The genetic architecture of natural variation in flower morphology

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A pollination syndrome is defined as a suite of floral traits that are associated with the attraction of a specific group of animals as pollinators. Traits such as flower morphology, color, scent, and rewards contribute to the plant's reproductive success by attracting pollinators. Here we focus on the genetics of natural variation in flower morphology and how the adaptation between plants and their cognate pollinator class contributes to plant's reproductive success. We review recent work on the genetic basis of interspecific differences in reproductive organ morphology and discuss possible genetic mechanisms for coordinated changes in complex syndromes.

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Introduction

What constitutes a species and in conjunction what is the genetic basis of speciation? This is a field of longstanding controversy but surprisingly few 'speciation' genes have been identified. Higher plants offer at least three important practical advantages for the study of speciation genetics. First, much of the diversity in angiosperms has occurred recently, providing stunning examples of speciation in action [1–3]. Second, the opportunity to perform interspecific crosses allows the functional genetic analysis of a large variety of taxonomic traits. Third, the sessile nature of plants makes it feasible to study selected offspring of interspecific crosses in the field, and thereby assess the phenotypic effect of identified genes under natural conditions.

Pollination syndromes

Many plant species rely on animal pollinators for their reproduction. The adaptation to a specific pollinator promotes reproductive isolation and ultimately speciation. The use of animal vectors reduces pollen loss and enhances specific transfer to cognate stigmas, but also requires the plant to elaborate flowers that attract a specific guild of pollinators. Pollinators have different energetic requirements, morphologies, and sensory systems that cause directional selection towards a complex of phenotypes, such as certain floral architecture, color, scent, and reward characteristics to enhance reproductive success. According to Proctor *et al.* 'these patterns of common characters, to which flowers of quite different evolutionary origins may converge, are called pollination syndromes' [4]. However, pollination systems are often complex and this has led to criticism of the classical syndrome concept, mainly based on the fact that flowers attract a broader spectrum of visitors than expected from the pollination syndrome concept [5,6]. Nevertheless, an association between certain floral traits and functional groups of pollinators that exert similar selective pressures continues to have support [7,8]. Shifts in pollination syndromes have occurred many times, for example in the *Solanaceae* bird pollination evolved at least 10 times [9]. Such shifts require changes in multiple pollination syndrome traits and individual traits are likely to be encoded by multiple genes.

To identify the genes that underlie shifts in pollination syndromes, quantitative trait loci (QTL) approaches have been undertaken on the progeny of interspecific crosses. In several plant genera, QTL were introgressed to assess the effect of individual loci and to test for epistasis and pleiotropy. Furthermore, behavioral studies were performed to estimate how much pollinator shifts are conditioned by a single QTL. A remarkable example is provided by the pioneering work of Bradshaw and Schemske, who showed how reciprocal introgressions of the *YUP* locus in *Mimulus* affected pollinator behavior in the field [10].

Here, we give an update on the latest work on floral morphology, focusing on reproductive organs. For other pollination syndrome traits, the reader is referred to excellent reviews [8,11–19].

Flower morphology

In animal-pollinated plants, the pollination success depends on the compatibility between the morphology of the flower and that of its cognate pollinator. For example, strongly elongated styles with exserted stigmas are typical for flowers that display a hummingbird pollination syndrome [15]. These adaptations ensure pollen deposition on the hummingbird's forehead while it feeds on the flowers. Subsequently, pollinators enter the next flower where they contact the stigma before the anthers. This floral design, known as 'approach herkogamy', prevents loss of pollen on the often self-incompatible stigma.

Comparable sexual organ exsertion can be adapted to other functions [20]. One such example can be found within wind-pollinated angiosperms. Here, the most common change in the evolution of new mating behavior is the change from obligate cross-pollinating (allogamous) species to self-pollinating (autogamous) species. Such transitions require mutational losses of genetic self-incompatibility systems as well as changes in floral morphology. Flowers in which the stigma is exserted beyond its own anthers are more likely to receive pollen from neighbouring plants, whereas flowers in which the stigma is recessed relative to its own anthers are more likely to self-pollinate. Such morphological changes could be achieved either by shortening of the anther filaments or elongation of the style. Chen *et al.* mapped QTL for style and stamen length [21[••]] in an interspecific cross between two wind-pollinated *Solanum* species, the allogamous *Solanum pennellii* and the cultivated, autogamous *Solanum lycopersicon*. Five tightly linked loci controlling style and stamen length underlying a complex QTL designated *stigma exsertion* 2.1 were found [22]. The authors isolated *LO2*, a gene encoding a putative transcription factor that is responsible for elongated styles in *S. pennellii*. They found that allelic variation at the 5′ regulatory portion of the *LO2* gene modulates style length, and hence stigma exsertion. Within the 5′ region, a 450 bp deletion is specific to the short-style allele of *S. lycopersicon* and a candidate for the downregulation of *LO2* in short styles. The analysis of cell number and size in developing styles showed that *LO2* modulates style elongation through localized, differential cell elongation. So far, this study is the only example where a reproductive organ QTL was successfully traced to the nucleotide level. In japonica rice, Miyata *et al.* [23] found a major, highly significant QTL designated *qES3* that increased stigma exsertion. In this case, reproductive organ morphology is agronomically important and the aim was to generate autogamous-like stigma in rice plants that increase seed production in hybrid rice. The japonica variety Koshihikari and a breeding line showing exserted stigma, IR24, were used. The QTL explained 32% of the total phenotypic variance in the F_2 population and the IR24 allele at the QTL increased the rate of exserted stigma by 20%. So far, the underlying gene has not been identified but it will be of great interest to determine whether orthologs of the genes identified in tomato and rice will also underlie variation in style and stamen length that lead to the evolution of exserted sexual organs in bird pollination syndromes (Figure 1).

Functionally interesting stylar polymorphisms have been described in other animal-pollinated families as well. None of them, however, is genetically as well characterized as the one found in tomato yet. Barrett, Jesson and Baker give an excellent overview of heterostylic, stigma-height dimorphic or enantiostylic plant families [20]. In heterostyly, stigma and anther heights are positioned

Figure 1
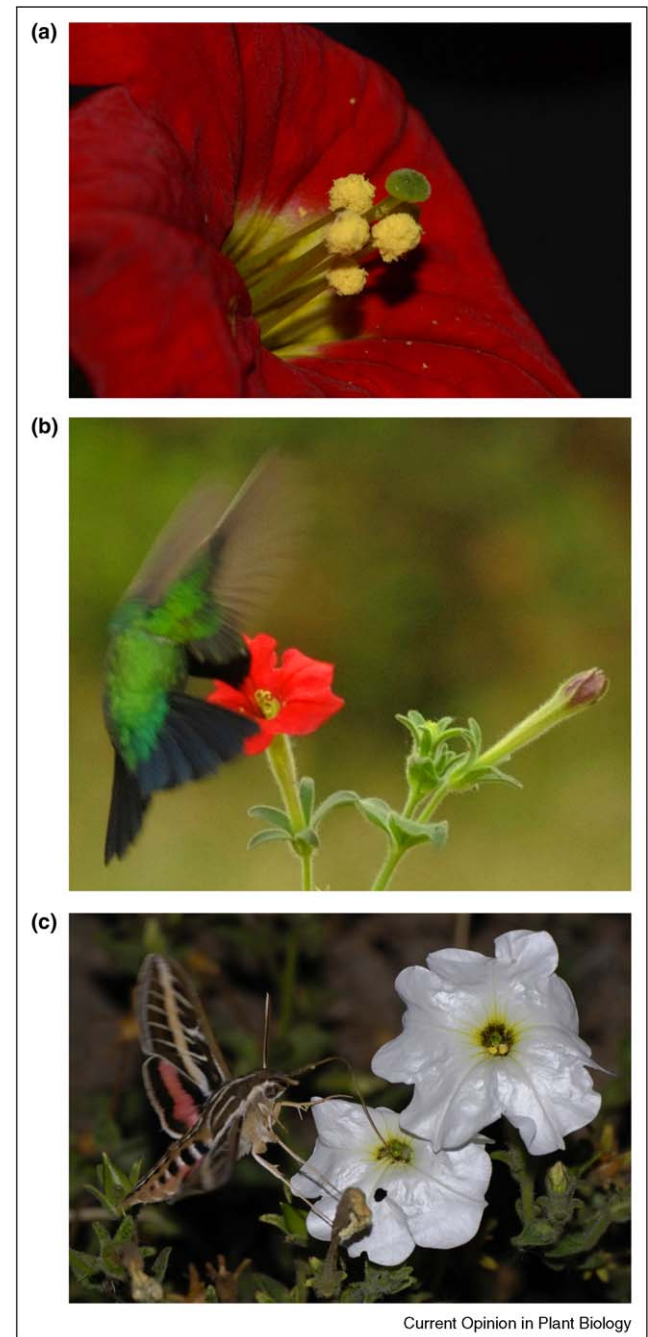
The rare species *Petunia exserta* with exserted sexual organs, adapted to hummingbird pollination **(a)** *Petunia exserta* pollinated by *Hylocharis chrysura* during choice experiments, Uruguay 2009; **(b)** *Petunia a.axillaris N* pollinated by *Hyles lineata*, Uruguay 2008; **(c)** all images are kindly provided by Alexandre Dell'Olivo.

reciprocally in two (distyly) or three (tristyly) morphs; in stigma-height dimorphism the two morphs vary in style length but not in anther height, whereas in enantiostyly the style is deflected either to the right or to the left side

of the flower. The existence of such complex and diverse sexual organ morphologies was explained by the finding that heterostyly promotes pollinator-mediated pollen dispersal between plants more effectively compared to uniform style length populations [20,24–26]. Pollen reception and pollen removal potentially interfere with one another leading to lost mating opportunities when stigmas and anthers are located at the same position within the flower. In species with a genetic incompatibility system, effective intermorph crosspollination further reduces pollen losses on incompatible stigmas.

Turnera subulata (white alder) and *Primula vulgaris* (primrose) are distylous species and possess a diallelic self-incompatibility system and intramorph incompatibility system that is determined by the *S-locus*. In *Primula*, the *S-locus* was designated a 'supergene' consisting of up to seven genes controlling both the dimorphism and the genetic incompatibility system [27,28]. The *S-locus* from dimorphic plant species can be regarded as one of the best examples of a co-adapted linkage group in plants. To date, the identity of the genes at the *S-locus* determining distyly is not known in any distylous species. However, a high-resolution map in *T. subulata* [29••,30] showed complete linkage of the *S-locus* with a sulfotransferase. The differential expression of this gene in long versus short styles may indicate a function in distyly [29••].

In none of the above-mentioned cases have the underlying genes been identified and thus it is not known whether some of them are homologous to the tomato *LO2* gene. If so, one might speculate about a cluster of genes that represent the vestiges of an ancient co-adapted gene complex in controlling mating behavior in all these cases.

Once they have arisen, stylar polymorphisms in animal-pollinated plants need to be maintained. *Narcissus papyraceus* displays stigma-height dimorphism and dimorphic populations with an equal percentage of long-style and short-style plants, but also long-style monomorphic populations exist. By contrast, short-style monomorphic populations have not been reported in the wild [31]. Conditions under which the polymorphism was maintained are illustrated in *Narcissus assoanus*, where long-style plants enhance female fitness when long-style plants operate as pollen donor, especially when the long-style phenotype is abundant. Pollination by long-tongued insects enables sufficient disassortative mating to maintain the style dimorphism [32], but the loss of the polymorphism and fixation of a morph should occur in the absence of long-tongued pollinators. In *N. papyraceus* it has indeed been shown that pollinator shifts can cause a loss of style polymorphism [33].

The sizes and shapes of other floral organs also determine reproductive success by affecting pollinator behavior. A fascinating co-adaptation between pollinator morphology, pollinator preference and flower morphology was described by Whittall and Hodges [34] in a study in *Aquilegia*. Using a species-level phylogeny, they showed an evolutionary trend for an increase in spur length during directional shifts to pollinators with longer tongues, suggesting that *Aquilegia* nectar spurs rapidly evolve to fit adaptive peaks predefined by pollinator morphology. To date, nothing is known about the genetic basis of spur length, but it is a promising system to gain insight into the mechanism of rapid genetic evolution. Such 'speciation genes' [35] can offer clues regarding the ecological settings, evolutionary forces, and molecular mechanisms that drive the divergence of populations and species.

Venail *et al.* [36••] studied the two closely related (sub)-species *Petunia axillaris axillaris N* and *Petunia axillaris parodii*. The two accessions display a typical moth pollination syndrome with white flowers that produce scent during the night and a high amount of nectar. Thus, they are very similar in all traits relevant for pollinator behavior, except floral limb size and tube length. Both traits segregate in an interspecific F_2 population. Genetically these traits appear to be complex; three and four medium effect QTL were found for limb area and tube length. F_2 individuals that differed exclusively either in tube length or in limb area were used for pollinator choice experiments. The hawkmoth *Manduca sexta* was more attracted to flowers with larger limb size as well as to flowers with shorter tube length.

Colocalization of QTL for floral traits

Several studies were undertaken with the aim to identify 'speciation genes' underlying floral QTL and indeed many of those found QTL of medium to large effects [10,37••,38,39,40••]. What is very interesting is that co-segregation of floral QTL appears to be prevalent [37••,38,41,42]. Bouk *et al.* used an interspecific backcross population of *Iris fulva* and *Iris brevicaulis*, which display a hummingbird and bee pollination syndrome, respectively. They identified QTL for sepal shape, anther extension, nectar guide area, sepal blade hue, and sepal blade brightness clustered in a roughly 40 cM interval on linkage group four. On the basis of permutation tests of this result, the colocalization of four out of ten identified floral QTL within a single 40 cM map interval would be expected to occur less than 1% of the time by chance alone. In a study in *Leptosiphon*, Goodwillie *et al.* described one QTL affecting corolla tube length and corolla lobe length that map to adjacent locations in reciprocal backcross data sets (*L. jepsonii* and *L. bicolor*) [43]. In *Mimulus*, Fishmann *et al.* described shared QTL for corolla width and stigma-anther distance in a study addressing the association of floral traits with mating system divergence [39] and Hall *et al.* identified a QTL that had a large effect on corolla tube length and stamen length [41]. In *Aquilegia*, Hodges *et al.* [44]

described QTL for spur chroma that mapped close to QTL for blade chroma and also one for spur length mapping close to one for blade length. QTL affecting multiple floral traits have furthermore been described from studies of *Arabidopsis* [38,45] and *Solanum* [46,47].

Changes in allele frequencies due to selection on one trait generate a correlated selection response in other traits [42,48]. This implies that either single genes of individually large or pleiotropic effect or linked clusters of genes with a large cumulative effect might play a role in the evolution of reproductive isolation and speciation [49]. However, it must be pointed out that although these studies may hint at a non-uniform pollination syndrome QTL, far more detailed mapping and gene identification will be required to provide evidence for such a theory. The power to detect QTLs and the degree of bias toward overestimation of their effects is sensitive to sample size [50,51]. Furthermore, the colocalization of QTL obviously depends on the resolution of the genetic map and the type of molecular markers used for mapping [52,53]. At present, there is no possibility to distinguish between pleiotropic effects of a single gene and (tight) genetic linkage of different genes underlying one QTL.

Two studies show that single loci can cause shifts in pollinator behavior. Bradshaw and Schemske performed reciprocal introgressions of the *YUP* locus into bee-pollinated *Mimulus lewisii* and hummingbird-pollinated *Mimulus cardinalis*. They created plants that differed from their parental lines only in petal color. Importantly, the introgressed segment altered pollinator preference in either parental background [10,49]. In *Petunia*, *AN2* encodes a MYB-domain transcription factor that explains much of the petal limb color difference between the purple bee-pollinated *Petunia integrifolia* and the white moth-pollinated *P. axillaris*. *AN2* is inactive in *P. axillaris* due to mutations in its coding region [40••,54]. Hoballah *et al.* introduced an active *AN2* gene into *P. axillaris* and thereby restored anthocyanin production. The transgenic plant attracted more bumblebees and fewer hawkmoths than the isogenic *P. axillaris* parent. It can be expected that such mutations will be highly favourable when competition, disease or adverse climatic conditions cause a shift in local pollinator availability. In such a scenario, a gradual accumulation of mutations could eventually lead to distinct pollination syndromes.

Conclusions
Shifts between pollination syndromes involve coordinated changes in multiple complex traits. Yet, they have occurred frequently. To date, several genetic loci have been mapped and the first few genes have been isolated. Although data are still scarce, they allow us a first glimpse of the genetic architecture of these complex traits. First, loci of medium to major phenotypic effect are probably too prevalent to be explained by poor genetic resolution

alone. Second, there appears to be a non-random distribution of loci, which suggests pleiotropy or close linkage of multiple genes. Third, changes in the activity of a single gene can have strong effects on pollinator behavior. These findings imply that shifts between reproductive strategies may not require concerted modifications of multiple genes. Obviously, much of this remains speculative due to the scarcity of data.

High throughput genomics offer the promise to unravel the natural variation in floral traits involved in reproductive isolation in their entire complexity. Thereby it will be possible to arrive at well-founded theories about the evolutionary genetics and ecology of reproductive isolation in flowering plants in the future.

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