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Molecular Mechanisms of Pollination Biology

Róisín Fattorini and Beverley J. Glover

Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom; email: bjg26@cam.ac.uk

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Keywords

adaptation, flower, olfactory cue, pollination, transcriptional regulation, visual cue

Abstract

Pollination is the transfer of pollen grains from the stamens to the stigma, an essential requirement of sexual reproduction in flowering plants. Cross-pollination increases genetic diversity and is favored by selection in the majority of situations. Flowering plants have evolved a wide variety of traits that influence pollination success, including those involved in optimization of self-pollination, attraction of animal pollinators, and the effective use of wind pollination. In this review we discuss our current understanding of the molecular basis of the development and production of these various traits. We conclude that recent integration of molecular developmental studies with population genetic approaches is improving our understanding of how selection acts on key floral traits in taxonomically diverse species, and that further work in nonmodel systems promises to provide exciting insights in the years to come.

Contents

Pollination: the transfer of pollen grains from the stamens to the stigma, the receptive region of the female reproductive organ

Autogamy: transfer of pollen from the stamen to the stigma of the same flower

Geitonogamy: transfer of pollen from the stamens of one flower to the stigma of another flower on the same plant

Apomixis: the development of an embryo and seed from the diploid tissues of the maternal plant

Fertilization: the fusion of a sperm nucleus with an egg nucleus to generate a zygote and subsequent embryo

INTRODUCTION

Pollination is the transfer of pollen grains (male gametophytes, carrying male gametes) from the stamens to the stigma, the receptive region of the female reproductive organ. It is essential for sexual reproduction in all seed plants, and without it reproduction can only occur through asexual, vegetative mechanisms. Pollination can occur within a single flower (a form of self-pollination known as autogamy), between flowers of an individual plant (another form of self-pollination, known as geitonogamy), and between flowers of different plants (cross-pollination) (**Figure 1**). Cross-pollination introduces the potential for the greatest genomic variation in the next generation. Self-pollination results in considerably more variation than asexual reproduction (including vegetatively reproducing systems and seed production through apomixis) due to the potential for recombination of alleles through chiasma formation during meiosis.

Historically, pollination biology has been studied from a whole-organism perspective, focused on understanding the relationships between different flowers and their agents of pollen transfer. More recently, we have gained the genetic understanding and technical abilities that allow us to explore the molecular mechanisms underpinning the development of floral features that influence pollination. These studies follow the developmental genetic revolution of the 1990s and early 2000s, when the molecular basis of flower development, including floral organ identity specification, was described. With those foundations in place, it is now possible to explore the molecular developmental basis of more subtle traits, such as the relative heights of stigma and stamens, patterns of pigmentation within petals, or the production of floral volatiles, all of which influence the ways that different pollen vectors interact with the flower.

In this review, we discuss recent insights from molecular and genetic studies that shed light on the cellular mechanisms facilitating pollination. We focus only on traits influencing pollen transfer itself and do not consider later processes, such as those related to fertilization or its inhibition.

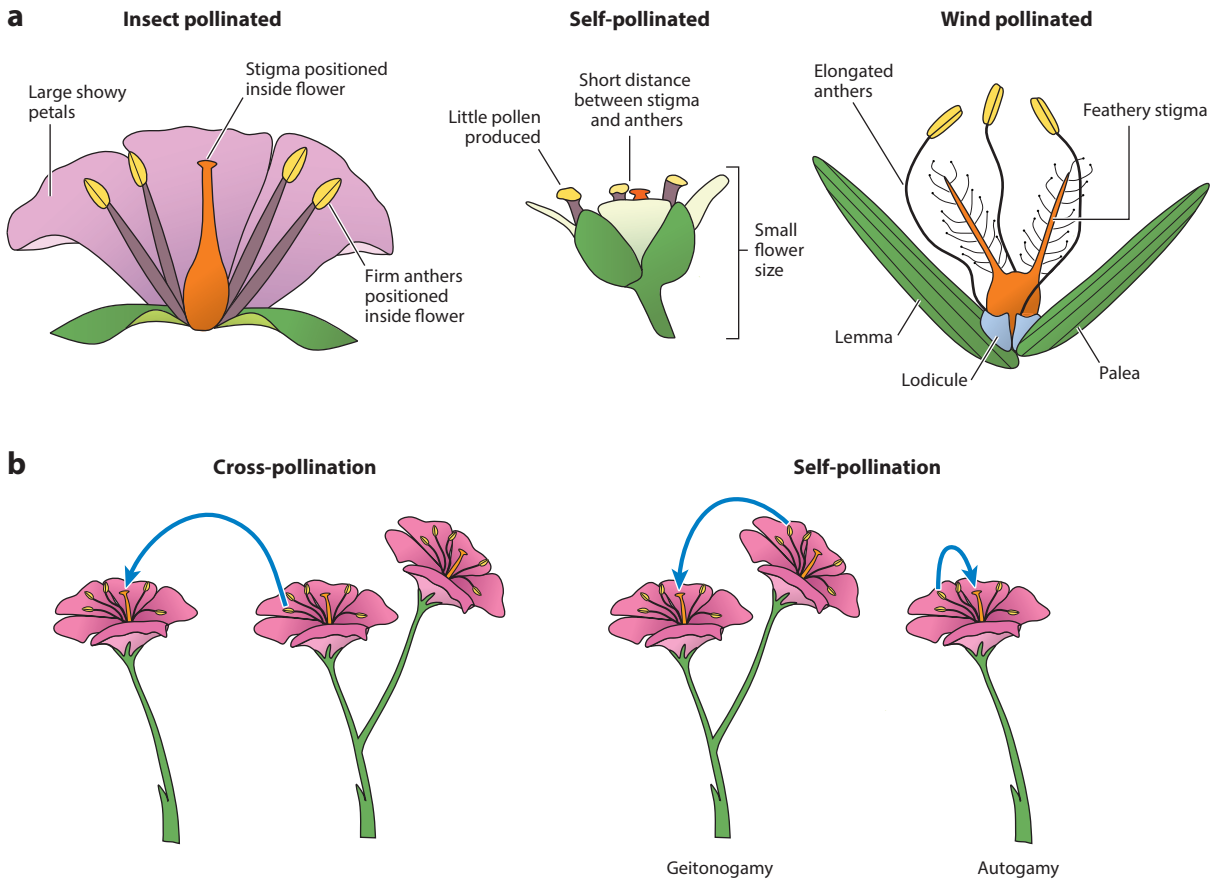

Figure 1

Comparative diagram indicating (*a*) key differences among flowers adapted for insect pollination, self-pollination, and wind pollination, and (*b*) mechanisms of cross-pollination and two forms of self-pollination.

ADAPTATION AND POLLINATION BIOLOGY

Establishing whether floral traits are adapted to attract pollinators is an important prerequisite to the investigation of molecular mechanisms underlying pollination biology. The basis of adaptation is the selective maintenance of genetic mutations that cause developmental change, resulting in heritable differences in phenotype that alter organism function and enhance fitness (12, 29). Nectar secondary metabolites may be adapted to deter ineffective pollinators and encourage specialists (37). However, secondary metabolites in leaves function in herbivore defense, and leaf secondary metabolite concentrations can be positively correlated with those found in nectar (89). This suggests that nectar secondary metabolite expression may simply result from pleiotropic constraints (1). Evidently, to avoid "proposing an adaptive story for each (trait) considered separately" (60, p. 581), the selective pressures contributing to floral adaptation must be conceptualized within the context of the whole organism. When a particular trait undergoes evolutionary developmental change, this has a functional effect on dependent traits. The trait burden of an individual trait is defined by the number and magnitude of the dependent traits (39). The trait burden concept, along with an understanding of other phenomena that drive evolutionary change in a whole-organism

context, allows us to deduce the most probable scenarios in trait evolution, which also requires an understanding of how developmental programs are modified (36). Derived traits can be altered from the ancestral form through changes in developmental programs: Heterochrony defines temporal changes in gene activity and organ development, and heterotopy describes spatial (positional) changes—a combination of both can occur. Identifying the molecular basis of phenotypic evolution requires positive feedback between hypotheses exploring floral diversification and empirical observations of gene expression, development, and structural constraints in a phylogenetic context (29).

Heterochrony: evolutionary change that occurs through an alteration to the timing of a developmental process

Heterotopy: evolutionary change that occurs through an alteration to the spatial position of a developmental process

Monoecious: having separate male flowers and female flowers on the same individual plant

Dioecious: producing separate male individuals and female individuals

One of the major factors determining patterns of floral evolution is the influence of external forces, including pollinators (36). Yuan et al. (175) commented that the links asserted from genotype to phenotype to pollinator response in the literature are based on "different standards of evidence" and that this can impede a comprehensive understanding of the genes underlying interactions between flowers and pollinators (p. 422). Ideally, a clear link between genotype and phenotype is established through experimentation, and pollinator response to the relevant trait is tested using foraging assays (175). This is not always possible for emerging developmental genetic systems, since pollination research may be conducted in parallel or not yet established in the relevant system. As such, we encourage critical application of the concepts outlined above in equating the data discussed here with floral phenotypes adapted for pollination.

WIND POLLINATION

The nonangiosperm seed plants are mainly pollinated by abiotic agents. Ancestral state reconstructions suggest that the earliest angiosperms were animal pollinated, although current estimates suggest that around 87.5% of extant angiosperm species are animal pollinated, with the rest having lost the ancestral trait (68, 105). Of these, self-pollination and wind pollination account for the large majority, and wind pollination is estimated to have evolved at least 65 times (83). Wind pollination is considered less efficient than animal pollination, so the frequency of this transition is unexpected (108). Friedman & Barrett (54) established that the shift to wind pollination occurs most frequently in lineages with unisexual flowers (monoecious or dioecious systems), in which a transition to self-pollination would be harder to achieve and/or less effective. They therefore hypothesized that the transition to wind pollination occurs as an adaptive response to limited pollinator availability.

The suite of morphological features associated with wind pollination is well defined (49), although there are very few molecular-genetic studies analyzing the development of these traits. These floral traits include a feathery style, elongated anthers, and many dry pollen grains of consistent size lacking surface ornamentation (**Figures 1** and **2*a–c***). In addition, absence of pollinator-attracting traits, namely petals, nectaries, and scent, is associated with wind-pollinated flowers. Wind-pollinated plants usually produce many flowers, and their flowering is often synchronous within populations (reviewed in 55). Work exploring the molecular mechanisms underpinning the development of this suite of traits is limited. Few conventional floral model species exhibit wind pollination, and comparative studies are hindered by the limited number of intrageneric systems in which there are both wind-pollinated and animal-pollinated species. One such system is *Thalictrum*, in the Ranunculaceae, where multiple evolutionary transitions have occurred between wind-pollinated species and small, generalist, animal-pollinated species (162). Phylogenetic analyses suggest repeated transitions in both directions within this genus, with associated changes in floral scent, floral organ size, and flower color creating the potential for a range of comparative molecular analyses (33, 162).

Despite the shortage of wind-pollinated systems in which flower development has been studied, molecular mechanisms that may underpin some of the key traits can be inferred from work

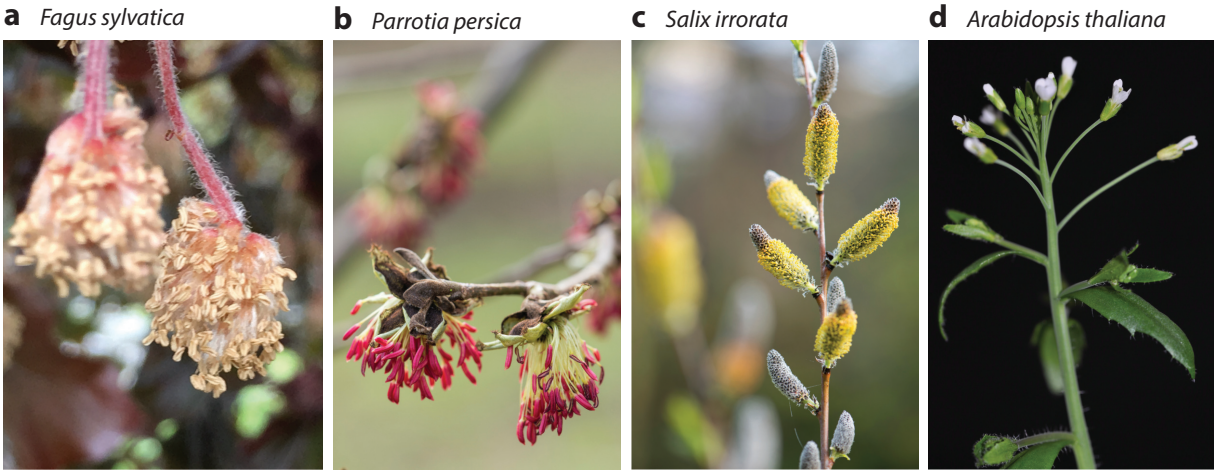

Figure 2

Wind-pollinated and self-pollinated flowers. (*a*) The male flowers of wind-pollinated *Fagus sylvatica* with many stamens. (*b*) Wind-pollinated flowers of *Parrotia persica*. (*c*) Male flowers of wind-pollinated *Salix irrorata*. (*d*) The self-pollinated flowers of *Arabidopsis thaliana*. Photos provided by Howard Rice and the Cambridge University Botanic Garden.

in animal-pollinated species. Studies in *Petunia exserta*, for example, have identified quantitative trait loci (QTLs) associated with the increase in cell number that leads to stigma and stamen exsertion (66). Many wind-pollinated species undergo synchronous or mast flowering to maximize the quantity of pollen that reaches stigmas (55). Studies in a number of systems, but most comprehensively in the self-pollinated *Arabidopsis thaliana*, have established a set of endogenous and exogenous pathways regulating the timing of flower induction (reviewed in 115). The relative contribution of each of these pathways to the initiation of flower development varies depending on environmental conditions. These pathways, particularly those responding to day length and temperature, may be part of the explanation for the synchronous or mast flowering of many wind-pollinated species (13). Tight connectivity between the environment and flowering response may act to ensure that local populations of the same species flower together, increasing the likelihood of successful wind pollination. Even in tropical habitats, where seasonal variation is limited and day-length changes are proportionally smaller, studies have shown that environmental triggers, such as the precise time of sunset or sunrise, may be sufficient to induce synchronous flowering (13).

Perhaps the best-studied example of a floral adaptation to wind pollination is the lodicule found in flowers of the grass family (Poaceae). These structures, composed of small scales, form in the floral whorl outside the stamens, where petals would be found in an animal-pollinated species. When the flower is mature, the lodicules swell rapidly, opening up the protective outer lemma and palea and exposing the reproductive structures to the wind. Early studies in maize demonstrated that *SILKY1*, a MADS box gene, is necessary for lodicule development, as the lodicules in the *silky1* mutant are converted to palea-like structures (6). Since *SILKY1* is the maize ortholog of *APETALA3* (*AP3*), which confers petal identity in *Arabidopsis*, the grass lodicule was interpreted as homologous to the eudicot petal, sharing key elements of development, including its master regulation. Since then, numerous studies in maize, rice, and barley have found overlaps between lodicule and petal development, particularly with respect to the activity of B and E class genes of the floral ABCDE model (reviewed in 171).

Mast flowering: synchronous flowering of large numbers of plants of the same species in a geographic region

Lodicule: the organs in the second floral whorl of grass flowers, which swell to open the flower

Lemma: the outer of two protective sepal-like organs in the first whorl of a grass flower

Palea: the inner of two protective sepal-like organs in the first whorl of a grass flower

ATTRACTING ANIMAL POLLINATORS

Floral Rewards

Volatile organic compound (VOC): lipophilic molecules with low boiling points and high vapor pressures at ambient temperatures, potentially detectable as scent

Nectary: the site of nectar secretion; may take various forms and occur on various organs

Angiosperm flowers and animal pollinators often have reciprocally beneficial interactions; when pollinators access floral rewards they can enhance plant reproductive success by collecting and exporting pollen. Nectar and pollen provide nutritive rewards for pollinators as well as additional benefits including heat sources, nesting materials, and sites for brooding, sleeping, and mating (9). Nectar is produced by many plant species, and its components include sugars, volatile organic compounds (VOCs), and amino acids (14, 35). The production and chemical composition of nectar, nectary morphology and development, and the regulation of these processes are the focus of a recent review by Roy et al. (128). Currently, we have limited understanding of the mechanisms regulating nectar secretion. A recent study in *A. thaliana* provided insight into how the plant hormone jasmonate contributes toward nectar production in coordination with auxin responses, demonstrating that nectar secretion may be induced by auxin acting downstream of jasmonates (135). *Arabidopsis* and *Nicotiana* spp. provide useful models of nectary development and nectar synthesis, but it is likely that molecular mechanisms differ between taxa because nectaries have evolved independently many times (128). As such, omics techniques may prove particularly informative in the identification of potential candidate genes and subsequent elucidation of nectary developmental pathways in nonmodel species. Solhaug et al. (141), for example, recently produced nectary transcriptomes of *Cucurbita pepo*, identifying the genes and corresponding metabolic processes temporally regulated as the nectary develops. The availability of these genetic resources and new innovations in genetic engineering should accelerate our ability to link genotype to phenotype.

Floral Signals

The mutualism between flowering plants and pollinators relies on effective communication, with floral signals optimized to inform the receiver and enhance signal detection within a noisy environment (48). Pollinators select flowers through recognition of floral displays (**Figure 3**), often involving multimodal signals including visual, olfactory, tactile, and thermal stimuli (reviewed in 120). Many pollinator choice experiments have confirmed the importance of visual and olfactory stimuli in pollinator attraction, and these stimuli often work synergistically (78). Both olfactory and visual cues are required to stimulate generalized nectar feeding responses in the hawkmoth *Manduca sexta* feeding on *Datura wrightii* (121). Attraction of mosquitoes to *Tanacetum vulgare* is heightened when plants emit visual and olfactory signals, relative to olfactory signals only (109). Whether floral traits are perceptible is dependent on pollinator sensory capabilities; for example, interpretation of floral coloration within bee color space demonstrated clusters of flower color in wavelengths where bee visual discrimination is greatest (75, 110). Understanding pollinator perception can provide insight into functionality; for example, bull's-eye patterning and nectar guides may facilitate insect landing and signal reward locality as they are only perceptible to insects at short distances from the flower (82). The attraction responses that flowers elicit are due to pollinator innate preferences, associative learning abilities, and pre-existing biases (9). Potential exploitation of these pre-existing biases is evident in insect pollinators' preferential attraction to floral volatiles that are also emitted by insects (118, 160). Quantification of pollinator responses to natural variants or experimentally altered floral phenotypes is an important component of assessing the relevance of candidate genes to pollination biology.

Mimulus as a Focal System

Although we reference multiple animal-pollinated species, *Mimulus* is our primary focus in exploring attractive floral features. This is due to particularly thorough experimental evidence linking

Figure 3

Photographs of different animal-pollinated flowers. (*a*) Fly-pollinated *Selinum carvifolia*. Features traditionally associated with fly pollination include small, pale-colored flowers. (*b*) Bee-pollinated *Phacelia tanacetifolia*. Features traditionally associated with bee pollination include blue color and visible pollen. (*c*) Moth-pollinated *Angraecum sesquipedale*. Features traditionally associated with moth pollination include long nectar spurs, zygomorphy and white color. (*d*) Butterfly-pollinated *Calendula officinalis*. Features traditionally associated with butterfly pollination include yellow color, a landing platform, and nectar held in short tubes. (*e*) Bird-pollinated *Aquilegia canadensis*. Features traditionally associated with bird pollination include red color and nectar held in short, wide spurs. (*f*). Bat-pollinated *Strongylodon macrobotrys*. Features traditionally associated with bat pollination include the position of the flowers hanging outside the vegetation and a plentiful supply of nectar. Photos provided by Howard Rice and the Cambridge University Botanic Garden.

genetic changes in floral phenotype to pollinator responses within this system, summarized by Yuan et al. (175). *Mimulus* contains over 120 species with a wide variety of floral phenotypes, mating systems, and pollinators (107). It is experimentally tractable with utility for investigating developmental genetics of floral traits (reviewed in 174), and studies of this genus imply a pollinator-mediated contribution toward reproductive isolation (123, 146). We focus on recent

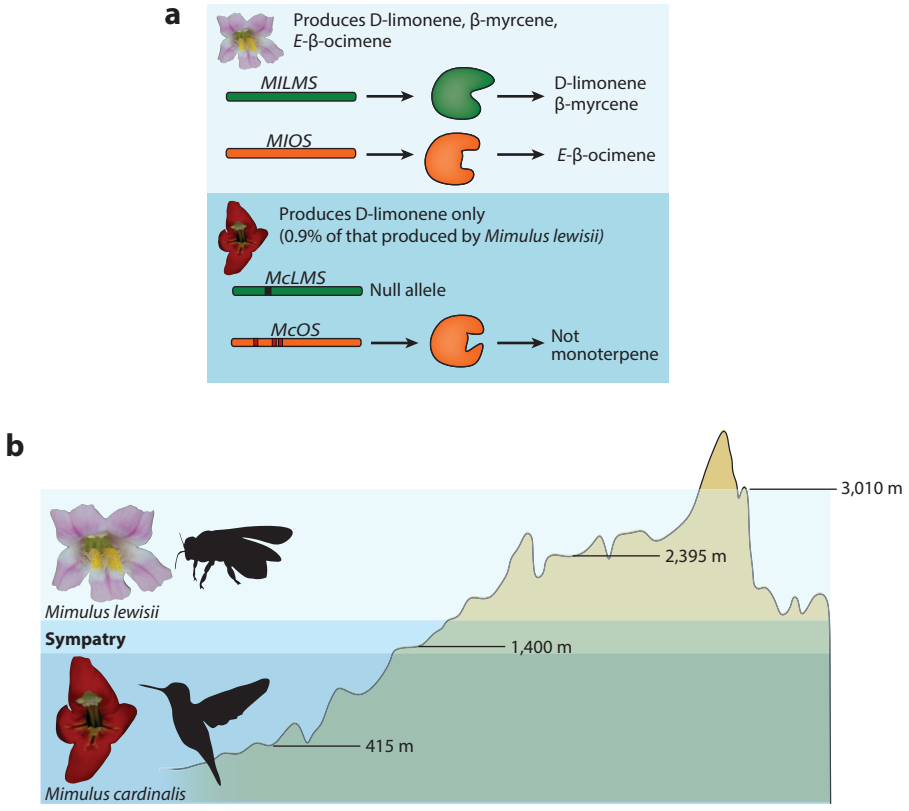

Figure 4

The diversification of floral form in *Mimulus* in response to selective pressure from different types of pollinators. (*a*) The genes underlying differences in volatile production between sister species (20). (*b*) A schematic representation of sister species *Mimulus cardinalis* and *Mimulus lewisii* elevation ranges in Yosemite National Park. Panel *b* adapted with permission from Angert & Schemske (7), copyright Society for the Study of Evolution.

developments in genetic regulation of pollinator-attracting features, particularly pigmentation and volatile emission (**Figures 4** and **5**). Our discussion is not exhaustive, and other reviews with additional information and perspectives are referenced throughout.

Carotenoid: terpenoid-derived lipid-soluble pigment in the yellow-red range

Betalain: tyrosine-derived pigment found only in the Caryophyllales, an order including beets, cacti and carnations; can be yellow or red-purple

Floral Pigmentation

Flower color is established predominantly through pigmentation with flavonoids, carotenoids, and betalains (34). Flavonoids produce the widest spectrum of pigments, including white or ivory flavones, flavonols, and flavanones; yellow chalcones and aurones; and anthocyanins (61). Anthocyanins have the broadest distribution of any floral pigment (61); accumulating in cell vacuoles, they produce red, pink, purple, black, and blue coloration. The anthocyanic petal hue is partly dependent upon vacuolar pH, which can alter the redox state of flavonoid molecules, causing changes in wavelengths of light absorbed (180). The anthocyanin biosynthesis pathway begins with chalcone synthase catalyzing the formation of tetrahydroxychalcone. The pathway is well characterized in many systems and explained in detail by Grotewold (61). Transcriptional

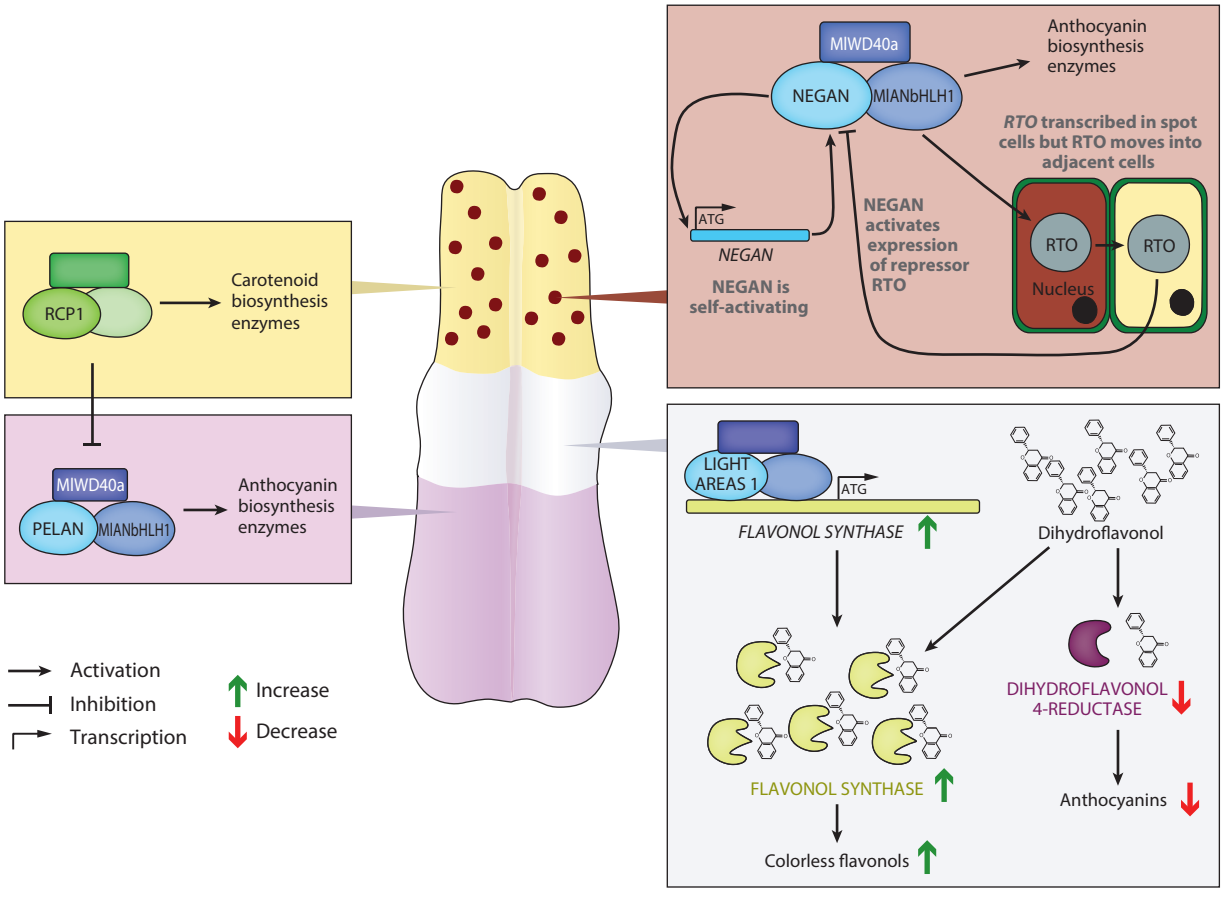

Figure 5

The genetic processes thought to underlie pigmentation traits in *M. lewisii* petals are outlined for (*clockwise from the top right*) anthocyanin spots (38), reduction in anthocyanin in the white region of the corolla throat (176), anthocyanin pigmentation in the petal lobe (177), and carotenoid pigmentation in nectar guides (130). Black arrows indicate regulation of a gene/protein or synthesis of a product, green and red arrows indicate a relative increase or decrease in a particular substrate/enzyme/expression of a gene/product. RCP1 is depicted as a component of an MBW complex; however, currently no partners have been identified. Abbreviations: MBW, MYB-bHLH-WD repeat; NEGAN, NECTAR GUIDE ANTHOCYANIN; PELAN, PETAL LOBE ANTHOCYANIN; RCP1, REDUCED CAROTENOID PIGMENTATION 1; RTO, RED TONGUE.

regulation of anthocyanin synthesis is understood in a diverse range of species. Anthocyanin production is controlled by members of R2R3 MYB and basic helix-loop-helix (bHLH) families that form a complex with WR-repeat (WDR) proteins to activate enzymes within the anthocyanin biosynthesis pathway (34, 61). The interactions and functions of MYB-bHLH-WDR complexes in anthocyanin production, conserved across divergent taxa, are reviewed by Xu et al. (168), and negative regulation is discussed by Chen et al. (22).

Anthocyanin: phenylpropanoid-derived water-soluble pigment in the red-blue range

Betalains only occur within the *Caryophyllales* (25), where their production is mutually exclusive with that of anthocyanins (88). Betalains can be subdivided into betacyanins, which produce violet coloration, and betaxanthins, which form yellow pigment (56). Betalains are defined by inclusion of the chromophore betalamic acid (18); both anthocyanins and betalains use arogenate as a precursor, but anthocyanins are phenylalanine derived and betalains are tyrosine derived

Transcription factor: a protein that binds specific DNA sequences in gene regulatory regions and affects gene expression as a consequence

(18, 88). Structural genes within the betalain biosynthetic pathway are described by Polturak & Aharoni (114). Located in the vacuoles, betalains can maintain red coloration regardless of vacuolar pH (72). The transcriptional regulation of betalains remains largely unknown, although MYB transcription factors have been implicated; for example, an anthocyanin MYB-like protein (MYB1) regulates betalain synthesis in *Beta vulgaris* (63).

Carotenoids are lipophilic isoprenoid compounds responsible for yellow, orange, and red coloration; they also contribute to brown and bronze hues in combination with anthocyanins (14, 53). A diverse array of species have carotenoid floral pigmentation, including *Gerbera*, *Lilium*, and *Narcissus* (10, 156, 157). Carotenoids functioning in floral pigmentation are stored in specialist plastid structures called chromoplasts where they accumulate in high quantities (101). The 2-*C*-methyl-D-erythritol 4-phosphate (MEP, or simply methylerythritol) pathway synthesizes carotenoids and also produces apocarotenoids, including volatiles involved in pollinator attraction (92). The carotenoid biosynthesis pathway is outlined by Sun et al. (149).

Carotenoid pigmentation functions in pollinator attraction in *Mimulus*. The presence or absence of yellow carotenoids is controlled by the locus YELLOW UPPER (YUP) in *Mimulus cardinalis*, a red-flowered species pollinated predominantly by hummingbirds, and *Mimulus lewisii*, which has pink flowers and is mainly bee-pollinated (16). These species were crossed to form hybrids (132), and near-isogenic lines (NILs) were produced, where *M. cardinalis* NILs containing the *M. lewisii* YUP allele had dark pink flowers and *M. lewisii* NILs with the *M. cardinalis* YUP allele had yellow-orange flowers (15). The bee visitation rate was negatively correlated with petal carotenoid and anthocyanin concentration in the hybrid study, and, consistent with this, bees had a strong preference for NIL pink *M. cardinalis* over red-flowered wild types. The hummingbird visitation rate was positively associated with petal anthocyanin concentration in crossing experiments, but hummingbirds preferred yellow-orange-flowered NIL *M. lewisii* over the pink-flowered wild type (16, 132). Evidently, a shift in pollinator guild can be induced by altering a single locus involved in floral carotenoid pigmentation within these species (**Figure 4**). Subsequently, a study investigating an *M. lewisii* mutant lacking nectar guide carotenoid pigmentation and a mutant with reduced petal-color pattern found that trait loss significantly reduced the bee visitation rate relative to wild-type plants (107). This functional evidence underpins the *Mimulus* floral genetic studies discussed below.

Variation in carotenoid pigmentation of flowers can be due to differential expression of genes in the carotenoid pathway; however, our knowledge of this regulation is still somewhat rudimentary (93). Sagawa et al. (130) identified the first transcription factor known to regulate carotenoid biosynthesis during flower development: Reduced Carotenoid Pigmentation 1 (RCP1) regulates carotenoid pigmentation in the yellow nectar guides (107) of *M. lewisii* (**Figure 4**). This subgroup 21 R2R3 MYB transcription factor was identified from bulk segregant analysis using a mutant with reduced carotenoid concentration in nectar guides (*rcp1-1*). In wild-type plants, *RCP1* was upregulated in nectar guide positions prior to structural gene expression, suggesting that RCP1 may be a transcriptional activator of carotenoid biosynthesis pathway enzymes. RNA interference was used to downregulate *RCP1* expression and, together with overexpression of the gene in *rcp1-1* mutants, demonstrated that *RCP1* is both necessary and sufficient for induction of a strong carotenoid phenotype. Stanley et al. (147) followed a similar line of inquiry characterizing the *RCP2* locus. *rcp2* mutants also exhibit reduced nectar guide carotenoid content due to downregulation of carotenoid biosynthesis genes. *RCP2* was mapped to a tetratricopeptide repeat protein (TPR), a family known to be involved in chloroplast development (69). Transgenic plants with inhibited *RCP2* expression had a carotenoid phenotype resembling the *rcp2-1* mutant, although *RCP2* sufficiency for inducing the wild-type carotenoid phenotype was not investigated (147). The authors speculated that RCP2 may be involved in chromoplast development, due to

a lack of properly differentiated chromoplasts in *M. lewisii rcp2* mutants compared to wild type. As chromoplasts are crucial for accumulation of floral carotenoids, the regulation of chromoplast biogenesis is an important aspect in carotenoid production (149), and this has been investigated in *Arabidopsis thaliana* (173) calli and *Cucumis melo* (153) fruit. Investigating the regulation of floral carotenoids in the context of chromoplast development and within taxonomically broad species will provide important insights.

Floral Patterning

Typically, floral patterns are due to differences in pigmentation within the corolla between petal cells (34). These petal patterns include stripes, spots, and bicolor flowers that can mediate plant–pollinator interactions (57, 82, 137). Floral patterns can also be created by differential coloration between floral organs; for example, *Commelina communis* L. has blue petals with contrasting yellow anthers that increase the number of pollinator landings and aid orientation toward landing points (155). The genetic mechanisms underlying floral patterns (reviewed in 34) spatially restrict pigmentation through various means. In *M. lewisii*, competition for substrates between the anthocyanin pathway and flavonol pathway causes color patterning (**Figure 4**). In the predominantly pink corolla of *M. lewisii*, an R2R3 MYB transcription factor (LAR1) indirectly represses anthocyanin synthesis in a section of white encircling the corolla throat—a phenotype thought to be important in bumblebee pollination (107, 176). LAR1 activates the expression of a gene encoding a flavonol synthase within the white petal area, which diverts dihydroflavonol substrates from the anthocyanin pathway to colorless or ivory flavonol production (176). Spot development in *Clarkia gracilis* is also initiated by spatially restricted expression of a gene coding for an enzyme. Dihydroflavonol-4-reductase is an anthocyanin biosynthesis enzyme activated by an R2R3 MYB transcription factor (CgMyb1). Variation in corolla spot position between *Clarkia* species is due to *cis*-regulatory differences in the promoter sequences of each *CgMyb1* allele (90, 91). Behavioral preferences of insect pollinators for petal spots and potential fitness gains associated with spotted phenotypes have been demonstrated in *Clarkia* (46). It would be interesting to determine whether spot position also influences pollinator interactions.

Interspecific comparisons of genetic pathways provide insight into evolutionary aspects of patterning. However, gaining a broader developmental perspective requires understanding of how adjacent cells adopt distinct fates within a petal during tissue development. A recent *Petunia* study characterized a putative R2R3 MYB repressor and incorporated these findings into a model of the anthocyanin gene regulatory network, also utilizing previous research on floral pigmentation patterning by MYB-bHLH-WD repeat (MBW) protein complexes and R3 MYB repressors (3). The model suggests that cell-specific anthocyanin production can be achieved through a set of activators and repressors acting together through a series of feedback loops (3). Similarly, in *M. lewisii* and *Mimulus guttatus* an R2R3 MYB activator and an R3 MYB repressor were identified that regulate petal anthocyanin spots (**Figure 4**) (38). The mode of action of these *Mimulus* genes during development is compatible with the classic Turing instability known as the reaction–diffusion model (152) [outlined by Yuan (174)], but this remains to be demonstrated. This model can explain spatial patterns in tissues through interactions between a self-activating activator and a repressor protein that is capable of activator inhibition along a diffusion gradient (94, 142). Regulation of anthocyanin synthesis by the MBW complex has been found in many systems. Within *Antirrhinum majus*, anthocyanin-pigmented venation induces similar pollinator visitation rates as full red phenotypes (137). Venation is regulated by an R2R3 MYB transcription factor (VENOSA) with circum-vein expression and, most likely, a bHLH regulator produced in the epidermis (**Figure 6**). This results in an anthocyanin phenotype restricted to epidermal cells above

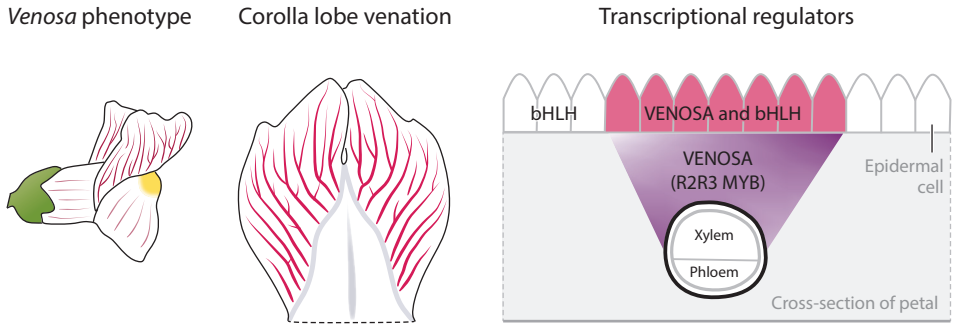

Figure 6

Diagram of *Antirrhinum majus* flower with the *Venosa* phenotype, close-up of corolla lobe venation patterning, and transcriptional regulators inducing pigmentation. Anthocyanin production is thought to occur in the epidermis above floral veins due to overlap in the expression between an R2R3 MYB transcription factor (VENOSA) and a basic helix-loop-helix (bHLH) transcription factor (e.g., DELILA) (137). Figure adapted from Davies et al. (34), with permission from CSIRO Publishing.

veins. R3 MYB repressor proteins are unlikely to inhibit *VENOSA* expression in unpigmented regions, as overexpression of *VENOSA* within these areas induces anthocyanin production. Rather, *Antirrhinum* venation is thought to occur due to spatial overlap in the expression of transcriptional regulators (137). The abundance of information available on anthocyanin synthesis and regulation provides valuable data that can be integrated into these models. Increasing characterization of the developmental genetics underlying patterning phenotypes will contribute to the formation of mechanistic models that can be validated experimentally to explore floral patterning.

Floral Scent Characteristics

Floral scents are blends of VOCs. These lipophilic molecules have low boiling points and high vapor pressures at ambient temperatures (44). Floral VOCs have many known signaling functions, and these are not restricted to plant–pollinator interactions (14). When emitted from flowers, volatiles can attract pollinators from long distances and stimulate landing and probing behaviors (8, 32, 121). Floral VOCs also repel florivores, prevent nectar and pollen robbers, and provide protection from yeast and bacteria (14, 77, 119). A striking example of pollinator attraction by volatiles is food deception through reward signal mimicry, which occurs in close to a third of all orchid species (100, 131). Understanding floral scent evolution is challenging because fragrances are usually composed of blends of many VOCs derived from different biosynthetic pathways (118, 133) and specialized metabolites are often multifunctional. The multifunctionality of specialized terpenes is discussed by Pichersky & Raguso (113) and that of linalool is discussed by Raguso (119). The emission of VOCs is often restricted to specific floral organs (41) and flower developmental stages (124), with the release of *Hedychium coronarium* main volatiles peaking when the flower is mature (178). Many floral VOCs have been identified from nearly 1,000 flowering plant species (79), and several studies have researched genes underlying floral scent profiles (43, 78, 111). In some cases, gene expression has been associated with ecological roles; for example, blocking the expression of biosynthetic genes necessary for the production of the floral attractant benzyl acetone and repellent nicotine impacts *Nicotiana attenuata* pollinator visitation (76). Elucidating the genetic mechanisms underlying VOCs is particularly challenging due to the intricacies of characterizing floral scent composition, context-dependent volatile functions, and complex spatiotemporal dynamics of VOC release (119).

Volatile Organic Compounds

Plant VOCs originate from a small number of primary metabolic pathways and are grouped according to biosynthetic origin. The major classes of floral volatiles are terpenoids, benzenoids/phenylpropanoids, and fatty acid derivatives (96), derived from pathways summarized by Dudareva et al. (42). The VOC pathways are reasonably well established, although several aspects still require further research, such as the identification of biosynthetic genes (14, 96, 120). The second-largest class of plant VOCs comprises benzenoids/phenylpropanoids, with many floral volatiles described (74, 80, 81). These compounds are derived from the amino acid phenylalanine synthesized in the shikimate pathway described by Maeda & Dudareva (88). The first committed step in phenylpropanoid and benzenoid synthesis is catalyzed by L-phenylalanine ammonia-lyase (96). Olfactory properties and the volatility of phenylpropanoids and benzenoids can be altered through methylation, hydroxylation, and acetylation (96). In *Silene latifolia*, veratrole (1,2-dimethoxybenzene) attracts the pollinating noctuid moth *Hadena bicruris* (40) and is produced through methylation of guaiacol by *O*-methyltransferases (2, 62). Fatty acid derivatives compose the smallest group of floral volatiles. They are biosynthesized by lipoxygenases via oxygenation of an octadecanoid precursor, mainly C18 fatty acids such as linoleic and linolenic acid (described in 164). The products of this reaction enter into one of two lipoxygenase pathway branches that synthesize volatiles (52), including *cis*-3-hexenol, nonanal, and methyl jasmonate (42). Our knowledge regarding floral fatty acid pathways is somewhat limited, although recent studies have identified additional genes involved in fatty acid biosynthesis in orchid species (134).

Terpenoids are the largest and most diverse floral volatile class and are derived from two interconvertible five-carbon (C5) precursors, each of which originates from an independent pathway that is species and/or organ specific (161). The mevalonic acid pathway produces sesquiterpenes (C15), and the methylerythritol (MEP) pathway synthesizes precursors to volatile hemiterpenes (C5), monoterpenes (C10), and diterpenes (C20) (42). The genes and enzymes involved in both pathways are reviewed by Vranová et al. (161). Floral volatile terpenoid biosynthesis has been investigated in a number of species, including *Clarkia breweri* (112) and *A. majus* (98). Bumblebee pollinators are attracted to the terpenoids D-limonene, β-myrcene, and *E*-β-ocimene produced by *Mimulus* (19). The bumblebee-pollinated *M. lewisii* flowers emit all three compounds, while hummingbird-pollinated *M. cardinalis* flowers emit only D-limonene in significantly lower quantities (19). Byers et al. (20) found that allelic variation was likely responsible for these interspecific differences in *OCIMENE SYNTHASE* (*OS*), affecting *E*-β-ocimene emission, and *LIMONENE-MYRCENE SYNTHASE* (*LMS*) responsible for the emission of D-limonene and β-myrcene (**Figure 4**). Eliminating *E*-β-ocimene production in transgenic *M. lewisii* plants decreased bumblebee visitation modestly but significantly, whereas inhibiting *LMS* had no effect on bumblebee visits (20). Within *Mimulus* section *Erythranthe*, *M. lewisii* is the only species that emits *E*-β-ocimene. Independent mutations in *OS* prevent *E*-β-ocimene production in other *Erythranthe* species, suggesting parallel evolution of loss-of-function alleles (111).

Currently, our understanding of the regulation of floral scent biosynthesis is limited (97), but several transcription factors associated with the benzenoid/phenylpropanoid network of *Petunia* have been characterized. The majority of these transcription factors are R2R3 MYB proteins (26, 27, 144). However, recently an ethylene response factor (PhERF6) was identified that negatively regulates *Petunia hybrida* floral volatile benzenoid/phenylpropanoid compounds. PhERF6 physically interacts with the R2R3 MYB activator EMISSION OF BENZENOIDS I (EOBI) by suppressing its binding to the promoter of *ODORANT I* (*ODO1*) (144); this prevents the activation of genes encoding shikimate pathway enzymes by ODO1, causing a decrease in benzenoid production (159). ODO1 has also been identified as a potential regulator of enzymes

in the shikimate pathway within *Lilium* (172). In *Petunia*, ethylene negatively regulates genes required for floral scent emission (154), and PhERF6 expression was upregulated after ethylene treatment (84). Basic helix-loop-helix transcription factors activate terpenoid pathway enzymes in flowers of *Phalaenopsis bellina* (PbbHLH4) (23). Additional studies in leaves and fruit have found variable types of transcription factors regulating terpene biosynthesis (87, 95, 181), but more research into floral terpenoid regulation is required.

Floral scent often consists of volatile blends from different biochemical pathways, released from flowers during the time at which pollinators are active. Coordinated regulation of these pathways requires control of both volatile production and emission patterns (125). Precursor availability has been shown to play a key role in volatile regulation (27, 81). As a diverse array of volatiles originate from phenylalanine, coordinated regulation can be achieved by targeting specific points within the phenylpropanoid pathway. The *Petunia* protein EMISSION OF BENZENOIDS II (EOBII) upregulates *ODO1* expression and promotes the production of a wide range of phenylpropanoid volatiles from different pathway branches, including benzaldehyde and eugenol (145). Downregulation of EOBII is correlated with decreased expression of enzymes in the shikimate pathway and phenylpropanoid pathway, as well as decreases in enzymes involved in the production of specific volatiles (145). The rhythmicity of floral scent emission is correlated with pollinator activity in multiple systems (67, 148) and also tends to be transcriptionally regulated (64, 81). Volatile emission in *P. hybrida* is regulated by *LATE ELONGATED HYPOCOTYL* (*PhLHY*), a gene associated with the circadian clock and thought to inhibit volatile production through the repression of multiple genes, including *ODO1*. PhLHY expression levels decrease in the evening when ODO1 is active in promoting volatile synthesis (50). The role of the circadian clock in regulating volatile emissions was reviewed by Zeng et al. (179). The fitness consequences of altering floral circadian rhythms, affecting traits such as volatile emission timing, have been investigated in both *Petunia* (51) and *N. attenuata* (170). In these studies, *Manduca sexta* behavioral assays suggested that disrupting plant circadian rhythm impacted both pollinator visitation preference and seed set (produced using outcrossed pollen).

Genome-wide analyses revealing the chromosomal location of floral scent biosynthesis genes would further knowledge of the evolution of floral VOCs. However, the relative contributions of genetic and environmental factors to variability in floral scent emissions are not well characterized, impeding evolutionary understanding (120). Recently, our ability to manipulate quantities of specific volatiles within plants, through the production of transgenics, has improved. This should enable behavioral assays identifying the key volatiles for pollinator attraction and how the function of these volatiles may vary in natural conditions (42).

Coordinated Regulation of Volatiles and Pigmentation

Attracting pollinators can require a combination of floral signals acting synergistically (121) or sequentially, for example, when an insect pollinator is initially attracted by odor, but visual cues guide it to the nectar source (117). Ehrlén et al. (47) found that in *Primula veris* both optical and olfactory traits influenced plant fitness. The evolution of floral color and scent is coupled because of both this shared function in attracting pollinators and shared precursors for volatile and pigment synthesis (14, 125). Modifications to these shared pathways, such as the phenylpropanoid pathway, can influence both volatile and pigment production (**Figure 7**). The MYB transcription factor PRODUCTION OF ANTHOCYANIN 1 (PAP1) has a dual role, activating floral anthocyanin and volatile production in *A. thaliana*, *P. hybrida*, and *Rosa* (variety Pariser Charme) (182). In *Rosa*, the expression of *PAP1* in transgenic plants increased anthocyanin concentration in flowers over

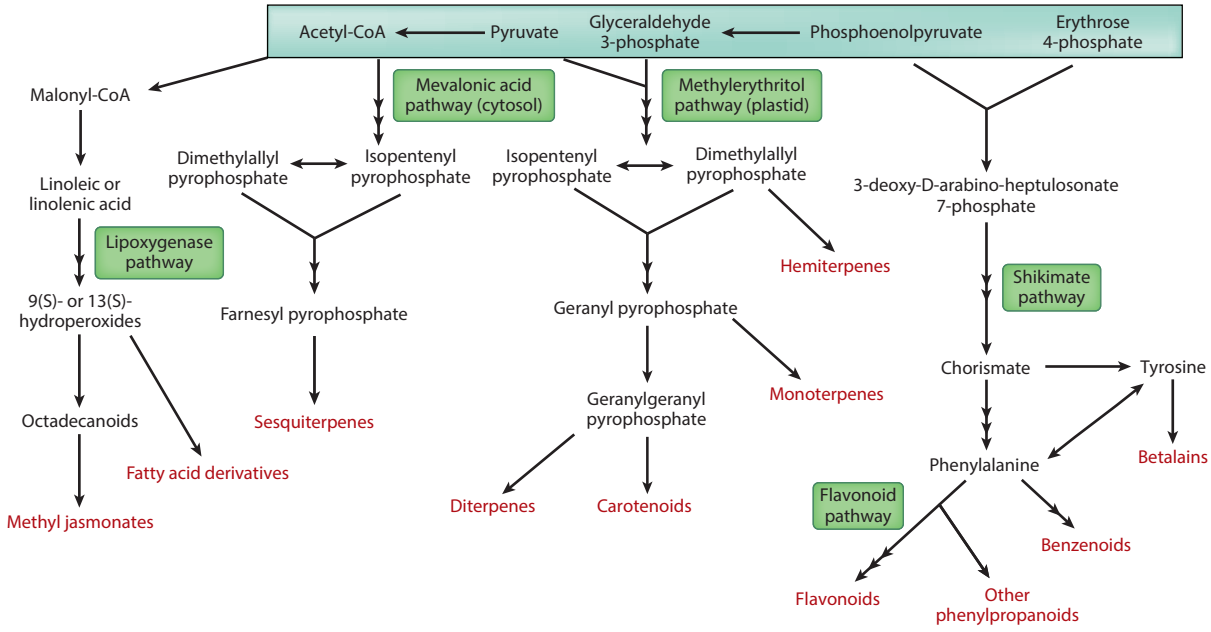

Figure 7

Simplified diagram of the biosynthetic pathways leading to the production of pigments and volatile organic compounds (VOCs). Multiple enzymatic reactions are represented by stacked arrows. The long box at the top represents substrates for plant VOC production that come from primary metabolism, the green boxes illustrate pathways, and the red compounds are types of VOCs. Figure adapted with permission from Dudareva et al. (42), copyright 2013 the authors; *New Phytologist* copyright 2013 New Phytologist Trust.

2.5-fold and phenylpropanoid compound eugenol 20-fold, compared to wild type. This increase in scent production was detectable to honey bees (*Apis mellifera*) (182). PH4 is the MYB component of an MBW complex activating the transcription of P-type H^+-ATPase proton pumps early in flower development; these pumps alter the vacuole pH, influencing petal hue (26). Following the completion of anthocyanin synthesis, PH4 also regulates volatile emission in mature flowers in a manner unrelated to vacuole pH, but targets have not yet been identified. Cna'ani et al. (26) suggest that coregulation of these traits may involve switches that activate or inhibit shunts, determining production of pigments or volatiles. The timing of metabolic flux into pathways that produce either volatile phenylpropanoids or flavonoids is regulated by gibberellic acid (GA) in *P. hybrida* (125). GA levels are high during early bud development, promoting the transcription of enzymes involved in anthocyanin synthesis (165). Phenylpropanoid volatile production coincides with a decrease in GA as the flower develops. Hormone treatments and transgenic assays indicate that GA actively suppresses scent production by downregulating transcriptional activators of pathways involved in phenylpropanoid volatile synthesis, for example, ODO1 and EOBI (125). The examples discussed above require shared pathways, but regulatory mechanisms operate at different times in development. In the absence of temporal separation of trait production, trade-offs between color and scent can occur and have been observed in several species (14). Functional trade-offs in floral phenotypes adapted for pollinator attraction are also apparent, as antagonistic interactions with herbivores and parasites may result in optimal phenotypes that do not maximize signal intensity (14).

OPTIMIZING FLOWER HANDLING AND POLLEN PLACEMENT

Actinomorphic: radially symmetrical; a description of a flower with many axes of symmetry

Zygomorphy: bilaterally symmetrical; a description of a flower with a single axis of symmetry

Once attracted by floral cues, a pollinator must interact with the flower physically to extract the reward. The nature of this interaction varies according to the type of pollinator and its morphology and behavior. Pollinators that land on the flower to feed often have substantial physical contact with the flower, while those that feed while hovering, such as hummingbirds and moths, may have less contact with the flower. In either case, the floral morphology can have a direct effect on how much physical interaction occurs, where pollen is placed on the animal's body, and how time- and energy-efficient the pollinator foraging is. In recent years, our understanding of the molecular mechanisms that underlie these aspects of floral morphology has been developed largely through work in the bee-pollinated *A. majus* (70) and in the genus *Petunia*, which includes a variety of pollination systems (58).

Macroscale floral morphology has a significant effect on pollinator landing and pollen placement. The symmetry of a flower influences the extent to which pollen deposition can be controlled, with actinomorphic (radially symmetrical) flowers less able to determine pollen placement than zygomorphic (bilaterally symmetrical) flowers. In many floral systems, dorsal stamens deposit pollen on the back of a pollinator, limiting the chances of the pollen being groomed or eaten and maximizing the probability of pollen transfer to the dorsally positioned stigma of another flower. Zygomorphy has also been proposed as a feature that improves pollinator access to the flower, such as by providing a landing platform, thus enhancing foraging efficiency (99). Floral symmetry can be regulated independently in any of the floral organ whorls and is produced through a gradient of expression of transcriptional regulators in the floral meristem. The bilateral symmetry found in *A. majus* depends on the actions of both dorsalizing regulators, primarily the TCP transcription factor CYCLOIDEA (CYC), and ventralizing regulators such as DIVARICATA (DIV), an MYB transcription factor (5, 86). *CYC* has a gradient of expression peaking in the dorsal part of the floral meristem and fading toward the ventral part. The primary function of CYC is to activate expression of RADIALIS (RAD), another MYB transcription factor that acts as an antagonist to the ventralizing activity of DIV (28). The antagonistic interaction occurs through competition between DIV and RAD to bind DRIF (DIV AND RAD INTERACTING FACTOR) proteins, which are necessary for DIV to activate downstream genes involved in the ventral development program (122). While floral symmetry has been studied in most detail in *Antirrhinum*, elements of the same program are known to have been recruited repeatedly during angiosperm evolution, with zygomorphy evolving multiple times (30). In particular, dorsal expression of CYC and related genes encoding TCP transcription factors is a common theme in many zygomorphic floral systems (reviewed in 143). The study of zygomorphy in multiple floral systems will be important in our understanding of the different ways in which floral symmetry can influence pollinator behavior and the effectiveness of pollination. Most studies focus on the corolla, but there are examples where the zygomorphy is extremely localized. For example, in *Solanum citrullifolium* only the ventral-most of the five anthers is dramatically elongated to position pollen on the back of pollen-feeding bees (158). This presents an exciting opportunity to explore whether the overall developmental basis of zygomorphy is conserved even where only specific organs are affected (**Figure 8**).

A more subtle method of controlling pollen placement involves altering the relative lengths of the reproductive organs and the structures containing a nectar reward. Both corolla tubes and nectar spurs (tubular outgrowths of the petal) collect nectar at the base, which increases the distance between the pollinator point of entry to the flower and the reward. This ensures that pollinators, using beaks or proboscises, must make contact with plant reproductive organs in order to access the reward. Natural selection is predicted to favor relative organ lengths that maximize this contact. These floral traits are particularly important in mediating interactions between flowers and hovering pollinators, such as moths and hummingbirds.

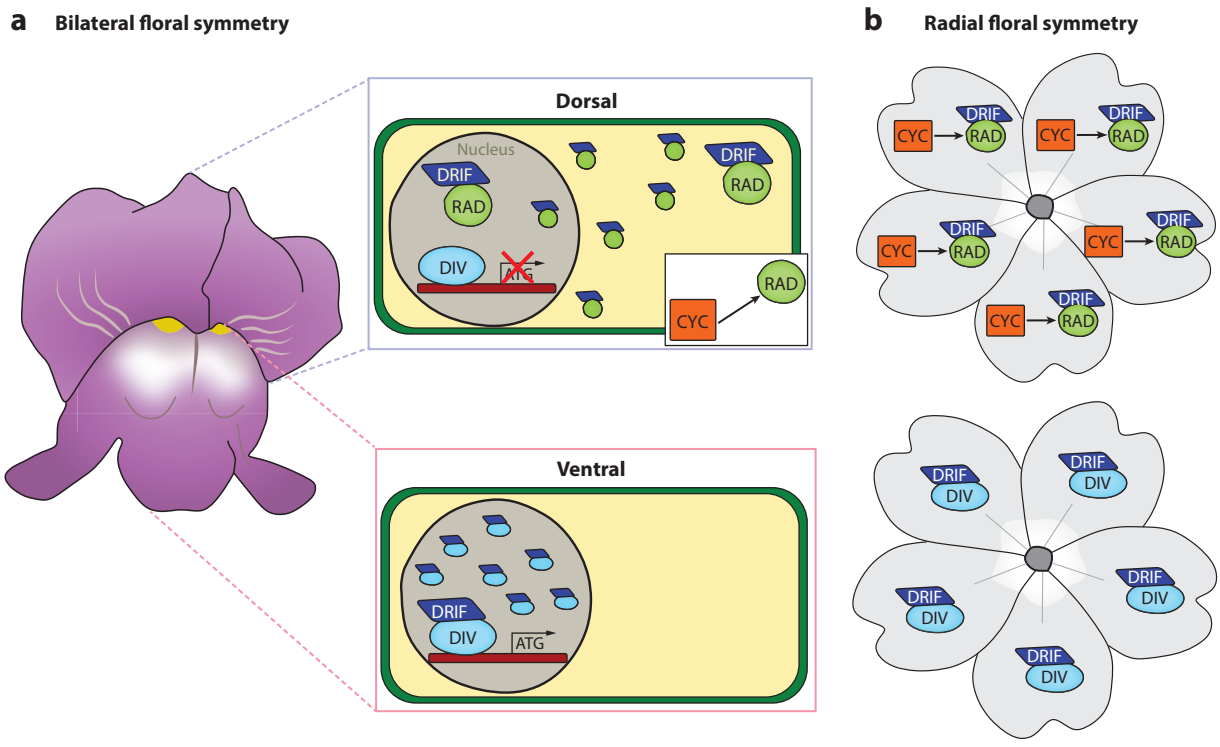

Figure 8

(*a*) Illustration of the genes involved in producing bilateral floral symmetry in *Antirrhinum majus*. CYCLOIDEA (CYC) is a dorsalizing regulator that activates RADIALIS (RAD). RAD competes with DIVARICATA (DIV) for binding of DIV AND RAD INTERACTING FACTOR (DRIF) proteins. DIV is a ventralizing regulator that needs to bind to DRIF to activate expression of genes involved in ventral petal development. (*b*) Theoretical flowers are presented with two possible mechanisms resulting in radial symmetry.

The cellular and molecular basis of nectar spur development has been reported in *Aquilegia* and *Linaria*. Puzey et al. (116) identified cell expansion and cellular anisotropy as the driving forces behind the development of longer nectar spurs in *Aquilegia*, and followed this up with a transcriptomic analysis that pointed to regulators of auxin signaling and organ polarity as the key factors determining spur length (169). In contrast, Cullen et al. (31) showed that the difference in spur length between two sister species of *Linaria* could be attributed to an increase in cell number in the longer-spurred species, pointing to a molecular mechanism centered on the regulation of cell division. The increase in *Petunia exserta* style and stamen length (relative to corolla length) is also due to an increase in cell number, rather than cell length. Hermann et al. (66) analyzed the exserted reproductive organs of *P. exserta*, which control pollen placement on, and removal of pollen from, hovering hummingbird pollinators (85, 136). *P. exserta* style and stamen length were significantly longer than those of related species with different pollinators. QTL analyses revealed three main loci, with one responsible for the majority of variation in stamen, stigma, and corolla tube length. It is likely that this locus contains a regulator of cell division that is responsible for the change in pollen placement associated with the pollinator shift (66). Characterizing the development of these floral traits has revealed different underlying strategies for floral structure elongation.

For pollinators that land, the efficiency of flower handling is influenced by the slipperiness of the flower surface. Whitney et al. (166) used both artificial surfaces varying in texture and *A. majus*

lines with different epidermal cell shapes to demonstrate that the conical epidermal cells found on most angiosperm petals provide grip to bees, enabling more efficient foraging. In choice tests, bees preferred conical-celled surfaces to flat-celled surfaces, and that preference increased when flowers were difficult to handle (4, 166). The molecular mechanism underpinning conical epidermal cell development was described in *A. majus*, where the flat-celled *mixta* mutant results from insertion of a transposable element into the *AmMIXTA* locus (102). *AmMIXTA* encodes an MYB transcription factor from subgroup 9 of the R2R3 MYB family and was shown to be both necessary and sufficient to induce epidermal cell outgrowth (59). Members of this subfamily regulate petal epidermal identity in a number of other systems (reviewed in 17). Given the significance of conical petal epidermal cells in the interaction between flower and bee, it is interesting that evolutionary transitions to nonlanding pollinators often involve loss of the conical cell form (103, 104). Ojeda et al. (104) showed that all of the Canary Island species of *Lotus* had lost conical cells on the dorsal petal in association with a shift to bird pollination. A broader study of bird-pollinated species across the Macaronesian Islands found that species that had shifted to a complete dependence on birds as pollinators had all lost conical petal cells (103), demonstrating the evolutionary lability of this particular trait.

Herkogamy: spatial separation of the stamens and stigma within a flower, which may minimize self-pollination

SELF-POLLINATION

The evolution of self-pollination has occurred multiple times within the angiosperms. Indeed, the evolution of multiple selfing species within a family has been described as "an almost universal feature of herbaceous plant families" (11, p. 281). Several different selective pressures have been hypothesized to explain this transition, which carries the negative consequence of reducing outbreeding and, therefore, genetic diversity. These selective pressures include limited availability of animal pollinators and escape from the pressure of floral herbivory. Selective pressure for rapid growth and reproduction in marginal habitats might also encourage the evolution of selfing, which can allow faster reproductive cycling. These pressures can also act in concert (reviewed in 139). Self-pollinating species exhibit a number of traits that set them apart from outcrossing relatives; most essential is the breakdown of any biochemical self-incompatibility system, allowing self-fertilization. A change in the allocation of resources to different parental functions is also apparent, most commonly a reduction in the pollen:ovule ratio resulting from reduced pollen production. Changes to flower morphology in selfing species often include a reduction in the size of petals and changes to the relative positions of anthers and stigma, reducing herkogamy and increasing the likelihood of within-flower pollination (reviewed in 139).

While there are few genera or even families in which both wind pollination and animal pollination occur, as discussed above, the transition to selfing has occurred frequently within families, genera, and even populations of a single species (11). These evolutionary transitions facilitate comparative analysis and thus isolation of molecular mechanisms responsible for key changes. Post-pollination processes such as self-incompatibility are beyond the scope of this article, and little is known about mechanisms to reduce pollen number (139), but recent studies have provided insight into morphological floral features associated with self-pollination.

Duncan & Rausher (45) showed that flower size and stigma/anther position were under selective pressure in selfing *Ipomoea lacunosa*. The genetic structure of *I. lacunosa* was compared to that of its outcrossing relative *Ipomoea cordatotriloba*; comparing the distribution of genetic diversity and morphological diversity between the species demonstrated that the evolution of reduced corolla size, smaller anther–stigma distance, and a shorter style length was attributable to natural selection acting on the selfing species, *I. lacunosa*.

The molecular mechanism governing corolla size reduction in selfing species has been explored in *Capsella*, where selfing *Capsella rubella* has flowers with an 85% reduction in petal size compared to outcrossing *Capsella grandiflora*. In 2011, Sicard et al. (140) showed that a segregating population derived from a cross between these two species recovered the full range of flower sizes with continuous variation, and therefore followed lines through multiple generations to generate recombinant inbred lines. A QTL approach using these lines revealed that the difference in petal area between parental species could largely be explained by five QTLs of major effect, indicating a complex genetic basis to the reduction in petal size. Morphological analyses demonstrated that the difference in petal area between *C. rubella* and *C. grandiflora* is due to reduced cell numbers, resulting from the premature termination of *C. rubella* petal growth relative to the rest of the plant (140). Sicard et al. (138) took a mapping approach to identify one of these QTLs. They established that a region in the intron of the *STERILE APETALA* (*SAP*) gene, which encodes an F-box protein (21), was responsible for a 25% reduction in petal area of *C. rubella* compared to *C. grandiflora*. Allelic variation in this intron is thought to modify gene expression, with polymorphisms in the *C. rubella* variant reducing *SAP* expression in the developing petal. In *Arabidopsis*, AtSAP is known to act as a regulator of organ growth and also specifies floral organ boundaries, operating as an F box protein that targets negative regulators of cell proliferation for degradation (163). Variation in regulatory regions of *SAP* alleles, present within pools of nonselfing plants, specifies a change in flower morphology associated with the transition to selfing. This recruitment of variation is an exciting example of evolution tinkering with existing genetic diversity (71). Recent results from Woźniak et al. (167) suggest that repeated evolution of selfing in *Capsella* species may operate through convergent genetic mechanisms. Floral transcriptomes of selfing *C. rubella* and *C. orientalis* were more similar to each other (in comparison to the outcrossing *C. grandiflora*) than were vegetative transcriptomes. Crosses between the two selfing species did not recover outcrossing petal sizes, indicating that the same genetic mechanisms or loci were at work in both species. This was confirmed by mapping petal area size QTLs in *C. orientalis*, several of which mapped to the same position as the previously studied *C. rubella* QTLs (167).

Sicard et al. (140) established that the absolute length of both stamens and gynoecium was smaller in selfing *C. rubella* than in outcrossing *C. grandiflora*, although the ratio between them was not altered. In contrast, Tedder et al. (150) investigated selfing and outcrossing populations of *Arabis alpina* and found a reduction in herkogamy (separation of stamens and stigma) associated with the selfing populations. In this example, the change in the ratio of stamen to stigma length was attributed to a reduction in stamen size in the selfing populations. Toräng et al. (151) explored the consequences of this difference in herkogamy for seed set between *A. alpina* populations in a pollinator-limited common garden site (in Sweden) and a pollinator-rich site (in Spain). They found that both herkogamy, operating through stamen length, and anther orientation were subject to strong directional selection when pollinators were limiting. While the molecular basis of this herkogamy remains to be explored, the presence of intraspecific variation provides the opportunity for a variety of comparative genetic approaches.

CONCLUSIONS

Recent advances in our understanding of molecular processes underpinning plant–pollinator interactions have occurred on multiple fronts. We have focused on genes controlling floral phenotypes, including evolutionary changes in structural genes, such as those responsible for differences in volatile emissions between *M. cardinalis* and *M. lewisii* (20). Cases of alterations to transcriptional regulation are also prevalent in the field, for example, mutations in regulatory elements causing differences in spot position between *C. gracilis* subspecies (91). Gene identification

in systems not conventionally thought of as models has been enabled by underpinning work in *A. thaliana*, providing a solid frame of reference for exploration in other species. Understanding the emerging properties of the floral phenotype requires synthesis of genetic data with morphological, developmental, and phylogenetic research. Our abilities to integrate these fields have been enhanced by numerous recent technical advances in genetics and genomic studies, enabling the identification of genes and processes important for generating biological diversity. Projects such as the 1000 Plant Transcriptome Project (**http://www.onekp.com**) (106) and the 5000 Insect Genome Project (126) provide a comparative framework that can be utilized for understanding plant–pollinator evolution. These resources will enable the analysis of many candidate genes and gene complexes, creating a more comprehensive context for the exploration of evolutionary trends, such as homoplasy and the generation of novelty (24).

These extensive genomic and transcriptomic resources must be utilized to establish causal relationships equating changes in genotype to those in phenotype, through the use of experimental procedures to establish evolved gene functions. Floral development is controlled by a cascade of gene-regulatory networks acting sequentially, mediated by phytohormones, and influenced by physical parameters and external factors (36). Environmental factors are transduced and activate genes controlling development, with continual feedback between developmental genetic processes and environmental variables (127). This interchange between genotype and environment is also apparent phylogenetically, as phenotypic evolution is shaped by ecological context through the process of natural selection (29). The physical colocation of genes regulating multiple floral traits at the same locus can coordinate pollinator-relevant floral traits (65), also impacting evolutionary trajectories. Of particular note has been the integration of molecular developmental studies with population genetic approaches, as variability occurring in natural systems can reveal mutations with "genuine evolutionary potential" (129, p. 76). Comparative studies can then consider how genetic changes contribute to morphological transitions from ancestral to derived states, while transition frequency can be assessed within the clade (129). A comprehensive understanding of the mechanisms controlling plant–pollinator interactions requires skills and knowledge drawn from many disciplines. The synergy of this information is being greatly advanced by improvements in genetic techniques and increased data availability, enabling pertinent genetic questions to be addressed from an unbiased perspective in ecologically relevant floral systems.

SUMMARY POINTS

1. Pollination, the transfer of pollen grains from stamens to stigmas, is essential for sexual reproduction in flowering plants, and it occurs through a variety of routes, including self-pollination, wind pollination, and animal pollination.
2. Recent technological advances have allowed insight into the molecular basis of the development and production of floral traits relevant to pollination.
3. We focus on traits that are thought to be adaptive in pollination biology, and, therefore, we provide criteria on the information needed before a floral phenotype can be considered adaptive.
4. Adaptations to wind pollination have been poorly studied at the molecular level, with the exception of the development of grass lodicules.
5. Detailed dissections of visual and olfactory signals that attract pollinating animals are emerging, with some studies showing considerable overlap in both metabolism and regulation between different pathways.

6. Floral morphology at the macroscale and microscale influences pollinator handling, and recent advances have provided insight into the regulation of these traits.
7. Adaptations that enhance self-pollination, including floral size and floral organ positioning, are comparatively well understood from recent comparative genomic work in *Capsella*.
8. We conclude that recent improvements in genetic techniques and increased data availability are advancing research into these traits by enabling an unbiased perspective in ecologically relevant floral systems.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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