarcation between the two states was difficult to define and was at least partly influenced by the degree of hydration of the bulbs.

2.3.1.2 FLORAL CHARACTERS

10. Flowers per scape (usually one 0, usually two 1, many 2)

Scapes of *Lycaste*, *Anguloa* and *Maxillaria* are generally single-flowered. Those of *Bifrenaria* frequently bear two flowers. *Xylobium* and *Rudolfiella* bear many flowers per scape.

11. Floral bract morphology (close fitting 0, loose fitting 1)

The floral bract surrounds the pedicel and ovary. Among some species of *Lycaste* sect. *Deciduosae*, such as *L. aromatica and L. campbellii*, the bract is close-fitting and its length is much shorter than that of the pedicel and ovary. Species of *Anguloa* and *Lycaste* sects. *Fimbriatae, Lycaste and Longisepalae* possess a relatively wider bract, whose length frequently equals or exceeds that of the pedicel and ovary and may extend over part of the dorsal sepal.

12. Sepal habit (not connivent 0, connivent 1)

The sepals of all species of *Anguloa* are connivent, giving the flowers a tulip-like appearance. Other Lycastinae species have lateral sepals which touch at the base of the column foot and then spread apart.

13. Sepal and petal colour (the same colouring 0, different in colour 1)

The base colour of the sepals of *Maxillaria picta, Maxillaria tenuifolia, Neomoorea wallisii*, *Rudolfiella aurantiaca* and all species of *Anguloa* and *Lycaste* sect. *Fimbriatae* is the same as that of the petals. Most species of *L.* sects. *Deciduosae* and *Lycaste* have sepals that are a different colour from the petals.

14. Sepal colour uniformity (uniform 0, light spotting 1, heavy spotting 2)

Anguloa clowesii, all species from *Lycaste* sect. *Fimbriatae* and many from sect. *Deciduosae*, for example *L. aromatica*, *L. campbellii* and *L. suaveolens*, have sepals that are a uniform colour. Other species have sepals that are either lightly spotted, such as *L. brevispatha* and *L. candida*, or very heavily spotted such as *A. hohenlohii*, *L. dowiana* or *L. deppei*.

15. Sepal indumentum (absent 0, present 1)

Most species of *Lycaste* sects. *Deciduosae* and *Lycaste* have a region of very short fine hair near the base of their sepals. Of the species examined here, the exceptions are *L. leucantha* and *L. macrophylla* subsp. *xanthocheila*. This character is absent from *Anguloa* and from all species of *L.* sects. *Fimbriatae* and *Longisepalae*, apart from *L. nana* Oakeley.

16. Lateral sepals (not falcate 0, falcate 1)

The lateral sepals of species *L.* sect. *Fimbriatae* have a pronounced right-angle bend near their base and lie parallel to the scape. Species from the other sections of *Lycaste*

do not have this character and spread horizontally, i.e. perpendicular to the scape.

17. Lateral sepal apex shape (truncate 0, obtuse 1, acute 2, falcate 3)

The lateral sepal apices of specimens of *Bifrenaria harrisoniae* are truncate, those of *Neomoorea wallisii*, *Rudolfiella aurantiaca*, *Xylobium latilabium* and the four species of *Maxillaria*, acute (less than 90 degrees) and those of all specimens of *Anguloa*, falcate. Within *Lycaste*, the apices are either acute, such as those of *L. aromatica* and *L. trifoliata*, or obtuse (more than 90 degrees), for example *L. candida* and *L. costata*; there appeared to be little correlation between the observed character state and the sections defined by Fowlie (1970).

18. Dorsal sepal apex shape (truncate 0, obtuse 1, acute 2)

This character was variable within both *Lycaste* and *Anguloa*. Of the other genera examined, the dorsal sepal apex of *Bifrenaria harrisoniae* was truncate, that of the *Rudolfiella* and *Xylobium* species was obtuse and those of the species of *Maxillaria* and *Neomoorea*, acute.

19. Petal colour uniformity (0 uniform, 1 light spotting, 2 heavy /overlay)

A few species whose sepals are a uniform colour have spotted petals. These include *Anguloa dubia* and *Lycaste cruenta*. Others, particularly from *L.* sect. *Lycaste*, have petals which are considerably less spotted than their sepals.

20. Petal indumentum (absent 0, present 1)

Not all species of *Lycaste* which have hair near the base of their sepals have a similar region on their petals. Exceptions include *L. candida*, *L. consobrina*, *L. deppei*, *L. lasioglossa*, *L. tricolor* and *L. xytriophora*.

21. Petal apex shape (truncate 0, obtuse 1, acute 2, falcate 3)

This character was variable within the genera and sections examined.

22. Petal attitude (no overlap 0, touching/overlapping at apex only 1, overlaps for most of the length 2)

The petals of all species from *Lycaste* sects. *Deciduosae*, *Longisepalae* and *Lycaste*, apart from *L. cochleata*, overlap for some of their length, whereas those of sect. *Fimbriatae* do not. Of the other species examined, the petals of *A. dubia* overlap along their length; those of *A. cliftonii* and *M. violaceopunctata* touch at the apex only.

23. Pronounced "step" near the back of the lip (absent 0, present 1)

Three of the species, *Anguloa brevilabris*, *A. virginalis* and *Lycaste cruenta* have a pronounced "step" at the back of the lip.

24. Lip colour (same as petals 0, different from petals 1)

Species of *Anguloa* and *Lycaste* sects. *Deciduosae*, *Longisepalae* and *Lycaste*, apart from *A. brevilabris*, *L. deppei* and *L. leucantha*, have a mid-lobe that is the same base-colour as the petals. That of species of *L.* sect. *Fimbriatae*, apart from *L. dyeriana*, *L. fowliei* and *L. locusta*, is a different colour from the petals. Of the other genera, *Bifrenaria harrisoniae*, *Neomoorea wallisii* and three of the *Maxillaria* species have differently coloured mid-lobes, whilst *M. violaceopunctata*, *Rudolfiella aurantiaca* and *Xylobium latilabium* have mid-lobes that are the same colour as the petals.

25. Disc colour uniformity (uniform 0, differently coloured veins 1, spotting 2)

The main body of the lip, the disc, of all species of *Lycaste* sect. *Fimbriatae* has a uniform colour. Among the other sections and genera examined, some species have discs that are either spotted, e.g. *L. macrophylla* subsp. *desboisiana*, or have veins that are a different colour, e.g. *Bifrenaria harrisoniae*.

26. Disc indumentum (absent 0, present 1)

Lycaste aromatica, *L. crinita*, *L. cruenta* and *Bifrenaria harrisoniae* all have long hair present on the mesochile of the lip. *Maxillaria tenuifolia*, *M. umbratilis* and *Neomoorea wallisii* have much shorter, velvety hairs.

27. Lip mid-lobe morphology (not prominent 0, prominent 1)

The lips of all the species used in the analysis apart from those of *Anguloa* and *Neomoorea* have a prominent mid-lobe.

28. Mid-lobe apex shape (truncate 0, rounded/obtuse 1, retuse 2, acute 3)

Four character states were recognised: truncate, e.g. *Lycaste dyeriana*; rounded or obtuse, e.g. *L. barringtoniae* or *L. trifoliata*; retuse, e.g. *L. locusta* or *L. dowiana* and acute, such as *Anguloa clowesii* or *Neomoorea wallisii*

29. Mid-lobe margin fimbriation (absent 0, present 1)

All species of *Lycaste* sect. *Fimbriatae* have a mid-lobe which has a fimbriate margin. Of the other species and genera examined, only *L. dowiana and L. tricolor* have this characteristic.

30. Mid-lobe texture (thin 0, fleshy 1)

All species of *Maxillaria*, *Anguloa* and *Lycaste* sect. *Fimbriatae*, apart from *M. tenuifolia*, *L. ciliata* and *L. fragrans*, appear to have a fleshy mid-lobe.

31. Mid-lobe indumentum (absent 0, velvety 1, hairy 2)

Lycaste lasioglossa is unique among the species sampled in that the mid-lobe of the lip is covered with long hairs. The mid-lobes of *Anguloa* species from sect. *Guloanga* are covered with very short hairs giving them a velvety texture, a characteristic they share with *L. crinita*, *L. leucantha*, *Bifrenaria harrisoniae* and *Maxillaria umbratilis*.

32. Mid-lobe colour uniformity (uniform 0, veins a different colour 1, spotted 2)

The mid-lobe of *Bifrenaria harrisoniae* has veins that are a different colour from the base-colour of the lip. Those of *Rudolfiella aurantiaca*, *Neomoorea wallisii*, and the four *Maxillaria* species are spotted. Within *Lycaste*, all species of *L.* sect. *Fimbriatae* have unmarked mid-lobes, those of the other three sections are either spotted, e.g. *L. brevispatha*, or unspotted, e.g. *L. aromatica*. Similarly, both character states exist within *Anguloa*.

33. Callus apex overhanging (absent 0, present 1)

Three of the species examined, *Bifrenaria harrisoniae*, *Maxillaria picta* and *Xylobium latilabium*, had a callus whose apex appeared to be fused to the lip. Those of all the other species were not. The state of the callus apex of *Rudolfiella aurantiaca* could not be determined.

34. Callus apex shape (entire 0, two or three lobed 1)

The callus apex of all species of *Anguloa* and *Lycaste* sect. *Fimbriatae* is emarginate (2-lobed); that of *Bifrenaria harrisoniae* is tridentate. All other species examined had callus apices that were entire.

35. Callus indumentum (absent 0, present 1)

Maxillaria tenuifolia, *Anguloa clowesii*, *A hohenlohii* and *Lycaste suaveolens* all have regions of short, velvet-like hair on the callus. Two other species, *Bifrenaria harrisoniae* and *L. aromatica*, have hair that is much longer.

36. Callus ridges (absent 0, present 1)

All species of *Lycaste* sect. *Fimbriatae*, apart from *L. mathiaseae*, have prominent ridges (keels) which run from the back of the lip to the front of the callus. Of the remaining species of *Lycaste*, *L. candida* and *L. macrobulbon* have prominent ridges

at the callus margins, as do *Anguloa cliftonii*, *A. dubia*, *A. eburnea*, *A. virginalis*, *Maxillaria umbratilis* and the species of *Neomoorea*, *Bifrenaria* and *Rudolfiella*.

37. Column hairs under the stigma (absent 0, present 1)

All species of *Lycaste* sects. *Deciduosae*, *Longisepalae* and *Lycaste*, apart from *L. tricolor*, have a region of hair on the front of the column, directly beneath the stigma. Most species from *L.* sect. *Fimbriatae* and the other genera lack this character; the exceptions are *L. fimbriata*, *Anguloa cliftonii*, *A. dubia* and *Rudolfiella aurantiaca*.

38. Column hairs over the ovary (absent 0, present 1)

Many species of *Lycaste* from sects. *Deciduosae*, *Fimbriatae* and *Lycaste* have a discrete region of hair on the front of the column, directly over the position of the top of the ovary. This character was absent in species from the other genera.

39. Column-foot indumentum (absent 0, present 1)

The front of the column foot of *Bifrenaria harrisoniae*, and seven of the species assigned to *Lycaste* sect. *Fimbriatae*, including *L. dyeriana,* have a region of hair.

40. Rostellum mid-lobe attitude (flat or ridged 0, recessed 1)

This character refers to the part of the rostellum immediately underneath the stipe (see Figures 1.2-1.4). In species of *Anguloa* it is slightly depressed, so that the stipe appears to sit in a groove.

41. Rostellum mid-lobe apex (flush 0, acute 1, truncate/slightly bidentate 2)

The apex of the mid-lobe of the rostellum of *Lycaste aromatica*, *L. deppei* and all species from sect. *Fimbriatae*, apart from *L. dyeriana*, is either truncate or slightly bidentate. That of *Anguloa*, *Bifrenaria harrisoniae*, *Rudolfiella aurantiaca* and the other

Lycaste species is acute. In *Maxillaria*, *Neomoorea*, *Xylobium latilabium* and *L. dyeriana*, the mid-lobe appears to form a continuum with the rest of the rostellum.

42. Rostellum side-lobe shape (not elongate 0, elongate 1)

The side-lobes of the rostellum of species of *Anguloa* sect. *Anguloa* (e.g. *A. eburnea* and *A. virginalis*) are elongate. Those of all the other species examined are not.

43. Anther-cap texture (smooth 0, velvety 1)

Most of the species examined have a smooth anther-cap, however two of the species of *Maxillaria*, *M. umbratilis* and *M. violaceopunctata*, and four species of *Lycaste*, *L. dowiana*, *L. macrophylla* subsp. *xanthocheila*, *L. macrobulbon* and *L. schilleriana,* have anther-caps with a velvety texture.

44. Number of stipites (one 0, two 1)

Species of *Anguloa*, *Lycaste* and *Neomoorea* have a single long stipe connecting the four pollinia to the viscidium. *Bifrenaria*, *Rudolfiella* and *Xylobium* have two stipites, each attached to two pollinia, sharing a common viscidium,

45. Stipe shape (short and triangular 0, oblong and saccate 1, linear 2)

The stipes of the species examined fell into three categories. Those of *Anguloa*, *Lycaste*, *Neomoorea* and *Rudolfiella* were linear with no obvious curvature across their width. Those of *Maxillaria umbratilis* and *M. violaceopunctata* were shorter, broader and saccate. The stipes of *Bifrenaria harrisoniae*, *Maxillaria picta* and *M. tenuifolia* were extremely short and either oblong (*B. harrisoniae*) or more triangular (*M. picta*, *M. tenuifolia*).

46. Viscidium shape (oblong/broadly elliptic 0, ovate 1, V- or M-shaped 2, lunate or linear 3)

The viscidium of specimens stored in spirit does not always preserve as well as the other floral parts. Of those specimens where it was still intact, four character states could be recognised. *Bifrenaria harrisoniae* and *Lycaste deppei* have a viscidium that is oblong to broadly elliptic. *Anguloa*, *Neomoorea* and all other species of *L.* sects. *Deciduosae*, *Longisepalae* and *Lycaste* have an ovate viscidium (e.g. Figures 1.2 and 1.4). In species of *L.* sect. *Fimbriatae* it was elongate and angled from the base of the stipe to form a V- or M-shape (e.g. Figure. 1.3), whilst that of *Rudolfiella aurantiaca* and the *Maxillaria* species was horseshoe -shaped.

47. Viscidium apex shape (truncate 0, acute 1)

Apart from *Lycaste deppei*, the viscidia of all species of *Anguloa*, *Lycaste* and *Neomoorea* had acute apices. Those of *Bifrenaria harrisoniae*, *Rudolfiella aurantiaca*, *Lycaste deppei* and the four *Maxillaria* species were truncate.

Excluded floral characters.

Two sets of characters were excluded. The first were descriptors of overall shape, such as "elliptic" or "lanceolate"; it was not always possible to delimit different character states. The second set were descriptors of colour, such as "red" or "yellow". Although these have frequently been used as an informal means of grouping species of both *Lycaste* and *Anguloa* (e.g. Sparrow, 1964; Oakeley, 1994), the various floral pigments involved are unknown.

2.3.1.3 SEED CHARACTERS

Due to the limited amount of material available, no characters were included in the analysis. A brief description of the seed morphology follows the data analysis.

PLATE 2.1

Lycaste sect. *Deciduosae*

A. *Lycaste aromatica*

B. *Lycaste campbellii* (from the Kew slide collection)

C. *Lycaste cochleata*


A


B

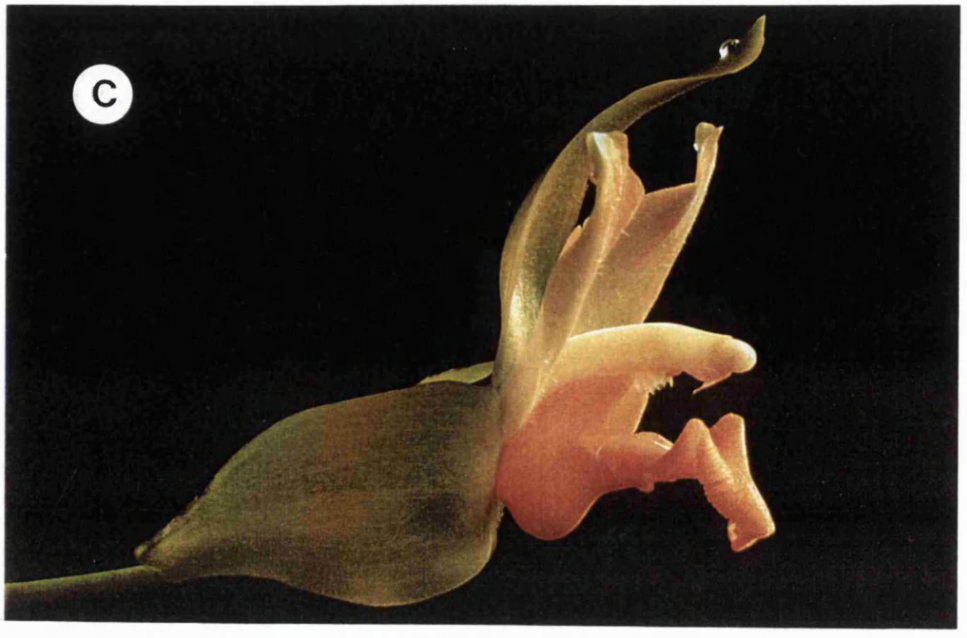

C

PLATE 2.2

Lycaste sect. *Deciduosae* cont.

A. *Lycaste cruenta*

B. *Lycaste cruenta*, showing spined pseudobulbs

C. *Lycaste deppei*


A


B


C

PLATE 2.3

Lycaste sect. *Deciduosae* cont.

A. *Lycaste macrobulbon*

B. *Lycaste bradeorum*

C. *Lycaste candida*


A


B


C

PLATE 2.4

Lycaste sect. *Lycaste*

A. *Lycaste dowiana*

B. *Lycaste macrophylla* subsp. *desboisiana*

Lycaste sect. *Longisepalae*

C. *Lycaste schilleriana* (photographed by Dr Henry Oakeley)


A


B


C

PLATE 2.5

Lycaste sect. *Fimbriatae*

A. *Lycaste locusta*

B. *Lycaste dyeriana* (photographed by Dr Henry Oakeley)

C. *Lycaste trifoliata* (photographed by Dr Henry Oakeley)


A


B


C

PLATE 2.6

Anguloa

A. *Anguloa cliftonii* (photographed by Dr Henry Oakeley)

B. *Anguloa cliftonii* (photographed by Dr Henry Oakeley)

C. *Anguloa hohenlohii*


A


B

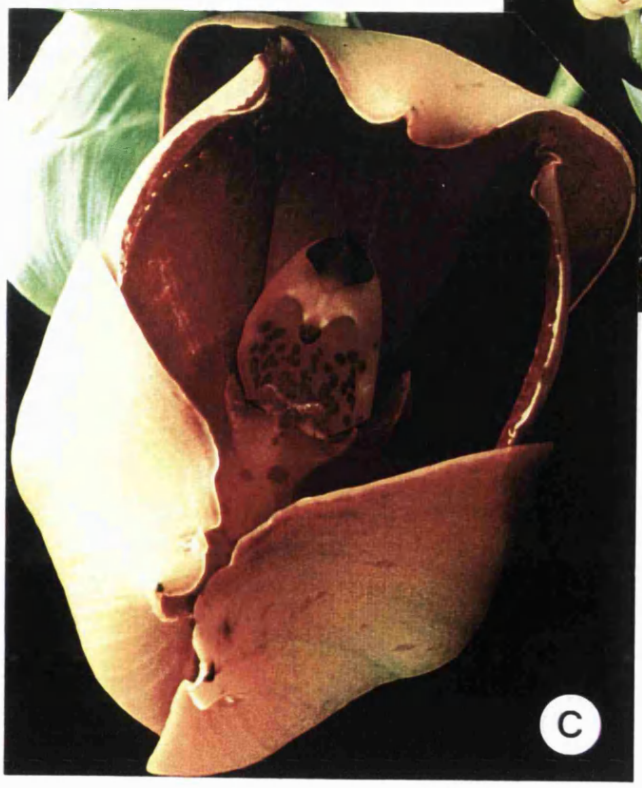

C

PLATE 2.7

Lycastinae species used for morphological and molecular analyses

A. *Neomoorea wallisii* (photographed by Dr Phillip Cribb)

B. *Bifrenaria harrisonii*

C. *Rudolfiella aurantiaca* (from the Kew slide collection)


A


B


C

2.3.2 DATA ANALYSIS

The data matrix used for the analysis is shown in Appendix 3. Parsimony analysis yielded 10000 equally parsimonious trees of Fitch length 235, with a consistency index (CI) of 0.29 and retention index (RI) of 0.71. Successive weighting reduced the number of trees to 226, with weighted length 44986 (Fitch length 238), CI of 0.52 and RI of 0.86. One of the weighted trees is shown in Figure 2.2. Numbers above the branches indicate the number of character-state changes, those in boldface show percent support from bootstrap replicates. Arrowheads indicate branches that collapse in the strict consensus of all successively weighted trees.

The tree shows *Lycaste* to be split into two clades. One of these contains all those species currently ascribed to sect. *Fimbriatae*, including *L. dyeriana*. Species from the other three sections are placed together in an adjacent clade. There is weak bootstrap support (60%, 67%) for these two clades and less than 50% support for the monophyly of the genus *Lycaste*. Sister to *Lycaste* is a weakly supported clade containing *Anguloa* and *Neomoorea*; within this, there is strong (97%) support for the monophyly of *Anguloa*. The other Lycastinae species are placed together, sister to *Lycaste*/*Anguloa*/*Neomoorea*, and there is strong support (89%) for the separation of *Lycaste*/*Anguloa*/*Neomoorea* from these. The outermost clade comprises the four species of *Maxillaria* and again, there is strong support (96%) for the separation of these from Lycastinae. The consistency indices of individual characters before reweighting ranged from 0.09 (character 24) to 1.00, indicating a high degree of homoplasy in the data set.

The character states that were the most difficult to determine concerned the shape of the mid-lobe apex; this part of the flower was often damaged in herbarium specimens. The character was deleted from the data matrix and the analysis repeated using the same conditions.

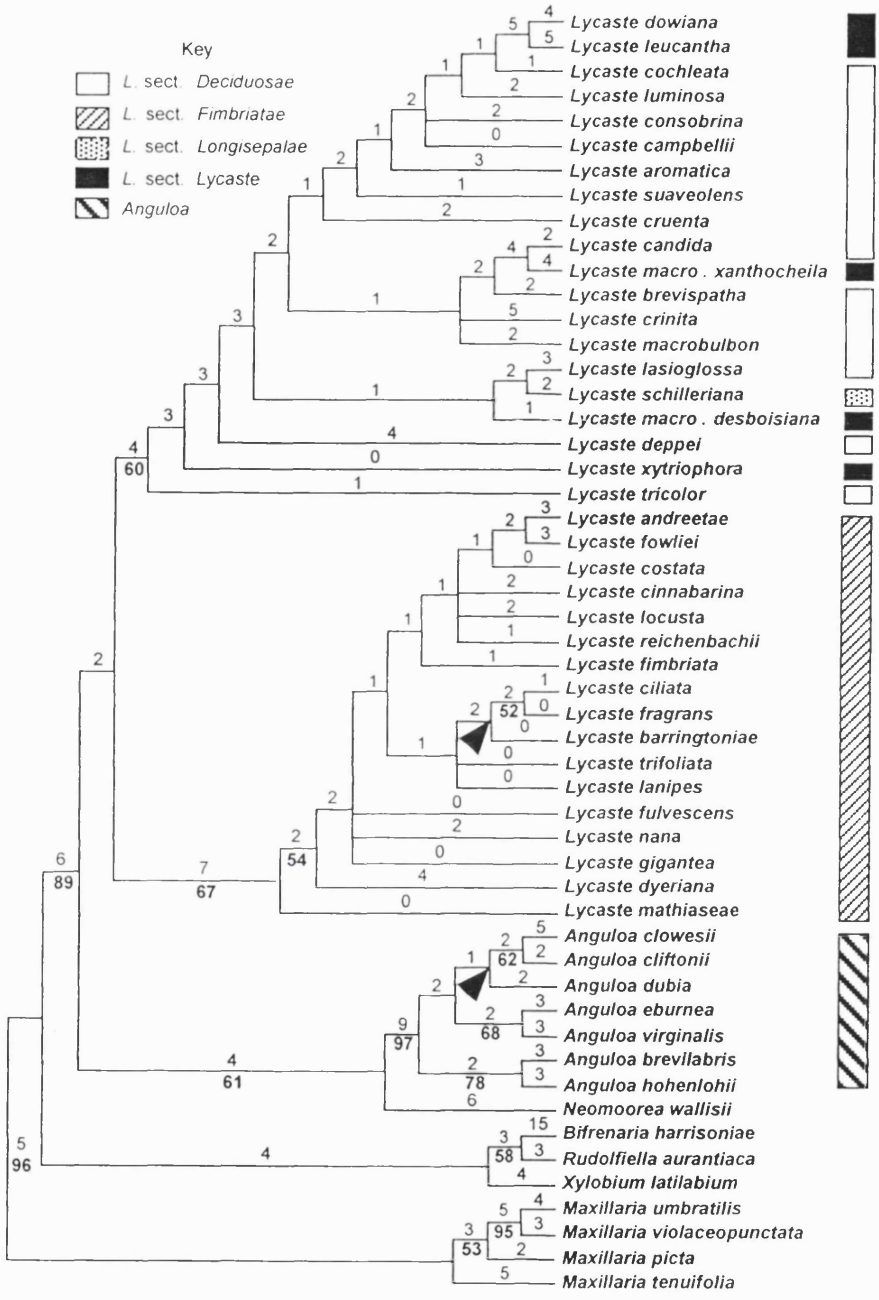


Figure 2.2 One of the 226 successively weighted most parsimonious trees showing cladistic relationships within subtribe Lycastinae, based on 47 morphological characters. Numbers above the branches are the estimated number of substitutions; numbers below are bootstrap percentages greater than 50%. Arrowheads indicate branches not present in all trees.

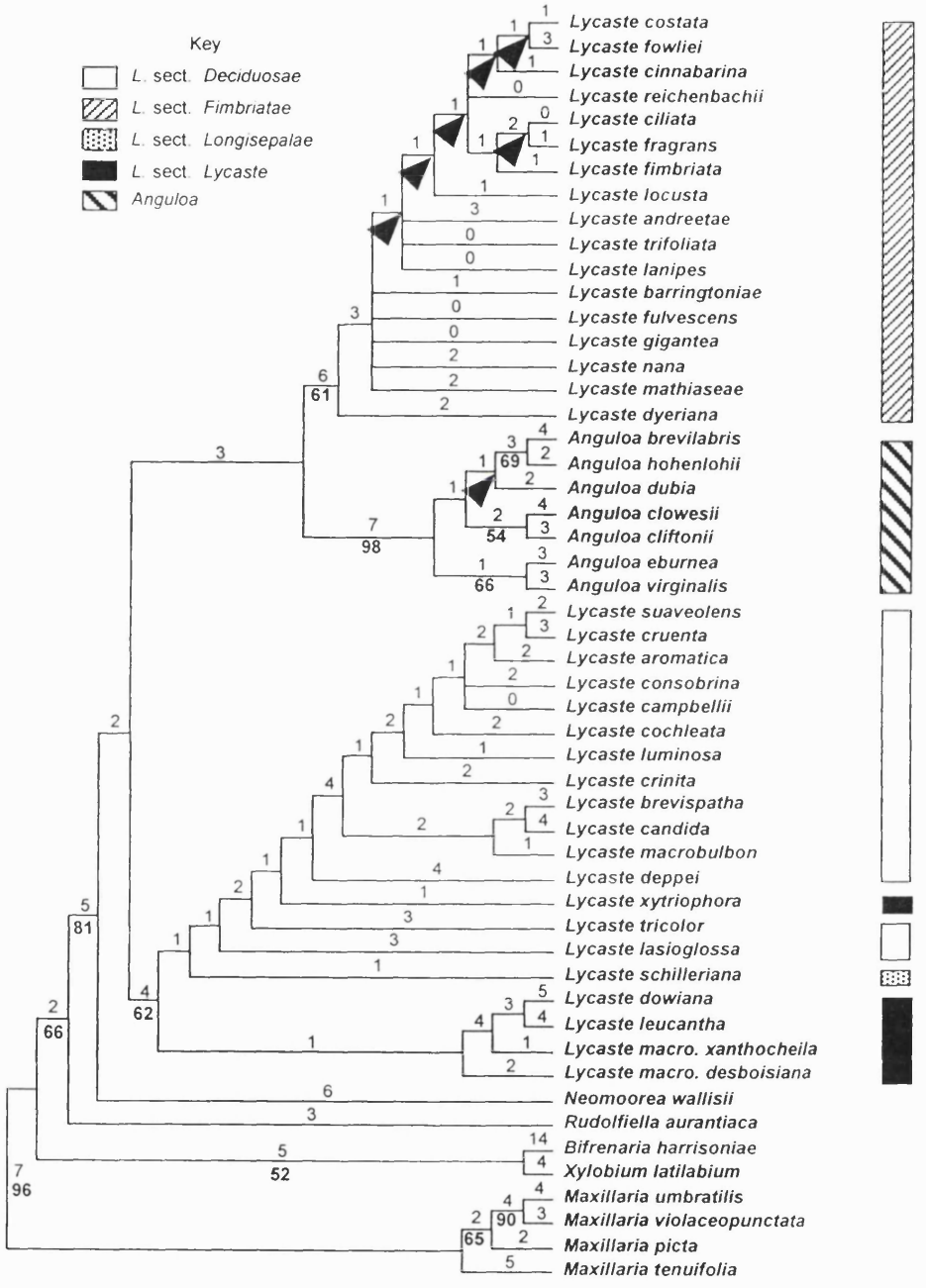


Figure 2.3 One of the 4164 successively weighted most parsimonious trees showing cladistic relationships within subtribe Lycastinae, based on 46 morphological characters. Numbers above the branches are the estimated number of substitutions; numbers below are bootstrap percentages greater than 50%. Arrowheads indicate branches not present in all trees.

Again, 10000 most parsimonoius trees were found, this time with Fitch length 221, CI 0.30 and RI 0.71. Successive weighting yielded 4164 trees of weighted length 42811 (Fitch length 222), CI 0.54 and RI 0.87. These trees have a different topology from those described above, as is shown in Figure 2.3. *Lycaste* is no longer monophyletic; the species have been placed in two clades. One of these comprises both *Anguloa* and species from *L.* sect. *Fimbriatae*, the other, as before, encompasses species from the other three sections of *Lycaste*. There is strong support for the monophyly of *Anguloa* (98%), but only weak support for that of both *L.* sect *Fimbriatae* and the clade containing species from the other sections of the genus. Sister to *Lycaste*/*Anguloa* is *Neomoorea* and as before there is strong support (81%) for the separation of these three genera from the other Lycastinae species. Within Lycastinae, there is weak support for the separation of *Rudolfiella* from *Bifrenaria* and *Xylobium*. As before, there is strong support (96%) for the separation of Lycastinae from the four species of *Maxillaria*.

2.3.3 SEED MORPHOLOGY

Scanning electron micrographs were obtained from seeds of 10 species and subspecies of *Lycaste* and two species of *Anguloa* (Plates 2.8-2.10). The seeds were fusiform, ranging in length from 174µm to 435µm and in width from 74µm to 156µm. Within each species there was some variation to the overall shape, length, width and length to width ratio; an example of the variation in size and shape of seeds of *L. fragrans* is illustrated in Plate 2.10A.

Testa cells were arranged spirally in seeds of *Lycaste brevispatha* (Plate 2.8A), *L. dowiana* (Plates 2.8B), *L. skinneri* (Plate 2.8E) and *L. bradeorum* (not illustrated here). Among the other taxa, cells were arranged parallel to the long axis of the seed. Generally, the medial cells were elongate and larger than the cells closer to the poles. The number of layers of smaller cells at the micropylar pole was variable (e.g. Plate

2.10B,C). In all of the seeds examined, there was clear demarcation between the anticlinal walls of adjacent testa cells (e.g. Plates 2.9B and 2.10B,C,D) and the transverse anticlinal walls were slightly elevated (e.g. 2.8E, 2.9E). Deep longitudinal striations were observed on the inner periclinal wall of *L. brevispatha* and *L. bradeorum* (Plate 2.8A, 2.10D). Among the other species, striation was less pronounced (e.g. Plate 2.10E) and among some species, such as *L. dowiana* (Plate 2.10B), appeared slightly verrucous.

2.4 DISCUSSION

Over the centuries, plant species have usually been described and delimited in terms of their morphological features. It therefore seemed appropriate to use such characteristics to examine phylogenetic relationships within *Lycaste* and *Anguloa*. To date, most published phylogenetic analyses have been based on DNA sequence data where every base in the gene or region of interest is considered a unique character and where the identity of each base (A, C, G or T) is the "character state". When dealing with morphological data, an initial character selection stage is required which introduces an element of subjectivity into the analysis. Inclusion or exclusion of characters can have a marked effect on tree topology as can be seen in Figures 2.2 and 2.3, where exclusion of a single character caused *Lycaste* sect. *Fimbriatae* to move from a clade comprising only species of *Lycaste* to a clade comprising *Anguloa*.

Traits common to both analyses (Figures 2.2 and 2.3) were the separation of *Maxillaria* from all the Lycastinae species, the monophyletic grouping of *Anguloa* and the separation of *Lycaste* into two clades. One of these comprised both *L. dyeriana* and species from sect. *Fimbriatae*, indicating that in spite of its pendent growth habit, *L. dyeriana* is in fact closer to sect. *Fimbriatae* than to *Bifrenaria*, as had been postulated by Fowlie (1970). The other clade comprised species from sects. *Deciduosae*, *Longisepalae* and *Lycaste*.

The analyses differed in the arrangement of taxa within each clade. The first analysis (Figure 2.2), for example, placed *L. macrophylla* subsp. *desboisiana* with *L. schilleriana* and *L. lasioglossa*. The second analysis (Figure 2.3) grouped it with *L. macrophylla* subsp. *xanthocheila*, *L. leucantha* and *L. dowiana*. Bootstrap support for the arrangement of taxa within the two *Lycaste* clades was low, generally less than 50%.

The differences in tree topology indicate that cladistic analysis based on "visible" morphological characters, i.e. those that can be seen with a dissecting microscope, is not a reliable method for examining phylogenetic relationships within *Lycaste*.

Examination of the micromorphology (anatomy) of these plants was outside the scope of this study, but may provide additional sources of characters. In the roots, for example, the innermost layer of velamen cells, adjacent to the root exodermis contains fibrous bodies called tilosomes. Pridgeon *et al.* (1983), have identified seven different forms of tilosome within the Orchidaceae. Those of *Lycaste* are termed "lamellate" and are plates consisting of parallel ridges and furrows. The function of the tilosomes was originally assumed to be for water absorbtion (Dietz, 1930), however Benzing *et al.* (1982) have since concluded that they probably act as protective plugs and barriers to transpiration. The number of layers of velamen cells differs between species of *Lycaste* (Pridgeon, 1987); *L. aromatica*, for example, has eight to ten layers whereas *L. cinnabarina* has only four or five.

PLATE 2.8

SEM photographs of seeds of *Lycaste*

Lycaste sect. *Deciduosae*

A. *L. brevispatha*

Lycaste sect. *Lycaste*

B. *L. dowiana*

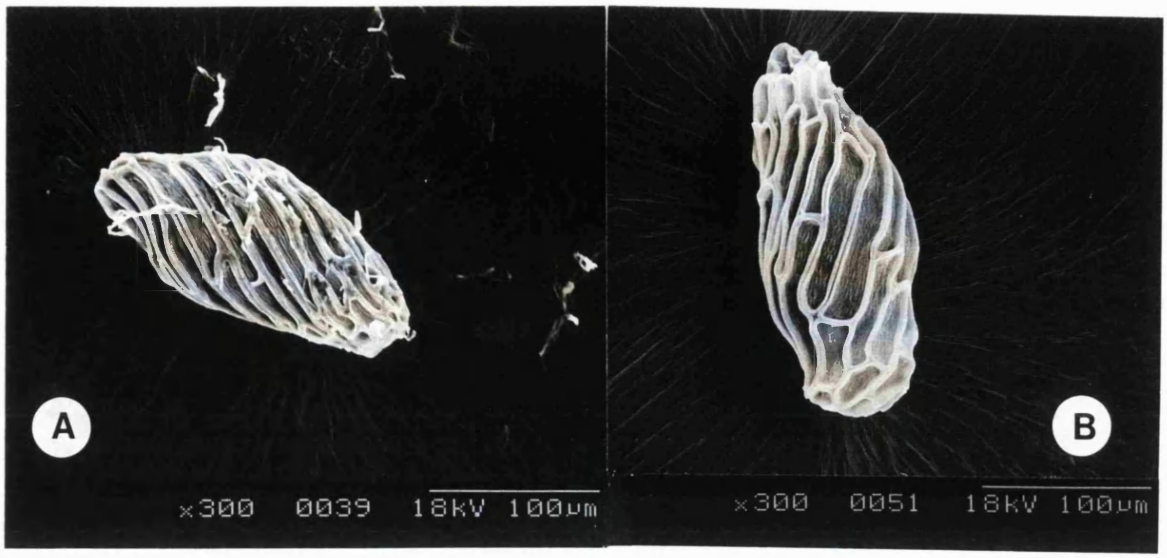
C. *L. macrophylla* subsp. *viridescens*

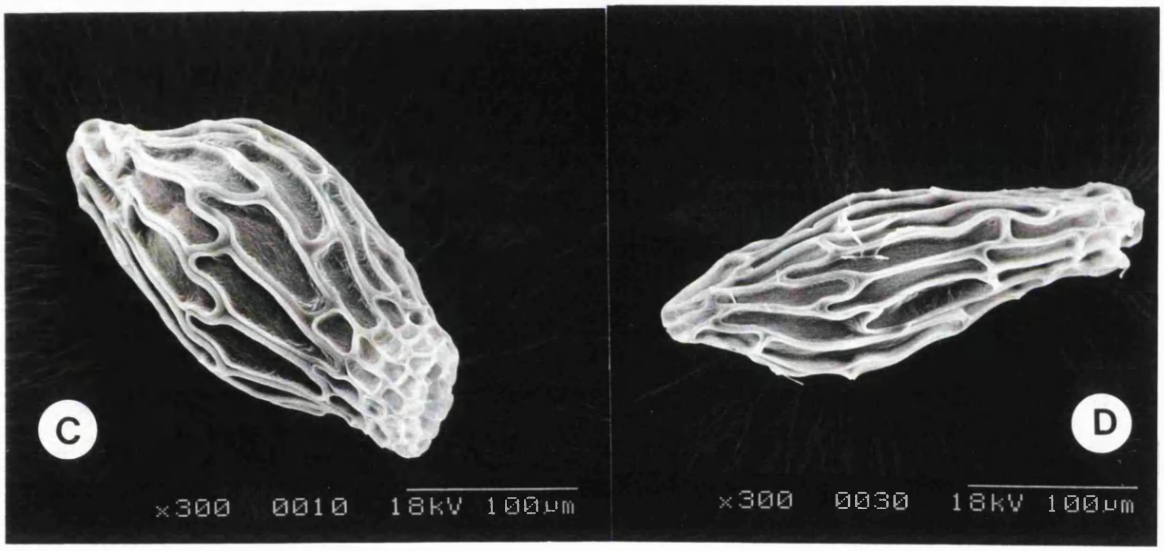
D. *L. macrophylla* subsp. *xanthocheila*

E. *L. skinneri*

Lycaste sect. *Longisepalae*

F. *L. schilleriana*


A
×300 0039 18kV 100µm
B
×300 0051 18kV 100µm


C
×300 0010 18kV 100µm
D
×300 0030 18kV 100µm


E
×300 0116 18kV 100µm
F
×300 0079 18kV 100µm

PLATE 2.9

SEM photographs of seeds of *Lycaste* sect. *Fimbriatae* and *Anguloa*

Lycaste sect. *Fimbriatae*

A. *L. fragrans*

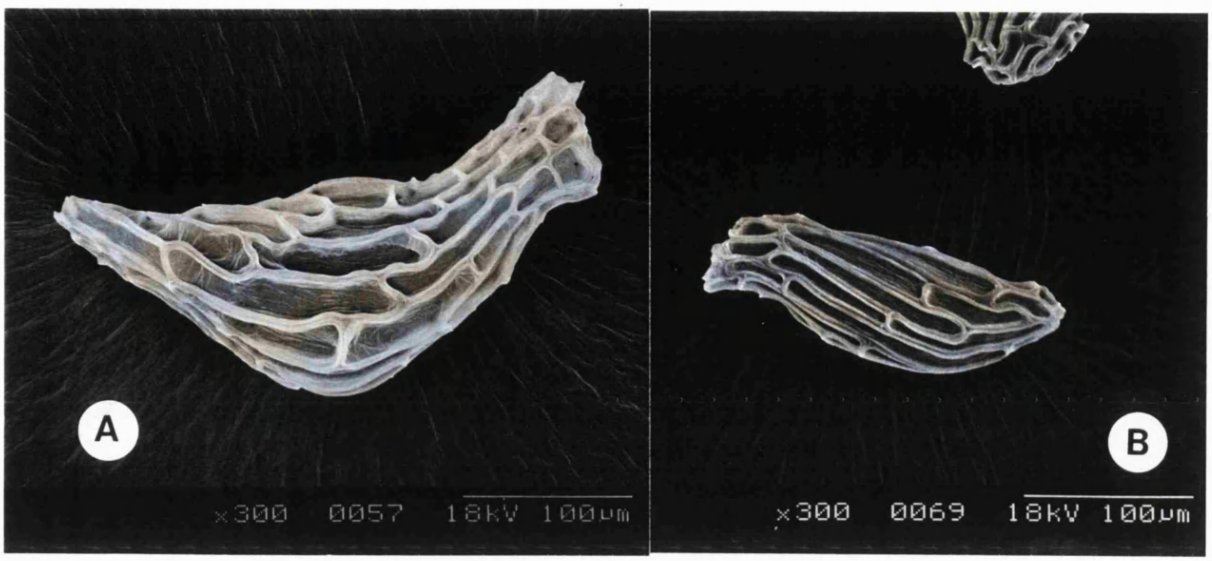
B. *L. dyeriana*

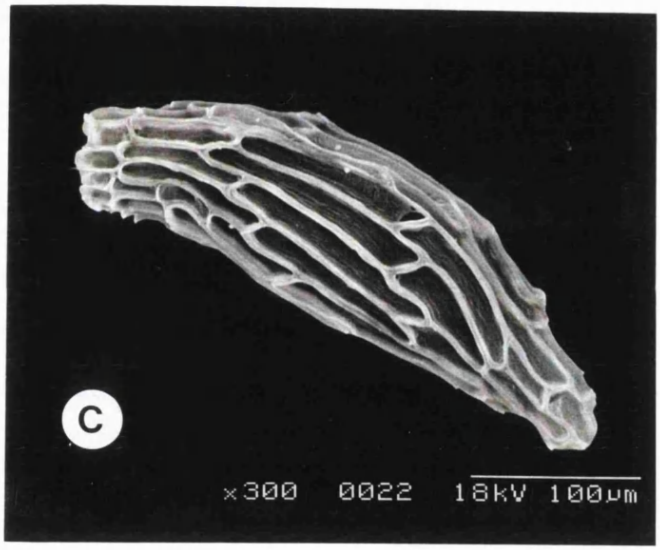
C. *L. lanipes*

Anguloa

D. *A. eburnea*

E. *A. brevilabris*


A
×300 0057 18kV 100µm
B
×300 0069 18kV 100µm


C
×300 0022 18kV 100µm

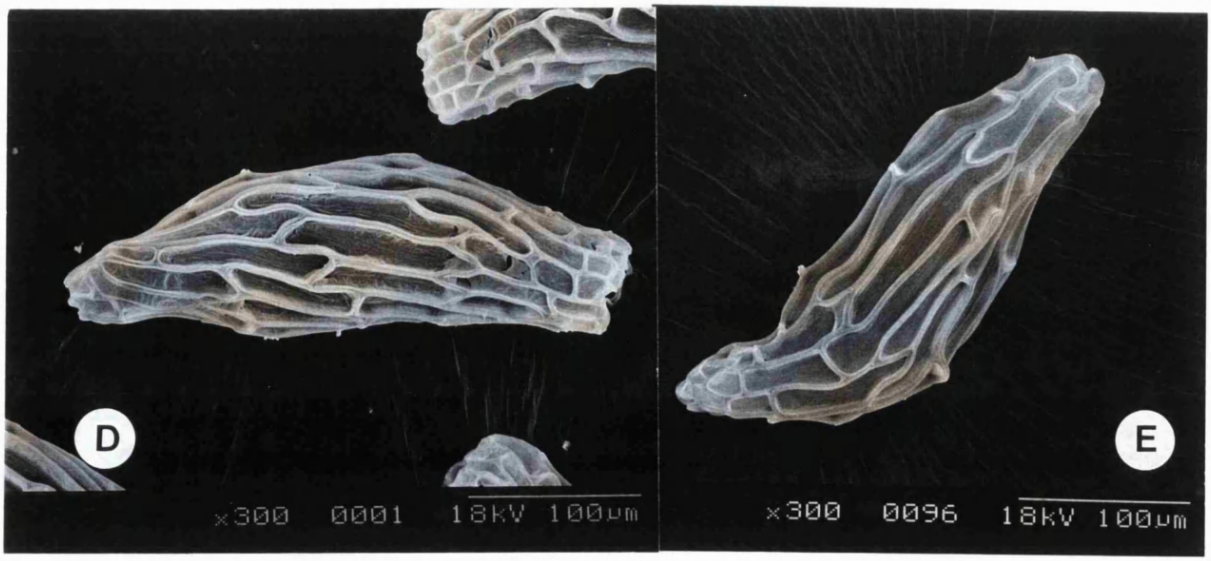

D
×300 0001 18kV 100µm
E
×300 0096 18kV 100µm

PLATE 2.10

SEM photographs of seeds of *Lycaste* and *Anguloa* cont.

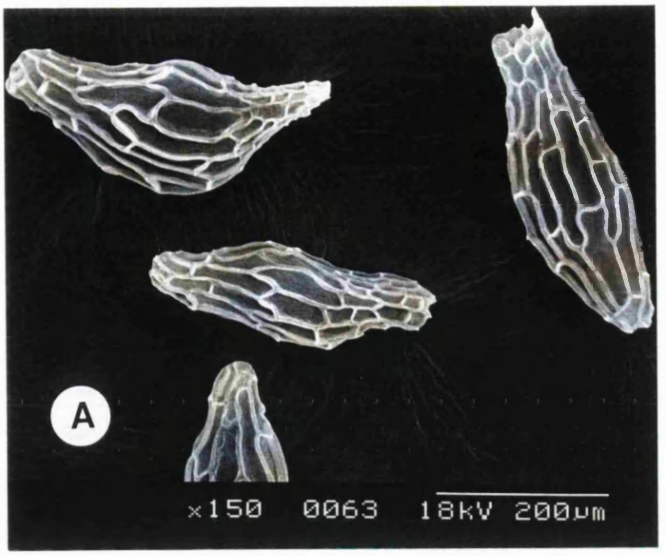
A. *L. fragrans*: Examples of variation in seed shape.

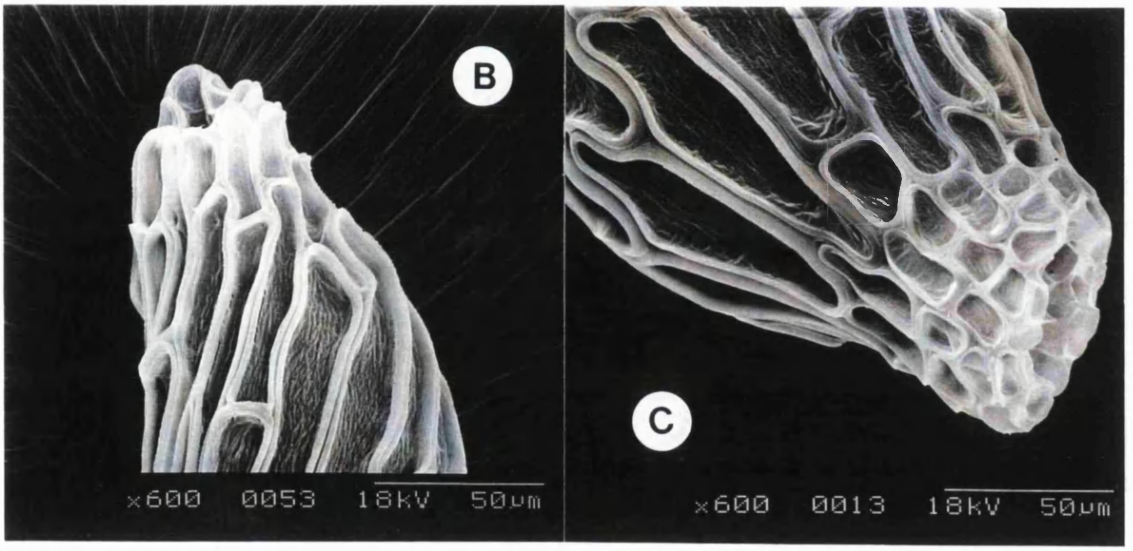
B. *L. dowiana*: Micropylar pole.

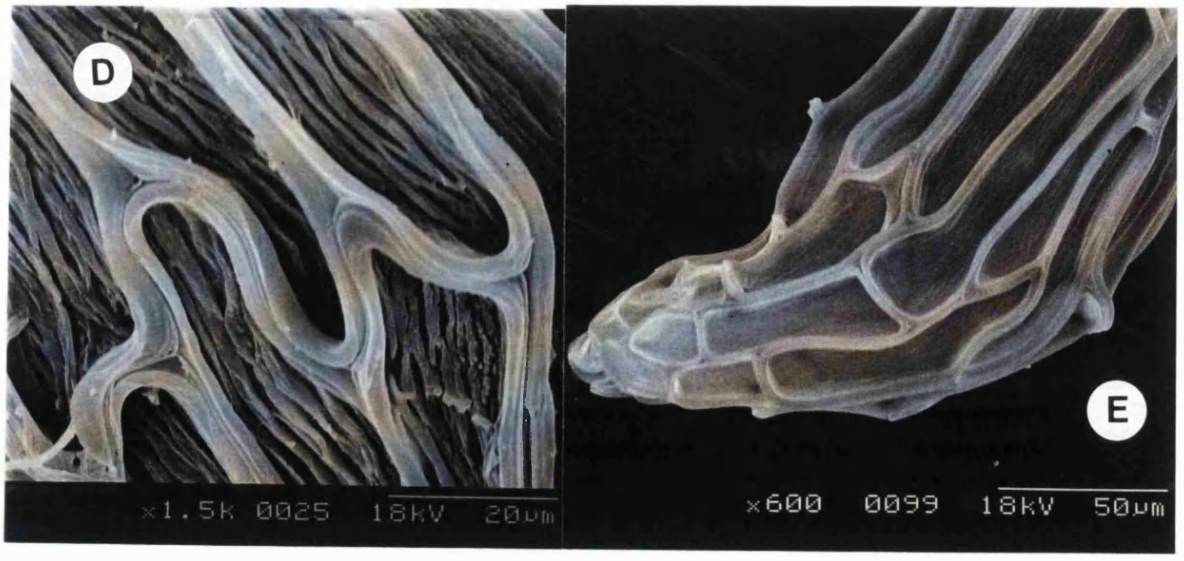
C. *L. macrophylla* subsp. *viridescens*: Micropylar pole.

D. *L. bradeorum*: Anticlinal and periclinal cell walls.

E. *A. brevilabris*: Anticlinal and periclinal cell walls.


A
×150 0063 18kV 200µm


B
×600 0053 18kV 50µm
C
×600 0013 18kV 50µm


D
×1.5k 0025 18kV 20µm
E
×600 0099 18kV 50µm

Chapter 3 MOLECULAR AND CYTOGENETIC ANALYSIS

The objectives of the work presented in this chapter were to investigate the infra- and intergeneric relationships of *Lycaste* and *Anguloa* and their relationship to other members of subtribe Lycastinae Schltr., using sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region and the plastid maturase-coding gene *matK*.

3.1 INTRODUCTION

Within Orchidaceae, ITS sequences have been used to examine the phylogeny of the tribe Diseae Dressler (Douzery *et al.*, 1999) and the subtribes Catasetinae Schltr. (Pridgeon and Chase, 1998) and Orchidinae (Pridgeon *et al.*, 1997). Both Catasetinae and its five component genera were found to be monophyletic as was subtribe Orchidinae (excluding *Holothrix* Lindl.). Dressler's (1993) recognition of two alliances within Orchidinae, those with elliptical to globose root tubers and those with palmate-attenuate root tubers, was also recognised. At genus level, sequence analysis has shown *Orchis* L. to be polyphyletic, with three strongly supported clades. These correlate with differences in somatic chromosome number, sepal fusion and lip morphology and suggest that a narrower circumscription of the genus is required (Pridgeon *et al.*, 1997).

The utility of ITS sequence data at the infrageneric level has also been demonstrated in a study of Cypripedioideae Lindl. (Cox *et al.*, 1997), which did not support the division of *Paphiopedilum* Pfitzer into the subgenera *Brachypetalum* and *Paphiopedilum* proposed by Atwood (1984). The latest revision of *Paphiopedilum* (Cribb, 1998) no longer recognises these subgenera.

Previously known as ORFK (Steele, 1991), *matK* has more variable sites than many other protein-coding regions of the plastid genome (Johnson and Soltis, 1995). Thus far it has been less extensively used for sequencing studies than ITS. Within Orchidaceae, Kores *et al.* (1997) used *matK* to examine phylogenetic relationships within the subfamily Orchidoideae. In a smaller study, Khayota (1995) used *matK* sequences to confirm that the African genus *Ansellia* Lindl. (Epidendroideae: Cymbidieae) is more closely related to *Grammatophyllum* Blume than to other genera in subtribe Cyrtopodiinae Benth. Its potential at the infrageneric level remains largely unexplored.

Many recent studies have shown that phylogenetic resolution and levels of support can be improved by combining independent molecular data sets (Chase and Cox, 1998; Soltis *et al.*, 1998; Savolainen *et al.*, 2000). Pertinent to the work presented here, Whitten *et al.* (2000) have combined DNA sequence data from ITS, *matK*, *trnL*, and *trnL-F* regions to examine the phylogenetic relationships and monophyly of tribe Maxillarieae Pfitzer. Dressler (1993) ascribed 164 genera to Maxillarieae, dividing it into eight subtribes: Cryptarrheninae Dressler, Zygopetalinae Schltr., Lycastinae, Maxillariinae Benth., Stanhopeinae Benth., Telipogoninae Schltr., Ornithocephalinae Schltr. and Oncidiinae Benth. Whitten *et al.* (2000) placed particular emphasis on subtribe Stanhopeinae, and their findings provided the basis for selection of appropriate outgroups.

Supplementary to the molecular analyses, published information on the chromosome number of Lycastinae species was examined. A correlation between chromosome number and orchid phylogeny has been acknowledged by, amongst others, Duncan (1959a), Jones (1966, 1974) and Dressler (1981, 1993). Extensive compilations of chromosome counts of orchids have been published by Duncan (1959b), and Tanaka and Kamemoto (1974, 1984).

Subtribe **Lycastinae**		
Bifrenaria	Chromosome number (2*n*)	Reference
B. harrisoniae (Hook.) Rchb.f.	40	Hoffmann (1929, 1930)
Anguloa		
sect. *Anguloa*		
A. virginalis Linden	40	*
sect. *Guloanga* Schltr.		
A. cliftonii Rolfe	40	*
A. clowesii Lindl.	40	*
Lycaste		
sect. *Fimbriatae* Fowlie		
L. barringtoniae (Smith) Lindl.	44	Aoyama & Karasawa (1988)
L.ciliata (Ruiz & Pav.) Lindl. ex Rchb.f.	44	Aoyama & Karasawa (1988)
L. cinnabarina Lindl.	50	Aoyama & Karasawa (1988)
L. dyeriana Sander ex Rolfe	48	Aoyama & Karasawa (1988)
L. linguella Rchb.f	48	Aoyama & Karasawa (1988)
L. locusta Rchb.f.	48	Aoyama & Karasawa (1988)
sect. *Deciduoseae* Fowlie		
L. aromatica (Graham ex Hook.) Lindl.	40	Aoyama & Karasawa (1988)
L. bradeorum Schltr.	40	Aoyama & Karasawa (1988)
L. brevispatha (Kl.) Lindl.	40	Aoyama & Karasawa (1988)
L. campbellii C.Schweinf.	40	Aoyama & Karasawa (1988)
L. cruenta Lindl.	40	Aoyama & Karasawa (1988)
L. deppei (Lodd.) Lindl.	40	Aoyama & Karasawa (1988)
L. tricolor (Klotzsch) Rchb.f.	40	Aoyama & Karasawa (1988)
sect. *Lycaste*		
L. dowiana Endres & Rchb.f.	40	Aoyama & Karasawa (1988)
L. macrophylla (Poepp. & Endl.) Lindl.	40	Aoyama & Karasawa (1988)
L. virginalis (Scheid.) Linden	40	Aoyama & Karasawa (1988)
Subtribe **Maxillariinae**		
Maxillaria		
M. picta Hook.	40	Blumenschein (1960)
M. tenuifolia Lindl.	40	Tanaka (1966)
Subtribe **Zygopetaliinae**		
Dichaea		
D muricata Sw. Lindl.	52	Woodard (in Duncan, 1959)
Koellensteinia		
K. graminea Rchb.f.	*ca.* 48	Hoffmann (1929)
Zygopetalum		
Z. mackaii Hook.	48	Hoffmann (1930)
Z. maxillare Lodd.	48	Blumenschein (1960)

Table 3.1 Chromosome counts for Lycastinae and related subtribes. *Anguloa* counts (*) were determined as part of this study.

The most common changes to chromosome number are caused by "polyploidy", a multiplication of the basic number due to chromosome replication without cell division. Smaller variations arise through "dysploidy", breakage and fusion of chromosomes without net gene loss or gain. Within a polyploid sequence, lower chromosome numbers are ancestral to higher ones; in a dysploid sequence, the numbers can run in either direction (Liklas, 1997).

Jones (1966) examined the variation in chromosome number within *Polystachya* Hook. (Polystachyinae). He found that there was no variation in the basic number (n = 20), and most species were diploid ($2n$ = 40). However, species of sect. *Affines* Kraenzl. were exclusively either tetraploid or hexaploid, and within sect. *Polystachya*, African species were exclusively diploid, American species exclusively tetraploid.

Within subtribe Catasetinae, Jones and Daker (1968) found almost all chromosome counts of *Catasetum* Kunth (which then included *Clowesia* Lindl. and *Dressleria* Dodson) and *Mormodes* Lindl. to be multiples of 54, or in a few cases, of 56. *Cycnoches* Lindl. showed a variation of base number: either $2n$ = 64 or $2n$ = 68.

Chase (1986b) has suggested that although chromosome number by itself is not a reliable indicator of taxonomic relationships, its pattern of distribution is not random. He observed two parallel trends among Oncidiinae genera which led to a reduction in chromosome number: the first, increased vegetative specialisation, the second increasing floral modification. He suggested that among these orchids, evolution has resulted in chromosome reduction from a primitive state of $2n$ = 60.

Aoyama and Karosawa (1988) undertook a karyomorphological study of 16 *Lycaste* species. They reported chromosome numbers of $2n$ = 40 for species from *Lycaste* sect. *Deciduosae* and sect. *Lycaste*, and $2n$ = 44, 48 or 50 within sect. *Fimbriatae*, as

shown in Table 3.1. To clarify intergeneric relationships, chromosome counts for three *Anguloa* species were determined by Margaret Johnson (RBG Kew) as part of the work presented here.

3.2 MATERIALS AND METHODS

Root tips were provided by Dr. Henry Oakeley. Leaf material was provided by Dr. Oakeley, RBG Kew, and the Herbario de la Asociación Mexicana de Orquideología. Vouchers and their locations are listed in Appendix 2. Six ITS and nine *matK* sequences were provided by Mark Whitten (Florida Museum of Natural History). These are marked with an asterisk in Appendices 2 and 4.

3.2.1 CYTOLOGY

Growing root-tips, (0.5cm long), were pretreated by slicing longitudinally and soaking in 8-hydroxyquinoline (0.002M, 4.5hr, 18°C). The material was fixed in freshly prepared absolute ethanol: acetic acid (3:1) and stored at 4°C until required. Hydrolysis, staining, and slide preparation followed standard cytological procedures as outlined in Johnson and Özhatay (1988). Photographs were taken on a Zeiss Photomicroscope III using PAN F film. Slides were made permanent by freezing with liquid CO_2 (Bowen, 1956) and are retained in the Cytogenetics Section of the Jodrell Laboratory, RBG Kew.

3.2.2 EXTRACTION OF DNA

The Mexican material was dried according to the method of Chase and Hills (1991). Leaves were sliced into pieces (0.5cm x 1cm), and placed in sealed polythene bags with silica gel (Sigma Aldrich 28-200 mesh, 22Å pore diameter). A small quantity of self-indicating silica gel was added to each bag to guard against water saturation.

Total DNA was extracted using a modified version of the 2xCTAB (hexadecyltrimethylammonium bromide) method of Doyle and Doyle (1987). Leaf material (1g fresh, 0.1-0.3g dried) was ground in a preheated pestle and mortar with 10ml warm (65°C) isolation buffer (100mM Tris-HCl pH8, 1.4M NaCl, 20mM EDTA, 2% CTAB) to which β-mercaptoethanol (40µl) had been added. The slurries were left to digest (65°C, 20min) before extraction with chloroform:isoamyl alcohol (24:1, 10ml, Luckham Rotatest shaker, 30min). The organic layer and plant debris were discarded. DNA was precipitated from the aqueous layer by adding cold absolute ethanol (-20°C). The DNA was spun into a pellet (3000rpm, 3min), washed with 70% ethanol (5ml) and allowed to dry. Caesium chloride solution (3 ml, 1.55g ml^{-1}) containing ethidium bromide was added and the pellets were left in the dark to dissolve. Typically this took 24 to 48hours.

DNA was separated from RNA and other residual material by gradient centrifugation through the CsCl - ethidium bromide (58000rpm, 5hr) using a Beckman XL-80 ultracentrifuge. The DNA band was observed under UV light and removed by pasteur pipette. Ethidium bromide was removed by extraction into an equal volume of butan-1-ol. The DNA solutions were transferred to dialysis tubing for the final stages of purification. The tubes were left in sucrose for up to two hours to remove some of the excess water. Caesium chloride and other remaining salts were removed by dialysis with water (5hr) and dialysis buffer (pH8, 19hr). Dialysis buffer contained 10mM TRIZMA® base (Sigma Aldrich) and 1mM EDTA, adjusted to pH8 with hydrochloric acid.

The quality and relative concentration of each DNA template was checked using agarose gel electrophoresis. Templates (5µl) were mixed with 3µl loading dye (0.025% bromophenol blue, 40% sucrose) prior to loading onto the gel. The gels, which contained ethidium bromide, were run at 70V for 15 minutes in TBE buffer (89mM

TRIZMA® base, 89mM boric acid, 2mM EDTA). DNA was detected by UV translumination. DNA templates were stored at –20°C while waiting for amplification. Long term storage was at –80°C.

3.2.3 PCR

The ITS region was amplified using the methods and primers (ITS4 and ITS5) described by Baldwin (1992). For the purposes of this study, ITS includes the 5.8S gene as well as the two non-coding spacer regions ITS1 and ITS2, see Figure 1.7. The *matK* amplification and sequencing primers were developed at RBG Kew, for use with Epidendroideae (Orchidaceae). Two *matK* amplification products were prepared for each taxon: the first using the –19F and 556R primers, the second 458F and either 1592R or *trnK*2R. All primer sequences are presented in Table 3.2.

	Primer	Sequence
ITS	ITS4	TCC TCC GCT TAT TGA TAT GC
	ITS5	GGA AGT AAA AGT CGT AAC AAG G
matK	-19F	CGT TCT CAT ATT GCA CTA TG
	163F	AGT TTA GTR CTT GTG AAA CG
	458F	CTA CTA ATA CCC YAT CCC ATC
	556R	GAA GRA ACA TCT TTK ATC CA
	1155F	TTC ACT TTT GGT YTC ACC CT
	1592R	TCA TGA ATG ATC CAC CGA
	*trnK*2R*	AAC TAG TCG GAT GGAATA G

Table 3.2 ITS and *matK* primer sequences (* From Johnson and Soltis, 1995).

Reaction mixes (100µl) were prepared in accordance with the basic principles of Saiki *et al.* (1988), and typically contained DNA template (1-2 µl), magnesium chloride (1.5-2.5mM), dNTPs (200µM each), primers (1ng μl^{-1} each), and Taq polymerase (0.75units, Promega). Bovine serum albumin (0.4%, 1µl) was used to facilitate

amplification of degraded DNA. A Cetus 480 Thermal Cycler (PE Applied Biosystems) was used for all reactions; the conditions are listed in Table 3.3.

	ITS		*matK*	
	Temp (°C)	Time (min)	Temp (°C)	Time (min)
Premelt	97	1	97	1
Denature	97	1	94	1
Anneal	48	1	52	0.75
Extension	72	3	72	2.5
Segment time increase	none		8sec/cycle	
Number cycles	40		30	
Final extension	72	6	72	7
Soak	4		4	

Table 3.3 PCR cycle sequence conditions for ITS and *matK*.

Amplified double stranded DNA products were purified by solid phase extraction through Wizard® PCR minicolumns (Promega) using the manufacturer's protocols. Final elution was with water (35-50µl). Cleaned PCR products were stored at –20°C.

3.2.4 SEQUENCING

The PE Applied Biosystems dyeDeoxy terminator mix was used for all sequencing reactions. This system uses ddNTP terminators that have fluorescent dyes incorporated into them. Four different dyes, each emitting light at a different wavelength are used, one for each nucleotide.

The reactions (5µl) were carried out in a Cetus Thermal Cycler, using 35 cycles at the following temperatures:

Denaturing	94°C	30 seconds
Annealing	50°C	15 seconds
Chain extension	60°C	4 minutes

The tubes were left to soak at 4°C until they could be cleaned.

Sequencing products were separated from unextended primers, dNTPs and ddNTPs by precipitation. Sufficient water was added to the sequencing tubes to increase the volume to 20µl and the contents transferred to a mixture of absolute ethanol (50µl) and 3M sodium acetate pH 4.6 (2µl). The mixtures were vortexed, allowed to stand at room temperature (5min) and then placed on ice (15-20min). Precipitated products were spun into a pellet by centrifugation (13000rpm, 25min). Supernatent liquid was drained and the pellet washed twice with 70% ethanol; after each wash, the pellet was reformed by centrifugation (13000rpm, 15min) and the ethanol decanted. Final drying was either in a vacuum oven (80°C, 30min) or under ambient conditions (12-24hr). Cleaned products were stored in the dark at room temperature.

Sequences were determined using either a 373 or 377 DNA Sequencing System (PE Applied Biosystems). The DNA fragments were separated by polyacrylamide gel electrophoresis. The identity and order of the terminator bases was determined by scanning the end of the gel with a laser (typically 600scans hr^{-1}). Laser excitation caused the terminator dyes to fluoresce. Detection was by a photomultiplier. Sequence Navigator software (PE Applied Biosystems Inc.) was used to edit the sequences, and the complementary strands were re-assembled using Autoassembler (PE Applied Biosystems Inc.). Each base position was examined to determine that complementary strands agreed.

3.2.5 DATA ANALYSIS

Sequences were aligned using Clustal W (Thompson *et al.*, 1994) and by eye; the aligned matrices are presented in Appendix 4. Four genera from the Zygopetalinae (*Batemannia* Lindl., *Dichaea* Schltr., *Koellensteinia* Rchb.f. and *Zygopetalum* Hook.) were specified as the outgroup, based on the results of Whitten *et al.* (2000). Heuristic searches and successive approximations weighting were performed using the method and programmes described in Section 2.2. Gaps in the matrix were again coded as missing values. Internal support was assessed from 1000 bootstrap replicates (Felsenstein, 1985) with simple stepwise addition, the nearest-neighbor interchanges (NNI) algorithm and the successive weightings applied.

3.3 RESULTS

Some of the results presented here have already been published (Ryan *et al.*, 2000). A copy of the paper can be found inside the back cover of this thesis.

3.3.1 CYTOLOGY

Chromosome counts of $2n = 40$ were obtained for three species of *Anguloa*: *A. cliftonii* and *A. clowesii* from sect. *Guloanga*, and *A. virginalis*, from sect. *Anguloa*. These agree with the counts for *Lycaste* sect. *Deciduosae* and sect. *Lycaste* (Aoyama and Karasawa, 1988) as shown in Table 3.1. Chromosome numbers in *Lycaste* sect. *Fimbriatae* range from $2n = 44$ to 50 (Table 3.1).

3.3.2 ANALYSIS OF THE ITS REGION

Sequences were obtained from 46 taxa. The length of the aligned matrix was 660 base pairs, of which 312 were variable and 123 potentially parsimony informative. Cladistic analysis yielded more than 6000 equally parsimonious trees (the maximum permitted by available computer memory) with a Fitch length of 515 steps, consistency index (CI)

of 0.75, including autapomorphies and retention index (RI) of 0.77. Successive weighting again yielded 6000 trees, of weighted length 303536 (Fitch length 515) and both CI and RI of 0.94. One of the weighted trees is shown in Figure 3.1. Numbers above the branches indicate the number of nucleotide substitutions, those in boldface show percent support from bootstrap replicates. Arrows indicate branches that collapse in the strict consensus of all SW trees.

The tree shows that *Lycaste*, as currently circumscribed, is not monophyletic. Species from sects. *Deciduosae*, *Lycaste* and *Longisepalae* have been placed in a single well-supported clade. Sister to that, again with strong support, are the species of *Anguloa* and *L.* sect. *Fimbriatae*, including *L. dyeriana*. Within this clade the monophyly of *Anguloa* is well supported; that of sect. *Fimbriatae* is not. *Neomoorea* is strongly supported as the sister group to *Lycaste* and *Anguloa*.

ITS sequences were obtained from three of the five genera that Dressler (1993) grouped as the *Bifrenaria* alliance (Figure 2.1). Of these, *Xylobium pallidiflorum* (Hook.) Nichols was placed in a moderately supported clade with genera of Maxillariinae, *Maxillaria* and *Cryptocentrum* Benth. The other two, *Bifrenaria harrisoniae* and *Rudolfiella aurantiaca*, were placed together in a separate clade sister to Maxillariinae. Zygopetalinae were used as an outgroup, as shown in Whitten *et al.* (2000).

3.3.3 ANALYSIS OF *matK*

Sequences were obtained from 22 taxa to compare with the general patterns revealed by ITS. The aligned matrix, Appendix 4, contained two indels, three and nine bases long, which were coded as additional characters. There was less variation between sequences than had been found for ITS. Of the 1578 characters included in the analysis, only 160 were variable; 60 of these were potentially parsimony informative.

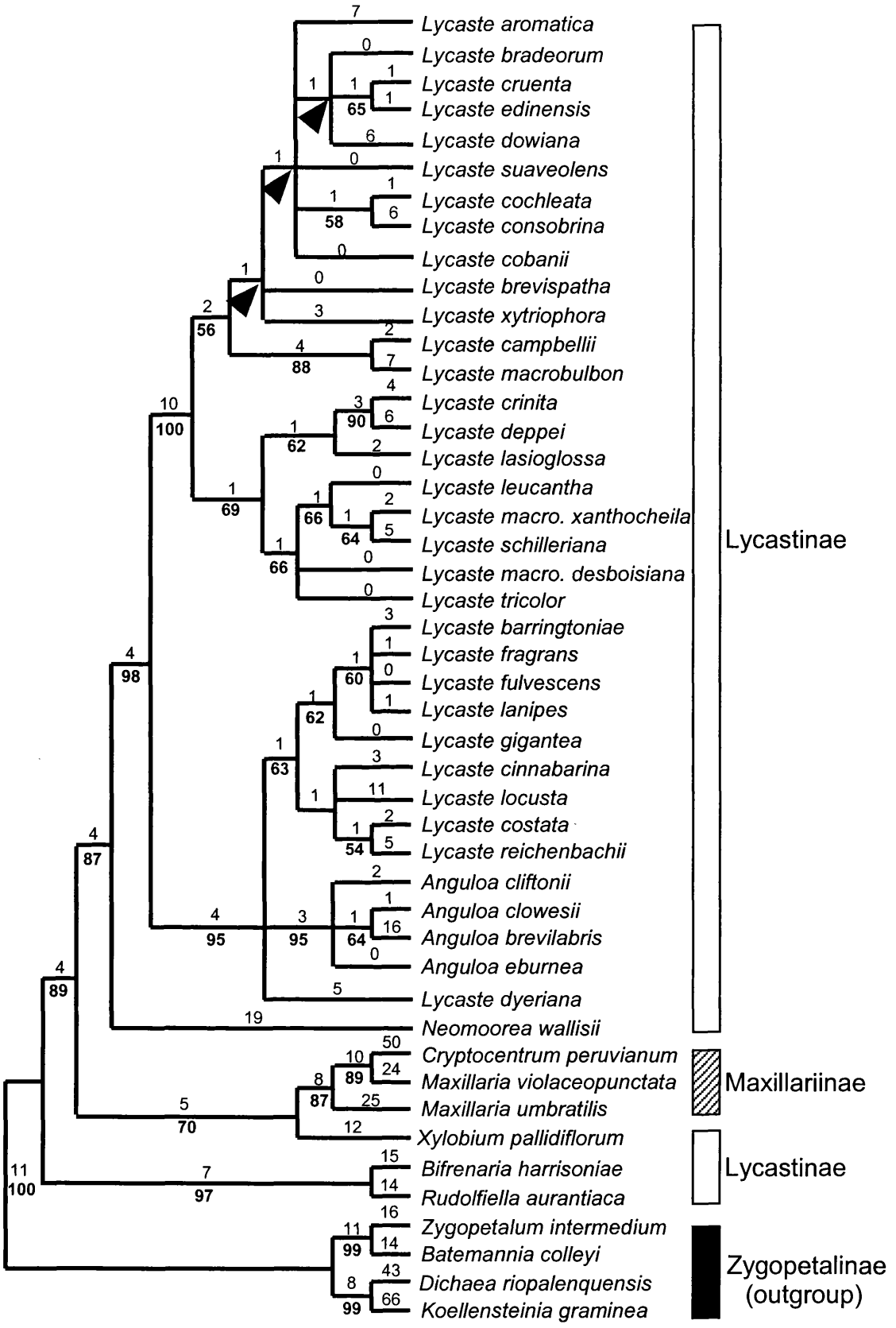


Figure 3.1 One of more than 6000 successively weighted most parsimonious trees showing cladistic relationships within Lycastinae based on ITS sequence data.

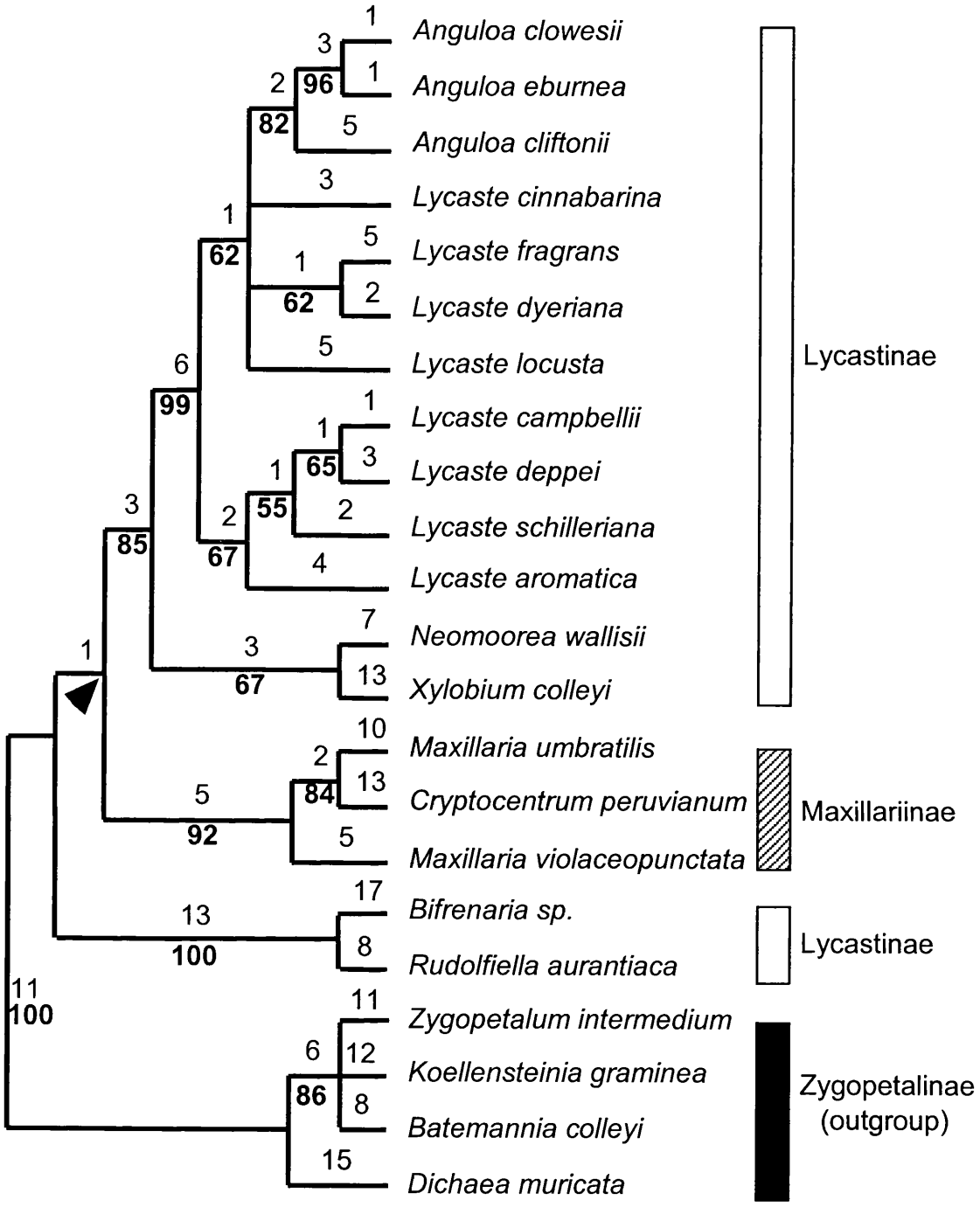


Figure 3.2 One of the nine successively weighted most parsimonious trees showing relationships within Lycastinae based on *matK* sequence data.

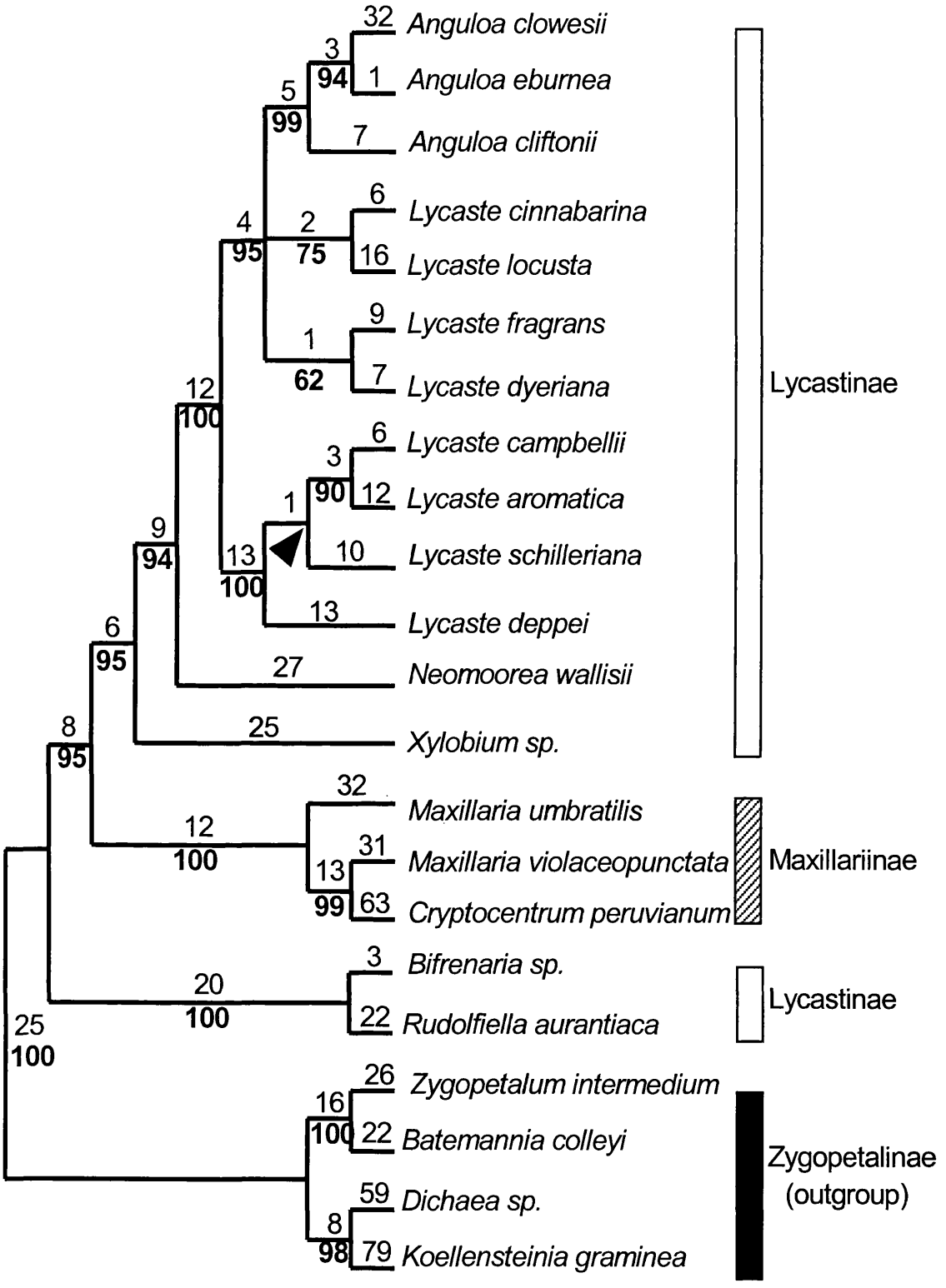


Figure 3.3 One of the six successively weighted most parsimonious trees showing relationships within Lycastinae based on ITS/*matK* sequence data.

The search yielded 108 equally parsimonious trees with a Fitch length of 212 steps, CI of 0.80 (including autapomorphies) and RI of 0.74. Successive weighting yielded nine trees of length 145579 (Fitch length 212), CI 0.95, and RI 0.93.

One of the reweighted trees is shown in Figure 3.2. *Lycaste* is again shown to be paraphyletic, with species from sect. *Fimbriatae* placed in the same clade as *Anguloa*. Support for this topology is much lower than was found from the ITS analysis, not surprising given the shorter branch lengths. One difference between the two sets of results is the position of *Xylobium*, which *matK* places in the same clade as *Neomoorea*; bootstrap support for this clade is weak. The position of *Bifrenaria* and *Rudolfiella* is the same as was found for ITS.

3.3.4 COMBINED SEQUENCE ANALYSIS

The same 22 taxa as in the *matK* analysis were used here. In three instances, outside *Lycaste/Anguloa*, the ITS sequence data which has been incorporated was obtained from a different species. These are labelled on Figure 3.3 as *Bifrenaria sp.*, *Dichaea sp.* and *Xylobium sp.*

In the combined analysis 2238 characters were included, of which 448 were variable and 167 potentially parsimony informative. The Fitch search found 60 equally parsimonious trees with a length of 669 steps, CI of 0.77 (including autapomorphies) and RI of 0.67. Successive weighting yielded six trees, with length of 415059 (Fitch length 669), CI of 0.95 and RI of 0.92. The topology of the reweighted tree shown in Figure 3.3 is in close agreement with that found by analysing ITS sequence data alone and shows higher levels of support. The single exception is *Xylobium*, which is separate (with a high bootstrap percentage) from both the genera of the Maxillariinae and from *Neomoorea*.

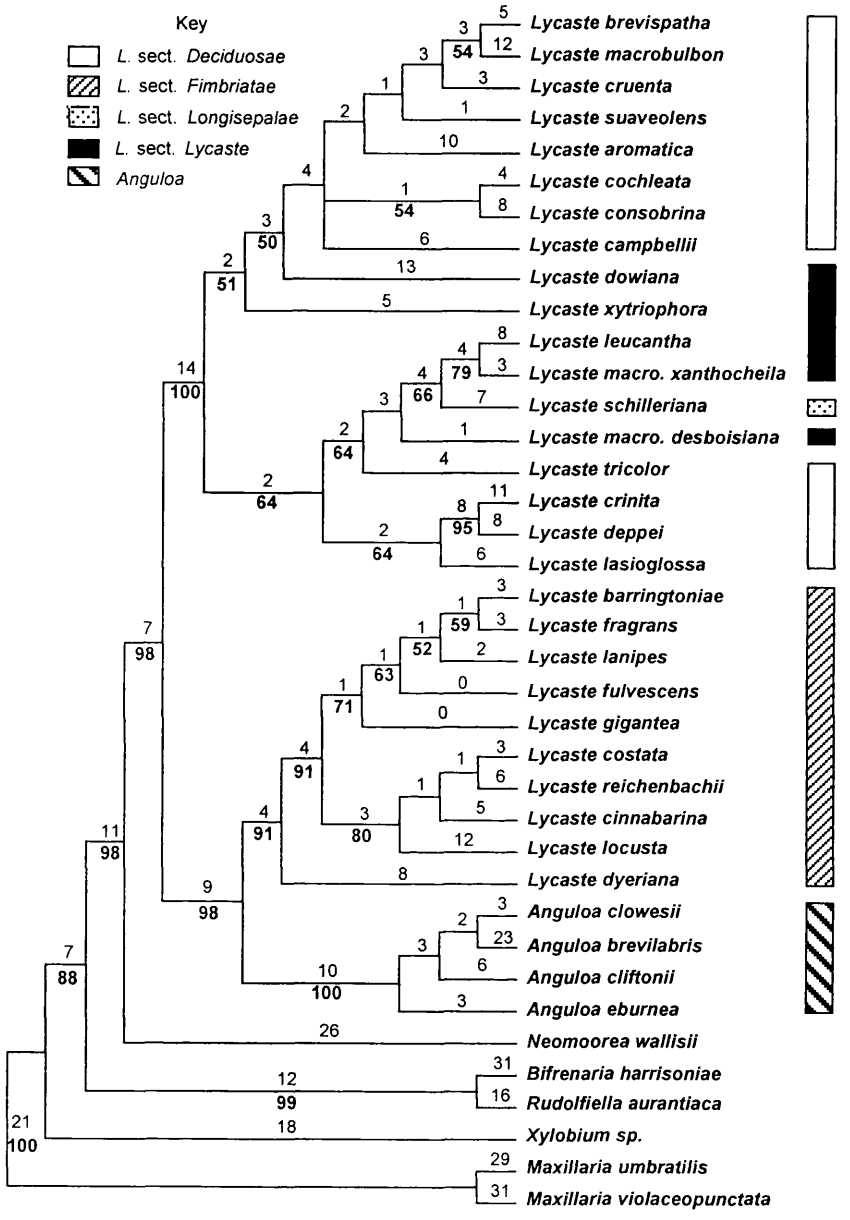


Figure 3.4 One of the three successively weighted most parsimonious trees showing cladistic relationships within subtribe Lycastinae, based on 47 morphological characters and ITS sequence data. Numbers above the branches are the estimated number of substitutions, numbers below are bootstrap percentages greater than 50%.

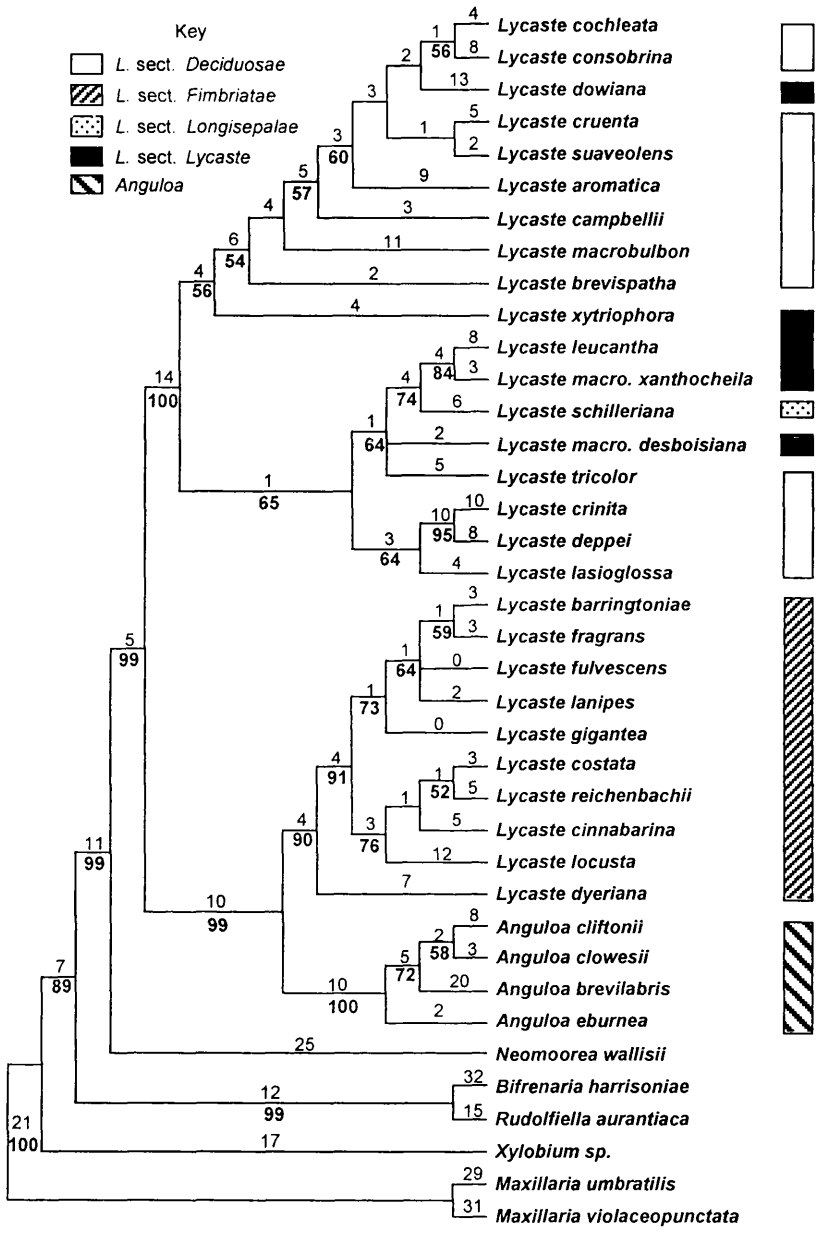


Figure 3.5 One of the two successively weighted most parsimonious trees showing cladistic relationships within subtribe Lycastinae, based on 46 morphological characters and ITS sequence data. Numbers above branches are the estimated number of substitutions; numbers below are bootstrap percentages greater than 50%.

3.3.5 COMBINED SEQUENCE AND MORPHOLOGICAL ANALYSIS

There has been some debate concerning the treatment of heterogeneous data, such as that from morphological and molecular analyses. Should the data sets be treated separately, always combined or combined only in certain circumstances?

In a recent study of phylogenetic relationships of subtribe Pogoniinae, Cameron and Chase (1999) used individual and combined analyses of *rbcL*, ITS and morphological data to demonstrate that the genus *Cleistes* L.C.Rich. is paraphyletic and that the infra- and inter-generic relationships reflect the geographical distribution of the species. The results obtained from each of the analyses were very similar.

Both ITS and morphological data were available for 36 species of Lycastinae. Combining the datasets provided 739 characters for cladistic analysis. Of these 458 were constant and 116 potentially parsimony informative. The two species of *Maxillaria*, were selected as the outgroup.

The analysis yielded 48 equally parsimonious trees with a length of 400 steps, CI of 0.61 and RI of 0.74. Successive weighting found three trees with a length of 238901 steps (Fitch length of 500 steps), CI of 0.89 and RI of 0.92. One of these is shown in Figure 3.4.

The analysis was repeated with morphological character 28 excluded (see sections 2.3.1 and 2.3.2). 150 equally parsimonious trees were found with a length of 488 steps, CI of 0.62 and RI of 0.75. Successive weighting found two trees of length 237260 steps (Fitch length 494), CI of 0.90 and RI of 0.93. One of these is shown in Figure 3.5. Both the topology and bootstrap values are highly congruent with that of Figure 3.4.

The two trees have similar topology to that obtained from ITS data alone, placing *Lycaste* sect. *Fimbriatae* and *L. dyeriana* closer to *Anguloa* than to the other species of *Lycaste*. They show stronger support for the monophyly of sect. *Fimbriatae* than was obtained from the three sequence analyses and place *L. dyeriana* closer to sect. *Fimbriatae* than to *Anguloa*. *Xylobium* is again separated from both *Bifrenaria* and *Rudolfiella.*

3.4 DISCUSSION

These analyses have shown that neither *Lycaste* nor subtribe Lycastinae, as defined by Dressler (1993), are monophyletic. All provide strong support for the separation of *Lycaste* sect. *Fimbriatae* from the rest of the genus. Further support is provided by the variation in chromosome number from the base number n = 20, found in other members of Lycastinae.

There are three possible taxonomic solutions. The first would be to include all *Lycaste* and *Anguloa* species within a single genus comprising three sections. According to the rule of priority, this would be called *Anguloa.* A second possibility would be to transfer only *L.* sect. *Fimbriatae* to *Anguloa.* Given the differences in floral morphology, pollination mechanism and chromosome number that exist between the three clades, neither of these options is completely satisfactory.

A third possibility would be to create a new genus for *L.* sect. *Fimbriatae.* This may be the most practical short-term solution as it would solve the morphological and cytological discrepancies, and also keep the name *Lycaste*, which is in common horticultural use. Given the low levels of bootstrap support, the monophyly of such a genus could not be guaranteed on the basis of sequence data alone. Nevertheless it was not contradicted by either the individual or the combined ITS/*matK* analyses. Strong support for this solution was obtained from the combined ITS/morphological

analysis. It is also the most compatible with previous taxonomic schemes as it would require the least number of nomenclatural transfers.

The position of *Lycaste dyeriana* remains ambiguous. All the analyses placed it in the same clade as *Anguloa* and *L.* sect. *Fimbriatae*, rather than with *Bifrenaria,* as had been suggested by Fowlie (1970). Its position within the clade has not been fully resolved. Given the similarity in both floral morphology and chromosome number to other members of *L.* sect. *Fimbriatae*, at the present time it seems logical to keep it within that section.

None of the analyses provided sufficient resolution to accept or refute Fowlie's concept of the other three sections. To answer these questions, comparison of sequences from a faster evolving region of DNA, or inclusion of sequence data from other DNA regions is required.

Fowlie (1964, 1970) treated some taxa within *Lycaste* as subspecies of *L. macrophylla.* Two of these, *L. macrophylla* subsp. *desboisiana* and *L. macrophylla* subsp. *xanthocheila*, both from Costa Rica, were included in both the ITS and combined ITS/morphology analyses. None of the trees (Figures 3.1, 3.4 and 3.5) places them adjacent to each other, indicating that their taxonomic status needs to be reviewed.

Within subtribe Lycastinae, Dressler's (1993) view of *Neomoorea* as nearest neighbour to *Anguloa* and *Lycaste* is supported. Unlike other members of Lycastinae, these three genera all have a single long stipe.

The position of *Xylobium* was ambiguous: ITS placed it with Maxillariinae, *matK* with *Neomoorea* and the combined analyses in a clade by itself. The topology of the combined ITS/*matK* analysis was well supported, the others were not.

All three sequence analyses identified Maxillariinae as being closer to *Lycaste*, *Anguloa*, *Neomoorea* and *Xylobium* than to the two Lycastinae genera, *Bifrenaria* and *Rudolfiella*. Dressler (1981) had noted the floral resemblance between *Xylobium* and *Maxillaria*, and thought it was a convergence caused by their pollination syndromes. Whether this issue is best resolved by reinstating subtribe Bifrenariinae Dressler (1979) without *Xylobium* or by expanding Maxillariinae is the subject of a more comprehensive study by Whitten *et al.* (2000). Their results, together with those presented here, indicate that a broader circumscription of Maxillariinae would be a more reasonable solution to the polyphyly of Lycastinae.

Chapter 4 OSMOPHORES

In many flowers, fragrance emission is not diffuse but is restricted to discrete areas of glandular tissue, termed "osmophores". Although detailed anatomical study of such structures was outside the scope of this thesis, some preliminary investigations were undertaken in an attempt to answer the following questions:

- Do *Lycaste* and *Anguloa* species have osmophores?
- Where are they located?
- Are they morphologically similar to each other?

4.1 INTRODUCTION

The name osmophore was first used by Arcangeli (1883) to describe the "fragrant" spadix of *Dracunculus vulgaris* Schott (Araceae) and was subsequently adopted by Vogel (1962) for his seminal work on the role of scent glands in pollination. In defining the term, Vogel (1966) specified certain criteria. The tissue should be glandular, multicellular, differentiated and well exposed to the atmosphere. During a relatively short period it produces strong or faint smells that are highly specific attractants; production of these odours is characterised by fast consumption of large quantities of reserve carbohydrates.

Most early reports of localised fragrance emission were based on olfactory observations alone. Among the Orchidaceae, for example, Pohl (1927) found the source of fragrance of *Stanhopea* flowers to be the hypochile of the lip, whereas Vogel (1954) identified the petals of *Habenaria polyphylla* Kraenzl. and the lip of *Herschelia rugens* (H.Bolus) Kraenzl. (as *Disa rugens* H.Bolus) as the sources of their scent. A rare example of chemical analysis was provided by Porsch (1908), who used phloroglucinol/hydrochloric acid to confirm the presence of vanillin in the epithelial cells of the margin and lower surface of the lip of *Maxillaria rufescens* Lindl.

Anatomical studies by Germ (1947, 1954) showed the fragrance-emitting areas of *Platanthera bifolia* (L.) L.C.Rich., *Gymnadenia conopsea* (L.) R.Br. and *G. nigra* (L.) Rchb. (as *Nigritella nigra*) to be characterised by distinct areas of glandular epithelium. Subsequent studies such as those by Vogel (1962), Pridgeon and Stern (1983) and Curry *et al.* (1988) have shown that these regions encompass epidermal and sub-epidermal cells and may extend into the parenchyma. In general, the cells are thin-walled, with large nuclei, dense cytoplasm and abundant amyloplasts with numerous starch grains.

The role of the starch is unknown. Based on his own observations, Vogel (1962) speculated that the carbohydrates serve as basic structural material as well as providing an energy source, such as for reduction and ring formation of carbon atoms during the synthesis of unsaturated terpenes. However, Stern *et al.* (1987) have monitored the development of osmophore tissues in *Stanhopea wardii* Lodd. ex Lindl. and *S. oculata* (Lodd.) Lindl. and found no difference in the number of starch grains at bud and anthesis stages. They concluded that the energy required for fragrance synthesis was provided by sugars from the phloem.

Little is known of where the fragrance chemicals are synthesized or their mode of transport to the surface. The smooth endoplasmic reticulum (SER) is associated with terpenoid biosynthesis (Brooker and Russell, 1975). Pridgeon and Stern (1983) and Stern *et al.* (1987) have suggested that it may also be involved in the transport process. Curry (1987) identified carnetine acetyl transferase in the SER and between the inner and outer mitochondrial membranes of *Stanhopea anfracta* Rolfe. He speculated that this enzyme might be directly linked to fragrance production.

In most cases, the fragrance evaporates from the cuticle, but there are exceptions. For example, Vogel (1962) has observed oil droplets on the surface of the osmophore of

both *Catasetum* and *Stanhopea* species. Williams *et al.* (1985) have detected crystals of (E)-methyl 4-methoxycinnamate on the lip and lateral sepal bases of *Gongora quinquinervis* Ruiz & Pav. These crystals are only present in the morning when fragrance production is strongest.

Among Orchidaceae, both the morphology of osmophores and their location show considerable variation at the level of genus and species (e.g. Vogel, 1962; Curry *et al.*, 1991), and it has been suggested by Pridgeon and Stern (1985) that this variation contributes to pollinator specificity.

Pridgeon and Stern (1983, 1985) examined the ultrastructure of the glands in the fly-pollinated genera *Restrepia* H.B.K. and *Scaphosepalum* Pfitz. Within *Restrepia*, the glands are papillose with cuticular pores, and are located on the petal apices and on the adaxial surface of the dorsal sepal. In *Scaphosepalum*, osmophores occur on the adaxial surface of the lateral sepal apices, and/or the dorsal sepal apex depending on the species. Like *Restrepia*, they are papillose but, with the exception of *S. microdactylum*, lack pores.

Vogel (1962) surmised that the osmophores on the sepals of *Restrepia* and *Scaphosepalum* serve as long-distance attractors, and that a secondary mechanism operates closer to the pollination mechanism. He also suggested that whereas there is an ultrastructural distinction between the adaxial and abaxial perianth surfaces of these genera, no such distinction exists among moth-pollinated flowers, in which the entire flower stains and pollinators are attracted over long distances.

Restrepia flowers are highly odiferous and only last for a few days; those of *Scaphosepalum* have a weaker odour and anthesis is more prolonged. Pridgeon and Stern (1983, 1985) have shown that in both genera, secretory products accumulate

between the plasma membrane and the cell wall. They suggested that in *Restrepia*, the fast build-up of these products exerts sufficient pressure on the cuticle to rupture it, hence the pores. In *Scaphosepalum*, the rate of accumulation is much slower and the cuticle remains intact.

Curry *et al.* (1991) studied the development of osmophores in several species of *Stanhopea* and *Sievekingia*, from late bud stage through to postanthesis. Within *Stanhopea*, the structures are contained in a pouch at the proximal end of the labellum (Stern *et al.*, 1987; Curry *et al.*, 1988). Those of *Sievekingia* are located in a similar position, but the flowers lack a sharply defined pouch. Within the two genera, they identified three different forms of osmophore.

In his interpretation of the term "osmophore", Vogel (1966) stated that the tissue was morphologically distinct, and this is certainly true for some genera of orchids, such as *Restrepia* (Pridgeon and Stern, 1983), *Scaphosepalum* (Pridgeon and Stern, 1985) and some species of *Stanhopea* (Curry *et al.*, 1991). However Stern *et al.* (1986) observed that there is little or no modification of tissue to identify the sites among species of *Gongora* Ruiz & Pav., *Trichopilia* Lindl. and *Xylobium*, and that the use of a dye to indicate areas of fragrance production is a necessary preliminary step.

Vogel (1962) found neutral red to be an effective and specific stain for osmophore tissue. He based this assertion on odour studies of dissected stained flowers, observing that only stained portions were fragrant. Although Stern *et al.* (1986) subsequently found Sudan black B to be more effective for wax-embedded material, neutral red remains the popular choice for initial screening and was used for the work presented here.

The mechanism by which both dyes operate is unknown. Stern *et al.* (1986) suggested that they are taken up by lipid secretions, and that as the chromogens of both contain amine groups, they form hydrogen bonds with acidic lipid groups such as hydroxyls.

4.2 MATERIALS AND METHODS

Plant material was provided by Henry Oakeley. Voucher numbers and their locations are listed in Appendix 2. Three species of *Lycaste* and one species of *Anguloa* were selected for this work:

L. cochleata (sect. *Deciduosae*)

L. trifoliata (sect. *Fimbriatae*)

L. macrophylla subsp. *xanthocheila* (sect. *Lycaste*)

A. virginalis

Freshly cut flowers were immersed in aqueous neutral red (0.1%w/v) for 30 to 40 minutes. The flowers were washed with distilled water and examined for permanent staining.

Fresh flowers of each species were used for the SEM. Those areas where staining had indicated the presence of osmophores were cut into strips (10mm x 2-3mm) and the cell structure fixed in Karnovsky's solution (24 hr), post-fixed with osmium tetroxide (1%w/v in 0.05M phosphate buffer) and dehydrated through an ethanol gradient. Karnovsky's solution contains paraformaldehyde (2%w/v) and glutaraldehyde (2.5%w/v) in phosphate buffer (0.05M). The full procedure, shown in Table 4.1, was a modification of that used by Curry *et al.* (1991).

Specimens were transferred to 100% acetone and dried to critical point using a Balzers 030 critical point drier, prior to platinum coating with an EMScope SC500A sputter coater. Coated specimens were examined by SEM (Cambridge Instruments

Stereoscan 240) using an accelerating voltage of 18kV and working distance of 10-11mm. Scanning electron micrographs were recorded on Kodak Plus-X film.

Step no.		Time	Temp.
1	Karnovsky's fixative	24 hr	25°C
2	0.1M phosphate buffer wash	5 min	25°C
3	0.1M phosphate buffer wash	5 min	25°C
4	1%w/v osmium tetroxide in 0.05M phosphate buffer	2 hr	25°C
5	0.1M phosphate buffer wash	10 min	25°C
6	0.1M phosphate buffer wash	10 min	25°C
7	0.1M phosphate buffer wash	10 min	25°C
8	0.1M phosphate buffer wash	30 min	25°C
9	30%v/v ethanol	5 min	25°C
10	50%v/v ethanol	10 min	25°C
11	70%v/v ethanol	36 hr	25°C
12	90%v/v ethanol	30 min	25°C
13	100%v/v ethanol	30 min	25°C
14	100%v/v ethanol	30 min	25°C
15	100%v/v ethanol	1 hr	25°C

Table 4.1 Fixation and dehydration protocol for SEM examination of osmophores.

4.3 RESULTS

4.3.1 STAINING WITH NEUTRAL RED

Areas of all four taxa were stained by neutral red, indicating the presence of secretory tissue. In each case the pattern of staining was different.

Staining of *Lycaste cochleata* was restricted to the petal margins, particularly near the apex, the abaxial surface of the column foot and discrete areas of the lip: The back of the lip, the callus margins, the side-lobes, the isthmus and the proximal area of the mid-lobe, as shown in Plate 4.1A.

Plate 4.1

The use of neutral red solution for locating sites of secretory tissue of *Lycaste* flowers.

A. *Lycaste cochleata*: Comparison of unstained (LHS) and stained flowers.

B. *L. trifoliata*: Whole flower after immersion in neutral red solution.

C. *L. trifoliata*: Dissected flower after immersion in neutral red solution.

D. *L. trifoliata*: Lip mid-lobe and callus apex after immersion in neutral red solution.


A


B


C


D

Plate 4.2

The use of neutral red solution for locating sites of secretory tissue in *Lycaste macrophylla* subsp. *xanthocheila*.

A. Side by side comparison of unstained (LHS) and stained flowers.

B. Side by side comparison of stained (LHS) and unstained flowers; the lip has been excised to show the column.

C. Comparison of stained (top) and unstained lips.


A


B


C

Plate 4.3

The use of neutral red solution for locating sites of secretory tissue in *Anguloa virginalis*.

A. Complete flower after immersion in neutral red solution.

B. Flower after immersion in neutral red solution, with lateral sepal and petal removed .

C. Lip after immersion in neutral red solution.


A


B


C

For *Lycaste trifoliata,* sites of secretory tissue were tentatively identified as the entire inner surface of the petals, the back of the lip, the side-lobe margins and most of the mid-lobe, especially a ridge immediately adjacent to the fimbriate margin (Plate 4.1B,C,D). The fimbriations themselves did not absorb neutral red and there was little evidence of osmophore tissue on either the callus or the column.

The pattern of staining on *Lycaste macrophylla* subsp. *xanthocheila* was more extensive than that of the other three taxa, encompassing the entire upper surface of the lip and callus, the distal region of the petals and much of the column (Plate 4.2).

The presence of secretory tissue was indicated on both surfaces of the lip of *Anguloa virginalis* (Plate 4.3). The other perianth parts were unaffected. On the inner surface of the lip, intensive staining was observed on the undersurface of the callus, the distal portion of mid-lobe underneath it, and the central portion of the lip to either side of the callus. The callus of *A. virginalis* has a central ridge running from the mid-lobe to the column foot. Although the ridge itself did not absorb dye, the "valleys" on either side did. On the outer lip surface, the only areas which did not absorb dye were the apices of the side-lobes, the mid-lobe and a small area immediately adjacent to the column foot.

4.3.2 SEM

A SEM photograph of the column base of *Lycaste cochleata* (Plate 4.4A) shows the osmophores to be conical with flat to rounded apices. Similarly shaped structures were also found on the side-lobes and mid-lobe of the lip where secretory tissue had been identified.

Fragrance glands from the mid-lobe of the lip of *L. trifoliata* were morphologically similar to those of *L. cochleata* (Plate 4.4B), with a broader base.

Plate 4.4

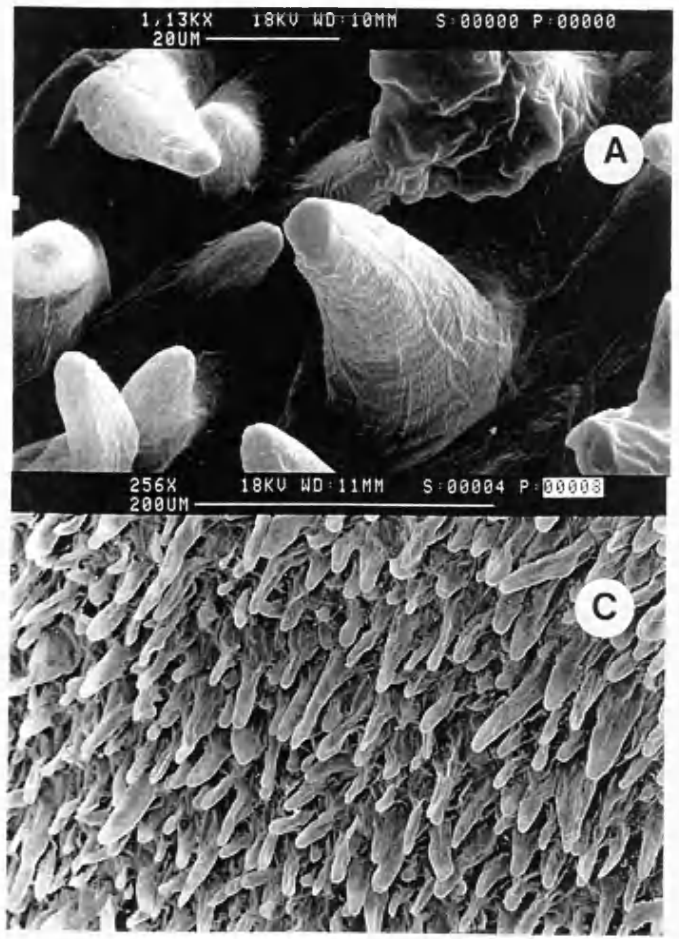
SEM photographs of the osmophores of three species of *Lycaste*.

A. *Lycaste cochleata*: column base.

B. *L. trifoliata*: Lip mid-lobe.

C. *L. macrophylla* subsp. *xanthocheila*: Central portion of the lip.

D. *L. macrophylla* subsp. *xanthocheila*: The region of lip adjacent to the callus.


1,13KX 18KV WD:10MM S:00000 P:00000
20UM
A
256X 18KV WD:11MM S:00004 P:00003
200UM
C

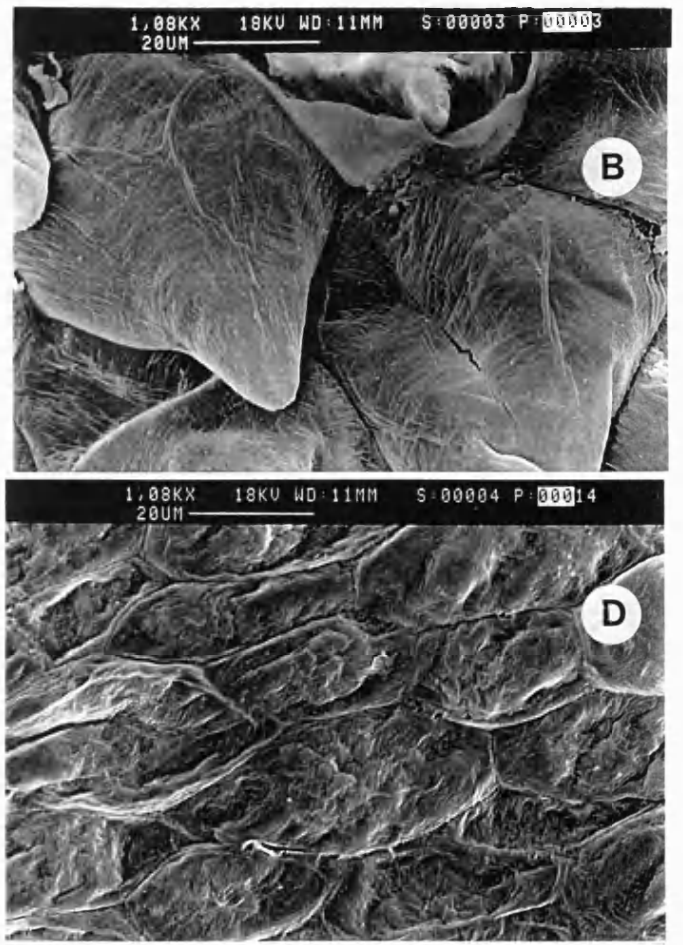

1,08KX 18KV WD:11MM S:00003 P:00003
20UM
B
1,08KX 18KV WD:11MM S:00004 P:00014
20UM
D

Plate 4.5

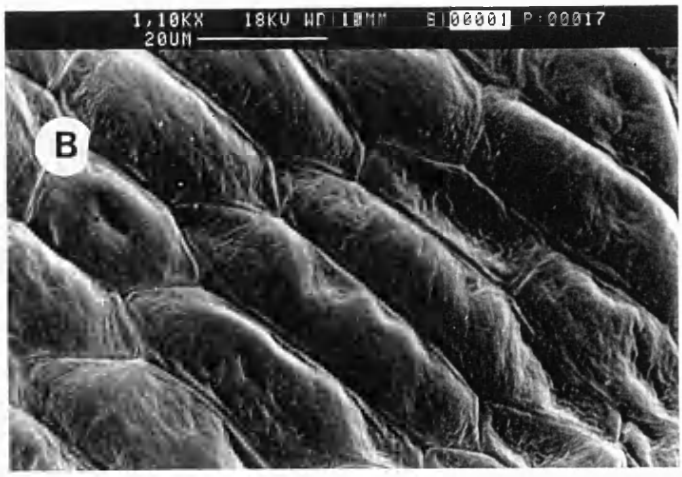
SEM photographs of the osmophores of *Lycaste macrophylla* subsp. *Xanthocheila* and *Anguloa virginalis.*

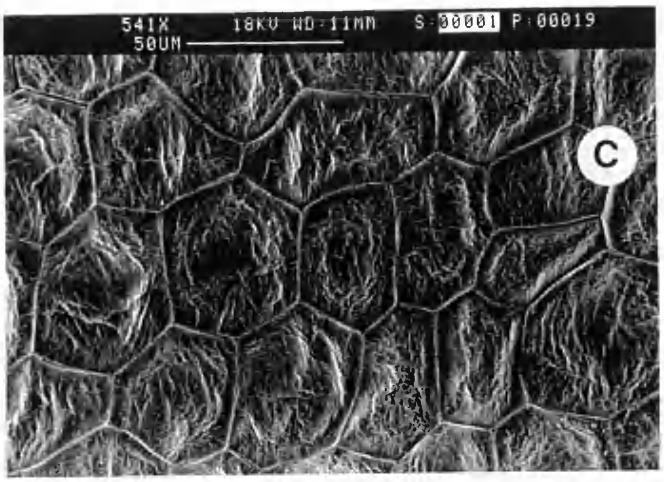
A. *L. macrophylla* subsp. *xanthocheila*: Callus edge.

B. *A. virginalis:* Callus undersurface.

C. *A. virginalis:* Lip mid-lobe underneath the callus.


2,10KX 18KV WD:11MM S:00004 P:00011
20UM
A


1,10KX 18KV WD:11MM S:00001 P:00017
20UM
B


541X 18KV WD:11MM S:00001 P:00019
50UM
C

Unlike *L. cochleata*, there was some morphological variation among osmophores from different sites of *L. macrophylla* subsp. *xanthocheila* (Plates 4.4C,D and 4.5A). Those from the central portion of the lip (Plate 4.4C) were longer and narrower and are probably best described as "villiform". Adjacent to the callus (Plate 4.4D) they were much less prominent and the surface assumed a slightly rugose appearance, while on the edge of the callus (Plate 4.5A) the surface appeared concave.

The fragrance glands of *Anguloa virginalis* were less conspicuous than those of the three species of *Lycaste* (Plate 4.5B,C). The structures on the under-surface of the callus were bullate (Plate 4.5B) with a smooth, slightly convex surface. Those on the mid-lobe, underneath the callus (Plate 4.5C) were a similar size but their surface was more rugose.

4.4 DISCUSSION

These preliminary investigations have shown that species of both *Lycaste* and *Anguloa* possess osmophores and that their location on the flower is species-dependent. Secretory tissue was observed on the mid-lobe of all three species of *Lycaste* and this may be used for long-distance pollinator attraction. The presence of glands at the base of the column and the lip of *L. cochleata* corroborates the description of *Euglossa viridisima* backing out of a flower of *L. xytriophora*, provided by Dodson and Frymire (1961b). Similarly, the location of fragrance glands on the under-side of the callus and the mid-lobe of *A. virginalis* supports the pollination mechanism described by Dodson (1966), who observed *Eulaema bolivensis* scratching this area of a flower of *A. clowesii*.

There have been no publications concerning the pollination of species from *Lycaste* sect. *Fimbriata*, and no conclusions could be drawn from this work.

Given the small sample size used for these investigations, it would be unwise to comment on the morphological similarities and differences observed. However, such features raise several questions concerning the relationship between osmophore morphology and pollination.

This work has highlighted a relatively unexplored area for further research. Little is known about the physiological processes of fragrance production and transport, or even whether all osmophores on an individual flower produce qualitatively and/or quantitatively similar fragrances. Given the technological advances that have occurred in the fields of analytical chemistry, anatomy and molecular biology over the last decade, more of these questions can now be addressed.

Chapter 5 THE ANALYSIS OF FLORAL FRAGRANCES

This study set out to answer three questions:

- Do *Lycaste* and *Anguloa* flowers have species' specific fragrances?
- Can floral fragrance analysis be used to establish phylogenetic and other trends?
- How do the fragrances of inter-species and inter-generic hybrids relate to those of their parents?

5.1 INTRODUCTION

The first published account of floral fragrance analysis was by Dodson and Hills (1966), who reported that fragrances of different species of tropical American *Catasetum*, *Gongora* and *Stanhopea* had different profiles when analysed by gas chromatography (GC). They also noted that different species of *Catasetum* which were pollinated by the same bee, *Eulaema cingulata*, had similar GC profiles. In a subsequent study, Hills *et al.* (1972) showed that differential fragrance production was the primary barrier to hybridisation among otherwise interfertile *Catasetum* species. For different species with the same fragrance, they found the most important isolating mechanisms were ecological and mechanical.

Dodson's method involved placing a plexiglass box around the inflorescences, then, following an equilibrium time of 30 minutes, withdrawing a sample of air (10ml) and injecting it onto a packed Carbowax 20M GC column. Detection was by flame ionisation.

Identification of compounds followed. Hills *et al.* (1968) used a combination of the relative retention time on two different GC phases and a sniffer port on the column outlet to identify α-pinene, β-pinene and 1,8-cineole from *Anguloa cliftonii* Rolfe. The

use of mass spectrometry (MS) was first reported by Holman and Heimermann (1973) in their analysis of the Asiatic epiphytic orchid *Neofinetia falcata* (Thunb.) H.H.Hu.

The early 1980s showed a move from packed to capillary GC columns (Bergström *et al.,* 1980; Williams and Whitten, 1982) and by the mid 1980s capillary GC-MS had become the routine analytical method.

Floral fragrance composition of orchids changes both qualitatively and quantitatively with time. In an early experiment, Hew *et al.* (1978) observed that the periods of maximum fragrance production for flowers of several tropical orchids coincided with those of maximum CO_2 production. The same year, Nilsson (1978) demonstrated that the composition of odours emitted by the European terrestrial *Platanthera chlorantha* (Cust.) Rchb. during the day differed from that produced at night.

More recently, Matile and Altenburger (1988) studied the rhythms of fragrance emission from several non-orchidaceous plants including two Apocynaceae: *Stephanotis floribunda* Brongs and *Hoya carnosa* R.Br. They found that the emission cycles of the five major components from *H. carnosa* were synchronised, with maximum production at 3am; *S. floribunda* showed seven different emission cycles. In a second paper, Altenburger and Matile (1988) showed that *H. carnosa* is able to adapt its cycle of fragrance emission very quickly to changes in light conditions.

Hills and Williams (1990) took four hourly headspace samples from *Clowesia rosea* Lindl. over a three day period, using an environmental chamber to ensure constant 12hour light and 12hour dark cycles. They found that emission of terpenoids such as α-pinene, 1,8-cineole and β-ocimene, which are produced by the mevalonic acid pathway, reached a maximum earlier in the day than that of shikimic acid produced methyl cinnamate.

Mookherjee *et al.* (1989) have also shown that the fragrances of "living" and "cut" flowers of many genera have significantly different profiles. Many hundreds of floral fragrance analyses have now been published. A comprehensive review has been compiled by Knudsen *et al.* (1993).

5.1.1 THE ORIGINS OF FLORAL FRAGRANCE

The chemical constituents of floral fragrances are biosynthesised by three distinct routes as shown in Figure 5.1. Terpenoids are derived from mevalonic acid, fatty acids from acetyl and malonyl coenzyme A, and aromatics primarily from shikimic acid.

Work on the terpenoids was pioneered by Wallach (1914), who hypothesised that dipentene (now known as limonene) was formed by the reaction of two molecules of isoprene (2-methylbuta-1,3-diene) and that sesquiterpenes were probably formed from three isoprene molecules. Ruzicka built on this work, publishing the "isoprene rule", which was subsequently modified to the "biogenetic isoprene rule" (Ruzicka *et al.*, 1953; Ruzicka, 1959). The principles of this rule, illustrated in Figure 5.2, are that each member of a terpenoid subgroup is derived from a single parent compound unique to that group and that these "parents" are related in a homologous fashion. Ruzicka identified the parents as 3,3-dimethylallyl pyrophosphate, which gives rise to hemiterpenoids (C_5), geranyl pyrophosphate to monoterpenoids (C_{10}), (2E,6E)-farnesyl pyrophosphate which yields the sesquiterpenoids (C_{15}) and geranylgeranyl pyrophosphate, the diterpenoids (C_{20}). Up to C_{25}, progression from one subgroup to the next is by "head to tail" condensation with isopentenyl pyrophosphate. For the purposes of floral fragrance work, only monoterpenoids and sesquiterpenoids have sufficiently low vapour pressure to be of interest.

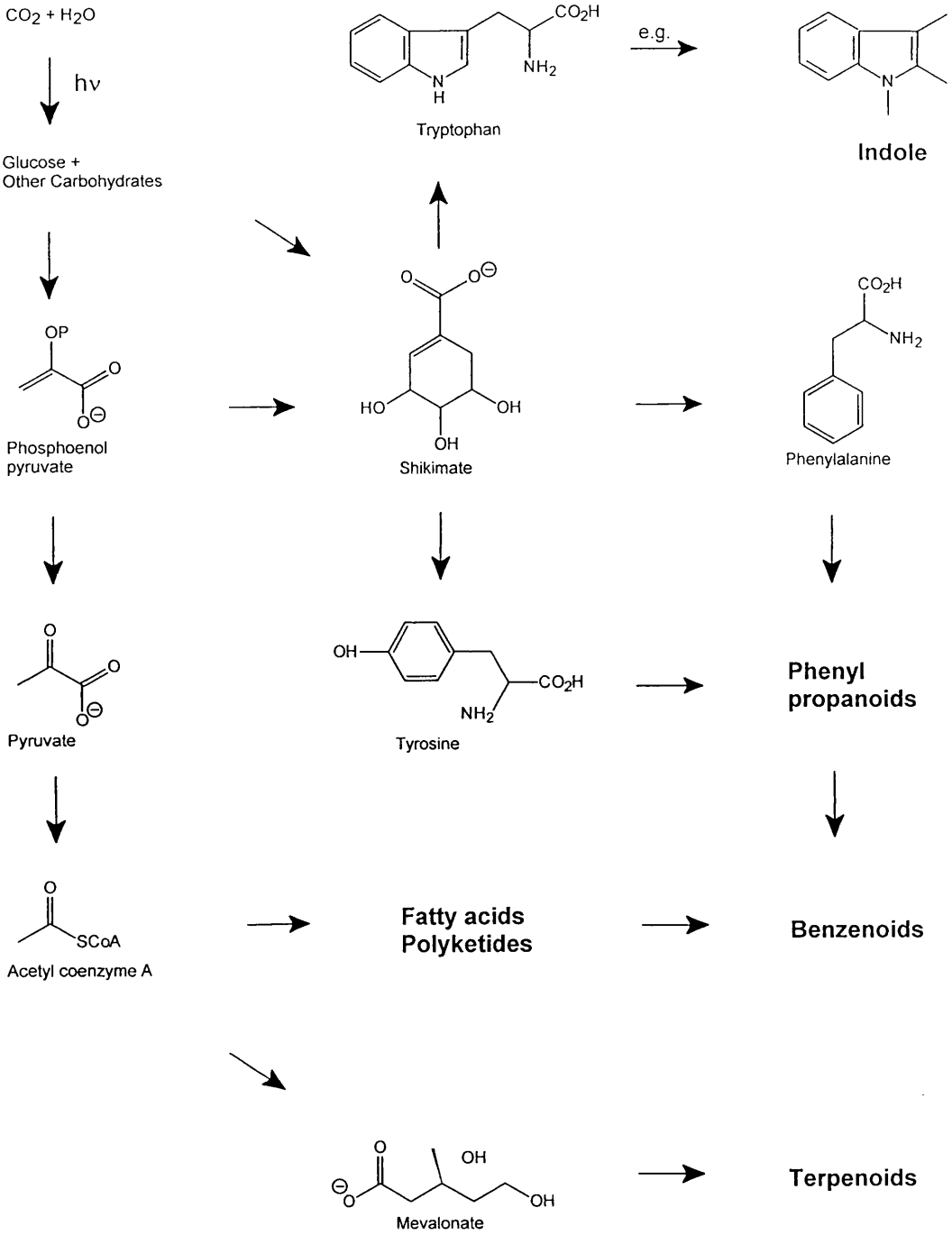


Figure 5.1 The biosynthesis of fragrance chemicals by plants, after Mann (1994).

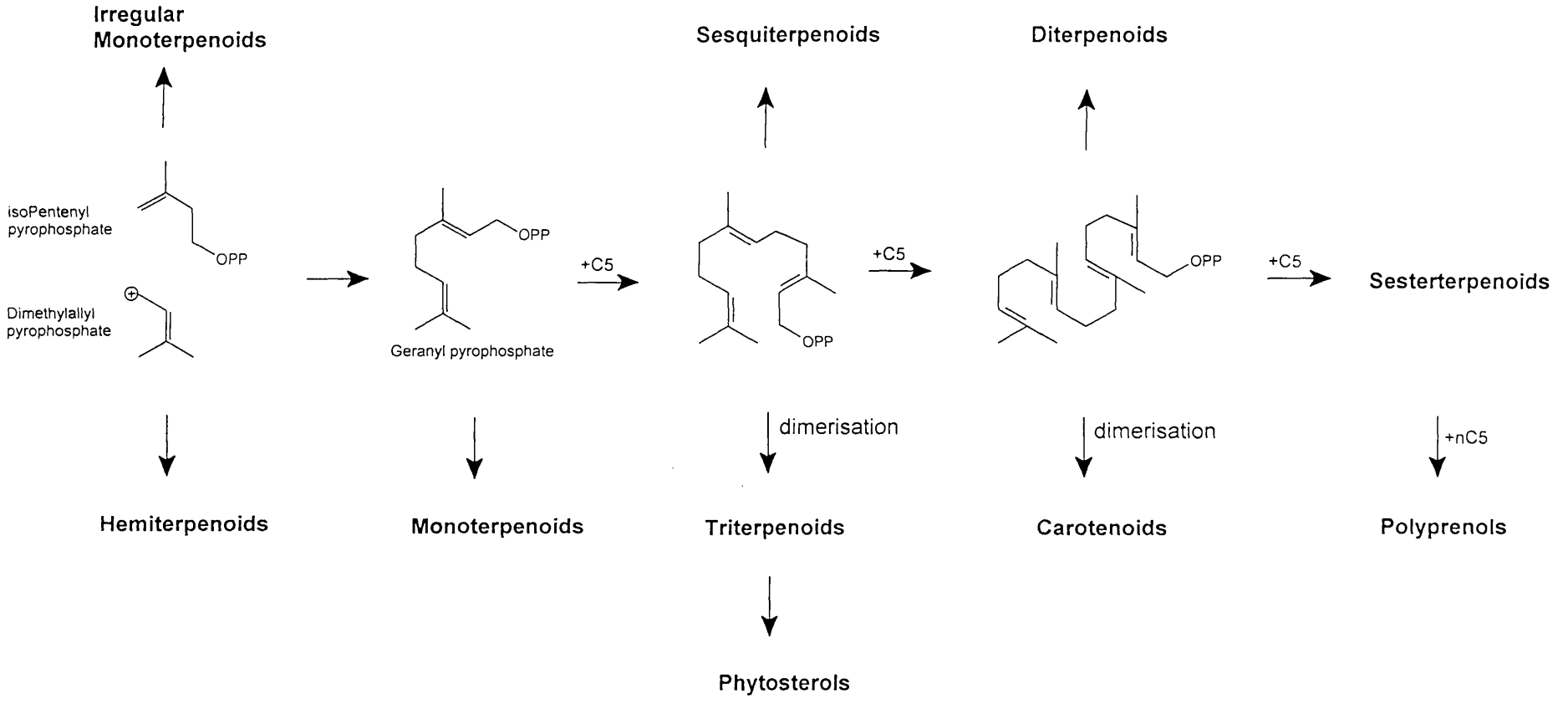


Figure 5.2 The biosynthesis of terpenoids, from Banthorpe and Charlwood (1980).

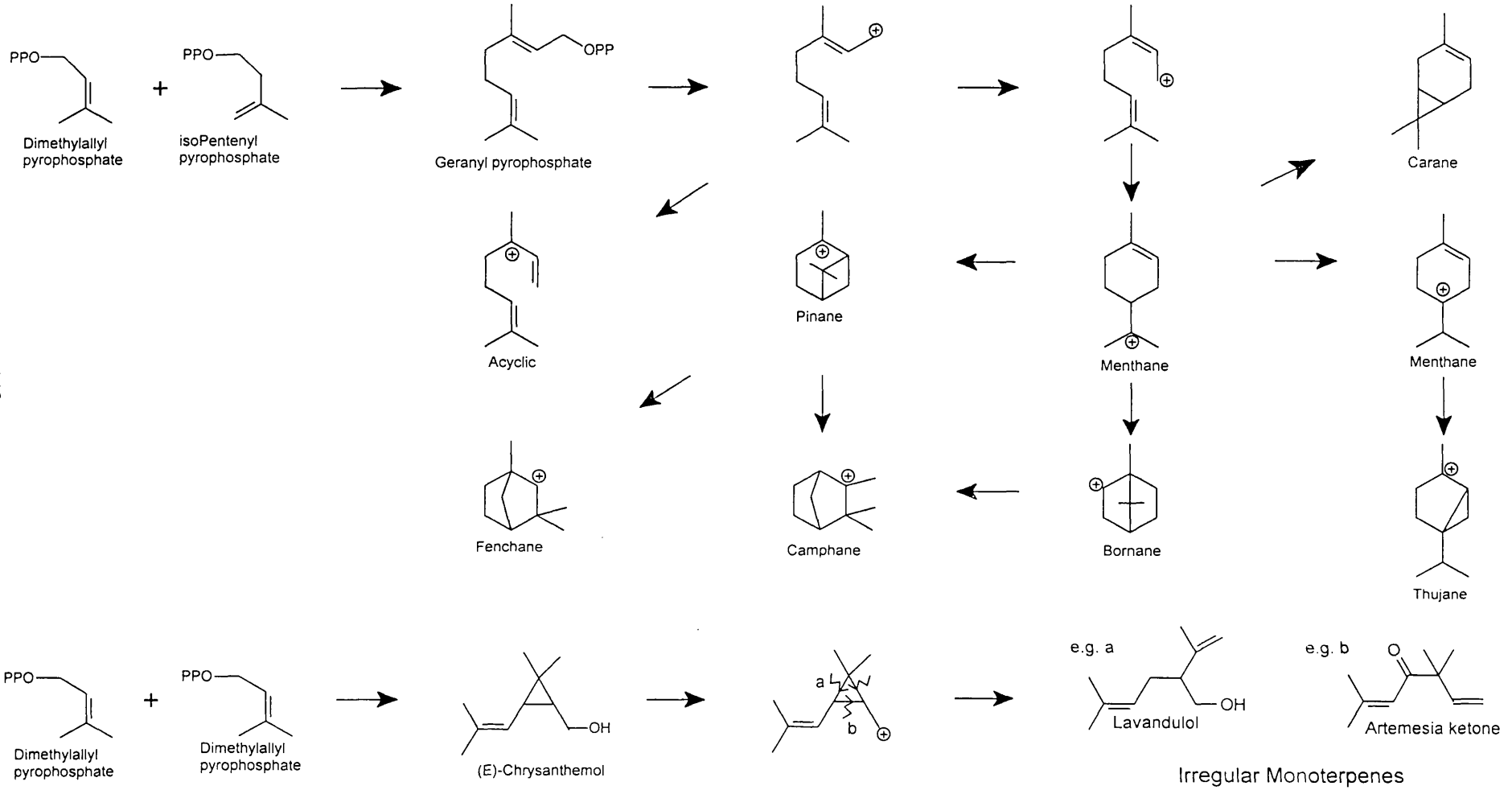
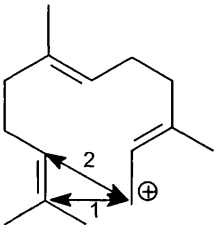


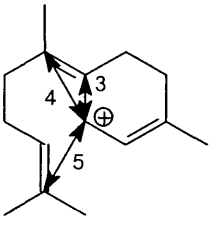
Figure 5.3 The biosynthesis of monoterpenoid skeletons, after Banthorpe (1994) and Croteau and Karp (1991).

Within the monoterpenoids, eight major skeletal types, all derived from geranyl pyrophosphate have been recognised. Their formation has been rationalized by Ruzicka *et al.* (1953) and Banthorpe (1994) in terms of a series of additions, eliminations and rearrangements of hypothetical carbocations as shown in Figure 5.3. The validity of these pathways has been confirmed by both ^{14}C and enzymic studies; comprehensive reviews have been published by Banthorpe *et al.* (1972) and Croteau (1987). In addition, there is a group of "irregular" monoterpenoids, thought to be formed from the condensation of 2 molecules of dimethylallyl pyrophosphate (Banthorpe *et al.,* 1977; Banthorpe, 1994). This last group includes two important perfumery chemicals lavandulol and artemesia ketone.

Over 200 sesquiterpene skeletal types have been recognised, ranging in complexity from acyclic (2E,6E)-α-farnesene to tetracyclics such as longifolene. Although there is no universally accepted system of classification, Banthorpe (1994) has suggested that Ruzicka's rationalisation according to reactions of hypothetical carbocations can be applied. Using this technique, five major cyclisation routes can be identified, which all derive from (2E,6E)-farnesyl pyrophosphate, or its (2Z,6E) and allyl isomers, as shown in Figure 5.4 below.



ex (2E,6E)-Farnesyl pyrophosphate



ex (2Z,6E)-Farnesyl pyrophosphate
or Nerolidyl pyrophosphate

Figure 5.4 Biosynthetic routes to the formation of cyclic sesquiterpenoids, after Banthorpe (1994).

These reaction pathways are shown in more detail in Figures 5.5 and 5.6.

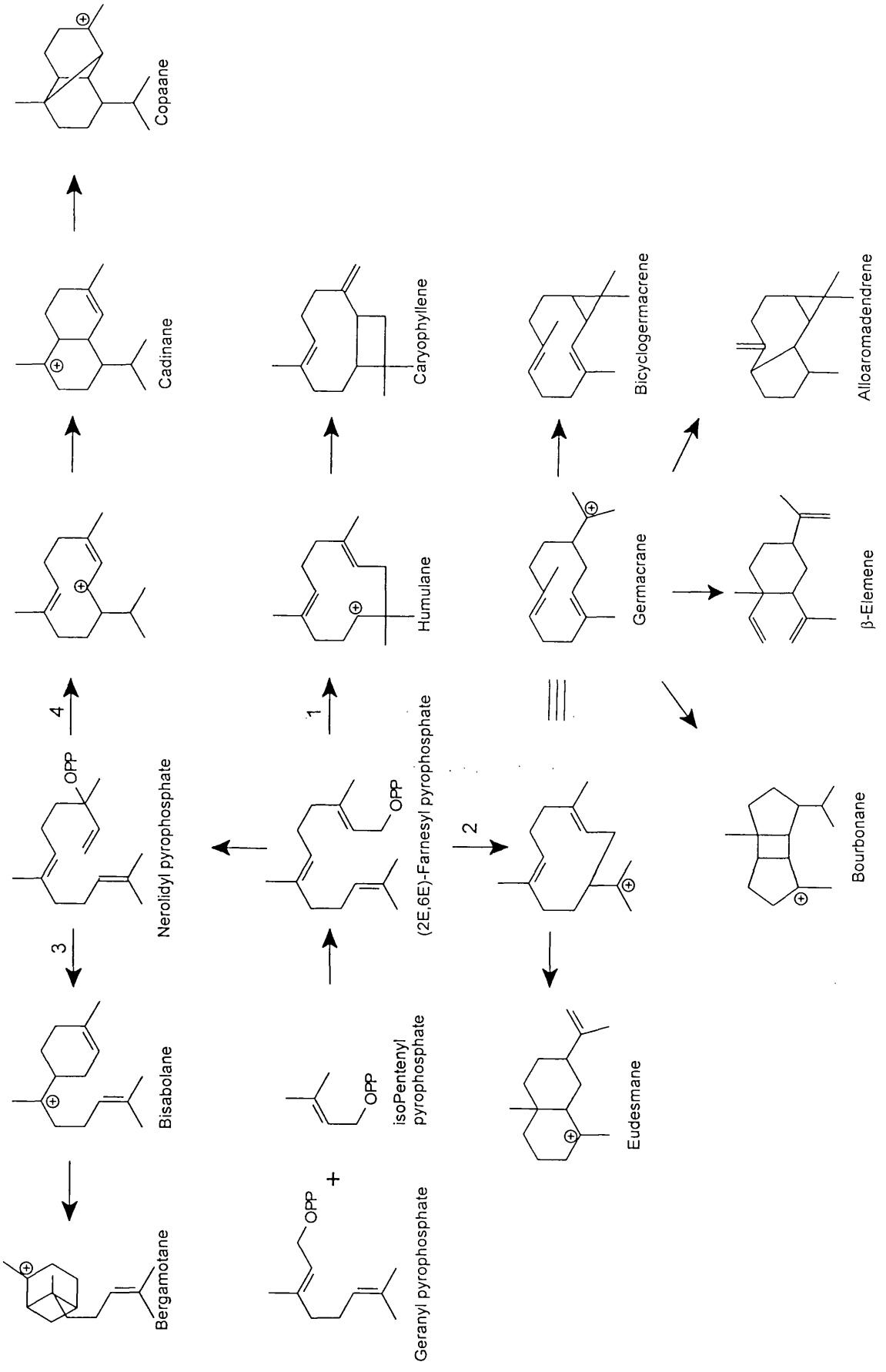


Figure 5.5 The biosynthesis of sesquiterpenoid skeletons, after Banthorpe (1994).

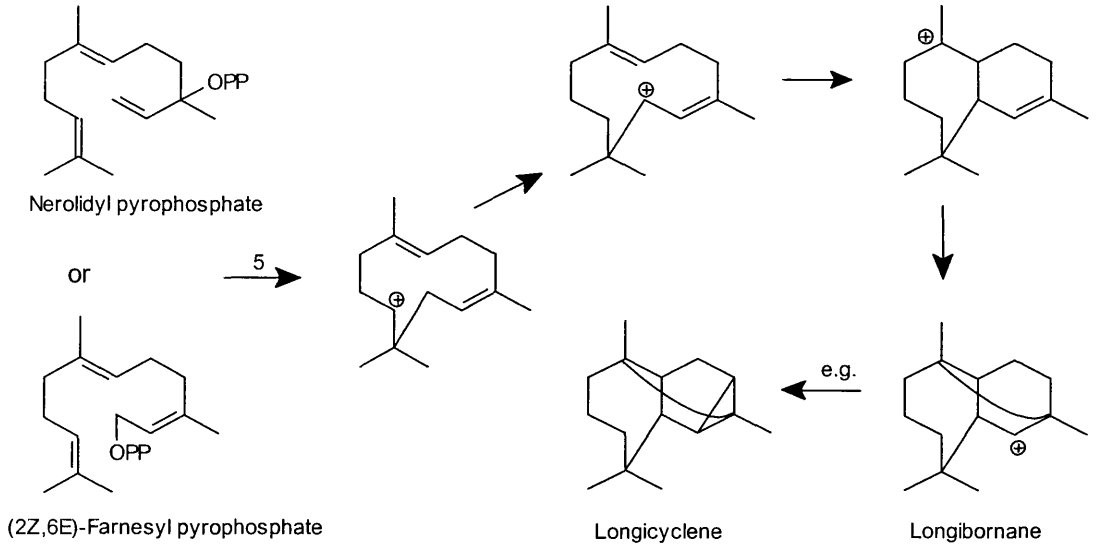


Figure 5.6 The biosynthesis of longibornanes, from Banthorpe and Charlwood (1980).

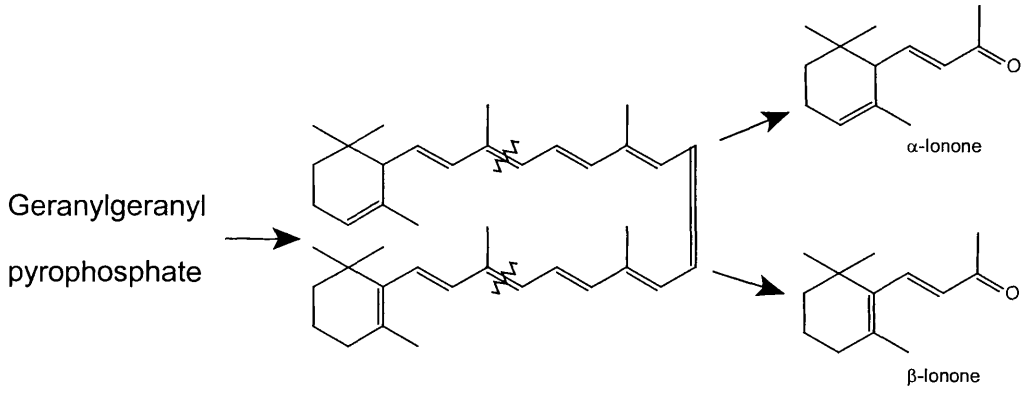


Figure 5.7 The formation of ionones, from Croteau and Karp (1991).

An additional group of odoriferous terpenoids which fall outside these classifications are the ionones and damascenones, formed by oxidative degradation of carotenoids as shown in Figure 5.7.

A key stage in the biosynthesis of aliphatic fatty acids is the transformation of acetyl coenzyme A into malonyl coenzyme A by acetyl coenzyme A carboxylase (Figure 5.8).

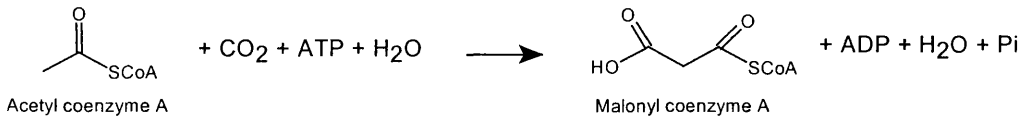


Figure 5.8 Biosynthesis of malonyl coenzyme A, from Luckner (1990).

Hydrolysis of acetyl coenzyme A yields acetic acid. Other saturated acids with an even number of carbon atoms are built by addition of molecules of malonyl coenzyme A to acetyl coenzyme A. For odd carbon numbered acids, acetyl coenzyme A is substituted by propionyl coenzyme A.

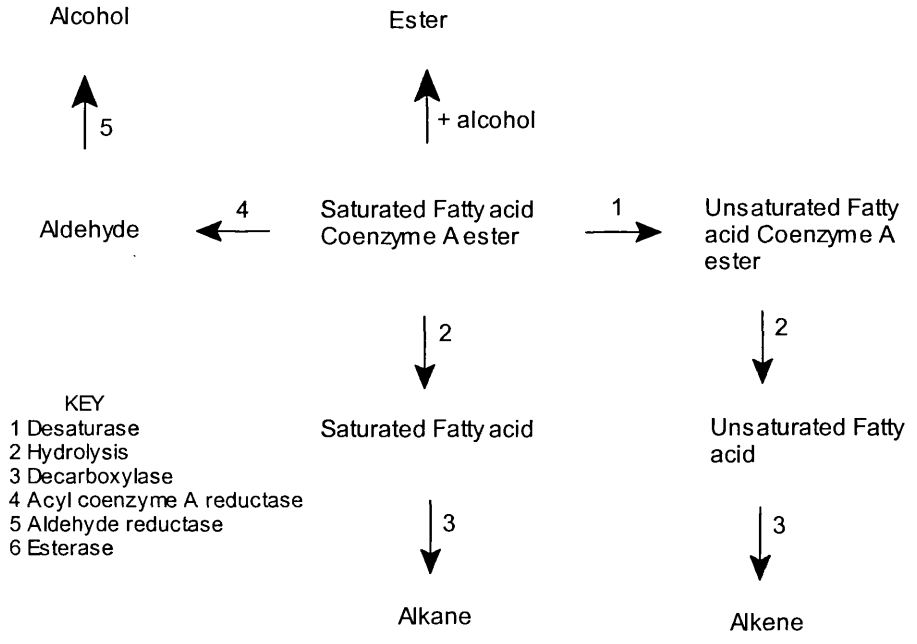


Figure 5.9 A mechanism for the biosynthesis of aliphatic compounds, after Luckner (1990).

The precursors of branched fatty acids are branched aliphatic amino acids such as leucine, isoleucine and valine. These are converted into acyl coenzyme A esters by transamination and oxidative decarboxylation.

Figure 5.9 illustrates one of the mechanisms by which other aliphatic compounds are derived.

Phenylpropanoids originate from the aromatic amino acids phenylalanine and tyrosine (Luckner, 1990), products of the shikimic acid pathway. Phenylalanine lyase (PAL) converts phenylalanine into (E)-cinnamic acid, the precursor of many aromatic compounds, as shown in Figure 5.10. Similar lyase action on tyrosine yields p-coumaric acid.

The origin of aromatic compounds with a smaller side-chains such as phenylacetates, benzoates and salicylates is less specific. Like phenylpropanoids, they can be formed from products of the shikimic acid pathway. Luckner (1990) has proposed that phenylacetic and 4-hydroxyphenylacetic acids are formed by decarboxylation of phenylalanine and tyrosine by amino acid decarboxylases (Figure 5.11). However benzoic, salicylic and 4-hydroxybenzoic acids are more likely to be formed by side-chain degradation of cinnamic and o- and p-coumaric acids than directly from the amino acids (Harborne, 1980; Croteau and Karp, 1991).

An alternative route to the formation of these lower homologues and analogues is via acetyl and malonyl coenzyme A (Harborne, 1980; Luckner, 1990).

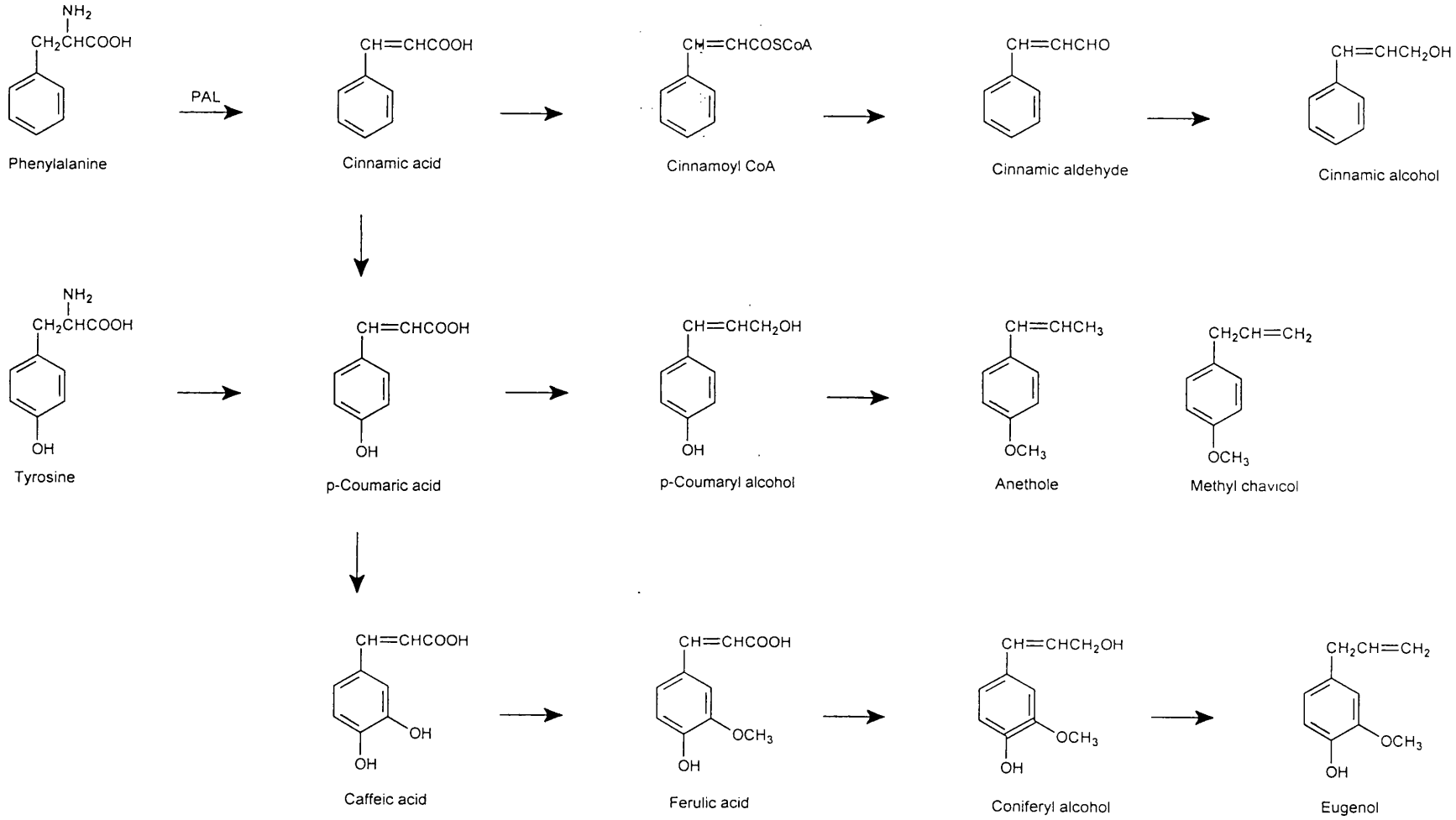


Figure 5.10 The biosynthesis of phenylpropanoids, from Croteau and Karp (1991).

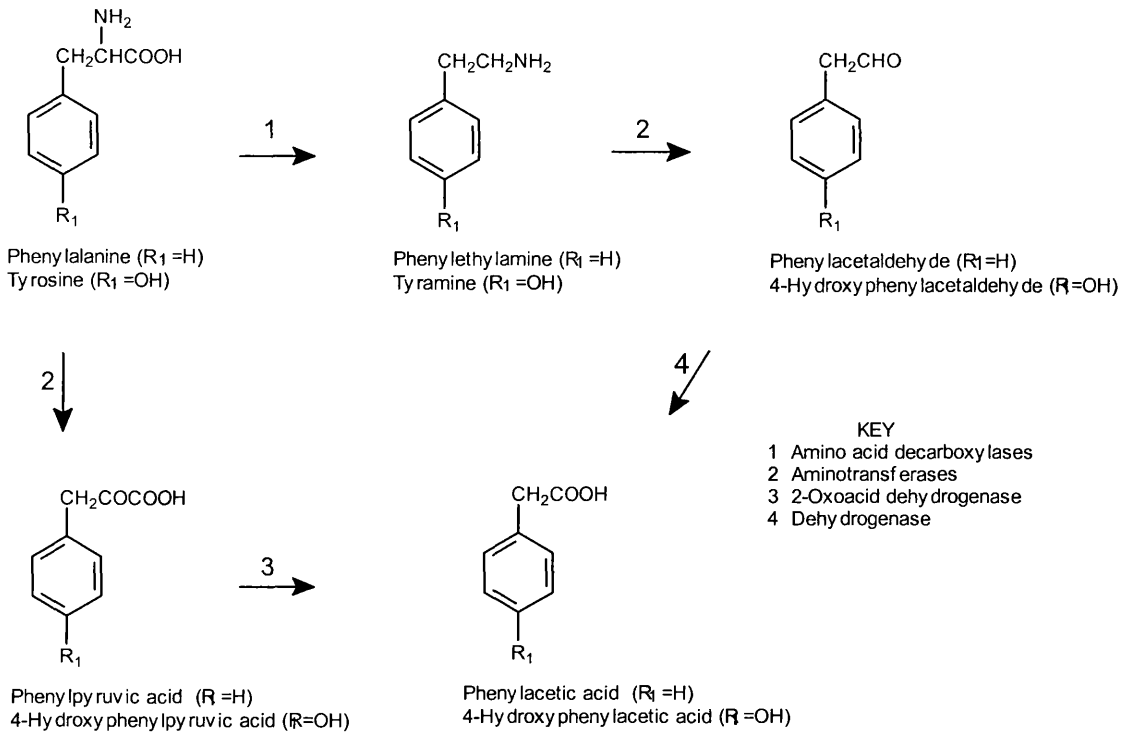


Figure 5.11 The biosynthesis of phenylacetic and 4-hydroxyphenylacetic acids, from Luckner (1990).

5.1.2 FRAGRANCE COMPOSITION AND POLLINATION STRATEGY

The role of fragrance in moth-pollinated taxa has been studied by Knudsen and Tollsten (1993), who analysed the floral fragrance composition of 15 moth- pollinated species from nine families including *Epidendrum ciliare* L. (Orchidaceae). They found the fragrances to be characterised by acyclic terpene hydrocarbons and their alcohols, benzenoid alcohols and esters and small quantities of nitrogen-containing compounds. None of the species studied contained significant quantities of either phenylpropanoid or fatty acid derivatives. Similar or structurally closely related compounds were found in temperate and tropical species from both the Old and New World, suggesting that all moths have similar sensory preferences. The fragrance composition of non-nectar containing flowers was not significantly different from the others, suggesting that these flowers rely on a deception pollination strategy.

The sex ratio of moths that visit flowers is strongly male biased, measured as 49:2 for *Epidendrum anceps* Jacq. (Goss and Adams, 1976). However there appears to be no relationship between the structure of female moth pheromones and floral scent compounds. Janzen (1983) concluded that the bias was due to differences in nutritional requirements between the sexes, with males requiring more energy for mate-seeking flights.

Ophrys species use a combination of chemical, visual and tactile stimuli to attract male bees and digger wasps of the superfamilies Apoidea, Scoliodea and Spheciodea; in some localities, male beetles (Elateridae and Scarabaeidae) are also attracted. The insects become sexually excited, land on the labellum and attempt copulation. During the copulation attempt, the pollinia become attached either to the insect's head or, with some *Ophrys* species, to its abdomen.

Studies on the chemical composition of *Ophrys* fragrances have been reviewed by Borg-Karlson (1990). The fragrances are dominated by fatty acid derivatives, terpenoid hydrocarbons and oxygenated terpenoids. No nitrogen-containing compounds have been detected. A few species contain up to 5% 2-phenylethyl alcohol, but no other benzenoid or phenylpropanoid compounds. In addition, fragrances from morphologically different forms of *Ophrys fusca* Link show qualitative and quantitative variation of aliphatic compounds and oxygenated monoterpenoids. *Ophrys fusca* has subsequently been split up into a number of closely allied species (Delforge, 1995).

The fragrances of *Ophrys lutea* Cav. and *O. fusca* contain the terpenoids nerol, neral, geraniol, geranal and (2E,6E)-farnesol as well as a series of primary and secondary aliphatic alcohols. These chemicals have been found in the mandibular and Dufour glands of many species of *Andrena* bees (Borg-Karlson *et al.*, 1985) suggesting they may be utilised as attractants or excitants.

Another genus assumed to use deception as the pollination strategy is *Cypripedium*. The primary source of attraction is the staminode (Bergström *et al.,* 1992), located on the proximal border of the central opening of the labellum. In trying to gain access, insects, primarily *Andrena* and *Megachile* bees, fall in. The only exit route, marked by a trail of false nectar guides and trichomes, is through the rear of the labellum past the pollen.

There have been two studies of *Cypripedium* fragrances. The first, by Bergström *et al.* (1992), looked at taxa of *C. calceolus* from three different geographical locations and found them to have distinctly different fragrance profiles. Barkman *et al.* (1997) analysed fragrances from eight different species and found their compositions to be both qualitatively and quantitatively very different. Identified compounds included terpenoids, aromatics and fatty acid derivatives.

To date, the widest survey of "euglossine" fragrance composition has been by Gerlach and Schill (1991). They analysed the fragrances of 101 plant species, from four families: Orchidaceae, Araceae, Gesneriaceae and Bromeliaceae, more than 75% of which were known to be pollinated by male euglossine bees. They found that although there were no fragrance patterns common to all euglossine bee pollinated flowers, most contained high levels of monoterpenoids and aromatic esters, whereas nitrogen-containing compounds were rare. They contrasted this with their findings for melittophilous (nectar-producing) flowers, where sesquiterpenoids were found to be the predominant group. In addition, they found that the same sets of chemical compounds were present in "euglossophilous" flowers from different families, suggesting convergent evolution.

An in-depth study of the floral fragrances of *Stanhopea* (Orchidaceae) and their euglossine pollinators has been undertaken by Whitten and Williams (1992) who found

the fragrances to be characterised by monoterpenoids and aromatic compounds; fatty acid derivatives and sesquiterpenoids were rare. In contrast to the work of Gerlach and Schill (1991), the nitrogen-containing compound indole was found in many species. Of the 34 species examined, only seven had species' specific fragrances. The others either shared a fragrance composition with other species or showed intraspecific variation. Species with similar fragrances were usually allopatric and sometimes shared pollinators.

There have been four reports of the fragrance composition of *Lycaste* and *Anguloa* species. Following the early work of Hills *et al.* (1968), Williams *et al.* (1981) used direct injection to analyse *Lycaste aromatica*, *L. ciliata*, *L. cruenta*, *Anguloa cliftonii*, *A. clowesii* and *A. ruckeri*. Identification of components was solely by GC retention index and was therefore limited.

Gerlach and Schill (1991) analysed fragrances from *Anguloa uniflora*, *A. virginalis* and *Lycaste deppei*. The compositions of the two *Anguloa* fragrances were markedly different. *A. uniflora* was dominated by 1,4-dimethoxybenzene (95%); *A. virginalis* by the monoterpenoids 1,8-cineole (66%), limonene (15%), myrcene (6%) and α-pinene (7%). *L. deppei* was again rich in monoterpenoids: myrcene (33%), limonene (27%), α-pinene (13%) and (E)-ocimene (9%).

Finally, Kaiser (1993) used adsorption on activated charcoal followed by elution with carbon disulphide for detailed analyses of *Anguloa clowesii*, *Lycaste aromatica*, *L. cruenta*, and *L. locusta*. His results showed all four fragrances to be qualitatively and quantitatively different. Like *Anguloa uniflora,* the major component of the fragrance of *A. clowesii* was 1,4-dimethoxybenzene (92%). Fragrances from the two deciduous lycastes, *L. aromatica* and *L.* cruenta had high levels of both monoterpenoids and phenylpropanoids, both of which were absent from *L. locusta*.

5.1.3 FLORAL FRAGRANCE AND PHYLOGENY

There have been few reports of numerical analyses based on floral fragrance data. Many of these have been based on similarity coefficients, clustering techniques or principal component analysis (PCA).

One of the earliest examples was by Williams (1981), who used "paired affinity indices" (PA) to show that the two moth-pollinated orchids, *Rhyncholaelia digbyana* (Lindl.) Schltr. and *R. glauca* (Lindl.) Schltr., were more closely related to each other than to *Brassavola* species. These two species had initially been placed within *Brassavola* but were subsequently transferred to *Bletia*, *Laelia* and finally *Rhyncholaelia*. Their current status remains within *Rhyncholaelia*.

Gregg (1983) used PCA to identify two distinct chemotypes of *Cycnoches densiflorum* Rolfe, characterised by high and low α-pinene contents.

Moya and Ackerman (1993) adopted a similar method to Williams (1981) to examine infraspecific variation within *Epidendrum ciliare*. They found unexpectedly high variation and suggested that this was an important strategy for non-rewarding flowers; the more dissimilar the fragrances, the longer it would take a pollinator to learn to avoid them.

More recently, Barkman *et al.* (1997) tried both similarity coefficients and the clustering algorithm UPGMA (unweighted pair group method average) to assess population-level variation versus species-level variation within *Cypripedium*. To compensate for quantitative variation caused by changes in headspace sampling conditions, the fragrance compounds were coded as present/absent. They showed that although there was variation within populations, it was considerably less than that observed between species.

In their study, some attempt was made to group chemicals together. Within the "fatty acids", homologues of alcohols, methyl esters and ethyl esters were treated as single characters. Within the terpenoids and aromatics, compounds differing by simple oxidation changes, such as benzyl alcohol and benzaldehyde, were also classed as single characters. Stereoisomers were grouped together, but geometric isomers such as (Z)-ocimene and (E)-ocimene were not.

Dobson *et al.* (1997) used a similar approach to examine interspecific variation within *Narcissus* (Amaryllidaceae), in this case using arcsine transformed GC area percent values. The resulting dendrogram showed good agreement with currently recognised taxonomic groupings at and above sectional level. Agreement was enhanced when volatiles reputedly sharing the same biosynthetic pathway were grouped. Sixteen categories were used for the analysis: fatty acid derivatives, miscellaneous isopentenoids, monoterpenoids (subdivided into compounds derived from geranyl pyrophosphate, myrcene, camphene, β-ocimene, linalol, pinene, bornyl pyrophosphate), sesquiterpenoids, carotenoids, benzenoids (subdivided according to the position of methoxylation) and nitrogen-containing compounds.

Cladistic analyses using floral fragrance data are rare. One example is by Barkman (1993), who found the *Cypripedium calceolus* complex to be monophyletic and suggested that it should include *C. macranthum*. However, analysis of ITS sequences indicate that the *C. calceolus* complex is not monophyletic (Cox *et al.*, 1997).

5.1.4 SAMPLE PREPARATION TECHNIQUES

Alongside the applications mentioned above, since the 1970s there have been significant improvements to the processes of sample collection and sample transfer onto a GC column. These developments have been partly driven by the chemical industry, where environmental monitoring is now a statutory requirement, and partly by

the flavour and fragrance industry in its search for new aroma chemicals. They will be considered in detail below.

The first stage of all floral headspace analysis has been to enclose the inflorescence. This allows a build up of fragrance chemicals from the plant and limits intrusion of chemicals from other sources. The three popular choices for this are glass (Bicchi *et al.*, 1987; Mookherjee, 1989; Kaiser, 1993), plexiglass (Hills *et al.*, 1968, Matile *et al.*, 1988; Raguso *et al.*, 1995) and polyacetate bags (Borg-Karlson *et al.*, 1985). Some workers have filtered the incoming air using either charcoal (Borg-Karlson, 1990) or Porapak Q (Dobson *et al.*, 1990). The system is then allowed to equilibrate, typically for 30 minutes to one hour.

Methods for sample collection fall into four categories:

a. Direct injection
b. Cold trapping
c. Static adsorption
d. Dynamic adsorption

a. Direct Injection

Direct injection onto packed columns as described by Hills *et al.* (1968), has been used to identify fragrance components of species of *Brassavola* (Williams, 1981), *Catasetum* (Hills *et al.*, 1972; Murrell, 1981), *Anguloa*, *Lycaste* and *Galeottia* (as *Mendonçella*, Williams *et al.*, 1981). The technique has been less successful with capillary columns, as was reported by Bergström *et al.* (1980), who found that they were unable to inject enough sample when experimenting with fragrances of *Ophrys* species. This lack of sensitivity has lead to the development of concentration techniques.

b. Cold trapping

Loper and Webster (1971) described a technique for analysing alfalfa (*Medicago*

farfara) volatiles. Florets were placed in the bottom of a syringe barrel (100ml) and the plunger inserted to the 100ml graduation. After an equilibration time of 30min, the headspace was injected into a dry ice/acetone cooled condensing coil fitted into the GC injector. Transfer to the GC column was via an eight-port sampling valve.

Later, Mack and Köpsel (1973) reported the use of both a humidified nitrogen purge and a vacuum pump to separate plant volatiles from the plant matrix of lily of the valley and lilac blossoms. The volatiles were condensed in cold traps at –70°C and, after an 8hr collection period, recovered by extraction with Frigen 11. Vacuum stripping has also been reported by Brunke *et al.* (1992), who used liquid nitrogen cooled traps (-196°C). The crucial disadvantages of vacuum techniques are that they cannot be used with living material or in the field.

c. Static Adsorption

Holman and Heimermann (1973) used glass-fibre paper impregnated with diffusion pump oil to trap volatiles from *Neofinetia falcata.* The flowers and adsorbent paper were enclosed in a glass container for a few days, after which the paper was removed and inserted directly into the injecton port of a gas chromatograph. They identified linalol, methyl benzoate and caryophyllene as the major components. The same technique has been used to analyse fragrance from North American *Magnolia* species (Thein *et al.*, 1975).

Moya and Ackerman (1993) put loose Tenax in an Erlenmeyer flask containing living inflorescences to passively adsorb volatiles from *Epidendrum ciliare*. They experienced problems in identifying the fragrance constituents, particularly terpenoids, but did not say whether this was due to a lack of sensitivity or to the quality of their spectral library.

Within the perfumery industry, the traditional method of extracting fragrance from flowers with a low essential oil content such as jasmine or tuberose was by "enfleurage". This technique, developed by Passy (1897), involves placing freshly picked flowers on a layer of odourless fat for 24 to 36 hours, during which time essential oil from the flowers is absorbed by the fat. The flowers are then replaced and the process repeated until the fat becomes saturated. The raw product, a "pomade", has perfumery applications; alternatively the essential oil can be recovered by ethanol extraction. The process is extremely labour intensive as the flowers have to be removed before decomposition sets in. It also only works with flowers that continue to produce fragrance for several hours after harvesting. Nowadays, apart from the production of tuberose pomade, it is obsolete.

Bergström *et al.* (1980) tried to replicate the technique by placing *Ophrys* labella directly onto Chromosorb W AW loaded with 10% silicone high vacuum grease for 4 to 5 days. The relative concentration of sesquiterpene hydrocarbons was higher than that collected by either direct injection or cold trapping. However they suggested there was a risk of chemical changes on the enfleurage surface caused by the long exposure to air.

Moving away from static adsorption, Joulain (1987) took the enfleurage method a step further, using an unheated portable hair drier to blow air through a grid supporting a layer of flowers and onto a layer of purified fat. Using this technique, the main component of the headspace of *Robinia pseudoacacia* was identified as 2-aminobenzaldehyde, which had not been detected using conventional extraction.

d. Dynamic adsorption

This is by far the most popular technique. Headspace is pushed or pulled through adsorbent traps and the fragrance chemicals recovered either by thermal desorption or

by solvent extraction. In most cases, "new" air has been allowed to enter the system, however some workers have opted for closed systems, using a pump to recirculate the air between the flower and the adsorption trap. Brunke *et al.* (1992) used such a system to investigate the headspace composition of roses and lilac. A very different application has been described by Sommerville *et al.* (1994), who designed a recirculating system to fingerprint human sweat.

A major problem has been to find adsorbents that will not only cope with the wide ranges of molecular mass and polarity found in plant volatiles, but will also release all trapped material during the desorption process. The three most commonly used are Porapak Q (e.g. Dobson *et al.*, 1990), Tenax (e.g. Williams *et al.*, 1982) and various forms of carbon, such as Carbopack B™ (Gerlach and Schill, 1991) or activated charcoal (e.g. Kaiser 1993). Some workers have used mixtures of Tenax and carbon (Knudsen and Tollsten, 1993; Whitten and Williams, 1992).

Other adsorbents which have been used are the Chromosorb "Century" series and XAD ion exchange resins. Murray (1977) used Chromosorb 105 and 106 to look at volatiles from the foliage of *Lantana camara* and of purple and yellow passion fruit *Passiflora edulis* Sims. (Passifloraceae) and *P. edulis* var. *flavicarpa*. He concluded that they perform better than Tenax for low molecular weight materials, however, there are limitations on the desorption temperature. Barkman (1993) used XAD-2 for his work on the phylogeny of *Cypripedium.*

Although most work on flowers has been performed using packed traps. Grob and Habich (1985) developed two designs of open tubular capillary traps, one coated with a thick apolar (12-15µm PS255) film and the other with carbon particles melted into the inner walls. The thick-film design was subsequently adopted by Bicchi *et al.* (1989) to examine the volatile composition of healthy and infected *Mentha* species. Embedded

carbon traps and also embedded Porapak Q traps were used by Burger *et al.* (1988) to collect headspace from *Hydnora africana* Thunb. (Hydnoraceae), which has an extremely fetid odour. Both designs have proved to be temperature stable, but their use has been limited due to a lack of capacity and, one suspects, the technical expertise required to make them to a consistent quality.

As mentioned above, there are two methods of recovering volatiles from adsorbents: solvent extraction and thermal desorption.

Solvent extraction has been the preferred method in published accounts of floral fragrance analysis. Various solvents have been used; the problem has been to find a solvent that will elute a wide range of polarities but will not co-elute with one of the fragrance components. Dichloromethane dissolves Tenax. Kaiser (1991, 1993) advocates the use of carbon disulphide. Other solvents which have been used are pentane (Dobson *et al.*, 1990; Knudsen and Tollsten, 1991), hexane (Raguso and Pichersky, 1995; Whitten and Williams, 1992), acetone (Gerlach and Schill ,1991), diethyl ether (Bergström *et al.*, 1992) and 1:1 pentane:ethyl acetate (Borg-Karlson *et al.*, 1985). The disadvantage with most of these is their high volatility which can cause severe problems in sample storage. Hexane is less volatile but is also more selective than the other solvents and may need to be followed by an ethanol wash (Hills and Schultzman, 1990).

Thermal desorption uses a combination of carrier gas and heat to transfer trapped volatiles to the front of a GC column, usually via a cold trap where they are re-concentrated prior to separation. The advantages of this method are that it can be automated (e.g. Perkin Elmer, Chrompack) and also that it does not discriminate between chemicals of differing polarity. The technique has been used successfully for the analysis of basil, sage and mint volatiles (Bicchi *et al.*, 1988) and, more recently

Epidendrum ciliare (Moya and Ackerman, 1993). However, it does have disadvantages. The choice of adsorbent is limited; personal experience has shown that both Porapak and the Chromosorb Century series are unsuitable due to their high levels of bleed. More importantly, it is a "single shot" technique, leaving no material for further analysis.

5.2 MATERIALS AND METHODS

Plant material used for this work was provided by Dr Henry Oakeley, The Royal Botanic Gardens Kew, AMO, Celia Lamas and Horencio Jarvio. A full list of vouchers and their locations is given in Appendix 2.

In designing a method, two significant constraints had to be considered. The first was that many of the plants came from private collections and were grown for exhibition purposes; damage was not an option! The second concerned the analyses which were carried out at Bush Boake Allen Ltd., a flavour, fragrance and aroma chemical company. Solvent extraction was considered inappropriate for recovering volatiles due to the possibility of contamination and the lack of odour-free low-temperature storage. Thermal desorption allowed the traps to be thoroughly cleaned and checked by GC immediately before sample collection.

5.2.1 SAMPLE COLLECTION

To eliminate anomalies caused by different sample collection and transfer procedures, a single method was used as outlined in Figure 5.12.

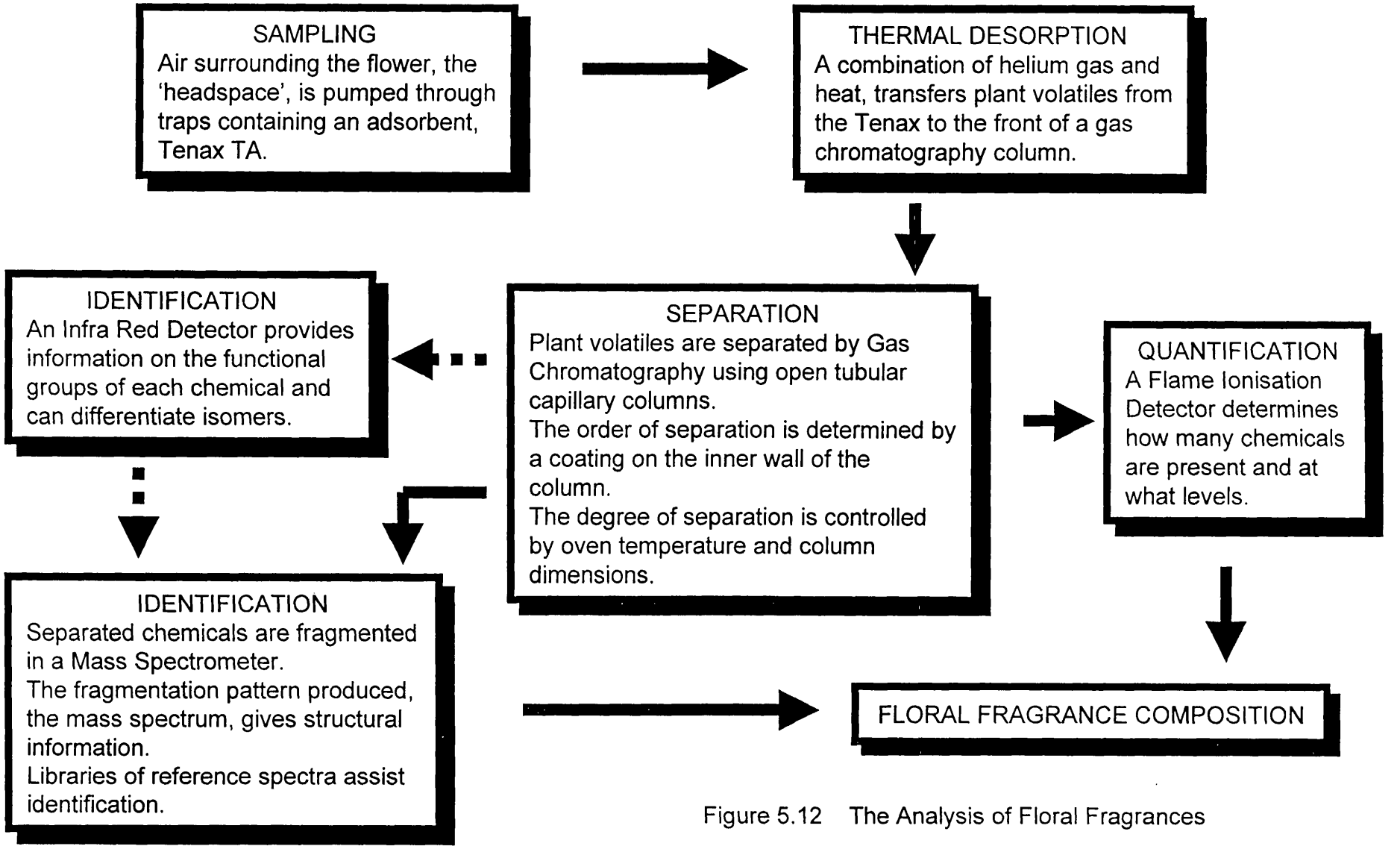


Figure 5.12 The Analysis of Floral Fragrances

Plants were sampled *in situ*. Growing inflorescences were enclosed in Rilsan bags (Whittaker Knight Consultants). Rilsan is a natural nylon material, which is light, flexible and semi-permeable. The flexibility meant that the bags could be shaped and cut to accommodate the wide range of shapes and sizes of flower. Semipermeability prevented a build-up of condensation and associated mal-odours; Table 5.1 demonstrates that it did not cause a loss of early boiling fragrance components from the headspace.

	Area %	
	Glass flask	Rilsan™ bag
2-Methylbutan-1-ol	0.2	0.3
Prenol	0.2	0.4
(Z)-Hex-3-enol	0.4	0.4
Car-3-ene	0.1	0.2
Benzyl alcohol	1.0	0.5
Phenylacetaldehyde	0.2	0.2
1,8-Cineole	0.5	0.9
Limonene	0.1	0.2
(E)-Ocimene	2.2	1.9
Methyl benzoate	43.2	56.9
Linalol	2.6	3.8
Phenylethyl alcohol	0.4	0.3
Benzyl cyanide	1.0	1.1
Linalol oxide (pyran)	0.3	0.2
Methyl salicylate	1.8	1.8
Phenylethyl acetate	0.1	0.2
Indole	40.0	26.9
Eugenol	3.2	1.0
α-Copaene	0.4	0.6
(2E,6E)-α-Farnesene	1.2	1.1
(Z)-Hex-3-enyl benzoate	0.5	0.2
Total	99.4	99.1

Table 5.1 The headspace composition of *Stephanotis floribunda* (Apocynaceae) when sampled from a glass flask and Rilsan bag.

The opening to the bags was partially restricted using wire or paperclips and the enclosed air allowed to equilibrate for 30 minutes. Portable air sampling pumps (Ametek S200, 60ml min^{-1}) were used to pump the air around the flower simultaneously through two Tenax traps. Apart from the fragrance cycle experiment, all samples were collected for 24 hours in order to minimise effects caused by changes in chemical composition with time. New bags were used for each sample. "Blanks" were taken from empty Rilsan bags. All flowers were sampled within the first week of anthesis.

The traps were stainless steel tubes (Perkin Elmer, 89mm x 5mm i.d) packed with Tenax TA (Chrompack, 60-80 mesh, 160mg), to give a bed length of approximately 35mm. The packing was held in place with stainless steel gauzes and a retaining spring (Perkin Elmer). Before and after use, traps were sealed with stainless steel end caps. Prior to packing, new batches of Tenax were conditioned with nitrogen for 24 hours (60ml min^{-1}, 300°C), using a glass GC column (SGE, 2.74m x 4mm i.d). The back pressure of each trap was monitored at regular intervals using a flow controlled supply of nitrogen (60ml min^{-1}) and a water manometer. Traps with similar back-pressures were paired. This ensured that the same amount of headspace would be pulled through each during the sampling process. Four traps (two pairs) were used for each flower, either by using two pumps or by sampling on consecutive days. One trap was used to optimise the split flows during thermal desorption, a second for quantitative analysis, the others were for qualitative analysis by mass spectrometry and, if enough sample was present, infra-red spectroscopy (IR).

5.2.2 SAMPLE ANALYSIS

Plant volatiles were transferred to a GC column by thermal desorption. Initially a Perkin Elmer ATD50 was used; subsequently replaced by an ATD400. In all cases, the Tenax traps were heated for five minutes in a stream of helium (220°C, 40ml min^{-1}), allowing the adsorbed volatiles to be transferred to an on- line cold trap (-30°C). At the end of

the desorption period, the cold trap was ballistically heated to 220°C and the volatiles discharged through a fused silica transfer line (150°C) onto the GC columns.

The ATD50 cold trap was a U-shaped stainless steel tube, with the central portion packed with Tenax. That used in the ATD400 was a straight quartz tube (150mm x 3mm i.d) packed with 20mg Tenax. With both designs, the Tenax plug was kept within the confines of the desorption oven by pesticide grade glass wool (Hewlett Packard).

For quantitative analysis, two 50m fused silica capillary columns were connected in parallel to the transfer line using a two-holed graphetised vespel ferrule (SGE). The stationary phases were BP1, a non-polar methyl silica and BP21, which has a similar polarity to Carbowax 20M or FFAP. Quantification was based on area counts from the BP1 column. The BP21 results were used to calculate ratios of co-eluting peaks such as benzaldehyde and α-pinene, or benzyl alcohol, limonene and 1,8-cineole.

Two mass spectrometers were used, a VG 7070H magnetic sector instrument and a Hewlett Packard 5671A MSD, which has a quadropole analyser. The choice of mass spectrometer was based purely on available instrument time. Identification was by comparison with spectral libraries, either BBA's own or commercial libraries: NBS, NIST and Wiley.

Some concentrated samples such as *Lycaste locusta* and *L. aromatica* were also analysed by GC-IR. It was hoped that the IR spectra obtained would provide sufficient functional group information to aid identification of unknowns. The instrument used was a dedicated GC detector (HP5565). The cell was a gold plated light pipe 120mm x 1mm i.d, fitted with KBr windows at each end and connected directly to the end of the GC column. To accommodate the high flow requirement of the light pipe, the GC column had a wider internal diameter (0.32mm) than that used for stand alone GC-MS.

ANALYTICAL CONDITIONS

THERMAL DESORPTION

INSTRUMENT	Perkin Elmer ATD400
DESORPTION	5min, 200°C
COLD TRAP	Tenax, -30°C to 220°C
COLD TRAP SPLITTERS	inlet: 40ml min^{-1}
	outlet: 20ml min^{-1} *
	purge: 2ml min^{-1}
TRANSFER LINE	uncoated fused silica, 150°C

* the outlet splitter was turned off for desorption of traps from less odiferous samples.

GAS CHROMATOGRAPHY

INSTRUMENT	Hewlett Packard 5890A
COLUMNS	50m FSOT BP1 ex SGE, 0.22mm i.d, 0.25µm film
	50m FSOT BP21 ex SGE, 0.22mm i.d, 0.25µm film
PROGRAMME	40°C to 240°C at 3C min^{-1}
DETECTORS	flame ionisation, 275°C
CARRIER GAS	helium, 34psi
INTEGRATION	Hewlett Packard 3365 Chemstation

GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS)

INSTRUMENT	Varian 3400 GC interfaced to a VG 7070H mass spectrometer
or	Hewlett Packard 5890A GC interfaced to a HP 7671A mass seletive detector
COLUMNS	as above
PROGRAMME	as above
CARRIER GAS	as above
SOURCE	electron impact, 70eV, 200°C
ACCELERATING VOLTAGE	4kV (VG7070H only)

GAS CHROMATOGRAPHY - INFRA-RED SPECTROSCOPY (GC-IR)

INSTRUMENT	Hewlett Packard 5890A GC interfaced to a Hewlett Packard 5565 IRD
COLUMN	50m FSOT HP1 ex Hewlett Packard 0.32mm i.d, 0.5µm film
PROGRAMME	as above
DETECTORS	wide band MCT, 750-4000 wave numbers
	Hewlett Packard 5971A MSD (as above)
CARRIER GAS	helium, 20psi
LIGHT PIPE TEMP.	200°C
TRANSFER LINE TEMP.	200°C

Table 5.2 Analytical conditions for floral fragrance analysis

Spectra were acquired at 3 scans sec^{-1}. Reference spectra were acquired from baseline sections of the total response chromatogram (TRC). Three libraries of vapour phase IR spectra were used for on-line searches: BBA, Robertet and EPA. A capillary

open splitter connected the light pipe outlet to the MSD allowing (almost) simultaneous acquisition of mass spectra.

Four sets of experiments were undertaken. To determine the optimum sample collection parameters, the first set concerned the variation of fragrance with time. The second set examined fragrance variation within species, the third, variation between species; and the last, the composition of fragrances from hybrids. Full analytical conditions are listed in Table 5.2.

5.2.3 PHYLOGENETIC ANALYSIS

Parsimony analyses were performed using PAUP version 4.0b2a (Swofford, 1999). The protocol was similar to that adopted for analysing morphological and DNA sequence data, using heuristic searches.

Each primary search comprised 1000 random stepwise addition replicates, with tree bisection-reconnection as the branch swapping algorithm and the Fitch (1971) criterion of unordered states and equal weights. The trees generated were used as starting trees for successive approximations weighting, as described previously. Internal support for "reweighted" trees was assessed from 1000 bootstrap replicates (Felsenstein, 1985) using simple stepwise addition, with NNI as the branch swapping algorithm.

5.3 RESULTS AND DISCUSSION

One of the problems associated with collecting floral fragrances over a 24 hour period is that atmospheric pollutants are collected simultaneously. Completely sealing the flower from the environment was inappropriate as it appeared to cause some stress; condensation built up on the inside of the sampling container and malodours were produced. The following "background" materials were consistently found in blanks and

have been removed from the data matrices: acetone, trichlorotriflouroethane, benzene, toluene, naphthalene, alkylbenzenes with relative molecular masses (RMM) 106 and 120, p-dichlorobenzene and n-alkanes. In addition, hexanal, which was detected in the headspace of many unopened buds, has been omitted.

Although considered an artifact, the alkanes provided a useful internal reference for calculating "Kovats" style retention indices, designated KI*. The time frame for this work meant that more than one pair of columns was employed; actual retention times varied. The use of retention indices compensated for these variations, facilitating construction of the main data table (Appendix 5). In addition, they provided support for MS identifications.

A conventional Kovats index (I_x) assumes constant temperature where the difference in retention time between two n-alkanes with consectutive carbon numbers will increase as the carbon number increases. The formula used is:

$$I_x = 100 \frac{(\log t_x - \log t_z)}{(\log t_{z+1} - \log t_z)} + z$$

where t_x is the retention time of the unknown, t_z the retention time of the n-alkane eluting before, t_{z+1} the retention time of the alkane eluting after and z the number of carbons in the alkane eluting before. For these analyses, the columns were temperature programmed; for the temperature range over which the fragrance compounds eluted, the difference in elution time between consectutive n-alkanes was constant. The formula was therefore modified:

$$KI^* = 100 \frac{(t_x - t_z)}{(t_{z+1} - t_z)} + z$$

With the exception of the fragrance cycle experiment, the results which follow are expressed as percentages of the total fragrance based on GC area; no correction factors have been applied. The reporting threshold was set at 0.1%.

In some of the tables that follow, compounds have been grouped according to their biosynthetic origin. The groups and their associated code are listed in Table 5.3. Unidentified compounds were coded "UK".

Esters of mixed origin such as prenyl benzoate or benzyl hexanoate were coded separately and included in both biosynthetic groups. Exceptions were compounds such as benzyl acetate, whose biosynthesis involved acetylation, methylation, ethoxylation or methoxylation. These are considered primary, ubiquitous metabolic processes and were ignored.

			Code
Fatty acids			FA
Terpenoids	Prenoids		C5
	Monoterpenoids	Acyclics	AC
		Caranes	CA
		Menthanes	ME
		Pinanes	PI
		Thujanes	TH
	Sesquiterpenoids		SQ
	Ionones		IO
Aromatics	Benzenoids		BE
	Phenylpropanoids		PR
Mixed esters	Prenoid / Fatty acid		F5
	Benzenoid / Fatty acid		BF
	Prenoid / Benzenoid		B5
Nitrogen compounds			NI

Table 5.3 A key to the biosynthetic groups identified in fragrances of *Lycaste* and *Anguloa*.

5.3.1 VARIATION OF FRAGRANCE WITH TIME

The fragrance of euglossine pollinated *Stanhopea pulla* changes qualitatively and quantitatively with time, with maximum fragrance production in the morning, after dawn

(Hills,1989). To see whether *Lycaste* species behaved in the same way, samples of fragrance from a *L. aromatica* flower were collected at hourly intervals during daylight. The results, presented graphically in Figure 5.13 show significant variation in the levels of both (E)-ocimene and 1,8-cineole, suggesting that for short sampling times, the time of day at which samples are collected is important.

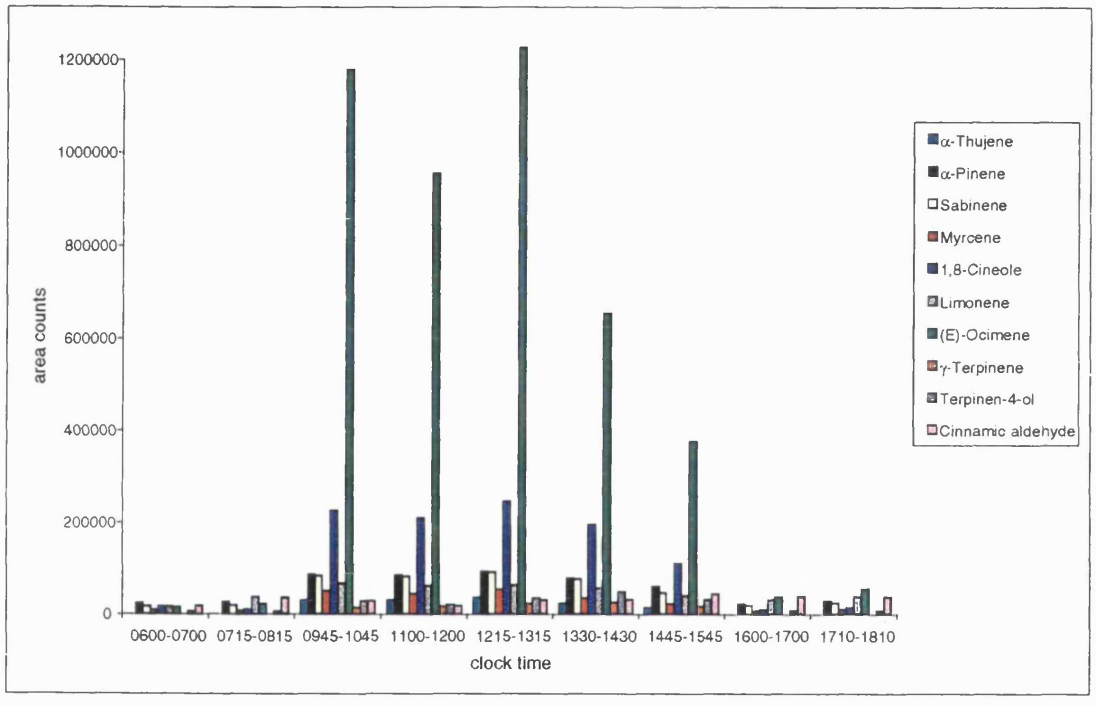


Figure 5.13 The daytime fragrance cycle of *Lycaste aromatica* (raw area counts).

These results are based on raw GC area counts and may have been affected by variations in the performance of both the instruments and the traps. Normally, an internal standard would compensate for such fluctuations, however the logistics of finding an appropriate standard, determining the appropriate concentration and the optimum method for introducing it onto the trap made this route impractical.

As a compromise, use of toluene, a degradation product of Tenax, was considered. Consistent amounts of toluene were released from the ATD cold trap each time it was fired. Its use as a reference would eliminate variations in area count due to instrument

performance. These results, Figure 5.14, illustrate a similar trend to those based on raw area counts.

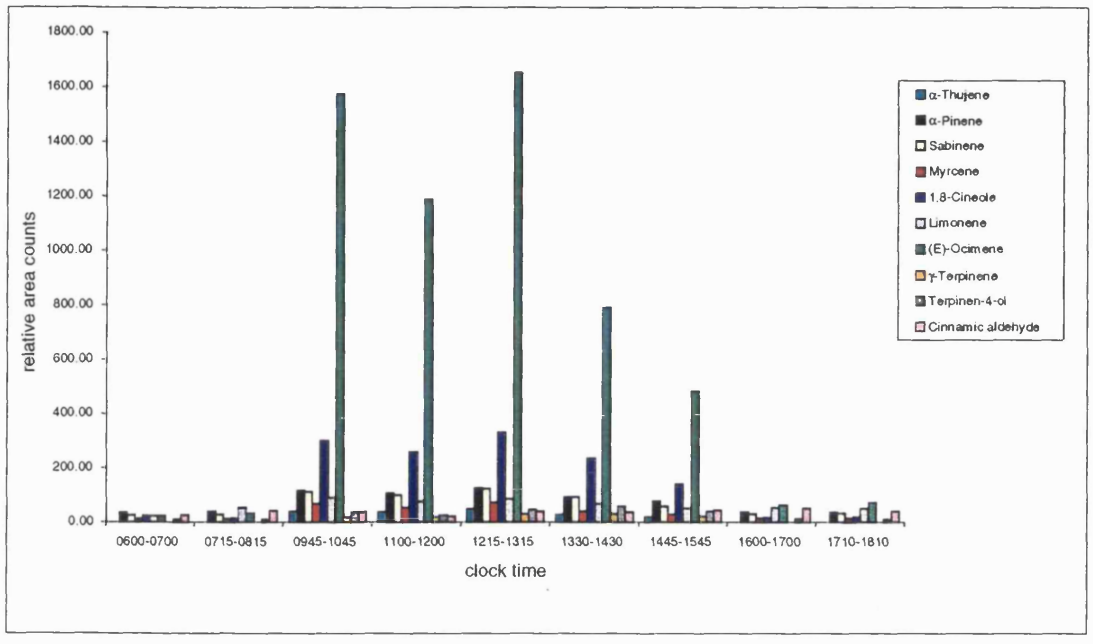


Figure 5.14 The daytime fragrance cycle of *Lycaste aromatica* (area counts relative to toluene).

Table 5.4 shows the variation in fragrance composition of a more typical *L. aromatica* during the first seven days of anthesis. The mean and standard deviation have been calculated for each chemical. The results are presented graphically in two formats: Figure 5.15 "A" illustrates quantitative variation of the major compounds, "B" that of groups of compounds derived by the same biosynthetic pathway.

The sample collected on Day 1 had a lower (E)-cinnamic aldehyde content than the others. Samples from Day 2 to Day 7 were quantitatively similar, indicating that the day on which the sample is collected is less crucial than for genera such as *Stanhopea* (Hills, 1989).

	Code	Day1	Day2	Day3	Day4	Day5	Day6	Day 7	Mean	Std Dev
α-Thujene	TH	0.3	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.07
α-Pinene	PI	0.7	0.3	0.3	0.4	0.4	0.3	0.2	0.4	0.14
Benzaldehyde	BE	1.6	1.5	1.5	1.4	1.2	1.3	1.2	1.4	0.17
Sabinene	TH	1.5	0.9	0.8	1.1	1.0	0.9	0.6	1.0	0.28
β-Pinene	PI	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.04
Myrcene	AC	1.7	1.4	1.3	1.4	2.0	1.8	2.0	1.7	0.29
α-Terpinene	ME	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.07
p-Cymene	ME	0.6	0.5	1.0	1.0	0.9	1.0	0.9	0.8	0.20
1,8-Cineole	ME	4.4	2.0	1.8	2.6	2.1	2.1	1.4	2.4	0.96
Limonene	ME	3.0	2.2	2.4	2.4	2.3	2.7	2.0	2.4	0.31
(Z)-Ocimene	AC	1.2	0.6	0.5	0.5	0.4	0.5	0.4	0.6	0.30
(E)-Ocimene	AC	20.0	15.8	9.6	10.9	17.3	15.1	19.4	15.4	3.97
γ-Terpinene	ME	0.8	0.9	1.4	1.4	1.9	1.9	2.1	1.5	0.50
(Z)-Sabinene hydrate	TH	0.2	0.1	0.2	0.3	0.3	0.3	0.2	0.2	0.06
Methyl benzoate	BE	0.6	1.0	0.9	0.8	0.6	0.7	0.6	0.7	0.15
Terpinolene	ME	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.07
Nonanal	FA	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.06
Linalol	AC	0.7	0.5	0.4	0.4	0.5	0.4	0.4	0.5	0.10
Unknown BP91,119,152	UK	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.3	0.08
2,3-Epoxy-2,6-dimeocta-5,7-diene	AC	0.9	0.7	0.5	0.5	0.5	0.5	0.5	0.6	0.14
Phenylpropylaldehyde	PR	0.2	0.5	0.6	0.5	0.4	0.5	0.4	0.5	0.13
1,4-Dimethoxybenzene	BE	1.6	1.5	1.8	1.8	2.1	1.6	1.4	1.7	0.23
Terpinen-4-ol	ME	2.4	3.3	5.9	5.7	7.7	7.6	7.1	5.7	2.09
Unknown BP95,93,91,150	UK	0.6	0.5	0.2	0.3	0.1	0.1	0.1	0.3	0.21
Methyl salicylate	BE	0.2	0.3	0.2	0.3	0.2	0.2	0.1	0.2	0.05
α-Terpineol	ME	0.3	0.3	0.2	0.4	0.2	0.3	0.2	0.3	0.08
(Z)-Cinnamic aldehyde	PR	7.1	7.1	8.5	7.4	4.2	5.2	3.8	6.2	1.78
Unknown (Terpinyl acetate)	ME	0.3	0.3	0.5	0.3	0.4	0.4	0.4	0.4	0.07
(E)-Cinnamic aldehyde	PR	43.4	53.9	56.0	55.3	51.1	51.4	51.7	51.8	4.19
Unknown BP97,72,41	UK	1.5	0.9	0.8	0.7	0.3	0.7	0.5	0.8	0.39
Methyl 3-phenylpropionate	PR	1.8	0.9	1.1	0.5	0.2	0.6	0.3	0.8	0.56
Unknown Safranal like	UK	0.4	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.08
Benzyl benzoate	BE	0.3	0.4	0.2	0.3	0.3	0.3	0.5	0.3	0.09
Total		99.7	99.9	99.9	99.9	99.8	99.9	99.9		

Fatty acid derivatives (FA)		0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.06
Benzenoids (BE)		4.2	4.6	4.7	4.7	4.3	4.1	3.9	4.4	0.30
Phenylpropanoids (PR)		52.5	62.4	66.3	63.7	55.9	57.7	56.3	59.2	4.93
Acyclic monoterpenes (AC)		24.5	19.1	12.3	13.7	20.6	18.3	22.8	18.8	4.81
Menthanes (ME)		12.4	10.1	13.5	14.1	15.9	16.5	14.5	13.8	2.16
Pinanes (PI)		0.9	0.4	0.4	0.5	0.5	0.4	0.3	0.5	0.18
Thujanes (TH)		2.0	1.2	1.1	1.6	1.6	1.4	1.0	1.4	0.34
Others		3.0	1.9	1.5	1.5	0.8	1.2	0.9	1.5	0.73

Table 5.4 *Lycaste aromatica*: Variation in fragrance composition over a seven day period. 24hr samples collected from 07.06.94 to 14.06.94.

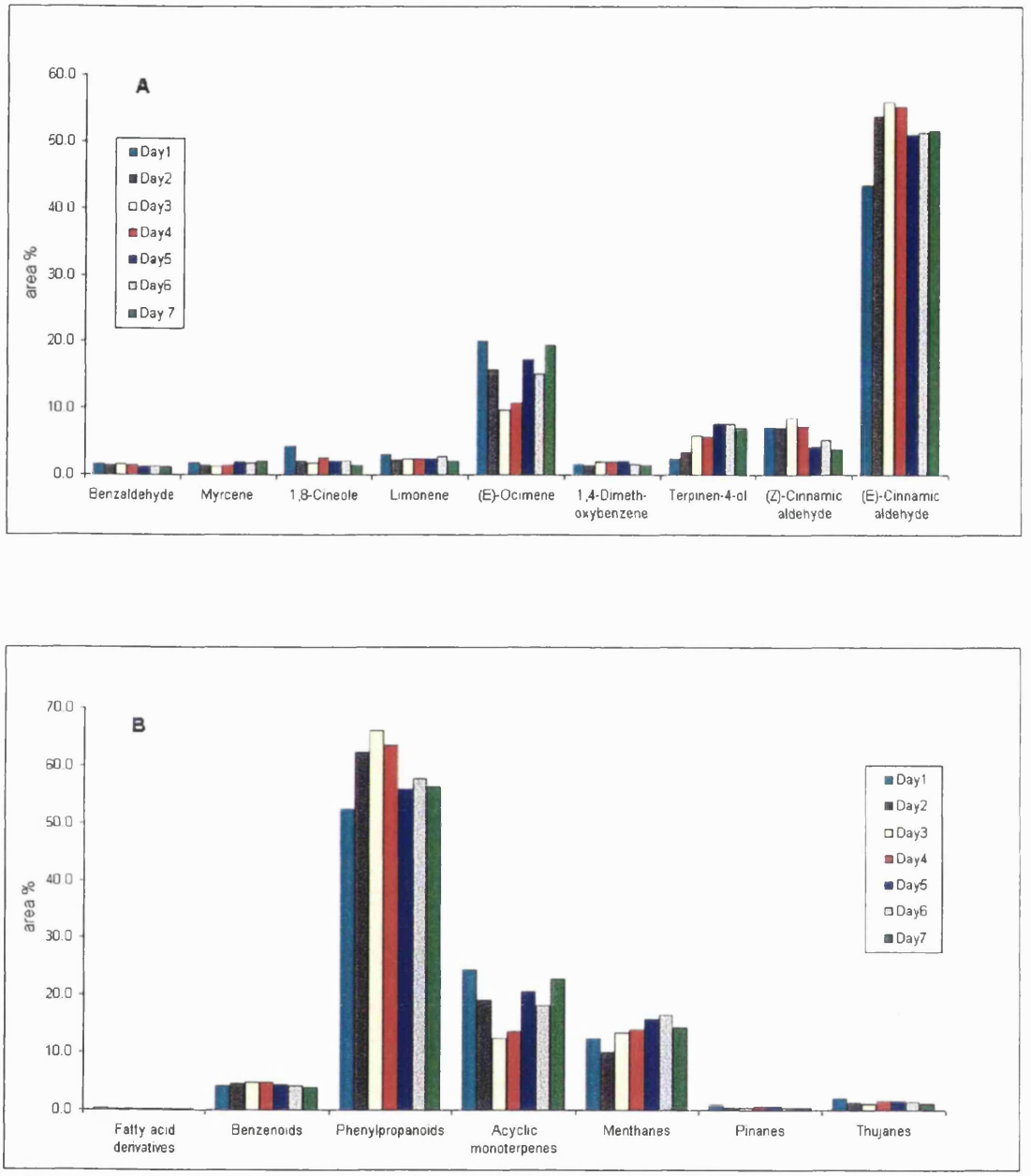


Figure 5.15 *Lycaste aromatica*: Variation in fragrance composition during the first seven days of anthesis. 24hr samples taken from 7.6.94 to 14.6.94.

Anguloa virginalis Linden emits a powerful eucalyptus / methyl salicylate fragrance. The headspace composition of a single flower sampled on two consecutive days is shown in Table 5.5. The results are in agreement with those of Gerlach and Schill (1991) identifying 1,8-cineole (eucalyptol) as the major component. Methyl salicylate

was present, but at a much lower level. The results also show variation in the percentage composition of the two major components.

	Area%	
	1	2
(2-Methylbut-3-en-2-ol)	0.2	0.0
α-Thujene	0.2	0.1
α-Pinene	2.7	1.3
Benzaldehyde	0.2	0.3
Sabinene	2.7	2.0
β-Pinene	0.8	0.5
Myrcene	16.5	26.1
α-Phellandrene	0.3	0.1
p-Cymene	0.1	0.2
Phenylacetaldehyde	0.1	0.2
1,8-Cineole	69.1	60.6
Limonene	2.4	2.1
(E)-Ocimene	0.3	0.3
γ-Terpinene	0.3	0.2
(E)-Sabinene hydrate	0.2	0.2
Methyl benzoate	0.6	1.7
Terpinolene	0.2	0.2
Linalol	1.6	2.6
Phenylethyl alcohol	0.1	0.1
Benzyl cyanide	0.6	0.5
Methyl salicylate	0.1	0.2
α-Terpineol	0.0	0.1
Verbenone	0.2	0.2
Carvone	0.0	0.1
Phenylethyl ester	0.1	0.0
Farnesol like	0.1	0.1
Total	99.9	100.0

Table 5.5 Fragrance composition of *Anguloa virginalis* Linden: replicate analyses from the same flower.

5.3.2 INFRASPECIES FRAGRANCE VARIATION

Fragrances from seven different *Lycaste aromatica* plants were analysed. The results, presented in Table 5.6 and Figure 5.16, show major quantitative differences, which are reflected both when considering each chemical individually and when grouping together those derived by the same biosynthetic route.

	Code	1	2	3	4	5	6	7	Mean	Std dev
α-Thujene	TH	0.5	0.9	1.0	0.9	0.2	1.4	1.3	0.9	0.42
α-Pinene	PI	0.6	2.6	0.8	0.7	0.3	2.1	2.7	1.4	1.04
Benzaldehyde	BE	1.2	0.3	0.9	0.2	1.5	0.2	4.2	1.2	1.42
Sabinene	TH	0.9	1.0	1.0	1.3	0.9	2.0	4.3	1.6	1.24
β-Pinene	PI	0.1	0.4	0.1	0.1	0.1	0.1	0.4	0.2	0.15
Myrcene	AC	2.3	1.9	0.7	1.4	1.4	1.3	0.6	1.4	0.60
α-Terpinene	ME	1.8	0.4	1.2	0.2	0.0	0.6	0.0	0.6	0.68
p-Cymene	ME	1.5	0.5	0.9	0.4	0.5	1.2	2.4	1.1	0.71
1,8-Cineole	ME	1.4	15.7	2.4	1.7	2.0	4.2	7.9	5.0	5.21
Limonene	ME	2.4	1.6	1.0	0.7	2.2	0.5	1.4	1.4	0.73
(Z)-Ocimene	AC	0.3	0.4	0.4	0.6	0.6	0.4	0.0	0.4	0.20
(E)-Ocimene	AC	48.0	41.8	52.4	68.6	15.8	55.2	1.3	40.4	23.63
γ-Terpinene	ME	3.3	0.7	2.4	0.5	0.9	1.0	0.5	1.3	1.09
Sabinene hydrate (E)- & (Z)-	TH	0.9	0.2	1.1	0.4	0.1	0.3	0.0	0.4	0.42
Methyl benzoate	BE	3.8	0.2	0.1	0.1	1.0	0.1	0.5	0.8	1.35
Terpinolene	ME	0.6	0.2	0.5	0.2	0.4	0.2	0.0	0.3	0.19
(3,5-Dimethylcyclohex-2-en-1-one)	FA	0.0	0.2	0.0	0.0	0.0	0.3	0.0	0.1	0.12
Nonanal	FA	0.1	0.3	0.2	0.4	0.2	0.3	1.7	0.5	0.56
Linalol	AC	0.6	1.0	0.2	0.6	0.5	0.4	0.0	0.5	0.33
Unknown BP91,150	UK	0.0	0.3	0.2	0.0	0.0	0.9	0.0	0.2	0.33
Unknown BP91,119,134	UK	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.06
2,3-Epoxy-2,6-dimethylocta-5,7-diene	AC	0.6	0.4	0.7	0.4	0.7	0.4	0.5	0.5	0.15
Phenylpropylaldehyde	PR	0.0	0.2	0.0	0.0	0.5	0.0	0.0	0.1	0.19
1,4-Dimethoxybenzene	BE	0.2	0.1	0.7	0.3	1.5	0.5	0.4	0.5	0.47
Benzoic acid	BE	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.2	0.53
Terpinen-4-ol	ME	9.9	1.5	7.1	1.3	3.3	3.3	2.6	4.1	3.19
Unknown BP95,93,91,150	UK	0.1	0.3	0.0	0.4	0.5	0.5	0.5	0.3	0.20
Methyl salicylate	BE	0.0	0.1	0.0	0.0	0.3	0.2	0.0	0.1	0.12
α-Terpineol	ME	0.3	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.14
(Z)-Cinnamic aldehyde	PR	0.5	5.7	3.0	8.3	7.1	2.4	33.0	8.6	11.12
(E)-Cinnamic aldehyde	PR	16.9	19.1	18.4	7.6	53.9	4.0	30.1	21.4	16.63
Unknown (Safranal like)	UK	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.2	0.42
Unknown BP97,72	UK	0.0	0.7	0.1	0.0	0.9	1.3	0.0	0.4	0.54
Carboxylic acid	FA	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.2	0.49
Methyl 3-phenylpropionate	PR	0.0	0.0	0.5	0.9	0.9	0.0	0.0	0.3	0.43
(Z)-Methyl cinnamate	PR	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.4	1.13
Unknown BP43,125,168	UK	0.0	0.1	0.1	0.0	0.0	0.2	0.0	0.1	0.08
Eugenol	PR	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.1	0.08
(E)-Methyl cinnamate	PR	0.2	0.0	0.2	0.0	0.0	7.4	0.0	1.1	2.77
Unknown (Safranal like)	UK	0.0	0.0	0.0	0.2	0.0	0.4	0.0	0.1	0.16
Unknown (Safranal like)	UK	0.3	0.5	0.3	0.7	0.2	1.8	0.0	0.5	0.60
Benzyl benzoate	BE	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.15
Total		99.7	99.7	98.9	99.6	99.4	99.5	99.4		

Fatty acid derivatives (FA)	0.1	0.5	0.2	0.4	0.2	0.6	3.0	0.7	1.2
Benzenoids (BE)	5.3	0.6	1.7	0.6	4.7	1.0	6.5	2.9	4.0
Phenylpropanoids (PR)	17.6	25.1	22.2	17.0	62.4	16.8	63.1	32.0	32.4
Acyclic monoterpenes (AC)	51.9	45.5	54.3	71.6	19.0	57.7	2.4	43.2	24.9
Menthanes (ME)	21.1	20.6	15.5	5.0	9.6	11.0	14.8	13.9	11.9
Pinanes (PI)	0.6	3.0	0.9	0.8	0.4	2.2	3.1	1.6	1.2
Thujanes (TH)	2.3	2.1	3.2	2.6	1.2	3.7	5.6	3.0	2.1
Others	0.8	2.2	0.9	1.6	1.9	6.5	0.9	2.1	2.4

Table 5.6 *Lycaste aromatica*: Variation in fragrance composition of seven different specimens.

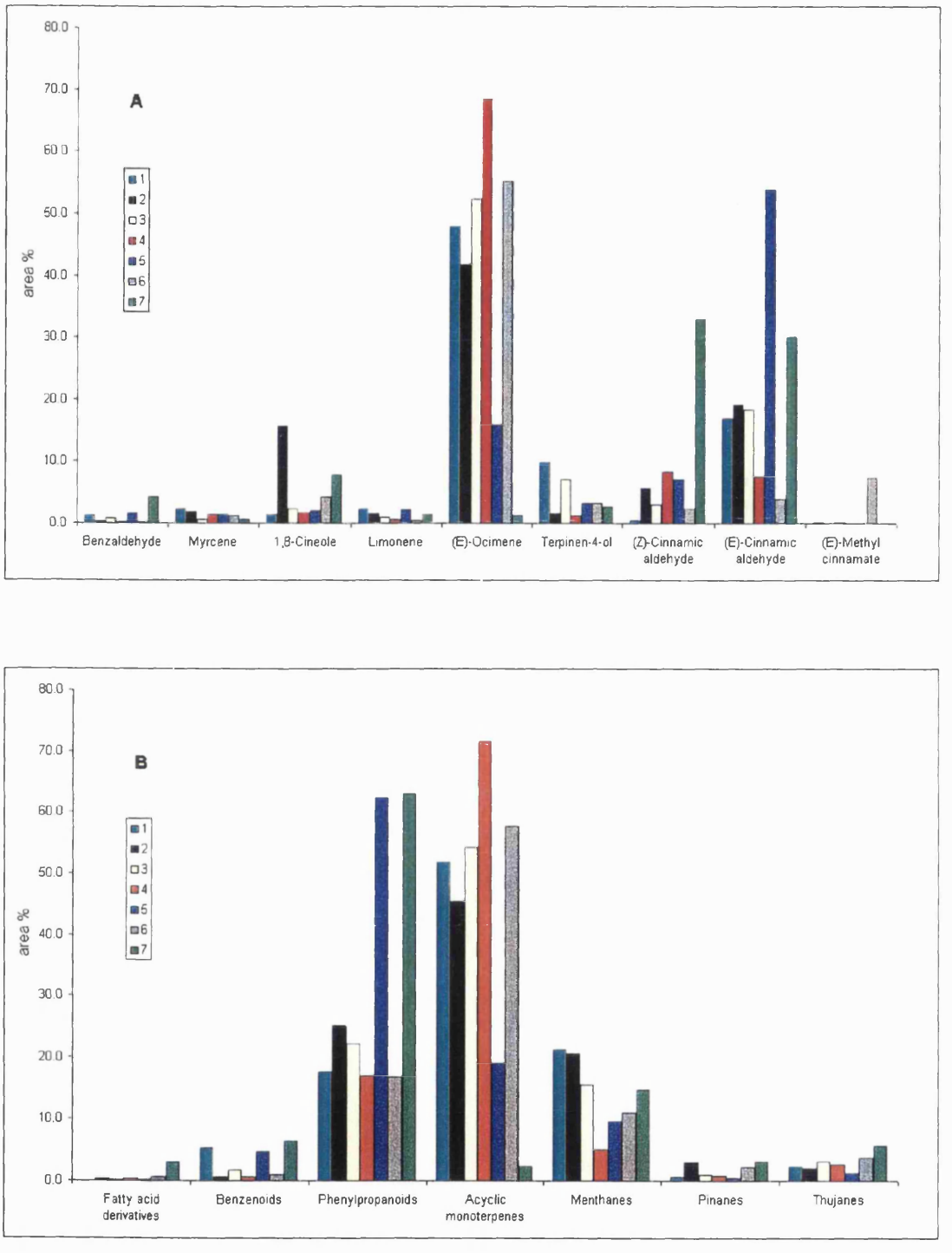


Figure 5.16 *Lycaste aromatica*: Variation in fragrance composition of seven different specimens.

Lycaste aromatica flowers smell strongly of cinnamon, hence the species name and also the Mexican local name for the plants, "canela". Fragrances from all seven individuals showed high levels of cinnamic aldehyde, with the (E) isomer ranging from 4% to 54%. The (Z) isomer was detected in all the samples (0.5% - 33%), but the Z/E

ratio was not constant. Other compounds with a wide variation in composition were the acyclic monoterpenoid (E)-ocimene and the menthanes 1,8-cineole and terpinen-4-ol.

Two of the samples, numbers "4" and "6" were from Santo Domingo, a wooded area on the outskirts of Huatusco, Vera Cruz. In this locality *L. aromatica and L. deppei* co-exist with some overlap in the flowering period. Although the two samples had a low cinnamic aldehyde content compared to the other five, no sesquiterpenoids were detected and there was no increase to the levels of myrcene, limonene or 1,4-dimethoxybenzene, all of which characterise the fragrance of *L. deppei.*

Fragrances from seven different *Lycaste deppei* plants showed similar quantitative variation (Table 5.7, Figure 5.17). The fragrance compositions are characterised by high levels of myrcene, limonene, 1,4-dimethoxybenzene, and the sesquiterpenoids germacrene-D and (2E,6E)-α-farnesene. To the human nose, the predominant note is that of methyl benzoate, which smells like marshmallow.

5.3.3 INTERSPECIES FRAGRANCE VARIATION

Fragrances were collected from 48 specimens, representing 22 *Lycaste* and six *Anguloa* species and subspecies. A selection of chromatograms is given in Appendix 6.

The full numerical data set is shown in Appendix 5. The table lists 252 chemicals in BP1 retention index order. "Trivial" names have been used for all the terpenoids; their chemical structures are shown in Figures 5.18 and 5.19. The term "unknown" is either followed by the MS base peak and major fragment ions or by the name of a compound with a similar mass spectrum; tentative identifications are in parentheses. Note that compound 168 was identified as an artefact and has been removed.

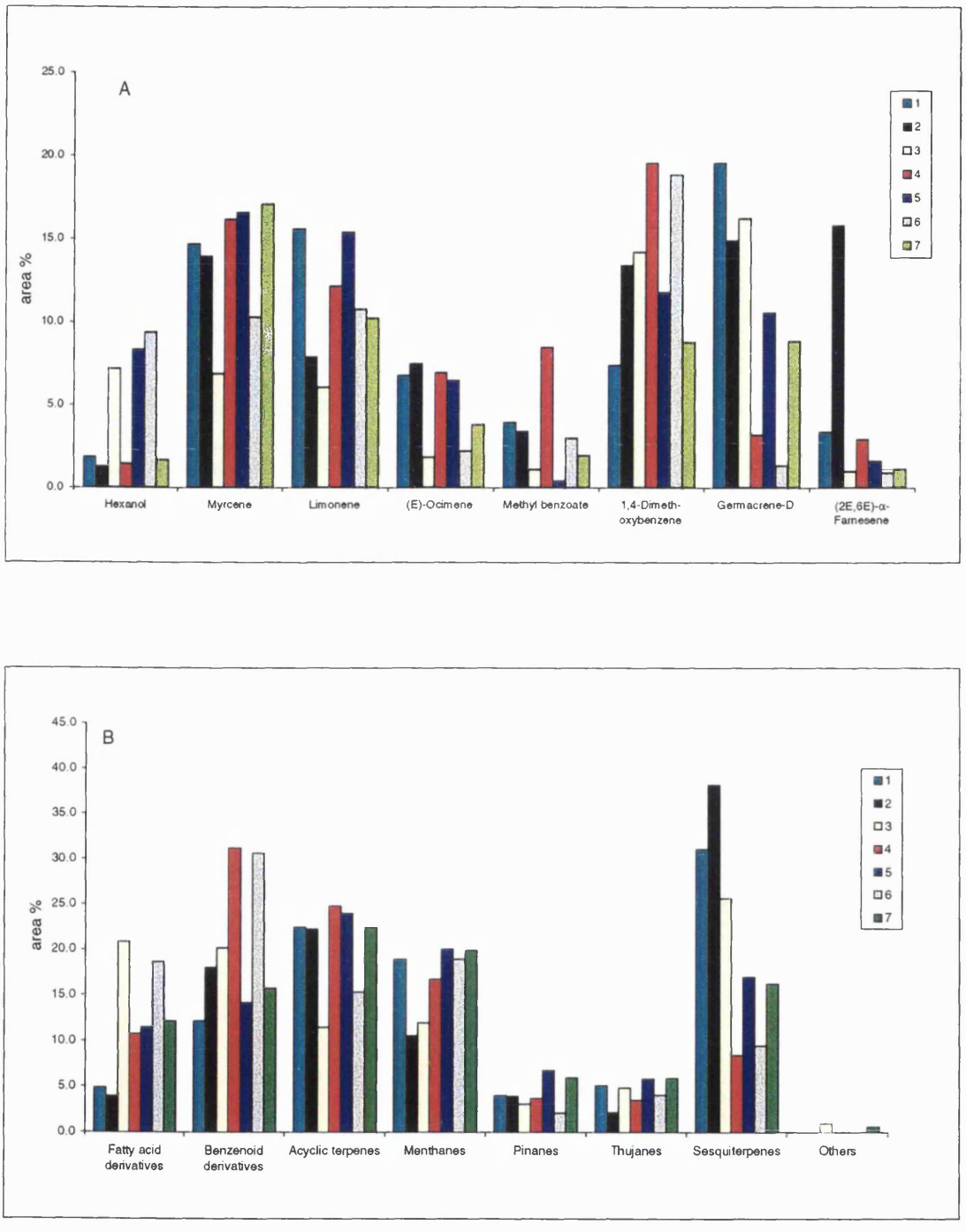


Figure 5.17 *Lycaste deppei*: Variation in fragrance composition of seven different specimens.

	Code	1	2	3	4	5	6	7	Mean	Std Dev
2-Methylbut-3-en-2-ol	FA	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.23
Valeraldehyde	FA	0.2	0.4	2.7	8.0	0.2	2.2	0.3	2.0	2.83
Pent-2-enal & alkane	FA	0.2	0.0	1.7	0.0	0.0	0.0	0.0	0.3	0.63
Methyl 2-methylbutyrate	FA	1.0	0.2	0.4	0.1	0.1	0.3	0.4	0.3	0.30
Hex-3-enol-cis	FA	0.0	0.1	0.5	0.0	0.3	0.2	0.0	0.2	0.18
Methyl pent-2-enoate (Me tiglate?)	FA	0.4	0.4	4.8	0.2	0.1	2.4	5.3	1.9	2.27
Hexanol	FA	1.8	1.3	7.2	1.5	8.4	9.4	1.7	4.5	3.67
α-Thujene	TH	1.2	0.7	0.5	0.9	1.6	0.8	1.0	1.0	0.37
α-Pinene	PI	2.5	2.8	1.7	2.6	4.4	1.3	4.3	2.8	1.19
Benzaldehyde	BE	0.1	0.1	0.6	0.2	0.1	1.3	0.6	0.4	0.44
6-Methyl hept-5-en-2-one	FA	0.3	0.5	0.6	0.4	0.4	1.7	1.6	0.8	0.59
Sabinene	TH	1.7	0.9	3.7	1.3	2.1	1.9	3.1	2.1	0.97
β-Pinene	PI	1.6	1.2	1.5	1.2	2.4	0.8	1.7	1.5	0.50
Myrcene	AC	14.7	14.0	6.9	16.2	16.6	10.3	17.1	13.7	3.76
α-Phellandrene	ME	0.0	0.2	0.2	0.1	0.1	0.0	0.2	0.1	0.08
α-Terpinene	ME	0.1	0.1	0.5	0.3	0.1	0.8	1.7	0.5	0.58
Benzyl alcohol	BE	0.2	1.0	1.3	1.7	1.2	2.4	0.4	1.2	0.74
p-Cymene	ME	0.3	0.4	0.9	0.3	0.9	1.5	1.0	0.7	0.43
1,8-Cineole	ME	0.2	0.4	0.9	0.2	0.3	0.5	1.2	0.5	0.39
Limonene	ME	15.6	7.9	6.1	12.2	15.4	10.8	10.3	11.2	3.55
β-Phellandrene	ME	0.2	0.4	0.7	0.3	0.2	0.1	1.0	0.4	0.33
(Z)-Ocimene	AC	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.04
(E)-Ocimene	AC	6.8	7.5	1.9	7.0	6.5	2.2	3.8	5.1	2.40
γ-Terpinene	ME	0.2	0.2	0.2	0.3	0.3	0.7	0.0	0.3	0.20
Sabinene hydrate (E)- & (Z)-	TH	2.2	0.6	0.7	1.3	2.2	1.5	1.8	1.3	0.51
Octanol	FA	0.1	0.1	1.6	0.2	0.6	0.0	0.7	0.5	0.56
Methyl benzoate	BE	4.0	3.4	1.1	8.5	0.4	3.0	2.0	3.2	2.66
Terpinolene	ME	0.4	0.3	0.0	0.3	0.5	0.0	0.3	0.3	0.19
Nonanal	FA	0.2	0.4	0.9	0.3	0.9	1.4	1.3	0.8	0.49
Linalol	AC	0.5	0.5	2.1	0.7	0.5	1.7	0.9	1.0	0.66
Phenylethyl alcohol	BE	0.0	0.1	2.5	0.1	0.3	1.7	1.9	1.0	1.04
1,4-Dimethoxybenzene	BE	7.4	13.4	14.2	19.6	11.8	18.9	8.8	13.4	4.64
Terpinen-4-ol	ME	0.2	0.1	1.2	0.4	0.1	0.9	1.6	0.6	0.59
α-Terpineol	ME	1.7	0.6	1.1	2.2	1.9	3.3	2.4	1.9	0.88
Decanal	FA	0.2	0.2	0.7	0.3	0.7	1.0	0.9	0.6	0.34
Nerol	AC	0.1	0.1	0.3	0.5	0.1	0.6	0.3	0.3	0.22
Carvone	ME	0.1	0.1	0.3	0.3	0.2	0.6	0.3	0.3	0.17
Methyl geranate	AC	0.1	0.0	0.2	0.1	0.0	0.3	0.0	0.1	0.10
1,2,4-Trimethoxybenzene	BE	0.3	0.1	0.2	0.2	0.0	0.8	0.0	0.2	0.27
Methyl anisate	BE	0.1	0.0	0.2	1.0	0.4	2.7	2.1	0.9	1.07
α-Copaene	SQ	0.2	0.2	0.4	0.1	0.2	0.4	0.2	0.2	0.11
β-Bourbonene	SQ	0.1	0.1	0.1	0.1	0.2	1.5	1.1	0.5	0.60
β-Elemene	SQ	0.4	0.3	0.4	0.2	0.2	0.4	0.4	0.3	0.10
Caryophyllene	SQ	2.9	1.6	0.6	0.2	0.4	0.7	0.6	1.0	0.94
Geranyl acetone	AC	0.2	0.1	0.3	0.1	0.1	1.0	0.2	0.3	0.34
(E)-β-Farnesene	SQ	1.1	1.8	1.2	0.6	1.1	0.7	1.2	1.1	0.39
α-Humulene	SQ	0.8	0.5	0.2	0.1	0.2	0.2	0.2	0.3	0.25
Alloaromadendrene	SQ	0.4	0.3	0.4	0.1	0.3	0.2	0.3	0.3	0.09
ar-Curcumene	SQ	0.1	0.1	0.3	0.1	0.1	0.4	0.4	0.2	0.13
Germacrene-D	SQ	19.6	14.9	16.3	3.2	10.6	1.4	8.9	10.7	6.76
β-Selinene	SQ	0.6	0.3	0.7	0.1	0.4	0.2	0.0	0.3	0.22
(2E,6E)-α-Farnesene	SQ	3.4	15.8	1.0	2.9	1.6	0.9	1.2	3.8	5.37
β-Bisabolene	SQ	0.9	1.5	3.0	0.5	1.0	1.1	1.0	1.3	0.82
γ-Cadinene	SQ	0.1	0.1	0.2	0.0	0.1	0.0	0.0	0.1	0.07
δ-Cadinene	SQ	0.3	0.5	0.3	0.1	0.4	0.2	0.3	0.3	0.11
Caryophyllene oxide	SQ	0.1	0.1	0.7	0.1	0.2	0.2	0.4	0.2	0.22
Total		99.0	99.3	98.3	99.5	99.4	99.5	98.7		

Table 5.7 *Lycaste deppei*: Variation in fragrance composition of seven different specimens.

	1	2	3	4	5	6	7	Mean	Std Dev
Fatty acid derivatives (FA)	4.9	4.0	20.9	10.8	11.5	18.7	12.2	11.9	6.33
Benzenoid derivatives (BE)	12.2	18.1	20.2	31.2	14.2	30.7	15.8	20.3	7.70
Acyclic monoterpenoids (AC)	22.5	22.3	11.5	24.8	24.0	15.4	22.5	20.4	4.96
Menthanes (ME)	19.0	10.6	12.0	16.8	20.1	19.0	20.1	16.8	3.93
Pinanes (PI)	4.0	4.0	3.1	3.8	6.8	2.1	6.0	4.3	1.62
Thujanes (TH)	5.2	2.2	4.9	3.6	5.9	4.1	5.9	4.5	1.35
Sesquiterpenes (SQ)	31.1	38.2	25.7	8.5	17.0	9.5	16.3	20.9	11.17

Table 5.7 cont. *Lycaste deppei*: Variation in fragrance composition of seven different individuals.

For comparative purposes, the taxa have been divided into three groups: *Lycaste* sect. *Fimbriatae* are first, followed by sects. *Deciduosae* and *Lycaste*, and finally *Anguloa*.

Fragrances of *Lycaste* appear to be species specific but there are no obvious patterns of biosynthetic pathways linking all species of a section. For example, the fragrance of *L. ciliata* contained about 50% linalol, whereas those of the *L. locusta* specimens contained almost no monoterpenoids. Prenyl compounds were detected in fragrances of *L. locusta* and *L. cinnabarina* but not in any of the other species. Some of the compounds detected are known to be chiral, but separation of enantiomers was not practical. GC-IR was found to be of limited use. Spectra are acquired in the vapour phase and although carbonyl groups were easily detected, alcohols were not.

The major components of the fragrance of *Anguloa virginalis* were the monoterpenoids myrcene and 1,8-cineole. The other species of the genus were characterised by high levels of benzenoids. 1,4-Dimethoxybenzene was identified as the dominant compound in fragrances of *A. cliftonii*, *A. clowesii*, *A. hohenlohii* and *A. eburnea*. *A. brevilabris* was characterised by high levels of methyl benzoate, methyl salicylate and methyl-2-methoxybenzoate.

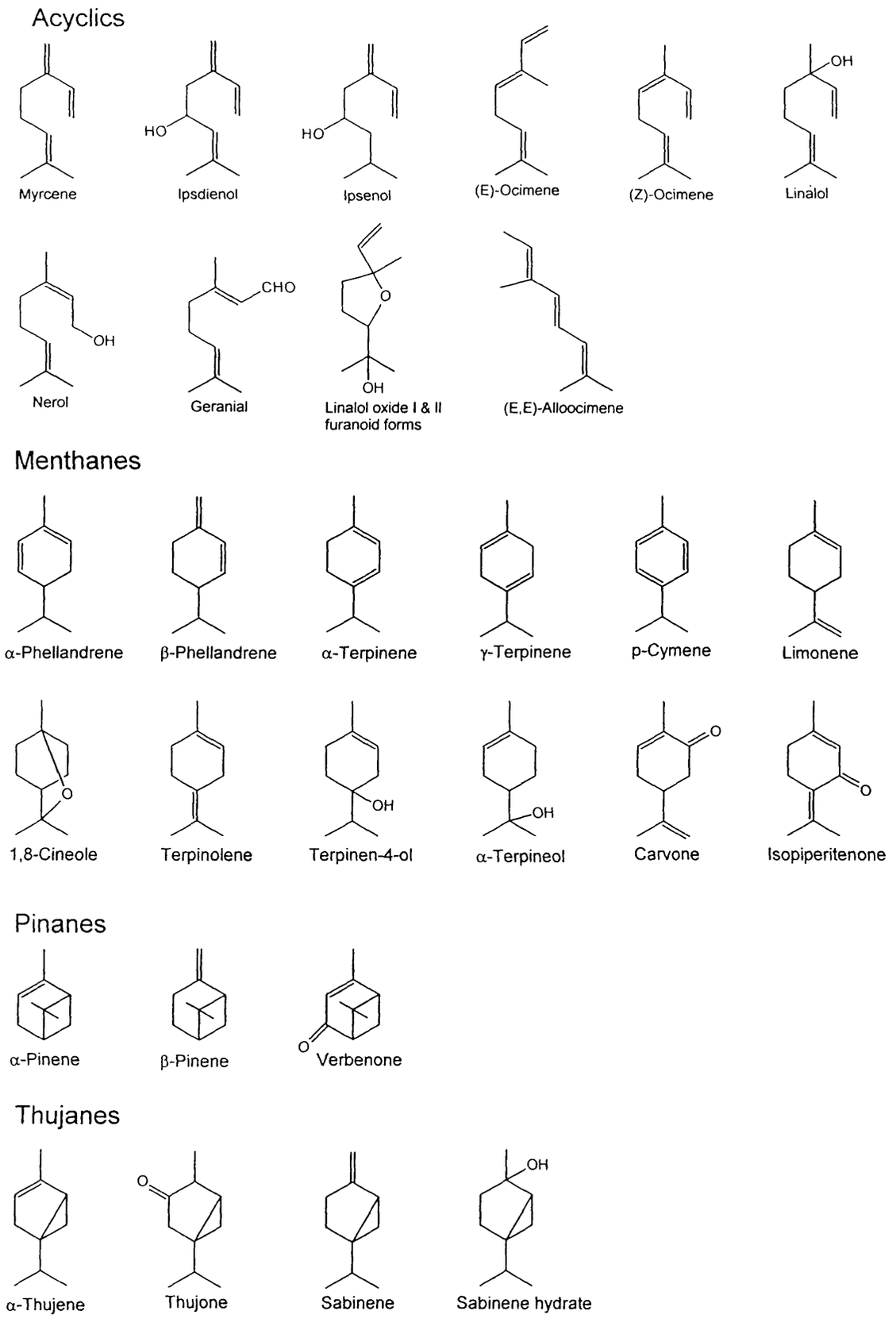


Figure 5.18 Monoterpenoids from the floral fragrances of *Lycaste* and *Anguloa*.

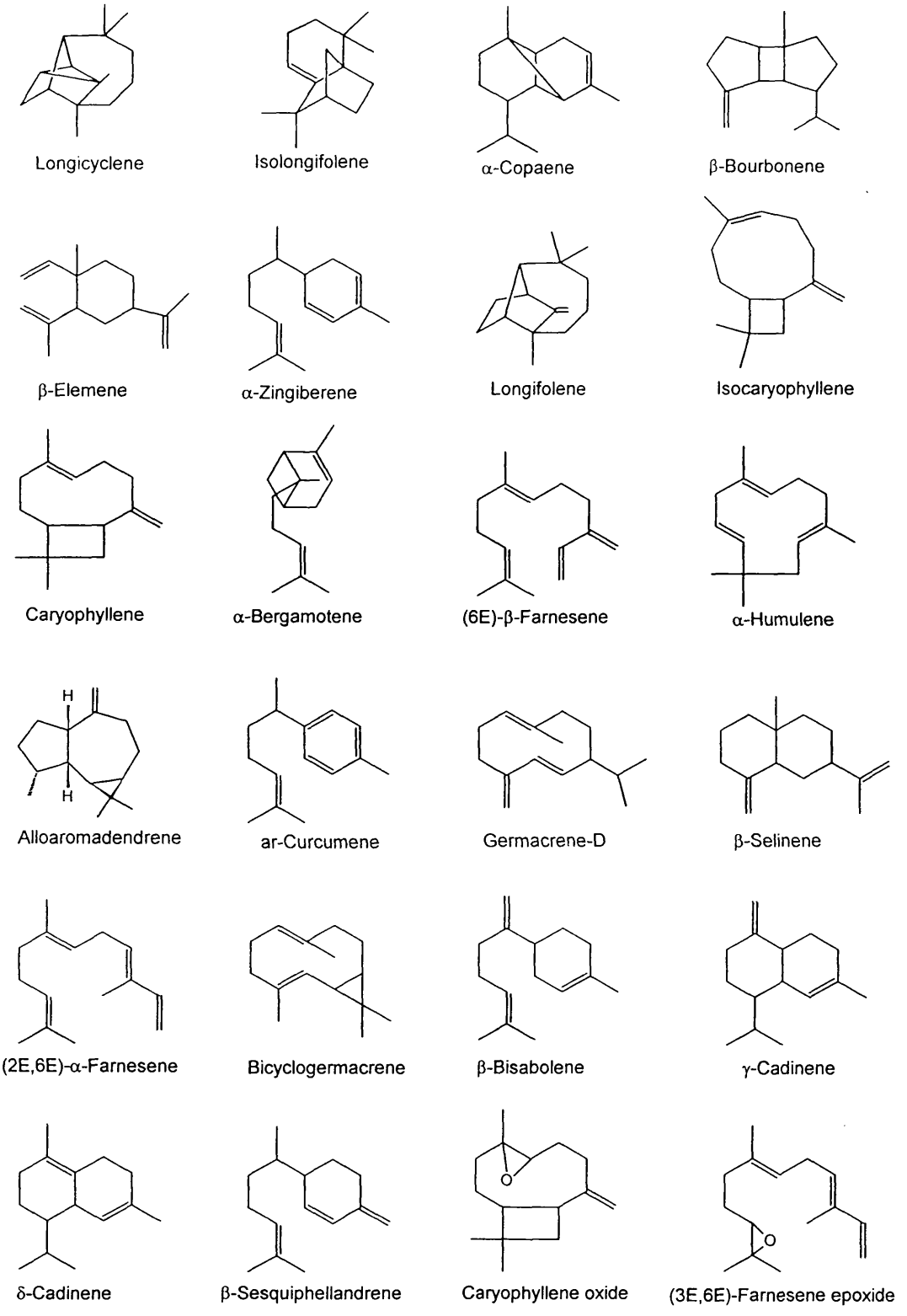


Figure 5.19 Sesquiterpenoids from the floral fragrances of *Lycaste* and *Anguloa*.

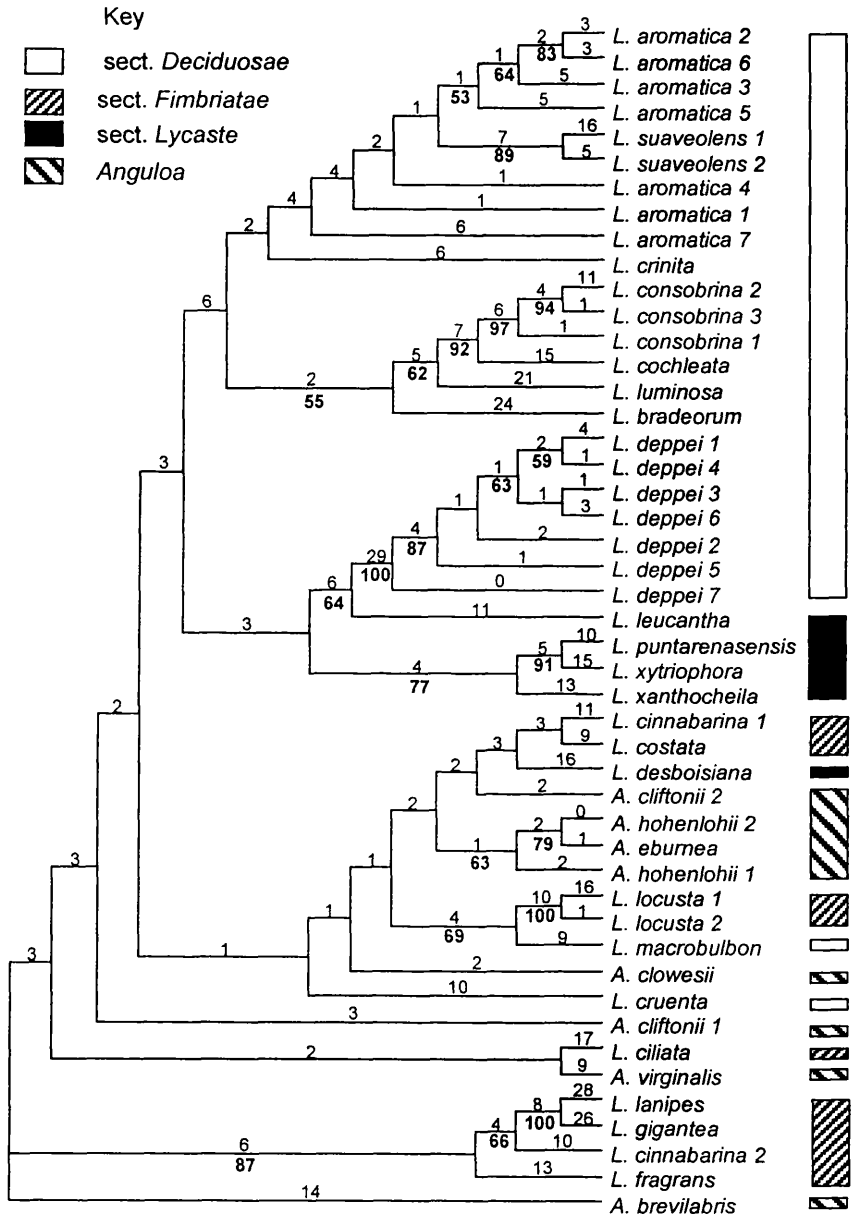


Figure 5.20 One of the 45 successively weighted most parsimonious trees showing cladistic relationships within *Lycaste* and *Anguloa*, based on individual floral fragrance compounds. Characters coded 0,1.

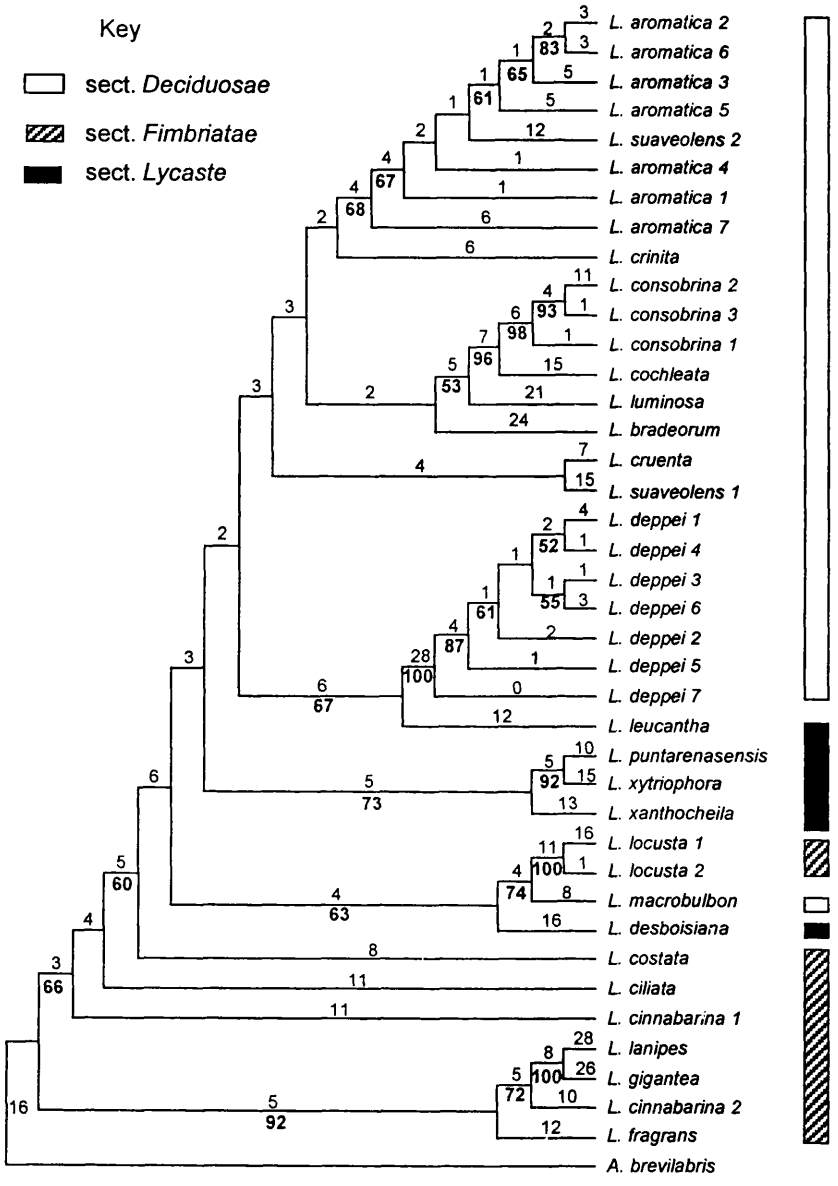


Figure 5.21 One of the three successively weighted most parsimonious trees showing cladistic relationships within *Lycaste*, based on individual floral fragrance compounds. Characters coded 0,1.

5.3.4 PHYLOGENETIC ANALYSIS

Data from Appendix 5 was used as the starting point for the analyses that follow. In each case the data has been coded for presence/absence (0,1).

Of the 252 characters identified in Appendix 5, 150 were potentially parsimony informative. An initial analysis using the method described in Chapter 2.2 yielded 54 equally parsimonious trees with a length of 554 steps, CI of 0.45 and RI of 0.65. Successive weighting again found 45 trees of length with a Fitch length of 554 steps, CI of 0.81 and RI of 0.85. One of these trees is shown in Figure 5.20. The eight specimens of *Anguloa* were specified as the outgroup.

The tree topology differs from that obtained by morphological and molecular analysis in that neither *Anguloa* nor *Lycaste* sect. *Fimbriatae* have been identified as monophyletic groups. Species of both are distributed through several clades. In most instances, specimens of the same species have been grouped together. Exceptions are *L. cinnabarina* and *A. cliftonii*. The two specimens of *L. cinnabarina* were very different and may be pollinated by different species of bee. In the case of *A. cliftonii*, the separation reflects the difference in number of compounds exceeding the GC-MS detection limit.

The analysis was repeated for the 28 specimens of *Lycaste*, with *A. brevilabris* specified as the outgroup. One hundred and eighty six equally parsimonius trees were found, of length 524 steps, CI 0.48 and RI of 0.65. Successive reweighting yielded three trees of length 17685 (Fitch length 524), CI 0.83 and RI 0.86. One of these is shown in Figure 5.21. Again, sect. *Fimbriatae* was not recognised as a monophyletic group.

This is not the first time that a disparity between molecular and fragrance analysis has been identified. Williams and Whitten (1999) recorded similar findings for *Stanhopea*. In both cases the characters used have been individual fragrance compounds.

One of the disadvantages of the coding system adopted here is that no segregation has been made between compounds representing a large proportion of the fragrance and those at trace levels.

Barkman (2001) has recently advocated the use of "step-matrices" in which independent biogenetic pathways are considered as characters and related enzymatic conversions the character states. He has suggested that such an approach would result in cladistic relationships that are more congruent than those obtained from DNA studies. This may indeed be true but current understanding of these enzymatic processes may not be sufficient to code these pathways correctly. Terpene synthases have been shown to catalyse formation of multiple products. Colby *et al.* (1993) have shown that (-)-4S-limonene synthase from peppermint and spearmint will produce small amounts of α-pinene, β-pinene and myrcene as well as limonene. Steele *et al.* (1998) have shown that δ-selinene synthase from *Abies grandis* will catalyse the synthesis of 34 sesquiterpenoids.

5.3.4 THE FRAGRANCE COMPOSITION OF HYBRIDS

The ideal scenario for any study of this nature would be the propagation of small populations of hybrids from vouchered parents, together with a record of the identity of the pod and seed parents. As *Lycaste* and *Anguloa* hybrids typically take from three to five years to develop to flowering size, this approach was not feasible.

	A. cliftonii	*A.* x *rolfei*	*A. brevilabris*
Valeraldehyde			0.2
Methyl hexanoate		0.1	1.0
α-Pinene	0.9	0.3	2.1
Benzaldehyde	0.7	0.4	0.3
Sabinene	0.5	0.3	0.5
β-Pinene	0.4		0.5
Myrcene	1.0	0.6	0.1
Methyl heptanoate			0.3
1,8-Cineole	10.0	1.9	2.3
Limonene	2.8	2.8	0.6
(E)-Ocimene	1.2	0.4	
(E)-Sabinene hydrate		0.1	
Methyl benzoate	2.3	22.3	27.7
(Z)-Sabinene hydrate		0.1	
Linalol	0.1	0.4	0.2
Phenylethyl alcohol			0.3
Benzyl cyanide		0.1	1.5
Limonene epoxide		0.3	
2,3-Epoxy-2,6-dimethy-octa-5,7-diene		0.1	
Benzyl acetate		1.3	2.8
1,4-Dimethoxybenzene	78.0	1.2	
Ethyl benzoate		0.3	
Methyl salicylate	1.4	38.2	8.8
Verbenone		0.1	
Anisaldehyde	0.6		
Phenylethyl acetate			0.6
Benzyl propionate			0.2
Methyl 3-phenylpropionate		5.0	0.3
Benzyl isobutyrate			0.3
Methyl 2-methoxybenzoate		3.4	12.5
Benzyl butyrate			0.6
(E)-Methyl cinnamate		20.0	35.1
Longifolene			0.2
Phenylethyl butyrate			0.2
Ethyl cinnamate		0.2	
Total	99.9	99.9	99.2

Table 5.8 Fragrance composition of *Anguloa* x *rolfei* (*A. cliftonii* x *A. brevilabris*), sampled from 03.08.92 to 04.08.92.

Tables 5.8 to 5.14 show the fragrance composition of seven hybrids together with example analyses of their putative parents, taken from Tables 5.6, 5.7 and Appendix 5. Average values have been used for parent species such as *Lycaste aromatica*, for which more than one specimen was sampled.

Anguloa x *rolfei* Sander (Plate 5.1A) was originally described as a natural hybrid of *A. cliftonii* and *A. ruckeri* (Rolfe, 1915). In the same year, Rolfe (1915) amended the parentage to *A. cliftonii* x *A. brevilabris*. The fragrance composition, shown in Table 5.8 certainly supports this change, with *A. brevilabris* contributing the high levels of methyl benzoate, methyl salicylate and (E)-methyl cinnamate. However, the level of 1,4-dimethoxybenzene is low compared with that of *A. cliftonii*.

	A. uniflora	*A. uniflora* x *A. clowesii*	*A. clowesii*	*A. virginalis*
α-Thujene		0.1		0.1
α-Pinene		1.3	0.6	1.3
Benzaldehyde		0.9	0.5	0.3
Sabinene		1.3	0.5	2.0
β-Pinene		0.4	0.2	0.5
Myrcene		6.9	1.0	26.1
α-Phellandrene		0.1		0.1
p-Cresyl methyl ether		0.2	0.4	
α-Terpinene		0.1		
p-Cymene		0.1		0.2
1,8-Cineole		32.2	7.7	60.6
Limonene		0.7	2.2	2.1
(E)-Ocimene		0.1		0.3
γ-Terpinene		0.1		0.2
(E)-Sabinene hydrate		0.1		0.2
Methyl benzoate		0.3		1.7
Terpinolene				0.2
Linalol				2.6
Benzyl cyanide				0.5
1,4-Dimethoxybenzene	100.0	54.5	86.8	
Methyl salicylate		0.6	0.1	0.2
Total	100.0	100.0	100.0	99.2

Table 5.9 Fragrance composition of the artificial hybrid *Anguloa uniflora* x *A. clowesii* and its putative parents sampled from 07.07.93 to 08.07.93

Reichenbach (1882) described *Anguloa* x *dubia* as the natural hybrid *A. clowesii* x *A. uniflora*. This combination was subsequently registered in the Sander's List by Veitch

(1910). *Anguloa dubia* is now considered a valid species (Schlechter 1916, Oakeley 1999). The plant used for this work (Plate 5.1B) is an artificial hybrid of *A. uniflora* x *A. clowesii*, made by Marcel Lecoufle. The fragrance composition, shown in Table 5.9 shows high levels of myrcene and 1,8-cineole suggesting that its true parentage is actually *Anguloa virginalis x A. clowesii.*

	L. aromatica	*L. x hartleyi*	*L. suaveolens*
α-Pinene	1.4	0.5	0.6
Benzaldehyde	1.2	0.1	0.6
Sabinene	1.6	0.3	0.9
β-Pinene	0.2	0.1	0.3
Myrcene	1.4	0.9	0.5
1,8-Cineole	5.0	5.2	0.6
Limonene	1.4	1.5	0.3
(Z)-Ocimene	0.4	0.2	
(E)-Ocimene	40.4	38.1	17.8
Guaiacol		0.2	0.2
Methyl benzoate	0.8		1.4
6-Methylhepta-3,5-dien-2-one		0.2	0.1
3,4-Epoxy-3,7-dimethylocta-1,6-diene		0.2	
Linaloi	0.5	0.2	0.2
Unknown BP91,43		0.3	
1,2-Dimethoxybenzene		0.9	2.0
Unknown BP91,119,134	0.3	0.1	
2,3-Epoxy-2,6-dimethylocta-5,7-diene	0.5	0.3	
1,4-Dimethoxybenzene	0.5	1.8	7.0
Unknown BP95,93,91,150	0.3	0.4	
Terpinen-4-ol	4.1		2.2
α-Terpineol	0.1	0.1	
(Z)-Cinnamic aldehyde	8.6		0.2
Unknown		0.2	
(Crysanthenyl acetate)		0.3	
(E)-Cinnamic aldehyde	21.4		3.0
p-Menth-1,3-dien-7-al		1.0	
Methyl 3-phenylpropionate	0.3	9.7	7.5
(Z)-Methyl cinnamate	0.4	0.4	0.1
Unknown BP43,55,95,125		0.1	
Unknown BP43,55,95,125		0.2	
Eugenol	0.1	9.2	1.3
(E)-Methyl cinnamate	1.1	26.0	50.8
Safranal like	0.5	0.9	
Total	92.5	99.6	97.6

Table 5.10 Fragrance composition of *Lycaste* x *hartleyi* Oakeley (*L. aromatica* x *L. suaveolens*), sampled April 1994.

Oakeley (1993) considers *L.* x *hartleyi* (Plate 5.2A) to be a natural hybrid of *Lycaste aromatica* x *L. suaveolens*. The fragrance composition, presented in Table 5.10, is characterised by high levels of 1,8-cineole, (E)-ocimene, methyl 3-phenylpropionate, eugenol and (E)-methyl cinnamate. The three aromatics, methyl 3-phenylpropionate, eugenol and (E)-methyl cinnamate, are all present at significant levels in *Lycaste suaveolens*. Of the two major monoterpenoids, (E)-ocimene is found at high levels in both putative parents, whereas 1,8-cineole is more characteristic of *L. aromatica*. Other compounds that characterise *L. aromatica* fragrance, cinnamic aldehyde and terpinen-4-ol, although found in *L.* Groganii, were not detected in this hybrid.

Lycaste Groganii (*L. aromatica* x *L. deppei*; Plate 5.2B) described by Rolfe (1904) and also by Cooper (1931), has a fragrance which reflects both parent plants as shown in Table 5.11. The high levels of (E)-ocimene and (E)-cinnamic aldehyde are attributable to *Lycaste aromatica*, whereas myrcene, limonene and the sesquiterpene hydrocarbons are inherited from *L. deppei*.

Table 5.12 shows the fragrance composition of *Lycaste* Wyld Forest (*L. locusta* x *L.* Groganii; Plate 5.2C) registered by Oakeley (1992). It shows high levels of the terpenoids myrcene, limonene and (E)-ocimene, all of which are present in significant quantities in *Lycaste* Groganii. The sesquiterpenoids α- and β-farnesene and β-bisabolene have all been identified in *Lycaste deppei* fragrances (a parent of *L.* Groganii), although at higher levels. Evidence of *L. locusta* parentage is provided by the esters butyl acetate, butyl butyrate, hexyl acetate, benzyl acetate, butyl benzoate and prenyl benzoate. However, two of the major components of *L. locusta* fragrance, ethyl acetate and diethyl carbonate, were not detected.

	L. aromatica	*L.* Groganii	*L. deppei*
2-Methylbut-3-en-2-ol		0.1	0.1
Valeraldehyde			2.0
Methyl pent-2-enoate			1.9
Hexanol			4.5
α-Thujene	0.9	1.0	1.0
α-Pinene	1.4	0.5	2.8
Benzaldehyde	1.2	0.9	0.4
Sabinene	1.6	1.7	2.1
β-Pinene	0.2	0.2	1.5
Myrcene	1.4	8.9	13.7
α-Phellandrene		0.1	0.1
α-Terpinene	0.6	0.3	0.5
p-Cymene	1.1	0.3	0.8
Benzyl alcohol		0.3	1.2
1,8-Cineole	5.0	0.2	0.5
Limonene	1.4	9.0	11.2
(Z)-Ocimene	0.4	0.1	0.3
(E)-Ocimene	40.4	44.5	5.1
γ-Terpinene	1.3	0.8	0.3
(E)-Sabinene hydrate	0.4	1.0	1.3
Guaiacol		0.1	
Methyl benzoate	0.8	18.2	3.2
Terpinolene	0.3	0.4	0.3
Nonanal	0.5		0.8
Linalol	0.5	1.7	1.1
Phenylethyl alcohol			1.0
1,2-Dimethoxybenzene		0.2	
Unknown BP91,119,134	0.3	0.3	
2,3-Epoxy-2,6-dimethylocta-5,7-diene	0.5	0.1	
1,4-Dimethoxybenzene	0.5	0.6	13.5
Terpinen-4-ol	4.1	1.5	0.6
Unknown BP95,93,91,150	0.3	0.4	
α-Terpineol	0.1	0.3	1.9
Methyl chavicol		0.2	
(Z)-Cinnamic aldehyde	8.6	0.1	
Nerol		0.1	0.3
Anisaldehyde		0.1	
(E)-Cinnamic aldehyde	21.4	4.2	
Unknown BP41,53,72,97	0.4	0.3	
Methyl anisate		0.2	0.9
Unknown (Safranal like)	0.5	0.1	
Caryophyllene		0.2	1.0
(E)-β-Farnesene		0.1	1.1
α-Humulene		0.1	0.3
Germacrene-D			10.9
(2E,6E)-α-Farnesene		0.4	3.9
β-Bisabolene		0.1	1.3
Total	96.1	99.9	93.4

Table 5.11 Fragrance composition of *Lycaste* Groganii (*L. aromatica* x *L. deppei*), sampled from 24.05.93 to 25.05.93.

	L. locusta	*Lycaste* Wyld Forest	*L.* Groganii
Ethyl acetate	68.7		
2-Methylbut-3-en-2-ol		0.4	0.1
Diethyl carbonate	5.5		
Ethyl butyrate	1.8		
Butyl acetate	7.8	0.5	
Prenyl acetate-I & II	3.5		
α-Thujene		1.6	1.0
α-Pinene	0.5	2.9	0.5
Benzaldehyde	0.2	4.2	0.9
Sabinene		2.6	1.7
β-Pinene		1.6	0.2
Butyl butyrate	0.8	3.2	
Myrcene		7.4	8.9
Ethyl hexanoate	1.5		
Hexyl acetate	1.2	0.1	
α-Terpinene		0.2	0.3
p-Cymene		0.3	0.3
Benzyl alcohol		1.6	0.3
1,8-Cineole		0.3	0.2
Limonene	0.2	23.5	9.0
β-Phellandrene		0.2	
(Z)-Ocimene		0.3	0.1
(E)-Ocimene		32.8	44.5
Prenyl butyrate-I & II	3.9		
γ-Terpinene		0.4	0.8
(E)- & (Z)-Sabinene hydrate		1.5	1.0
Methyl benzoate			18.2
Terpinolene		0.7	0.4
Linalol		0.6	1.7
1,2-Dimethoxybenzene			0.2
Unknown BP9,119,134		0.2	0.3
2,3-Epoxy-2,6-dimethylocta-5,7-diene		0.2	0.1
Benzyl acetate	0.4	1.4	
Ethyl benzoate	0.2		0.6
Terpinen-4-ol		0.9	1.5
Unknown BP95,93,91,150			0.4
Prenyl tiglate	0.5		
Butyl hexanoate	0.3		
α-Terpineol		4.5	0.3
Methyl chavicol			0.2
Geraniol		0.2	
(Z)- & (E)-Cinnamic aldehyde		0.2	4.3
Safranal like		0.3	0.1
Unknown BP97,72,43		0.4	0.3
Prenyl hexanoate-I & II	1.1		
Benzyl butyrate		0.6	
Methyl anisate			0.2
Butyl benzoate	0.3	0.7	
Safranal like		0.2	
Caryophyllene			0.2
(E)-β-Farnesene		0.2	0.1
Prenyl benzoate-I & II	0.2	0.1	
(2E,6E)-α-Farnesene		0.4	0.4
β-Bisabolene		0.1	0.1
Benzyl benzoate		1.5	
Total	98.6	99.0	99.4

Table 5.12 Floral fragrance composition of *Lycaste* Wyld Forest *(L. locusta* x *L.* Groganii), sampled from 05.04.95 to 06.04.95.

	A. clowesii	*Angulocaste* Whatcroft	*L. cruenta*
α-Pinene	0.6	1.0	0.6
Benzaldehyde	0.5	0.1	0.1
Sabinene	0.5	0.6	0.3
β-Pinene	0.2	0.3	0.2
Methyl 2-hydroxy-3-methylpentanoate			0.3
Myrcene	1.0	1.3	0.6
p-Cresyl methyl ether	0.4		
Car-3-ene		0.1	
α-Terpinene		0.1	
p-Cymene		0.2	
1,8-Cineole	7.7	12.3	9.7
Limonene	2.2	3.0	1.4
(Z)-Ocimene		0.1	0.1
(E)-Ocimene		14.7	14.7
Nonanal		0.2	
Linalol		0.1	
1,2-Dimethoxybenzene		0.4	0.4
1,4-Dimethoxybenzene	86.8	60.7	2.1
Unknown BP95,93,91,150		0.1	0.2
Methyl salicylate	0.1	0.6	
Decanol		0.1	
p-Menth-1,3-dien-7-al		0.3	0.3
Methyl 3-phenylpropionate			8.3
Unknown		0.4	
(Z)-Methyl cinnamate			2.6
Eugenol		2.6	4.2
1,2,4-Trimethoxybenzene		0.3	
(E)-Methyl cinnamate			53.5
Methyl eugenol		0.5	
Unknown Safranal like		0.2	0.3
Total	100.0	100.0	99.9

Table 5.13 Fragrance composition of *Angulocaste* Whatcroft (*A. clowesii* x *L. cruenta*), Sampled from 17.07.92 to 19.07.92

The intergeneric hybrid *Angulocaste* Whatcroft (Plate 5.3A) was registered by Dunning (1965) as *Anguloa clowesii* x *Lycaste macrobulbon*. It emits a strong, spicy, clove-like odour. Oakeley (1991) considers it identical to the older *Angulocaste* Joiceyii (*Lycaste cruenta* x *Anguloa clowesii*), registered by Sanders in 1922. The fragrance composition, shown in Table 5.13, is rich in 1,8-cineole, (E)-ocimene, 1,4-dimethoxybenzene and eugenol, suggesting that the *Lycaste* parent is in fact *L. cruenta*.

	A. clowesii	*Angulocaste* Wyld Dragon	*L. locusta*
Ethyl acetate			68.7
Valeraldehyde + alkane		1.1	
Methyl 2-methylbutyrate		0.6	
Diethyl carbonate			5.5
Ethyl butyrate			1.8
Butyl acetate			7.8
Prenyl acetate-I & II			3.5
Unsaturated hydrocarbon		0.4	
α-Pinene	0.6	2.3	0.5
Benzaldehyde	0.5	29.5	0.2
Sabinene	0.5		
(β-Pinene)	0.2	0.5	
Butyl butyrate			0.8
Myrcene	1.0		
Ethyl hexanoate			1.5
Octanal		0.2	
Hexyl acetate			1.2
p-Cresyl methyl ether	0.4		
Benzyl alcohol		1.8	
p-Cymene		0.4	
1,8-Cineole	7.7	0.9	
Limonene	2.2	0.8	0.2
Prenyl butyrate-I & II			3.9
Methyl benzoate		15.9	
Nonanal		0.8	
Limonene epoxide-I & II		0.5	
Benzyl acetate		21.1	0.4
1,4-Dimethoxybenzene	86.8	17.3	
Ethyl benzoate		0.8	0.2
Methyl salicylate	0.1	0.6	
Prenyl tiglate			0.5
Butyl hexanoate			0.3
Verbenone		0.4	
Propyl benzoate		0.4	0.1
Benzyl propionate		0.2	
Prenyl hexanoate-I & II			1.1
Benzyl butyrate		0.2	
1,2,4-Trimethoxybenzene		1.7	
1-Hydroxy-2,2,4-trimepent-3-yl 2-mepropionate		0.3	
Butyl benzoate		0.1	0.3
3-Hydroxy-2,2,4-trimepent-1-yl 2-mepropionate		0.2	0.1
Longifolene		0.3	
Prenyl benzoate-I		0.4	0.3
Prenyl benzoate-II		0.1	
Total	100	99.8	98.9

Table 5.14 Fragrance composition of *Angulocaste* Wyld Dragon (*A. clowesii* x *L. locusta)*, sampled from 25.5.93 to 26.5.93.

Angulocaste Wyld Dragon (Plate 5.3B) was registered by Oakeley (1995) as *A. clowesii* x *L. locusta.* The fragrance is characterised by high levels of benzaldehyde, methyl benzoate, benzyl acetate and 1,4-dimethoxybenzene, as shown in Table 5.14. As with *Lycaste* Wyld Forest, ethyl acetate and diethyl carbonate were not detected. Clues to *L. locusta* parentage are the presence of ethyl propionate, benzyl acetate, butyl benzoate and prenyl benzoate.

These analyses demonstrate that fragrance is inherited from both parents. They also show that past confusion in assigning names to plants has caused errors when registering hybrid parents.

PLATE 5.1

Hybrids of *Anguloa*

A. *Anguloa* x *rolfei*

B. *A. uniflora* x *A. clowesii*


B


A

PLATE 5.2

Hybrids of *Lycaste*

A. *Lycaste* x *hartleyi*

B. *Lycaste* Groganii

C. *Lycaste* Wyld Forest


A


B


C

PLATE 5.3

Angulocaste hybrids

A. *Angulocaste* Whatcroft

B. *Angulocaste* Wyld Dragon


A


B

Chapter 6 CONCLUSIONS

Parsimony analysis has been used to examine the phylogenetic relationships of *Lycaste* and *Anguloa*. Data from three different sources was evaluated: Morphological examination, comparison of DNA sequences from two regions, ITS and *matK*, and analysis of the floral fragrance composition.

The most conclusive results came from the molecular analysis, which showed that *Lycaste* was paraphyletic and that species currently ascribed to sect. *Fimbriatae* are closer to *Anguloa* than to the other sections of *Lycaste*. This was corroborated by a combined analysis of ITS and morphological data. Within subtribe Lycastinae, *Neomoorea* was identified as the nearest neighbour to *Lycaste* and *Anguloa*. *Maxillaria* and *Cryptocentrum* were identified as being more closely related to *Lycaste* and *Anguloa* than the Lycastinae genera, *Bifrenaria* and *Rudolfiella*. Further sampling of these genera will be necessary to clarify their taxonomic status. Neither of the DNA regions selected for this work provided sufficient resolution to clarify relationships within the other three sections of *Lycaste*. Selection of a faster evolving region of DNA or use of a fingerprinting technique such as AFLPs will be required.

Forty seven morphological characters were selected for cladistic analysis. The results again show *Lycaste* sect. *Fimbriatae* to be separated from the other species of the genus. The analysis was less robust than that of either ITS or *matK* and removal of a single character was shown to affect the topology of the final tree. As with the sequence data, there was insufficient resolution to clarify relationships amongst the other species of the genus. Subjectivity is inherent in this type of analysis. However the quality of the result could be improved by increasing the number of characters. Examination of the micro-morphology of vegetative parts, such as the structure of the roots, might provide the source for these.

Both morphological and molecular analyses placed the single pendent species *Lycaste dyeriana* closer to *Lycaste* and *Anguloa* than to *Bifrenaria*, as had been proposed by Fowlie (1970). They also showed clear separation between the subspecies of *L. macrophylla*. The floral fragrance compositions of these subspecies also differ and their taxonomic status may need to be reviewed.

The floral fragrance composition of 22 species of *Lycaste* and six species of *Anguloa* was determined. Fragrance emission from *L. aromatica* does not exhibit the complex diurnal rhythms previously identified in *Stanhopea* (Hills, 1989) and the composition appears to stay constant during the first seven days of anthesis. This makes the time of sampling less critical than for other genera.

The floral fragrances of *Lycaste* are species specific. However, quantitative variation was observed in species for which multiple sampling was possible (*L. aromatica*, *L. deppei* and *L. consobrina*). Two hundred and fifty two compounds were identified from the fragrances of both genera and some of these, such as 1,8-cineole, benzyl acetate, methyl salicylate and eugenol, are recognised as attractants for euglossine bees (e.g. Williams and Dodson, 1972).

Parsimony analysis of individual fragrance compounds resulted in a different set of relationships from that of either the morphological or molecular data set. In most instances, specimens of the same species were grouped together. However neither *Anguloa* nor *Lycaste* sect. *Fimbriatae* were recognised as monophyletic groups. Williams and Whitten (1999) have suggested that such analyses reflect pollinator selection. Little is known of the pollinators of these genera and this may be an area for future investigation.

Cladistic analysis is concerned with character state changes and for the fragrance work two states were recognised, presence or absence. Barkman (2001) has suggested the use of "step matrices" to code compounds according their position along a biosynthetic chain. Current understanding of the necessary stages is restricted. However, Raguso and co-workers (e.g. Raguso and Pichersky, 1999) are exploring the activity of various synthases using *Clarkia breweri* as a model system. It is hoped that their findings will also be relevant to the Orchidaceae.

Analysis of the floral fragrances of hybrids suggests that fragrance is biparentally inherited. This could have a horticultural application for confirming the identity of putative parents.

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Appendix 1 A CLASSIFICATION OF *LYCASTE* AND *ANGULOA* SPECIES

after Schlechter (1916), Fowlie (1970) and Oakeley (1993,1994).

The distribution and locality data cited below was obtained from herbarium specimens at AMES, AMO, BM, CR, K, K-LINDL., MA, W, USJ and from illustrations in the following: Atwood (1989), Bennett & Christenson (1995), Dodson & Dodson (1982), Vásquez & Dodson (1989), Fowlie (1970), Hamer (1985). An exclamation mark (!) has been used to indicate specimens seen. Collections at AMES and MA were viewed in microfiche format; those at Herb. Vasq., LASCA and SEL have been seen as published illustrations only.

***Lycaste* Lindl.** in *Bot. Reg.* 29: misc. 14 (1843); Bentham and Hooker, *Genera Plantarum* 3: 547 (1880-83); De Wolfe in *Amer Orchid Soc. Bull.* 22: 334 (1953); Fowlie, *The Genus Lycaste*: 7 (1970); Oakeley, *Lycaste Species: the Essential Guide* (1993). Type species: *Lycaste plana* Lindl. in *Bot. Reg.* 28: misc. 85 (1842) *nom. nud.* and in *Bot. Reg.* 29: 15, t. 35 (1843).

I. **Sect. *Deciduosae*** Fowlie, *The Genus Lycaste*: 7 (1970), *nom. nud.* No type specified.

A. Subsect. *Xanthanthae* Fowlie, *The Genus Lycaste*: 7 (1970), *nom. nud.* Type species: *Lycaste aromatica* (Graham ex Hook.) Lindl.

1. *Lycaste aromatica* (Graham ex Hook.) Lindl. in *Bot. Reg.* 29: misc. 16 (1843). Type: illustration in Hook., *Exotic Flora* 3: t. 219 (1826).
Maxillaria aromatica Graham in *Edinb. N. Phil. J.* 1: 173-174 (1826) *nom. nud.*; Graham ex Hook. in Hook., *Exot. Fl.* 3: t. 219 (1826); Lindl. in *Bot. Reg.* 22: t. 1871 (1836).
Colax aromaticus Spreng., *Syst. Veg.* ed. 16, 4: Cur. Post. 307 (1827).

DISTRIBUTION. Mexico, Belize, Guatemala, Honduras & Nicaragua.
COLLECTIONS. Mexico: Chiapas, El Sumidero, Tuxtla Gutiérrez, 2000m, *Kennedy* F62M19 (LASCA); Chiapas, Bajando Teopisca, *Hágsater* 1359 (AMO, K!); Oaxaca, Totontepec (AMO!); Vera Cruz, Orizaba, *Meisner* 1333 (K-LINDL.!); V.C., Tarmapan, *Purpus* 16459 (K!); V.C., Coetzala, 700m, *Ryan* 8 (K!); V.C., Naranjal, 740m, *Ryan* 11 (K!); V.C., Huatusco, 1285m, *Ryan* 42 (K!); V.C., Fortín de las Flores (AMO!); Coscomatepec (AMO!). Belize: Toledo district, *Adams* 257 (K!). Honduras: Dept. Cortes, Santa Cruz de Yojon, 610m, *Edwards* 500 (K!). Nicaragua: *Araquistain &*

Sandino 1462 (SEL); Matagalpa Prov., *Zelaya* 3955 (BM!). Unknown origin: cult. *Oakeley* 1957 (K!); cult. *RBG Kew* 582-1931 (K!); cult. *Sparrow* s.n. (K!).

ETYMOLOGY. From the Latin, "*aromaticus*", aromatic or spicy, in reference to the cinnamon-like scent of the flowers.

2. *L. bradeorum* Schltr. in *Fedde, Repert. Beih.* 19: 138 (1922). Type: Costa Rica, near Guanacaste, *Brade* 1326 (B†, illustration on sheet 31613 AMES!).

DISTRIBUTION. Honduras, Nicaragua & Costa Rica.
COLLECTIONS. Costa Rica: Prov. Guanacaste, Hacienda María, Parque Nacional Rincón de la Vieja, 800m, *de Vries* sheet 84747 (CR!); Cartago, Turrialba, 1100m, *G.Rivera et al.* sheet 160996 (CR!). Nicaragua: Matagalpa Prov., Finca Milwaukee, *Heller* 2362 (LASCA); Jinotega Prov., Macizos de Peñas Blancas, 1200m, *Heller* 8426 (SEL).

ETYMOLOGY. Named after A.B. Brade, collector of the type material.

3. *L. campbellii* C.Schweinf. in *Sargentia* 8: 103 (1949). Type: Panama, Perlas Archipelago, San José Island, *Johnston* 1371 (holo. AMES!).

DISTRIBUTION. Panama & possibly Colombia.
COLLECTIONS. Panama: cult. *Severin* K6 (K!). Unknown origin: cult. *Oakeley* 13.8.94 (K!); cult. *Oakeley* 8.93 ex Cedric Maunder (K!).

ETYMOLOGY. Named after the horticulturalist, Major William Wesley Campbell.

4. *L. cochleata* Lindl. in *Lindl. & Paxt. Flow. Gard.* 1: 126 (1850-51). Type: sheet LYCASTE 013L (K-LINDL.!).
Maxillaria cochleata (Lindl.) Beer, *Prakt. Stud. Orch.* 264 (1854).

DISTRIBUTION. Mexico, Belize, Guatemala, El Salvador, Honduras & Nicaragua.
COLLECTIONS. Mexico: Chiapas, El Sumidero Canyon, near Tuxtla Gutiérrez *White* 59.636 (LASCA); Chiapas, 120km SE of Palenque, *Chater et al.* 162 (BM!); Chiapas, 24km S of Las Casas, *Berliner* B67M1 (LASCA). Belize: Toledo District, Blue Creek, 40m, *Adams* 257 (K!). El Salvador: *Hamer* 421 (SEL). Unknown origin: *Oakeley* 31.7.90 (K!).

ETYMOLOGY. Named for its shell-shaped lip.

5. *L. consobrina* Rchb.f. in *Bot. Zeit.* 10: 669 (1852). Type: sheet 36818 (lecto. W!).

DISTRIBUTION. Mexico & Guatemala.
COLLECTIONS. Mexico: Vera Cruz, Amatlan de los Reyes, 650m, *Ryan* 2 (K!); V.C., Coetzala, 700m, *Ryan* 23 (K!); V.C., Naranjal, 740m, *Ryan* 13 (K!).

ETYMOLOGY. From the Latin "consobrina", a cousin, presumably after its similarity to the other yellow-flowered species.

6. *L. crinita* Lindl. in *Bot. Reg.* 30: misc. 39 (1844). Type: Mexico, Oaxaca, *Loddiges* 955 (holo. K-LINDL.!).
L. micheliana Cogn. in *Rev. Hort.* 72*:* 264 (1900).
DISTRIBUTION. Southern Mexico.
COLLECTIONS. Mexico: Jalisco, Manzanillo Mts., N. of Manzanillo, 1370m, *Kennedy* sheet 55.482-1 (K!); Guerrero, Mina, Carraceras, 1100m, *Hinton et al.* 10101 (K!, BM!); Guerrero, Mts. above Acapulco, 1070m, *Marsalis* 62P344 (LASCA); Montes de Oca, San Antonio, *Hinton et al.* 14047 (K!); Oaxaca, Km188 Oaxaca to Puerto Angel, 1400m, *Ryan* 81 (in cult. K!); Oaxaca, Km41 Pochula to Oaxaca, 1100m, *Ryan* 84 (in cult K!).

ETYMOLOGY. From the Latin "crinita", long-haired, a reference to the lip.

7. *L. cruenta* Lindl. in *Bot. Reg.* 29: misc. 16 (1843). Type: Guatemala, *Skinner* s.n. (holo. K-LINDL.!).
Maxillaria cruenta Lindl., *Bot. Reg.* 28: t.13 (1842).
L. balsamea A.Rich. in *Portef. Hort. Paris,* 1: 131 (1847) *nom. nud.;* A.Rich. ex Lindl. in *Paxt. Fl. Gard.* 1: 126 (1851-52). Type: *Richard* "Hort. Par. Jan 1850" (K-LINDL.!).
L. rossiana Rolfe in *Orchid Rev.* 1: 239 (1893) *nom. nud.* Type: cult. *Ross* 1.1894 (K!).

DISTRIBUTION. Mexico, Guatemala & El Salvador.
COLLECTIONS. Mexico: Chiapas, Teopisco, *Kennedy* sheet 57.417-1 (K!); Chiapas, 128km SW of Comitan, 910m, *Giridlian* G63M1 (LASCA); road to Motozin, above Huixtla, 450m *Salazar* 5288 (in cult. K!); *Hart* 9 (K!); *Oakeley* 57385 (K!). Guatemala:

Dept. Santa Rosa, Santa Rosa, 600m, *Ames* 3501 (K!). Unknown origin: *Weathers* 13.1.90 (BM!); cult. *Oakeley* HFO4 (K!); cult. *Pechell* 14206 (K!).

ETYMOLOGY. From the Latin "cruenta", meaning blood stained, a reference to the dark red spot at the base of the lip.

8. *L. deppei* (Lodd.) Lindl. in *Bot. Reg.* 29: misc. 15 (1843). Type: Mexico, Vera Cruz, Orizaba, *Meisner* 471 (lecto. K-LINDL.!).
Maxillaria deppei Lodd., *Bot. Cab.* 17: t.1612 (1830).
L. leiantha Richard ex Beer, Prakt. Orch. 263 (1854).
M. leiantha (Richard ex Beer) Beer, Prakt. Orch. 265 (1854).

DISTRIBUTION. Mexico, Guatemala, El Salvador, Honduras & Nicaragua.
COLLECTIONS. Mexico: Chiapas, Comitan 1980m, *Kennedy* F62M7 (LASCA); Vera Cruz, Huatusco, 1250m, *Ryan* 40 (K!); V.C., Orizaba, *Bourgeau* 3080 (K!); V.C., near Xalapa, 1220m, *Pringle* 8190 (BM!, K!). Nicaragua: Jinotega, Filete Caballo Blanco, 1650m, *Heller* 11826 (SEL).

ETYMOLOGY. Named after the collector of the type, Mr Deppe.

9. *L. lasioglossa* Rchb.f. in *Gard. Chron.*: 215 (1872) and in *Bot. Mag.* 102: t. 6251 (1872). Type: cult. *Veitch* 2/72 (K!).
L. macropogon Rchb.f. in *Gard. Chron.* ser.3 3: 200 (1888). Type: Costa Rica, *Hubsch,* illustration, sheet 36671 (W!).

DISTRIBUTION. Guatemala only.
COLLECTIONS. Guatemala: Lake Izabal, *Hiatt* Hi63G25 (LASCA); Montebello Lakes *Kenn*edy F62G1 (LASCA); *Oakeley* 12.2.91 (K!); *Oakeley* 4.91 (K!). Unknown origin: cult. *RBG Kew* 15.2.1915 (K!); cult. *RBG Kew* 7.2.1916 (K!); cult. *RBG* Kew 9.10.1916 (K!); cult. *RBG* Kew 28.4.1925 (K!).

ETYMOLOGY. Named after the hairy lip.

10. *L. macrobulbon* (Hook.) Lindl. in *Lindl. & Paxt. Flow. Gard.* 1: 126 (1850-51). Type: Colombia: Sierra Nevada, Santa Marta, *Purdie* (K missing).
Maxillaria macrobulbon Hook. in *Bot. Mag.* 72: t. 4228 (1846).
L. pleiochroma Rchb.f. in *Hamb. Gartenz.* 14: 179 (1860).

DISTRIBUTION. Colombia.
COLLECTIONS. Colombia: Ocaña, N. of Santander, Orilles de Rio Abrego SM65C5 (LASCA). Unknown origin: *Oakeley* D75B (K!), *Oakeley* D77 (K!), *Oakeley* G51 (K!), *Oakeley* G52A (K!).

11. *L. suaveolens* Summerh. in *Bot. Mag.* 154: t. 9231 (1931). Type: cult. *Kew 22.6.1926* (holo. K!).
L. aromatica var. *maius* B.K. in *Gard. Chron.* ser.3 79: 421 (1926).

DISTRIBUTION. Mexico, Guatemala & Nicaragua.
COLLECTIONS. Mexico: Vera Cruz, Tamaulipas, Rancho del Cielo, N. of Gómez Farías, *Dressler* 2588 (K!). Nicaragua: Zelaya, Cerro Musún, 1350m, *Heller* 11655 (SEL). Unknown origin: *Oakeley* A60/67 (K!).

ETYMOLOGY. Latin for "fragrant".

B. Subsect. *Paradeciduosae* Fowlie, *The Genus Lycaste*: 7 (1970), *nom. nud.* No type specified.

12. *L. brevispatha* Klotzsch, ex Rchb.f. in *Walp. Ann.* 6: 604 (1861). Type: *Warscewicz* (holo. B†).
Maxillaria (L.) brevispatha Klotzsch in *Allg. Gartenzeit.* 19: 217 (1851).
L. lawrenciana Rchb.f. in *Ann. Bot. Sys.* 6: 603 (1861).

DISTRIBUTION. Nicaragua, Costa Rica & Panama.
COLLECTIONS. Costa Rica: Headwaters of Río Porrosati, above San José de la Montaña, 1400m, *Horich & Fowlie* 60P2216 (LASCA); Cordillera de Talamanca, Vjarras, *Oakeley* CR7 (K!); cult. *RBG Kew* 252-1934 (K!); *Oakeley* CR24 (K!).

ETYMOLOGY. From the Latin, alluding to the short floral bract.

13. *L. candida* Lindl. in *Lindl. & Paxt. Flow. Gard.* 2: 37 (1851-52) (as *L. leucantha*). Type: Costa Rica, *Warscewicz* s.n. 1.1851 (lecto. K-LINDL.!).
Maxillaria candida Lindl. *Bot. Reg.* 27: misc. 23, No 59 (1841).

DISTRIBUTION. Costa Rica & Panama.

COLLECTIONS. Costa Rica: San José, camino Alto de San Juan, sheet 51071 (USJ!). San Cristobal, 1830m, *Lehmann* H.K.712 (K!). Panama: Chiriqui, 1220–1320m, *Powell* 140 (K! as *L. brevispatha*).

ETYMOLOGY. Latin for white.

14. *L. luminosa* Oakeley in *Amer. Orchid Soc. Bull.* 60: 638 (1991). Type: cult. *Oakeley* 57387 (holo. K!).

DISTRIBUTION. Unknown.

ETYMOLOGY. The flower colour was described as radiating "a warm luminous glow of pastel green and cream".

15. *L. tricolor* (Klotzsch) Rchb.f. in *Walp. Ann.* 6: 603 (1861). Type: *Warscewicz* (holo. B†); Panama, Volcán Chiriqui, 2130-3780m, *Rchb.f.* 10 (lecto. W!).
Maxillaria (L.) tricolor Klotzch in *Allg. Gartenz.* 20: 185 (1852).

DISTRIBUTION. Nicaragua, Costa Rica & Panama.
COLLECTIONS. Costa Rica: Prov. de Alajuela, San Pedro de San Ramón, 1075m, *Brenes* sheet 25930 (CR!); San Ramón, along path to refuge, 1055m, sheet 03991 (USJ!); Río Zacero Gorge, nr. Laguna, 900-1000m, *Horich* s.n. sheet 58.345-2 (K!). Unknown origin: cult. *Sainsbury* 47705 (K!).

II. Sect. *Fimbriatae* Fowlie, *The Genus Lycaste:* 7 (1970) *nom. nud.* No type specified.

16. *L. andreettae* Dodson in *Ic. Pl. Trop. (Orch. Ecuador)* 5: t. 443 (1982). Type: Ecuador, Morona Santiago, Wapú and Yukipa, 1000m, *Andreetta* 027 (holo. SEL).

DISTRIBUTION. Ecuador.
COLLECTIONS. Ecuador: *Oakeley* s.n. (K).

ETYMOLOGY. Named in honour of the Ecuadorian botanist Padre Angel Andreetta.

17. *L. barringtoniae* (J.E. Smith) Lindl. in *Bot. Reg.* 30: misc. 43 (1844). Type: *Purdie* 4 Apr.1844 (lecto. K!).

Epidendrum barringtoniae J.E. Smith in *Ic. Pict.* t. 15 (1793).
Dendrobium barringtoniae (J.E. Smith) Swartz in *Nov. Act. Ups.* 6: 82 (1799).
M. barringtoniae (J.E. Smith) Lodd., *Bot. Cab.* 19: t. 1824 (1832).

DISTRIBUTION. West Indies.
COLLECTIONS. Cuba: *Wright* 634(K!); Edo. Palenquito, 800m, *Eggers* 1889 (K!); Gran Piedra, 1070m, *Hawkes* 14 (K!); Oriente Prov., near Santiago de Cuba, *Clement* 1 (BM!). Dominican Republic: Santo Domingo, Cordillera Central, Prov. Monte Cristi, 1200m, *Ekman* H12736 (K!); Santo Domingo, 1250m, *Türkheim* 3474 (K!); Santiago Prov., Dist. San José de las Matas, 500-600m, *Valeur* 632 (K!). Jamaica: *Levy* 287-1932 (K!); Blue Mountains, near Radnor, *Gossil* 6.1909 (BM!). Unknown origin: cult. *RBG Kew* 1910 (K!), *Oakeley* 6&7 1993 (K!).

ETYMOLOGY. Named after Mrs Barrington.

18. *L. ciliata* (Ruiz & Pav.) Lindl. ex Rchb.f. in *Bonplandia* 4: 324 (1856). Type: *Ruiz & Pavón* (holo. M).
M. ciliata Ruiz & Pav., *Syst. Veg. Fl. Per. et Chil.* 221 (1798).

DISTRIBUTION. Colombia, Ecuador, Peru & Bolivia.
COLLECTIONS. Ecuador: Tungurahua, 4km E of Baños on Río Blanco, 1600m, *Dodson & Thein* 880 (SEL); Tungurahua, Baños, 1800m, *Embree* F62E9 (LASCA) and *Oakeley* s.n. (K!). Peru: Prov. Moyobamba, Moyabamba, *Woytkowski* 71 (K!); Dept. Junin, Prov. Tarma, Agua Dulce, *Woytkowski* 37 (K!). Bolivia: Santa Cruz, Sara, Río Macuñucú, *Vásquez* 950 (Herb. Vasq.).

19. *L. ciliata* supsp. *rossyi* (Hoehne) Fowlie, *The Genus Lycaste*: 71 (1970). Type: Brazil, São Paulo, *H.Rossy* 32167 (holo. Instituto Biologico, São Paulo).
L. rossyi Hoehne in *Engl. Bot. Jarb.* 68: 138 (1937).

DISTRIBUTION. Brazil.

ETYMOLOGY. Named after the collector, Heinrich Rossy.

20. *L. cinnabarina* (Lindl.) Rolfe in *Orch. Rev.* 1: 303 (1893) and *Lindenia*, 9: 23, t. 394 (1893). Type: Peru, Sources of the Marañon, *Warscewitz* 58 (holo. K-LINDL!).

M. cinnabarina Lindl. in *Gard. Chron.* 292 (1853) *nom. nud.*; and in Stevens' Sale Catalogue for that year.
L. denningiana Rchb.f. in *Gard. Chron.* ser.2 6: 808 (1876).

DISTRIBUTION. Ecuador, Peru.
COLLECTIONS. Unknown origin: cult. *Parc. Léopold* 1893 (K!); *Oakeley* D19 (K!); *Oakeley*, G15 (K!).

ETYMOLOGY. From the Latin "cinnabarinus", alluding to the flower colour

The first reference to this species, by Lindley, advertised a sale of Warscewicz's imported plants at which *"M. cinnabarina"* was to be sold for between £2.2s. and £4. There is a printed description with the holotype specimen, presumably from the sale catalogue: *"108 Maxillaria cinnabarina (Lindley) a new species with pale yellowish green flowers and a rich apricot lip."* Neither the library at Kew nor the RHS have the original catalogues, so the source of the description can't be verified. No other description of *L. cinnabarina* was published until 1893. In the interim, Reichenbach described the same species as *L. denningiana*, working from a specimen provided by Veitch & Sons.

21. *L. costata* Lindl. in *Bot. Reg.* 29: misc. 15 (1843). Type: Peru, *Mathews* 1026 (holo. K-LINDL.!).
M. costata Lindl. in Bot. Reg. 24: misc.175 (1838).
L. longiscapa Rolfe in *Gard. Chron.* ser.3 84: (1928); E.Cooper in *Orchid Rev.* 38: 168 (1930) and Summerh. in *Bot. Mag.* 158: t. 9400 (1935).

DISTRIBUTION. Colombia & Peru
COLLECTIONS. Colombia: Western Cordillera, Las Paras, 1400-1800m, *Lehman* 2945 (BM!). Peru: Huanuco, *McLean* s.n. (K!); *Woytkowski* 25 (K!).

22. *L. diastasia* D.E.Benn. & Oakeley in *Brittonia,* 46(3): 243 (1994). Type: Peru, Huanuco, Leoncio Prado, along road to Monzon, *Jara P.* 3797 (holo. USM).

DISTRIBUTION. Peru.
COLLECTIONS. Peru: San Martín, Moyobamba, along Rio Mayo *Bennett et al.* 3226 (USM); along road to Lamas *Villena* 4548 (USM).

ETYMOLOGY. From the Greek "diastasis", meaning separation, a reference to the outer pair of callus ribs.

23. *L. dyeriana* Sander in *Gard. Chron.* ser.3 18: 49 (1895), *Kew Bull.* 195 (1898) and *Bot. Mag.* 132: t. 8103 (1906). Type: *Sander & Co.* July 1895 (holo. K!).

DISTRIBUTION. Peru
COLLECTIONS. Peru: *Mason* 2064 (K!); *Dodson ex Oakeley* D28 (K!); San Martín, Tarapoto, 950m, *Bennett* 3945 (USM). Unknown origin: cult. *RBG Kew* 28.7.24 (K!); cult. *Oakeley* (K!).

ETYMOLOGY. Named to honour Sir William Turner Thistleton-Dyer, then Director of Kew.

24. *L. fimbriata* (Poepp. & Endl.) Cogn. in *Martius Fl. Bras.* 3(5): 455 (1902). Type: *Poeppig* 1732 (W!).
M. fimbriata Poepp. & Endl., *Nov. Gen. et Sp.* 1: 38, t. 62 fig.3 (1836).

DISTRIBUTION. Peru & Bolivia.
COLLECTIONS. Peru: Dept. San Martín, Zepalacio, nr. Moyobamba, 1100m, *Klug* 3621 (K!); Pasco, Oxapampa, nr Progreso, 1770m, *Bennett* 5534 (K).

ETYMOLOGY. A reference to the heavily fringed lip.

25. *L. fowliei* Oakeley in *Orchid Dig.* 58: 23 (1994). Type: cult. *Oakeley* D22 (holo. K!).

DISTRIBUTION. Unknown.

ETYMOLOGY. Named after Jack Fowlie, author of "The Genus *Lycaste*".

26. *L. fragrans* Oakeley in *Orchid Dig.* 58(1): 20 (1994). Type: cult. *Oakeley* s.n. (holo. K!).

DISTRIBUTION. Ecuador.
COLLECTIONS. Ecuador: *Oakeley* D41 (K!).

ETYMOLOGY. The flowers are night-scented.

27. *L. fulvescens* Hook. in *Bot. Mag.* 71: t. 4193 (1845). Type: *Hooker*, Kew Neg. 7399 (K-LINDL.!).
L. crocea Linden in *Orch. Linden* 21 *in nota.*

DISTRIBUTION. Venezuela.
COLLECTIONS. Venezuela: Bolivar, Summit of Cerro Java, 1800m, *Dunsterville* 1374 (K!); Tachira, Headwaters of Río Quinmarí, 2000m, *Dunsterville* 1073 (K!); *Tograth* s.n. (K); *Cubillos* s.n. (K). Unknown origin: cult. *RBG Kew* 3.1862 (K!).

ETYMOLOGY. A reference to the yellow/brown flower colour.

28. *L. gigantea* Lindl. in *Bot. Reg.* 29: misc. 14 (1843) and *Bot. Reg.* 31: t. 34 (1845). Type: *Hartweg* 51 (holo K!).
L. longipetala (Ruiz & Pav.) Garay in *Caldasia*, 8: 524 (1962).
M. longipetala Ruiz & Pav., *Syst. Veg. Fl. Per. et Chil.* 1: 220 (1798).
Dendrobium longipetalum Pers. *Sym. Pl.* 2: 523 (1807).
L. gigantea Lindl. in *Bot. Reg.* 29: misc. 14 (1843) and *Bot. Reg.* 31: t. 34 (1845).
L. barbifrons Lindl. in *Ann. Nat. Hist.* ser.1 15: 383 (1845). *M. heynderycxii* E.Morr. in *Ann. Soc. Bot. Gard.* 1 97 (1845).
M. gigantea Beer in *Prakt. Stud. Orch.* 265 (1854).

DISTRIBUTION. Ecuador & Peru.
COLLECTIONS. Ecuador: Tungurahua, NE slope of Volcán Tungurahua, 2500m *Dodson & Thein* 2047 (SEL); Western slopes of the Andes, *Pearce* s.n. sheet 61612 (BM!). Peru: Dept. Cuzco, Urubamba Prov., 2740m, *Saunders* 443 (BM); *Oakeley* 57541 (K).

Under the rules of priority, *M. longipetala* Ruiz & Pav. is the older name and should take precedence. However, there appears to be some confusion over the status of the holotype. Is it a *Lycaste* or a *Maxillaria*? For the purposes of this thesis, the name *L. gigantea* has been substituted throughout.

29. *L. grandis* Fowlie ex Oakeley, *Lycaste Species: the Essential Guide* 18 (1993). Type: *Oakeley* s.n. (K!)
L. longipetala sensu Fowlie, *The Genus Lycaste*: 82 (1970) *non* (Ruiz & Pav.) Garay.

DISTRIBUTION. Venezuela.

30. *L. hirtzii* Dodson in *Ic. Pl. Trop. (Orch. Ecuador)* 5: t. 444 (1982). Type: Ecuador, Tungurahua, cliffs above Baños, 2200m, *Dodson* 10722 (holo. SEL).
L. colombina Ospina in Mutisia 71: 9 (1988).

DISTRIBUTION. Ecuador.

ETYMOLOGY. Named after the Ecuadorian botanist Alexander Hirtz.

31. *L. jarae* D.E.Benn. & E.A.Christianson in *Orchid Dig.* 60(1): 14 (1996). Type: Peru, Huanuco, Leonicio Prado, 6km SE of Tingo María, 1100m, *Jara P.* 6433 (holo. USM).
DISTRIBUTION. Peru.

ETYMOLOGY. Named after the collector, Sr. Enrique Jara P. of Tingo María, Peru.

32. *L. lanipes* Lindl. in *Bot. Reg.* 29: misc.15 (1843) and in *Gard. Chron.* 212 (1843). Type: Ecuador, Paccha, *Hartweg* Jan 1843 (holo. K-LINDL.!).
L. mesochlaena Rchb.f. & Warsc. in *Bonplandia* 2: 98 (1854).
L. cobbiana B.S.Williams in *Orchid Growers Manual* ed. 6: 376 (1885).

DISTRIBUTION. S.W. Ecuador.
COLLECTIONS. Ecuador: Loja, Cerro Redondo, above Olmedo, 2500m *Dodson et al.* 10535 (SEL).

ETYMOLOGY. A Latin reference to the wooly column foot.

33. *L. linguella* Rchb.f. in *Gard. Chron.* 738 (1871). Type: Veitch (holo. W!).

DISTRIBUTION. Colombia, Ecuador & Peru.
COLLECTIONS. Colombia: Caja Marca, Roldanillo, 1200-1600m, *Lehmann* 715 (K!). Ecuador: Morona-Santiago, Gurumales, Río Paute, 2000m, *Andreetta* 1510 (SEL).

ETYMOLOGY. A reference to the prominent lip.

34. *L. locusta* Rchb.f., in *Gard. Chron.* ser.2 11: 524 (1879). Type: *Rchb.f.* 36784 (holo. W!).

DISTRIBUTION. Peru.
COLLECTIONS. Peru: *Mason* 2763 (K!); 2000m, *Oakeley* A25/46 (K!); Dept. Junin, Prov. Tarma, Utucuyacu, *Woytkowski* 54.355-1 (K!). Unknown origin: *Koopowitz* Sept 95 (K!); *Oakeley* Mar 93 (K!); *Oakeley* s.n. (K!).

ETYMOLOGY. Named for its colour: "as green as a green grasshopper".

35. *L. mathiasiae* G.C.Kenn. in *Orchid Dig.* 42: 59-61 (1978). Type: Peru, Tingo María, *M.Mathias* (holo. AMES).

DISTRIBUTION. Peru.
COLLECTIONS. Unknown origin: *Oakeley* s.n. (K!).

ETYMOLOGY. Named after the collector, Dr Mildred Mathias.

36. *L. maxibractea* D.E.Benn. & Oakeley in *Brittonia* 46(3): 246 (1994). Type: Peru, Cuzco, La Convención, Quillabamba, *D.McSoreley & L.Moore* 3572 (holo. K!).

DISTRIBUTION. Peru.

ETYMOLOGY. A reference to the uncommonly large floral bract.

37. *L. mesochlaena* Rchb.f. in *Bonplandia* 2: 98 (1854). Type: Colombia, Antioquia, 57 (holo. W!)

DISTRIBUTION. Colombia & Eastern Ecuador.
COLLECTIONS. Ecuador: Zamora, Chinchipe, Km42 Loja to Zamora, 1700m, *Dodson* (SEL).

ETYMOLOGY. A reference to the uncommonly large floral bract.

38. *L. mezae* D.E.Benn. & Oakeley in *Brittonia*, 46(3): 246 (1994). Type: Peru, Amazonas, Chachapoyas, above Leimebamba, *J.Meza* 5417 (NY).

DISTRIBUTION. Peru & Ecuador.

COLLECTIONS. Ecuador: Morona-Santiago, Chiquinda, Aguacate, *Andreetta* 41 (SEL).

ETYMOLOGY. Named after the collector, Jorge Meza.

39. *L. nana* Oakeley in *Orchid Dig.*, 58: 23 (1994). Type: Hort. Oakeley (holo. K!).

DISTRIBUTION. Unknown.

40. *L. peruviana* Rolfe in *Kew Bull.* 160 (1910). Type: sheet 61602 (neo. BM!)

DISTRIBUTION. Peru.

41. *L. reichenbachii* Gireoud ex Rchb.f. in *Bonplandia* 4: 324 (1856). Type: Gireoud No4 & Illustration on sheet 36792 (holo. W!).

DISTRIBUTION. Peru.
COLLECTIONS. Peru: *Oakekey* A54 (K!). Unknown origin: *Oakeley* 27.9.96 (K!).

ETYMOLOGY. Named in honour of the orchid taxonomist H.G.Reichenbach.

42. *L. trifoliata* Lehmann ex Masters in *Gard. Chron.* ser.3 17: 529 (1895). Type: Ecuador, Morona-Santiago, Chiquinda, 1600-1800m *F.C.Lehmann* 708 (holo. K!).
L. lata Rolfe in *Kew Bull.* 370 (1910).

DISTRIBUTION. Colombia, Ecuador, Peru & Bolivia.
COLLECTIONS. Colombia: Canca & Antioquia, Forests of the Montaña de Caramanta, 2500-2900m, *Lehmann* 7220 (K!). Ecuador: Tungurahua, Baños Pujo, 1400m, *Hirtz* s.n. (SEL). Peru: Huanuco, Leoncio Prado, below Mirador, 1800m, *Bennett* 2585 (USM). Bolivia: Cochabamba, Río Limatambo, Chapare, *Vásquez* 904 (Herb. Vasq.). Unknown origin: *Oakeley* A56 ex Dix (K!).

ETYMOLOGY. Three leaved.

III. Sect. *longisepalae* Fowlie, *The Genus Lycaste:* 8 (1970), *nom. nud.* Monotypic. Type: Herb. *Rchb.f.* sheet 36629 (holo. W!).

43. *L. schilleriana* Rchb.f. in *Bonplandia* 3: 215 (1855). Type: Herb. *Rchb.f.* sheet 36629 (holo. W!).

L. hennisiana Kränzl. in *Orchis* 1: 33 (1906).

L. longisepala C.Schweinf. in *Bot. Mus. Leafl. Harvard Univ.* 15: 157 (1952).

DISTRIBUTION. Peru.

COLLECTIONS. Peru: Huanuco, Sariapampa, brow of jungle, 3200m, *Woytkowski* 50.1896-1 (K!). Unknown origin: *Holmes* 300-1928 (K!); cult. *RBG Kew* Dec 1925 (K!); *Oakeley* D80 ex Zakharoff (K!); *Woytkowski* 51.824-2 (K!); *Woytkowski* 51.824-3 (K!).

ETYMOLOGY. Named after Consul Schiller.

IV. Sect. *Lycaste*

L. sect. *macrophyllae* Fowlie, *The Genus Lycaste: 8* (1970), *nom. nud.* Type species: *L. macrophylla* (Poepp. & Endl.) Lindl.

44. *L. dowiana* Endres & Rchb.f. in *Gard. Chron.* 2(ser.2): 194 (1874). Type: *Endres & Rchb.f.* 89, 44616 & 35945 (holo. W!).

DISTRIBUTION. Nicaragua, Costa Rica, Panama, South America.

COLLECTIONS. Nicaragua: Granada, Volcán Mombacho, 1400m, *Heller* 2925 (SEL). Unknown origin: *Tibbs* 63701 (K!).

ETYMOLOGY. Dedicated to Captain Dow, a friend of Endres.

45. *L. leucantha* Klotzsch as *Maxillaria (L.) leucantha* Klotzsch in *Allg. Gartenz.* 18: 402 (1850). Type: *Warscewicz* (holo. B†); Costa Rica, *Warscewicz* s.n. LYCASTE 021L (lecto. K-LINDL.!).

DISTRIBUTION. Costa Rica.

COLLECTIONS. Costa Rica: Puntarenas, Cordillera de Talamanca, between Cerro Frantzius and Cerro Pittier, 1500–1600m, *Davidse* sheet 108826 (CR!); Puntarenas, Buenas Aires, 1600-1800m, *Valerio* 146 (CR!); Cachí, sheet 038726 (USJ!); 2 km S. of Cachí, 1300m, *Fowlie* 60P2202 (LASCA); Sarapiqui, between La Cinchona and Vara Blanca, 1500m, *Horich* sheet 59.1020-1 (K!); cult. *Lankaster Gardens* s.n. (K!); cult. *Sanders* ex Lankester s.n. (K!). Unknown origin: cult. *RBG Kew* 7.1914 (K!); cult. *Wubben* 8.92 (K!); cult. *Wubben* 9.92 (K!).

ETYMOLOGY. From the Latin for "white flowered".

46. *L. macrophylla* (Poepp. & Endl.) Lindl. in *Bot. Reg.* 29: misc. 14 (1843). Type: Pöeppig L.022L (K-LINDL.!) as *Maxillaria macrophylla*.
M. macrophylla Poepp. & Endl. in *Nov. Gen et Sp.* 1: 37 t.64 (1836), and in *Bot. Reg.* 24: misc. 171 (1838).

DISTRIBUTION. Costa Rica, Panama, Venezuela, Colombia, Ecuador & Peru.
COLLECTIONS. Costa Rica: Reserva Vert Pacifico, 1600-1620m, *Dryer* 73814 (CR!). Ecuador: Imbabura, nr. Lita, 700m, *Hirtz* 272 (SEL). Peru: Amazonas, Peca, *Woytkowski* 50.1154 (K!); Bongara, 1500m, *Bennett et al.* 2007 (UC); Junin, Chanchamayo, nr. Huacapistana, 1600m, *Bennett et al.* 0340 (UC). Bolivia: La Paz, Nor Yungas, between Arapata and Cruz Loma, 2000m, *Vásquez et al.* 305 (Herb. Vasqz.). Unknown origin: *Mathews* 3191 (BM!).

ETYMOLOGY. Latin for large leaved.

47. *L. macrophylla* subsp. *desboisiana* (Cogn.) Fowlie in *Lasca Leaves* 41 (March 1964). Type: possibly *Fowlie* 60P2205 (LASCA).
L. macrophylla var. *desboisiana* Cogn. in *Chron. Orch.* 1: 4 (1897).

DISTRIBUTION. Nicaragua & Costa Rica.
COLLECTIONS. Nicaragua: Jinotega, Jinotega Grade, 1120m, *Heller* 11313 (SEL). Costa Rica: Alajuela, Reserva Forestal de San Ramón (spirit material, USJ!); *Oakeley* 63702 (K!); *Ruiz* CR8 (K!).

ETYMOLOGY. Named after the donor, M. Fr. Desbois, author of *La Monographie Cypripedium* (1888).

48. *L. macrophylla* subsp. *filomenoi* (Schltr.) Fowlie in *Lasca Leaves* 41 (March 1964). Type: (holo. B†).
L. filomenoi Schltr in *Fedde, Repert. Beih.* 9: 100 (1921).

DISTRIBUTION. Peru.

ETYMOLOGY. Named after Dr Serafin Filomeno.

49. *L. macrophylla* subsp. *fowliei* Oakeley in *Orchid Dig.* 58(1): 23 (1994). Type: Hort. Oakeley (K!).

DISTRIBUTION. Unknown.

ETYMOLOGY. Named after Jack Fowlie.

50. *L. macrophylla* subsp. *measuresiana* (B.S.Williams) Fowlie in *Lasca Leaves* 41 (March 1964). Type: *Woytkowski* 50.1154 (UC).
L. plana var. *measuresiana* B.S.Williams in *Orchid Alb.* 7: t. 306 (1887).

DISTRIBUTION. Ecuador, Peru, Bolivia.

51. *L. macrophylla* subsp. *panamensis* Fowlie in *Lasca Leaves* 41 (March 1964). Type: Panama, Cocle Prov., El Valle de Anton, 910m, *Fowlie* F62P8 (LASCA).

DISTRIBUTION. Panama & Colombia.

52. *L. macrophylla* subsp. *plana* (Lindl.) Fowlie in *Lasca Leaves* 41 (March 1964). Type: Bolivia, *Loddiges* (K-LINDL.!).
L. plana Lindl. in *Bot. Reg* 28: misc. 85 (1842) *nomen* and in *Bot. Reg.* 29: t. 35 and misc.15 (1843).

DISTRIBUTION. Peru & Bolivia.

ETYMOLOGY. A reference to the "even" petals, c.f. those of *L. macrophylla* which are "undulating".

53. *L. macrophylla* subsp. *puntarenasensis* Fowlie in *Lasca Leaves* 45 (March 1964), *The Genus Lycaste* 50 (1970). Type: Costa Rica, Puntarenas, Los Altos de San Juan, *Spencer & Fowlie* F62CR43 (holo. LASCA).

DISTRIBUTION. Costa Rica & Panama.
COLLECTIONS. Costa Rica: Alajuela, San Ramón, Cuenca del Río San Lorencito, 850-1000m, *anon.* 050252 (USJ!); San Vito de Java, *anon.* 11337 (USJ!).

54. *L. macrophylla* subsp. *viridescens* Oakeley in *Amer. Orchid Soc. Bull.* 60: 642 (1991). Type: Hort. *Oakeley* (holo. K!).

DISTRIBUTION. Unknown.

55. *L. macrophylla* subsp. *xanchocheila* Fowlie in *Lasca Leaves* 45 (March 1964). Type: Costa Rica, Puntarenas, San Vito de Java, *Wilson* H62CR53 (holo. LASCA).

DISTRIBUTION. Costa Rica.
COLLECTIONS. Costa Rica: San Vito de Coto Bruz, *anon.* (spirit material, USJ!); *Oakeley* 13.8.94 (K!).

ETYMOLOGY. A Greek reference to the yellow lip.

56. *L. neglecta* Schltr. in *Fedde, Repert.* 27: 66 (1929). Type: La Paz, *Buchtien* 3707 (holo. B†.).

DISTRIBUTION. Bolivia.

57. *L. powellii* Schltr. in *Fedde, Repert. Beih.* 17: 65 (1922). Type: Panama, Canal Zone, Balboa, *Powell* 15 (holo. B†, iso. K!, sheets 23869 & 26792 iso. AMES!).

DISTRIBUTION. Costa Rica & Panama.
COLLECTIONS. Panama: Cativa, Porto Bello, East Ridge, 300m, *Wood* sheet 56.759-1 (K!).

ETYMOLOGY. Named after the collector, C.W. Powell.

58. *L. skinneri* Lindl. in *Bot. Reg.* 29: misc. 15 (1843). Type: Guatemala, *Skinner* (holo. K-LINDL.!) as *Maxillaria skinneri.*
M. skinneri Bateman ex Lindl. in *Bot. Reg.* 26: misc. 48 (1840).
L. virginalis (Schweidw.) Linden in *Lindenia*, 4: 22 (1888).

DISTRIBUTION. Mexico & Guatemala.

COLLECTIONS. Mexico: Chiapas, *Linden* 36655 (W!); Chiapas, Montebello Lakes, near Tepancuapan, 1830m, *Kennedy* F62M20 (LASCA). Guatemala: Dept. Alta Verapaz, Cóban, 1370m, *Smith* 226 (K!); Cóban, Baja Verapaz, *Lehmann* 1340 (BM!).

ETYMOLOGY. Named after the collector, George Ure Skinner.

59. *L. xytriophora* Linden & Rchb.f. in *Saund. Refug. Bot.* 2: t. 131 (1872) and *Gard. Chron. ser 2.* 2: 194 (1874).Type: 86651 (holo. W!).

DISTRIBUTION. Ecuador.
COLLECTIONS. Ecuador: El Oro, near Zaruma, 1300m, *Dodson et al.* 8477 (SEL); 1000-1500m, *Lehmann* 6892 (K!); 1500-2000m *Lehmann* 6892 t. 508 (K!); Vilcabamba, Loja, *Dall* 102 (K-LCD); *Fraser* s.n. sheet 61618 (BM!).

Misapplied Names

L. aciantha Rchb.f. in *Bonplandia*, 3: 216 (1855). = *Maxillaria sciantha.*

L. acuminata Rchb.f. in *Bonplandia*, 3: 216 (1855) = *M. acuminata.*

L. farinosa Kränzl. *in Notizbl. Bot. Gart. Berlin,* 7: 427 (1920). - not seen.

L. grandiflora Beer in *Prakt. Orch. 265. = M. grandiflora.*

L. harrisoniae G. Don ex Loud. *Encyc. Pl. Suppl.* 2: 1468. = *Bifrenaria harrisoniae.*

L. tetragona Lindl. in *Bot. Reg.* 29: misc. 49 no64 (1843).

M. tetragona Lindl. *Bot. Reg.* t. 1428. = *B. tetragona.*

L. tyriantha Loud. *Hort. Brit. Suppl. 3: 582. = B. tyriantha.*

L. wittigi Rchb.f. in *Gard. Chron.* 10 ser. 2: 654 (1878). = *B. wittigi.*

Anguloa Ruiz & Pav., *Prodr. Fl. Per.* 118 t. 26 (1794); Schltr. in *Orchis* 10: 122-145 (1916); Oakeley in *Orchid Rev.* 102: 226-232 (1994).

I. Sect. *Guloanga* Schltr. in *Orchis* 10: 125 (1916).

1. *Anguloa brevilabris* Rolfe in *Orchid Rev.* 23: 292 (1915). Type: Colombia, Antioquia, between Cativo and Buritica, 1400-1600m, *Lehmann* 7235 (holo. K!).

A. goldschmidtiana Schltr. in *Orchis* 10: 139 (1916). Type: cult. *Goldschmidt,* ex Prothero & Morris, (B†).
A. sagittata Summerh. in *Kew Bull.* 102 (1931). Type: Colombia, *Hay* s.n. (holo. K!).

DISTRIBUTION. Colombia.
COLLECTIONS. Unknown origin: cult. *Briton* 7.1918 (K!); cult. *Hugo* 8.94 (K!); cult. *Oakeley* s.n. (K!).

ETYMOLOGY. A reference to the short lip.

2. *Anguloa cliftonii* Rolfe in *Kew Bull.* 160 (1910) and in *Bot. Mag.* 143: t. 8700 (1917). Type: cult. RBG Kew 8.1914 (K!).
DISTRIBUTION. Colombia.
COLLECTIONS. Colombia: Valle, Río Bravo, NW of Darien, *Hugh-Jones* 75 (K!). Unknown origin: *Oakeley* 25.7.91 (K!); *Oakeley* 1994 (K!).

ETYMOLOGY. Named after Mr J. Talbot Clifton of Lytham Hall, from whose collection it was described.

3. *Anguloa clowesii* Lindl. in *Bot. Reg.* 30: misc. 26 (1844). Type: Venezuela, Prov. Merida, Taji, 1676m, *Linden* 622, 1842 (holo. K-LINDL!).

DISTRIBUTION. Colombia, Venezuela.
COLLECTIONS. Colombia: *M. Castello* 1887 (K!); *Oakeley* s.n. (K!). Venezuela: Merida, *Dunsterville* 737 (illustration, K!). Unknown origin: cult. *RBG Kew* 7.1887 (K!); cult. *Oakeley* s.n. (K!); cult. *Oakeley* 27.9.96, ex "L&R Orchids", (K!); *Mason* 1212 (K!); *Purdie* 12.1845 (K!); *Veitch* 11. 1886 (K!).

ETYMOLOGY. Named after the Rev. J. Clowes, Broughton Hall, in whose collection it first flowered in the UK.

4. *Anguloa dubia* Rchb.f. in *Gard. Chron.* ser.2 17: 764 (1882). Type: Herb. *Rchb.f.* sheet 42501 (holo. W!).

DISTRIBUTION. Colombia, Venezuela
COLLECTIONS. Colombia: La Plata, Patico, 1300-1600m, *Lehmann* HK721 (K!); *Oakeley* 3.97, ex Cali Orchid Show, (K!). Venezuela: *McCorquodale* & Co (K!).

ETYMOLOGY. From the Latin "dubius", meaning doubtful.

5. *Anguloa hohenlohii* C. Morr. in *Belg. Hort.* 3: 201, t. 31 (1853). Type: Location of holotype unknown.

A. purpurea Linden in *Illustr. Hortic.* 28: t. 427 (1881).

A. macroglossa Schltr. in *Orchis* 10: 132 (1916). Type: cult. *Goldschmidt.* (B†). The plant has not been found in the wild; the only remaining record seems to be the drawing and description in *Orchis,* cited above. Oakeley (1999) thinks that this may be a variety of *A. hohenlohii.*

DISTRIBUTION. Colombia & Venezuela.

COLLECTIONS. Venezuela: *Oakeley* a & b (K!). Unknown origin: *Oakeley* 6.93; *Tibbs* 63937 (K!).

ETYMOLOGY. Named to honour Prince Frédéric-Charles-Joseph de Hohenlohe-Waldenbourg-Schillingsfurst.

II. Sect. *Anguloa.* Type species: *A. uniflora* Ruiz & Pavón.

Sect. *Euanguloa* Schltr. in *Orchis* 10: 125 (1916).

6. *A. eburnea* B.S.Williams in *Orchid Alb.* 3: t133 (1884). Type: Warscewicz 1854 (lecto, K!).

DISTRIBUTION. Colombia, Ecuador.

COLLECTIONS. Ecuador: cult. *Oakeley.* Unknown origin: *Oakeley*, ex Vacherot & Lecoufle, 3.7.94 (K!); *Oakeley* 6.8.91 (K!).

ETYMOLOGY. From the Latin "eburneus", ivory, a reference to the colour of the flower.

7. *A. tognettiae* Oakeley in *Orchids* (Tokyo) 38: 26 (1999); in *Orchidee* 50: 28 (1999); in *Orchideologia* 21: 173 (1999). Type: Hort. *Tognetti* (holo. K).

DISTRIBUTION. Venezuela.

ETYMOLOGY. Named after Vilma Tognetti of La Puerta, Venezuela, in whose collection it first flowered.

8. *A. uniflora* Ruiz & Pav., *Syst. Veg. Fl. Peruv. et Chil.* 228. Type: *Ruiz & Pavón* (holo. MA).
A. mantini Hort. ex *Illustr. Hortic.* 187 (1895).

DISTRIBUTION. Colombia, Ecuador & Peru.
COLLECTIONS. Colombia: *Oakeley* 6.8.91 (K!). Ecuador: Morona-Santiago, above Gualaquiza, *M.Fiske* OIC5237 (SEL). Unknown origin: *Veitch* 1890 (BM!).

9. *Anguloa virginalis* Linden in *Gard. Chron.* 392 (1851); Schltr., *Die Orchideen* 404 (1914). Type: Colombia, Ocaña, 1550m, *Linden* 43 (holo. K-LINDL!).
A. turneri B.S.Williams, *Orchid Grow. Man.* 7: 103 (1894).

DISTRIBUTION. Venezuela, Colombia, Ecuador, Peru & Bolivia.
COLLECTIONS. Colombia: Caracas, *Linden* 622 (BM!). Peru: Huacapistana, 1830m, *Sandemann* 4390 (K!); Junin, Concepción, above Calabazas, 2400m, *Bennett et al.* 3969 (USM); Sources of the Marañon, *Warscewitz* 38 (K-LINDL.!). Bolivia: Cochabamba, Chapare Prov., between Limbo and Palmar, 1900m, *Vásquez* 117 (Herb. Vasq.). Unknown origin: *Pearce* s.n. sheet 61640 (BM!); *Oakeley* A50 (K!); *Oakeley* 6.93 (K!); *Oakeley* 12.6.93 (K!).

Misapplied Names

Anguloa grandiflora H. B. & K. in *Nov. Gen et Sp.* 1: 345 (1836). = *Stanhopea bucephalus.*
Anguloa hernandezii Kunth in *Syn.* 1: 332. = *Stanhopea tigrina.*
Anguloa lurida Link in *Preuss. Gart. Ver.* 1: 289 t. 6. = *Catasetum luridum.*
Anguloa squalida Poepp. & Endl. in *Nov. Gen. et Sp.* 1: 74 (1836). = *Lycomormium squalida.*
Anguloa superba H. B. & K. in *Nov. Gen. Et Sp.* 1: 343, t. 93 (1836). = *Acineta humboldtii.*

Appendix 2 VOUCHER INFORMATON

Information is presented in the form:

Species name	DNA template or analysis ref.	Collector/ identifier	Location / media

O-xxx **and MWC**xxxx numbers refer to DNA templates banked at Kew. Those of the form "Xxxx**NP**", relate to floral fragrance data files, archived at Bush Boake Allen. Vouchers have been archived at the Herbario de la Asociación Mexicana de Orquideología (AMO), the Florida Museum of Natural History (FLAS), the Marie Selby Botanical Garden (SEL) and the Royal Botanic Gardens, Kew (K). "O" refers to the collection of Dr Henry Oakeley. In most cases the vouchers are either dried herbarium specimens (d) or plant material preserved in alcohol (s); "sl" denotes a photographic record; "c" refers to plants in cultivation for which vouchers are missing.

Much of the material used for these studies was obtained from cultivated plants, or from plants which lacked individual collection numbers. In such cases an alternative identifier, such as a date, a supplier or the accession number of the Kew spirit collection has been used.

ILLUSTRATIONS

Lycaste aromatica (Graham ex Hooker) Lindl.	*Oakeley* 63859	K s
L. cinnabarina Lindl.	*Oakeley* 64032	K s
Anguloa clowesii Lindl.	*Oakeley* 57535	K s

MORPHOLOGICAL ANALYSIS

Subtribe **Lycastinae** Pfitz.

1. *Bifrenaria* alliance

Bifrenaria harrisoniae (Hook.) Rchb.f.	*Mansell & Hatchon* 14275	K s
B. harrisoniae	*Hatschbach* 11671 (2)	K d
B. harrisoniae	*Sanders* 25.4.1898	K d
Rudolfiella aurantiaca (Lindl.) Hoehne	*Broadway* 4114	K d
R. aurantiaca	*Broadway* 9387	K d
R. aurantiaca	*Hance* 5323	K d
R. aurantiaca	*Jenman* 719	K d
R. aurantiaca	*Lehmann* HK636	K d
R. aurantiaca	*Sothers & Pereira* 601	K s
R. aurantiaca	*Thurn* 550	K d

Xylobium latilabium C.Schweinf.	*Hodgson* 53492	K s
X. latilabium	*Mason* 1510	K d

2. *Lycaste* alliance

a. ***Anguloa*** Ruiz & Pav.

A. brevilabris Rolfe	*Briton* 16.7.1918	K d
A. brevilabris	*Hugo* 8.1994	K d
A. brevilabris	*Lehmann* 7235	K d
A. brevilabris	*Oakeley* 63703	K s
*A. brevilabris***	*Oakeley* ex Colombia	O c
A. cliftonii Rolfe	cult. RBG Kew 8.1914 (2)	K d
A. cliftonii	*Oakeley* 25.7.1991	K d
A. cliftonii	*Oakeley* 1994	K d
A. clowesii	cult. RBG Kew 1887	K d
A. clowesii	*Mason* 1212	K d
A. clowesii	*Oakeley* (L&R)	K d
A. clowesii	*Oakeley* 57535	K s
A. clowesii	*Oakeley* 63933	K s
A. clowesii	*Purdie* 1845	K d
A. dubia Rchb.f.	*Oakeley* (Cali 1)	K d
A. dubia	*Oakeley* (Cali 2)	K d
A. dubia	*Oakeley* (McCorquodale)	K d
A. eburnea Williams	*Oakeley* 6.8.1991	K d
*A. eburnea***	*Oakeley* ex Ecuador	O c
A. eburnea	*Vacherot & Lecoufle* 3.7.94	K d
A. hohenlohii C.Morr.	*Oakeley* a	K d
A. hohenlohii	*Oakeley* b	K d
A. virginalis Linden	*Oakeley* 6.93	K d
A. virginalis	*Oakeley* A50	K d
A. virginalis	*Sandemann* 4390	K d

b. ***Lycaste*** Lindl.

Sect. ***Deciduosae*** Fowlie

Subsect. ***Xanthanthae*** Fowlie

Lycaste aromatica	*Oakeley* 22.5.1994	K d
L. aromatica	*Oakeley* 3.7.1995	K d
L. aromatica	*Oakeley* 27.9.1996	K d
L. aromatica	*Oakeley* 63959	K s
L. aromatica	*Purpus* 16459	K d
L. aromatica	*Sheppe* 13702	K s
L. aromatica	*Sparrow* 13295	K s

L. bradeorum Schltr.**	*Oakeley* B9	K d
L. campbellii C.Schweinf.	*Maunder* 8.1983	K d
L. campbellii	*Oakeley* 13.8.1994	K d
L. campbellii	*Severin* K6	K s
L. cochleata Lindl.	*Adams* 257	K d
L. cochleata	*Oakeley* 56123	K s
L. cochleata	*Oakeley* 56442	K s
L. consobrina Rchb.f.	*Ryan* 23	K s
L. consobrina	*Ryan* 2	K s
L. crinita Lindl.	*Hinton* 14047	K d
L. crinita	*Kennedy* 55.482-1	K d
L. crinita	cult. Swiss Orchid Soc.	K d
L. cruenta Lindl.	*Oakeley* 63700	K s
L. cruenta	cult. RBG Kew 14206	K s
L. deppei (Lodd.) Lindl.	*Oakeley* "St Thomas"	K d
L. deppei	*Ryan* 27	K s
L. lasioglossa Rchb.f.	*anon.* 15.2.1915	K d
L. lasioglossa	*anon.* 7.2.1916	K d
L. lasioglossa	*anon.* 28.4.1925	K d
L. lasioglossa	*Oakeley* 57514	K s
L. lasioglossa	*Oakeley* 12.2.1991	K d
L. lasioglossa	*Oakeley* 2.1992	K d
L. lasioglossa	*Veitch* 2.1872 (lecto)	W d
L. macrobulbon (Hook.) Lindl.	*Oakeley* D75B	K d
L. macrobulbon	*Oakeley* D77	K d
L. macrobulbon	*Oakeley* G51	K d
L. macrobulbon	*Oakeley* G52A	K d
L. suaveolens Summerh.	*Oakeley* 57306	K s
L. suaveolens	cult. RBG Kew 22.6.1926(type)	K d
L. suaveolens	cult. RBG Kew 1984-2244	K s

Subsect. **Paradeciduosae** Fowlie

L. brevispatha Klotzsch	cult. RBG Kew 13299	K s
L. brevispatha	*Oakeley* CR7 A5	K d
L. brevispatha	*Oakeley* CR24	K s
*L. brevispatha***	*Oakeley* ex San Isidro	O c
L. candida Lindl.	*Oakeley* 63698	K s
L. luminosa Oakeley	*Oakeley* 57387 (holo)	K s
L. tricolor (Klotzsch) Rchb.f.	*Gin* 7.1.1995	K d
L. tricolor	*Horich* 58.345-2(5)	K d
L. tricolor	*Sainsbury* 47705	K s

Sect. ***Fimbriatae*** Fowlie

L. andreetae Dodson	*Oakeley* s.n.	K d
L. barringtoniae (Smith) Lindl.	*Elkman* 12736	K s
L. barringtoniae	*Hawkes* 51.1335-1	K d
L. barringtoniae	*Levy* 14203	K s
L. barringtoniae	*Oakeley* D18	K d
L. barringtoniae	*Valeur* 632	K d
L. barringtoniae	*anon.* 1910	K d
L. ciliata (Ruiz & Pav.) Lindl.	*Oakeley* s.n.	K d
L. cinnabarina Lindl.	*Gower* 1895	K d
L. cinnabarina	*Oakeley* D19	K d
L. cinnabarina	*Oakeley* G15	K d
L. cinnabarina	*Oakeley* (Wyld Court)	K d
L. costata Lindl.	*Oakeley* A62/A67	K d
L. costata (as *L. longiscapa* Rolfe)	cult. Glasnevin	K d
L. dyerana Sander ex Rolfe*	*Dodson* (ex. Quito)	K d
L. dyeriana	*Oakeley* D28	K d
L. dyeriana	cult. RBG Kew 25.6.1923	K d
L. dyeriana	*Mason* 2064	K s
L. dyeriana	*Sander & Co.* 1895 (holo)	K d
L. dyeriana	*Woytkowski* 25	K d
L. fimbriata (Poepp. & Endl.) Cogn.	*Mason* 80	K s
L. fowliei Oakeley	*Oakeley* D22 (holo)	K d
L. fragrans Oakeley*	*Oakeley* D41	K d
L. fulvescens Hooker	*Oakeley* 14.5.95	K d
L. fulvescens	*Oakeley* 26.5.95	K d
L. fulvescens	*Oakeley* s.n.	K s
L. fulvescens	*Oakeley* (Java ex Tograth)	K d
L. fulvescens	*Oakeley* (dwarf ex Merida)	K d
L. gigantea Lindl.	*Hartweg* 51 (holo)	K d
L. gigantea	*Oakeley* 57541	K s
L. lanipes Lindl.	*Oakeley* 19.2.1994	K d
*L. lanipes**	*Oakeley* 1.1992	K d
L. lanipes "Green Ice"	*Oakeley* 5.12.1994	K d
L. locusta Rchb.f.	*Davis* 36784 (holo)	W sl
L. locusta	*Koopowitz* 17.9.93	K d
L. locusta	*Mason* 34443	K s
L. locusta	*Oakeley* 3.1993	K d
L. locusta	*Oakeley* s.n.	K s
L. mathiasiae G.C.Kenn.	*Oakeley* s.n.	K d
L. nana Oakeley	*Oakeley* s.n. (holo)	K d

L. reichenbachii Gireoud ex Rchb.f.	*Oakeley* 57314	K s
L. reichenbachii	*Oakeley* 27.9.96	K d
L. trifoliata Lehmann ex Masters	*Dix* 8.1992	K d
L. trifoliata	*Oakeley* 57251	K s

Sect. ***Longisepalae*** Fowlie

L. schilleriana Rchb.f.	*Hay* 14216	K s
L. schilleriana	cult. RBG Kew 12.1925	K d
L. schilleriana	*Woytkowski* 51.824-2	K d
L. schilleriana	*Woytkowski* 51.824-3	K d
*L. schilleriana**	*Zakharoff* D80	K d

Sect. ***Lycaste*** Fowlie

L. dowiana Endres & Rchb.f.**	*Oakeley* s.n.	K d
L. dowiana	*Tibbs* 63701	K s
L. leucantha Klotzsch	cult. Lankaster Gardens *s.n*	K d
L. leucantha	*Sanders* 14211	K s
L. leucantha	*Wubben* 8.1992	K d
L. leucantha	*Wubben* 9.1992	K d
L. macrophylla subsp. *desboisiana* Fowlie	cult. McBeans 3.1991	K d
L. macrophylla subsp. *desboisiana*	*Oakeley* 63702	K s
L. macrophylla subsp. *desboisiana*	*Oakeley* 3.1993	K d
L. macrophylla subsp. *desboisiana*	*Ruiz* CR8	K d
L. macrophylla supsp. viridescens Oakeley**	*Oakeley* (holo)	K d
L. macrophylla subsp. *xanthocheila* Fowlie	*Andreetae* 23.4.1992	K d
L. macrophylla subsp. *xanthocheila**	*Oakeley* 13.8.1994	K d
L. macrophylla subsp. *xanthocheila*	*Oakeley* G21	K d
L. macrophylla subsp. *xanthocheila*	*Oakeley* 1.5.1991	K d
L. skinneri Lindl. var. ipala**	*Oakeley* s.n.	O c
L. xytriophora Linden & Rchb.f.	cult. RBG Kew 9.1913	K d
L. xytriophora	cult. RBG Kew 7.2.1916	K d
L. xytriophora	cult. RBG Kew 14.10.1918	K d
L. xytriophora	cult. RBG Kew 63697	K s

3. *Neomoorea* Rolfe

N. wallisii (Rchb.f.) Schltr.	cult. Glasnevin 1892	K d
N. wallisii	*Moore* (holo)	K d

Subtribe **Maxillariinae** Benth.

Maxillaria picta Hook.	*Hunt* 6319	K d

M. picta	*Leppard* 318	K s
M. tenuifolia Lindl.	*Gentle* 2189	K d
M. tenuifolia	*Matuda* 1400	K d
M. tenuifolia	*Taylor* 52108	K s
M. umbratilis L.O.Williams	*Mason* 1	K s
M. violaceopunctata Rchb.f.	*Mason* 263	K s

OSMOPHORES

A. virginalis	*Vacherot & Lecouffle*	K s
L. cochleata	*Oakeley* s.n.	K sl
L. trifoliata	*Oakeley* s.n.	K sl
L. macrophylla subsp. xanthocheila	Oakeley s.n.	K sl

CHROMOSOME COUNTS

A. cliftonii	97-229/230	*Oakeley* 70000.008	K s
A. clowesii	97-228	*Oakeley* 63933	K s
A. virginalis	97-226	*Oakeley* 63934	K s

TOTAL DNA TEMPLATES

Subtribe **Lycastinae** Pfitz.

1. *Bifrenaria* alliance

Bifrenaria harrisoniae	O-95	*Chase* O-95 (ITS)	K s
Bifrenaria sp.°	W97	*Whitten* 93197 (*matK*)	FLAS
Rudolfiella aurantiaca	O-178	*Chase* O-178	K s
Xylobium aff. *colleyi* (Lindl.) Rolfe°	W136	*Hills* F1662 (*matK*)	FLAS
Xylobium pallidiflorum (Hook.) Nichols°	W60	*Whitten* 90241 (ITS)	FLAS

2. *Lycaste alliance*

a. *Anguloa*

A. brevilabris	MWC7675	*Oakeley* D10	K d
A. cliftonii	O-88	*Chase* O-88	K s
A. clowesii	MWC7677	*Oakeley* A49	K d
A. eburnea	MWC7676	*Oakeley* A52	K d

b. *Lycaste*

Sect. ***Deciduosae***

Subsect. ***Xanthanthae***

L. aromatica	MWC7644	*Oakeley* 1957	K s
L. bradeorum	MWC7646	*Oakeley* B9	K d
L. campbellii	MWC7649	*Severin* K6	K s

L. cobani Lindl.	MWC7659	*Oakeley* D7	K d
L. cochleata	MWC7668	*Oakeley* D27	K d
L. consobrina	MWC7651	*Ryan* 67	K s
L. crinita	MWC7648	*Ryan* 82	K s
L. cruenta	MWC7652	*Oakeley* 17.11.95	K d
L. deppei	MWC7643	HMKN 049.85 01092	K c
L. edinensis Hort.	MWC7658	*Oakeley* A58	K d
L. lassioglossa	MWC7660	*Mason* 73	K s
L. macrobulbon	MWC7666	*Oakeley* D74	K d
L. suaveolens	MWC7650	*Oakeley* A60	K d
Subsect. ***Paradeciduosae***			
L. brevispatha	MWC7664	*Oakeley* A5	K d
L. candida	MWC7665	*Oakeley* 63698	K s
L. tricolor	MWC7661	*Oakeley* H1	K d
Sect. ***Fimbriatae***			
L. barringtonii	MWC7656	*Oakeley* D18	K d
L. cinnabarina	MWC7641	*Oakeley* G15	K d
L. dyeriana	MWC7655	*Oakeley* D28	K d
L. costata	MWC7671	*Oakeley* A62/A67	K d
L. fragrans	MWC7647	*Oakeley* D41	K d
L. fulvescens	MWC7674	*Oakeley* D47	K d
L. gigantea	MWC7657	*Oakeley* D63	K d
L. lanipes	MWC7673	*Oakeley* D36/G38	K d
L. locusta	MWC7667	*Oakeley* A25	K d
L. reichenbachii	MWC7672	*Oakeley* 10.95	K d
Sect. ***Longisepalae***			
L. schilleriana	MWC7642	*Oakeley* D80/H75	K d
Sect. ***Lycaste***			
L. dowiana	MWC7645	*Tibbs* 63701	K s
L. leucantha	MWC7670	*Oakeley* 15.10.95	K d
L. macrophylla subsp. *desboisiana*	MWC7669	*Oakeley* A8	K d
L. macrophylla subsp. *xanthocheila*	MWC7663	*Oakeley* H70	K h
L. xytriophora	MWC7654	cult. RBG Kew 1973.13936	K s
3. Neomoorea			
N. wallisii	O-503	*Chase* O-503	K s

Subtribe **Maxillariinae** Benth.

Cryptocentrum sp.°	O-108	*Chase* O-108 (*matK*)	K s
C. peruvianum (Cogn.) C.Schwienf.	O-115	*Chase* O-115 (ITS)	K s
Maxillaria umbratilis °	W-69	SEL 1995-0397	SEL
M. violaceopunctata °	W-80	SEL 1981-2139	SEL

Subtribe **Zygopetalinae** Schltr.

Batemannia colleyi Lindl.°	O-77	*Chase* O-177	K s
Koellensteinia graminea Rchb.f.°	O-501	*Chase* O-501	K s
Zygopetalum intermedium Lindl.	O-160	*Chase* O-160	K s
Dichaea muricata (Sw.) Lindl.°	O-93	*Chase* O-93 (*matK*)	K s
D. riopalenquensis Dodson	O-114	*Chase* O-114 (ITS)	K s

FLORAL FRAGRANCE ANALYSES

a. ***Anguloa***

A. brevilabris	T265NP	*Oakeley* s.n.	K s
A. cliftonii	P161NP	*Oakeley* 25.7.91	K d
A. cliftonii	T448NP	*Oakeley* 1994	K d
A. clowesii	P162NP	*Oakeley* 57535	K s
A. hohenlohii	T358NP	*Oakeley* 6.93	K d
A. hohenlohii	V548NP	*Tibbs* 63937	K s
A. eburnea	S357NP	*Oakeley* 6.8.91	K d
A. virginalis	T357NP	*Oakeley* 12.6.93	K d

b. ***Lycaste***

Sect. ***Deciduosae***

Subsect. ***Xanthanthae***

L. aromatica♠	V485NP	*Oakeley* 57700	K s
L. aromatica 1	T359NP	*Oakeley* 6.93	K d
L. aromatica 2	V390NP	*Ryan* 11	K s
L. aromatica 3	V427NP	*Oakeley* HF09	K sl
L. aromatica 4	V524NP	*Ryan* 52	K s
L. aromatica 5♠♠	V527NP	*Oakeley* 1957	K s
L. aromatica 6	V541NP	*Salazar* 5331	AMO
L. aromatica 7	V543NP	*Ryan* 88	K s
L. bradeorum	X099NP	*Oakeley* B9	K d
L. cochleata	X237NP	*Oakeley* D27	K sl
L. consobrina 1	V382NP	*Ryan* 12	K s
L. consobrina 2	V389NP	*Ryan* 10	K s
L. consobrina 3	V424NP	*Ryan* 17	K s

L. crinita	W456NP	*Soto* 6522-A	AMO
L. cruenta	P169NP	*Oakeley* A42	K d
L. cruenta	W330NP	*Oakeley* HF04	K s
L. deppei 1	V443NP	*Ryan* 41	K s
L. deppei 2	V447NP	*Ryan* 48	K s
L. deppei 3	V450NP	*Ryan* 40	K s
L. deppei 4	V444NP	*Ryan* 28	K s
L. deppei 5	V445NP	*Salazar* GAS5330	AMO
L. deppei 6	V448NP	*Ryan* 89	K s
L. deppei 7	V449NP	*Ryan* 29	K s
L. macrobulbon	X098NP	*Oakeley* 3.95	K sl
L. suaveolens	T441NP	cult. RBG Kew 1984-2244	K s
L. suaveolens	T634NP	*Oakeley* 7.93	K s
Subsect. ***Paradeciduosae***			
L. luminosa	T360NP	*Oakeley* 57387	K s
Sect. ***Fimbriatae***			
L. ciliata	R459NP	*Oakeley* 7.92	O sl
L. cinnabarina	T535NP	*Oakeley* 57536	K s
L. cinnabarina	Y184NP	*Oakeley* G15	K d
L. costata	U026NP	*Oakeley* A62/A67	K d
L. fragrans	W802NP	*Oakeley* 63699	K s
L. gigantea 'Baeza'	Y183NP	*Oakeley* 10.95	K d
L. lanipes 'Bergström'	Y185NP	*Oakeley* 10.95	K d
L. locusta 1	R087NP	*Oakeley* A25	K d
L. locusta 2	X397NP	*Oakeley* 6.95	K sl
Sect. ***Lycaste***			
L. leucantha	T533NP	*Oakeley* 15.10.95	K d
L. macrophylla subsp. desboisiana	W706NP	*Oakeley* 63702	K s
L. macrophylla subsp. *puntarenasensis*	W801NP	*Oakeley* 2.95	K d
L. macrophylla subsp. *xanthocheila*	T534NP	*Oakeley* 7.93	K d
L. xytriophora	T266NP	*Oakeley* 5.93	K d

c. Hybrids

Anguloa x *rolfei*	S358NP	*Oakeley* 8.92	K sl
Anguloa virginalis x *A. clowesii*	T575NP	*Oakeley* 9.93	K sl
Lycaste Groganii	T239NP	*Tibbs* 5.93	K sl
Lycaste x *hartleyi*	V428NP	*Oakeley* 4.94	K sl
Lycaste Wyld Forest	X238NP	*Oakeley* 4.95	K sl

Angulocaste	Whatcroft	R456NP	*Oakeley* 7.92	K sl
Angulocaste	Wyld Dragon	T264NP	*Oakeley* 5.93	K sl

* Parsimony analysis and seed SEM work.

** Seed SEM work only.

° DNA sequence(s) provided by Mark Whitten

♠ Fragrance cycle work

♠♠ Fragrance sampled on seven consecutive days

Appendix 3 MORPHOLOGICAL CHARACTER STATES

A key is given at the end of the table

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bifrenaria harrisoniae	1	1	0	0	1	1	1	1	1	1	0	0	1	1	0	1	0	0	0	0
Rudolfiella aurantiaca	1	1	0	0	1	1	0	1	1	2	0	0	1	1	0	0	2	1	1	0
Neomoorea wallisii	1	1	0	1	1	1	0	1	1	2	0	0	1	2	1	0	2	2	2	1
Maxillaria umbratilis	1	0	0	0	0	0	2	1	0	0	1	0	1	0	0	0	2	2	0	0
Maxillaria violaceopunctata	1	1	0	0	0	0	2	1	0	0	0	0	1	0	0	0	2	2	0	0
Maxillaria tenuifolia	1	0	0	0	0	0	2	1	0	0	0	0	1	1	0	0	2	2	2	0
Maxillaria picta	1	1	0	1	0	0	2	1	0	0	1	0	1	1	0	0	2	2	1	0
Xylobium latilabium	1	1	0	0	1	1	2	1	1	2	?	0	1	1	1	0	2	1	1	1
Anguloa brevilabris	1	1	1	1	1	1	0	0	1	0	1	1	1	2	0	0	3	2	2	0
Anguloa clowesii	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	0	3	2	0	0
Anguloa cliftonii	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	0	3	2	1	0
Anguloa dubia	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	0	3	1	1	?
Anguloa hohenlohii	1	1	2	1	1	1	0	0	1	0	1	1	1	2	0	0	3	1	2	?
Anguloa eburnea	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	0	3	1	0	0
Anguloa virginalis	1	1	2	1	1	1	0	0	1	0	1	1	1	1	0	0	3	2	1	0
Lycaste brevispatha	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	0	1	2	1	1
Lycaste aromatica	1	1	2	1	1	1	0	0	1	0	0	0	0	0	1	0	2	2	0	1
Lycaste suaveolens	1	1	2	1	1	1	0	0	1	0	0	0	0	0	1	0	2	2	0	1
Lycaste consobrina	1	1	2	1	1	1	0	0	1	0	1	0	0	0	1	0	2	2	0	0
Lycaste deppei	1	1	1	1	1	1	0	0	1	0	1	0	0	2	1	0	2	2	1	0
Lycaste cruenta	1	1	2	1	1	1	0	0	1	0	1	0	0	0	1	0	2	2	1	1
Lycaste lasioglossa	1	1	0	1	1	1	0	1	1	0	0	0	0	2	1	0	2	2	0	0
Lycaste cochleata	1	1	2	1	1	1	0	0	1	0	0	0	0	0	1	0	1	2	0	1
Lycaste tricolor	1	1	0	1	1	1	0	0	1	0	1	0	0	2	1	0	2	2	1	0
Lycaste campbellii	1	1	2	1	1	1	0	0	1	0	0	0	0	0	1	0	2	2	0	1
Lycaste candida	1	1	2	1	1	1	0	0	1	0	1	0	0	1	1	0	1	1	1	0
Lycaste luminosa	1	1	2	1	1	1	0	0	1	0	0	0	0	0	1	0	2	1	0	1
Lycaste andreetae	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0
Lycaste barringtoniae	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	2	1	0	0
Lycaste ciliata	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste cinnabarina	1	1	2	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste costata	1	1	1	1	1	1	0	1	1	0	1	0	1	1	0	1	1	2	0	0
Lycaste fimbriata	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste fowliei	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0
Lycaste fragrans	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	2	1	0	0
Lycaste fulvescens	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste gigantea	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste locusta	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste nana	1	1	0	1	1	1	0	1	1	0	1	0	1	0	1	1	2	2	0	1
Lycaste reichenbachii	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste trifoliata	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste xytriophora	1	1	1	1	1	1	0	?	1	0	1	0	0	2	1	0	2	2	1	0
Lycaste dowiana	1	1	0	1	1	1	0	?	1	0	1	0	0	2	1	0	1	0	0	1
Lycaste leucantha	1	1	0	1	1	1	0	1	1	0	1	0	0	0	0	0	1	0	0	0
Lycaste macro. desboisiana	1	1	0	1	1	1	0	1	1	0	1	0	0	2	1	0	2	2	1	1
Lycaste dyeriana	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	2	2	0	0
Lycaste crinita	1	1	2	1	1	1	0	0	1	0	1	0	0	0	1	0	2	2	0	1
Lycaste lanipes	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	2	2	0	0
Lycaste mathiaseae	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	?	?	?	0	0
Lycaste macrobulbon	1	1	2	1	1	1	0	0	1	0	1	0	0	0	1	0	2	2	1	1
Lycaste schilleriana	1	1	0	1	1	1	0	1	1	0	1	0	0	2	0	0	2	2	1	0
Lycaste macro. xanthocheila	1	1	?	1	1	1	0	1	1	0	1	0	0	2	0	0	1	1	1	0

Character	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Bifrenaria harrisoniae	0	0	0	1	1	2	1	2	0	0	1	1	0	1	2	1	0	0	1
Rudolfiella aurantiaca	2	0	0	0	2	0	0	2	0	0	0	2	?	0	0	1	1	0	0
Neomoorea wallisii	2	0	0	1	2	1	0	3	0	0	0	2	1	0	0	1	0	0	0
Maxillaria umbratilis	2	0	0	1	2	1	1	3	0	1	1	2	1	0	0	1	0	0	0
Maxillaria violaceopunctata	2	1	0	0	2	0	1	3	0	1	0	2	1	0	0	0	0	0	0
Maxillaria tenuifolia	2	0	0	1	2	1	1	2	0	0	1	2	1	0	1	0	0	0	0
Maxillaria picta	2	0	0	1	2	0	1	1	0	1	0	2	0	0	0	0	0	0	0
Xylobium latilabium	1	0	0	0	1	0	1	2	0	0	0	?	0	0	0	0	0	0	0
Anguloa brevilabris	1	?	1	1	2	0	0	1	0	1	1	2	1	1	0	0	0	0	0
Anguloa clowesii	3	0	0	0	0	0	0	3	0	1	1	0	1	1	1	0	0	0	0
Anguloa cliftonii	3	1	0	0	2	0	0	3	0	1	1	0	1	1	0	1	1	0	0
Anguloa dubia	2	2	0	0	2	0	0	3	0	1	1	2	1	1	0	1	1	0	0
Anguloa hohenlohii	2	0	0	0	2	0	0	1	0	1	1	2	1	1	1	0	0	0	0
Anguloa eburnea	2	?	0	0	0	0	0	3	0	1	0	2	1	1	0	1	0	0	0
Anguloa virginalis	2	0	1	0	2	0	0	3	0	1	0	2	1	1	0	1	0	0	0
Lycaste brevispatha	1	2	0	0	2	0	1	1	0	0	0	2	1	0	0	0	1	1	0
Lycaste aromatica	2	2	0	0	2	2	1	1	0	0	0	0	1	0	2	0	1	0	0
Lycaste suaveolens	2	2	0	0	2	0	1	1	0	0	0	0	1	0	1	0	1	1	0
Lycaste consobrina	2	2	0	0	0	0	1	2	0	0	0	0	1	0	0	0	1	0	0
Lycaste deppei	2	2	0	1	0	0	1	1	0	0	0	2	1	0	0	0	1	1	0
Lycaste cruenta	2	2	1	0	2	2	1	1	0	0	0	0	1	0	0	0	1	1	0
Lycaste lasioglossa	2	2	0	0	2	0	1	1	0	0	2	2	1	0	0	0	1	0	0
Lycaste cochleata	1	0	0	0	0	0	1	2	0	0	0	0	1	0	0	0	1	0	0
Lycaste tricolor	2	2	0	0	0	0	1	2	1	0	0	2	1	1	0	0	0	0	0
Lycaste campbellii	2	2	0	0	0	0	1	2	0	0	0	0	1	0	0	0	1	0	0
Lycaste candida	0	2	0	0	2	0	1	2	0	0	0	2	1	0	0	1	1	1	0
Lycaste luminosa	1	2	0	0	0	0	1	2	0	0	0	0	1	0	0	0	1	1	0
Lycaste andreetae	2	0	0	1	0	0	1	3	1	1	0	0	1	1	0	1	0	1	0
Lycaste barringtoniae	2	0	0	1	0	0	1	1	1	1	0	0	1	1	0	1	0	1	0
Lycaste ciliata	2	0	0	1	0	0	1	1	1	0	0	0	1	1	0	1	0	?	?
Lycaste cinnabarina	1	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	0	0	1
Lycaste costata	1	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	0	1	1
Lycaste fimbriata	2	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	1	1	1
Lycaste fowliei	1	?	0	0	0	0	1	2	1	1	0	0	1	1	0	1	0	0	1
Lycaste fragrans	2	0	0	1	0	0	1	1	1	0	0	0	1	1	0	1	0	1	1
Lycaste fulvescens	2	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	0	1	0
Lycaste gigantea	2	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	0	1	0
Lycaste locusta	1	0	0	0	0	0	1	2	1	1	0	0	1	1	0	1	0	1	0
Lycaste nana	2	0	0	1	0	0	1	2	1	1	0	0	1	1	0	1	0	1	0
Lycaste reichenbachii	1	0	0	1	0	0	1	1	1	1	0	0	1	1	0	1	0	1	1
Lycaste trifoliata	2	0	0	1	0	0	1	1	1	1	0	0	1	1	0	1	0	?	0
Lycaste xytriophora	2	2	0	0	0	0	1	2	0	0	0	2	1	0	0	0	1	0	0
Lycaste dowiana	0	2	0	0	0	0	1	2	1	0	0	0	1	0	0	0	1	0	0
Lycaste leucantha	1	2	0	1	0	0	1	2	0	0	1	0	1	0	0	0	1	1	0
Lycaste macro. desboisiana	2	2	0	0	2	0	1	1	0	0	0	2	1	0	0	0	1	1	0
Lycaste dyeriana	2	0	0	0	0	0	1	0	1	1	0	0	1	1	0	1	0	1	1
Lycaste crinita	1	2	0	0	0	2	1	3	0	0	1	2	1	0	0	0	1	1	0
Lycaste lanipes	2	0	0	1	0	0	1	1	1	1	0	0	1	1	0	1	0	1	0
Lycaste mathiaseae	?	0	0	1	0	0	1	2	1	?	0	0	1	0	0	0	?	?	?
Lycaste macrobulbon	1	2	0	0	2	0	1	1	0	0	0	2	1	0	0	1	1	1	0
Lycaste schilleriana	2	2	0	0	2	0	1	1	0	0	0	2	1	0	0	0	1	0	0
Lycaste macro. xanthocheila	1	2	0	0	2	0	1	2	0	0	0	2	1	0	0	0	1	1	?

Character	40	41	42	43	44	45	46	47
Bifrenaria harrisoniae	0	1	0	0	2	0	0	0
Rudolfiella aurantiaca	0	1	0	0	2	2	3	0
Neomoorea wallisii	0	0	0	0	1	2	1	1
Maxillaria umbratilis	0	0	0	1	1	1	3	0
Maxillaria violaceopunctata	0	0	0	1	1	1	3	0
Maxillaria tenuifolia	0	0	0	0	1	0	3	0
Maxillaria picta	0	0	0	0	1	0	3	0
Xylobium latilabium	0	0	0	0	1	0	?	?
Anguloa brevilabris	1	1	0	0	1	2	1	1
Anguloa clowesii	1	1	0	0	1	2	1	1
Anguloa cliftonii	1	1	0	0	1	2	1	1
Anguloa dubia	1	1	0	0	1	2	1	1
Anguloa hohenlohii	1	1	0	0	1	2	1	?
Anguloa eburnea	1	1	1	0	1	2	1	1
Anguloa virginalis	1	1	1	0	1	2	1	1
Lycaste brevispatha	0	1	0	0	1	2	1	1
Lycaste aromatica	0	2	0	0	1	2	1	1
Lycaste suaveolens	0	?	0	0	1	2	1	1
Lycaste consobrina	0	1	0	0	1	2	1	1
Lycaste deppei	0	2	0	0	1	2	0	0
Lycaste cruenta	0	1	0	0	1	2	1	1
Lycaste lasioglossa	0	1	0	0	1	2	1	1
Lycaste cochleata	0	1	0	0	1	2	1	1
Lycaste tricolor	0	?	0	0	1	2	1	1
Lycaste campbellii	0	1	0	0	1	2	1	1
Lycaste candida	0	0	0	0	1	2	1	1
Lycaste luminosa	0	1	0	?	1	2	1	1
Lycaste andreetae	0	1	0	0	1	2	2	1
Lycaste barringtoniae	0	2	0	0	1	2	2	1
Lycaste ciliata	0	2	0	?	1	2	?	?
Lycaste cinnabarina	0	2	0	0	1	2	2	1
Lycaste costata	0	2	0	0	1	2	2	1
Lycaste fimbriata	0	2	0	0	1	2	?	1
Lycaste fowliei	0	?	0	0	1	2	?	?
Lycaste fragrans	0	2	0	0	1	2	2	1
Lycaste fulvescens	0	2	0	0	1	2	2	1
Lycaste gigantea	0	2	0	?	1	2	?	?
Lycaste locusta	0	2	0	0	1	2	2	1
Lycaste nana	0	?	0	0	1	2	?	?
Lycaste reichenbachii	0	2	0	0	1	2	?	?
Lycaste trifoliata	0	2	0	?	1	2	?	?
Lycaste xytriophora	0	0	0	0	1	2	1	1
Lycaste dowiana	0	0	0	1	1	2	1	1
Lycaste leucantha	0	0	0	0	1	2	1	1
Lycaste macro. desboisiana	0	0	0	0	1	2	1	1
Lycaste dyeriana	0	0	0	0	1	2	?	?
Lycaste crinita	0	?	0	0	1	2	?	?
Lycaste lanipes	0	2	0	0	1	2	?	?
Lycaste mathiaseae	0	?	0	?	1	2	?	?
Lycaste macrobulbon	0	1	0	1	1	2	1	1
Lycaste schilleriana	0	1	0	1	1	2	1	1
Lycaste macro. xanthocheila	0	?	0	1	1	2	1	1

1. Growth habit (pendent 0, erect 1)
2. Rhizome habit (ascending 0, creeping 1)
3. Pseudobulb spines (absent 0, vestigial 1, prominent 2)
4. Leaf number (single leaf 0, more than one 1)
5. Leaf petiole (absent 0, present 1)
6. Leaf vernation (conduplicate 0, plicate 1)
7. Leaf texture (papery 0, leathery 1)
8. Leaf persistence (persisting for one year or less 0, more than one year 1)
9. Leaf shape (not-linear 0, linear 1)
10. Flowers per scape (usually one 0, usually two 1, many 2)
11. Floral bract morphology (close fitting 0, loose fitting 1)
12. Sepal habit (not connivent 0, connivent 1)
13. Sepal and petal colour (the same colouring 0, different in colour 1)
14. Sepal colour uniformity (uniform 0, light spotting 1, heavy spotting 2)
15. Sepal indumentum (absent 0, present 1)
16. Lateral sepals (not falcate 0, falcate 1)
17. Lateral sepal apex shape (truncate 0, obtuse 1, acute 2, falcate 3)
18. Dorsal sepal apex shape (truncate 0, obtuse 1, acute 2)
19. Petal colour uniformity (0 uniform, 1 light spotting, 2 heavy /overlay)
20. Petal indumentum (absent 0, present 1)
21. Petal apex shape (truncate 0, obtuse 1, acute 2, falcate 3)
22. Petal overlap (none 0, at apex only 1, most of the length 2)
23. Pronounced "step" near the back of the lip (absent 0, present 1)
24. Lip colour (same as petals 0, different from petals 1)
25. Disc colour uniformity (uniform 0, differently coloured veins 1, spotting 2)
26. Disc indumentum (absent 0, present 1)
27. Lip mid-lobe morphology (not prominent 0, prominent 1)
28. Mid-lobe apex shape (truncate 0, rounded/obtuse 1, retuse 2, acute 3)
29. Mid-lobe margin fimbriation (absent 0, present 1)
30. Mid-lobe texture (thin 0, fleshy 1)
31. Mid-lobe indumentum (absent 0, velvety 1, hairy 2)
32. Mid-lobe colour uniformity (uniform 0, veins different colour 1, spotted 2)
33. Callus apex overhanging (absent 0, present 1)
34. Callus apex shape (entire 0, two or three lobed 1)
35. Callus indumentum (absent 0, present 1)
36. Callus ridges (absent 0, present 1)
37. Column hairs under the stigma (absent 0, present 1)
38. Column hairs over the ovary (absent 0, present 1)
39. Column-foot indumentum (absent 0, present 1)
40. Rostellum mid-lobe attitude (flat or ridged 0, recessed 1)
41. Rostellum mid-lobe apex (flush 0, acute 1, truncate/ bidentate 2)
42. Rostellum side-lobe shape (not elongate 0, elongate 1)
43. Anther-cap texture (smooth 0, velvety 1)
44. Number of stipites (one 0, two 1)
45. Stipe shape (short and triangular 0, oblong and saccate 1, linear 2)
46. Viscidium shape (oblong/elliptic 0, ovate 1, V-/M-shaped 2, lunate /linear 3)
47. Viscidium apex shape (truncate 0, acute 1)

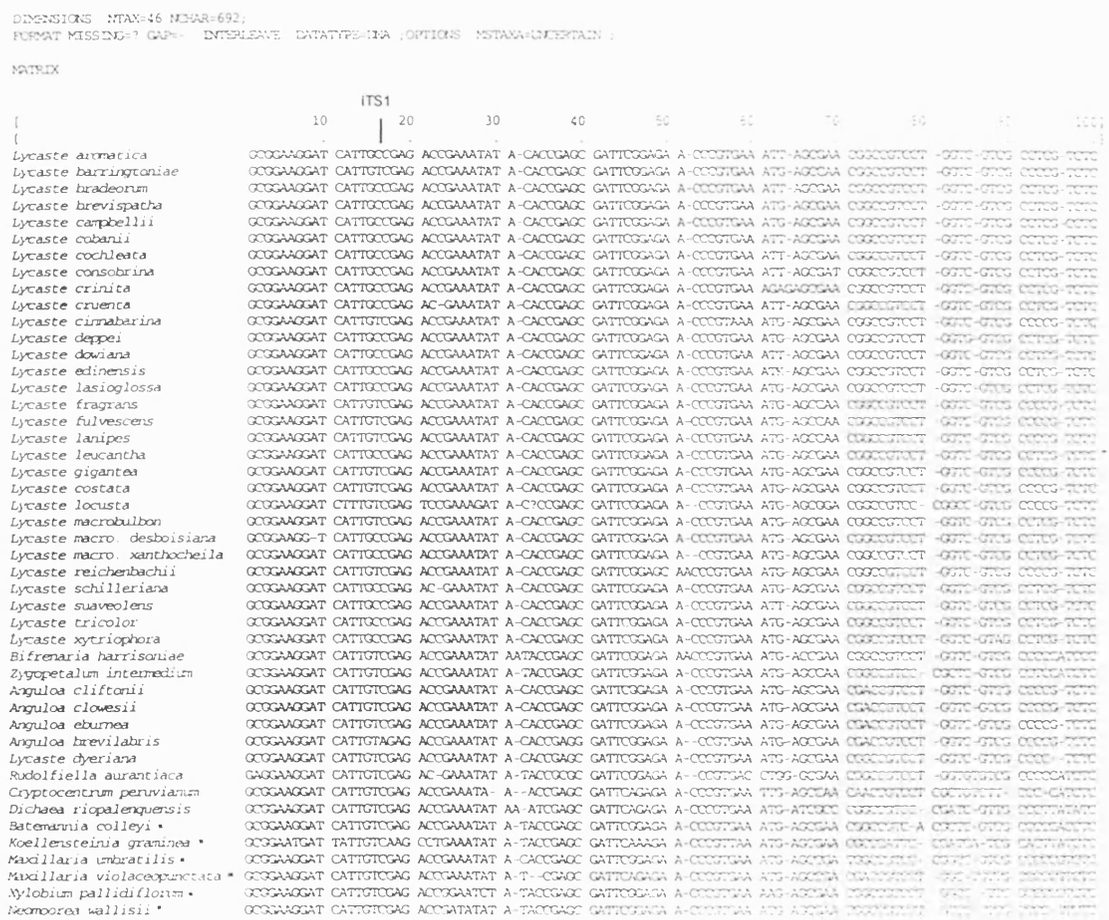
A summary of the characters and codes used for morphological analysis

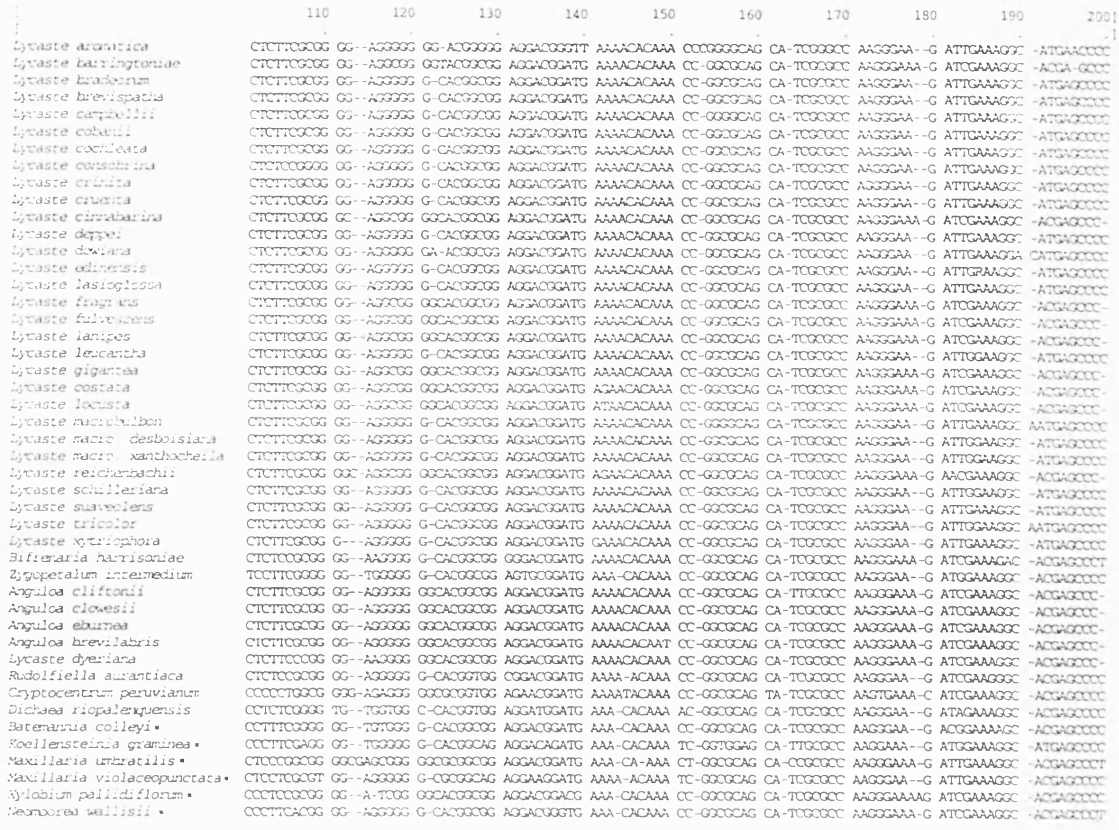
Appendix 4 MOLECULAR DATA

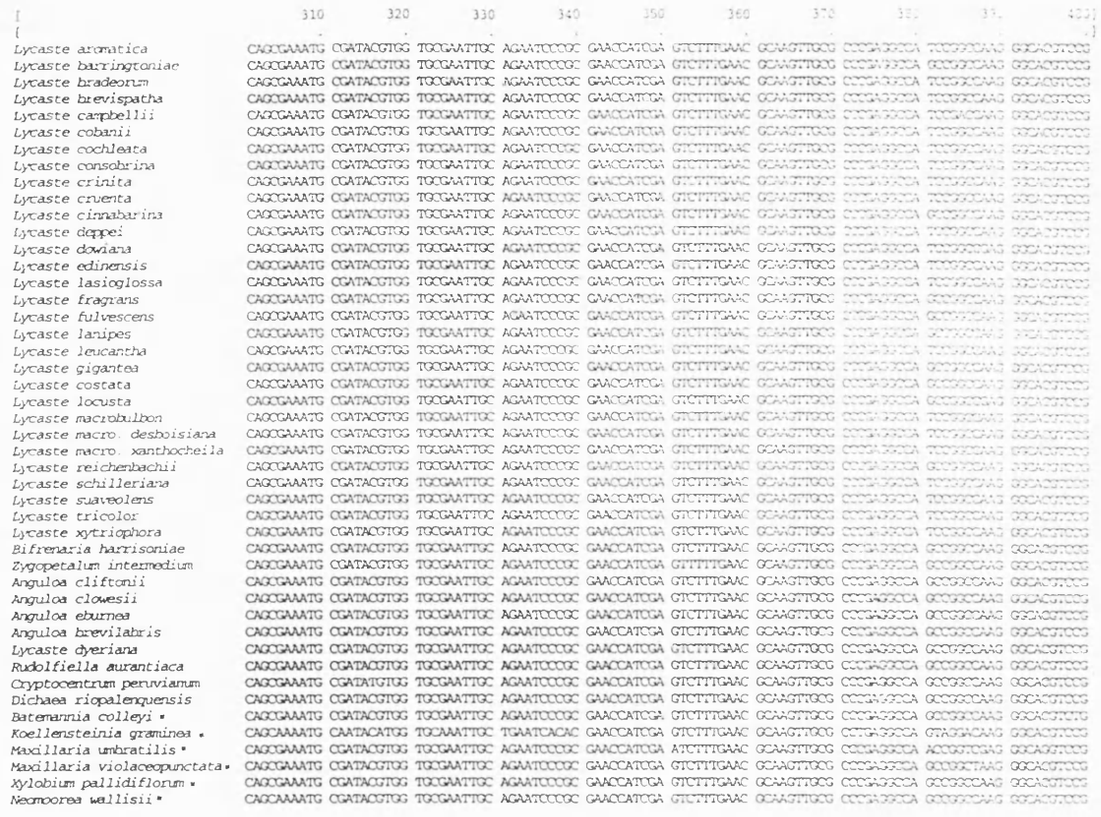
Sequences marked * were provided by Mark Whitten

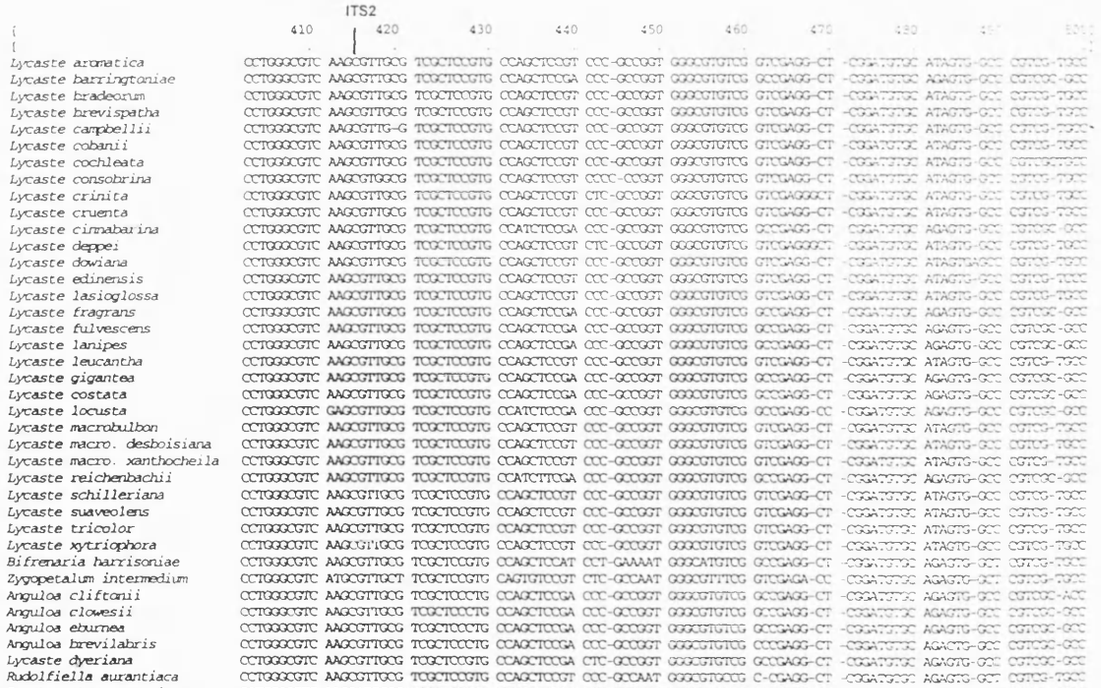
ITS SEQUENCES

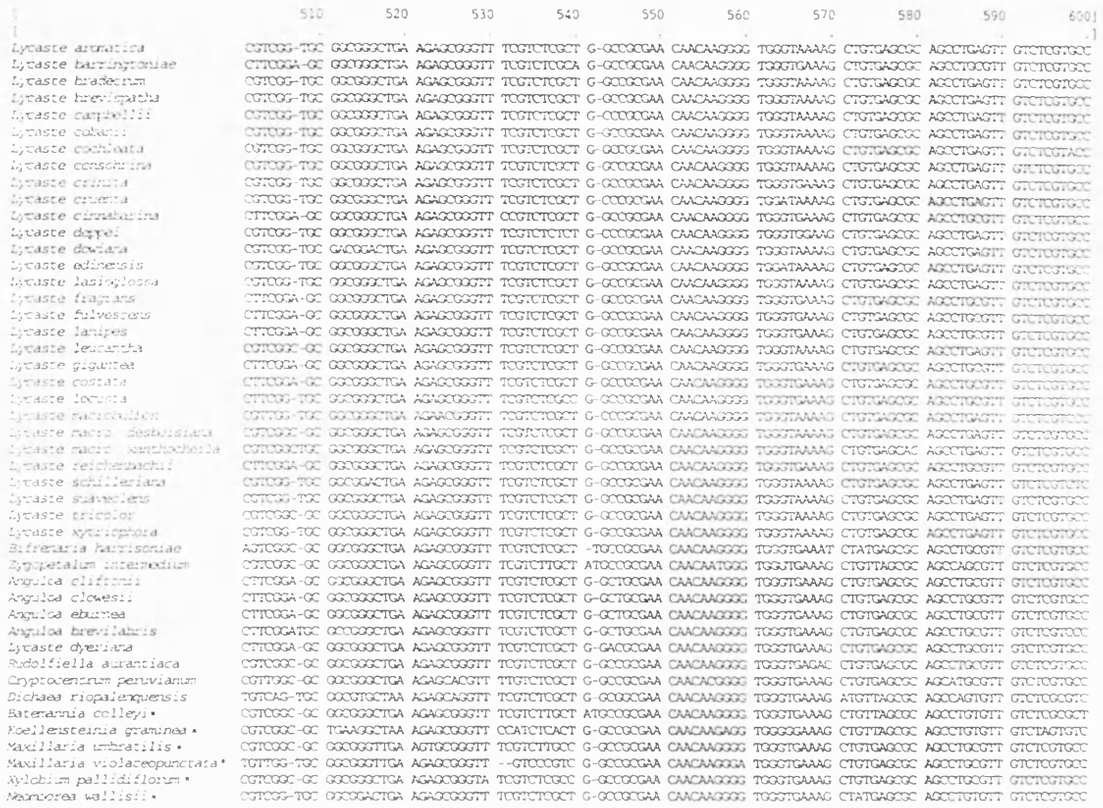
Characters 1 to 15 and 676 to 692 were excluded from the data set.




5.8S


310
320
330
340
350
360
370
380
390
400
Lycaste aromatica
Lycaste barringtoniae
Lycaste bradeorum
Lycaste brevispatha
Lycaste campbellii
Lycaste cobanii
Lycaste cochleata
Lycaste consobrina
Lycaste crinita
Lycaste cruenta
Lycaste cinnabarina
Lycaste deppei
Lycaste dowiana
Lycaste edinensis
Lycaste lasioglossa
Lycaste fragrans
Lycaste fulvescens
Lycaste lanipes
Lycaste leucantha
Lycaste gigantea
Lycaste costata
Lycaste locusta
Lycaste macrobulbon
Lycaste macro. desboisiana
Lycaste macro. xanthocheila
Lycaste reichenbachii
Lycaste schilleriana
Lycaste suaveolens
Lycaste tricolor
Lycaste xytriophora
Bifrenaria harrisoniae
Zygopetalum intermedium
Anguloa cliftonii
Anguloa clowesii
Anguloa eburnea
Anguloa brevilabris
Lycaste dyeriana
Rudolfiella aurantiaca
Cryptocentrum peruvianum
Dichaea riopalenquensis
Batemannia colleyi
Koellensteinia graminea
Maxillaria umbratilis
Maxillaria violaceopunctata
Xylobium pallidiflorum
Neomoorea wallisii


ITS2
410
420
430
440
450
460
470
480
490
500
Lycaste aromatica
Lycaste barringtoniae
Lycaste bradeorum
Lycaste brevispatha
Lycaste campbellii
Lycaste cobanii
Lycaste cochleata
Lycaste consobrina
Lycaste crinita
Lycaste cruenta
Lycaste cinnabarina
Lycaste deppei
Lycaste dowiana
Lycaste edinensis
Lycaste lasioglossa
Lycaste fragrans
Lycaste fulvescens
Lycaste lanipes
Lycaste leucantha
Lycaste gigantea
Lycaste costata
Lycaste locusta
Lycaste macrobulbon
Lycaste macro. desboisiana
Lycaste macro. xanthocheila
Lycaste reichenbachii
Lycaste schilleriana
Lycaste suaveolens
Lycaste tricolor
Lycaste xytriophora
Bifrenaria harrisoniae
Zygopetalum intermedium
Anguloa cliftonii
Anguloa clowesii
Anguloa eburnea
Anguloa brevilabris
Lycaste dyeriana
Rudolfiella aurantiaca
Cryptocentrum peruvianum
Dichaea riopalenquensis
Batemannia colleyi
Koellensteinia graminea
Maxillaria umbratilis
Maxillaria violaceopunctata
Xylobium pallidiflorum
Neomoorea wallisii



*mat*K SEQUENCES

Characters 1577 to 1619 were excluded from the analysis.

The final two characters (1620 and 1621) code for the two indels.

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DIMENSIONS  NTAX=22 NCHAR=1621;
FORMAT MISSING=? GAP=-  INTERLEAVE  DATATYPE=DNA ; OPTIONS  MSTAXA=UNCERTAIN ;

MATRIX

[                                    10         20         30         40         50         60         70         80         90        100]
[                                     .          .          .          .          .          .          .          .          .          .]
Zygopetalum intermedium      ATGTATCATT TCATAACACA AGAAGTGCCT CTTTTTTTTG GTTACAGTCG AAATGGATTT CAATGGCAGA ATTACAAGTA TATTTAGATT TAAAAAAGAG
Rudolfiella aurantiaca       ??GCA-GATT -GAGAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAT AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT GAAAAAAGAT
Anguloa clowesii             A-GTATCATT TCATAACACA AGAAGTGC-T CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Anguloa eburnea              ATGTATCATT -CATAACACA AGAAGTGC-T CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Anguloa cliftonii            ATGTATCATT TCATAACACA AGAAGTGCCT CCCTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste cinnabarina          ATGTATCATT TCATAACACA AGAAGTGCCT CTTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste fragrans             ATGTATCATT TCATAACACA AGAAGTGCTT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste locusta              ATGTATCATT TCATAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste dyeriana             ATGTATCATT TCATAACACA AGAAGTGCTT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste campbellii           ATGTATCATT TCATAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Lycaste aromatica            ATGTATCATT TCATAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAG
Lycaste deppei               ATGTATCATT TCATAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAG
Lycaste schilleriana         ATGTATCATT TCATAACACA AGAAGTGCCT CCTTTTTTTG GTTACAGTAG AAATGTATTT AAATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAT
Maxillaria umbratilis *      ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Maxillaria violaceopunctata *?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Koellensteinia graminea *    ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Bifrenaria sp. *             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Cryptocentrum peruvianum *   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Neomoorea wallisii *         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??ATGGCAGA ATTACAAGGA TATTTAGATT TAAAAAAGAG
Dichaea muricata *           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Batemannia colleyi *         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Xylobium colleyi *           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

[                                   110        120        130        140        150        160        170        180        190        200]
[                                     .          .          .          .          .          .          .          .          .          .]
Zygopetalum intermedium      AGATTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTTGTT TTTTATGAAC
Rudolfiella aurantiaca       AGATTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Anguloa clowesii             ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Anguloa eburnea              ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Anguloa cliftonii            ATATTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste cinnabarina          ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste fragrans             ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste locusta              ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste dyeriana             ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste campbellii           ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste aromatica            ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste deppei               ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Lycaste schilleriana         ATTTTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Maxillaria umbratilis *      ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? GCTCATTATC ATAGCTTCAA TAGTTTTATT TTTTATGAAC
Maxillaria violaceopunctata *?????????? ?????????? ?????????? ?????????? ?????????? ?????????T GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Koellensteinia graminea *    ?????????? ?????????? ?????????? ?????????? ?????????? ??ACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGCTT TTTTATGAAC
Bifrenaria sp. *             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????T GCTCATTATC ATAACTTCAA TAGTTTGATT TTTTATGAAC
Cryptocentrum peruvianum *   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? GCTCATTATC ATAGCTTCAA GAGTTTTCTT TTTTATGAAC
Neomoorea wallisii *         ATATTTTGGC AACAAAACTT CCTCTATCCG CTACTCCTTC AGGAGTATAT TTACTCACTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC
Dichaea muricata *           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Batemannia colleyi *         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Xylobium colleyi *           ?????????? ?????????? ?????????? ?????????? ?GGAGTATAT TTACTC?CTT GCTCATTATC ATAGCTTCAA TAGTTTGATT TTTTATGAAC

[                                   210        220        230        240        250        260        270        280        290        300]
[                                     .          .          .          .          .          .          .          .          .          .]
Zygopetalum intermedium      CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTGGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Rudolfiella aurantiaca       CTATGGAAAT TCTCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TTTTTGATTT CTTCGGTGAA
Anguloa clowesii             CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Anguloa eburnea              CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Anguloa cliftonii            CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste cinnabarina          CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste fragrans             CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste locusta              CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste dyeriana             CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste campbellii           CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste aromatica            CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste deppei               CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Lycaste schilleriana         CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Maxillaria umbratilis *      CTGTGGAAAT TATTGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Maxillaria violaceopunctata *CTGTGGAAAT TATTGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Koellensteinia graminea *    CTGTGGAAAT TATCGGTTAT GACAATAAAT CTAGTTTGGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTTAA
Bifrenaria sp. *             CTATAGAAAT TCTCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TTTTTGATTT CTTCGGTGAA
Cryptocentrum peruvianum *   CTGTGGAAAT TATTGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Neomoorea wallisii *         CTGTAGAAAT TATCGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
Dichaea muricata *           ?????????? ??????TTAT GACAATAAAT CTAGTTTGGT ACTTGTGAAA CGTTTAATTA TTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Batemannia colleyi *         ?????????? ????????AT GACAATAAAT CTAGTTTTGT ACTTGTGAAA CGTTTAATTA TTCGAATGTA TCAACAGAAA TCTTTGATTT CTTCGGTGAA
Xylobium colleyi *           CTGTGGAAAT TATTGGTTAT GACAATAAAT CTAGTTTAGT ACTTGTGAAA CGTTTAATTA CTCGAATGTA TCAACAGAAA TATTTGATTT CTTCGGTGAA
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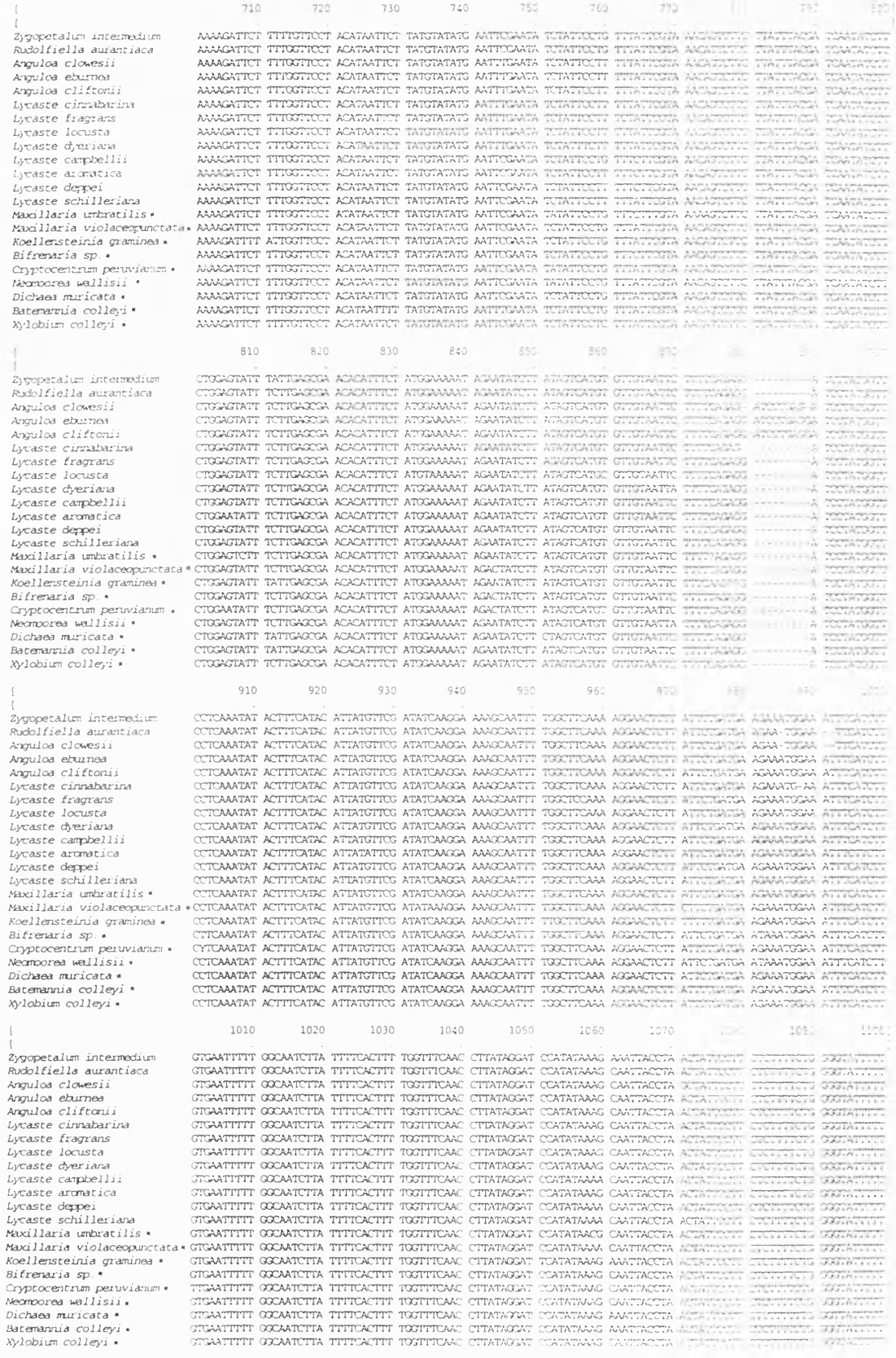

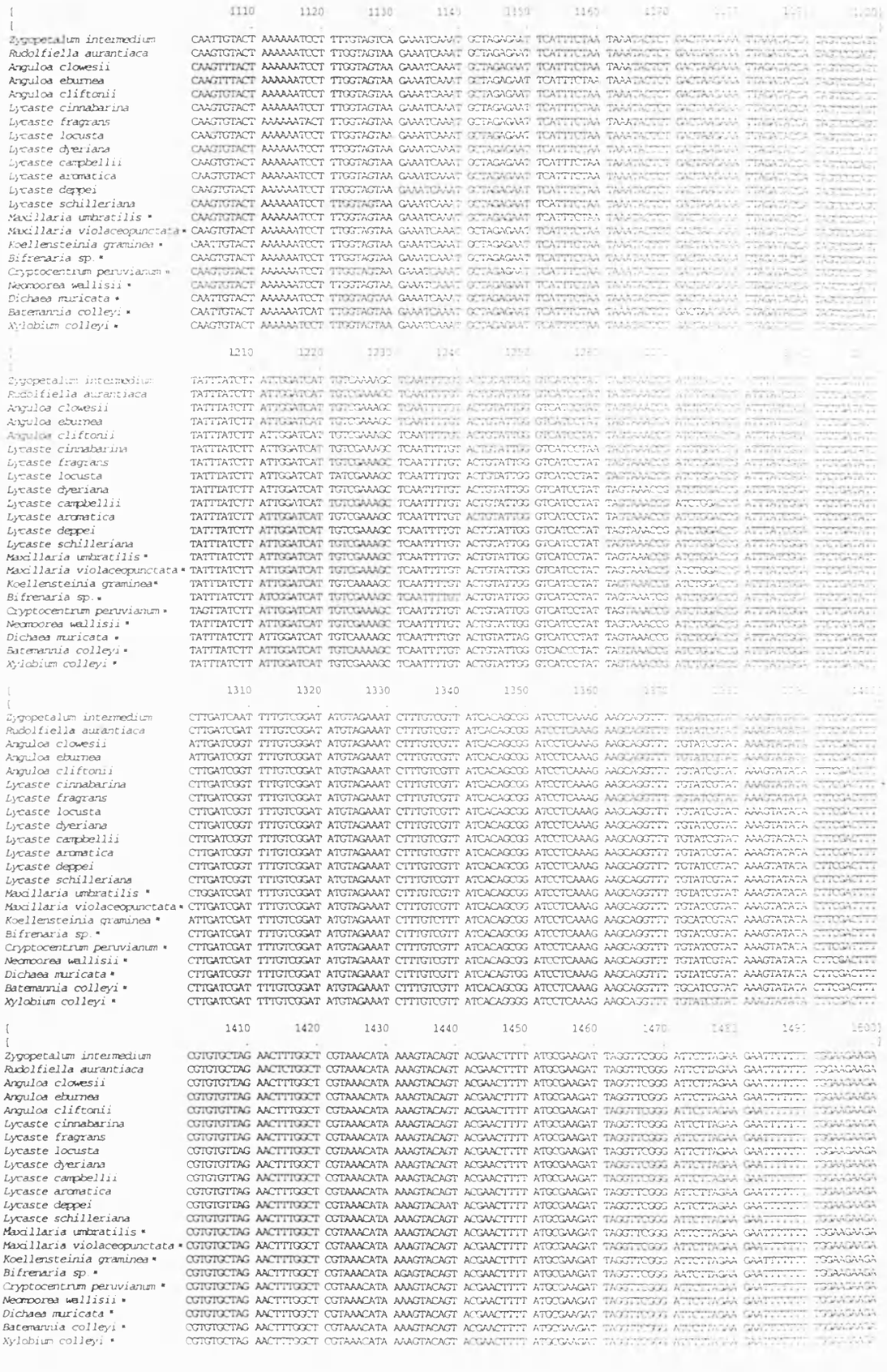

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[                      .           .           .           .           .           .           .           .           .           .]
Zygopetalum intermedium  AGAATCTCTT TCTTTAATCT TCCTACAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG ?????????? ??????????
Rudolfiella aurantiaca   ACAATATCTT TCTTTAATCT TCCTCCAAAA AATACCTTTC TATTTTACAC GGATTACATA -AGA-CGTAT TTG-TATT-G A-CAT-ATCC GTATCACTGA
Anguloa clowesii         ACAATCTCTT TCTTTAATCT TCCTCC-AAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG A-CAT-ATCA G??????????
Anguloa eburnea          ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT T-GGTATTTG GACATTATCC GTATCAATGA
Anguloa cliftonii        ACAATCTCTT TCTTTAATCT TCCTCC-AAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGAT???? ?????????? ??????????
Lycaste cinnabarina      ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC ??????????
Lycaste fragrans         ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC GTATCAATGA
Lycaste locusta          ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC GTATCAATGA
Lycaste dyeriana         ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC GTATCAATGA
Lycaste campbellii       ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGA-CGTAT T-GGTATTTG GACATTATCC GTATCAATGA
Lycaste aromatica        ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC G-ATCAATGA
Lycaste deppei           ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAT TTGGTATTTG GACATTATCC G??????????
Lycaste schilleriana     ACAATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTACATA GAGAACGTAC TTGGT????? ?????????? ??????????
Maxillaria umbratilis *  AGAA?????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Maxillaria violaceopunctata * A????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Koellensteinia graminea *  A????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Bifrenaria sp. *          ACAATATCTT TCTTTAATCT TCCTCCAAAA AATACCTTTC TATTT????? ?????????? ?????????? ?????????? ??????????
Cryptocentrum peruvianum *  ACAATCTCTT TCTTTAATGT TCCTCCAAAA AATCCCTTT- T?????????? ?????????? ?????????? ?????????? ??????????
Neomoorea wallisii *     ACCATCTCTT TCTTTAATCT TCCTCCAAAA AATCCCTTT- TATTTTACAC GGATTATATA GAGA-CGTAT TTGGTATTTG GACATTATCC G??????????
Dichaea muricata *       AGAA?????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Batemannia colleyi *     AGAATCTCTT TCTTTAATCT TCCTCCAAAA AA???????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
Xylobium colleyi *       AAC??????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????

[                    1610        1620]
[                      .           .]
Zygopetalum intermedium  ?????????? ????????? AA  [1606]
Rudolfiella aurantiaca   TCTG-TGGAT CATTCATGA  AA  [1594]
Anguloa clowesii         ?????????? ????????? AT  [1604]
Anguloa eburnea          TCTGGTGGAT CATTCAT??  AT  [1612]
Anguloa cliftonii        ?????????? ????????? AT  [1614]
Lycaste cinnabarina      ?????????? ????????? AA  [1604]
Lycaste fragrans         TCTGGTGGAT CATTCATGA  AA  [1606]
Lycaste locusta          TCTGGTGGAT CATTCATGA  AA  [1604]
Lycaste dyeriana         TCTGGTGGAT CATTCATGA  AA  [1605]
Lycaste campbellii       TCTGGTGGTA CAT?????  TA  [1607]
Lycaste aromatica        TCTGG????? ????????? TA  [1607]
Lycaste deppei           ?????????? ????????? TA  [1609]
Lycaste schilleriana     ?????????? ????????? TA  [1609]
Maxillaria umbratilis *  ?????????? ????????? AA  [1607]
Maxillaria violaceopunctata * ?????????? ????????? AA  [1607]
Koellensteinia graminea *  ?????????? ????????? AA  [1607]
Bifrenaria sp. *         ?????????? ????????? AA  [1606]
Cryptocentrum peruvianum *  ?????????? ????????? AA  [1606]
Neomoorea wallisii *     ?????????? ????????? AA  [1605]
Dichaea muricata *       ?????????? ????????? AA  [1607]
Batemannia colleyi *     ?????????? ????????? AA  [1607]
Xylobium colleyi *       ?????????? ????????? AA  [1607]

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END;
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Appendix 5 THE FLORAL FRAGRANCE COMPOSITION OF *LYCASTE* AND *ANGULOA* SPECIES

		Code	KI*	*L. ciliata*	*L. cinnabarina 1*	*L. cinnabarina 2*	*L. fragrans*	*L. lanipes*	*L. locusta 1*	*L. locusta 2*	*L. gigantea*	*L. costata*	*L. aromatica 1*	*L. aromatica 2*	*L. aromatica 3*	*L. aromatica 4*	*L. aromatica 5*	*L. aromatica 6*	*L. aromatica 7*	*L. bradeorum*	*L. cochleata*	*L. consobrina 1*
1	Ethyl acetate	FA	602	-	-	-	-	-	50.8	86.6	-	-	-	-	-	-	-	-	-	-	-	-
2	(2-Methylbut-3-en-2-ol)	FA	602	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	0.2	-	-
3	Acetic acid	FA	608	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-
4	Isoprene	C5	628	-	0.6	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Valeraldehyde	FA	676	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Ethyl propionate	FA	700	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Propyl acetate	FA	702	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Unknown	FA	733	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Methyl 2-methylbutyrate	FA	760	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Diethyl carbonate	FA	762	-	-	-	-	-	6.8	4.2	-	-	-	-	-	-	-	-	-	-	-	-
11	Ethyl butyrate	FA	783	-	-	-	0.1	4.4	1.8	1.8	-	-	-	-	-	-	-	-	-	-	-	-
12	Butyl acetate	FA	798	-	-	0.1	0.4	0.2	11.4	4.1	2.8	-	-	-	-	-	-	-	-	0.1	-	-
13	Unknown BP43,72,57,58	FA	819	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	-
14	Propyl isobutyrate	FA	826	-	-	-	-	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Ethyl 2-methylbutyrate	FA	835	-	-	-	-	0.1	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
16	(Z)-Hex-3-enol	FA	843	-	-	0.4	0.2	7.2	-	-	2.3	-	-	-	-	-	-	-	-	-	-	-
17	(Methyl tiglate)	FA	846	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Hexanol	FA	856	-	-	-	-	0.4	-	-	7.5	-	-	-	-	-	-	-	-	-	-	-
19	isoAmyl acetate	FA	862	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Methyl 2-hydroxy-3-methylbutyrate	FA	862	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Prenyl acetate-I	C5	865	-	-	-	-	-	5.8	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Cyclohexanone	FA	865	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	Heptanal	FA	878	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3
24	Propyl butyrate	FA	882	-	-	-	-	0.2	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-
25	2-Ethoxyethyl acetate	FA	884	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	Butyl propionate	FA	893	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
27	Amyl acetate	FA	898	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-
28	Prenyl acetate-II	C5	906	-	-	-	-	-	1.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
29	Methyl hexanoate	FA	908	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	α-Thujene	TH	923	0.7	-	-	-	-	-	-	-	-	0.5	0.9	1.0	0.9	0.2	1.4	1.3	-	-	-

		Code	KI*	*L. ciliata*	*L. cinnabarina 1*	*L. cinnabarina 2*	*L. fragrans*	*L. lanipes*	*L. locusta 1*	*L. locusta 2*	*L. gigantea*	*L. costata*	*L. aromatica 1*	*L. aromatica 2*	*L. aromatica 3*	*L. aromatica 4*	*L. aromatica 5*	*L. aromatica 6*	*L. aromatica 7*	*L. bradeorum*	*L. cochleata*	*L. consobrina 1*
31	Unsaturated hydrocarbon	HC	926	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	α-Pinene	PI	930	0.6	-	-	2.4	0.3	0.3	0.7	0.9	-	0.6	2.6	0.8	0.7	0.3	2.1	2.7	4.6	-	3.5
33	Benzaldehyde	BE	933	17.3	63.3	1.0	24.6	5.4	0.3	0.1	0.4	33.6	1.2	0.3	0.9	0.2	1.5	0.2	4.2	-	0.2	0.2
34	Methyl 3-methyl-2-oxopentanoate	FA	946	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
35	6-Methylhept-5-en-2-one	AC	965	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	3.8	1.6
36	Sabinene	TH	965	0.6	-	-	0.8	0.3	-	-	0.4	0.6	0.9	1.0	1.0	1.3	0.9	2.0	4.3	0.5	-	-
37	β-Pinene	PI	970	-	-	-	0.6	0.2	-	-	0.2	-	0.1	0.4	0.1	0.1	0.1	0.1	0.4	0.5	0.7	0.7
38	Unknown (Me 2-hydroxy-3-mepentanoate like)	FA	974	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	Methyl 2-hydroxy-3-methylpentanoate	FA	981	2.3	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	1.1	0.3
40	Butyl butyrate	FA	981	-	-	0.5	3.0	7.6	1.1	0.5	1.5	-	-	-	-	-	-	-	-	-	-	-
41	Myrcene	AC	983	1.1	0.2	-	1.4	0.9	-	-	2.2	1.0	2.3	1.9	0.7	1.4	1.4	1.3	0.6	0.4	0.1	0.3
42	Ethyl hexanoate	FA	983	-	-	-	-	1.0	2.8	0.2	-	-	-	-	-	-	-	-	-	-	-	-
43	Octanal	FA	984	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
44	Ethyl hex-3-enoate	FA	988	-	-	-	-	6.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Hex-3-enyl acetate	FA	995	-	-	3.7	-	0.3	-	-	16.8	-	-	-	-	-	-	-	-	-	-	-
46	Unknown BP59	FA	997	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	α-Phellandrene	ME	997	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Hexyl acetate	FA	998	-	-	-	-	0.3	1.8	0.6	9.5	-	-	-	-	-	-	-	-	-	-	-
49	p-Cresyl methyl ether	BE	1000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	Car-3-ene	CA	1001	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	0.9
51	Methyl heptanoate	FA	1007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	α-Terpinene	ME	1008	-	-	-	-	-	-	-	-	-	1.8	0.4	1.2	0.2	-	0.6	-	0.2	-	-
53	p-Cymene	ME	1009	-	-	-	-	-	-	-	0.4	0.1	1.5	0.5	0.9	0.4	0.5	1.2	2.4	0.1	0.2	0.1
54	Phenylacetaldehyde	BE	1013	1.1	0.7	0.4	1.4	5.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	Benzyl alcohol	BE	1015	0.4	2.3	-	0.3	-	-	-	0.2	0.1	-	-	-	-	-	-	-	-	-	-
56	o-Hydroxybenzaldehyde	BE	1015	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	1,8-Cineole	ME	1015	4.7	-	-	15.0	0.5	-	-	14.0	-	1.4	15.7	2.4	1.7	2.0	4.2	7.9	1.1	-	-
58	β-Phellandrene	ME	1015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	Limonene	ME	1015	0.8	-	-	3.2	0.9	0.3	0.1	2.3	0.7	2.4	1.6	1.0	0.7	2.2	0.5	1.4	1.1	2.0	0.7
60	(Z)-Ocimene	AC	1015	-	-	-	-	0.9	-	-	0.1	-	0.3	0.4	0.4	0.6	0.6	0.4	-	0.3	0.1	0.5
61	Acetophenone	BE	1028	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-
62	(E)-Ocimene	AC	1039	0.2	-	0.2	0.3	1.7	-	-	0.1	-	48.0	41.8	52.4	68.6	15.8	55.2	1.3	23.5	8.2	66.8
63	Unknown BP43,72,84,99,109	FA	1047	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-

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64	Prenyl butyrate-I	F5	1048	-	-	-	-	-	6.7	-	-	-	-	-	-	-	-	-	-	-	-	-
65	γ-Terpinene	ME	1050	-	-	-	0.2	-	-	-	0.1	0.2	3.3	0.7	2.4	0.5	0.9	1.0	0.5	0.3	-	-
66	(E)-Sabinene hydrate	TH	1057	-	-	-	-	0.3	-	-	0.7	0.2	0.9	0.2	1.1	0.4	0.1	0.3	-	0.3	-	-
67	Alkane	AK	1057	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	Octanol	FA	1059	-	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-	0.4	-
69	Guaiacol	BE	1066	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	Unknown BP85,152	AC	1067	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
71	(Ipsenol)	AC	1068	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	Linalol oxides I & II	AC	1068	-	0.9	-	0.2	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
73	Methyl benzoate	BE	1071	-	2.1	67.0	1.1	0.4	-	-	-	41.5	3.8	0.2	0.1	0.1	1.0	0.1	0.5	-	-	-
74	Amyl butyrate	FA	1078	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	6-Methylhepta-3,5-dien-2-one	AC	1079	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	3,4-Epoxy-3,7-dimethylocta-1,6-diene	AC	1080	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-
77	Terpinolene	ME	1080	-	-	-	-	0.2	-	-	-	-	0.6	0.2	0.5	0.2	0.4	0.2	-	0.1	-	-
78	(3,5-Dimethylcyclohex-2-en-1-one) BP82,81,43	FA	1082	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	0.3	-	-	-	-
79	C11 alkene	HC	1082	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	Hex-3-enyl propionate	FA	1082	-	-	-	-	0.4	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
81	Prenyl butyrate-II	F5	1084	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-
82	(Z)-Sabinene hydrate	TH	1084	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-
83	Nonanal	FA	1088	-	-	-	-	-	-	-	1.2	-	0.1	0.3	0.2	0.4	0.2	0.3	1.7	0.3	0.1	0.3
84	Linalol	AC	1090	50.7	0.2	15.4	19.4	1.6	-	-	1.9	8.7	0.6	1.0	0.2	0.6	0.5	0.4	-	0.9	-	0.3
85	Phenylethyl alcohol	BE	1092	2.3	0.2	-	0.8	2.9	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
86	Unknown BP119,134	UK	1095	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	Benzyl cyanide	PN	1097	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.8
88	Thujone	TH	1100	-	-	-	-	0.5	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
89	Methyl nicotinate	NI	1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	Unknown BP91,150	BE	1102	-	-	-	-	-	-	-	-	-	-	0.3	0.2	-	-	0.9	-	0.3	-	-
91	Unknown BP69,41	AC	1106	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	Prenyl ester (angelate?) & unsat H/C	F5	1109	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
93	1,2-Dimethoxybenzene	BE	1117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	Unknown BP91,119,134	UK	1119	-	-	-	-	-	-	-	-	-	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.1	0.4
95	(E,E)-Alloocimene	AC	1119	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	2,3-Epoxy-2,6-dimethylocta-5,7-diene	AC	1123	-	-	-	-	-	-	-	-	-	0.6	0.4	0.7	0.4	0.7	0.4	0.5	0.5	0.2	0.8

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97	Ipsdienone	AC	1123	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-
98	Ocimene epoxide	AC	1124	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	Phenylpropylaldehyde	BE	1126	-	-	-	-	-	-	-	-	-	-	0.2	-	-	0.5	-	-	-	-	-
100	((E)-Pin-3-en-2-ol)	PI	1130	-	-	-	-	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-
101	(Ipsdienol)	AC	1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	Benzyl acetate	BE	1135	0.2	0.2	2.5	1.4	-	0.5	0.3	2.8	-	-	-	-	-	-	-	-	-	-	-
103	1,4-Dimethoxybenzene	BE	1141	-	-	-	-	-	-	-	-	-	0.2	0.1	0.7	0.3	1.5	0.5	0.4	-	1.2	4.7
104	Unknown BP109,124,152	UK	1144	-	-	-	-	1.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	(Verbenone like)	PI	1147	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-
106	Methyl phenylacetate	BE	1148	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
107	Ethyl benzoate	BE	1151	-	-	-	0.1	2.3	0.2	0.1	-	-	-	-	-	-	-	-	-	-	-	-
108	isoValeraldehyde-hexanal condensation product	FA	1151	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
109	Benzoic acid	BE	1156	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	-	-	-
110	Octanoic acid	FA	1157	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	Unknown BP95,93,91,150	UK	1160	-	-	-	-	-	-	-	-	-	0.1	0.3	-	0.4	0.5	0.5	0.5	0.3	-	-
112	Terpinen-4-ol	ME	1160	-	-	-	-	-	-	-	-	0.6	9.9	1.5	7.1	1.3	3.3	3.3	2.6	-	-	-
113	2-Ethylhexyl acetate	FA	1162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
114	Methyl salicylate	BE	1163	-	26.0	0.8	10.3	0.9	-	-	1.2	9.4	-	0.1	-	-	0.3	0.2	-	-	-	-
115	Unknown	UK	1166	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
116	Hex-3-enyl butyrate	FA	1167	-	-	1.5	0.6	26.1	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-
117	Prenyl tiglate	F5	1169	-	-	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-
118	Butyl hexanoate	FA	1169	-	-	-	-	0.7	0.5	-	0.4	-	-	-	-	-	-	-	-	-	-	-
119	Hexyl butyrate	FA	1169	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-	-	-	-	-	-
120	Butyl hex-3-enoate	FA	1171	-	-	-	-	3.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
121	α-Terpineol	ME	1175	1.2	-	-	1.0	0.9	-	-	3.6	2.2	0.3	-	-	-	0.3	-	-	0.7	-	-
122	Methyl chavicol	PR	1175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-
123	Verbenone	PI	1179	-	-	-	-	0.6	-	-	7.4	-	-	-	-	-	-	-	-	-	-	-
124	Cinnamic aldehyde-l	PR	1185	-	-	-	-	-	-	-	-	-	0.5	5.7	3.0	8.3	7.1	2.4	33.0	-	-	-
125	Propyl benzoate	BF	1187	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
126	Cuminal	PR	1188	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
127	Decanal	FA	1189	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	0.2
128	Unknown (p-Mentha-2,8-dien-1-ol-tr)	ME	1189	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-

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129	2-Methyl-3-phenylpropanal	PR	1206	-	-	-	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	(Verbenone, no 80)	ME	1216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
131	Nerol	AC	1218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
132	Carvone	ME	1218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
133	Anisaldehyde	BE	1225	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
134	Neral	AC		0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	Phenylethyl acetate	BE	1226	-	-	0.6	0.5	-	-	-	4.8	-	-	-	-	-	-	-	-	-	-	-
136	Benzyl propionate	BF	1230	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
137	Cinnamic aldehyde-II	PR	1231	-	-	-	-	-	-	-	-	0.3	16.9	19.1	18.4	7.6	53.9	4.0	30.1	-	-	-
138	Geraniol	AC	1231	3.4	-	-	-	-	-	-	0.6	0.2	-	-	-	-	-	-	-	-	-	-
139	isoPiperitenone	ME	1239	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-
140	Unknown BP43,69,70,122	UK	1239	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1	-	0.6	-	-
141	(Dec-4-en-1-ol)	FA	1239	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-
142	Geranial	AC	1239	2.0	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
143	Unknown BP97,72,41,43	UK	1242	-	-	-	-	-	-	-	-	-	-	0.7	0.1	-	0.9	1.3	-	-	-	-
144	3,7-Dimethylocta-1,6-dien-3,5-diol	AC	1242	-	0.2	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
145	Ethyl salicylate	BE	1242	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
146	Prenyl hexanoate-I	F5	1249	-	-	-	-	-	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-
147	p-Mentha-1,3-dien-7-al	ME	1251	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-
148	Alkene	HC	1253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
149	Methyl 3-phenylpropionate	PR	1256	-	-	-	-	-	-	-	-	-	-	-	0.5	0.9	0.9	-	-	-	-	-
150	Phenylethyl ester	BE	1256	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
151	Carboxylic acid	FA	1257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	-	-	-
152	Hex-3-enyl valerate	FA	1264	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
153	Methyl cinnamate-I	PR	1267	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0	-	-	-	-
154	Benzyl isobutyrate	BF	1272	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
155	Prenyl hexanoate-II	F5	1283	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-
156	Undecanal	FA	1289	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157	Methyl nerate/geranate	AC	1300	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-
158	Methyl 2-methoxybenzoate	BE	1302	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
159	Triacetin	FA	1310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
160	Benzyl butyrate	BF	1328	-	-	3.0	0.2	1.1	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-

		Code	KI*	*L. ciliata*	*L. cinnabarina 1*	*L. cinnabarina 2*	*L. fragrans*	*L. lanipes*	*L. locusta 1*	*L. locusta 2*	*L. gigantea*	*L. costata*	*L. aromatica 1*	*L. aromatica 2*	*L. aromatica 3*	*L. aromatica 4*	*L. aromatica 5*	*L. aromatica 6*	*L. aromatica 7*	*L. bradeorum*	*L. cochleata*	*L. consobrina 1*
161	Phenylethyl propionate	BF	1325	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
162	Eugenol	PR	1333	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.2	-	-	-	-	-	-
163	Ethyl ester BP88,108,79,67,164	BF	1334	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
164	Methyl anisate	BE	1336	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
165	1,2,4-Trimethoxybenzene	BE	1337	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166	Phenylpropyl acetate	BE	1343	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-
167	1-Hydroxy-2,2,4-trimepent-3-yl 2-mepropionate	FA	1352	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
169	Methyl 3-phenyllactate	PR	1353	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.9	-
170	Longicyclene	SQ	1353	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
171	Butyl benzoate	BF	1357	-	-	1.1	-	-	-	0.5	0.1	-	-	-	-	-	-	-	-	-	-	-
172	3-Hydroxy-2,2,4-trimepent-1-yl 2-mepropionate	FA	1359	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
173	Geranyl acetate	AC	1362	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	6.4	-	-
174	Methyl cinnamate-II	PR	1364	-	-	-	-	-	-	-	-	-	0.2	-	0.2	-	-	7.4	-	6.5	-	-
175	Hex-3-enyl hexanoate	FA	1365	-	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
176	Hex-3-enyl hex-3-enoate	FA	1367	-	-	-	-	2.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
177	Hexyl hexanoate	FA	1370	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
178	Unknown BP43,84,69,109,128	UK	1371	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	0.6
179	isoLongifolene	SQ	1376	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
180	Phenylethyl isobutyrate	BF	1378	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
181	α-Copaene	SQ	1378	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
182	β-Bourbonene	SQ	1385	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
183	β-Elemene	SQ	1389	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
184	Unknown BP43,119,134 (Crysanthenyl acetate)	UK	1385	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
185	α-Zingiberene	SQ	1388	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
186	Unknown BP43,69,97	UK	1389	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
187	Dodecanal	FA	1390	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
188	Unknown (Safranal like)	UK	1390	-	-	-	-	-	-	-	0.2	-	0.3	0.5	0.3	0.7	0.2	1.8	-	-	-	1.3
189	Sesquiterpene hydrocarbon BP95	SQ	1403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
190	Butyl phenylacetate	BF	1405	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
191	Longifolene	SQ	1406	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
192	isoCaryophyllene	SQ	1408	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
193	α-Ionone	IO	1412	-	-	-	2.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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194	Neryl acetone	SQ	1413	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
195	Unknown BP163,206	SQ	1416	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
196	Phenylethyl butyrate	BF	1419	-	-	0.7	-	2.8	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-
197	3,4-Dihydrobetaionone	IO	1420	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
198	Prenyl benzoate-I	B5	1420	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-
199	Caryophyllene	SQ	1423	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	(2,3-Epoxyneryl acetate)	AC	1428	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
201	Sesquiterpene hydrocarbon BP94	SQ	1429	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
202	Geranyl acetone	SQ	1436	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
203	α-Bergamotene	SQ	1436	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
204	(E)-β-Farnesene	SQ	1448	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
205	Sesquiterpene hydrocarbon BP119	SQ	1450	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
206	Unknown	UK	1454	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
207	2,3-Epoxygeranyl acetate	AC	1455	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37.8	-	-
208	α-Humulene	SQ	1456	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
209	Alloaromadendrene	SQ	1460	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
210	Sesquiterpenoid BP161,203,218	SQ	1466	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	Phenylethyl isovalerate	BF	1466	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
212	β-Ionone	IO	1468	-	-	-	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
213	ar-Curcumene	SQ	1473	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
214	Pentadec-1-ene	HC	1479	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
215	Germacrene-D	SQ	1482	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
216	Sesquiterpene hydrocarbon BP119,93	SQ	1482	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
217	β-Selinene	SQ	1487	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
218	Unknown BP43,69,109,119 (Epoxy acetate?)	AC	1490	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-
219	BHT	BE	1494	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
220	(E,E)-α-Farnesene	SQ	1498	2.3	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	3.1	60.8	12.1
221	Bicyclogermacrene	SQ	1499	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
222	β-Bisabolene	SQ	1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
223	γ-Cadinene	SQ	1508	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
224	δ-Cadinene	SQ	1515	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
225	Unknown	UK	1505	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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226	β-Sesquiphellandrene	SQ	1513	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
227	Unknown BP69,41,81,137	UK	1519	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1	-
228	Unknown BP69,218	SQ	1520	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
229	Unknown	UK	1524	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	-	-
230	Methyl 4-methoxycinnamate-I	PR	1546	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-
231	Hex-3-enyl benzoate	BF	1550	-	-	0.3	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
232	Farnesol like	SQ	1550	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
233	Hexyl benzoate	BF	1558	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-I	SQ	1560	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-
235	Unknown BP41,85,93,119,159,220	SQ	1561	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	(E,E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	SQ	1572	0.3	2.1	0.6	4.8	0.2	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-
237	Caryophyllene oxide	SQ	1578	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5
238	Unknown BP41,85,93,119,159,220	SQ	1580	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
239	Unknown BP41,69,79,81,43 (Epoxyfarnesene?)	SQ	1587	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.9	9.7	-
240	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-II	SQ	1589	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
241	Unknown BP43,69,82,109	UK	1591	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-
242	Unknown BP41,82,81,69,220	SQ	1594	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-
243	Humulene-1,2-epoxide	SQ	1600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
244	Sesquiterpenoid BP81,59 MW218	SQ	1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
245	Hex-3-enyl phenylacetate	BF	1605	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
246	Phenylethyl hexanoate	BF	1618	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	Methyl 4-methoxycinnamate-II	PR	1618	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
248	Phenylethyl hex-3-enoate	BF	1620	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
249	Unknown BP173,99,84,55	UK	1655	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	Unknown BP99,194	UK	1677	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
251	Unknown BP99,152	UK	1710	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
252	Methyl farnesate	SQ	1768	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
253	(E,E)-Farnesyl acetate	SQ	1821	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.0	-
	TOTAL			98.6	99.3	100.0	99.0	99.7	100.0	100.0	99.3	100.0	99.6	99.8	99.7	99.4	99.0	98.9	99.7	100.0	99.9	99.2

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xythophora*
1	Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	5.9	-	-	-	-	-
2	(2-Methylbut-3-en-2-ol)	-	-	-	-	0.5	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Acetic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6	0.1	-	2.0	-
4	Isoprene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Valeraldehyde	-	-	-	-	0.2	0.4	2.7	8.0	0.2	2.2	0.3	-	-	0.2	-	-	-	0.2	-	-
6	Ethyl propionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Propyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Unknown	-	-	-	-	0.2	-	1.7	-	-	-	-	-	-	0.1	12.3	1.4	-	-	-	-
9	Methyl 2-methylbutyrate	-	-	0.2	-	1.0	0.2	0.4	0.1	0.1	0.3	0.4	-	-	-	-	3.7	-	-	0.4	26.0
10	Diethyl carbonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Ethyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.6	-	-	-	-	-
12	Butyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-
13	Unknown BP43,72,57,58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Propyl isobutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Ethyl 2-methylbutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	(Z)-Hex-3-enol	-	-	-	-	-	0.1	0.5	-	0.3	0.2	-	-	-	-	-	-	-	-	-	-
17	(Methyl tiglate)	-	-	-	-	0.4	0.4	4.8	0.2	0.1	2.4	5.3	-	-	-	-	-	-	-	-	-
18	Hexanol	-	-	-	-	1.8	1.3	7.2	1.5	8.4	9.4	1.7	-	-	-	-	-	-	-	-	-
19	isoAmyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	Methyl 2-hydroxy-3-methylbutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
21	Prenyl acetate-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Cyclohexanone	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-	-	-	-
23	Heptanal	0.4	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	Propyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	2-Ethoxyethyl acetate	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	1.7	-	-	-	-
26	Butyl propionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	Amyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	Prenyl acetate-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	Methyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	α-Thujene	-	-	0.9	-	1.2	0.7	0.5	0.9	1.6	0.8	1.0	-	-	-	-	-	-	0.4	-	-
31	Unsaturated hydrocarbon	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-	-	1.0	-	0.2	-	-
32	α-Pinene	3.4	1.1	0.9	0.6	2.5	2.8	1.7	2.6	4.4	1.3	4.3	-	5.0	0.2	4.6	3.5	0.2	0.9	18.1	10.3

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xytriophora*
33	Benzaldehyde	0.5	0.2	-	0.1	0.1	0.1	0.6	0.2	0.1	1.3	0.6	-	3.4	0.9	1.2	1.2	0.5	0.8	0.8	0.3
34	Methyl 3-methyl-2-oxopentanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	6-Methylhept-5-en-2-one	2.2	1.4	-	-	0.3	0.5	0.6	0.4	0.4	1.7	1.6	0.3	-	2.2	-	-	-	-	-	-
36	Sabinene	-	-	0.9	0.3	1.7	0.9	3.7	1.3	2.1	1.9	3.1	0.1	2.9	-	4.2	-	0.3	1.4	2.1	-
37	β-Pinene	0.8	0.3	0.1	0.2	1.6	1.2	1.5	1.2	2.4	0.8	1.7	-	3.0	-	-	-	0.1	0.5	3.4	-
38	Unknown (Me 2-hydroxy-3-mepentanoate like)	-	-	-	-	-	-	-	-	-	-	-	-	-	2.2	-	-	-	-	-	-
39	Methyl 2-hydroxy-3-methylpentanoate	0.8	0.8	-	0.3	-	-	-	-	-	-	-	-	-	5.9	-	-	0.1	-	-	-
40	Butyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	Myrcene	0.5	0.1	0.6	0.6	14.7	14.0	6.9	16.2	16.6	10.3	17.1	0.8	8.4	-	-	1.0	0.1	0.8	1.7	-
42	Ethyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	Octanal	0.2	0.3	-	-	-	-	-	-	-	-	-	-	-	-	10.6	-	-	-	-	-
44	Ethyl hex-3-enoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Hex-3-enyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	Unknown BP59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	α-Phellandrene	-	-	-	-	-	0.2	0.2	0.1	0.1	-	0.2	-	-	-	-	-	-	-	-	-
48	Hexyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	p-Cresyl methyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	Car-3-ene	0.9	0.4	-	-	-	-	-	-	-	-	-	0.1	2.5	-	-	-	-	0.2	1.3	2.8
51	Methyl heptanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	α-Terpinene	-	-	-	-	0.1	0.1	0.5	0.3	0.1	0.8	1.7	-	-	-	-	-	0.2	0.5	-	-
53	p-Cymene	0.1	-	1.2	-	0.3	0.4	0.9	0.3	0.9	1.5	1.0	-	1.3	0.3	-	1.2	0.2	0.5	1.0	1.4
54	Phenylacetaldehyde	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	Benzyl alcohol	-	-	-	-	0.2	1.0	1.3	1.7	1.2	2.4	0.4	0.3	2.2	-	-	-	-	-	-	-
56	o-Hydroxybenzaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	1,8-Cineole	0.1	-	0.3	9.7	0.2	0.4	0.9	0.2	0.3	0.5	1.2	0.4	3.9	0.9	1.2	1.4	0.3	0.9	3.8	1.8
58	β-Phellandrene	-	-	-	-	0.2	0.4	0.7	0.3	0.2	0.1	1.0	-	-	-	-	-	-	-	-	-
59	Limonene	0.8	0.2	1.1	1.4	15.6	7.9	6.1	12.2	15.4	10.8	10.3	0.2	5.2	0.9	20.0	11.3	0.2	0.3	3.2	5.9
60	(Z)-Ocimene	0.4	0.5	1.5	0.1	0.3	0.3	0.3	0.4	0.3	0.3	0.4	-	-	0.2	-	-	-	0.1	-	-
61	Acetophenone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
62	(E)-Ocimene	24.4	53.5	72.0	14.7	6.8	7.5	1.9	7.0	6.5	2.2	3.8	-	1.5	29.9	-	-	0.7	35.0	-	-
63	Unknown BP43,72,84,99,109	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	Prenyl butyrate-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xyrtiophora*
65	γ-Terpinene	-	-	4.0	-	0.2	0.2	0.2	0.3	0.3	0.7	-	-	-	-	-	-	-	0.8	-	-
66	(E)-Sabinene hydrate	-	-	-	-	1.8	0.6	0.7	1.0	1.6	1.5	1.8	-	-	-	-	-	-	0.5	-	-
67	Alkane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.3	-	-	-	2.2
68	Octanol	-	-	-	-	0.1	0.1	1.6	0.2	0.6	-	0.7	-	-	-	-	-	-	-	-	-
69	Guaiacol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.3	-	-
70	Unknown BP85,152	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	(Ipsenol)	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-
72	Linalol oxides I & II	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	-	-	0.5	-
73	Methyl benzoate	1.0	0.2	-	-	4.0	3.4	1.1	8.5	0.4	3.0	2.0	3.8	2.9	1.0	-	3.0	0.3	2.4	1.4	3.6
74	Amyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	6-Methylhepta-3,5-dien-2-one	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	0.2	-	-	-
76	3,4-Epoxy-3,7-dimethylocta-1,6-diene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77	Terpinolene	-	-	-	-	0.4	0.3	-	0.3	0.5	-	0.3	-	-	-	-	-	-	0.2	-	-
78	(3,5-Dimethylcyclohex-2-en-1-one) BP82,81,43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	C11 alkene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	-	-
80	Hex-3-enyl propionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	Prenyl butyrate-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	(Z)-Sabinene hydrate	-	-	-	-	0.4	-	-	0.3	0.6	-	-	-	-	-	-	-	-	-	-	-
83	Nonanal	0.5	0.3	0.6	-	0.2	0.4	0.9	0.3	0.9	1.4	1.3	0.2	7.2	0.6	4.1	1.2	0.2	0.2	1.4	2.2
84	Linalol	5.1	7.9	0.2	-	0.5	0.5	2.1	0.7	0.5	1.7	0.9	4.1	-	0.2	-	22.4	0.2	0.2	3.6	-
85	Phenylethyl alcohol	-	-	-	-	-	0.1	2.5	0.1	0.3	1.7	1.9	-	1.8	-	-	-	-	-	-	-
86	Unknown BP119,134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	-
87	Benzyl cyanide	1.0	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	Thujone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	Methyl nicotinate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	2.7
90	Unknown BP91,150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	Unknown BP69,41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	Prenyl ester (angelate?) & unsat H/C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	1,2-Dimethoxybenzene	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	3.6	0.5	-	-
94	Unknown BP91,119,134	0.3	0.7	0.2	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-	-	-
95	(E,E)-Alloocimene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.4	-	-
96	2,3-Epoxy-2,6-dimethylocta-5,7-diene	0.5	0.3	0.3	-	-	-	-	-	-	-	-	-	-	1.0	-	1.3	-	-	-	-

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthochelia*	*L. xytriophora*
97	Ipsdienone	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-
98	Ocimene epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
99	Phenylpropylaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	((E)-Pin-3-en-2-ol)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	(Ipsdienol)	-	-	-	-	-	-	-	-	-	-	-	3.2	-	-	-	-	-	-	-	-
102	Benzyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	6.8	-	-	1.2	-	-	-	-
103	1,4-Dimethoxybenzene	5.7	8.8	1.5	2.1	7.4	13.4	14.2	19.6	11.8	18.9	8.8	-	-	0.9	-	-	8.0	5.9	7.3	-
104	Unknown BP109,124,152	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	(Verbenone like)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Methyl phenylacetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
107	Ethyl benzoate	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-
108	isoValeraldehyde-hexanal condensation product	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
109	Benzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
110	Octanoic acid	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	9.5	-	-	-	-	-
111	Unknown BP95,93,91,150	0.4	0.1	-	0.2	-	-	-	-	-	-	-	-	-	0.5	-	1.3	-	-	-	-
112	Terpinen-4-ol	-	-	4.1	-	0.2	0.1	1.1	0.4	0.1	0.9	1.6	-	-	-	-	-	0.3	2.1	-	-
113	2-Ethylhexyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.3
114	Methyl salicylate	-	-	-	-	-	-	-	-	-	-	-	81.2	-	-	-	9.9	0.1	0.4	-	-
115	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-
116	Hex-3-enyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
117	Prenyl tiglate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
118	Butyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
119	Hexyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
120	Butyl hex-3-enoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
121	α-Terpineol	-	-	0.2	-	1.7	0.6	1.1	2.2	1.9	3.3	2.4	-	-	-	-	-	-	-	1.2	-
122	Methyl chavicol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	Verbenone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	-
124	Cinnamic aldehyde-l	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
125	Propyl benzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
126	Cuminal	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	0.1	-	-
127	Decanal	0.2	0.2	0.4	-	0.2	0.2	0.7	0.3	0.7	1.0	0.9	-	3.5	0.3	4.1	0.7	0.2	-	0.8	-
128	Unknown (p-Mentha-2,8-dien-1-ol-tr)	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xyfrtophora*
129	2-Methyl-3-phenylpropanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	(Verbenone, no 80)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-	-	-
131	Nerol	-	-	-	-	0.1	0.1	0.3	0.5	0.1	0.6	0.3	-	-	-	-	-	-	-	-	-
132	Carvone	-	-	-	-	0.1	0.1	0.3	0.3	0.2	0.6	0.3	-	-	-	-	-	-	-	-	-
133	Anisaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
134	Neral	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	Phenylethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-	-
136	Benzyl propionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
137	Cinnamic aldehyde-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	5.3	-	-
138	Geraniol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
139	isoPiperitenone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
140	Unknown BP43,69,70,122	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
141	(Dec-4-en-1-ol)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
142	Geranial	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
143	Unknown BP97,72,41,43	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	-	-	-	-	-	-
144	3,7-Dimethylocta-1,6-dien-3,5-diol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
145	Ethyl salicylate	-	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-	-	-	-	-
146	Prenyl hexanoate-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
147	p-Mentha-1,3-dien-7-al	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	Alkene	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
149	Methyl 3-phenylpropionate	-	-	-	8.3	-	-	-	-	-	-	-	-	-	-	-	-	12.1	2.8	-	-
150	Phenylethyl ester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
151	Carboxylic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
152	Hex-3-enyl valerate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
153	Methyl cinnamate-I	0.9	-	2.0	2.6	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
154	Benzyl isobutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
155	Prenyl hexanoate-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
156	Undecanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-
157	Methyl nerate/geranate	-	-	-	-	0.1	-	0.2	0.1	-	0.3	-	-	-	-	-	-	-	-	-	-
158	Methyl 2-methoxybenzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
159	Triacetin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.0	-	-	0.7	2.6
160	Benzyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xytriophora*
161	Phenylethyl propionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
162	Eugenol	-	-	-	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	2.7	-	-
163	Ethyl ester BP88,108,79,67,164	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-	-	-
164	Methyl anisate	-	-	-	-	0.1	-	0.2	1.0	0.4	2.7	2.1	-	-	-	-	-	-	-	-	-
165	1,2,4-Trimethoxybenzene	-	-	-	-	0.3	0.1	0.2	0.2	-	0.8	-	-	-	-	-	-	-	-	-	-
166	Phenylpropyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
167	1-Hydroxy-2,2,4-trimepent-3-yl 2-mepropionate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7	-	-	-	-	-
169	Methyl 3-phenyllactate	2.5	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
170	Longicyclene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.0
171	Butyl benzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
172	3-Hydroxy-2,2,4-trimepent-1-yl 2-mepropionate	-	-	-	-	-	-	-	-	-	-	-	-	1.3	-	-	-	-	-	0.7	-
173	Geranyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174	Methyl cinnamate-II	1.3	0.1	4.2	53.5	-	-	-	-	-	-	-	-	-	0.7	-	-	70.1	31.5	-	-
175	Hex-3-enyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
176	Hex-3-enyl hex-3-enoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
177	Hexyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
178	Unknown BP43,84,69,109,128	2.7	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
179	isoLongifolene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1
180	Phenylethyl isobutyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
181	α-Copaene	-	-	-	-	0.2	0.2	0.4	0.1	0.2	0.4	0.2	-	-	-	-	-	-	-	-	-
182	β-Bourbonene	-	-	-	-	0.1	0.1	0.1	0.1	0.2	1.5	1.1	-	-	-	-	-	-	-	-	-
183	β-Elemene	-	-	-	-	0.4	0.3	0.4	0.2	0.2	0.4	0.4	-	-	-	-	-	-	-	-	-
184	Unknown BP43,119,134 (Crysanthenyl acetate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
185	α-Zingiberene	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	-	-	-	-	-	-
186	Unknown BP43,69,97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
187	Dodecanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.6	-	-	-	-	-
188	Unknown (Safranal like)	0.7	0.4	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-
189	Sesquiterpene hydrocarbon BP95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.0
190	Butyl phenylacetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
191	Longifolene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.7
192	isoCaryophyllene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1	-
193	α-Ionone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xytriophora*
194	Neryl acetone	0.4	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
195	Unknown BP163,206	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9
196	Phenylethyl butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
197	3,4-Dihydrobetaionone	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-	-	-
198	Prenyl benzoate-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
199	Caryophyllene	-	-	-	-	2.9	1.6	0.6	0.2	0.4	0.7	0.6	-	-	0.7	-	-	-	-	17.7	-
200	(2,3-Epoxyneryl acetate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
201	Sesquiterpene hydrocarbon BP94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7
202	Geranyl acetone	3.9	0.3	-	-	0.2	0.1	0.3	0.1	0.1	1.0	0.2	-	-	-	0.6	-	-	-	-	-
203	α-Bergamotene	-	-	-	-	-	-	-	-	-	-	-	0.3	2.0	-	-	-	-	-	5.5	-
204	(E)-β-Farnesene	-	-	-	-	1.1	1.8	1.1	0.6	1.1	0.7	1.2	0.2	3.9	3.3	-	-	-	-	-	-
205	Sesquiterpene hydrocarbon BP119	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7
206	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	-	-	-	1.2
207	2,3-Epoxygeranyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
208	α-Humulene	-	-	-	-	0.8	0.5	0.2	0.1	0.2	0.2	0.2	-	-	-	-	-	-	-	4.4	-
209	Alloaromadendrene	-	-	-	-	0.4	0.3	0.4	0.1	0.3	0.2	0.3	-	-	-	-	-	-	-	-	-
210	Sesquiterpenoid BP161,203,218	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-	-	2.0	-	-	-	-
211	Phenylethyl isovalerate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
212	β-Ionone	0.3	-	-	-	-	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-
213	ar-Curcumene	-	-	-	-	0.1	0.1	0.3	0.1	0.1	0.4	0.4	-	1.2	4.4	-	-	-	-	-	-
214	Pentadec-1-ene	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-	-	-	-	-	-
215	Germacrene-D	-	-	-	-	19.6	14.9	16.3	3.2	10.6	1.4	8.9	-	-	-	-	-	-	-	-	-
216	Sesquiterpene hydrocarbon BP119,93	0.9	0.2	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-
217	β-Selinene	-	-	-	-	0.6	0.3	0.6	0.1	0.4	0.2	-	-	-	-	-	-	-	-	-	-
218	Unknown BP43,69,109,119 (Epoxy acetate?)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
219	BHT	-	-	-	-	1.0	0.5	0.1	0.1	0.1	0.1	0.1	-	3.3	-	-	-	-	-	-	-
220	(E,E)-α-Farnesene	25.6	18.2	-	-	3.4	15.8	1.0	2.9	1.6	0.9	1.2	-	-	3.0	-	2.8	-	-	-	-
221	Bicyclogermacrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
222	β-Bisabolene	-	-	-	-	0.9	1.5	3.0	0.5	1.0	1.1	1.0	0.4	11.1	24.8	-	-	-	-	-	-
223	γ-Cadinene	-	-	-	-	0.1	0.1	0.2	-	0.1	-	-	-	-	-	-	-	-	-	-	-
224	δ-Cadinene	-	-	-	-	0.3	0.5	0.3	0.1	0.4	0.2	0.3	-	-	-	-	-	-	-	-	-
225	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1

		L. consobrina 2	*L. consobrina 3*	*L. crinita*	*L. cruenta*	*L. deppei 1*	*L. deppei 2*	*L. deppei 3*	*L. deppei 4*	*L. deppei 5*	*L. deppei 6*	*L. deppei 7*	*L. desboisiana*	*L. leucantha*	*L. luminosa*	*L. macrobulbon*	*L. macro. puntarenasensis*	*L. suaveolens 1*	*L. suaveolens 2*	*L. macro. xanthocheila*	*L. xytriophora*
226	β-Sesquiphellandrene	-	-	-	-	-	-	-	-	-	-	-	-	-	2.7	-	-	-	-	-	-
227	Unknown BP69,41,81,137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
228	Unknown BP69,218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
229	Unknown	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
230	Methyl 4-methoxycinnamate-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
231	Hex-3-enyl benzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
232	Farnesol like	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
233	Hexyl benzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
234	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
235	Unknown BP41,85,93,119,159,220	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
236	(E,E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
237	Caryophyllene oxide	1.7	0.6	-	-	0.1	0.1	0.7	0.1	0.2	0.2	0.4	-	-	-	-	-	-	-	2.0	-
238	Unknown BP41,85,93,119,159,220	5.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
239	Unknown BP41,69,79,81,43 (Epoxyfarnesene?)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
240	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
241	Unknown BP43,69,82,109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
242	Unknown BP41,82,81,69,220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	Humulene-1,2-epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	-
244	Sesquiterpenoid BP81,59 MW218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
245	Hex-3-enyl phenylacetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
246	Phenylethyl hexanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	Methyl 4-methoxycinnamate-II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
248	Phenylethyl hex-3-enoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
249	Unknown BP173,99,84,55	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-
250	Unknown BP99,194	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.9	-
251	Unknown BP99,152	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-
252	Methyl farnesate	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-
253	(E,E)-Farnesyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-
	TOTAL	99.4	99.8	97.8	100.0	99.9	99.9	98.6	100.0	99.8	99.9	98.9	100.0	90.0	98.1	94.7	94.0	99.9	99.5	95.9	94.4

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
1	Ethyl acetate	-	-	-	-	-	-	-	-
2	(2-Methylbut-3-en-2-ol)	-	0.2	-	-	-	-	-	-
3	Acetic acid	-	-	-	-	-	-	-	-
4	Isoprene	-	-	-	-	-	-	-	-
5	Valeraldehyde	0.2	-	-	-	-	-	-	-
6	Ethyl propionate	-	-	-	-	-	-	-	-
7	Propyl acetate	-	-	-	-	-	-	-	-
8	Unknown	-	-	-	-	-	-	-	-
9	Methyl 2-methylbutyrate	-	0.2	-	-	-	-	-	-
10	Diethyl carbonate	-	-	-	-	-	-	-	-
11	Ethyl butyrate	-	-	-	-	-	-	-	-
12	Butyl acetate	-	-	-	-	-	-	-	-
13	Unknown BP43,72,57,58	-	-	-	-	-	-	-	-
14	Propyl isobutyrate	-	-	-	-	-	-	-	-
15	Ethyl 2-methylbutyrate	-	-	-	-	-	-	-	-
16	(Z)-Hex-3-enol	-	-	-	-	-	-	-	-
17	(Methyl tiglate)	-	-	-	-	-	-	-	-
18	Hexanol	-	-	-	-	-	-	-	-
19	isoAmyl acetate	-	-	-	-	-	-	-	-
20	Methyl 2-hydroxy-3-methylbutyrate	-	-	-	-	-	-	-	-
21	Prenyl acetate-I	-	-	-	-	-	-	-	-
22	Cyclohexanone	-	-	-	-	-	-	-	-
23	Heptanal	-	-	-	-	-	-	-	-
24	Propyl butyrate	-	-	-	-	-	-	-	-
25	2-Ethoxyethyl acetate	-	-	-	-	-	-	-	-
26	Butyl propionate	-	-	-	-	-	-	-	-
27	Amyl acetate	-	-	-	-	-	-	-	-
28	Prenyl acetate-II	-	-	-	-	-	-	-	-
29	Methyl hexanoate	1.0	-	-	-	-	-	-	-
30	α-Thujene	-	-	-	-	-	-	-	0.1
31	Unsaturated hydrocarbon	-	-	-	-	-	-	-	-
32	α-Pinene	2.1	1.8	-	0.6	-	-	-	1.3
33	Benzaldehyde	0.3	1.4	-	0.5	-	-	-	0.3

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
34	Methyl 3-methyl-2-oxopentanoate	-	-	-	-	-	-	-	-
35	6-Methylhept-5-en-2-one	-	-	-	-	-	-	-	-
36	Sabinene	0.5	0.8	0.1	0.5	-	-	-	2.0
37	β-Pinene	0.5	0.7	-	0.2	-	-	-	0.5
38	Unknown (Me 2-hydroxy-3-mepentanoate like)	-	-	-	-	-	-	-	-
39	Methyl 2-hydroxy-3-methylpentanoate	-	-	-	-	-	-	-	-
40	Butyl butyrate	-	-	-	-	-	-	-	-
41	Myrcene	0.1	1.6	0.3	1.0	0.3	-	-	26.1
42	Ethyl hexanoate	-	-	-	-	-	-	-	-
43	Octanal	-	-	-	-	-	-	-	-
44	Ethyl hex-3-enoate	-	-	-	-	-	-	-	-
45	Hex-3-enyl acetate	-	-	-	-	-	-	-	-
46	Unknown BP59	-	-	-	-	-	-	-	-
47	α-Phellandrene	-	-	-	-	-	-	-	0.1
48	Hexyl acetate	-	-	-	-	-	-	-	-
49	p-Cresyl methyl ether	-	-	-	0.4	0.2	-	-	-
50	Car-3-ene	-	-	-	-	-	-	-	-
51	Methyl heptanoate	0.3	-	-	-	-	-	-	-
52	α-Terpinene	-	-	-	-	-	-	-	-
53	p-Cymene	-	-	-	-	-	-	-	0.2
54	Phenylacetaldehyde	0.1	-	-	-	-	-	-	0.2
55	Benzyl alcohol	-	-	-	-	-	-	-	-
56	o-Hydroxybenzaldehyde	-	-	-	-	-	-	-	-
57	1,8-Cineole	2.3	19.1	0.8	7.7	0.4	0.3	-	60.6
58	β-Phellandrene	-	-	-	-	-	-	-	-
59	Limonene	0.6	5.0	0.6	2.2	0.6	0.0	-	2.1
60	(Z)-Ocimene	-	-	-	-	-	-	-	-
61	Acetophenone	-	-	-	-	-	-	-	-
62	(E)-Ocimene	-	1.8	0.5	-	-	-	-	0.3
63	Unknown BP43,72,84,99,109	-	-	-	-	-	-	-	-
64	Prenyl butyrate-I	-	-	-	-	-	-	-	-
65	γ-Terpinene	-	-	-	-	-	-	-	0.2
66	(E)-Sabinene hydrate	-	-	-	-	-	-	-	0.2

		A. brevilabris	*A. cliftonii* 1	*A. cliftonii* 2	*A. clowesii*	*A.hohenlohiii* 1	*A.hohenlohiii* 2	*A. eburnea*	*A. virginalis*
67	Alkane	-	-	-	-	-	-	-	-
68	Octanol	-	-	-	-	-	-	-	-
69	Guaiacol	-	-	-	-	-	-	-	-
70	Unknown BP85,152	-	-	-	-	-	-	-	-
71	(Ipsenol)	-	-	-	-	-	-	-	-
72	Linalol oxides I & II	-	-	-	-	-	-	-	-
73	Methyl benzoate	27.7	2.0	2.7	-	-	-	-	1.7
74	Amyl butyrate	-	-	-	-	-	-	-	-
75	6-Methylhepta-3,5-dien-2-one	-	-	-	-	-	-	-	-
76	3,4-Epoxy-3,7-dimethylocta-1,6-diene	-	-	-	-	-	-	-	-
77	Terpinolene	-	-	-	-	-	-	-	0.2
78	(3,5-Dimethylcyclohex-2-en-1-one) BP82,81,43	-	-	-	-	-	-	-	-
79	C11 alkene	-	-	-	-	-	-	-	-
80	Hex-3-enyl propionate	-	-	-	-	-	-	-	-
81	Prenyl butyrate-II	-	-	-	-	-	-	-	-
82	(Z)-Sabinene hydrate	-	-	-	-	-	-	-	-
83	Nonanal	-	-	-	-	-	-	-	-
84	Linalol	0.2	0.2	-	-	-	-	-	2.6
85	Phenylethyl alcohol	0.3	-	-	-	-	-	-	0.1
86	Unknown BP119,134	-	-	-	-	-	-	-	-
87	Benzyl cyanide	1.5	-	-	-	-	-	-	0.5
88	Thujone	-	-	-	-	-	-	-	-
89	Methyl nicotinate	-	-	-	-	-	-	-	-
90	Unknown BP91,150	-	-	-	-	-	-	-	-
91	Unknown BP69,41	-	-	-	-	-	-	-	-
92	Prenyl ester (angelate?) & unsat H/C	-	-	-	-	-	-	-	-
93	1,2-Dimethoxybenzene	-	-	-	-	-	-	-	-
94	Unknown BP91,119,134	-	-	-	-	-	-	-	-
95	(E,E)-Alloocimene	-	-	-	-	-	-	-	-
96	2,3-Epoxy-2,6-dimethylocta-5,7-diene	-	-	-	-	-	-	-	-
97	Ipsdienone	-	-	-	-	-	-	-	-
98	Ocimene epoxide	-	-	-	-	-	-	-	-
99	Phenylpropylaldehyde	-	-	-	-	-	-	-	-

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
100	((E)-Pin-3-en-2-ol)	-	-	-	-	-	-	-	-
101	(Ipsdienol)	-	-	-	-	-	-	-	-
102	Benzyl acetate	2.8	-	-	-	-	-	-	-
103	1,4-Dimethoxybenzene	-	63.5	92.5	86.9	98.2	99.7	100.0	-
104	Unknown BP109,124,152	-	-	-	-	-	-	-	-
105	(Verbenone like)	-	-	-	-	-	-	-	-
106	Methyl phenylacetate	-	-	-	-	-	-	-	-
107	Ethyl benzoate	-	-	-	-	-	-	-	-
108	isoValeraldehyde-hexanal condensation product	-	-	-	-	-	-	-	-
109	Benzoic acid	-	-	-	-	-	-	-	-
110	Octanoic acid	-	-	-	-	-	-	-	-
111	Unknown BP95,93,91,150	-	-	-	-	-	-	-	-
112	Terpinen-4-ol	-	-	-	-	-	-	-	-
113	2-Ethylhexyl acetate	-	-	-	-	-	-	-	-
114	Methyl salicylate	8.8	1.1	1.7	0.1	-	-	-	0.2
115	Unknown	-	-	-	-	-	-	-	-
116	Hex-3-enyl butyrate	-	-	-	-	-	-	-	-
117	Prenyl tiglate	-	-	-	-	-	-	-	-
118	Butyl hexanoate	-	-	-	-	-	-	-	-
119	Hexyl butyrate	-	-	-	-	-	-	-	-
120	Butyl hex-3-enoate	-	-	-	-	-	-	-	-
121	α-Terpineol	-	-	-	-	-	-	-	0.1
122	Methyl chavicol	-	-	-	-	-	-	-	-
123	Verbenone	-	-	-	-	-	-	-	0.2
124	Cinnamic aldehyde-l	-	-	-	-	-	-	-	-
125	Propyl benzoate	-	-	-	-	-	-	-	-
126	Cuminal	-	-	-	-	-	-	-	-
127	Decanal	-	-	-	-	-	-	-	-
128	Unknown (p-Mentha-2,8-dien-1-ol-tr)	-	-	-	-	-	-	-	-
129	2-Methyl-3-phenylpropanal	-	-	-	-	-	-	-	-
130	(Verbenone, no 80)	-	-	-	-	-	-	-	-
131	Nerol	-	-	-	-	-	-	-	-
132	Carvone	-	-	-	-	-	-	-	0.1

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
133	Anisaldehyde	-	0.4	0.7	-	0.4	-	-	-
134	Neral	-	-	-	-	-	-	-	-
135	Phenylethyl acetate	0.6	-	-	-	-	-	-	-
136	Benzyl propionate	0.2	-	-	-	-	-	-	-
137	Cinnamic aldehyde-II	-	-	-	-	-	-	-	-
138	Geraniol	-	-	-	-	-	-	-	-
139	isoPiperitenone	-	-	-	-	-	-	-	-
140	Unknown BP43,69,70,122	-	-	-	-	-	-	-	-
141	(Dec-4-en-1-ol)	-	-	-	-	-	-	-	-
142	Geranial	-	-	-	-	-	-	-	-
143	Unknown BP97,72,41,43	-	-	-	-	-	-	-	-
144	3,7-Dimethylocta-1,6-dien-3,5-diol	-	-	-	-	-	-	-	-
145	Ethyl salicylate	-	-	-	-	-	-	-	-
146	Prenyl hexanoate-I	-	-	-	-	-	-	-	-
147	p-Mentha-1,3-dien-7-al	-	-	-	-	-	-	-	-
148	Alkene	-	-	-	-	-	-	-	-
149	Methyl 3-phenylpropionate	0.3	-	-	-	-	-	-	-
150	Phenylethyl ester	0.5	-	-	-	-	-	-	-
151	Carboxylic acid	-	-	-	-	-	-	-	-
152	Hex-3-enyl valerate	-	-	-	-	-	-	-	-
153	Methyl cinnamate-I	-	-	-	-	-	-	-	-
154	Benzyl isobutyrate	0.3	-	-	-	-	-	-	-
155	Prenyl hexanoate-II	-	-	-	-	-	-	-	-
156	Undecanal	-	-	-	-	-	-	-	-
157	Methyl nerate/geranate	-	-	-	-	-	-	-	-
158	Methyl 2-methoxybenzoate	12.5	-	-	-	-	-	-	-
159	Triacetin	-	-	-	-	-	-	-	-
160	Benzyl butyrate	0.6	-	-	-	-	-	-	-
161	Phenylethyl propionate	-	-	-	-	-	-	-	-
162	Eugenol	-	-	-	-	-	-	-	-
163	Ethyl ester BP88,108,79,67,164	-	-	-	-	-	-	-	-
164	Methyl anisate	-	-	-	-	-	-	-	-
165	1,2,4-Trimethoxybenzene	-	-	-	-	-	-	-	-

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
166	Phenylpropyl acetate	-	-	-	-	-	-	-	-
167	1-Hydroxy-2,2,4-trimepent-3-yl 2-mepropionate	-	-	-	-	-	-	-	-
169	Methyl 3-phenyllactate	-	-	-	-	-	-	-	-
170	Longicyclene	-	-	-	-	-	-	-	-
171	Butyl benzoate	-	-	-	-	-	-	-	-
172	3-Hydroxy-2,2,4-trimepent-1-yl 2-mepropionate	-	-	-	-	-	-	-	-
173	Geranyl acetate	-	-	-	-	-	-	-	-
174	Methyl cinnamate-II	35.1	-	-	-	-	-	-	-
175	Hex-3-enyl hexanoate	-	-	-	-	-	-	-	-
176	Hex-3-enyl hex-3-enoate	-	-	-	-	-	-	-	-
177	Hexyl hexanoate	-	-	-	-	-	-	-	-
178	Unknown BP43,84,69,109,128	-	-	-	-	-	-	-	-
179	isoLongifolene	-	-	-	-	-	-	-	-
180	Phenylethyl isobutyrate	0.1	-	-	-	-	-	-	-
181	α-Copaene	-	-	-	-	-	-	-	-
182	β-Bourbonene	-	-	-	-	-	-	-	-
183	β-Elemene	-	-	-	-	-	-	-	-
184	Unknown BP43,119,134 (Crysanthenyl acetate)	-	-	-	-	-	-	-	-
185	α-Zingiberene	-	-	-	-	-	-	-	-
186	Unknown BP43,69,97	-	-	-	-	-	-	-	-
187	Dodecanal	-	-	-	-	-	-	-	-
188	Unknown (Safranal like)	-	-	-	-	-	-	-	-
189	Sesquiterpene hydrocarbon BP95	-	-	-	-	-	-	-	-
190	Butyl phenylacetate	-	-	-	-	-	-	-	-
191	Longifolene	0.2	-	-	-	-	-	-	-
192	isoCaryophyllene	-	-	-	-	-	-	-	-
193	α-Ionone	-	-	-	-	-	-	-	-
194	Neryl acetone	-	-	-	-	-	-	-	-
195	Unknown BP163,206	-	-	-	-	-	-	-	-
196	Phenylethyl butyrate	0.2	-	-	-	-	-	-	-
197	3,4-Dihydrobetaionone	-	-	-	-	-	-	-	-
198	Prenyl benzoate-I	-	-	-	-	-	-	-	-
199	Caryophyllene	-	-	-	-	-	-	-	-

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
200	(2,3-Epoxyneryl acetate)	-	-	-	-	-	-	-	-
201	Sesquiterpene hydrocarbon BP94	-	-	-	-	-	-	-	-
202	Geranyl acetone	-	-	-	-	-	-	-	-
203	α-Bergamotene	-	-	-	-	-	-	-	-
204	(E)-β-Farnesene	-	-	-	-	-	-	-	-
205	Sesquiterpene hydrocarbon BP119	-	-	-	-	-	-	-	-
206	Unknown	-	-	-	-	-	-	-	-
207	2,3-Epoxygeranyl acetate	-	-	-	-	-	-	-	-
208	α-Humulene	-	-	-	-	-	-	-	-
209	Alloaromadendrene	-	-	-	-	-	-	-	-
210	Sesquiterpenoid BP161,203,218	-	-	-	-	-	-	-	-
211	Phenylethyl isovalerate	-	-	-	-	-	-	-	-
212	β-Ionone	-	-	-	-	-	-	-	-
213	ar-Curcumene	-	-	-	-	-	-	-	-
214	Pentadec-1-ene	-	-	-	-	-	-	-	-
215	Germacrene-D	-	-	-	-	-	-	-	-
216	Sesquiterpene hydrocarbon BP119,93	-	-	-	-	-	-	-	-
217	β-Selinene	-	-	-	-	-	-	-	-
218	Unknown BP43,69,109,119 (Epoxy acetate?)	-	-	-	-	-	-	-	-
219	BHT	-	-	-	-	-	-	-	-
220	(E,E)-α-Farnesene	-	-	-	-	-	-	-	-
221	Bicyclogermacrene	-	-	-	-	-	-	-	-
222	β-Bisabolene	-	-	-	-	-	-	-	-
223	γ-Cadinene	-	-	-	-	-	-	-	-
224	δ-Cadinene	-	-	-	-	-	-	-	-
225	Unknown	-	-	-	-	-	-	-	-
226	β-Sesquiphellandrene	-	-	-	-	-	-	-	-
227	Unknown BP69,41,81,137	-	-	-	-	-	-	-	-
228	Unknown BP69,218	-	-	-	-	-	-	-	-
229	Unknown	-	-	-	-	-	-	-	-
230	Methyl 4-methoxycinnamate-I	-	-	-	-	-	-	-	-
231	Hex-3-enyl benzoate	-	-	-	-	-	-	-	-
232	Farnesol like	-	-	-	-	-	-	-	0.1

		A. brevilabris	*A. cliftonii 1*	*A. cliftonii 2*	*A. clowesii*	*A.hohenlohiii 1*	*A.hohenlohiii 2*	*A. eburnea*	*A. virginalis*
233	Hexyl benzoate	-	-	-	-	-	-	-	-
234	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-I	-	-	-	-	-	-	-	-
235	Unknown BP41,85,93,119,159,220	-	-	-	-	-	-	-	-
236	(E,E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	-	-	-	-	-	-	-	-
237	Caryophyllene oxide	-	-	-	-	-	-	-	-
238	Unknown BP41,85,93,119,159,220	-	-	-	-	-	-	-	-
239	Unknown BP41,69,79,81,43 (Epoxyfarnesene?)	-	-	-	-	-	-	-	-
240	2,6,10-Trimethyldodeca-2,6,8,10-tetraen-1-al-II	-	-	-	-	-	-	-	-
241	Unknown BP43,69,82,109	-	-	-	-	-	-	-	-
242	Unknown BP41,82,81,69,220	-	-	-	-	-	-	-	-
243	Humulene-1,2-epoxide	-	-	-	-	-	-	-	-
244	Sesquiterpenoid BP81,59 MW218	-	-	-	-	-	-	-	-
245	Hex-3-enyl phenylacetate	-	-	-	-	-	-	-	-
246	Phenylethyl hexanoate	-	-	-	-	-	-	-	-
247	Methyl 4-methoxycinnamate-II	-	-	-	-	-	-	-	-
248	Phenylethyl hex-3-enoate	-	-	-	-	-	-	-	-
249	Unknown BP173,99,84,55	-	-	-	-	-	-	-	-
250	Unknown BP99,194	-	-	-	-	-	-	-	-
251	Unknown BP99,152	-	-	-	-	-	-	-	-
252	Methyl farnesate	-	-	-	-	-	-	-	-
253	(E,E)-Farnesyl acetate	-	-	-	-	-	-	-	-
	TOTAL	99.9	99.8	100.0	100.0	100.0	100.0	100.0	100.0

Appendix 6 FLORAL FRAGRANCE CHROMATOGRAMS

The following chromatograms were obtained from a 50m BP1 column (SGE), according to the conditions specified in Table 5.2. The retention time scale is in minutes. Peak annotation follows that of Appendix 5.

A. *Lycaste ciliata*

B. *L. costata*

C. *L. cinnabarina* 1

D. *L. cinnabarina* 2

E. *L. fragrans*

F. *L. gigantea*

G. *L. lanipes*

H. *L. locusta* 2

I. *L. aromatica* 3

J. *L. bradeorum*

K. *L. cochleata*

L. *L. crinita*

M. *L. cruenta*

N. *L. deppei* 1

O. *L. luminosa*

P. *L. macrophylla* subsp. *desboisiana*

Q. *L. suaveolens* 2

R. *Anguloa brevilabris*

S. *A. cliftonii*

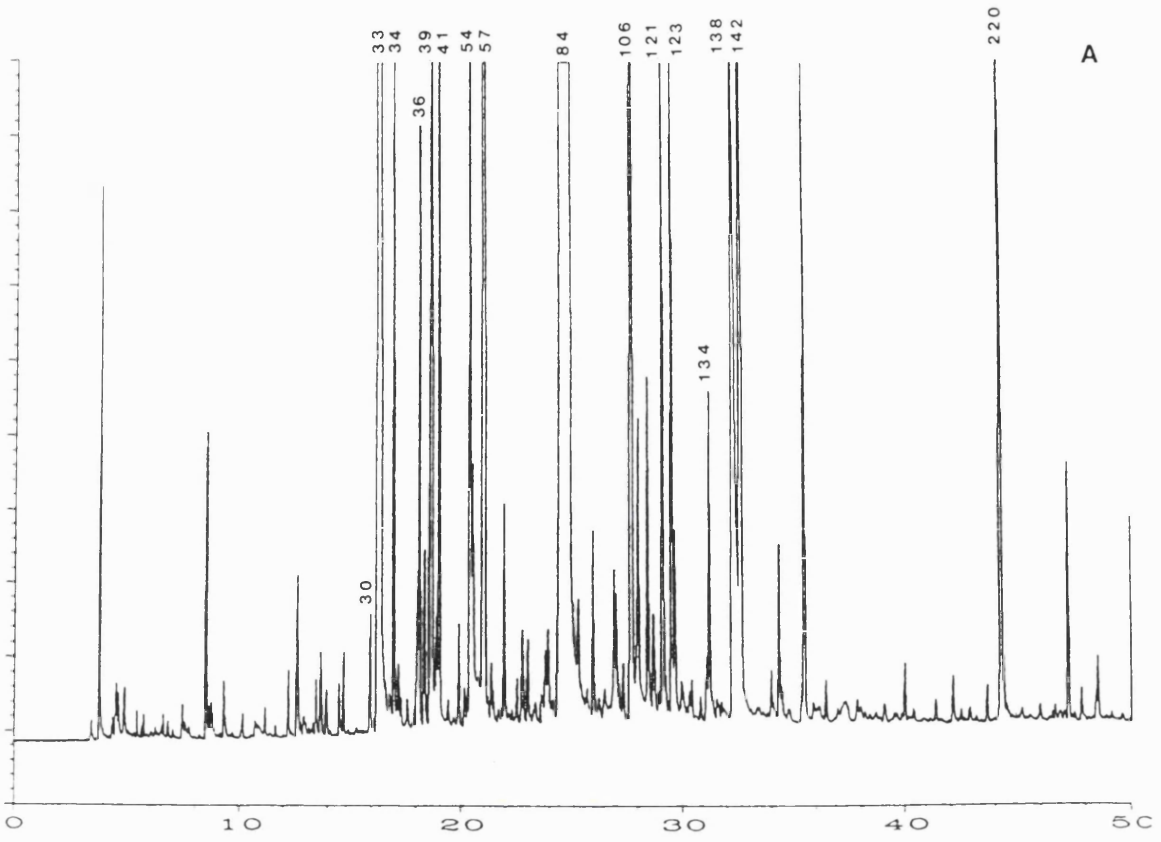
T. *A. clowesii*

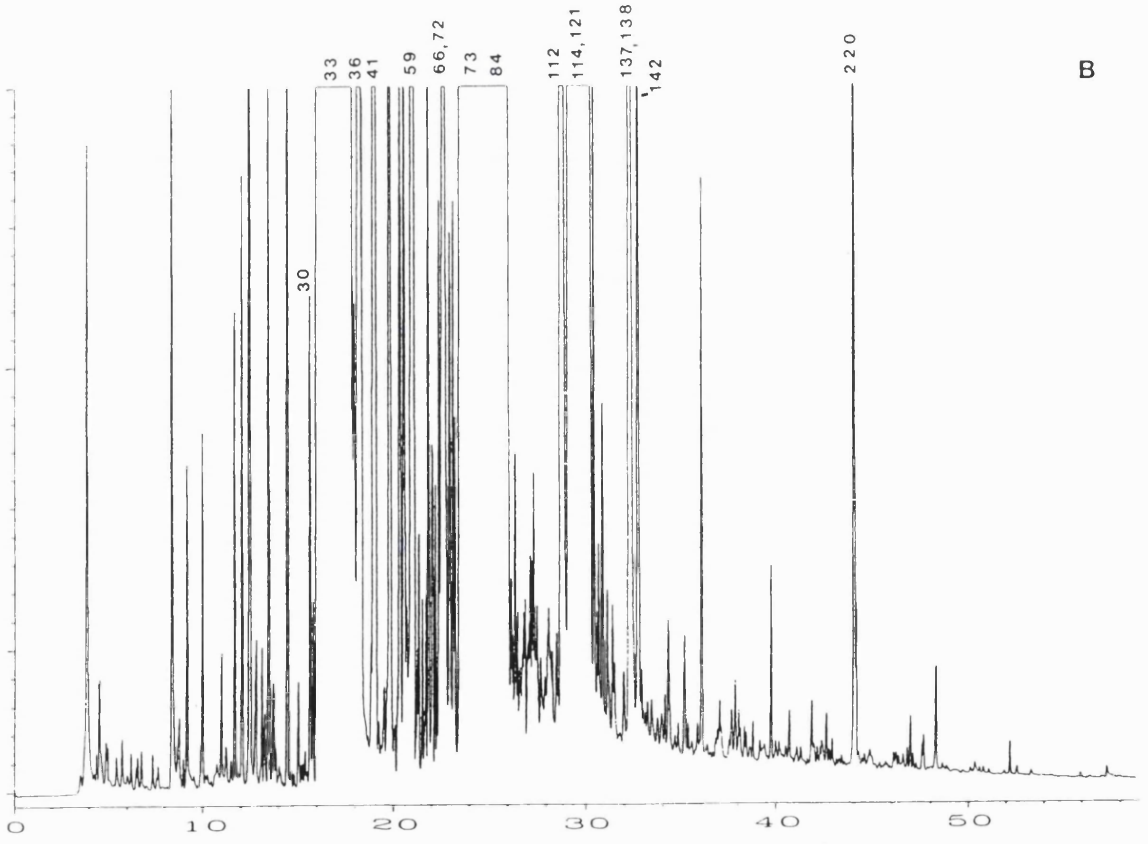
U. *A. hohenlohii*

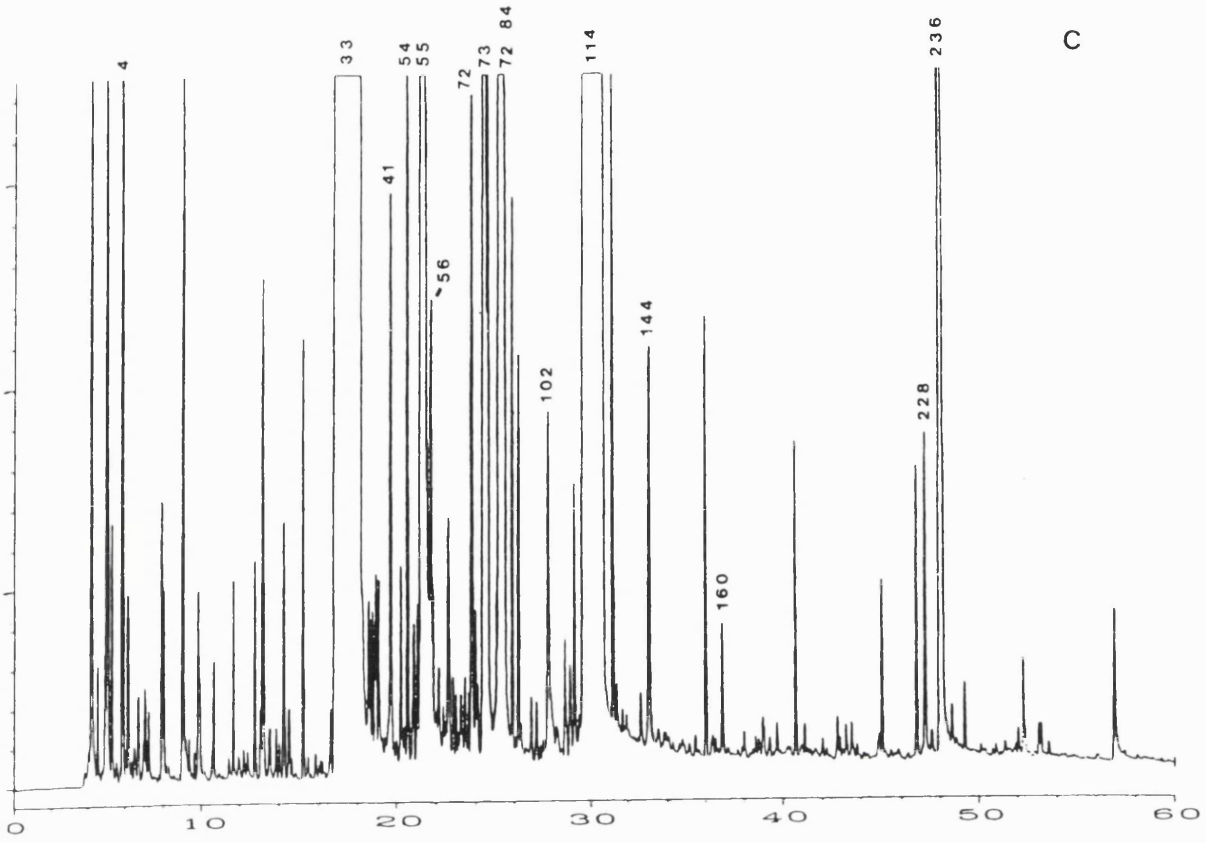
V. *A. eburnea*

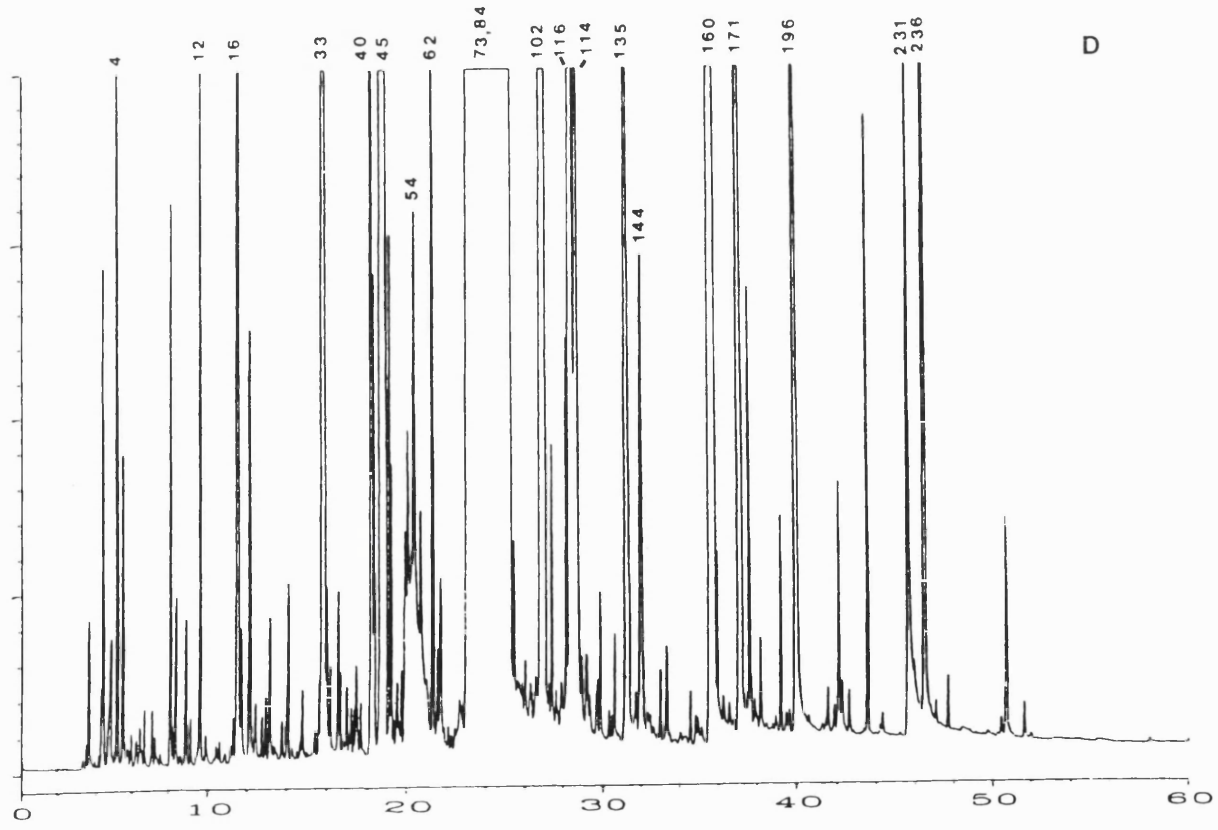
W. *A. virginalis*

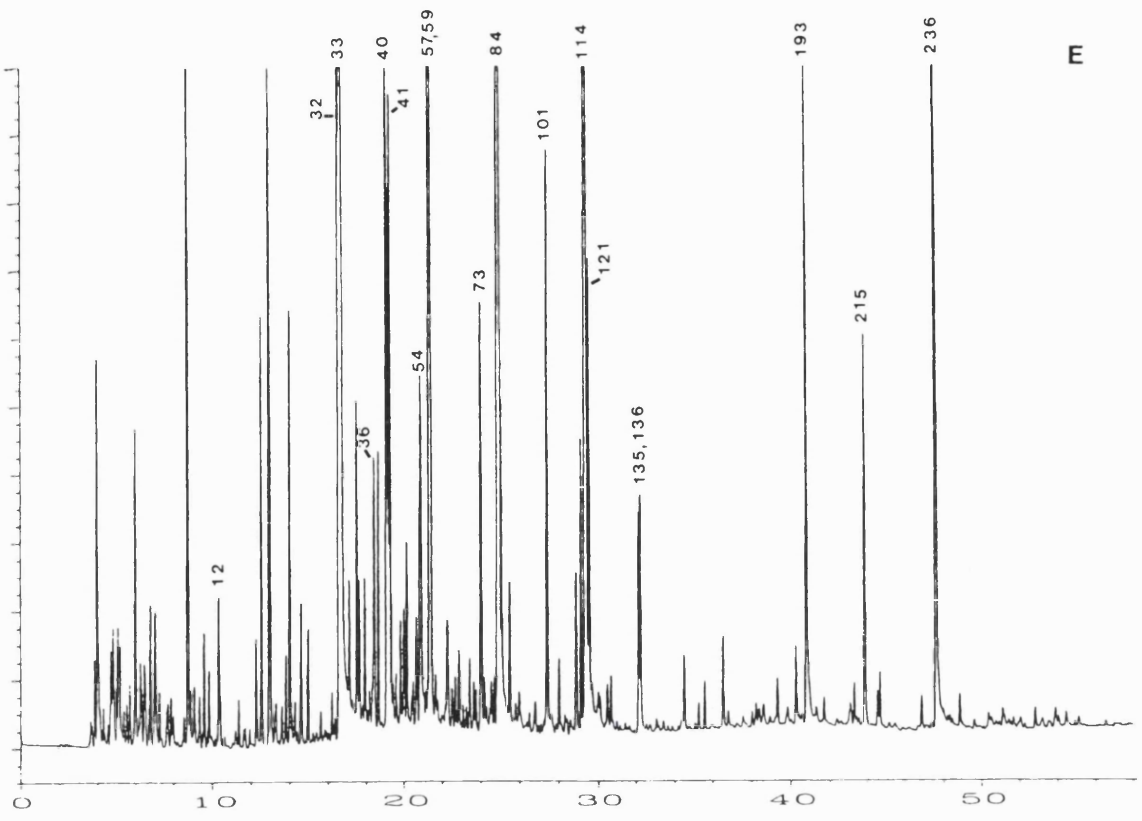
X. Blank

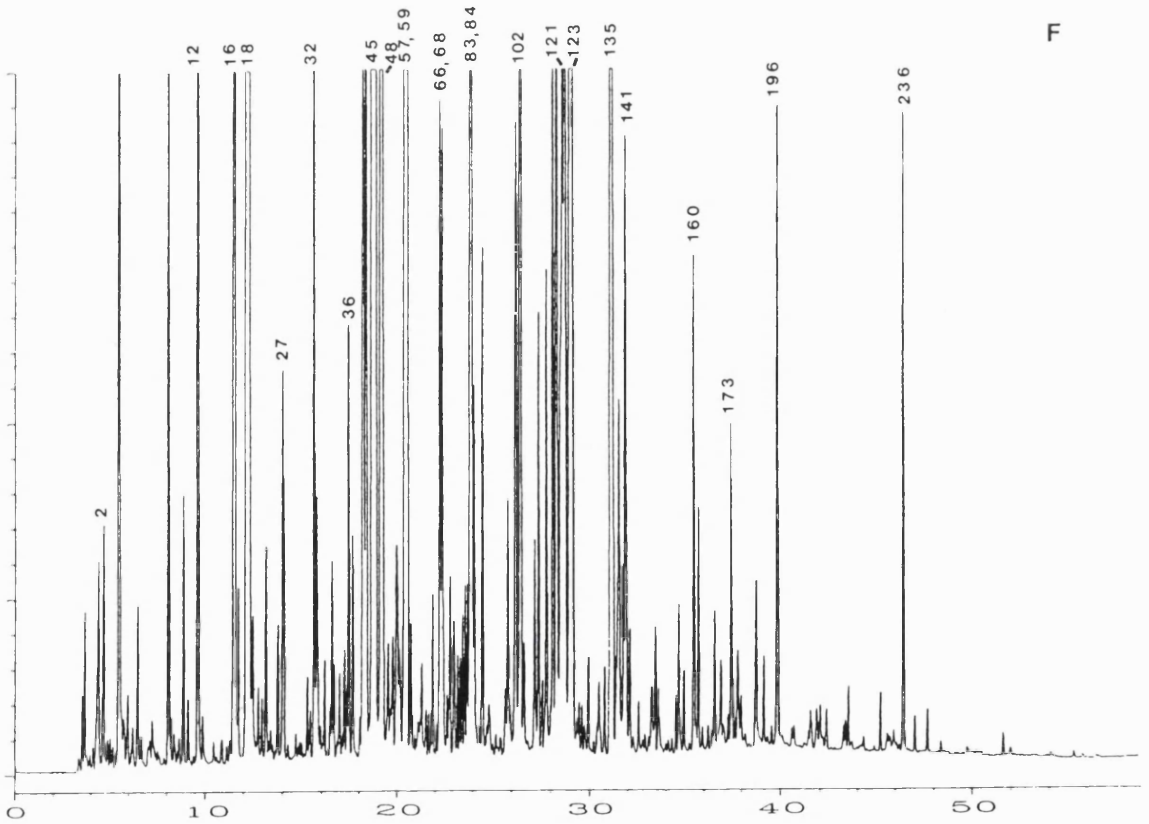

A
30
33
34
36
39
41
54
57
84
106
121
123
134
138
142
220
0
10
20
30
40
50

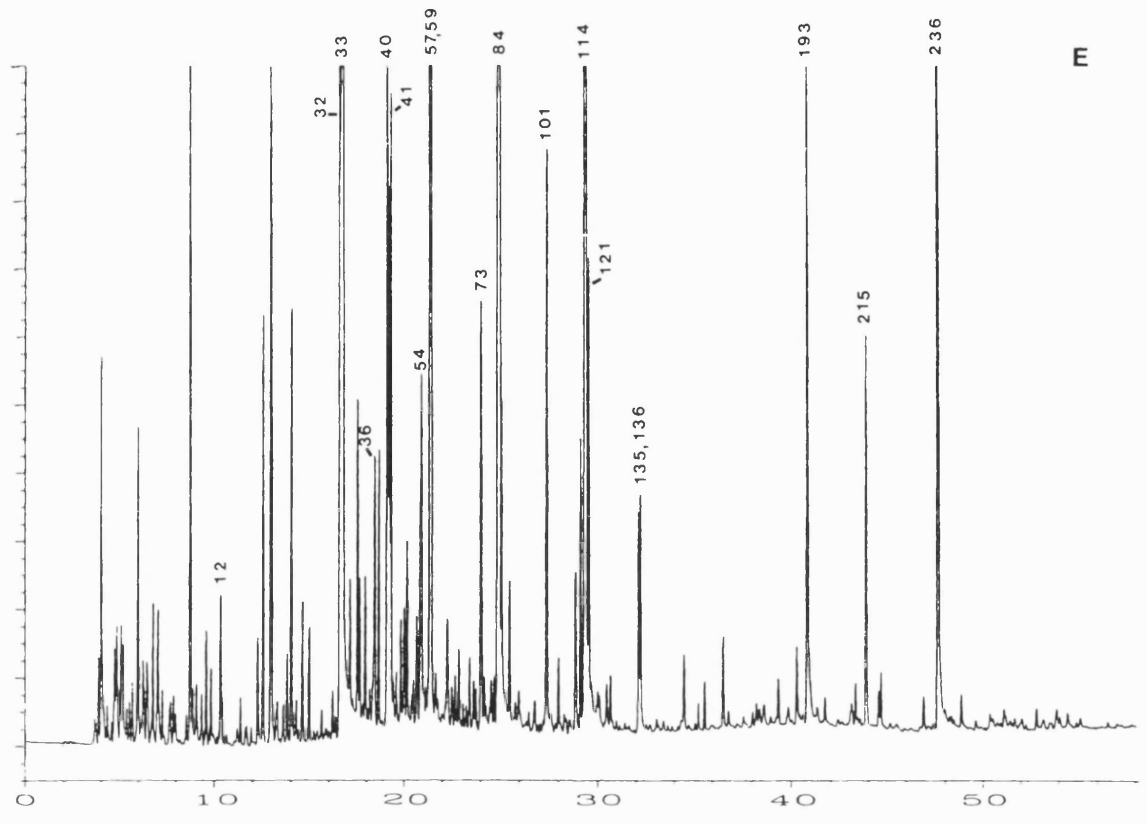

B
30
33
36
41
59
66,72
73
84
112
114,121
137,138
142
220
0
10
20
30
40
50


C
4
33
41
54
55
56
72
73
72
84
102
114
144
160
228
236
0
10
20
30
40
50
60


D
4
12
16
33
40
45
54
62
73,84
102
116
114
135
144
160
171
196
231
236
0
10
20
30
40
50
60


E
12
32
33
36
40
41
54
57,59
73
84
101
114
121
135,136
193
215
236
0
10
20
30
40
50


F
2
12
16
18
27
32
36
45
48
57, 59
66,68
83,84
102
121
123
135
141
160
173
196
236
0
10
20
30
40
50


E
12
32
33
36
40
41
54
57,59
73
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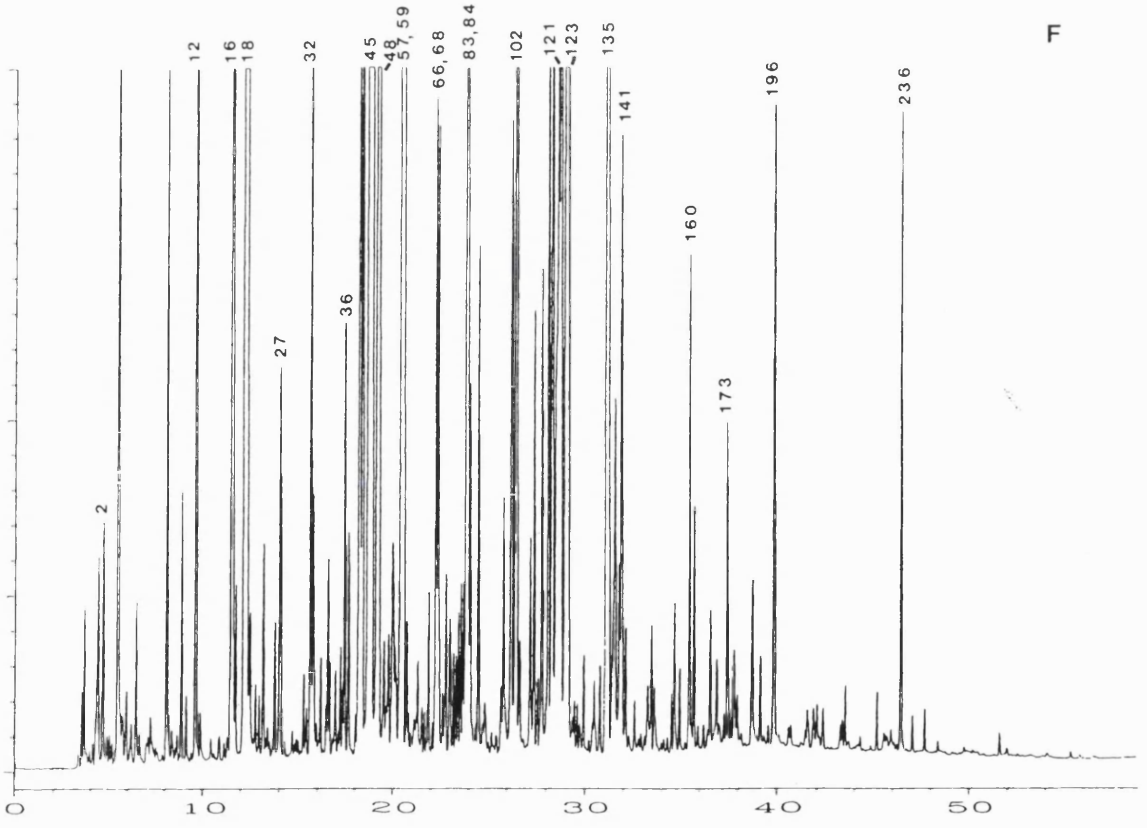
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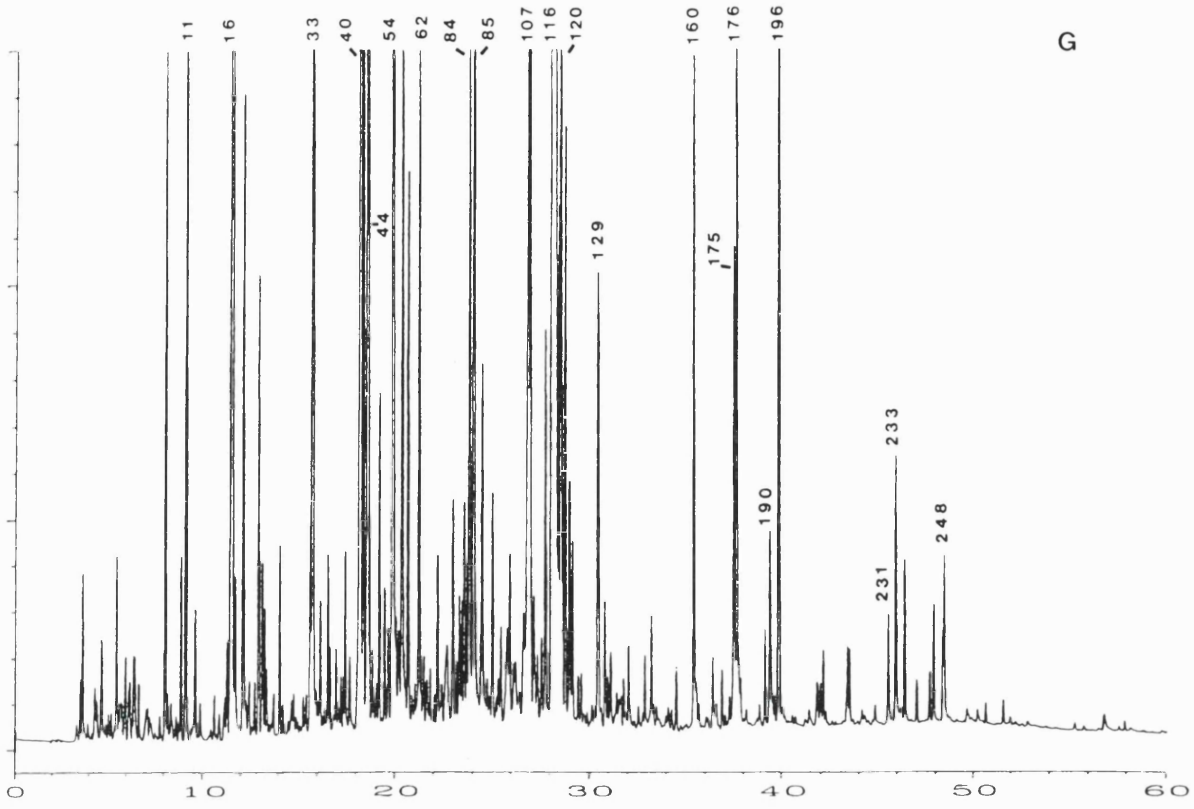
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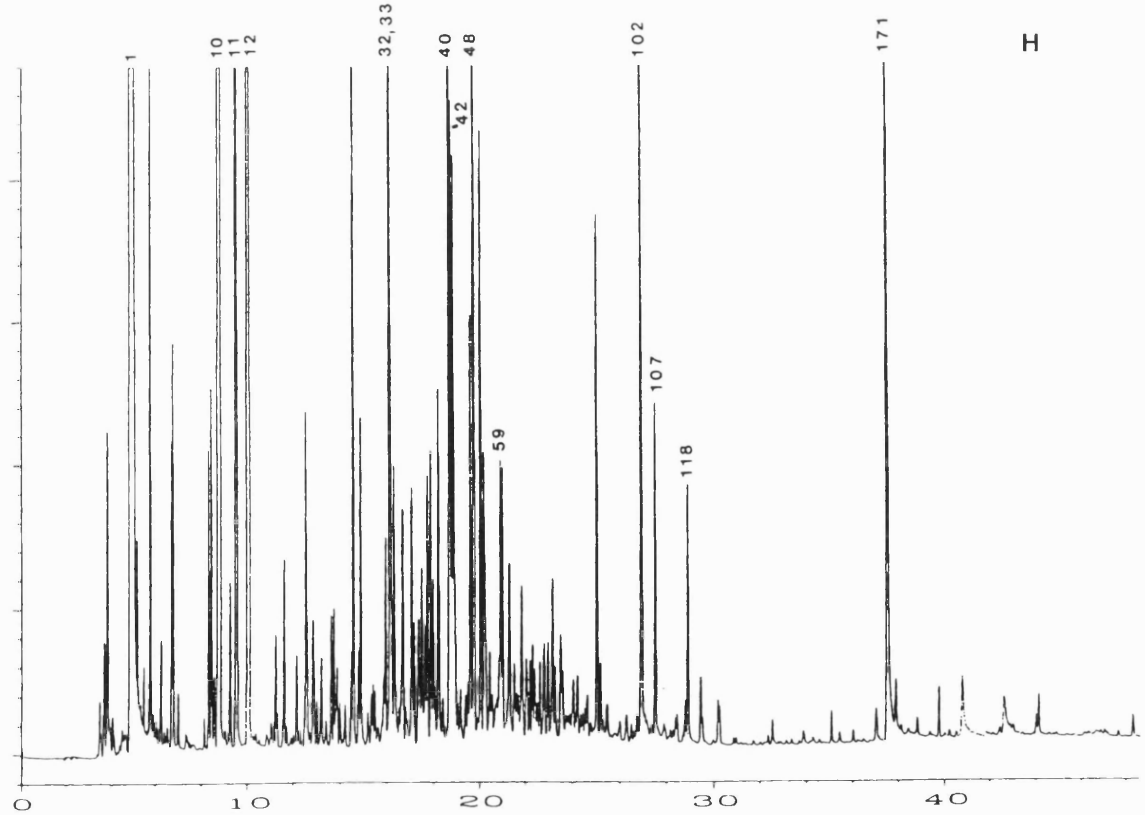
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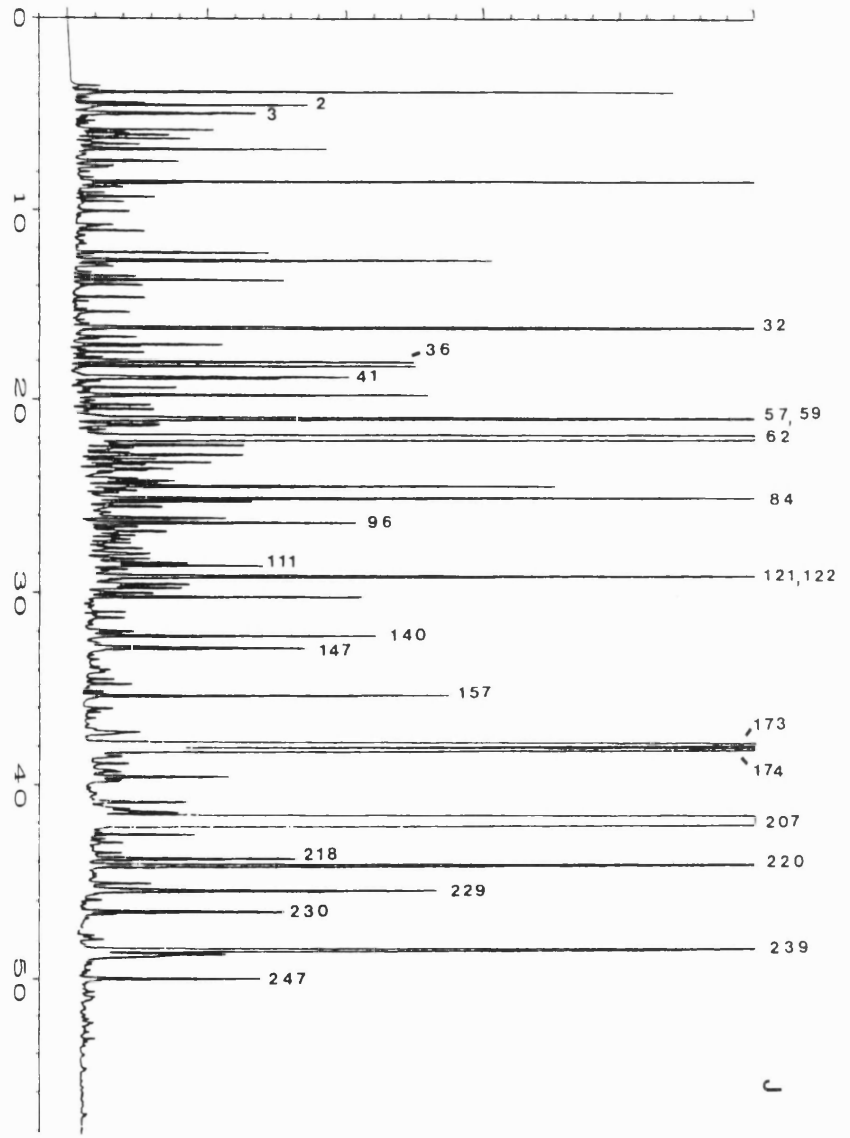
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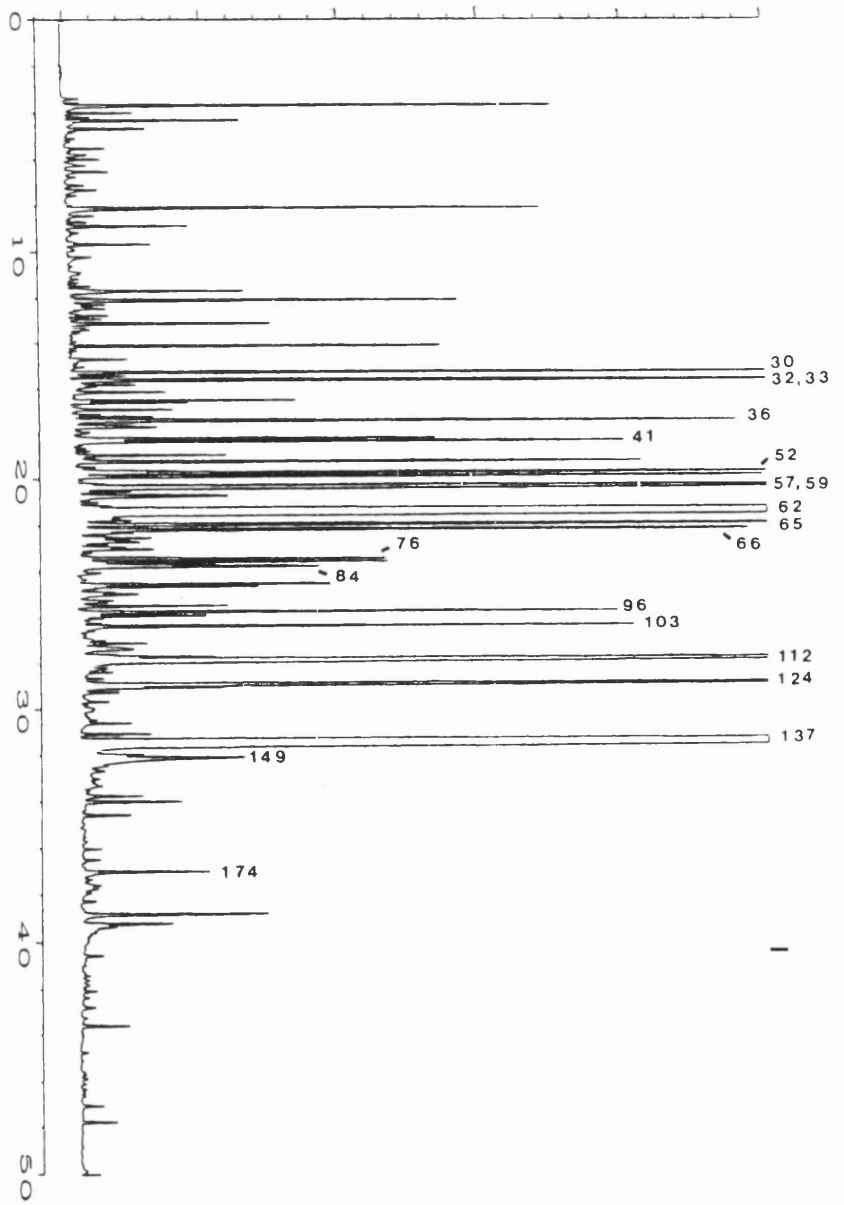
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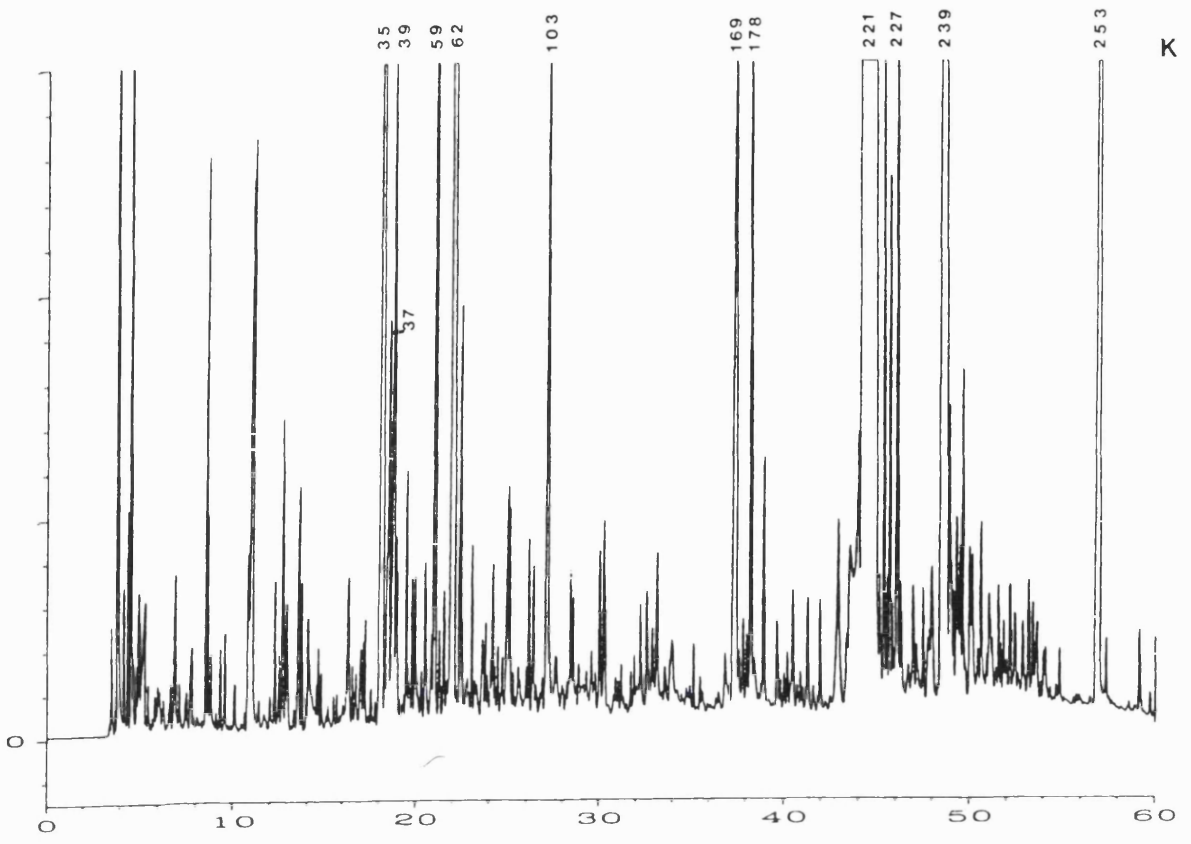
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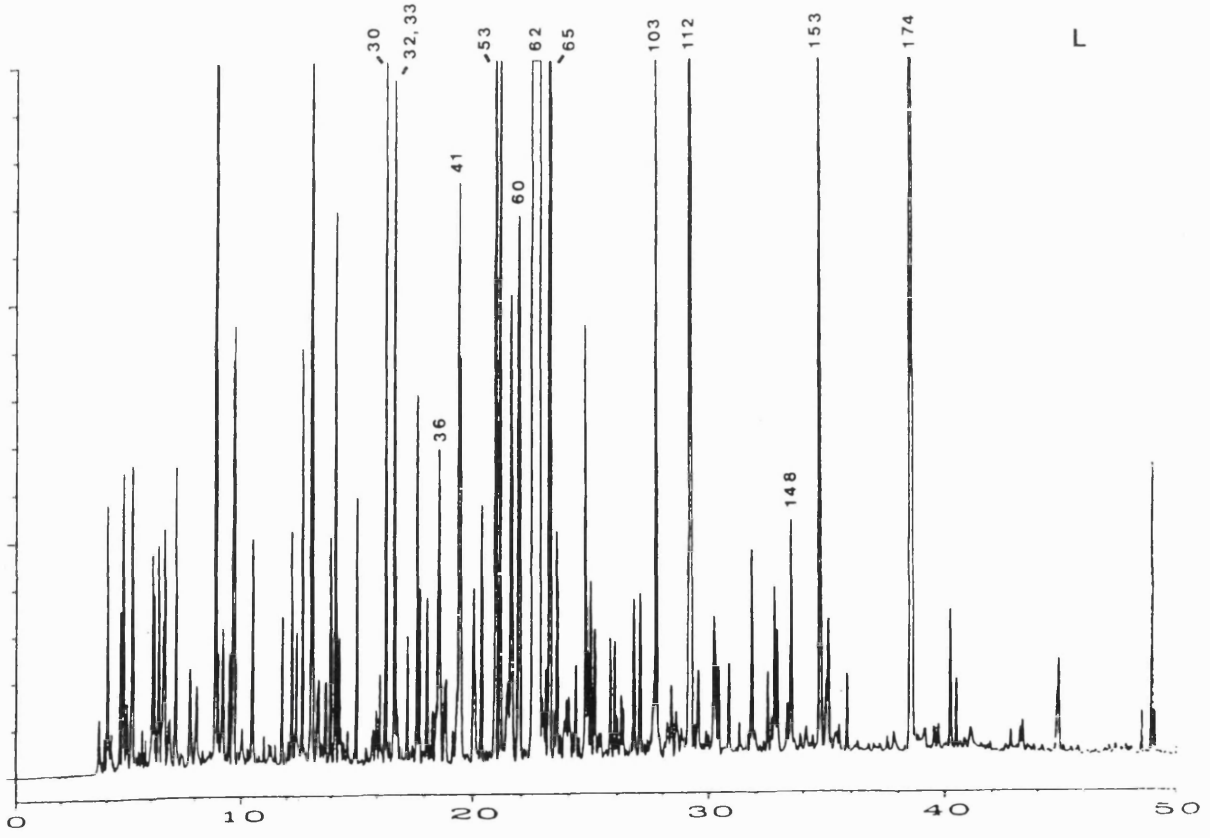
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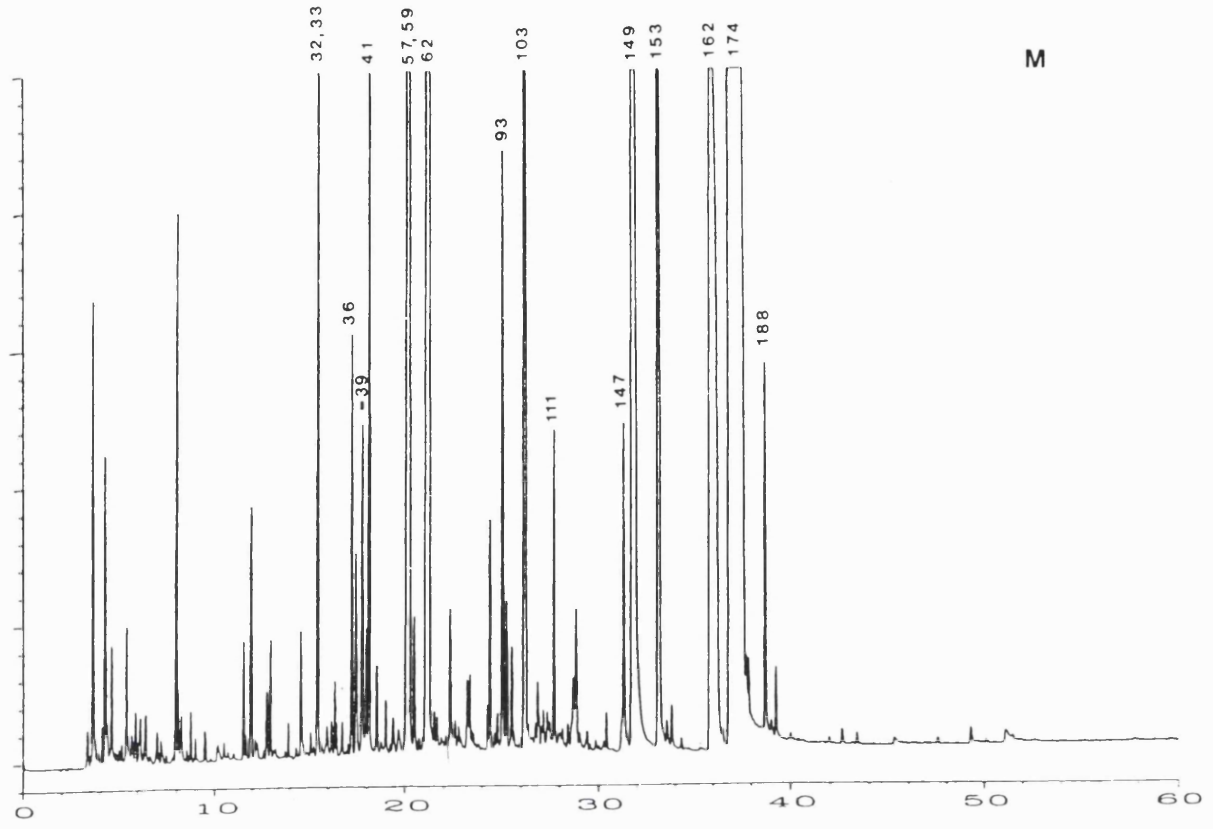
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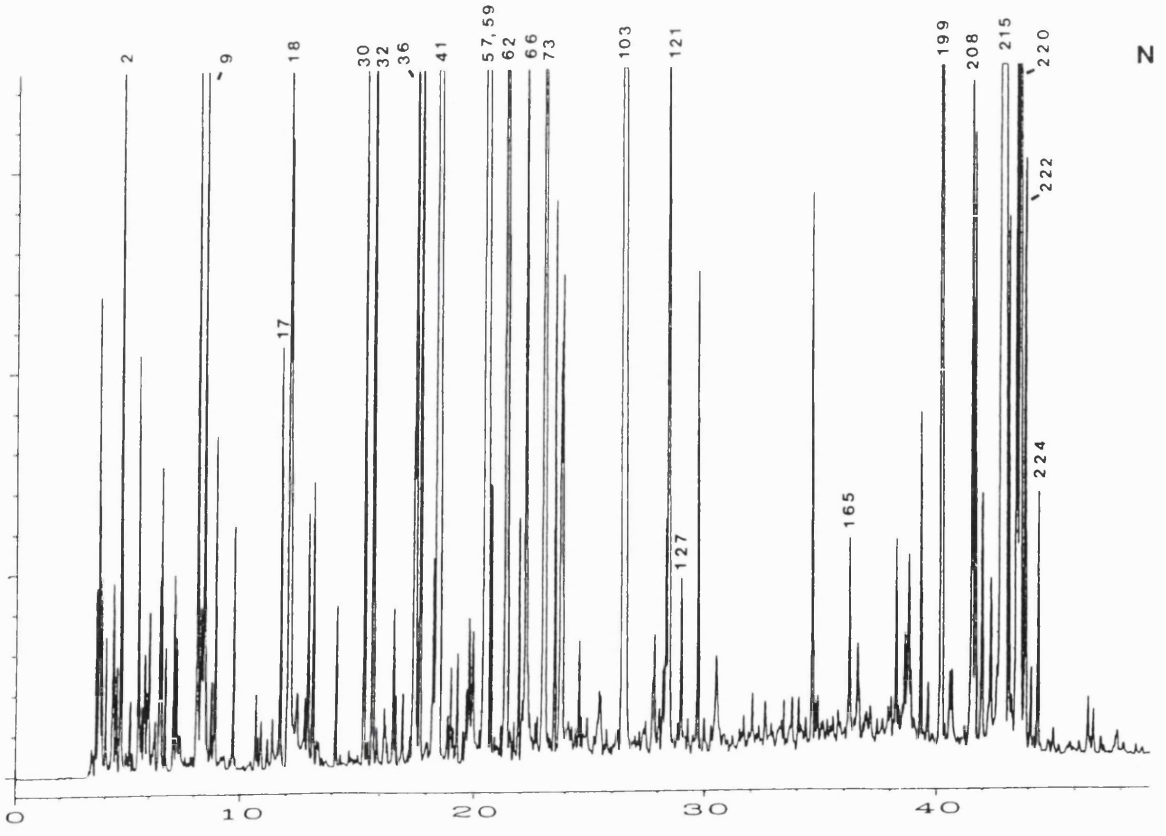
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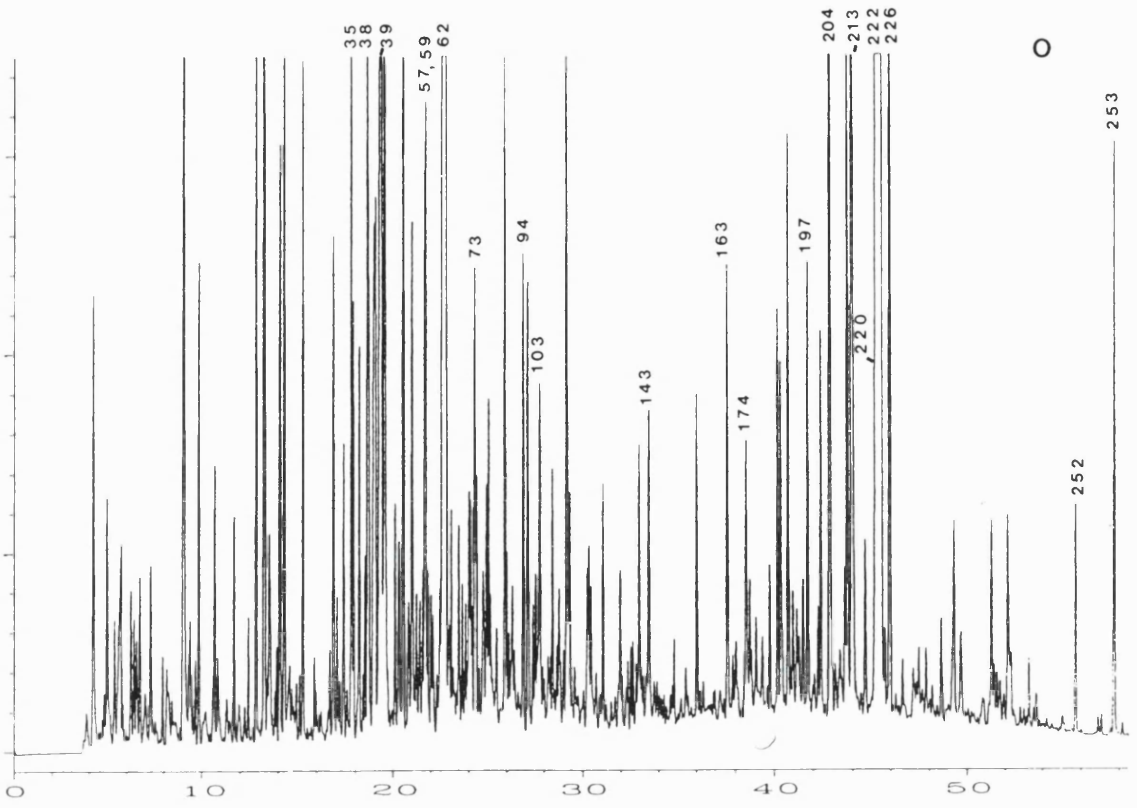
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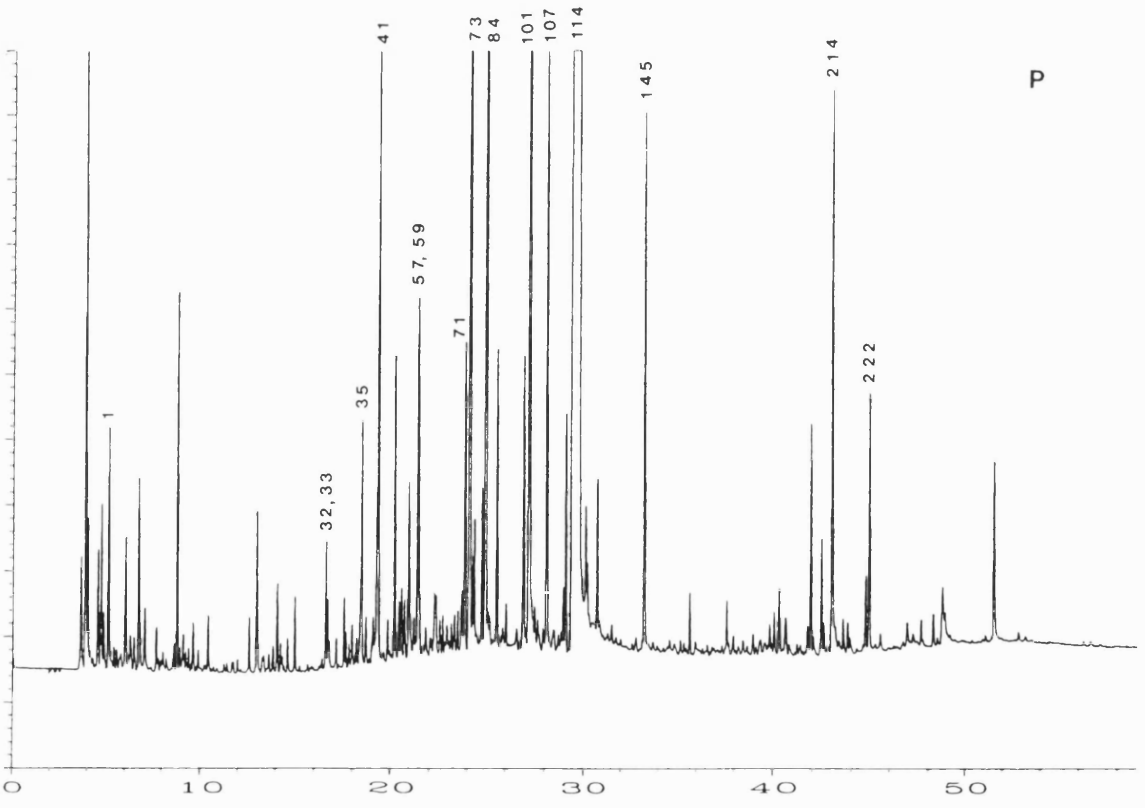
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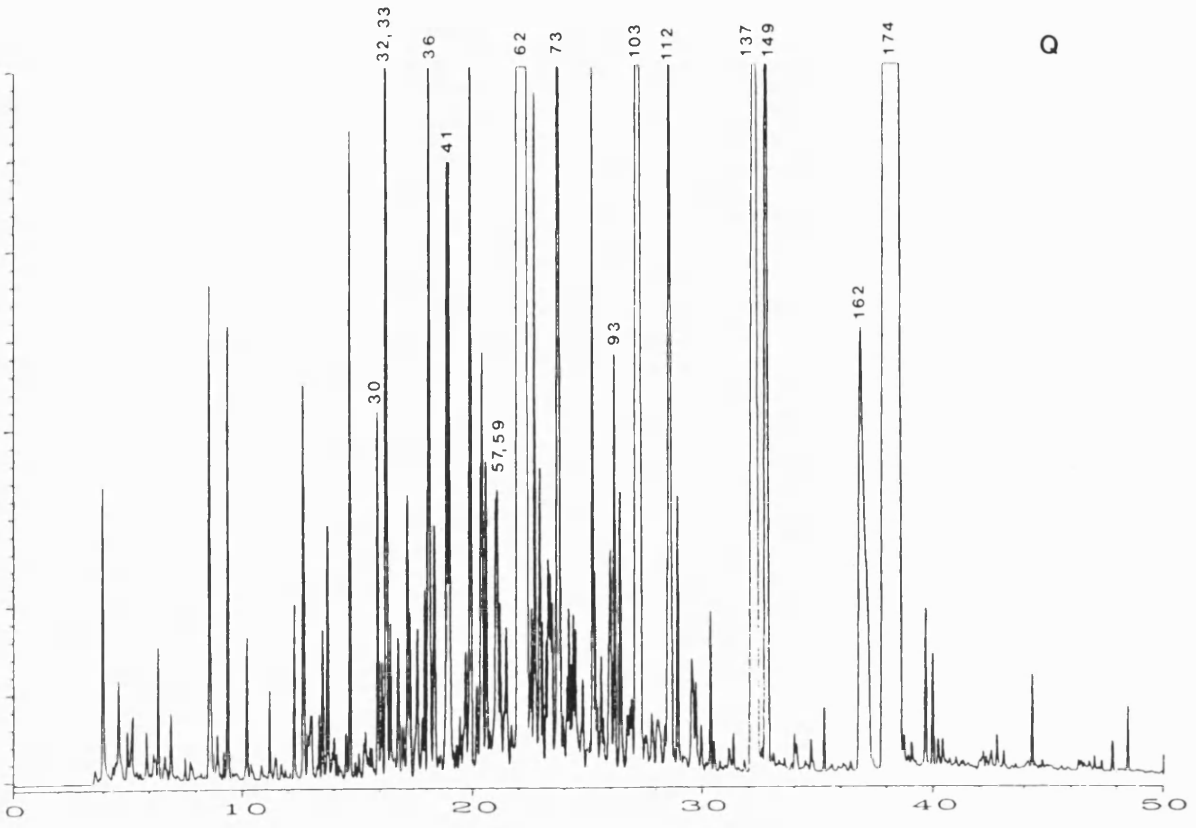
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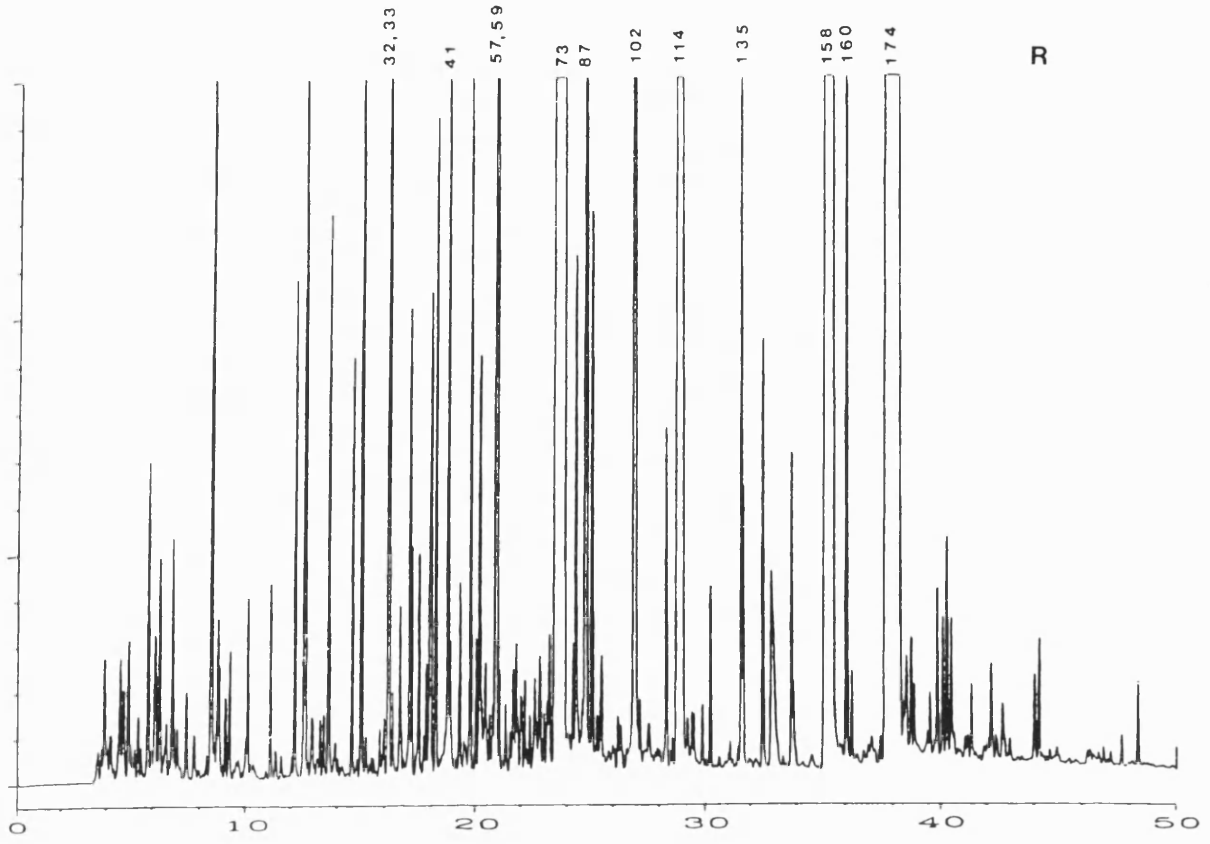
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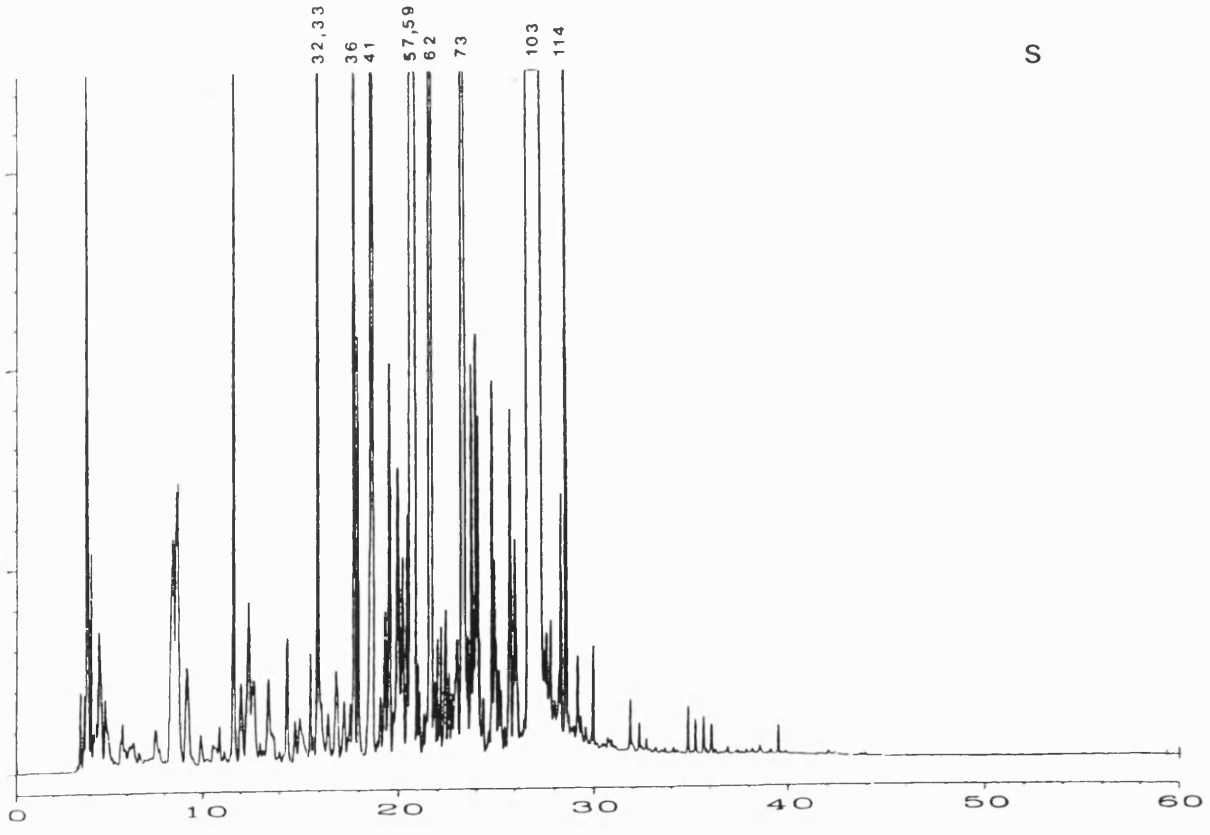
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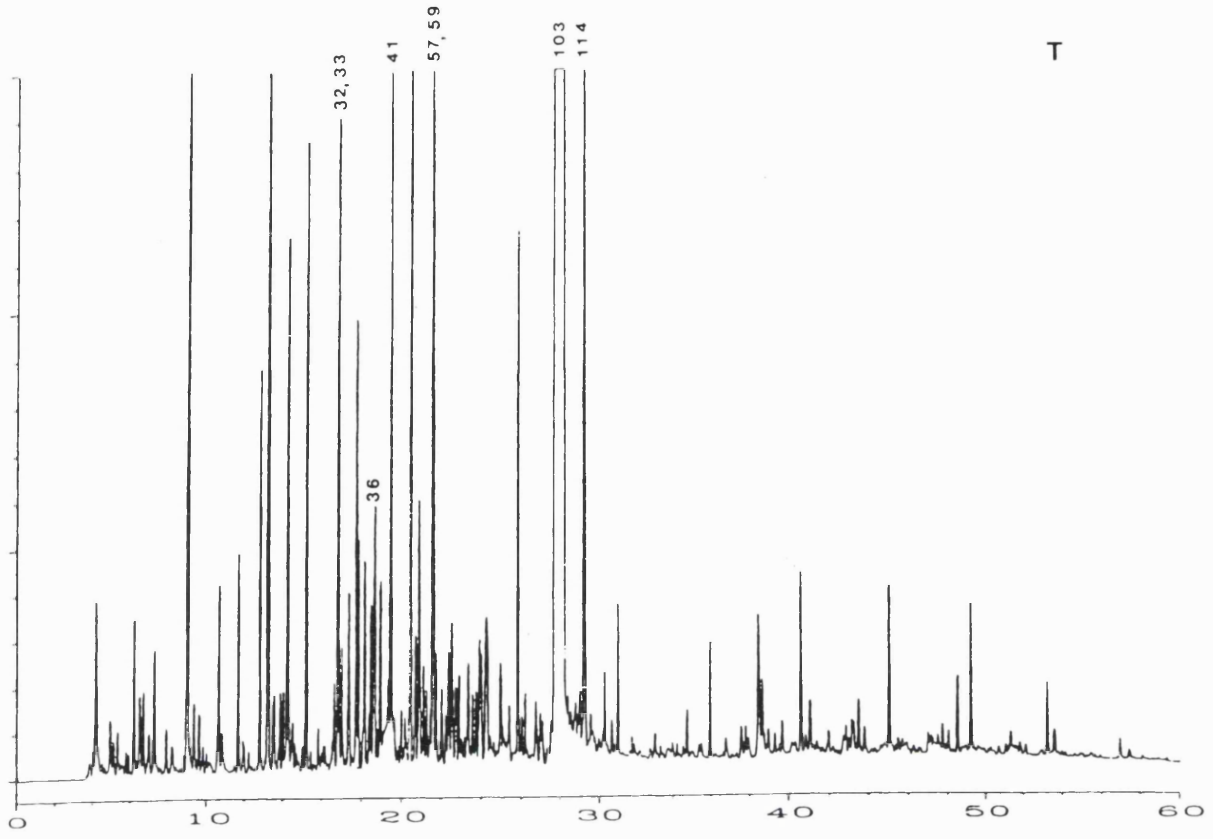
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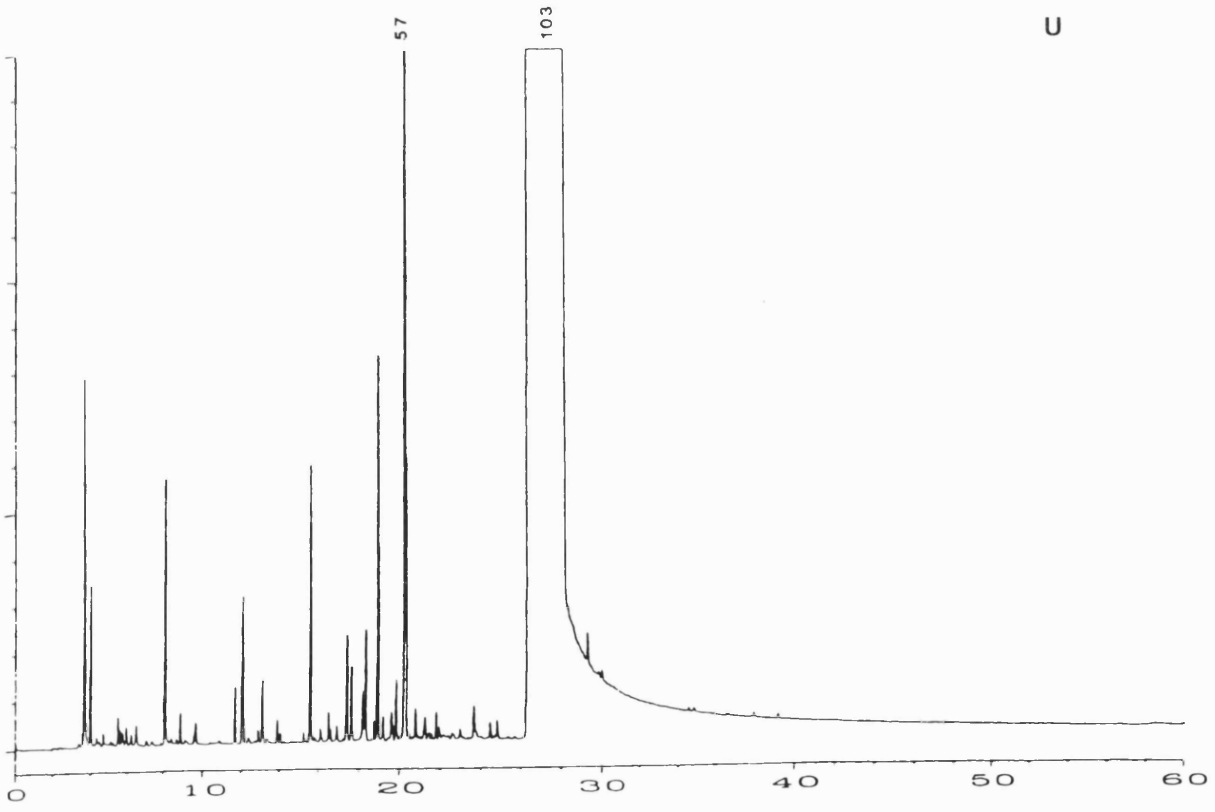
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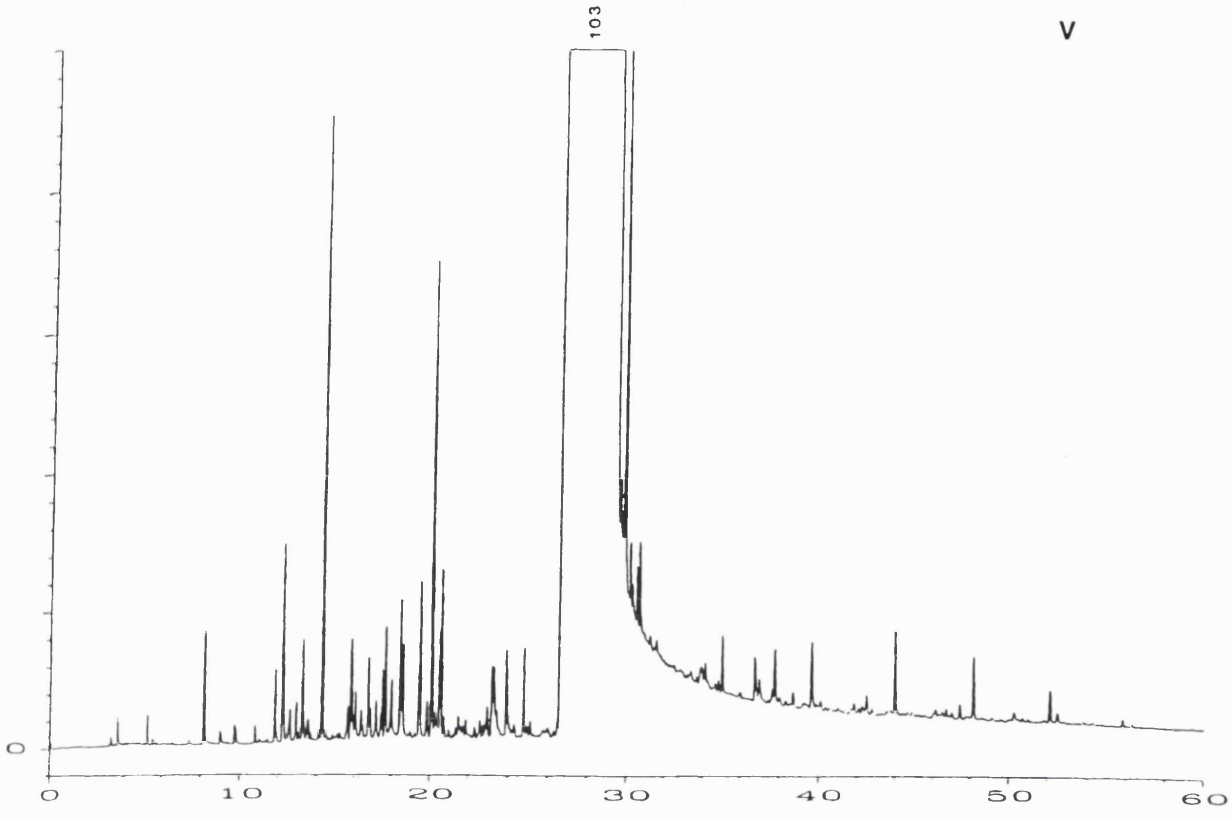
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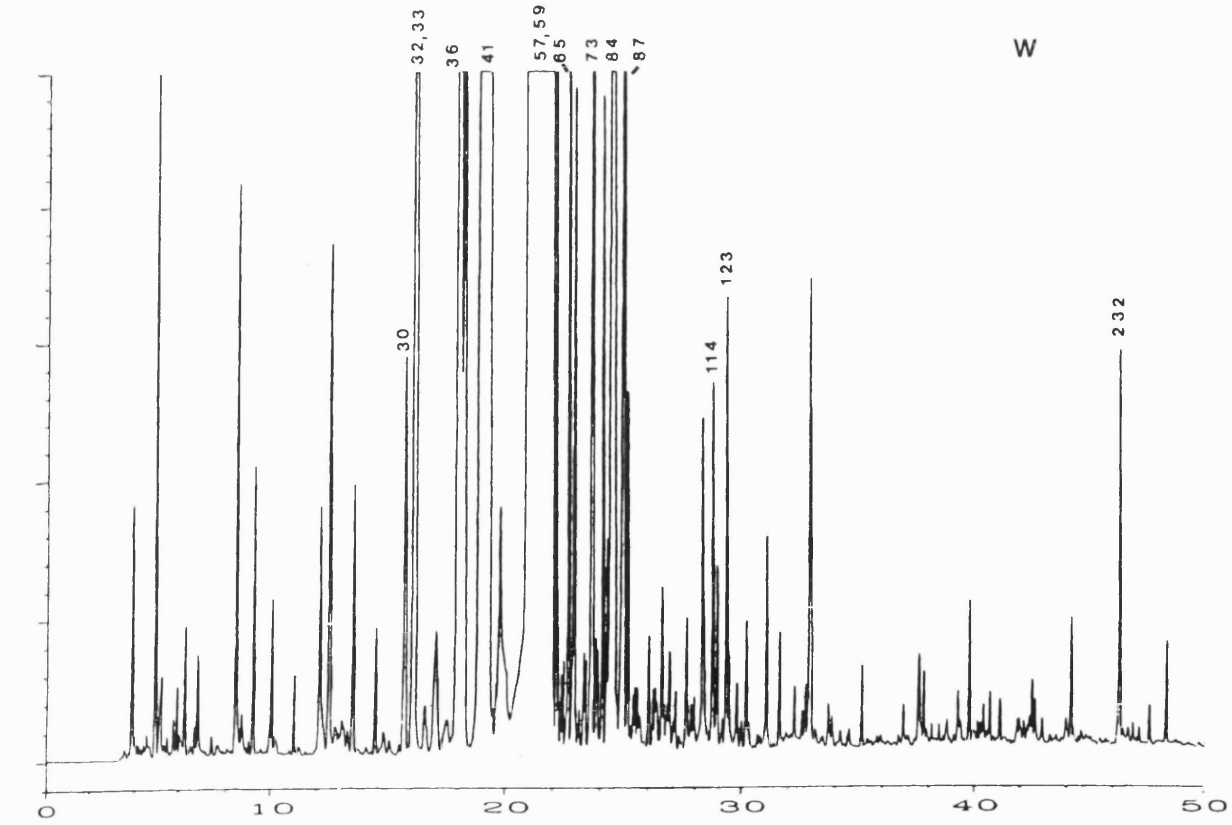
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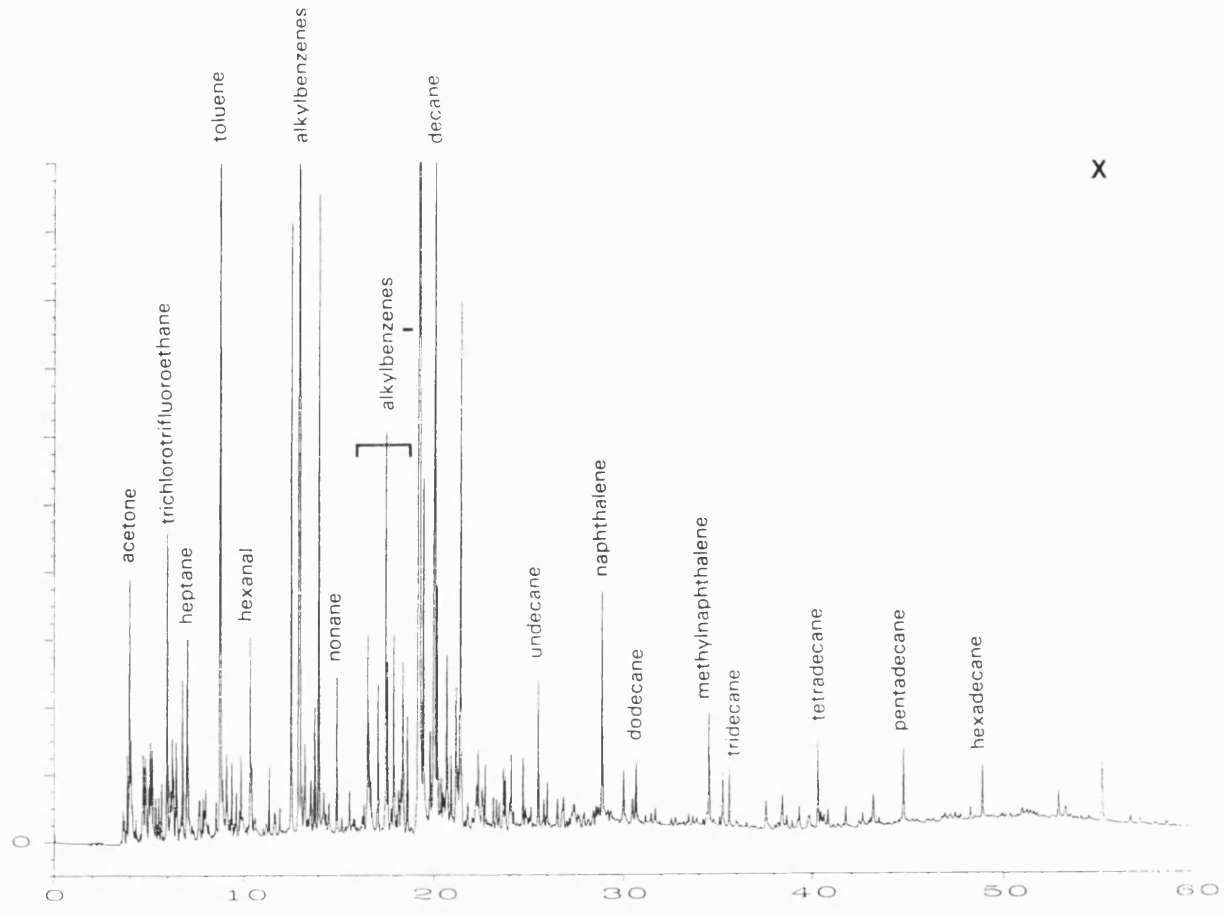
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Lindleyana 15(1): 33–45. 2000.

A PHYLOGENETIC ASSESSMENT OF *LYCASTE* AND *ANGULOA* (ORCHIDACEAE: MAXILLARIEAE)[1]

ANGELA RYAN[2], W. MARK WHITTEN[3], MARGARET A. T. JOHNSON[2], AND MARK W. CHASE[2]

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ABSTRACT: In this study, two DNA sequence regions, the internal transcribed spacer (ITS) of nuclear ribosomal DNA and the plastid gene *matK*, were used to examine the inter- and infrageneric relationships of *Lycaste*, *Anguloa*, and other members of subtribe Lycastinae. Parsimony analysis of both ITS and *matK* sequences places *Lycaste* sect. *Fimbriatae* in a clade with *Anguloa*, sister to the clade containing species from the other three sections. The analyses identified *Neomoorea* as the nearest neighbor to *Lycaste* and *Anguloa*. They also identified the Maxillariinae genera *Cryptocentrum* and *Maxillaria* as being more closely related to *Lycaste* and *Anguloa* than *Bifrenaria* and *Rudolfiella*, which have been placed in Lycastinae by some authors. There was insufficient variation among sequences of both ITS and *matK* to examine species relationships within sections. Chromosome counts for three *Anguloa* species were determined here as $2n = 40$.

LYCASTE AND ANGULOA are two closely related genera of tropical American orchids. *Lycaste* was first described by Lindley (1843) as a segregate of *Maxillaria* and is currently considered to comprise some 60 species, ranging from Mexico to Bolivia and southern Brazil, typically at elevations between 500 m and 2500 m. *Anguloa*, described by Ruiz and Pavón (1794), comprises about ten species (Oakeley, 1999) found in the Andes from Venezuela to Peru between 1500 m and 2500 m.

Species of *Lycaste* are epiphytic, lithophytic or terrestrial herbs, whereas species of *Anguloa* are generally lithophytic or terrestrial. Both genera are characterized by dark green fusiform pseudobulbs that are laterally compressed and may have spines at the apex, which are formed by the midrib in the deciduous species. New pseudobulbs bear 2–4 apical, plicate leaves narrowing at the base to form a petiole-like stalk. Inflorescences are lateral, one to several arising from the base of the previous year's pseudobulb either with the onset of new growth or, in the case of *Anguloa*, just afterwards. They are generally single-flowered and partially to completely covered by up to six leafy bracts. An additional floral bract sheathes the pedicel and six-ribbed ovary and may extend along the dorsal sepal. In common with many genera of tribe Maxillarieae, the entire base of the lateral sepals is joined to the column-foot to form a chin or "mentum."

There are significant differences between the morphology of the perianth of the two genera, particularly the structure of the lip, and these are summarized in Table 1. Superficially, the most obvious differences are in the arrangement of the sepals and petals. In the case of *Lycaste*, the lateral sepals are subequal and spreading. They are broader than the dorsal sepal, and among species from *L.* sect. *Deciduosae*, *L.* sect. *Longisepalae*, and *L.* sect. *Lycaste* usually recurved or reflexed. The petals are smaller than the sepals and lie parallel to the column, almost enclosing it. Species from *L.* sect. *Fimbriatae*, such as *L. cinnabarina*, tend to have petals and sepals of the same color, usually green, whereas species from the other sections of the genus often have petals of either a different color or with a different pattern of spotting from the sepals.

Sepals and petals of *Anguloa* species are con-

[1] Leaf material was provided by Henry Oakeley, the Herbario de la Asociación Méxicana de Orquideología A. C., and the Royal Botanic Gardens, Kew. Anette de Bruijn and Jeffrey Joseph provided technical assistance with automated sequencing. AR acknowledges her former employers, Bush Boake Allen Ltd, and supervisor Phillip Cribb for their support of her thesis studies. Funding for the molecular studies was provided by the Bentham-Moxon Trust and the Royal Botanic Gardens, Kew.

TABLE 1. Major differences in floral morphology between *Lycaste* and *Anguloa*.

	Anguloa	*L.* sect. *Fimbriatae*	*Lycaste* other sections
Sepals connivent	present	absent	absent
Sepals and petals same color(s)	present except *A. cliftonii*	present, usually green	absent
Lateral sepal apex sickle-shaped	present	absent	absent
Lateral sepal base falcate	absent	present	absent
Lip held upright	present	absent	absent
Lip mid-lobe smaller than side lobes	present	absent	absent
Mid-lobe margin fimbriate	absent	present	absent
Lip callus extending from base of lip to mid-lobe	absent	present	absent
Callus generally multi-keeled	absent	present	absent
Callus apex bifid	present	present	absent
Column hairy	absent	present	present
Viscidium generally ovate, may be barbed.	present	absent	present
Viscidium often elongate, may be "V-" or "M-" shaped	absent	present	absent

cave and fleshier than those of most species of *Lycaste*. They are held erect and overlap for most of their length to form a cup with a narrow opening, hence one of their popular names, "tulip orchid." One of the most striking features of *Anguloa* flowers is the action of the lip, which is held upright and is hinged to the tip of the column foot so that it rocks back and forth when touched, the basis for another vernacular name, "cradle orchid." There is no common name for *Lycaste*. Both genera have cap-shaped, slightly pointed, terminal anthers containing four laterally compressed pollinia adpressed in pairs and attached to a single stipe.

Most authors have concentrated on resolving species-level problems (e.g. Kennedy, 1976; Oakeley, 1994, 1999). The sectional classifications in current use (Fowlie, 1970; Schlechter, 1916) were based on only a few diagnostic characters, and little attention has been focused on phylogenetic relationships.

The first subgeneric classification of *Lycaste* was by De Wolfe (1953), who proposed that those species with a fimbriate margin to the mid-lobe of the lip and a bifid callus should be placed in a separate section. Fowlie (1970) took this concept a stage further, recognizing four sections and two subsections (Table 2), the treatment most commonly used. He concurred with De Wolfe in his definition of *L.* sect. *Fimbriatae*, considering this section to contain the most primitive species of the genus, predating the formation of the Andes. However, he differed in his treatment of the single pendent species, *L. dyeriana*; De Wolfe had treated it as a "fimbriate" *Lycaste*, but Fowlie considered it to be more closely allied to *Bifrenaria* Lindl. and excluded it from the genus. Apart from one isolated species, *L. barringtoniae*, which is found exclusively in the Greater Antilles, species of *L.* sect. *Fimbriatae* occur in the South American Andes, ranging from Venezuela to Peru. The species have unspined pseudobulbs, persistent leaves, and are distinguished from other *Lycaste* species by their lip and viscidium morphology (Table 1).

Species exhibiting vegetative growth during the rainy season, followed by a resting period during which they lose their leaves, were assigned to *L.* sect. *Deciduosae*. The pseudobulbs of all such species apart from *L. tricolor* have apical spines, formed when the leaves are shed. Two subsections of *L.* sect. *Deciduosae*, described at the same time, reflect differences in flower color and geographical range: species of the yellow-flowered *L.* subsect. *Xanthanthae* range from Mexico to northern Colombia, whereas those of the pink-flowered *L.* subsect. *Paradeciduosae* are found in the highlands of Nicaragua, Costa Rica, and Panama.

Lycaste sect. *Lycaste* (syn. *L.* sect. *Macrophyllae* Fowlie) ranges from Guatemala to Bolivia and was defined in an earlier publication (Fowlie, 1964) as having obscurely spined or unspined pseudobulbs, non-deciduous leaves, and a non-fimbriate lip with a spathulate callus. Fowlie considered some taxa within this section to be subspecies of *L. macrophylla* (Poepp. & Endl.) Lindl. rather than "full-blown" species. His criterion

TABLE 2. Chromosome counts for *Anguloa* and *Lycaste* species. *Anguloa* counts (*) were determined as part of this study.

	Chromosome number (*2n*)	Reference
Subtribe **Lycastinae**		
Bifrenaria		
B. harrisoniae (Hook.) Rchb.f.	40	Hoffmann (1929, 1930)
Anguloa		
A. sect. *Anguloa*		
A. virginalis Linden	40	*
A. sect. *Guloanga* Schltr.		
A. cliftonii Rolfe	40	*
A. clowesii Lindl.	40	*
Lycaste		
L. sect. *Fimbriatae* Fowlie		
L. barringtoniae (Smith) Lindl.	44	Aoyama and Karasawa (1988)
L. ciliata (Ruiz & Pav.) Lindl. ex Rchb.f.	44	Aoyama and Karasawa (1988)
L. cinnabarina Lindl.	50	Aoyama and Karasawa (1988)
L. dyeriana Sander ex Rolfe	48	Aoyama and Karasawa (1988)
L. linguella Rchb.f.	48	Aoyama and Karasawa (1988)
L. locusta Rchb.f.	48	Aoyama and Karasawa (1988)
L. sect. *Deciduoseae* Fowlie		
L. aromatica (Graham ex Hook.) Lindl.	40	Aoyama and Karasawa (1988)
L. bradeorum Schltr.	40	Aoyama and Karasawa (1988)
L. brevispatha (Kl.) Lindl.	40	Aoyama and Karasawa (1988)
L. campbellii C. Schweinf.	40	Aoyama and Karasawa (1988)
L. cruenta Lindl.	40	Aoyama and Karasawa (1988)
L. deppei (Lodd.) Lindl.	40	Aoyama and Karasawa (1988)
L. tricolor (Klotzsch) Rchb.f.	40	Aoyama and Karasawa (1988)
L. sect. *Lycaste*		
L. dowiana Endres & Rchb.f.	40	Aoyama and Karasawa (1988)
L. macrophylla (Poepp. & Endl.) Lindl.	40	Aoyama and Karasawa (1988)
L. virginalis (Scheid.) Linden	40	Aoyama and Karasawa (1988)
Subtribe **Maxillariinae**		
Maxillaria		
M. picta Hook.	40	Blumenschein (1960)
M. tenuifolia Lindl.	40	Tanaka (1966)
Subtribe **Zygopetaliinae**		
Dichaea		
D. muricata Sw. Lindl.	52	Woodard (in Duncan, 1959)
Koellensteinia		
K. graminea Rchb.f.	ca. *48*	Hoffmann (1929)
Zygopetalum		
Z. mackaii Hook.	48	Hoffmann (1930)
Z. maxillare Lodd.	48	Blumenschein (1960)

was their ability to form hybrid swarms in localities where ranges overlapped (Fowlie, 1964, 1970). He placed a single species, *Lycaste schilleriana* in *L.* sect. *Longisepalae.* This species occurs in the highland regions of Colombia, Ecuador, and northern Peru and resembles a long-sepaled member of *L.* sect. *Lycaste.* Subsequent publications have all used Fowlie's system uncritically (e.g. Tomlinson, 1984; Oakeley, 1991a,b,c, 1993).

Schlechter (1916) published a revision of *Anguloa* in which he recognized two sections: *A.* sect. *Anguloa* (syn. *A.* sect. *Euanguloa* Schltr.) and *A.* sect. *Guloanga.* The former was characterized by extended downward-pointing triangular lobes on either side of the rostellum and contained

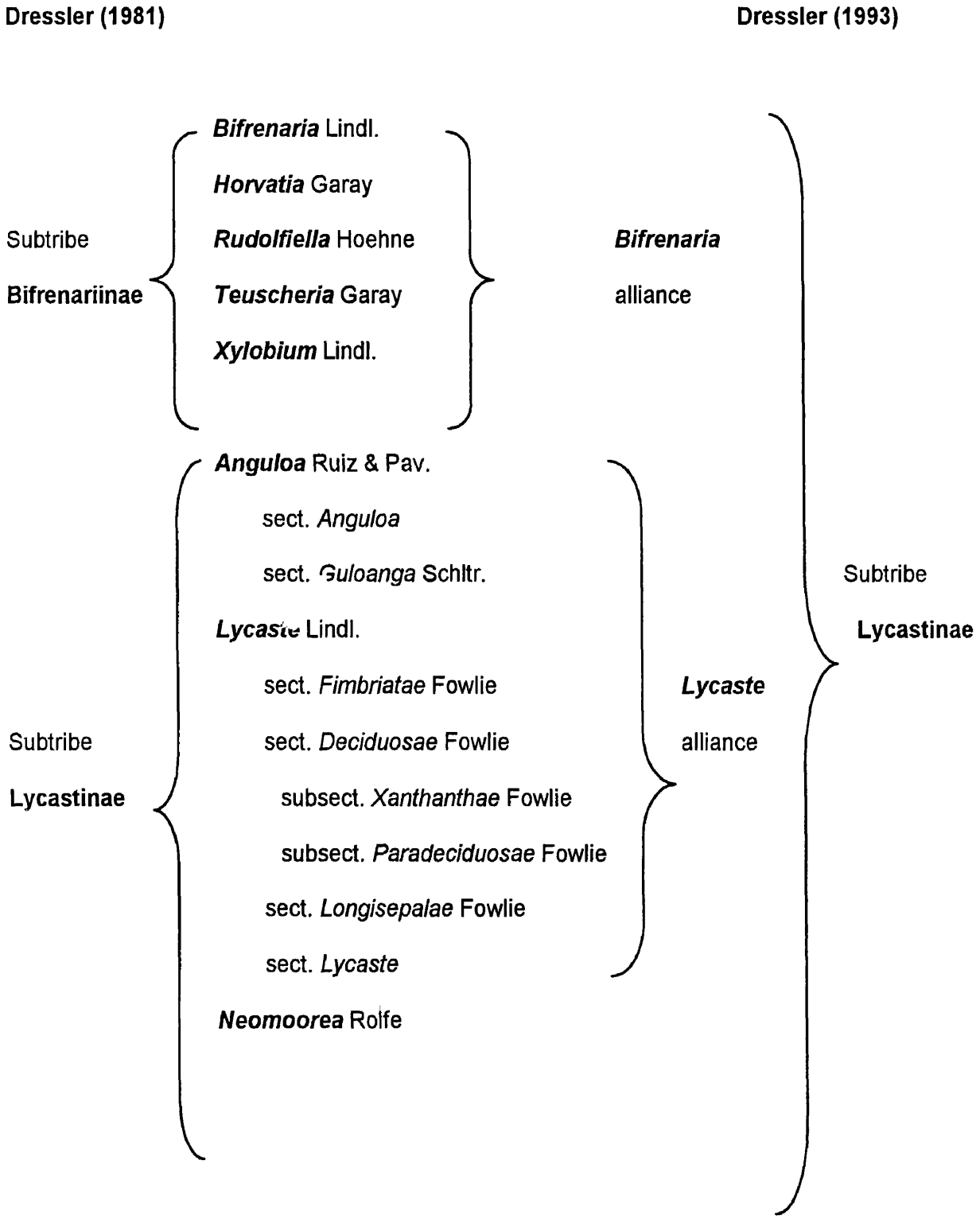

Fig. 1. Subtribe Lycastinae Schltr., after Dressler (1981, 1993), Fowlie (1970), and Schlechter (1916).

two species, *Anguloa uniflora* Ruiz & Pav. and *A. virginalis*. Species lacking these lobes were placed in *A.* sect. *Guloanga*. In his revision, individual species were keyed out according to differences in the structure of the lip, particularly the mid-lobe and callus. Recent treatments have continued to partition species within the genus according to the presence or absence of lateral rostellar lobes but have abandoned Schlechter's sectional names (Kennedy, 1976; Tomlinson, 1984; Oakeley, 1994, 1999).

Both *Lycaste* and *Anguloa* are ascribed to subtribe Lycastinae (Maxillarieae), within which there are eight genera, as shown in Fig. 1. In his

latest orchid classification, Dressler (1993) identified two tentative alliances: the *Lycaste* alliance, comprising *Lycaste* and *Anguloa,* and the *Bifrenaria* alliance comprising *Bifrenaria* Lindl., *Horvatia* Garay, *Rudolfiella* Hoehne, *Teuscheria* Garay, and *Xylobium* Lindl. The remaining genus, *Neomoorea* Rolfe, was treated as an isolated taxon.

A correlation between chromosome number and orchid phylogeny was acknowledged by, among others, Duncan (1959a), Jones (1966, 1974), and Dressler (1981, 1993). Extensive compilations were published by Duncan (1959b), and Tanaka and Kamemoto (1974, 1984). Aoyama and Karosawa (1988) undertook a karyomorphological study of 16 *Lycaste* species. They reported chromosome numbers of $2n = 40$ for species from *Lycaste* sect. *Deciduosae* and *L.* sect. *Lycaste* and numbers varying between $2n = 44–50$ within *L.* sect. *Fimbriatae* (Table 2). For comparison, chromosome numbers of three *Anguloa* species have been determined here.

The main objectives of this study were to investigate the infra- and intergeneric relationships of *Lycaste* and *Anguloa* and their relationships to other members of Lycastinae. We conducted parsimony analysis of DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region (Baldwin, 1992) and the plastid maturase-coding gene *matK* (Johnson and Soltis, 1995). Within Orchidaceae, ITS sequences have previously been used to examine the phylogeny of tribe Diseae (Douzery et al., 1999) and subtribes Orchidinae (Pridgeon et al., 1997) and Catasetinae (Pridgeon and Chase, 1998). Their utility at the subgeneric level was also demonstrated in a study of Cypripedioideae (Cox et al., 1997).

Previously known as ORFK (Steele, 1991), *matK* is one of the most rapidly evolving protein-coding regions within the plastid genome (Johnson and Soltis, 1995). Thus far it has been less extensively used for sequencing studies than ITS. Within the Orchidaceae, Kores, Molvray, and Chase (1997) used *matK* to examine phylogenetic relationships within subfamily Orchidoideae. In a smaller study, Khayota (1995) used *matK* sequences to confirm that the African genus *Ansellia* Lindl. (Epidendroideae: Cymbidieae) is more closely related to *Grammatophyllum* Blume than to other genera in subtribe Cyrtopodiinae. Its potential at the infrageneric level remains largely unexplored in orchids.

Pertinent to this work, Whitten, Williams, and Chase (in press) combined DNA sequence data from ITS, *matK*, *trn*L, and *trnL-F* to examine the phylogenetic relationships and assess the monophyly of tribe Maxillarieae, with particular emphasis on subtribe Stanhopeinae. We used their study as the basis for selection of appropriate outgroups for *Lycaste* and *Anguloa.*

MATERIALS AND METHODS

Vouchers have been deposited at FLAS, SEL, K or the Kew Spirit Collection and are listed in Table 3. Authority abbreviations follow Brummitt and Powell (1992).

For the chromosome counts, growing root-tips 0.5 cm long were pretreated by slicing longitudinally and soaking in 0.002 M 8-hydroxyquinoline (OQ) for 4.5 hr at 18 C. The material was fixed in freshly prepared 3:1 absolute ethanol: acetic acid and stored at 4 C until required. Hydrolysis, staining, and slide preparation followed standard cytological procedures as outlined in Johnson and Özhatay (1988). Photographs were taken on a Zeiss Photomicroscope III using Pan F film. Slides were made permanent by freezing with liquid CO_2 (Bowen, 1956) and are retained in the Cytogenetics Section of the Jodrell Laboratory at the Royal Botanic Gardens, Kew.

Total DNA was extracted from either fresh (1 g) or silica gel-dried (0.1–0.3 g) leaf material using the modified 2× CTAB procedure of Doyle and Doyle (1987). The DNA was precipitated in cold (-20 C) absolute ethanol, pelleted, and purified through a cesium chloride gradient (1.55 g ml^{-1}). Cesium chloride and other residual salts were subsequently removed by dialysis.

The ITS region was amplified using the methods and primers (ITS5 and ITS4) described by Baldwin (1992). For the purposes of this study, "ITS" includes the 5.8S gene as well as the two non-coding spacer regions ITS1 and ITS2. The thermal cycling protocol consisted of an initial premelt at 97 C for 1 min, followed by 40 cycles that each comprised 1 min denaturation at 97 C, 1 min annealing at 48 C, and 3 min chain extension at 72 C. The final stage of the protocol was a 7 min extension at 72 C.

The *matK* amplification and sequencing primers were developed at the Royal Botanic Gardens,

TABLE 3. Plant materials used in this study. * indicates material used for chromosome counts.

	Location	Voucher no.
Tribe **Maxillarieae** Pfitzer		
Subtribe **Lycastinae** Schltr.		
1. *Bifrenaria* alliance		
Bifrenaria harrisoniae (Hook.) Rchb.f.	K spirit	*Chase O-95* (ITS)
B. sp.	FLAS	*Whitten 93197* (*matK*)
Rudolfiella aurantiaca (Lindl.) Hoehne	K spirit	*Chase O-178*
Xylobium aff. *colleyi* (Batem. ex Lindl.) Rolfe	FLAS	*Hills F1662*
Xylobium pallidiflorum (Hook.) Nichols.	FLAS	*Whitten 90241*
2. *Lycaste* alliance		
Anguloa Ruiz & Pav.		
A. brevilabris Rolfe	K	*Oakeley D10*
A. eburnea Williams	K	*Oakeley A52*
A. cliftonii Rolfe	K spirit	*Chase O-88*
A. cliftonii Rolfe*	K spirit	*Oakeley 70000.008*
A. clowesii Lindl.	K	*Oakeley A49*
A. clowesii Lindl.*	K spirit	*Oakeley 63933*
A. eburnea Williams	K	*Oakeley A52*
A. virginalis Linden*	K spirit	*Oakeley 63934*
Lycaste Lindl.		
L. sect. *Fimbriatae* Fowlie		
L. barringtonii (Smith) Lindl.	K	*Oakeley D18*
L. cinnabarina Lindl.	K	*Oakeley G15*
L. dyeriana Sander ex Rolfe	K	*Oakeley D28*
L. fragrans Oakeley	K	*Oakeley D41*
L. fulvescens Hook.	K	*Oakeley D47*
L. gigantea Lindl.	K	*Oakeley D63*
L. lanipes Lindl.	K	*Oakeley D36/G38*
L. locusta Rchb.f.	K	*Oakeley A25*
L. costata Lindl.	K	*Oakeley A62/A67*
L. reichenbachii Gireoud ex Rchb.f.	K	*Oakeley 10.95*
L. sect. *Deciduosae* Fowlie		
L. aromatica (Graham ex Hook.) Lindl.	K spirit	*Oakeley 1957*
L. bradeorum Schltr.	K	*Oakeley B9*
L. brevispatha (Kl.) Lindl.	K	*Oakeley A5*
L. campbellii C. Schweinf.	K spirit	*Severin K6*
L. candida Lindl.	K spirit	*Oakeley 63698*
L. cobani Oakeley	K	*Oakeley D7*
L. cochleata Lindl.	K	*Oakeley D27*
L. consobrina Rchb.f.	K spirit	*A. Ryan 67*
L. crinita Lindl.	K spirit	*A. Ryan 82*
L. cruenta Lindl.	K	*Oakeley 17.11.95*
L. deppei (Lodd.) Lindl.	K living cult.	*HMKN 049.85 01092*
L. edinensis Hort.	K	*Oakeley A58*
L. lasioglossa Rchb.f.	K spirit	*Mason 73*
L. macrobulbon (Hook.) Lindl.	K	*Oakeley D74*
L. suaveolens Summerh.	K	*Oakeley A60*
L. tricolor (Klotzsch) Rchb.f.	K	*Oakeley H1*
L. sect. *Lycaste* (syn. sect. *Macrophyllae* Fowlie)		
L. dowiana Endres & Rchb.f.	K spirit	*M. Tibbs s.n.*
L. leucantha Klotzsch	K	*Oakeley 15.10.95*
L. macrophylla subsp. *desboisiana* Fowlie	K	*Oakeley A8*
L. macrophylla subsp. *xanthocheila* Fowlie	K	*Oakeley H70*
L. xytriophora Linden & Rchb.f.	K spirit	*RBG Kew 1973.13936*
L. sect. *Longisepalae* Fowlie		
L. schilleriana Rchb.f.	K	*Oakeley D80/H75*
3. *Neomoorea* Rolfe		
Neomoorea wallisii (Rchb.f.) Schltr.	K spirit	*Chase O-503*
Subtribe **Maxillariinae** Benth.		
Cryptocentrum sp.	K spirit	*Chase O-108* (*matK*)
C. peruvianum (Cogn.) C.Schweinf.	K spirit	*Chase O-115* (ITS)

TABLE 3. Continued.

	Location	Voucher no.
Maxillaria umbratilis L.O.Williams	SEL	*SEL 1995-0397*
M. violaceopunctata Rchb.f.	SEL	*SEL 1981-2139*
Subtribe **Zygopetalinae** Schltr.		
Batemannia colleyi Lindl.	K spirit	*Chase O-177*
Koellensteinia graminea Rchb.f.	K spirit	*Chase O-501*
Zygopetalum intermedium Lindl.	K spirit	*Chase O-160*
Dichaea muricata (Sw.) Lindl.	K spirit	*Chase O-93* (*matK*)
D. riopalenquensis Dodson	K spirit	*Chase O-114* (ITS)

Kew, for use with Epidendroideae; their sequences are presented in Table 4. Two amplification products were prepared for each taxon: the first using the −19F and 556R primers, the second 458F and either 1592R or *trnK*2R. Thermal cycling consisted of an initial premelt at 97 C, followed by 30 cycles under the following conditions: 1 min denaturation at 94 C, 0.75 min annealing at 52 C, and 2.5 min chain extension at 72 C with a segment time increase of 8 sec/cycle. The final extension was at 72 C for 7 min.

Double-stranded DNA products were purified by solid-phase extraction through Wizard PCR minicolumns (Promega) using the manufacturer's protocols. DNA sequences were determined using an ABI 377 automated sequencer (PE Applied Biosystems, Inc.) and standard dye-terminator chemistry. Sequence Navigator software (PE Applied Biosystems, Inc.) was used to edit the sequences, and the complementary strands were assembled using AutoAssembler (PE Applied Biosystems, Inc.).

Sequences were aligned using Clustal W (Thompson, Higgins, and Gibson, 1994) and by eye. Four genera from the Zygopetalinae (*Batemannia* Lindl., *Dichaea* Schltr., *Koellensteinia* Rchb.f., and *Zygopetalum* Hook.) were specified as the outgroup, based on the results of Whitten et al. (in press). Heuristic searches were performed using PAUP version 4.0b2a (Swofford, 1999). Gaps in the matrix were coded as missing values. Each matrix was subjected to 1000 random addition replicates, with tree bisection-reconnection (TBR) as the branch swapping algorithm under the Fitch (1971) criterion of unordered states and equal weights. All trees generated by this method were swapped to completion or until memory capacity (6000 trees) was exceeded. Successive approximations weighting (SW) using a base weight of 1000 (Farris, 1969) was applied to the resultant trees, and rounds of reweighting and analysis were continued until the tree length remained constant for two successive rounds. Internal support was assessed with 1000 bootstrap replicates (Felsenstein, 1985) with simple stepwise addition, the nearest-neighbor interchanges (NNI) algorithm, and SW weights applied.

TABLE 4. *matK* primer sequences (* From Johnson and Soltis, 1995).

−19F	CGT TCT CAT ATT GCA CTA TG
163F	AGT TTA GTR CTT GTG AAA CG
458F	CTA CTA ATA CCC YAT CCC ATC
556R	GAA GRA ACA TCT TTK ATC CA
1155F	TTC ACT TTT GGT YTC ACC CT
1592R	TCA TGA ATG ATC CAC CGA
*trnK*2R	AAC TAG TCG GAT GGAATA G*

RESULTS

Cytology—Chromosome counts of $2n = 40$ were obtained for three species of *Anguloa*: *A. cliftonii* and *A. clowesii* from *A.* sect. *Guloanga* and *A. virginalis,* from *A.* sect. *Anguloa.* These agree with the counts for *Lycaste* sect. *Deciduosae* and *L.* sect. *Lycaste* (Aoyama and Karasawa, 1988) as shown in Table 3. Chromosome numbers in *L.* sect. *Fimbriatae* range from $2n = 44$ to $2n = 50$ (Table 2).

Analysis of ITS—Sequences were obtained from 46 taxa. The length of the aligned matrix was 660 base pairs, of which 312 were variable and 123 potentially parsimony-informative. Analysis yielded more than 6000 equally parsimonious trees (the maximum permitted by available computer memory) with a Fitch length of 515 steps, consistency index (CI) of 0.75 (including autapomorphies), and retention index (RI) of 0.77. Successive weighting again yielded 6000 trees of

weighted length 303536 (Fitch length 515) and both CI and RI of 0.94. One of these SW trees is shown in Fig. 2. Numbers above the branches indicate the number of nucleotide substitutions; those in boldface show percent support from bootstrap replicates. Arrows indicate branches that collapse in the strict consensus of all SW trees.

The tree shows that *Lycaste,* as currently circumscribed, is not monophyletic. Species from *L.* sect. *Deciduosae, L.* sect. *Lycaste,* and *L.* sect. *Longisepalae* form a single well supported clade. Sister to that, again with strong support, are the *Anguloa* and *L.* sect. *Fimbriatae* species, including *L. dyeriana.* Within this clade the monophyly of *Anguloa* is well supported; and that of *L.* sect. *Fimbriatae* is not supported. *Neomoorea* is strongly supported as the sister group to *Lycaste/Anguloa.*

ITS sequences were obtained from three of the five genera that Dressler (1993) grouped as the *Bifrenaria* alliance. Of these, *Xylobium pallidiflorum* was placed in a moderately supported clade with genera of Maxillariinae, *Maxillaria* Ruiz & Pav. and *Cryptocentrum* Benth. The other two, *Bifrenaria harrisoniae* and *Rudolfiella aurantiaca,* were placed together in a separate clade sister to Maxillariinae. Zygopetalinae were used as an outgroup, as shown in Whitten et al. (in press).

Analysis of matK—Sequences were obtained from 22 taxa to confirm the general patterns revealed by ITS. The aligned matrix contained two indels, three and nine bases long, which were coded as additional characters. There was less variation among sequences than had been found for ITS. Of the 1578 characters included in the analysis, only 160 were variable, of which 60 were potentially parsimony-informative. The search yielded 108 equally parsimonious trees with a Fitch length of 212 steps, CI of 0.80 (including autapomorphies), and RI of 0.74. Successive weighting yielded nine trees of length 145579 (Fitch length 212), CI of 0.95, and RI of 0.93. One of the SW trees is presented in Fig. 3.

Lycaste is again shown to be paraphyletic, with species from *L.* sect. *Fimbriatae* placed in the same clade as *Anguloa.* Support for this topology is much lower than for the ITS analysis, not surprising given the shorter branch lengths. One difference between the two sets of results is the position of *Xylobium,* which *matK* places in the same clade as *Neomoorea,* but bootstrap support for this clade is weak. The position of *Bifrenaria* and *Rudolfiella* is the same as was found for ITS.

Combined analysis—The same 22 taxa as in the *matK* analysis were used here. In three instances outside *Lycaste/Anguloa,* the ITS sequence data was obtained from a different species than that for *matK.* These are labeled on Fig. 4 as *Bifrenaria* sp., *Dichaea* sp., and *Xylobium* sp.

In the combined analysis 2238 characters were included. The Fitch search found 60 equally parsimonious trees with a length of 669 steps, CI of 0.77 (including autapomorphies), and RI of 0.67. Successive weighting yielded six trees, with length of 415059 (Fitch length 669), CI of 0.95, and RI of 0.92. The topology of the SW tree shown in Fig. 4 is in close agreement with that found by analyzing ITS sequence data alone and shows higher levels of support. The single exception is *Xylobium,* which is separate (with a high bootstrap percentage) from both the Maxillariinae genera and *Neomoorea.*

DISCUSSION

These analyses show that neither *Lycaste* nor subtribe Lycastinae as defined by Dressler (1993) is monophyletic. All three data sets provide strong support for the separation of *L.* sect. *Fimbriatae* from the rest of the genus. Further support is provided by the variation in chromosome number from the base number $n = 20$, found in other members of Lycastinae. There are three possible taxonomic solutions. The first would be to include all *Lycaste* and *Anguloa* species within a single genus comprising three sections. According to the rules of priority, this would be called *Anguloa.* A second possibility would be to transfer only the species from *L.* sect. *Fimbriatae* to *Anguloa.* Given the differences in floral morphology, pollination mechanism, and chromosome number that exist between the three clades, neither of these options is completely satisfactory. A third option would be to create a new genus for *L.* sect. *Fimbriatae.* Major morphological features distinguishing such a genus from *Lycaste* and *Anguloa* (Table 1) are predominantly green flowers, falcate lateral sepals, a fimbriate or crenate margin to the mid-lobe of the lip, a lip callus which extends from the column base to the mid-lobe with a bifid apex, and an elongate viscidium. This may be the most practical solution as it would solve the mor-

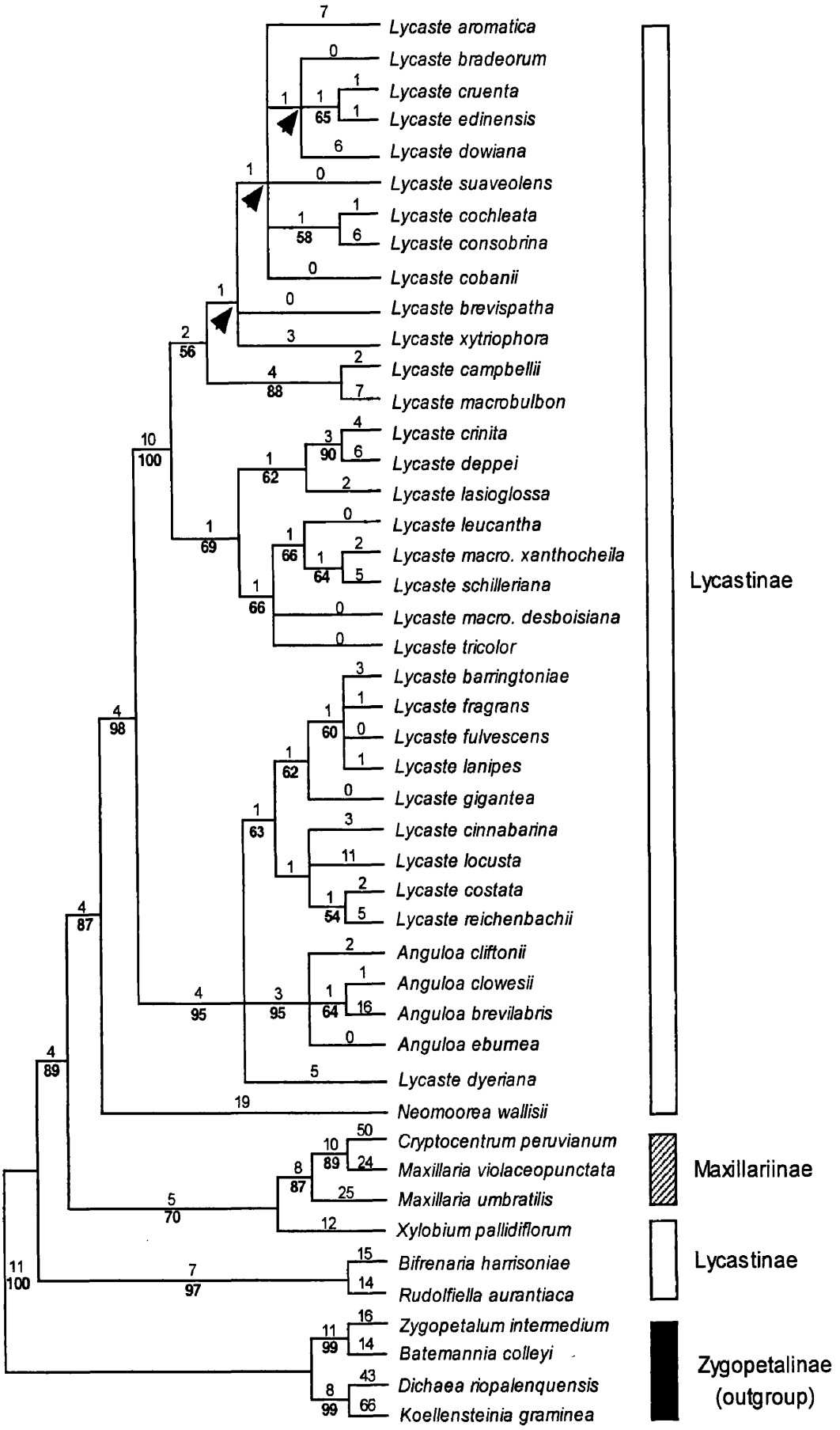

Fig. 2. One of the more than 6000 successively weighted most-parsimonious ITS trees showing cladistic relationships within Lycastinae. Numbers above the branches are the estimated number of substitutions (ACCTRAN optimization); numbers below the branches are bootstrap percentages greater than 50%. Arrowheads indicate groups not found in all 6000 trees.

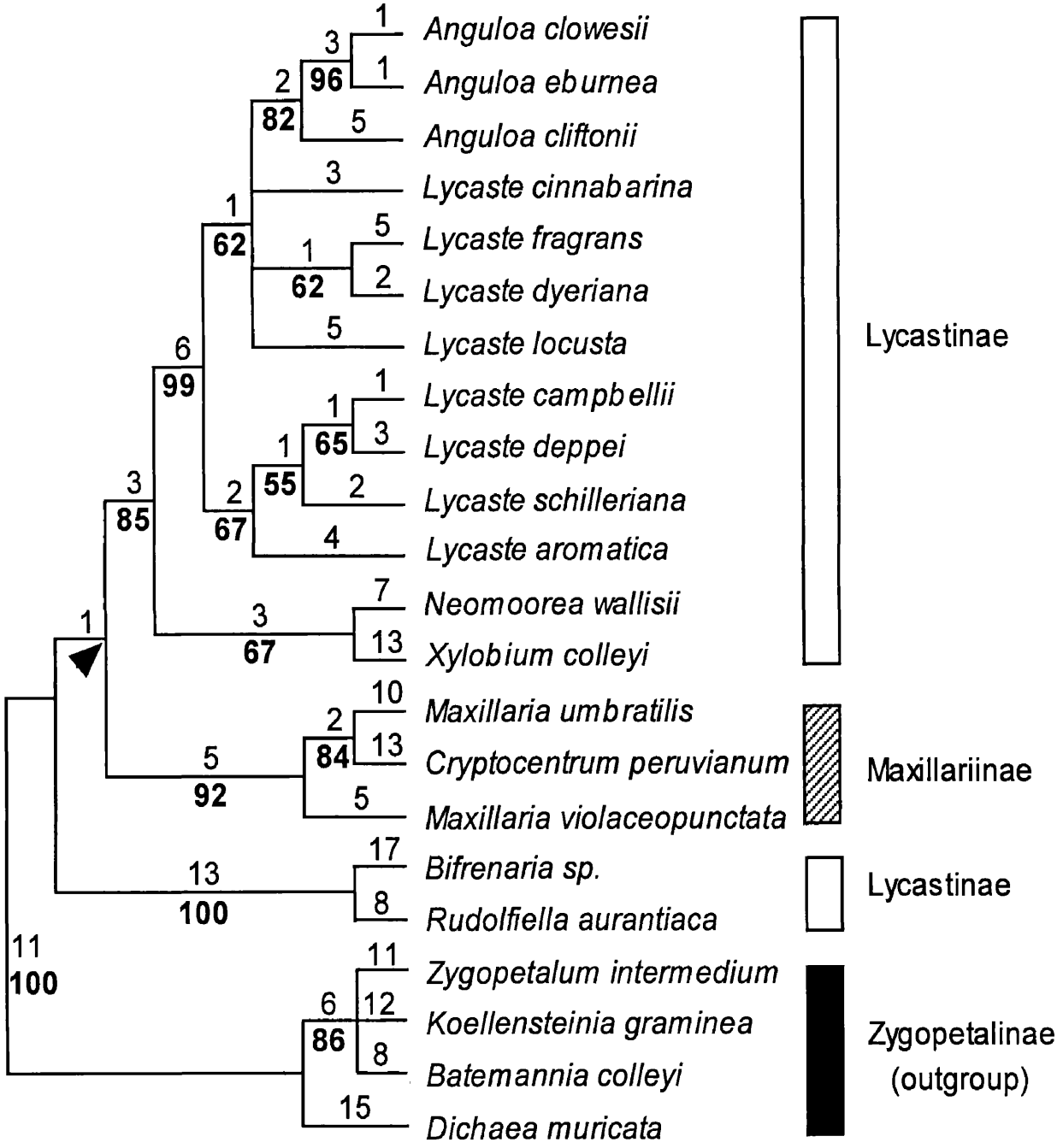

Fig. 3. One of the nine successively weighted most-parsimonious *matK* trees showing relationships within Lycastinae. Numbers above the branches are the estimated number of substitutions (ACCTRAN optimization); numbers below the branches are bootstrap percentages greater than 50%. Arrowheads indicate groups not found in all nine trees.

phological and cytological discrepancies and also retain the name *Lycaste,* which is in widespread horticultural use. Monophyly of such a genus is not currently established but is also not contradicted by either of the individual or the combined results. This last solution is also the most compatible with previous taxonomic schemes as it requires the least number of nomenclatural transfers. We have therefore followed this option (Oakeley and Ryan, in prep.).

The position of *L. dyeriana* remains problematic. All three analyses placed it in the same clade as *Anguloa* and *L.* sect. *Fimbriatae* and not with *Bifrenaria,* as had been suggested by Fowlie (1970). Its position within the clade was not clearly resolved. Given the similarity in both floral morphology and chromosome number to other members of *L.* sect. *Fimbriatae,* at the present time it seems appropriate to keep it within that section.

Neither ITS nor *matK* provided sufficient resolution to refute Fowlie's concept of the other three sections. To answer these questions, com-

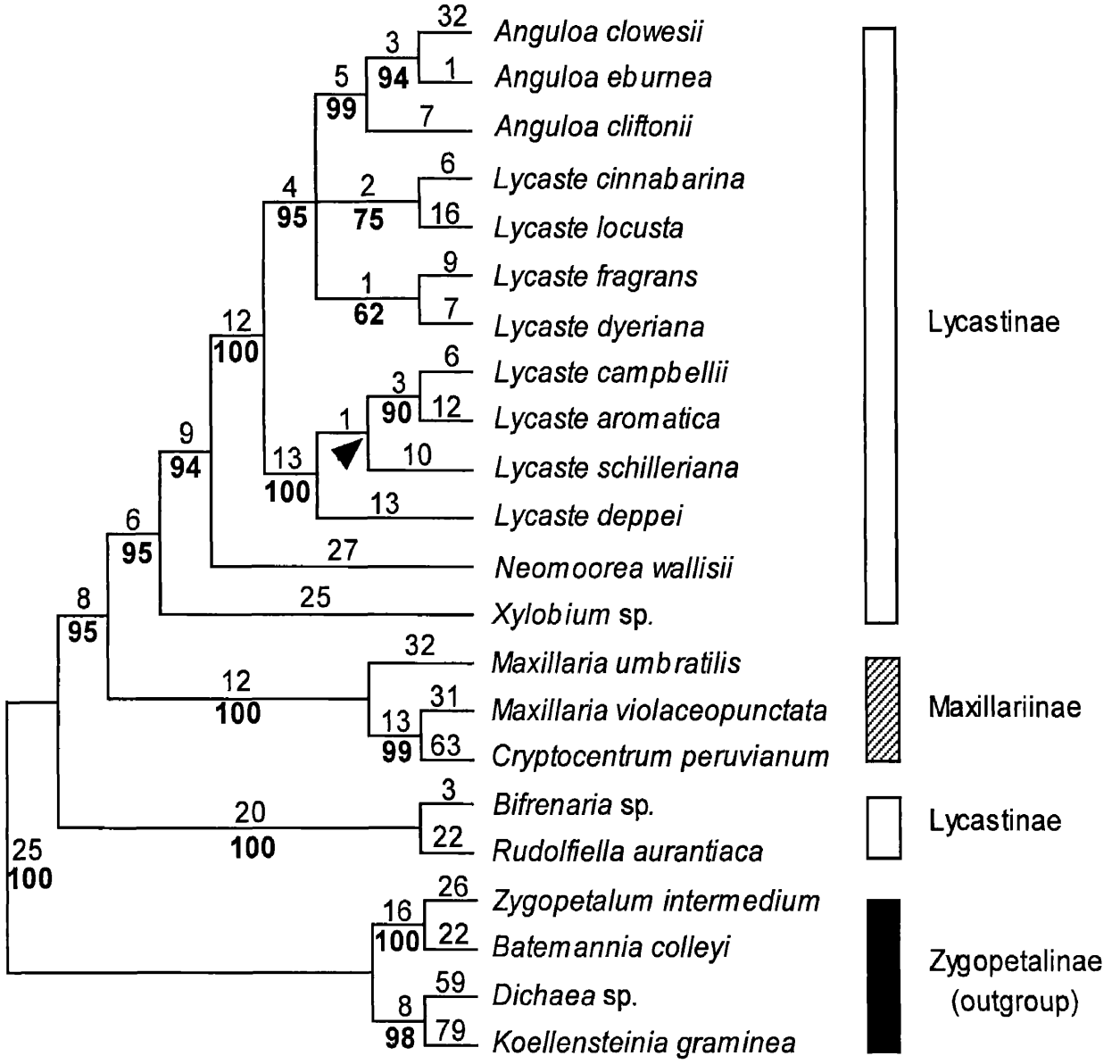

Fig. 4. One of the six successively weighted most-parsimonious combined trees showing relationships within Lycastinae. Numbers above the branches are the estimated number of substitutions (ACCTRAN optimization); numbers below the branches are bootstrap percentages greater than 50%. Arrowhead indicates groups not found in all six trees.

parison of sequences from a faster evolving region of DNA or inclusion of sequence data from other DNA regions is required. Fowlie (1964, 1970) considered some plants within the genus to be subspecies of *L. macrophylla.* The ITS sequences of two of these, *L. macrophylla* subsp. *desboisiana* and *L. macrophylla* subsp. *xanthocheila,* both from Costa Rica, were included in the ITS analysis. The results (Fig. 2) do not place these two subspecies adjacent to each other, indicating that their taxonomic status may need to be reviewed. Given the low bootstrap percentages within the clade, further sampling and more variable characters are needed before such changes are made.

The position of *Neomoorea* as nearest neighbor to *Anguloa* and *Lycaste* agrees with Dressler (1993). Unlike other members of Lycastinae, these three genera all have a single long stipe. The position of *Xylobium* was variable: ITS placed it with Maxillariinae, *matK* with *Neomoorea,* and the combined analysis in a clade by itself, sister to *Neomoorea/Anguloa/Lycaste.* However, the topology of the combined analysis was well supported, and the other two were not.

All three analyses placed *Maxillaria* and *Cryptocentrum* closer to *Lycaste, Anguloa, Neomoorea,* and *Xylobium* than to the two other genera of Lycastinae, *Bifrenaria* and *Rudolfiella.* Dressler (1981) noted the floral resemblance between

Xylobium and *Maxillaria* and thought it represented convergence caused by the pollination system. Whether this issue is best resolved by reinstating subtribe Bifrenariinae Dressler (1979) without *Xylobium* or by expanding Maxillariinae is the subject of a more comprehensive study by Whitten et al. (in press). Based on those results as well as ours, we would favor a broader circumscription of Maxillariinae as a more reasonable solution to the polyphyly of Lycastinae as shown here.

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