



SECOND EDITION

UNDERSTANDING FLOWERS & FLOWERING

An Integrated Approach

BEVERLEY GLOVER

Understanding Flowers and Flowering

Understanding Flowers and Flowering

An Integrated Approach

Second Edition

Beverley Glover

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Preface

Flowers are the features of plants that most endear them to human beings. We grow flowers in our gardens, we display them in our homes, and they feature prominently in our artistic history. Scientists, too, have been fascinated by flowers, and there is a long tradition of botanical study of floral structure and floral ecology. However, it is only in the last few decades that the tools of molecular genetics have been applied to flower induction, development, and morphology. The data arising from these studies, when combined with painstaking observation and analysis of the interactions of plants with their pollinators, are beginning to provide the first truly integrated understanding of both how and why flowers take the forms we so admire.

Traditionally, flowers are studied from one of a number of viewpoints. Molecular biologists may study the genetic control of flower induction or flower development, usually focusing on a single model species. Evolutionary biologists may study how flowers evolved, the forms of the earliest flowers, or the morphology of flowers of ancient lineages alive today. Pollination biologists may study the interactions between plants and their pollinating animals, the natural selection exerted by those pollinators, and how these pressures affect plant population dynamics. However, it is becoming clear that these various disciplines each have enormous power to inform and shape the work of the others. An understanding of how flower colour is controlled biochemically and genetically, for example, can be of great benefit when studying how pollinator-imposed selection might have influenced the evolution of petal colour within a group of plants. Similarly, an understanding of how time of flowering influences competition with other species in the same community can be of great help in

understanding why and how certain genetic components of the floral transition mechanism are conserved and others are less so.

I hope that this book might serve as a starting point for those interested in taking such an integrated approach to the study of flowers. I have written it, and revised it for this second edition, with the intention of helping to bridge the gaps between the different disciplines that work with flowers. My aim is to provide students and researchers studying one aspect of floral biology with an overview of other important aspects of flowers, both to help them to set their own work in context and to encourage them to consider experiments which might lead to greater integration of the field. In particular, I hope that this book will encourage dialogue between floral biologists of all varieties, with a long-term view to ensuring a continuing increase in interdisciplinary studies of flowers.

The book is divided into three main sections. Section I is introductory, giving some necessary background to the evolution of flowers and to the history of scientific thought on flowers and flowering. In this second edition the chapter on the evolution of flowers has been extensively revised and updated. Section II considers the molecular mechanisms that control flower induction, flower development, and floral mating type, providing coverage of the genetic material available for shaping by natural selection. This section is initially focused on a very few species of model plants, looking at the molecular similarities which unite all flowers. In the later chapters it considers the development and reproductive strategies of plants from a range of species, with a new chapter on the floral transition in diverse species, and extensive revision of the chapter on floral development in various groups. Section III extends

this analysis much further, considering the explanations for the differences between flowers, rather than their similarities. This section moves between molecular explanations for flower morphology and the ecological consequences of that morphology, in an attempt to integrate what we know both of how and why different flowers take their different forms. A new chapter on the lability of floral form considers how floral traits change within phylogenetic contexts. Finally, the epilogue attempts to draw out some themes which persist throughout the book, suggesting possible future directions for the field.

Many people have contributed to the development of both the first and second editions of this book, and I am particularly grateful to all members of my own research laboratory, past and present, for enthusiastic support and helpful discussions at many points in the process. The second edition has benefited from the suggestions and advice of reviewers of the first edition, and I am particularly grateful to Doug Soltis, Elena Kramer, and Martin Ingrouille for constructive comments. John Parker, Caroline Dean, David Hanke, Cathie Martin, Jeff Ollerton, Rob Raguso, and Nick Waser read various sections of the book in detail, and I must thank them all for the time and care that they took and for their excellent suggestions and advice. Many people were kind enough to provide me with images for figures. While these are acknowledged in the relevant figure legends, I particularly thank Nick

Waser, Enrico Coen, and the Cambridge University Botanic Garden for their great generosity in sharing images, Matthew Dorling and Heather Whitney for photographic assistance, Mike Webb for biochemical pathways, and Rosie Bridge for line drawings. For this second edition I owe a great debt to Alison Reed, whose brilliant drawings and photographs have greatly enhanced the figures throughout the book. Thanks also to Roy Barlow and Don Manning for their excellent cover design for the first edition, which has been adapted by the OUP team for edition 2. At Oxford University Press, Ian Sherman has provided steadfast support for this project, dealing with various changes to the schedule with calm good humour, while Helen Eaton, Christine Rode and Lucy Nash have kindly shepherded me through the production process. On a personal note I am still grateful to Jocelyn, Duncan, and Katie Taylor for lending me the space and quiet to really begin writing the first edition, rather than just thinking about it. In recent years, and particularly during the development of this second edition, I have relied heavily on Sam Brockington and Edwige Moyroud for the discussion of ideas and the development of new lines of thought, as well as for practical and personal support in day-to-day academic life. Thank you both. And finally, as with everything I do, the writing of a second edition has only been possible because of the patience and support of Stuart, Sam, and Katie—I do appreciate you all, really.

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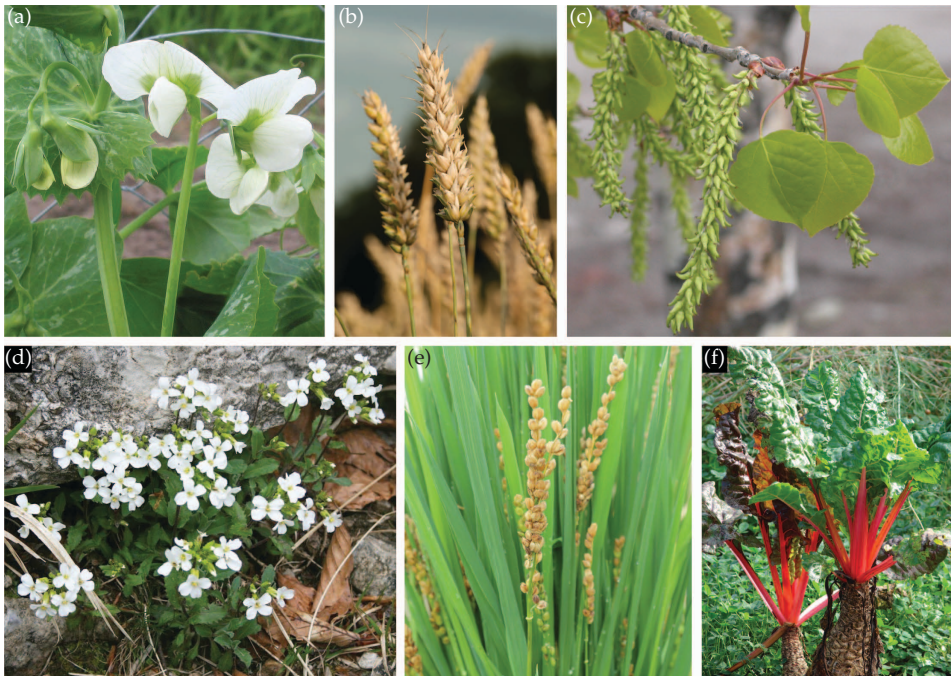


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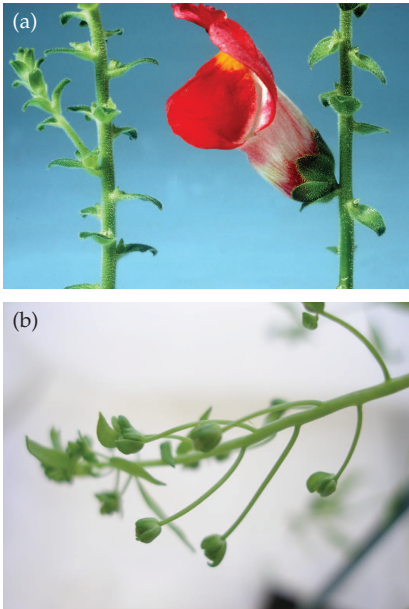


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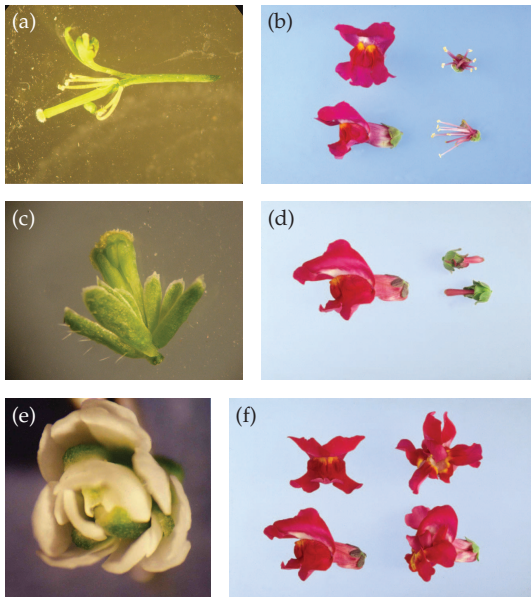


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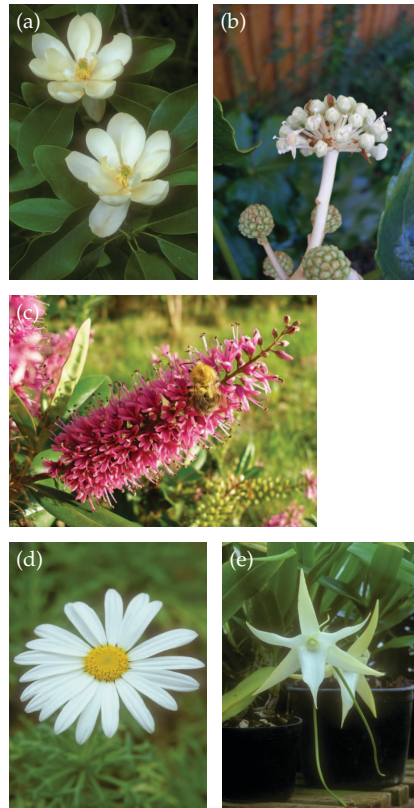


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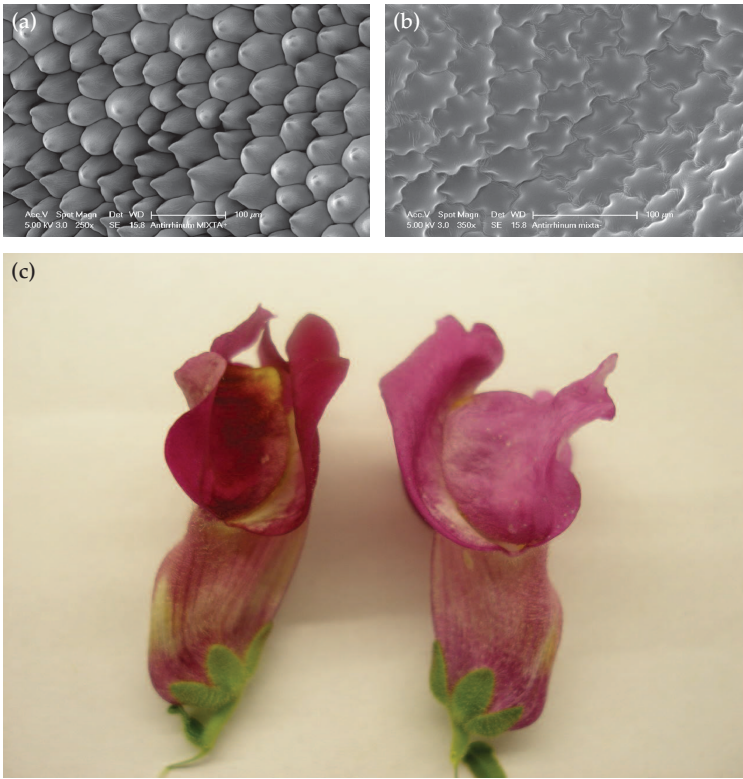


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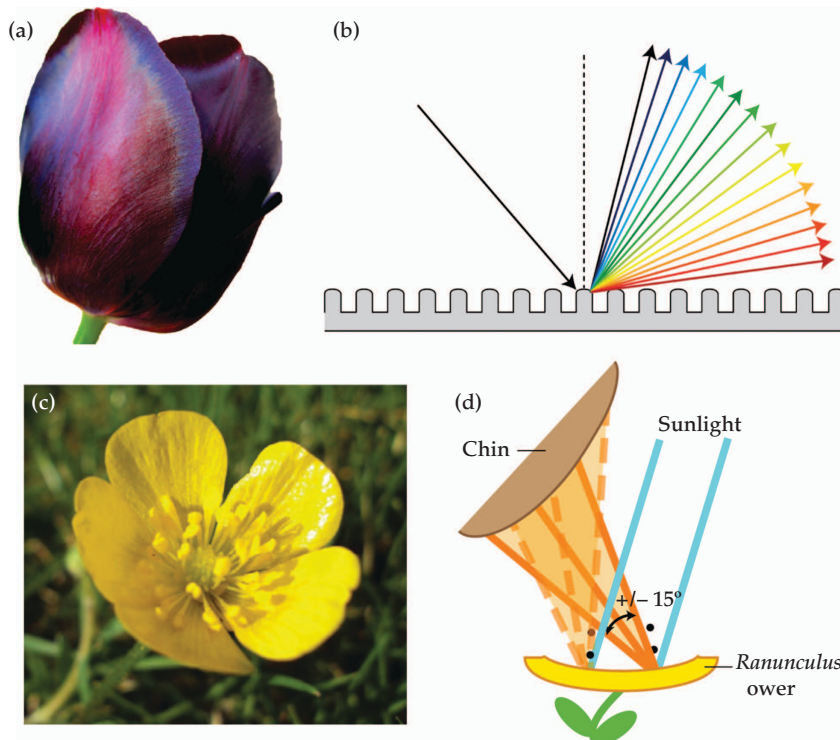


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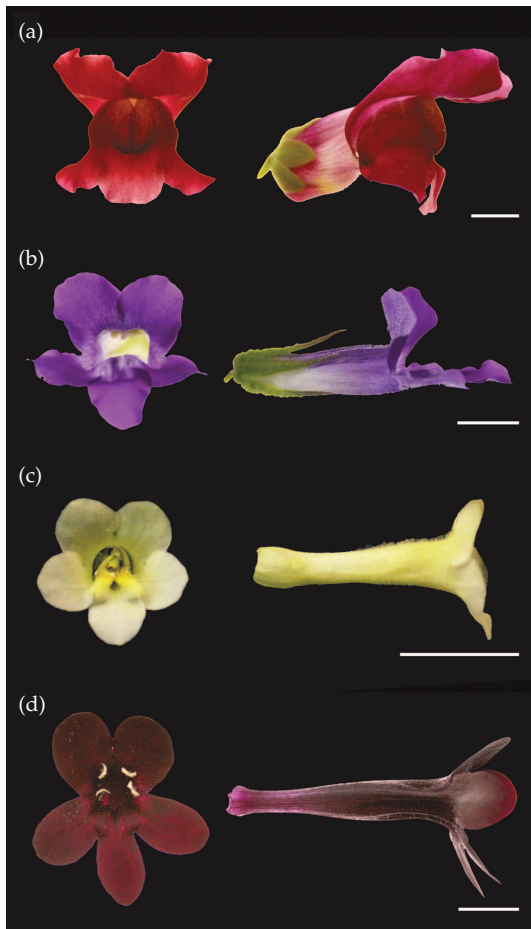


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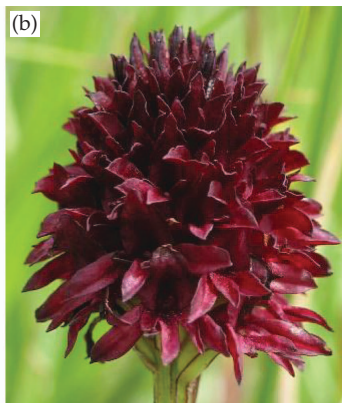
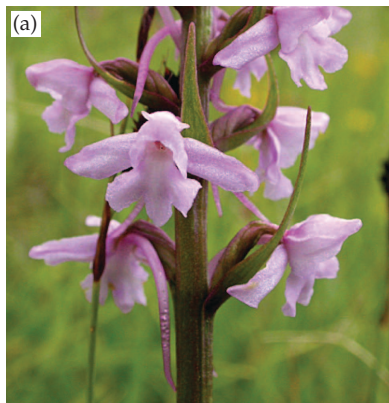


Plate 22 Variation in nectar spur length. (a) *Gymnadenia conopsea* flowers have very long nectar spurs. (b) *Gymnadenia rzellicani* flowers have almost no nectar spur. Images kindly provided by Matt Box (Sainsbury Laboratory, Cambridge University). See also Figure 18.3.

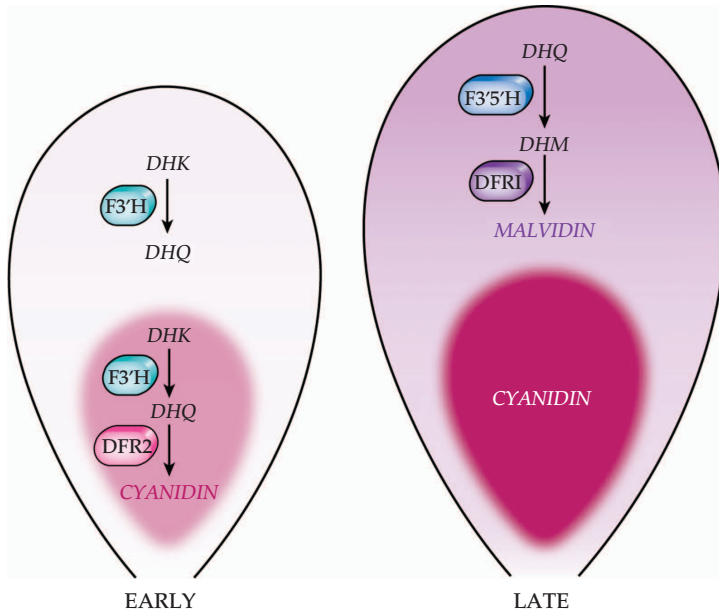


Plate 23 Development of the *Clarkia gracilis* petal spot. Early expression of *DFR2* in the spot region, in the presence of F3'H but not F3'5'H, results in red cyanidin pigment. Later expression of *DFR1* throughout the petal, in the presence of F3'5'H, results in mauve malvidin production. See also Figure 18.4.

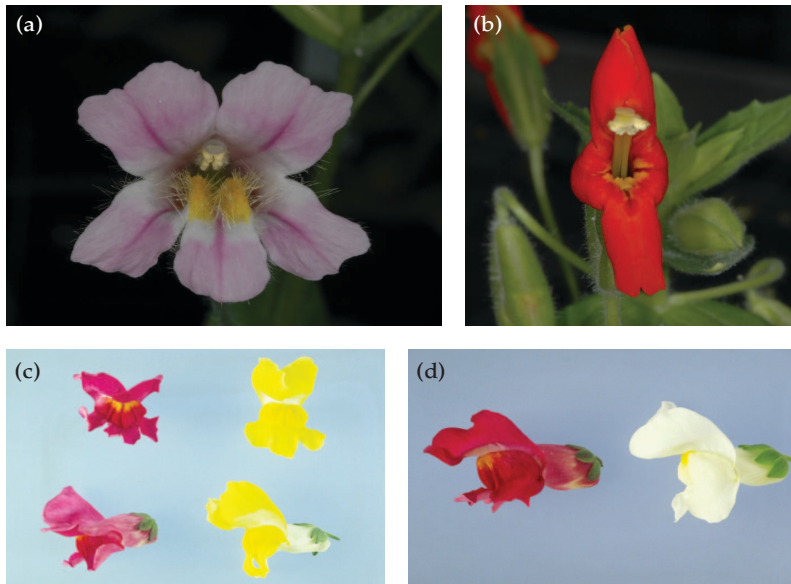


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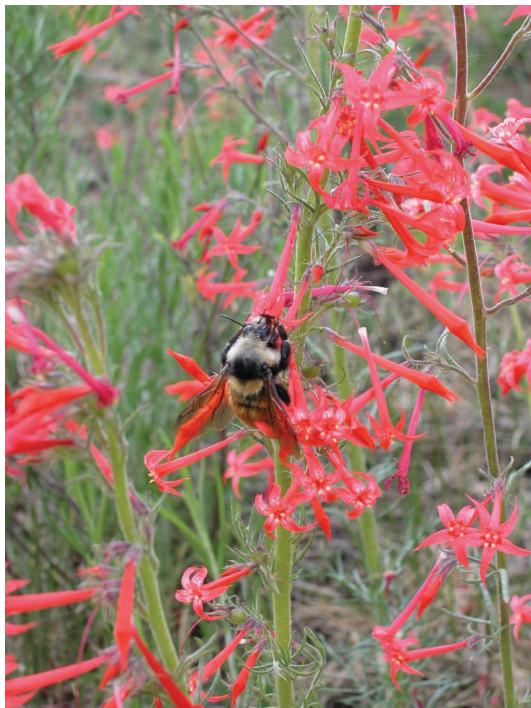


Plate 26 Flowers for which pollinators have been shown to discriminate on the basis of size. *Ipomopsis aggregata*. Image kindly provided by Nick Waser (University of California – Riverside). See also Figure 20.4.



Plate 27 Plants for which the main pollinator type can be accurately predicted from floral morphology. (a) *Penstemon centranthifolius* (hummingbird-pollinated). (b) *Penstemon heterophyllus* (bee-pollinated). Images (a) and (b) kindly supplied by Scott Armbruster (University of Portsmouth). See also Figure 21.1.



Plate 28 Plants that have cast doubt on the pollination syndrome concept. (a) *Viola cazorlensis*. Image kindly supplied by Carlos Herrera (Seville). (b) *Microlooma sagittatum* with its sunbird pollinator. Image kindly supplied by Anton Pauw (Stellenbosch). See also Figure 21.2.

SECTION I

Introduction

The evolution of flowers

The oldest fossil flower currently known is around 125 million years old. Flowers, and the plants that produce them (angiosperms or flowering plants), are relatively recent innovations in evolutionary terms. The first land plants, which did not possess flowers, arose around 470 million years ago, but fossil evidence indicates that only after another 340 million years did the angiosperms appear. However, following their appearance in the fossil record of the early Cretaceous period, the angiosperms spread geographically from their point of origin in the tropics and diversified dramatically to become the ecologically dominant plant group in the great majority of terrestrial habitats. This extraordinary geographic and morphological radiation took a mere 40 million years, and was even more extraordinary for the number of species it generated. The 250–400,000 species of extant angiosperms represent the most species-rich plant group by far, and are exceeded in numbers in the speciose animal kingdom only by the arthropods. Furthermore, the differences in growth habit, morphology, and life history within the angiosperms are vast, leading Darwin to describe the speed and scale of this recent radiation as ‘an abominable mystery’. It is not possible to provide here a full analysis of the extensive literature on the origins and radiation of angiosperms and their flowers. The aim of this chapter is to provide an overview of the key issues surrounding the origin of the flowering plants and their flowers, and to conclude with an introduction to the major groups of flowering plants, many of which will be referred to in later chapters.

1.1 The origin of flowering plants

Angiosperms are defined by a number of features, of which possession of a flower is only one. Some

of these traits are shown in Fig. 1.1. They have fully protected and enclosed ovules with two layers of protective integuments surrounding them, enclosed in a carpel within which the seed eventually develops. Their wood contains true, continuous vessels, as well as the more widespread tracheids in which water has to cross a membrane between individual cells. Their phloem consists of sieve tube elements and the unique companion cells, both derived from the same mother cell. Angiosperms have distinctive pollen, with columnar structures providing support for the surface layer. In addition, only angiosperms undergo double fertilization, whereby two genetically identical sperm cells are released into the ovary with one fertilizing the egg and the other fusing with the central cell to form the endosperm. Traditionally, fossil evidence was all that was available to probe the origins of the angiosperms, but, more recently, molecular data obtained from extant species have been used to inform the debate. The following two sections consider insights from these two types of evidence into the age and nature of the earliest angiosperms, and their relationships with other seed plants.

1.1.1 Fossil evidence for angiosperm origins

Fossil evidence dates the origin of the angiosperms to the early Cretaceous period, with the oldest fossil flowers (125 million years ago), angiosperm fruits (121 million years ago), angiosperm pollen (130 million years ago), and angiosperm leaves (120 million years ago) all supporting this conclusion (Hughes 1994; Krassilov and Dobruskina 1995; Brenner 1996; Friis *et al.* 1999, 2001; Sun *et al.* 2002). The oldest fossils suggest that the first angiosperms were aquatic plants. For example, *Archaeofructus*, a fossil dated to

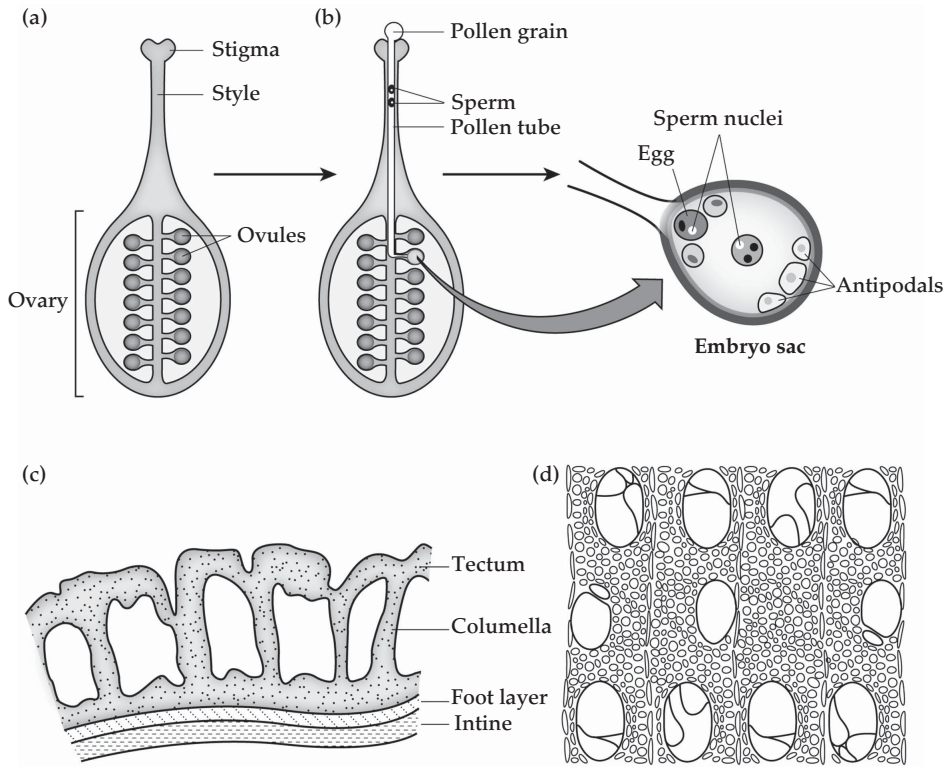


Figure 1.1 Some defining features of angiosperms. (a) Enclosed ovules, enfolded within the carpel. (b) Double fertilization, with two sperm nuclei arriving in the pollen tube. One fertilizes the egg cell and the other fertilizes the central cell with its two nuclei, generating the triploid endosperm. (c) Columnar pollen exine, shown in cross section. (d) Wood with true vessels as well as the narrower tracheids found in gymnosperms.

124.6 million years ago, has the long thin stems and highly dissected compound leaves that are typical of aquatic species. Perhaps even more convincingly, it is found with fossilized fish mixed in with the plant tissue (Sun *et al.* 2002). Similarly, Friis *et al.* (2001) identified a fossil flower from deposits up to 125 million years old as a member of the Nymphaeales (the modern water lilies), on the basis of its unique centrally protruding floral axis and its distinctive seeds with wavy cell walls in their seed coats.

The recent identification of *Leefructus mirus*, a fossil with features diagnostic of the eudicot order Ranunculales, in deposits dated at 122–125 million years old, suggests that the angiosperms might be older than paleontologists had previously thought (Sun *et al.* 2011). The eudicots are a more recently derived group of the angiosperms (see Section 1.6 and Fig. 1.5 for an overview of relationships within

the angiosperms). If the eudicots were already present in the early Cretaceous period, it is likely that the first angiosperms arose considerably earlier. Molecular analyses also date the origin of the angiosperms a little earlier than 130 million years ago (see Section 1.1.2 below).

The fossil record suggests that the angiosperms rapidly diversified from their aquatic origins to occupy understory and early successional niches on dry land, with this first diversification occurring during the early Cretaceous period (Friis *et al.* 2005). Their subsequent radiation over the course of the Cretaceous quickly led to the adoption of late successional ecological positions, presumably as their size and woodiness increased. The scarcity of angiosperm wood in the early Cretaceous fossil record, along with the apparently small size of early angiosperm seed and leaves, supports the conclusion that the early angiosperms were small herbaceous

species with a weedy lifestyle, whether on land or in fresh water. Some authors have speculated that this herbaceous habit followed an early loss of woodiness, since most gymnosperms are woody and most of the earliest diverging groups of extant angiosperms are also woody (Willis and McElwain 2002).

The first angiosperms appear to have originated in tropical regions, only spreading to higher latitudes after 20–30 million years (Barrett and Willis 2001). The earliest angiosperm fossils (of pollen) have been found in modern-day Israel and Morocco, land that lay just north of the equator in the early Cretaceous period. The subsequent spread of the angiosperms into higher latitudes was very rapid, accompanied by an increasing dominance of those areas already occupied (as measured by the relative proportions of angiosperm and other pollen types retained in the fossil record) (Willis and McElwain 2002).

The identity and morphology of the last common ancestor of the angiosperms and other land plant groups have long been a source of debate. The most closely related extant group is the gymnosperms, traditionally viewed as a cluster of three or four divisions. Of these divisions, the conifers (Coniferophytes) are familiar, and dominate many high-latitude forests. The cycads (Cycadophytes) are currently less prominent, but fossil records indicate that they were once ecologically highly significant. The Ginkgophytes are currently represented by only one surviving species, *Ginkgo biloba*, a commonly grown tree in parks and gardens. The remaining group, the Gnetophytes, was, until recently, considered likely to be the closest relative

of the angiosperms, on the basis of both fossil and morphological evidence (Crane *et al.* 1995). There are currently three extant genera of gnetophytes, namely *Gnetum*, *Ephedra*, and *Welwitschia*, which share a range of morphological similarities with some angiosperms (see Fig. 1.2). In particular, leaf morphology and venation in *Gnetum* closely resemble that of angiosperms, their xylem does contain vessels, and some gnetophytes produce reproductive structures containing both male and female parts. Double fertilization, often considered to be a defining feature of angiosperms, has also been documented in both *Ephedra* and *Gnetum* (Friedman 1990). However, recent molecular studies have refuted this hypothesis (see below), and have even questioned the status of the Gnetophytes as a division, suggesting that they are part of the Coniferophytes (Qiu *et al.* 1999; Chaw *et al.* 2000). These recent studies confirm the monophyletic nature of the extant gymnosperms, indicating that none of them provide a clear link to the angiosperms.

The fossil record informs the debate on the relationship of angiosperms to other land plant lineages by providing details of extinct groups. Two extinct gymnosperm groups in particular, the Bennettiales and the Mesozoic ‘seed ferns’ (such as *Caytonia*), have frequently been proposed as ancestors or close relatives of the angiosperms, and several studies based on fossil and extant morphology linked the Bennettiales, the Gnetophytes, and the angiosperms into a clade known as the anthophytes (Crane 1985; Doyle and Donoghue 1986). These conclusions were based on the distinctive morphologies of the extinct plants, analysed by painstaking

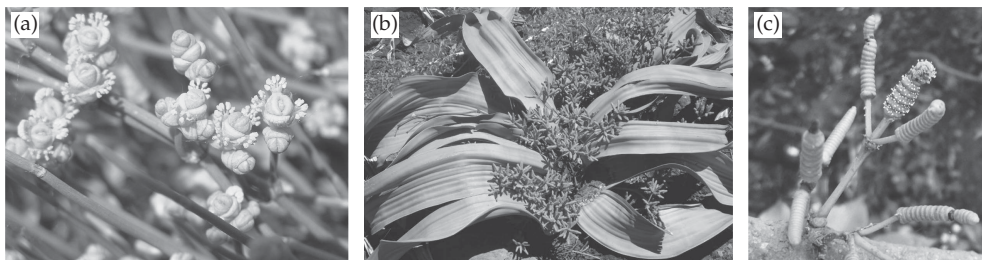


Figure 1.2 The Gnetophytes. (a) *Ephedra distachya* subsp. *monostachya* (male). Photo by Le.Loup.Gris (Wikimedia Commons). (b) *Welwitschia mirabilis* (male). Photo by Franzfoto (Wikimedia Commons). (c) *Gnetum latifolium* var. *funiculare*. Photo by Vinayaraj (Wikimedia Commons). See also Plate 1.

microscopical observation of fossils. *Caytonia*, for instance, has ovules almost entirely enclosed within cupules, bearing some similarity to angiosperm carpels. The Bennettitales have many morphological features in common with angiosperms, most notably the production of a bisexual reproductive shoot surrounded by sterile (possibly perianth-like) organs. Because these fossil taxa cannot be incorporated into molecular phylogenies, attempts to integrate them into our understanding of seed plant relationships requires a combined molecular/morphological approach. Such approaches often produce conflicting results, but it is certainly likely that some of these extinct lineages represent branches of the land plant phylogeny that diverged along the branch leading to angiosperms.

1.1.2 Molecular evidence for angiosperm origins

Molecular evidence for the origins of angiosperms is based on the comparison of DNA sequences or fingerprints from extant species. In addition, use of a molecular clock, which calculates the age of divergence of two sequences according to the number of differences between them, can provide estimates of the date of evolutionary events. Recent molecular dating studies have used methods to allow for divergent evolutionary rates, particularly those arising following branching of a lineage, when different branches might experience very different evolutionary rates according to whether they adopt a woody (long generation time) or herbaceous (short generation time) habit (Smith and Donoghue 2008).

Early molecular dating studies indicated an origin of the angiosperms considerably earlier than the 130 million years ago that the fossil record suggests. Studies indicated variously that the angiosperms arose in the late Carboniferous period, 290 million years ago (Kenrick 1999; Qiu *et al.* 1999), and that the monocotyledonous angiosperms diverged from the dicotyledonous species 250–200 million years (Wolfe *et al.* 1989) or 300 million years ago (Martin *et al.* 1989). However, improvements in dating technology are mainly responsible for the convergence of recent reports on an age for the angiosperms of 140–180 million years (Sanderson *et al.* 2004; Bell *et al.* 2005a). The most recent detailed study used a ‘relaxed clock’ method that allowed different lineages to experience

different evolutionary rates, and concluded that the angiosperms originated 167–199 million years ago (Bell *et al.* 2010). The same study dated the origin of key angiosperm clades, with the eudicots appearing around 130 million years ago, the Rosids arising 108–121 million years ago, and the Asterids appearing 101–119 million years ago (see Section 1.6). These dates are highly compatible with the fossil record, suggesting only that the very earliest angiosperms have not yet been retrieved from fossils. The dating of the eudicots at 130 million years old is particularly interesting, given the recent discovery of the eudicot fossil *Leefructus mirus* from deposits 122–125 million years old (Sun *et al.* 2011).

If the angiosperms originated 167–199 million years ago, the absence of angiosperm fossils from deposits earlier than 130 million years can be readily explained in a number of ways. It is possible that the fossil record does not contain the very earliest angiosperms because they were not woody, were relatively rare within their communities, or were predominantly found in dry or alpine environments not conducive to fossil formation. Some authors have suggested that their early Cretaceous diversification actually represents a migration event from a previous habitat less suited for fossil formation.

The molecular phylogenies of Qiu *et al.* (1999) and Chaw *et al.* (2000) have also shed light on questions of relatedness between gymnosperm groups and the early angiosperms (see Figs. 1.2 and 1.3).

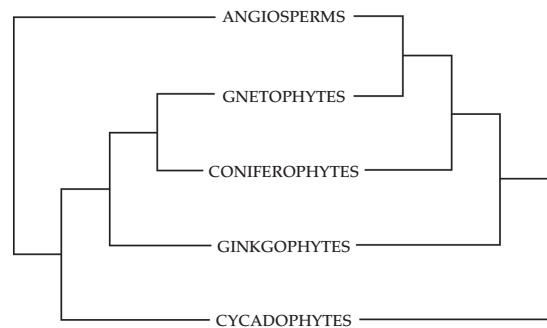


Figure 1.3 Molecular evidence and morphological evidence suggest different relationships between the angiosperms and the various groups of extant gymnosperms. Molecular data (left) place the angiosperms as sister group to the monophyletic gymnosperms, whereas traditional morphological analysis (right) placed the angiosperms and the gnetophytes as sister taxa.

To the astonishment of many botanists, the extant gymetophytes resolved in a single clade with the conifers, refuting the suggestion that their ancestors were the precursors of the angiosperms. Instead, the extant gymnosperms have been shown to be monophyletic and to have diverged from the total land plant lineage around 300 million years ago. They can therefore be thought of as the sister group to angiosperms, and do not necessarily provide much relevant information about the likely morphology of the ancestral angiosperm. Indeed, the derived nature of many extant gymnosperm reproductive structures, combined with the partial nature of the fossils of extinct gymnosperms, makes it very hard to draw conclusions even about the nature of ancestral gymnosperm reproductive structures.

The phylogeny of Qiu *et al.* (1999) provides useful information on the relationships of early diverging angiosperm clades, which can be used to infer features of ancestral angiosperm reproductive structures. The Nymphaeales (water lilies) are indeed ancestral to many other plant groups, but are not the sister group of all the other angiosperms. This position was awarded to *Amborella trichopoda*, the only extant species of the Amborellales, and a native of New Caledonia (see Section 1.6; Fig. 1.4). *Amborella* is a small weedy shrub, supporting the idea that the basal angiosperms were understorey or early successional species, but indicating that



Figure 1.4 The flower of *Amborella trichopoda*. Photograph kindly supplied by Sangtae Kim and Pam Soltis (University of Florida). See also Plate 2.

they were probably woody, not herbaceous, despite the relative scarcity of early fossilized angiosperm wood. Following the publication of the phylogeny of Qiu *et al.* (1999), the position of *Amborella* as the most basal extant angiosperm was controversial for a while. Goremykin *et al.* (2003) analysed 61 protein-coding genes common to 13 fully sequenced land plant chloroplast genomes, which placed the origin of *Amborella* later than the origin of the monocots. However, the controversy was short-lived and the position of *Amborella* as sister to the other angiosperms has been confirmed by numerous other studies (see detailed discussion in Stefanovic *et al.* 2004; Soltis and Soltis 2004; Soltis *et al.* 2004; Martin *et al.* 2005; Lockhart and Penny 2005). This controversy highlights some of the important considerations involved in designing approaches to reconstruct phylogeny, and also emphasizes the point that any phylogenetic tree can only be viewed as the current best hypothesis based on available data.

1.2 Seed plant reproductive structures

The reproductive structures of most plant lineages prior to the angiosperms, including most of the gymnosperms, were unisexual. The evolution of seeds freed plants to reproduce in the absence of a film of external water (previously necessary to allow fragile free-swimming male gametes to fuse with a static egg). In seed plants the female gametophyte is surrounded by parental sporophyte tissue, usually derived from bracts. These enfolding bracts act to protect the ovule and may also serve as a protective coat when the seed is dispersed. In gymnosperms the ovule is not completely enclosed in sporophyte tissue, but is protected within a chamber, into which wind-blown pollen is drawn after being trapped by a drop of secreted liquid.

In the cycads, dioecy is the rule, with female plants producing clusters of ovules on the edges of modified leaves called megasporophylls. The whole female reproductive structure forms an ovulate cone. Male plants produce cones of differently specialized leaves, the scale-like microsporophylls, arranged in a spiral phyllotaxis to produce a cone. These microsporophylls possess a pollen sac on their abaxial surface, each of which produces numerous pollen grains. A similar system operates

in *Ginkgo*, although the ovules are born in pairs on stems rather than as a cone. In contrast, most conifers are monoecious, producing male and female cones on the same individual plant, although some taxa within the group are dioecious (such as the junipers and yews). The Gnetophytes are usually dioecious, although some species of the genus *Ephedra* are monoecious. Analysis of cone structure has revealed that the pollen cone of conifers is a condensed branch with the microsporophylls representing the modified leaves along the branch. However, the seed cone of conifers, and both cone types in the Gnetophytes, are thought to be derived from a condensed branch with branches. In this scenario the central stem produces bracts with modified stems in their axils—the modified stems are the scales of the cone (Judd *et al.* 2007).

1.3 The first flowers

Despite the absence of a clear picture of the last common ancestor of angiosperms and gymnosperms, there are clearly some key innovations that arose in the angiosperm lineage and gave rise to flowers, such as the combining of male and female reproductive structures within a small space on the same shoot and the production of perianth organs (Theissen *et al.* 2002). Indeed, it is these innovations that give us the modern definition of a flower (*the bisexual reproductive shoot of an angiosperm, in which the reproductive organs are surrounded by whorls of sterile organs*). Analysis of the development of these innovations allows us to build a general picture of the evolution of the first flowers.

1.3.1 A bisexual reproductive shoot

The typical angiosperm flower is hermaphrodite, and this is believed to be the ancestral condition for flowers. Although there are many examples of derived unisexual flowers—either on hermaphroditic plants (monoecy) or on unisexual plants (dioecy)—the majority of angiosperm flowers produce both male and female reproductive organs. This is in marked contrast to the reproductive structures of most other plant lineages, where unisexual reproductive structures are the norm. In the gymnosperms, as described above, the two types of

unisexual reproductive cones occur either together on a single plant or each on a separate plant. The combination of male and female organs on a single shoot is an angiosperm innovation. The development of a single shoot containing both male and female reproductive organs was therefore a key event in the evolution of flowers.

Discussion of the development of a bisexual reproductive shoot is complicated by the lack of clear homology between gymnosperm cones and angiosperm flowers. Since the cones of cycads and the pollen cones of conifers are condensed branches with the scales representing leaves, they could be described as homologous to a flower (with the reproductive and perianth organs derived from leaves). However, the compound cones of the Gnetophytes and female conifers are clearly not homologous to flowers. In these cones the scales are modified stems, and so the structure is much more like an angiosperm inflorescence, where each flower is formed from a separate axillary meristem. Whatever the homology relationships, the evolution of the flower required the development of a bisexual shoot, which may then have required much subsequent reduction to form the flowers we see today. Assuming that the ancestor of angiosperms and gymnosperms produced its male and female reproductive structures on separate shoots, each composed of a spiral of organs, then the development of a bisexual shoot (and later flower) requires either the development of female organs at the top of the male shoot or the development of male organs at the base of the female shoot. All angiosperm flowers contain an outer whorl of male reproductive organs and an inner whorl of female reproductive organs, suggesting that this evolution of bisexual flowers occurred only once (Cronk 2001). A single exception to this rule (with male reproductive structures inside a whorl of female ones) has been identified in the inside-out flower of *Lacandonia schismatica* (Pandanales), as a result either of homeotic organ conversion or of reduction of the inflorescence to resemble a flower (for a review, see Garay-Arroyo *et al.* 2012).

An adaptive explanation for the combination of male and female reproductive organs in the same shoot was proposed by Frohlich (2002), who observed that ectopic sterile ovules are sometimes

produced by the male reproductive cones of the gnetophyte *Welwitschia*, and appear to attract pollinating insects to the male branches by exuding the same droplets of liquid that are secreted by fertile female ovules (Endress 1996). This would provide a selective advantage in terms of male fitness (pollen export) to a male shoot with additional female characters. From there it is easy to imagine the full feminization of the ectopic ovules into fertile structures. Bisporangiate cones are also occasionally produced by other gymnosperms, and the presence of ovule droplets is commonly reported in these cones (Rudall *et al.* 2011). Flores-Renteria *et al.* (2011) even observed that the bisporangiate cones of *Pinus johannis* were fully fertile, unlike the sterile ovules in male cones reported in *Welwitschia*. Other authors have noted that bisexual shoots facilitate selfing, which is potentially of great importance to plants (like the early angiosperms) invading new habitats.

Several models have been proposed to explain the evolution of the bisexual shoot from a molecular genetic perspective. These focus on two sets of genes—those controlling the conversion of the meristem to the reproductive form (particularly *LEAFY*; see Chapters 7 and 9), and those controlling the production of male organs in the angiosperm flower (B function genes; see Chapter 10). This section will outline these models, but the reader might find the models clearer when they have read later chapters on floral meristem and floral organ development.

The first model to take a genetic approach to the evolution of bisexual flowers was the Mostly Male Theory. In this model the bisexual shoot evolved by the production of ectopic ovules in the centre of a male cone, the cone retaining a ‘mostly male’ identity (Frohlich 2002). This hypothesis was based on analysis of sequence and expression patterns of genes predicted to regulate gymnosperm cone development, which suggested that an angiosperm gene required for flower production (*LEAFY*) is most closely related to a gymnosperm gene involved in male (but not female) cone development (Frohlich and Parker 2000). The *PrFLL* gene of *Pinus radiata* (and the equivalent gene in *Welwitschia* and *Ginkgo*) is very similar to the angiosperm *LEAFY* gene, which encodes a protein crucial in determining the floral nature of the meristem. In *Pinus*

radiata, *PrFLL* is expressed in male cones only, and a duplicate gene, *NEEDLY*, which has been lost from the angiosperm lineage, is expressed only in female cones. Frohlich interpreted these observations as implying that the angiosperm flower is descended from an ancestor of the male gymnosperm cone. In the ancestral angiosperm lineage the genes responsible for ovule development were recruited to the control of *PrFLL*, resulting in the production of ectopic ovules, and eventually the evolution of carpels. However, more recent data have largely disproved the exclusively male expression of *PrFLL* and the exclusively female expression of *NEEDLY* in gymnosperms. First, Shindo *et al.* (2001) showed that the *PrFLL*-like gene of *Gnetum parvifolium* is expressed in the female reproductive structure, rather than the male one. Dornelas and Rodriguez (2005) similarly showed that the *PrFLL*-like gene of *Pinus caribaea* is expressed in female, but not male, cones. Vazquez-Lobo *et al.* (2007) reported expression patterns for the *PrFLL*-like and *NEEDLY* genes from *Picea abies*, *Podocarpus recihii* and *Taxus globosa*, finding that both genes are expressed in both male and female reproductive structures of all three species. A recent comparison of transcriptome profiles of the male and female cones of *Ginkgo biloba*, the cycad *Zamia fisheri* and the gnetophyte *Welwitschia mirabilis* with the transcriptomes of flowers of *Arabidopsis thaliana* (Brassicales) and *Oryza sativa* (Poales) detected no significant differences between the proportion of gymnosperm orthologous genes that were expressed both in the male cone and in the angiosperm flowers, and the proportion of gymnosperm orthologous genes that were expressed both in the female cone and in the angiosperm flowers (Tavares *et al.* 2010). From this analysis the authors concluded that the angiosperm flower was not a ‘mostly male’ structure, as its transcript content did not more closely resemble a male cone than a female cone.

If the bisexual shoot did not evolve by the ectopic development of ovules on a male cone (or vice versa), it must have evolved by the conversion of organs at the axis of a male cone into the female form, or by the conversion of the organs at the base of a female cone into the male form. These homeotic models do not imply that the flower is predominantly male or female, but that conversion