Classification by molecules:
What's in it for field botanists?

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ABSTRACT

Following a brief summary of the author's views on the philosophy and practice of plant classification, with particular reference to molecular systematics, the impact of the use of DNA sequence data on the classification of a range of taxa in the British flora is assessed. The degree to which their classification has been affected is discussed under four headings: *No change here, then* (little or no impact); *Welcome back old friends* (changes that represent reversions to classifications that were in use some time ago but had fallen out of favour); *A cautious welcome to new friends* (changes that seem novel at first sight but which on study are readily explicable by previously available data); *Unwelcome newcomers* (changes that seem unavoidable but which are not supported by exomorphic characters). It is concluded that, far from being remote and of interest only to professional molecular biologists, molecular systematics is highly relevant to the study and enjoyment of plants by all botanists, from molecular biologists to plant hunters, who still have much to contribute.

KEYWORDS: Taxonomy, Classification, Molecular systematics, DNA sequencing, APG-III, Paraphyletic groups, British Flora

INTRODUCTION

WHY CLASSIFY

Plants (and animals) have been classified by man from the earliest times. It is a natural and sensible way to commence the study of organisms, because it enables the huge and bewildering range of their form to be comprehended in a logical way, rather than being simply marvelled at, and it provides a framework into which all known organisms can be fitted and their relationships assessed. Uncertainty is introduced when the type of classification to be adopted has to be decided. Since there are many types of classification possible, controversy is inevitable; it has existed from the first attempts at classification until the present day, and doubtless will always be with us. But arguments about the ideal type of classification are pointless unless the

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purpose of the classification is taken into consideration, because no single system of classification is ideal for all situations.

TYPES OF CLASSIFICATION

By and large there are two main categories of classification – artificial and natural, although most systems carry elements of both. Artificial classifications are generally based on one or few characters, and are usually devised for specific purposes. Good examples are the use of wood anatomy by timber technologists, growth-habit and flower-colour by landscape gardeners, and the number of stamens and pistils by Linnaeus. Although such classifications might be ideal for particular situations, they are usually totally inadequate when applied widely. This is because they do not indicate the *relationships* of plants, whether that relationship is defined phenetically (by appearance) or phyletically (in evolutionary terms). For example, some of the plants in Linnaeus's class Diandria (with two stamens) are *Anthoxanthum* (a grass), *Veronica* (speedwell) and *Cypripedium* (an orchid). Close relatives of all three of these belong to quite different classes. In other words, the placement of a taxon in an artificial group does not predict any further characters of that taxon.

In contrast, natural systems group together plants with overall similarity, using a wide range of characters, and provide a high level of predictivity. The statement that a taxon belongs to, say, the Poaceae, Lamiaceae or Fabaceae immediately informs us about many features of that taxon. If we discover a new species of grass, for example, we are able to predict accurately a large number of its characters before we examine the plant in detail. Classifications defining natural groups such as these are known as general purpose classifications, because they are the most useful ones in most situations, and they are considered the best sort to aim for. For a long time predictivity has been considered the ideal yardstick of a good classification (e.g. Stace 1989), and this is still true today (Stuessy 2009). Although other

criteria have been advocated as the best indication of a good natural classification, those views are in a minority and I personally am totally convinced that the best (most useful) classification is the most predictive one.

HOW TO CLASSIFY

The construction of a highly predictive general purpose classification has traditionally been attempted by two different routes: phenetic and phyletic. Both methods may vary from highly subjective to ones constrained by strict mathematical conventions. In the former (phenetic) method the aim is to use a large number of characters and classify together those taxa sharing the greatest number of common features. In the latter (phyletic) approach phylogenetic trends are analysed and the classification is based on the closeness of taxa in their evolutionary pathways. Classifications obtained by the rigorous application of each of these two approaches should be extremely similar, if not identical, because the degree of similarity between taxa is determined genetically by their closeness of evolutionary descent. (Consider, for example, your own family and your next-door neighbour's family). If there are significant differences between the two classifications then for some reason or other one method has (or both methods have) failed us. Early on (e.g. Nineteenth Century) both methods relied on highly subjective assessments, when such 'failures' were commonplace, but increasingly more sophisticated techniques have been applied and subjectivity has been concomitantly reduced; it will never, however, be eradicated. The introduction of many of these newer techniques was heralded by claims that we now had a robust methodology that gave us a classification that closely reflected the true (i.e. phylogenetic) relationships of the taxa. The data being used, however, were always secondary manifestations of the genetic code (e.g. morphology, chemistry, cytology), and inevitably sometimes gave misleading or (when two different sets of data were used) conflicting results. The ability to use the genetic code (sequence of bases in DNA) directly, therefore, was a massive advance when it became available in the 1990s, for at last, in theory at least, we had direct access to the genetic basis of evolution.

MOLECULAR CLASSIFICATIONS

Since we know in detail the mechanism of evolution (mutation) of DNA (the miscoding of the four bases during replication) we can be certain that a phylogeny based on it will truly reflect the course of evolution, and a classification derived from it will be the closest we can ever get to an accurate representation of the relationships (and therefore similarity) of taxa. Because of this we can be confident that molecular classifications based on DNA base sequences will not be bettered in the future, but will endure for centuries to come and become universally adopted. The latest version, known as APG-III, was published in September 2009 (APG 2009).

This is not the place to discuss the methodology of molecular systematics, but it must be admitted that considerable controversy still surrounds some aspects. These particularly involve the methods used to derive the evolutionary pathways of DNA from its base sequences, and the parts (inevitably a tiny fraction of the whole) of DNA that are sequenced. Real concerns exist because sometimes different classifications are obtained from the use of different techniques, or from the analysis of different DNA regions. In a recent analysis (Carlson *et al*. 2009) of Dipsacaceae, for example, *Scabiosa sensu lato* was defined as a monophyletic taxon using evidence from cpDNA data, but polyphyletic using nuclear ITS evidence unless the genus *Sixalix* (including *S. atropurpurea*) is separated. *Coeloglossum* has been found embedded within *Dactylorhiza* following most analyses (e.g. Bateman *et al*. 1997; Pridgeon *et al*. 1997), but it was separated from it by the analysis of Devos *et al*. 2006. Such experiences are, however, rather rare and cannot shake our belief in the philosophy of molecular classification, especially as we largely understand the causes of these so-called incongruences.

There is, however, one major aspect of classification for which DNA base sequences do not provide all the answers. Hybrids, and polyploids derived from them, should in theory carry the DNA sequences of both parents, but this is often not true with regard to the two most commonly utilised region of DNA: chloroplast DNA and nuclear rDNA (the small region of chromosomal DNA that codes the ribosomal RNA, which forms the structure of the cytoplasmic ribosomes, in which the proteins are coded). Chloroplasts are virtually always inherited from the female parent in angiosperms, although the reverse is true in gymnosperms (e.g. Ennos *et al*. 1999), and

rDNA, via a process known as concerted evolution, most often also represents only the female-derived sequence (e. g. Franzke & Mummenhoff 1999; Lihová *et al*. 2004). Hence the phylogenies of hybridogenous plants based on these regions are often in reality phylogenies of the female parents of the taxa being studied, rather than of the taxa themselves, and moreover results from the two regions of DNA are not suitable as checks on each other as they exhibit the same parental directional bias. There are, however, examples of rDNA sequences in a hybrid representing those of the male rather than female parent, and sometimes some populations of the hybrid or polyploid exhibit the male and others the female parent, or the hybrid derivative exhibits a chimaera of both parent types (Franzke & Mummenhoff 1999; Lihová *et al*. 2004 and other examples cited therein). A recent study of *Nymphaea* (Volkova *et al*. 2010) found that the r-DNA of the allopolyploid *N. candida* is constantly that of one of its parents, *N. alba*, although some populations of *N. candida* are derived from *N. alba* as the female parent and others from it as the male parent. Sometimes the hybrid/polyploid may appear in two different places in the resultant cladogram according to its male or female inclination, thus providing valuable clues as to its origin (P. Catálan, pers. comm. 2010). Using this reasoning it seems that the hexaploid *Vulpia myuros* is likely to have evolved from hybrids between the diploid *V. bromoides*/*V. muralis* group and the tetraploid *V. ciliata*/*Psilurus* group. Similarly the tetraploid *V. fasciculata* might have arisen from hybridisation between the diploid *V. membranacea*/*V. fontqueriana* group and the diploids in *Vulpia* section *Loretia* (Torrecilla *et al*. 2004; Stace 2005).

It is not known to what *extent* this is a problem in molecular classification at the lower levels of the hierarchy, but the classification of polyploids at the species level using molecular data clearly needs to be approached with great caution. Ideally polyploids should be omitted from the initial analyses, to be added later when the relationships of the diploids have been clarified. Probably, however, these problems are of negligible significance at the higher levels of the hierarchy.

A further consequence of the use of only a very small proportion of the DNA in deducing the phylogeny is that in some closely related taxa there might actually be no differences present in those regions. Hence the absence of any differences does not necessarily mean that the total sequences are identical. For example, apparently no differences in DNA sequences have been detected between *Platanthera chlorantha* and *P. bifolia* (Bateman & Sexton 2008) or between *Gentianella amarella* and *G. anglica* (Winfield & Parker 2000). Experienced taxonomists, especially field botanists, are well aware that in each of these two examples two separate taxa are involved, and therefore that molecular differences must exist.

CLASSIFICATIONS FOR FLORAS

Although there is consensus regarding the best classification to be adopted for general purposes, a decision on which system should be followed in Floras, or in other floristic works arranged in list form, is another matter. If the most closely related plants are required to appear close together then obviously the current taxonomic classification should be utilised. But some authors have chosen other arrangements, of which alphabetical is commonest, as for example in *Vascular Plants of the Pacific Northwest* (Hitchcock *et al*. 1955–1969) and *Flora of Canada* (Scoggan 1978–1979). I find it difficult to see any real advantage in an alphabetical arrangement, because a full index or cross-referencing in the appropriate place in the text is still necessary due to the existence of synonyms, and future name-changes often necessitate radical reordering. The user will not know whether watercress is under *Nasturtium* or *Rorippa*, so both need to be listed. And surely it is better if, say, the grasses are all together in a generic list. In Britain, *Scarce Plants in Britain* (Stewart *et al*. 1994) is alphabetically arranged; there is, fortunately, a complete index, but *Anacamptis morio* and *Neotinea ustulata* do not appear in it and as time goes on more and more standard names will be found lacking. The text of the *RHS Gardeners' Encyclopedia of Plants and Flowers* (Brickell 1989) is alphabetical, but the coloured illustrations are arranged according to plant habit, flowering time and flower colour, etc., for ease of identification. A dual approach was also used in *The Pocket Guide to Wild Flowers* (McClintock & Fitter 1956), the text here being in systematic order. These are special solutions for particular objectives, but for any technical Flora I consider a systematic order mandatory.

In the past it has often been difficult to decide which of several currently available systems of classification should be adopted in a

Flora. Sometimes, e.g. *Flora Europaea* (Tutin *et al.* 1964–1980), *Flora of Turkey* (Davis 1965–1985) and *Flora Nordica* (Jonsell 2000–), rather old and outdated systems were deemed the most suitable, and in other cases, e.g. *Flora of the British Isles* (Clapham *et al.* 1952), an essentially new sequence was concocted. Most authors consider that a Flora is not the ideal place to adopt a new or very recently devised classification, for fear of its being short-lived or soon greatly modified. A well-known and well-tested system is far better. For that reason it could be argued that APG is too new and unfamiliar to be suitable for use in an identification manual, and certainly most new Floras being produced, at least in Europe, still do not adopt it. However, as pointed out above, APG is certain to endure for the foreseeable future; in this respect it is unique, and the more it is promoted the sooner it will become familiar and its universal logic and usefulness demonstrated. If it is accepted that a Flora should be arranged systematically then the adoption of the APG system is inevitable. Diggs & Lipscomb (2002) and Stace (2009) have argued for the adoption by Flora-writers of a pragmatic compromise between the extremes of a strict cladist interpretation and the use of only phenotypically observable characters.

A further weighty consideration operating when adapting a phylogenetic classification for use in a floristic work is how to express the latter as a linear sequence. A phylogeny is obtained from DNA sequence data in the form of a branching pathway, usually each branch-point giving rise to only two (or very few) sub-branches, not as a main axis with side-branches. In converting this form to a linear sequence either of the two sub-branches at each branch-point may with equal justification be placed before the other. Usually the smaller or shorter sub-branch is placed first, or (allowing for much subjectivity) the one with more obviously highly evolved taxa is placed second; often these two informal criteria coincide. In the LAPG-III system (LAPG 2009) the monocots precede all but a few of the most 'primitive' dicots, reversing the common situation. However, the opposite is equally faithful to the DNA data, and for that reason I as well as others (e.g. Heywood *et al.* 2007, Hawthorne & Hughes 2008) prefer to place the monocots (ending with the grasses) after the dicots, retaining the more familiar sequence.

When adopting the APG system there are further legitimate possibilities of deviating from the classifications published by APG (2009) and LAPG (2009). Two will be briefly mentioned. Firstly there is the vexed question of whether paraphyletic groups should be recognised as taxa. Paraphyletic groups are those whose members are all derived from a common ancestor, but unlike monophyletic groups they do not include **all** of the derivatives of that ancestor. Arguments for and against still rage, and will for the foreseeable future. Although it is probably true that the majority view is against the recognition of paraphyletic taxa, they are accepted by many. I subscribe to this latter view because, as stated previously, I consider that the over-riding criterion of a good classification is a high degree of predictivity. There can be no doubt that a paraphyletic Araceae (with Lemnaceae excluded), for example, is a more highly predictive taxon than a monophyletic Araceae (with Lemnaceae included). The duckweeds differ from the rest of the Araceae in morphology, chromosome number, ecology, physiology and reproductive behaviour, and I believe that there is no over-riding reason why they should not be separated at the family level.

Secondly, I do not believe that data from DNA sequences should be used to deduce the **absolute** level (rank) at which taxa should be recognised, only their **relative** levels. For example, in APG-III the Sparganiaceae and Typhaceae are amalgamated (under the latter), but since only the two genera *Sparganium* and *Typha* are involved the recognition of both families is equally in accordance with the data. Similarly, whether or not *Orchis* should be drawn wide enough to include its sister group *Aceras* is largely a matter of preference (Bateman 2006; Bateman *et al.* 1997; Pridgeon *et al.* 1997). All our centuries of experience in taxonomic research has shown that at a particular taxonomic rank any character is capable of showing wide variation in the degree of divergence of its character-states in different taxonomic groups. For example ovary position (superior, inferior, etc.) is frequently constant at the family level (e.g. Ranunculaceae), sometimes at the tribal or generic level (e.g. Rosaceae), and rarely not above the sectional or species level (e.g. *Saxifraga*). The fact that such a minuscule part of the total DNA sequences of related taxa can be compared emphasises the inadvisability of using sequence data as an **absolute** measure of relationship.

RELEVANCE OF MOLECULAR CLASSIFICATIONS TO
THE FIELD BOTANIST

GENERAL CONSIDERATIONS

There is undoubtedly a considerable resistance to the adoption of new classifications, part of a natural tendency to conservatism in *Homo sapiens*. Most people prefer the familiar to the novel, and this particularly applies to classifications because they summarise the patterns of variation and it is difficult to adopt and learn a different system. Sometimes it is claimed in mitigation that the new system is more difficult to absorb or remember, or is less successful at interpreting the plant relationships. These are, however, hollow excuses; in reality the reluctance to change is due not to 'difficulty' or inadequacy, but simply to unfamiliarity (or even laziness!). If the new system is a good (highly predictive) one, better than the replaced one, perseverance with it will soon reveal its advantages. There is also the point that the new generation of botanists that is constantly being recruited on a rolling basis does not have knowledge or experience of the older systems, and therefore it lacks the reticence of the older generations. The latter cannot afford to get left behind.

Two important yet contrary points concerning the molecular APG classification are relevant here. Firstly, there can be no doubt it that will prove more highly predictive than any previous system, and that it will endure for the foreseeable future. Secondly, it has to be admitted that some of the close relationships indicated are counter-intuitive and would not have been predicted by other means. There is naturally a reluctance to accept cryptic characters over visible ones, and hence to group plants of very different appearance (e.g. *Callitriche*, *Plantago*, *Linaria*) together, or to separate very similar ones (e.g. *Cormus*, *Sorbus*).

The main purpose of this paper is to address the above conundrum. It seeks to demonstrate that the perceived negative features of the APG system are in fact mostly spurious, and are in any case far outnumbered by overtly positive aspects. In fact the apparent anomalies can be used didactically to demonstrate the value of the APG classification, and it is important that field botanists along with all other taxonomists be exposed to the new system as soon as possible. Older systems, albeit as diverse as those of Linnaeus, Bentham & Hooker, Engler,

Hutchinson or Cronquist, remain instructive, interesting and eminently worthy of study, but they are essentially extinct fossilised forerunners of the new molecular classification. It is simply not the case that the classification adopted by botanists is unimportant; use of the APG system will prove beneficial to all users, even to the extent of increasing the enjoyment of discovering plants in the field. The following four sections are intended to address these points, using the whole range of situations with which the APG system confronts the (often unsuspecting) botanist.

1. NO CHANGE HERE, THEN

It must be emphasised that ***most*** aspects of the main classifications to which British botanists have been exposed in the past half-century (e.g. Clapham *et al*. 1952; Tutin *et al*. 1964–1980; Cronquist 1981) are equally evident in the APG system at family, genus and species levels. This is testament to the powers of deduction that taxonomists have made from detailed studies over three centuries of many aspects of the variation of plants. Also demonstrated is the fact that the lower the level of taxonomic rank (especially below the genus level) the fewer changes to the classification are made by the APG system.

Large well-known families such as Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Lamiaceae, Orchidaceae and Poaceae are identically or almost identically defined using molecular and phenetic data. Given the distinctive features of these families, making them instantly recognisable, it would be most surprising if this were not so, and it would justifiably shake our confidence in molecular data. Even some less easily defined families, in which one might have feared disruption using molecular data, remain unaltered. To illustrate the morphological diversity of just three families, in *New Flora of the British Isles* (Stace 2010) Ranunculaceae (note the range in perianth organisation and fruit type) appear 11 times in the family keys, Saxifragaceae (wide range in flower structure, especially ovary position) appear 12 times, and Rosaceae (huge variation in all aspects of flower and fruit morphology) 17 times. Yet, apart from the exclusion of *Parnassia* from the Saxifragaceae, all three remain with the same familiar circumscriptions.

More surprisingly, there are some pairs of families whose distinction in the past has often been debated, yet their traditional delimitations

TABLE 1. FRAGMENTATION OF THE LILIACEAE *SENSU LATO*

ALISMATALES
      Tofieldiaceae (*Tofieldia*)
      (with 12 other families)

DIOSCOREALES
      Nartheciaceae (*Narthecium*)
      (with Dioscoreaceae)

LILIALES
      Melanthiaceae (*Paris*)
      Alstroemeriaceae (*Alstroemeria*)
      Colchicaceae (*Colchicum*)
      Liliaceae *sensu stricto* (5 genera; *Gagea*, incl. *Lloydia*, native)

ASPARAGALES
      Xanthorrhoeaceae (5 genera; *Simethis* native)
      Alliaceae (13 genera; *Allium*, *Leucojum* & *Narcissus* native)
      Asparagaceae (14 genera, 9 native, incl. Agavaceae)
      (with Orchidaceae & Iridaceae)

are vindicated by molecular methods. A good example is Lamiaceae/Verbenaceae. Another is Apiaceae/Araliaceae. In the latter the traditional distinction is maintained apart from the position of *Hydrocotyle*, which should be transferred from the former to the latter family (Plunkett 2001). An alternative, surely preferable, solution is to separate the Hydrocotylaceae as a third family. Another would be to amalgamate the two families. All three choices are equally in accordance with the molecular data.

2. WELCOME BACK OLD FRIENDS

One of the most interesting aspects of the new molecular classification comes from an analysis of the apparently new relationships that it proposes, especially as upon inspection many of these turn out to be not so novel after all. I wish to cite several examples. The first two concern the two families that have been by far the most radically transformed by molecular data, Liliaceae and Scrophulariaceae.

Liliaceae

Our representatives of the Liliaceae *sensu* Cronquist (1981) have not only been divided into a minimum of nine families (some of which could be further split), but these families are dispersed across four different orders in which they are often linked with other families that were not formerly in the Liliaceae (Table 1). Some of the new arrangements were probably not predictable, e.g. *Tofieldia* and *Narthecium* fall into different families in different orders, but in fact the rudiments of this new

classification were foreseen in the pioneering work of Rolf Dahlgren (e.g. Dahlgren 1980, 1983), who studied a wide range of characters and classified from first principles without regard to previous classifications, which therefore did not influence him. This new system of lilioid classification has been built up over the past 30 years or so in several publications (e.g. Dahlgren & Clifford 1982, Dahlgren *et al*. 1985), preparing us for the radical changes now seen in the APG classification. In our flora the Liliaceae *sensu stricto* contain only three native species, now all in the genus *Gagea*. These are difficult to distinguish morphologically as a group from the five bulb-bearing genera of Asparagaceae subfamily Scilloideae, but blue flowers are ubiquitous in the latter apart from *Ornithogalum*, and absent in the former.

Scrophulariaceae

The traditional Scrophulariaceae are now represented in our flora by five families, Scrophulariaceae *sensu stricto* containing only three native genera (Table 2). In addition the semi-parasitic genera (*Rhinanthus* etc.) are removed to the Orobanchaceae, and Buddlejaceae are included in the new Scrophulariaceae. Moreover, in the APG system three other very diverse families are subsumed into one of the five Scrophulariaceous families: Plantaginaceae, Hippuridaceae and Callitrichaceae are grouped with eleven of our genera including *Veronica* and *Linaria* in a family variously known as Veronicaeae or Plantaginaceae. An alternative treatment would

TABLE 2. REDISTRIBUTION OF THE SCROPHULARIACEAE *SENSU LATO* (LAMIALES)

CALCEOLARIACEAE
>   *Calceolaria*

*VERONICACEAE
>   *Digitalis*, *Erinus*, *Veronica* (incl. *Hebe*), *Sibthorpia*, plus Antirrhineae

*PLANTAGINACEAE
>   *Plantago* & *Littorella*

*HIPPURIDACEAE
>   *Hippuris*

*CALLITRICHACEAE
>   *Callitriche*

SCROPHULARIACEAE *sensu stricto*
>   *Verbascum*, *Scrophularia*, *Phygelius*, *Sutera* ('Bacopa'), *Limosella*, plus Buddlejaceae (*Buddleja*)

PHRYMACEAE
>   *Mimulus*

PAULOWNIACEAE
>   *Paulownia*

OROBANCHACEAE
>   Traditional Orobanchaceae, plus semi-parasitic Scrophulariaceae

* or these four amalgamated

continue to recognise the three very distinctive families, leaving a paraphyletic Veronicaceae. It is instructive to recognise, however, that several aspects of these radical realignments had been foreshadowed by earlier work. For example, the closeness of *Buddleja* to Scrophulariaceae *sensu stricto* in terms of their secondary metabolites was documented by Jensen (1992) and others, and the floral and embryological similarities of *Lathraea* and *Orobanche* and the semi-parasitic Scrophulariaceae have often been noted (e.g. Kuijt 1969). Several Coleoptera and Lepidoptera are known to use only *Plantago* and *Linaria* as their foodplants (Drummond 1957; Allen 1960, 1961), presumably due to chemical similarities, which led Airy Shaw (1958) to comment that he had long considered their respective families to be more closely related than current classifications indicated. How right he was!

Pteridophytes
The traditional division of the pteridophytes into four phyla (psilophytes, lycophytes, calamophytes, filicophytes) has been transformed by the discovery that the lycophytes, not the psilophytes, were the first group to be differentiated from the rest, which are together known informally as monilophytes (Smith *et al*. 2006). The Nineteenth Century morphologists had discovered that in the true ferns there are two major types of sporangia: those with thick walls (eusporangiate ferns) and those with thin walls (leptosporangiate ferns); this character is correlated with others (e.g. subterranean mycorrhizal versus surface green gametophytes respectively) and was used to define the major division of the true ferns right up to the middle of the Twentieth Century (e.g. Smith 1938). If this character is extended to the rest of the monilophytes, the psilophytes are seen to be eusporangiate and the calamophytes (horsetails) are leptosporangiate. In fact molecular data have demonstrated that the first division of the monilophytes was into eusporangiates (psilophytes and eusporangiate true ferns) and leptosporangiates (calamophytes and leptosporangiate true ferns) (Fig. 1). Hence the horsetails are more closely related to the vast majority of the true ferns than are the eusporangiate ferns (in our flora just the Ophioglossaceae). This gives us an unfamiliar sequence in pteridophyte classification, but one which agrees with the strong emphasis on the eusporangiate/leptosporangiate distinction formerly held by pteridologists but somewhat relegated in more recent times.

Primitive angiosperms
The revelation that angiosperms are not simply separable into dicotyledons and mono-cotyledons represents a major departure from
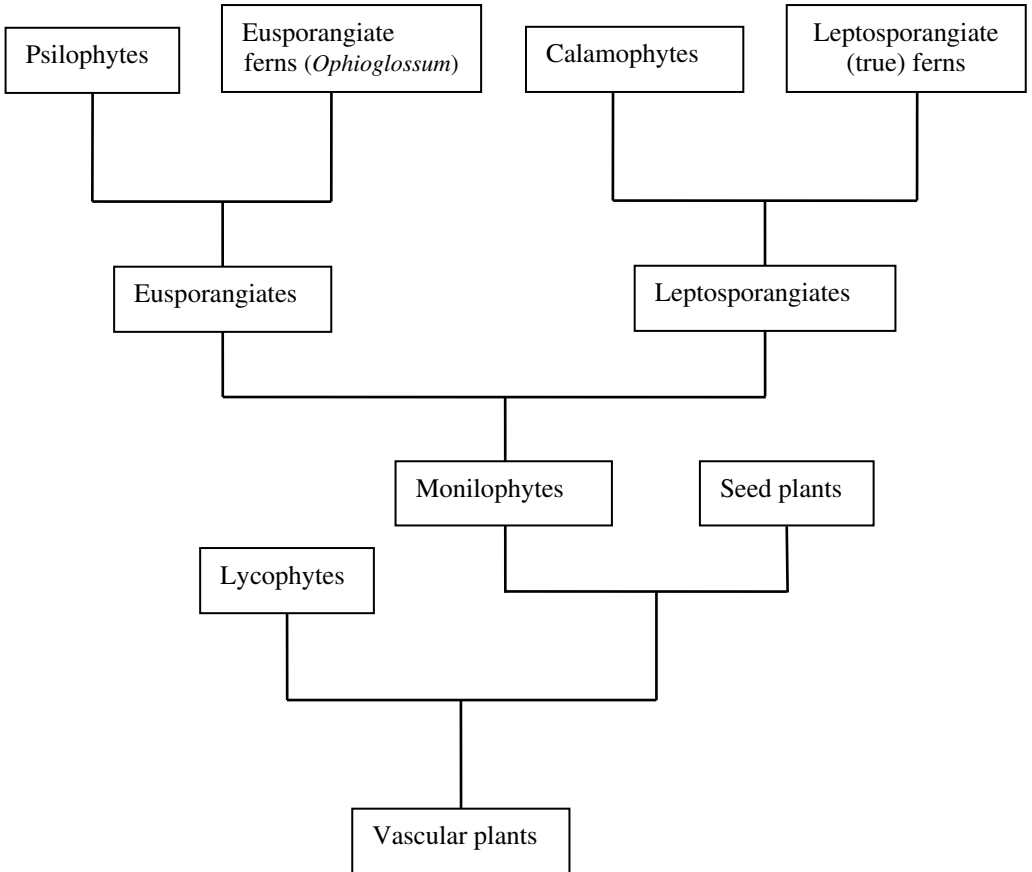
FIGURE 1. Cladogram of relationships of the major groups of pteridophytes, simplified and adapted from Smith *et al*. (2006). Based on DNA sequences from several separate studies.

most previous classifications. Before the division of the angiosperms into dicots and monocots a number of primitive families split off (Fig. 2). There are five such families in our flora, the Nymphaeaceae being the only native one; they can be usefully known as pre-dicots. But this is actually not a totally novel concept. Many early workers commented on the monocot-like features of the Nymphaeaceae and allies (e.g. vascular cambium, pollen-grain and root-hair character-states); this is well discussed and referenced by Dahlgren (1983). The pre-dicots are largely those families placed by Cronquist (1981) at the start of the dicotyledons, before the Ranunculaceae. The Ceratophyllaceae were at first placed by the APG system in the pre-dicots, but are now thought to be the most primitive (i.e. earliest divergent) true dicotyledon family.

Sapindaceae

The amalgamation of the extremely different Hippocastanaceae (*Aesculus*) and Aceraceae (*Acer*) with the tropical family Sapindaceae, whose only representative in our flora is the introduced tree *Koelreuteria*, which has alternate pinnate leaves and strongly inflated capsules, seems at first like the joining of disparate entities. However, a wide look at the former Sapindaceae *sensu stricto* shows a considerable range of structure, including, for example, the presence in the Malaysian *Atalaya* of fruits extremely like those of *Acer* (Heywood *et al*. 2007). Flowers with five petals and eight stamens are characteristic for all three former families. Moreover, many old works (e.g. Hooker 1884, Bonnier 1917, Fiori 1923–1929) recognised the Sapindaceae *sensu lato*, so that the APG classification is not brand new but a re-adoption of a much older idea.
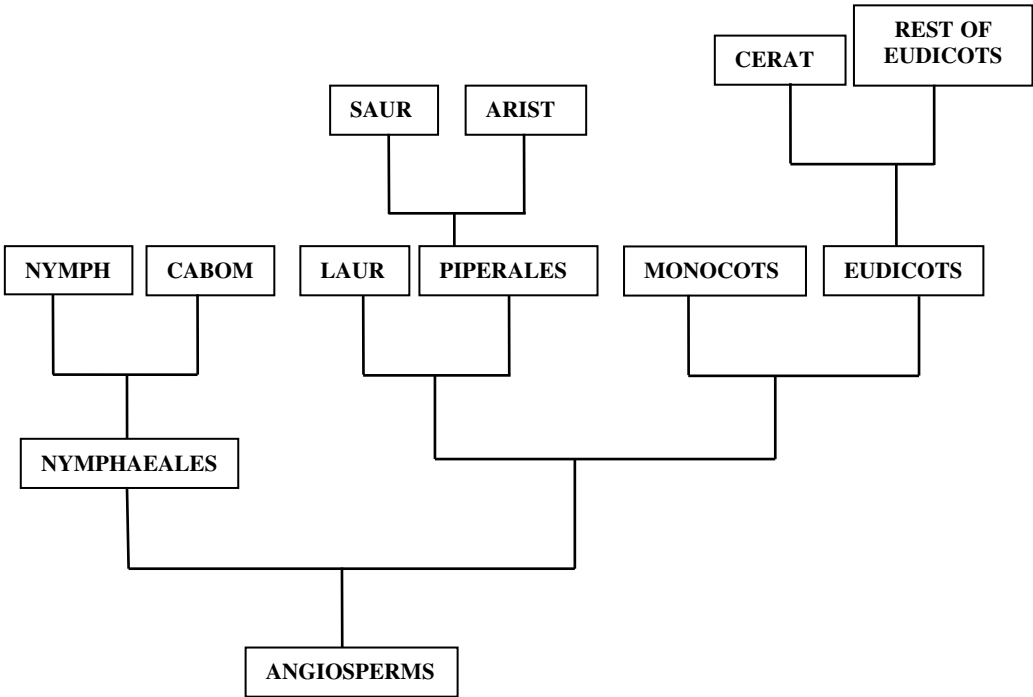
FIGURE 2. Cladogram of relationships of the major groups of angiosperms as represented in our flora, simplified and adapted from APG (2009). Based on DNA sequences from many separate studies.
Abbreviated family names: NYMPH = Nymphaeaceae; CABOM = Cabombaceae; LAUR = Lauraceae; SAUR = Saururaceae; ARIST = Aristolochiaceae; CERAT = Ceratophyllaceae.

Helictotrichon

The genera *Avenula* and the Australian *Amphibromus* have to be split from *Helictotrichon*, but this is actually only a reversion to former opinions. *Amphibromus* (including our *A. neesii*) was traditionally a separate genus, but was amalgamated with *Helictotrichon* by Clayton & Renvoize (1986) in *Genera Graminum*, a work that in general lumps rather than splits wherever possible. *Avenula* (containing our two native species) has a chequered history, being recognised or not and with a range of conflicting diagnoses, but was established in the current sense by Holub (1962, as *Avenochloa*; 1980). It is primarily separated by aspects of root and leaf epidermis anatomy. Despite these cryptic characters, which led to many taxonomists not recognising the split, molecular data clearly vindicate Holub's conclusions. Hence we no longer have any *Helictotrichon* in our flora.

Three resurrected genera

Here are three more examples of 'new' classifications resulting from molecular systematics

actually being cases of reversion to older thinking.

Molecular data indicate that the genus *Apium* should be divided into *Apium sensu stricto* (only *A. graveolens* in our flora) and *Helosciadium* (Spalik *et al*. 2009). Although the British floras of the Nineteenth Century treated *Apium sensu lato*, at the start of the Twentieth Century the two segregates were recognised, e.g. by Riddelsdell (1906, 1917), and this continued right up to Hutchinson (1948); it was Clapham *et al*. (1952) who re-amalgamated them.

Watercress was placed in the genus *Nasturtium* by Aiton (1812) and remained there until it was amalgamated with *Rorippa* by Hayek (1905), and this was almost universally adopted. However, DNA sequences show that *Nasturtium* is closer to *Cardamine* than to *Rorippa sensu stricto* (Fig. 3), and the re-segregation of *Nasturtium* and *Rorippa* is now accepted (Franzke *et al*. 1998).

Similarly, *Ranunculus* is polyphyletic if *Ficaria* is retained within it (Paun *et al*. 2005). Lesser Celandine was first placed in the genus
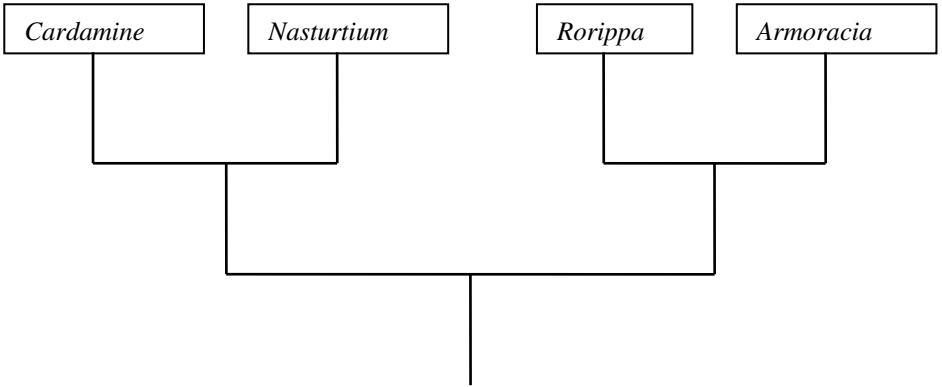
FIGURE 3. Cladogram of relationships of *Rorippa* and *Nasturtium*, simplified and adapted from Franzke *et al*. (1998). Based on DNA sequences from cp-DNA and r-DNA ITS regions.
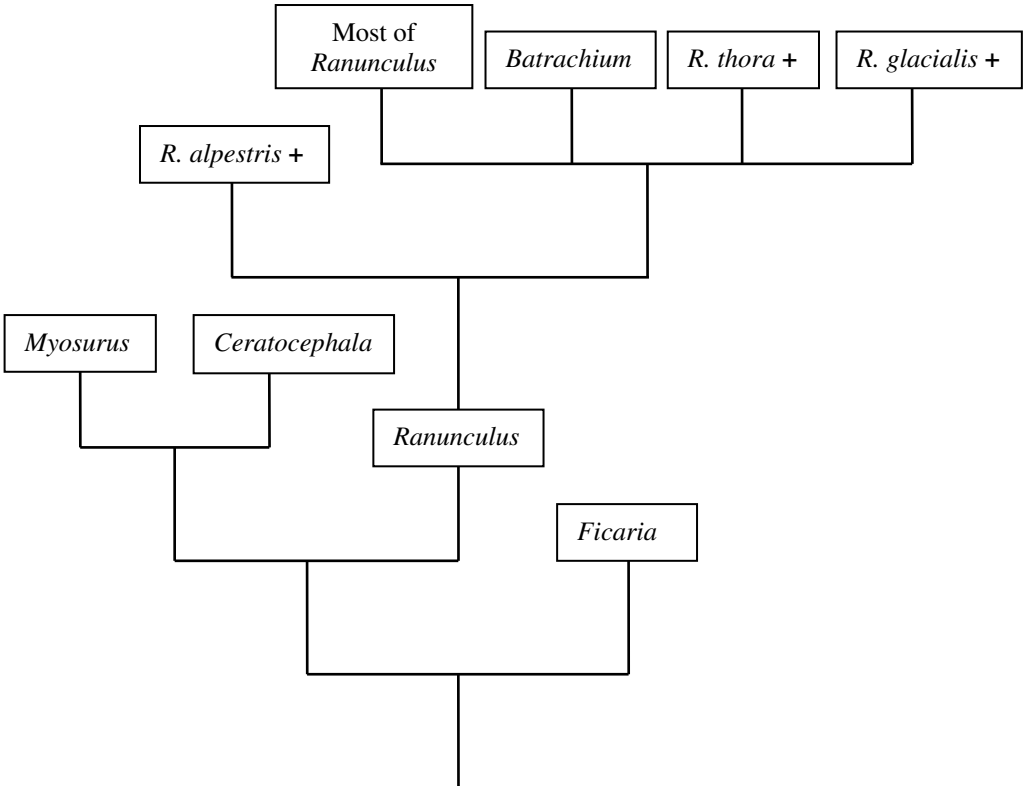

FIGURE 4. Cladogram of relationships of *Ranunculus* and its closest relatives, simplified and adapted from Paun *et al*. (2009). Based on DNA sequences from cp-DNA and r-DNA ITS regions.

Ficaria by Hudson (1762), although this was followed by very few authors except Hutchinson (1948). Again, the recognition of two genera is a reversion to a much earlier opinion. According to the molecular evidence *Ficaria* can be retained in *Ranunculus* only if *Myosurus* and *Ceratocephala* are as well. It is of interest, on the other hand, that the Water-crowfoots, which have often been segregated into the genus *Batrachium*, are shown by molecular data to be true members of *Ranunculus* (Fig.4).

3. A CAUTIOUS WELCOME TO NEW FRIENDS

As previously implied, it is often difficult at first to become persuaded that novel relationships suggested by a new classification are genuine. However, those that at first sight seem implausible, yet on closer scrutiny indicate hidden truths, are perhaps the most instructive of all. The following examples are intended to illustrate this point.

Legumes and Milkworts

It must be admitted that the rather extraordinary flowers of these two families do bear an overall resemblance; how many of us I wonder in our very early days thought that *Polygala* might be some diminutive legume? In almost all classifications, however, the two have been placed far apart in different orders. Despite this they are keyed out adjacently in the family keys in both Clapham *et al.* (1952) and Stace (1997, 2010). So it is perhaps not so surprising that in the new APG classification the Polygalaceae and Fabaceae are classified along with just two other very small Southern Hemisphere families in the order Fabales. Superficial resemblances often do indicate some underlying close genetic relationships.

Malva/Lavatera

When two quite variable genera are delimited by a single character (fusion of epicalyx), and there seem to be a number of parallelisms between them, it is likely that a new look might result in a quite different classification. Molecular data do not support the epicalyx character as an indicator of the major lines of evolution in this group (Escobar García *et al.* 2009). The cladograms (Fig. 5) contructed from these data can be interpreted as a classification in several diverse ways. It is still possible to recognise *Malva* and *Lavatera* as separate genera, as did Linnaeus, but several species would need to be moved from one to the other and additional genera would be needed. For example, *Malva moschata* would become a *Lavatera*, and *Lavatera cretica* and *L. arborea* would be transferred to *Malva*. In general appearance the flowers of *Malva moschata* resemble those of *Lavatera* species such as *L. trimestris* at least as closely as those of most *Malva* species, and the similarity of *Lavatera cretica* to *Malva sylvestris* is well known, leading to frequent misidentifications by the inexperienced. In this family there is another pair of similar species, often misidentified by beginners in Mediterranean field work, viz. *Althaea hirsuta* and *Malva cretica*. It is most instructive, therefore, to find that in molecular terms these two species fall into the same subclade, not in the main *Malva* or *Althaea* subclades (Fig. 5). Once again the molecular evidence more strongly supports superficial resemblances than the traditional taxonomic framework. In practical terms it seems better to recognise an enlarged *Malva* (to include *Lavatera* and *Althaea hirsuta*) than to indulge in the considerable swapping of species that would be needed to retain *Malva* and *Lavatera*, especially as additional genera would be needed as well.

Peucedanum

Considering how narrowly most umbellifer genera are drawn, the three species (two native) of *Peucedanum* in our flora are remarkably diverse, and have completely unrelated English names. The differences can be seen in Table 3. Several of the characters that distinguish the species are more usually constant at the generic level. For this reason the three species had to be keyed out in separate places in the generic key in both Clapham *et al.* (1952) and Stace (1997, 2010). It is perhaps not surprising, then, to find that the genus is not monophyletic; in fact our three species fall in separate clades and are now placed in separate genera. The conundrum is explained by the fact that the fruits of the three species (and of the non-British ones too) are very similar, and traditionally fruit morphology and anatomy are given great emphasis in umbellifer classification. Once again, general appearance is a better signal of evolutionary relationship than the perceived most important diagnostic characters.

Festuca/Schedonorus

The large diverse genus *Festuca* has been divided up many times in different ways, but the most frequent group to be split off is the
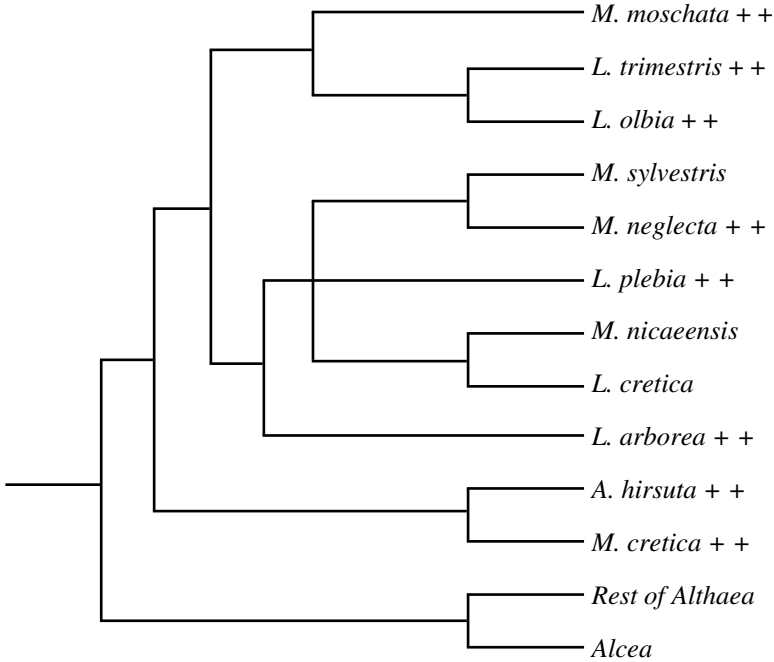
FIGURE 5. Cladogram of relationships of *Malva*, *Lavatera* and allied genera, simplified and adapted from Escobar García *et al.* (2009). Based on DNA sequences from five cp-DNA and r-DNA ITS regions.

TABLE 3. CHARACTERS OF THE BRITISH SPECIES OF *PEUCEDANUM* (APIACEAE)

P. palustre	*P. officinale*	*P. ostruthium*
Stems hollow	Stems solid	Stems hollow
Leaves fully decompose	Leaf-bases remain as dense sheath of fibres	Leaves fully decompose
Bracts >3	Bracts 0-few	Bracts 0-few
Sepals very small, not persistent	Sepals conspicuous, persistent	Sepals very small, not persistent
Petals white	Petals yellow	Petals white
Milk-Parsley	*Hog's Fennel*	*Masterwort*
THYSELIUM	*PEUCEDANUM*	*IMPERATORIA*
Fruits strongly dorsally compressed, somewhat longer than wide, glabrous, with low dorsal and winged lateral ridges	Ditto	Ditto

'broad-leaved fescues' (*F. pratensis*, *F. arundinacea* and *F. gigantea*). These species are distinct from the rest in morphology (e.g. long pointed leaf-auricles), cytology (pattern of chromosome banding) and hybridisation behaviour. While they do not hybridise with other species of *Festuca*, they cross very readily with *Lolium perenne* and *L. multiflorum* in all six combinations (Stace 1975). Despite this there has been great resistance to the redrawing of generic boundaries, and the broad-leaved fescues are retained in *Festuca* in virtually all floras. However, molecular data clearly show them to be separated from the rest
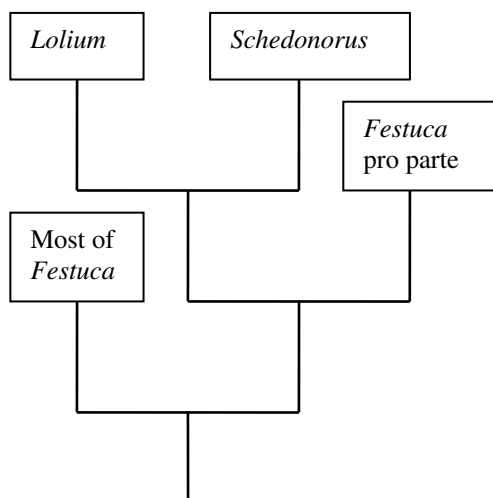
FIGURE 6. Greatly simplified cladogram of relationships of some major groups of *Festuca sensu lato*, adapted from Catalán *et al*. (2006). Based on DNA sequences from r-DNA ITS region.

of *Festuca*, in fact sister to *Lolium* (Fig. 6), and confirm the need to segregate them into the genus *Schedonorus* (or else transfer them to *Lolium*, or to amalgamate all three) (Gaut *et al*. 2000, Catalán *et al*. 2006). In this case the molecular evidence has simply been the final nail in the coffin. The hybrids with *Lolium* go under × *Schedolium*.

Thlaspi and relatives
Several generic relationships in the Brassicaceae are indicated for the first time by DNA sequence analysis. In a family where fruit morphology alone has always been considered of paramount importance, this is with hindsight not surprising (cf. *Malva/Lavatera* above). *Thlaspi* and relatives provide one example. The five species in our flora should be segregated into four genera: *Thlaspi* (*T. arvense* and *T. alliaceum*), *Pachyphragma* (*T. macrophyllum*), *Noccaea* (*T. caerulescens*) and *Microthlaspi* (*T. perfoliatum*). The first two of these four are notable for containing species that smell of garlic when fresh. Two other British crucifers possess the same feature: *Alliaria*, which has a totally different fruit (siliqua rather than silicula) and has always been placed far from *Thlaspi*, usually close to *Sisymbrium*; and *Peltaria*, an eastern European alien recently found naturalised on Skye, also usually placed

far from *Thlaspi*. The fruits of *Peltaria* are different again, being pendent, indehiscent, flattened 'fried eggs', usually with only one seed. Extraordinarily, these four garlic-smelling genera are found to be close together on molecular data, forming, together with *Teesdalia*, the tribe Thlaspideae (Al-Shehbaz *et al*. 2006). The two non-garlic-smelling genera formerly in *Thlaspi* are placed in a related tribe, Noccaeeae. Clearly, the presence of the garlic-smelling compounds is a much better indicator of relationships than is fruit morphology.

Taxa with reduced morphology
Many taxa exhibit a reduced structure compared with their relatives due to the adoption of a particular life-style, e.g. parasitism, an annual autogamous habit, or occurrence in water. In many cases in the past we have been quick to segregate such taxa because of their very distinctive features, but, where the molecular data show that these taxa are nested within (rather than being sister to) their 'unreduced' relatives, consideration of amalgamation is warranted. Examples are Cuscutaceae with Convolvulaceae; Viscaceae with Santalaceae; Lemnaceae with Araceae; Pyrolaceae, Monotropaceae and Empetraceae with Ericaceae; *Coronopus* with *Lepidium*; and
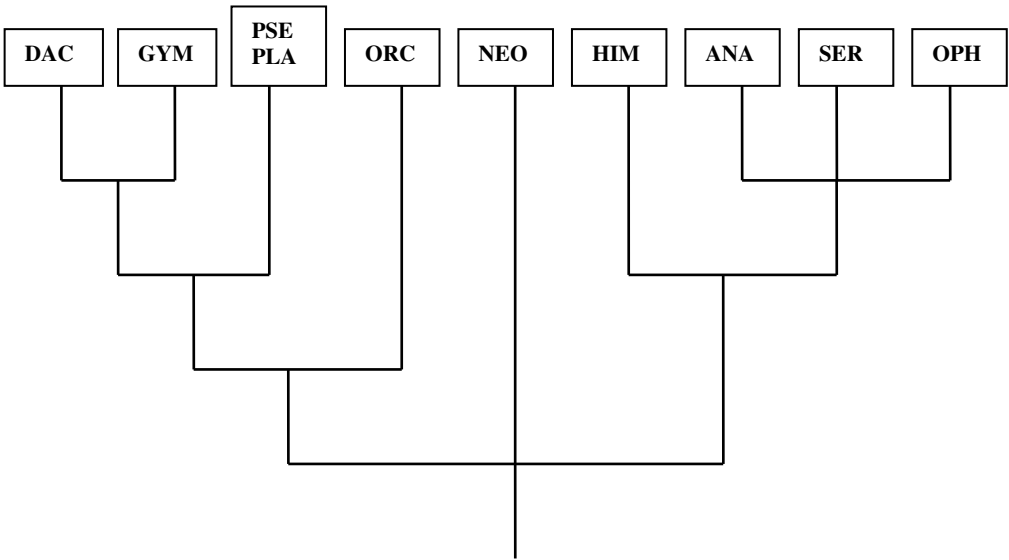
Figure 7. Cladogram of relationships of British genera of orchids of subtribe Orchidinae, simplified and adapted from Bateman (2006). Based on DNA sequences from cp-DNA and r-DNA ITS regions.
Abbreviated generic names: DAC = *Dactylorhiza*; GYM = *Gymnadenia*; PSE = *Pseudorchis*; PLA = *Platanthera*; ORC = *Orchis sensu stricto*; NEO = *Neotinea*; HIM = *Himantoglossum*; ANA = *Anacamptis*; SER = *Serapias*; OPH = *Ophrys*.

Callitrichaceae with Plantaginaceae/Veronicaceae. It is instructive to search for other examples, and these relatively novel alignments should help us to pinpoint wider evolutionary trends more clearly.

4. UNWELCOME NEWCOMERS

It would be disingenuous to imply that all changes signalled by molecular research are either obviously, or after study can clearly be seen to be, changes for the good. Because of the nature of the data we must assume that this *is* the case, but it is often hard to accept it, and when there are no exomorphic characters to support the new classification real practical problems arise. The number of examples, although rising, is mercifully still small. It is not, however, a new problem. There have always been 'strange bedfellows', where the association of two taxa is at first difficult to understand. *Circaea* and *Epilobium*, *Ruta* and *Citrus*, *Primula* and *Anagallis*, *Galium* and *Coprosma*, and *Malus*, *Rosa*, *Aphanes* and *Rubus* are a few of many examples. It is just that we have come to grips with these over a long period, whereas the new examples, e.g. *Veronica* and *Plantago*, or *Veratrum* and *Paris*, still seem very strange.

Orchis

By far the best-known example, and perhaps the first to emerge, is the dismemberment of the genus *Orchis*. A monophyletic *Orchis* is maintained only by removing some species to the genus *Anacamptis* and others to *Neotinea* (Bateman *et al.* 1997; Pridgeon *et al.* 1997) (Fig. 7). *Orchis ustulata* actually fits well into *Neotinea*, and the newly enlarged latter genus is easily recognised and keyed as an entity (small flowers), but this is not true of the extended *Anacamptis* (including *Orchis laxiflora* and *O. morio*). The latter two species are starkly different in appearance from *A. pyramidalis*, and it is difficult to see how the new *Anacamptis* and *Orchis* can now be readily recognised, still less keyed. The best practical answer to the latter is to key out the species to the two genera together. The very useful character concerning the stance of the two lateral sepals cuts across the new generic boundary. Hybridisation patterns to some extent support the new generic separation, but not entirely so, because apparently well substantiated hybrids have been found between *O. mascula* and *Anacamptis morio* (Godfery 1933).
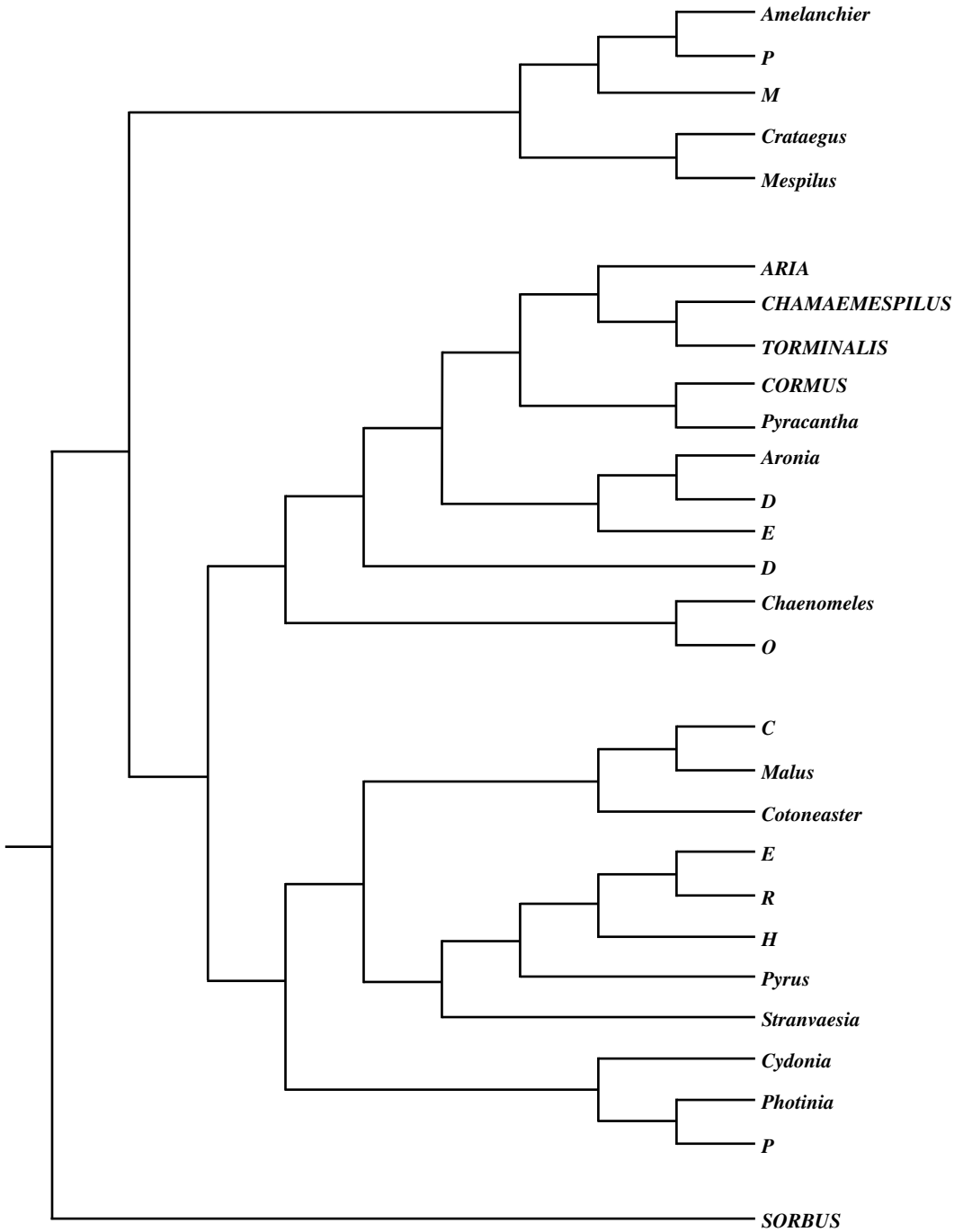
FIGURE 8. Cladogram of relationships of the genera of the former Rosaceae subfamily Maloideae, simplified and adapted from Potter *et al*. (2007). Genera in the British flora are named; others are indicated by their initial letter only. *Sorbus* segregates are in capital letters. Based on DNA sequences from six nuclear and four chloroplast loci.

Senecio

The huge genus *Senecio* has been divided in many different ways in the past, but the separation of a relatively small group of species centred on *S. jacobaea* (also including *S. cineraria*, *S. aquaticus*, *S. erucifolius* and *S. paludosus* in our flora) following molecular studies (Pelser *et al.* 2007) is unprecedented. This group, now known as the genus *Jacobaea*, is supported by hybridisation behaviour, because there exist several hybrids within it but none is known between the genera *Jacobaea* and *Senecio*. There appear, however, to be no distinguishing exomorphic characters; as the above authors euphemistically state "clear morphological synapomorphies for *Jacobaea* have not been identified to date". The species of the two genera, as with *Anacamptis*/*Orchis*, are best keyed out together.

Anagallis/Lysimachia

These two genera of Primulaceae have not yet been examined sufficiently widely in terms of DNA sequences to enable a thorough reclassification, but there is enough evidence to make us certain that the generic limits will require revision (Anderberg *et al.* 2007). One particular surprise to emerge is that *Lysimachia nemorum* appears to fall within *Anagallis*, not *Lysimachia*, despite its yellow corolla, valvate capsule, and glabrous filaments borne suprabasally on the corolla; all four character-states are otherwise absent from *Anagallis*. So why is *L. nemorum* called Yellow or Wood Pimpernel in English? Can we spot other characters to link it with *Anagallis*? Conversely, and as a matter of interest, this same research shows that *Centunculus* should be re-separated from *Anagallis*.

Sorbus

Molecular evidence indicates that *Sorbus* is polyphyletic, and should be divided into up to five genera: *Sorbus sensu stricto* (*S. aucuparia*), *Cormus* (*S. domestica*), *Torminalis* (*S. torminalis*); *Aria* (most of our other species); and the Alpine *Chamaemespilus* (Potter *et al.* 2007). The most surprising (and perhaps even suspicious) aspect of this is that *Sorbus sensu stricto* and *Cormus* are not only separated, but placed very far apart in the old subfamily Maloideae; the other three segregates are placed close to *Cormus* (Fig. 8). These restricted genera are readily distinguished, but there is a major problem in practice because of the extent of hybridisation. Hybrids not only occur between most of these generic segregates, but also involve other genera such as *Aronia*, *Cotoneaster*, *Amelanchier*, *Crataegus*, *Mespilus*, *Malus* and *Pyrus*. Most of these hybrids are sterile and occur only in cultivation, but many of those between the segregates of *Sorbus* are apomictic, have reproduced to form taxa with distinctive distributions, and are now recognised at the rank of species. In our flora there are three such groups of agamospecies, derived from *Aria × Torminalis*, *Aria × Sorbus* and *Aria × Sorbus × Torminalis* (Rich *et al.* 2010). If the five segregate genera are recognised then the latter three groups would also need to be accorded separate generic status. In addition there are a number of hybrids that are not considered as species because they are non-apomictic and sterile, e.g. *S. aria × S. aucuparia = S. × thuringiaca* and *S.aria × S.torminalis = S. × tomentella* (*S. × vagensis*). In our flora these cover two segregate generic combinations, which would nomenclaturally require separate nothogeneric recognition. Hence in our flora alone in place of *Sorbus* there would need to be seven genera plus two nothogenera. Of the 37 native species now recognised in our flora only one would remain in *Sorbus*, and 13 would fall into the three new genera of hybrid origin. This system is workable but vastly more complex than the present one. According to the data of Potter *et al.* (2007) *Sorbus* could be retained in its present wide sense only if almost all of the Maloideae were also amalgamated with it!

CONCLUSIONS

The APG system of classification of angiosperms is here to stay. It is not just another scheme in a long line of attempts to find the answer to flowering plant family relationships, but is *the* answer itself, or something pretty close to it. Undoubtedly adjustments will be made over the years, but they will concern minor detail and not represent radical realignments.

The view is expressed here that perceived difficulties or dissatisfaction with the new system are due principally to unfamiliarity and a residual conservatism among taxonomists. If the APG system had been in common use for the past 50 years, and now the Cronquist classification, say, were being proposed, I have no doubt that the same reluctance to change would be apparent. It is frankly that we have

been indoctrinated by the traditional classifications, e.g. that fruit morphology is the most important character in the crucifers, and that abandonment of these prejudices will take time.

Partly because of the intrinsic detail of the new system, and partly because any new system will provoke its potential users into thinking carefully about it, often from different angles from previously, the APG molecular classification has much to teach us. New associations as well as new dissociations of taxa are very informative. For instance, the *Thlaspi* example above should prompt us to take the smell of garlic more seriously as an important character. It should lead field botanists, if they don't already, to sniff a fresh crucifer as one of the primary facts to gather about it. How many of us know immediately which of our five former species of *Thlaspi* are garlic-scented? Not all of us I guess. The case of *Peucedanum* already mentioned should reinforce our understanding of the important generic characters, so that we get our priorities right when trying to determine, say, a sterile umbellifer. Not a few field botanists will rejoice in the discovery that sometimes really obvious features like scent, colour and leaf-shape have turned out to be more revealing of relationships than much more cryptic characters. And plant breeders will be pleased to see that the significance of the ability of taxa to hybridise in indicating relationships (in recent years questioned by some, e.g. Seberg & Petersen 1998) is reinforced.

The use of the molecular classification is far from a final answer to all problems. For example, the true relationships of the Boraginaceae (i.e. which order the family belongs to) are still uncertain (or they were when I last investigated). Diggs & Lipscomb (2002) and Stace (2009), as summarised above, have advocated the adoption of a pragmatic compromise. Molecular data alone are not the answer to everything, and the following caveats should in my opinion, be heeded.

- Extremely distinctive taxa that markedly change the circumscription of the group to which they are closest should be considered candidates for separate recognition, leaving a more tightly defined albeit paraphyletic taxon. I see no merit in the dogma that all taxa must be monophyletic.
- Relatively weak molecular evidence should not be relied upon to change old classifications; changes should be made only once the data are unequivocal. *Coeloglossum* is best retained as a separate genus until its amalgamation with *Dactylorhiza* becomes certain (compare Pridgeon *et al.* 1997 and Devos *et al.* 2006).
- Degrees of similarities/differences in DNA sequences should not be used as an absolute criterion of relationships, only as a relative one. As for all other taxonomic data, differences of a degree that indicate family distinction in one area of angiosperms might be better expressed at the generic level in another area.
- There will always be scope for argument and disagreement. Decisions should always be reached by considering a great range of evidence in addition to the molecular data. Botanists who know the plants concerned intimately, particularly in the living state in the field, are usually better placed to judge the evidence objectively. There is a feeling among some botanists that the scientists who investigate molecular systematics are divorced from whole plants – they know them simply as DNA sequences. This was recently expressed in a heartfelt but cynical way by Robert Thorne (Thorne 2010) in his obituary of one of his peers (Armen Takhtajan), both doyens of angiosperm classification in the second half of the Twentieth Century:

  > "This was before the age of molecular taxonomy when we thought it important to have close contact with the plants we were cataloguing for their phyletic relationships."

  Some of us still do!

All botanists, from molecular biologists to plant hunters, have much to learn from and much to contribute to the field of molecular systematics. The new molecular classification will lead us to look at our plant finds in a different way, and thereby add to our enjoyment and understanding.

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