



Tansley review

Pollination syndromes in the 21st century: where do we stand and where may we go?

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Summary

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Pollination syndromes, recurring suites of floral traits appearing in connection with specific functional pollinator groups, have served for decades to organise floral diversity under a functional–ecological perspective. Some potential caveats, such as over-simplification of complex plant–animal interactions or lack of empirical observations, have been identified and discussed in recent years. Which of these caveats do indeed cause problems, which have been solved and where do future possibilities lie? I address these questions in a review of the pollination-syndrome literature of 2010 to 2019. I show that the majority of studies was based on detailed empirical pollinator observations and could reliably predict pollinators based on a few floral traits such as colour, shape or reward. Some traits (i.e. colour) were less reliable in predicting pollinators than others (i.e. reward, corolla width), however. I stress that future studies should consider floral traits beyond those traditionally recorded to expand our understanding of mechanisms of floral evolution. I discuss statistical methods suitable for objectively analysing the interplay of system-specific evolutionary constraints, pollinator-mediated selection and adaptive trade-offs at microecological and macroecological scales. I exemplify my arguments on an empirical dataset of floral traits of a neotropical plant radiation in the family Melastomataceae.

I. Introduction

Flowers are the defining structures of angiosperms (Sauquet & Magallón, 2018). While their organisation (bauplan) is relatively conserved across larger taxonomic units, flowers represent an astounding diversity in mode, i.e. architecture, colour, reward and scent, even among closely related species (Endress, 1996; Specht & Bartlett, 2009; Schiestl & Johnson, 2013; Sauquet *et al.*, 2017). This diversity in floral mode has largely been attributed to the

selection imposed by pollinating agents, and, to a lesser extent, by floral antagonists and abiotic factors (Strauss & Whittall, 2006; Harder & Johnson, 2009; van der Niet *et al.*, 2014; Gervasi & Schiestl, 2017; Campbell *et al.*, 2018; Caruso *et al.*, 2018). Over the past 150 years, the concept of pollination syndromes has been formulated and continuously refined to serve as a large-scale framework for structuring this diversity in floral mode from a functional, pollination-ecological perspective (Delpino, 1873–1874; Vogel, 1954; Stebbins, 1970; Faegri & van der Pijl, 1979;

Endress, 1996; Ollerton *et al.*, 2009; Rosas-Guerrero *et al.*, 2014). Generally, pollination syndromes represent convergent floral adaptations to specific functional pollinator groups (Smith & Kriebel, 2018; Dellinger *et al.*, 2019c). Importantly, there is no strict definition on how to delimit syndromes (e.g. which traits, which pollinator groups) and different authors have employed different classification systems throughout the years (reviewed in Fenster *et al.*, 2004; Ollerton *et al.*, 2009).

Regardless of the delimitation, the fundamental assumption of the pollination-syndrome concept is that flowers adapt to their single most efficient functional pollinator group (most efficient in removing and depositing pollen, see definition of 'efficiency' by Ne'eman *et al.*, 2009; Stebbins, 1970, Ashworth *et al.*, 2015). A functional pollinator group, as such, is defined as pollinators who select for the same combination of floral traits, while different functional groups will select for different trait combinations (Fenster *et al.*, 2004). Recent meta-analyses on pollination syndromes (Rosas-Guerrero *et al.*, 2014; Ashworth *et al.*, 2015) have used the following 11 functional groups: bee, bird, bat, fly, wasp, moth, butterfly, long-tongued fly, beetle, carrion fly and nonflying mammal. From here onwards, I refer to these as 'traditional syndromes' (also see discussion by Ollerton *et al.*, 2009).

Pollination syndromes are based on floral traits that are likely to underlie pollinator-mediated selection and capture differences in activity patterns, sensory abilities, dietary preferences, morphology and behaviour of pollinators (Fenster *et al.*, 2004; Muchhala, 2007; Schiestl & Johnson, 2013). Floral traits that are usually recorded (in traditional syndromes) include the timing of anthesis, flower colour and scent, flower orientation, flower size and symmetry, overall corolla shape, the position of sexual organs and the reward type (for a summary see Rosas-Guerrero *et al.*, 2014). Some studies also include flower exposure from foliage, sturdiness, subtler quantitative colour differences (e.g. UV patterning) or the more refined analyses of scent bouquets and nectar composition (Raguso *et al.*, 2003; Reynolds *et al.*, 2009).

As a means of classifying flowers under a functional–ecological perspective, pollination syndromes have often been used to predict pollinators for species for which pollinators are not known. These predictions were generally made by the experienced researcher by sorting flowers into syndromes based on the observed trait combinations (Faegri & van der Pijl, 1979). Over the past decades, more objective statistical classification methods have become available (Wilson *et al.*, 2004; Pichler *et al.*, 2020). The strongest associations between functional pollinator groups and flower traits have been reported for highly specialised systems in the tropics (Ashworth *et al.*, 2015).

The concept of pollination syndromes has received considerable attention and stimulated controversial debate in recent years (e.g. Waser *et al.*, 1996, 2011; Martén-Rodríguez *et al.*, 2009; Ollerton *et al.*, 2009; Armbruster *et al.*, 2011; Rosas-Guerrero *et al.*, 2014; Prieto-Benítez *et al.*, 2015). Much of the controversy related to pollination syndromes arose from the urgent desire of botanists and pollination biologists to devise a broadly applicable functional classification and explanation for the overwhelming diversity of flowers, thereby disregarding subtler aspects of pollination biology.

Some shortfalls have been identified and discussed extensively (Ollerton *et al.*, 2009; Rosas-Guerrero *et al.*, 2014), including the over-simplification of complex plant–animal interactions (i.e. lumping all visitors as pollinators regardless of differences in their efficiency in transferring pollen), the predominant focus on specialised systems only, or using syndromes to predict pollinators in plant clades in which no empirical pollinator observations are available. Further, the lack of a unified terminology, incongruences in geographic (e.g. worldwide vs regional) and taxonomic scale (e.g. across angiosperms vs single species) as well as the limited focus on a few, easy-to-code-for, floral traits make comparability between studies difficult and may render syndrome predictions unreliable (Ollerton *et al.*, 2009; Dellinger *et al.*, 2019a). Recent studies have shown a potential remedy against some of these shortcomings. The detailed assessment of pollination efficiency of a flower's visitor assemblage, for example, was found to be a reliable measure to correctly identify the 'most efficient pollinator' and hence correctly predict pollination syndromes (Rosas-Guerrero *et al.*, 2014). Also, more detailed and inclusive recording of floral traits helped to differentiate syndromes with higher accuracy and gave more information on syndrome evolution (Abrahamczyk *et al.*, 2017).

Pollination syndromes continue to stir controversial debate, however, with researchers questioning their validity and heuristic value in the (post)genomic era. Keeping all caveats in mind, how reliable and useful are pollination syndromes in predicting pollinators and understanding floral evolution? How have researchers dealt with the known caveats in recent years and which caveats/problems prevail? And how may modern microecological and macroecological and evolutionary questions make use of the syndrome concept?

In the following sections, I present a critical review of the current pollination-syndrome literature (2010–2019) in the light of the known shortcomings. First, I show that pollination syndromes are applied across a broad spectrum of disciplines, rendering a robust conceptual framework an absolute necessity. Then, I quantify how often pollination syndromes are used without knowledge of pollination biology, how pollinators are recorded and which traits are used most often to substantiate syndrome predictions. I further analyse how often predictions are erroneous and which traits are considered as most or least informative in differentiating syndromes. I discuss and summarise a variety of approaches that have been employed to objectively classify pollination syndromes and study flower diversification. Finally, I demonstrate what we may learn from the combination of a broader assessment of floral traits, multivariate statistics and comparative phylogenetic methods on an empirical trait dataset of the Melastomataceae tribe Meranieae. I conclude this review with an outlook on the opportunities to adopt new approaches for studying pollination syndromes.

II. A critical review of the pollination-syndrome literature from 2010 to 2019

The first millennial decade saw a number of highly influential conceptual publications on common shortfalls and possible improvements of the pollination syndrome concept, i.e. Fenster *et al.*'s (2004) review on functional pollinator groups and Ollerton

et al.'s (2009) critique on the (mis)use of pollination syndromes. What solutions have we sought since then to overcome these shortfalls, and where do current problems lie? To answer these questions, I conducted a literature search with the search terms 'pollination&syndrome*', considering title, abstract, author keywords and keywords plus, in the year range 2010–2019 in Web of Science 5.3.4. I screened 447 papers to extract information on the scope of the study, the approaches used to study pollinators, traits recorded and the types of analyses employed (Supporting Information Methods S1, S2). I excluded 101 papers that did not directly address pollination syndromes (e.g. theoretical or methodological articles, taxonomic descriptions, reviews), from further analyses, leaving 346 articles.

I first give an overview of the variety of objectives addressed by studies employing pollination syndromes. Then, working through eight major points of criticism on the pollination-syndrome concept, I quantify (when possible) and discuss whether and how these shortfalls may impact and bias our understanding of plant–pollinator interactions and floral evolution. Each section briefly introduces the point of criticism, provides an evaluation of the associated problems and is followed by a discussion on possible solutions.

1. The objectives of studies employing pollination syndromes

In the 346 papers reviewed, pollination syndromes were used in a broad variety of contexts and questions. I scored the major objectives of each study (each study may have multiple objectives; hence percentages of categories do not sum to 100%). The majority of studies aimed at generally describing the study system's pollination syndrome (52.6%), testing the concept (33.8%) and experimentally assessing, for example, the breeding system or relative effectiveness of different pollinators (30.3%). Here 21.7% of studies investigated whether pollination syndromes correlated with other factors such as the availability of flowering resources and 17.3% used syndromes to analyse whether and how pollinators affect/correlate with clade diversification. Pollination syndromes were also used to address conservation issues (7.8%), modelling and statistical prediction of pollinators (3.8% and 2.3%, respectively); 5.8% sought to better understand the evolution of pollination syndromes at the genomic level (e.g. evolution of colour genes).

2. The problem of scale

Traditional pollination syndromes were developed as a broad, functional–ecological classification system that would be applicable across angiosperms. At this broadest possible taxonomic scale, it follows that more general categorisations (e.g. widely distributed floral traits, which can be scored for all taxa) are used. It is not surprising that researchers have found mismatches between these idealised, traditional syndromes and the trait combinations realised in actual flowers (Ollerton *et al.*, 2009). Furthermore, it has become clear that pollination syndromes differ in different parts of the world (e.g. the 'bat' and 'bird' syndrome in the Old and New world tropics are characterised by different trait combinations, Fleming

et al., 2009). Unless the aim of a study is to test whether a (group of) plant species fits into 'traditional' syndromes, the overall conclusion was that studies at lower taxonomic scales (e.g. within families) should focus on taxon-specific traits to accurately describe syndromes in the group and region under study (Ollerton *et al.*, 2009).

The 346 papers screened indeed addressed pollination syndromes at various taxonomic scales (Fig. 1a). The majority, 59.8%, worked at small taxonomic units (single or few species), 21.4% addressed questions at generic, tribal or family level, 15.9% worked at the community level and the remaining 2.9% worked at other/higher scales. In total, 244 studies (70.5%) included floral traits. The majority of these (77.5%) recorded one or several of the traits included in traditional syndromes (as listed by Rosas-Guerrero *et al.*, 2014; Table S2; Fig. 1b); 90.6% included one or several additional traits such as quantitative measurements of nectar, scent, colour or flower morphology, pollen grain features, pollen to ovule ratios or shape of nectaries. Of the *c.* 10% of studies that did not include additional traits, the majority (37.8%) were community-level studies including many different taxa, followed by studies on single taxa or studies at the generic/tribal/family level (29.7% each).

These results suggested that particularly researchers working at small taxonomic scales have heeded the call to use more refined trait datasets to evaluate pollination syndromes. Ultimately, the lack of a single 'scale' at which pollination syndromes operate may turn out to be a strength rather than a weakness. As long as authors are clear about the scale at which they work, it offers the necessary flexibility to adjust character coding to functionally meaningful traits of the specific study system. In the long run, this may even prove valuable in understanding universal patterns of pollinator-mediated selection and angiosperm evolution (and hence angiosperm-wide pollination syndromes) as new traits and pollinator combinations hitherto not considered may emerge as important (also see section II.7 'There is more to pollination syndromes than a handful of floral traits').

3. The predominant focus on (few) specialised systems

Obviously, pollination syndromes were conceived for lineages comprising taxa with markedly diverging flower traits specialised on different functional pollinator groups. This focus on specialised systems came in for criticism as a large number of angiosperms indeed is generalised on being pollinated by several functional groups (Waser *et al.*, 1996).

In the literature reviewed, species from 76 different plant families were considered (Fig. 2; Table S1; note that I excluded community-level studies and studies at higher taxonomic or nonsystematic scales from this count). This represents 18.9% of the 416 angiosperm families currently recognised (The Angiosperm Phylogeny Group *et al.*, 2016). The plant families studied were mostly groups well known for enigmatic, specialised pollination syndromes, such as Orchidaceae (24 studies, Monocots), Gesneriaceae (17 studies), Solanaceae (i.e. *Petunia*, 17 studies), Plantaginaceae (i.e. *Penstemon*, 14 studies; all Lamiids) and Fabaceae (13 studies, Fabids). Some of the largest families, like Asteraceae, were only represented by a few (two) studies, and families known for

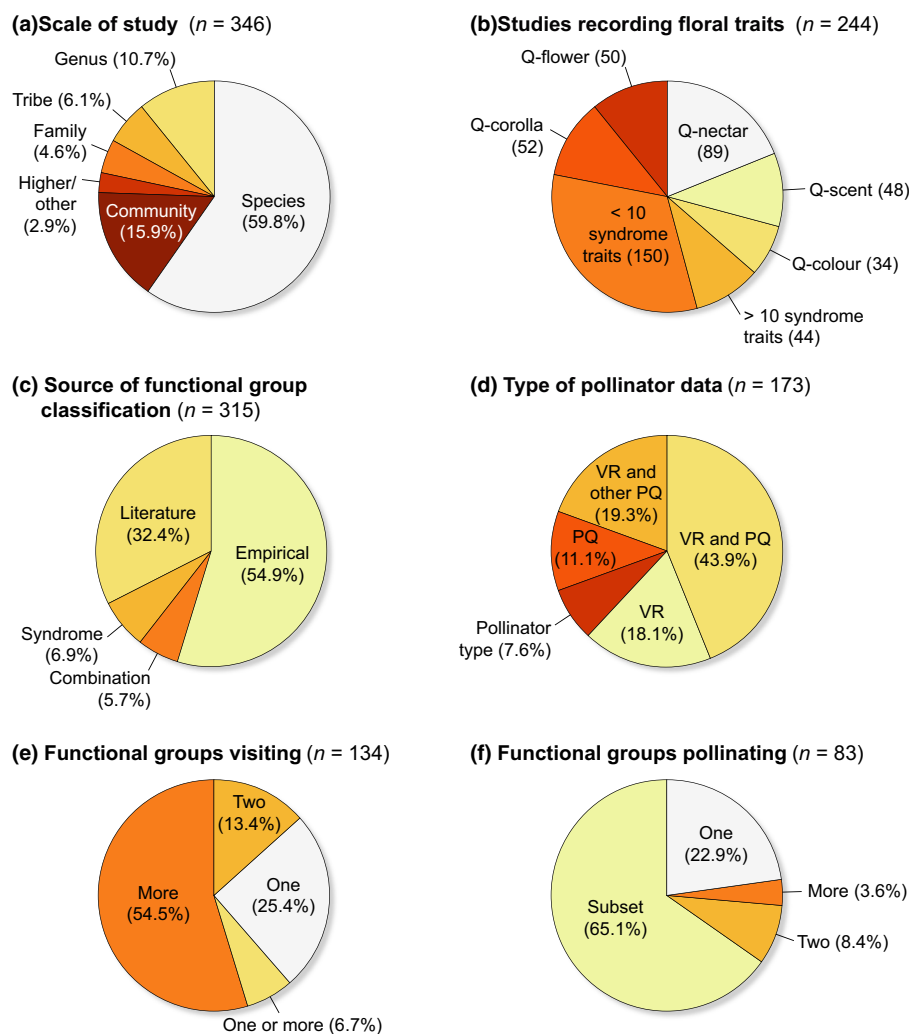


Fig. 1 Pollination-syndrome studies worked at different scales (a), were based on relatively few traits (b) and used different approaches to record pollinators (c–f). (a) The majority of studies worked at low taxonomic scales or the community context. (b) The majority of studies recorded relatively few traditional syndrome traits or quantitative measurements of reward, scent and corolla. (c) Most studies empirically observed pollinators or used literature reports to classify functional groups, c. 7% was only based on syndrome inference (floral traits). (d) More than 90% of studies quantitatively recorded pollinators (VR, visitation rate; PQ – pollinator quality (i.e. pollen deposition); other PQ, other approach to differentiate visitors from pollinators (i.e. morphological match between flower and pollinator; Supporting Information Methods S1); type, only recording functional group). (e) More than 60% of studies reported visitation by more than one functional pollinator group; ‘one or more’ refers to studies including more than one plant species. (f) More than 60% of studies reported only a subset of the observed visitors as effective pollinators.

generalised pollination systems such as Apiaceae or Brassicaceae were not included at all.

Quantifying our potential perception bias arising from the focus on (few) lineages with ‘exciting’ pollination biology will only be possible in future once we know more about hitherto neglected lineages and systems with seemingly ‘boring’ (often generalised) flowers (Vasconcelos *et al.*, 2019). Albeit not included in the literature reviewed, recent studies on generalised systems do exist (e.g. Gómez *et al.*, 2014, 2016; Zych *et al.*, 2019). These have documented geographic patterning in floral traits, and could partially link it with adaptation to the local pollinator community. Studies performed under experimental conditions could further demonstrate strong selection on scent mediated by a subset of the pollinator community even in seemingly generalised flowers (Gervasi & Schiestl, 2017). Investigating how, why and when generalised systems, and perhaps a ‘generalist syndrome’, are maintained despite specialisation on the local pollinator community will be one important approach to acquiring a more holistic understanding of the mechanisms underlying syndrome formation (Aigner, 2006). Furthermore, studying large plant groups with phenotypically ‘boring’ flowers is essential to understand potential intrinsic (system specific) and extrinsic (e.g. abiotic and biotic

environment) constraints which may have prevented floral diversification despite species diversification. Finally, the investigation of ‘new’ plant groups whose pollination biology is understudied may lead to exciting discoveries of novel pollination systems and associated floral trait changes such as pollination by marine invertebrates (van Tussenbroek *et al.*, 2016) or pollinator shifts in functionally highly specialised systems (Dellinger *et al.*, 2019a).

4. Pollinator observations and classifying pollinators into ‘functional groups’

Different pollinator species are pooled into ‘functional groups’ based on how they interact with flowers (Fenster *et al.*, 2004). Such groups may include a single species or many different species (Armbruster, 2017). Again, while it may be useful to work with the traditional categories (*sensu* Rosas-Guerrero *et al.*, 2014) at high taxonomic levels, the majority of studies investigates syndromes at lower taxonomic levels (Fig. 1a) and may find a more refined categorisation useful (Murúa & Espíndola, 2015). Only empirical observations allow us to correctly understand flower–pollinator interactions and hence devise meaningful functional categories (if necessary), disregarding taxonomic affinities (Fenster *et al.*, 2009a).

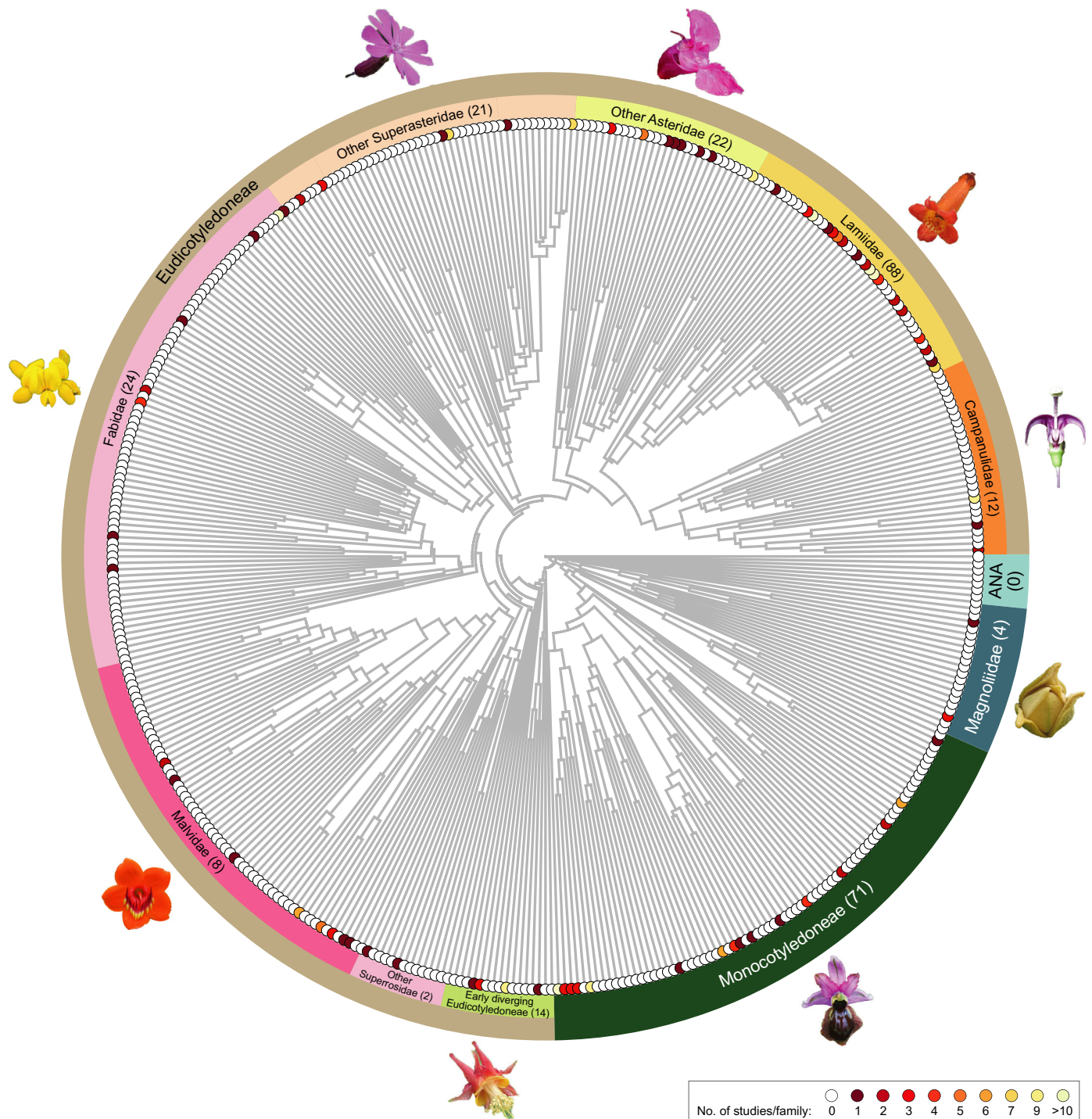


Fig. 2 Here, c. 20% of angiosperm families were represented in syndrome studies, with a strong focus on specialised interactions in the Lamiids and Monocots. Flower images represent enigmatic systems (clockwise, starting with Magnoliids: Annonaceae, Orchidaceae, Ranunculaceae, Melastomataceae, Fabaceae, Plantaginaceae, Solanaceae, Gesneriaceae, Campanulaceae); numbers in brackets give the number of studies conducted in each clade. Coloration of major clades follows Cole *et al.* (2017) (Supporting Information Methods S3).

How many current studies are indeed based on empirical pollinator observations and how have they classified pollinators?

In total, 315 of the 346 studies used functional groups for analyses. Of these, 54.9% had performed pollinator observations in the specific study and 32.4% were based on pollinator observations from the literature (Fig. 1c). Only 7% relied on inferences made

solely based on floral traits (syndromes) and the remaining 5.7% used combinations of observations, literature reports and syndrome inference. Here, 83.3% of the studies based on direct pollinator observations (a total of 173) worked at low taxonomic levels (one or a few species) and primarily aimed at describing and testing/verifying pollination syndromes (73.3%). Most of these studies

summarised pollinators into traditional functional groups despite identifying pollinators at the lowest taxonomic level possible (often species). Only 4.4% of studies created unusual functional groups based on observed or presumed behavioural or morphological differences between pollinators, such as phytophagous vs dung beetles (Schiestl & Dötterl, 2012), 15 insect groups (Koski *et al.*, 2015) or straight-bill and sickle-bill hummingbirds (Lagomarsino *et al.*, 2017).

These results showed that a large proportion of (particularly small-scale) studies indeed verified syndromes through empirical pollinator observations and only a small fraction of studies worked without empirical data. This does not mean, however, that we may reduce efforts to document pollinators. Several of the large-scale studies included in this review suffered from deficient pollinator observations and hence could not systematically analyse flower character evolution in relation to pollination (e.g. Reginato & Michelangeli, 2016; Heckenhauer *et al.*, 2018; Vasconcelos *et al.*, 2019). This observation is particularly important in the light of modern sequencing techniques and online search engines, which allow for the fast collation of large datasets, but leave us uninformed of the functional–ecological implications of flower mode (Ollerton *et al.*, 2015).

Regarding classification of functional groups, most studies adhered to traditional or taxonomic groups to summarise observed patterns. It is unclear whether a different grouping would have led to different conclusions. However, the fact that many authors have identified pollinators to species is promising, as it allows more refined assessments of flower–pollinator relationships (Murúa & Espíndola, 2015; Pichler *et al.*, 2020). In the past, only a few authors have made use of the flexibility of the functional-group idea in that it allows (and actually demands!) grouping based on the interaction differences observed in the study system, irrespective of taxonomic affinities of the pollinators. For example, Wilson *et al.* (2004) grouped individuals of the same bee species into nectar foragers vs pollen foragers and Armbruster (1993) differentiated fragrance-collecting male bees from resin-collecting female euglossine bees. At the other perspective, pollinators that we intuitively perceive as different may sometimes function as one group (e.g. nectar foraging hummingbirds, bats, rodents, flower piercers; Dellinger *et al.*, 2019a).

Generally, we have to be aware of the fact that any typological categorisation, such as functional groups and syndromes, leaves us at the boundaries of subjective interpretation of continuous, dynamic natural systems. While functional groups have been shown to be highly useful to summarise patterns, I urge that we continue to move on from typology to the (objective) quantification of syndromes when possible. I propose two approaches towards delineating functional pollinator groups and making classification less typological. The first approach starts with the pollinators. Quantifying the contribution of (different) flower visitors to pollination is the most powerful tool for understanding their importance as pollinators (see section II.5 'Visitation by multiple functional groups and selection by the most efficient pollinator'). Pollination efficiency may be combined with measurements of pollinator morphology (e.g. length of mouthparts). These matrices may be combined with floral trait matrices

to search for convergence patterns (Guzmán *et al.*, 2017; Pichler *et al.*, 2020) and may then be evaluated in the light of known or observed sensory/behavioural differences of pollinators to devise functional groups. Manipulative experiments may be conducted subsequently to identify the factors which render a specific set of taxa a 'functional' group and when and how this group may be narrowed or widened (e.g. through trait manipulation restricting pollen transfer). The second approach starts from the flowers. Particularly when working at macroevolutionary scales, authors may not want to classify flowers *a priori* but rather use statistical methods (Table 2) to detect patterns of floral trait convergence, and only then relate these to pollinator data (Pichler *et al.*, 2020).

5. Visitation by multiple functional groups and selection by the 'most efficient pollinator'

When formulating the 'most effective pollinator principle', Stebbins (1970) emphasised that 'character syndromes' (pollination syndromes) do not preclude the existence of secondary, potentially less efficient pollinators. Indeed, most flowers are visited by multiple functional pollinator groups (e.g. Waser *et al.*, 1996; Ollerton *et al.*, 2007). One major advancement that pollination-syndrome research has seen was to acknowledge this fact and to start measuring the relative contribution to pollination (pollination efficiency) of (diverse) pollinator assemblages instead of only documenting their flower visitation (Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014; Santiago-Hernández *et al.*, 2019). In specialised, and even in many generalised, flowers (which are easily accessible to various different pollinator groups), only a subset of all visitors was found to be effective as pollinators (Bartoš *et al.*, 2005; Rosas-Guerrero *et al.*, 2014). Recording pollinator efficiency requires more time and potentially laboratory equipment than assessing visitation only. How many authors have employed more refined measures to identifying 'most efficient pollinators' and what have they found? Does visitation by multiple visitors make syndrome predictions less accurate?

Of the 173 studies based on direct observations, the majority (63.2%) included both an evaluation of visitation rate and pollen transfer efficiency (Fig. 1d). In total, 18.1% only documented visitation rates, 11.1% only documented pollen transfer and 7.6% only reported types of pollinators. As expected, the majority of studies (61.2%) found more than two functional groups visiting; 13.4% reported visitation by two groups and 25.4% documented only one functional group visiting (Fig. 1e). The vast majority of studies documenting two or more functional groups as visitors reported only a subset of these visitors as pollinators (68.8% and 93.8%, respectively); 71.4% of studies reported the most frequent visitor also being the most important pollinator (but also see King *et al.*, 2013); 122 studies formulated a hypothesis about an expected pollinator/visitor, based either on previous observations or expectations derived from floral syndrome traits (see below). Here, 85.2% could at least partially confirm these expectations, while 13.9% found other pollinators/visitors than expected and one study did not find any legitimate pollinator. Syndrome expectations were confirmed or contradicted equally often, regardless of

whether multiple functional groups or only one was visiting (83.5% at least partly confirmed syndrome expectation with multiple functional groups visiting and 90% when one functional group was visiting).

These results underline findings reported previously: visitation by multiple functional pollinator groups is common but does not negatively impact the predictive power of pollination syndromes. It will be interesting, however, to further evaluate the impact of secondary/less efficient pollinators on floral trait evolution. Generally, pollination by multiple functional pollinator groups is believed to be disadvantageous, as it may induce strong fitness costs through adaptive trade-offs (Muchhala, 2008; Padyšáková *et al.*, 2013; Strelin *et al.*, 2017). It seems, however, that not all pollinator combinations are equally costly and trade-offs may sometimes remain small (Aigner, 2006; Ashworth *et al.*, 2015). An exciting and unanswered question in this context is whether some pollinator combinations (e.g. hummingbirds and bats, see Lagomarsino & Muchhala, 2019; Dellinger *et al.*, 2019b) are more likely to evolve into stable bimodal systems (i.e. systems with intermediate adaptations to two distinct functional pollinator groups *sensu* Goldblatt and Manning, 2006) than others (e.g. hummingbirds and bees, Castellanos *et al.*, 2004)? Why and how is bimodality maintained and under which conditions does generalisation evolve?

Furthermore, despite the predominance of pollinators as selective agents on flowers (Caruso *et al.*, 2018), other biotic interactions (e.g. herbivores, competition) are also important (Jogesh *et al.*, 2017). In the reviewed literature, only eight studies addressed and quantified other agents of selection than pollinators: six studied floral herbivory and two addressed reward robbing. Particularly when pollinators and antagonists are attracted through the same traits (e.g. large, colourful displays, Brothers & Atwell, 2014), this may induce conflicting selection on these traits and potentially lead to the evolution of defence/deterrence traits (Doubleday *et al.*, 2013). Incorporating antagonistic interactions (i.e. through fitness estimates) and potential defence traits into studies on pollination syndromes is important since defence traits may promote (preadaptations) or constrain shifts between pollination syndromes (Hanley *et al.*, 2009) and may also explain mismatches between traditional and observed syndromes (see section II.8 'Not all floral traits are equally informative').

6. Prediction of pollinators

Particularly in large plant clades in which time, logistic and budgetary constraints limit the number of species for which pollinators can be observed empirically, pollination syndromes are used for predicting pollinators (Lagomarsino *et al.*, 2017; Dellinger *et al.*, 2019a). Several authors have argued, however, that such predictions are risky, particularly when no (or few) pollinator observations exist for the respective plant group, when multiple functional groups visit flowers or when only very few floral traits are considered (Ollerton *et al.*, 2009; Fenster *et al.*, 2009a). In small-scale studies, pollination syndromes are often used to formulate a hypothesis about a species' most likely pollinator that is then evaluated through measurements of pollinator efficiency. I extracted this information from the literature to assess how often

authors verified/falsified their hypothesis and to understand which floral traits led them to their specific hypothesis.

In total, 64 studies hypothesised which functional group would pollinate the study species based on syndrome traits. Of those, 27.8% expressed doubt on classification into clear-cut syndromes because they observed floral trait combinations commonly assigned to different syndromes (Fig. 3a). Of those, 85% correctly predicted visitation by mixed assemblages, while 15% found different pollinators than expected from syndrome traits (Fig. 3b,c). Studies that did not express uncertainty about syndrome classification could (at least partially) confirm predictions in 86.3% of cases and 13.6% of studies reported other pollinators than expected (Fig. 3b, c). In this regard, 58 studies substantiated their syndrome expectations by naming one to three (traditional) syndrome traits in the introduction or materials and methods sections: colour (55.2%), reward (34.5%), scent (31.0%) and flower shape (25.9%) being the most commonly used traits (Fig. 4; Table S2).

These results demonstrated that authors could predict pollinators with high accuracy even when basing predictions on only a few traits. Importantly, these traits also allowed authors to correctly identify species that were likely to be pollinated by multiple functional groups. How can these results be extrapolated to larger scales for which empirical verification of syndrome assumptions is not possible? Promising advancements have been made, particularly in the field of Machine Learning (Pichler *et al.*, 2020). A series of powerful and mathematically flexible algorithms exists that may first be trained and evaluated on species for which empirical pollinator observations exist, and then be used to predict pollinators for (closely) related species that lack pollinator observations (Table 1; Dellinger *et al.*, 2019a; Pichler *et al.*, 2020).

7. There is more to pollination syndromes than a handful of floral traits

Several authors have criticised traditional pollination syndromes in being limited to a relatively narrow set of traits that are easy and fast to code (Fenster *et al.*, 2009a; Abrahamczyk *et al.*, 2017). This is particularly problematic in lineages in which pollination syndromes are best characterised by traits not included in traditional syndromes (Table 1; Dellinger *et al.*, 2019a). Furthermore, quantitative traits such as colour, scent or shape are difficult to discretise and may lead to subjective, erroneous categorisation (Abrahamczyk *et al.*, 2017). How many and which floral traits are generally included in pollination-syndrome studies? And how many of those are quantitative?

Of the 244 studies that included floral traits, the majority (77.2%) was indeed based on a relatively small set of traits (1–10; Fig. 1b). Here, 66.8% of studies included quantitative measurements of reward or attraction traits (nectar: 36.4%, scent 19.7%, colour 13.9%); 22.1% measured corolla dimensions quantitatively and 20.5% measured both sterile and reproductive floral organs quantitatively. Only 3.3% of studies were based on morphometric assessments of flower shape and only two studies quantified morphometric relationships between both corolla and reproductive organs.

(a) Doubt on clear-cut syndrome prediction ($n = 64$)

(b) Expectation from (a)

(c) Functional groups acting as efficient pollinators

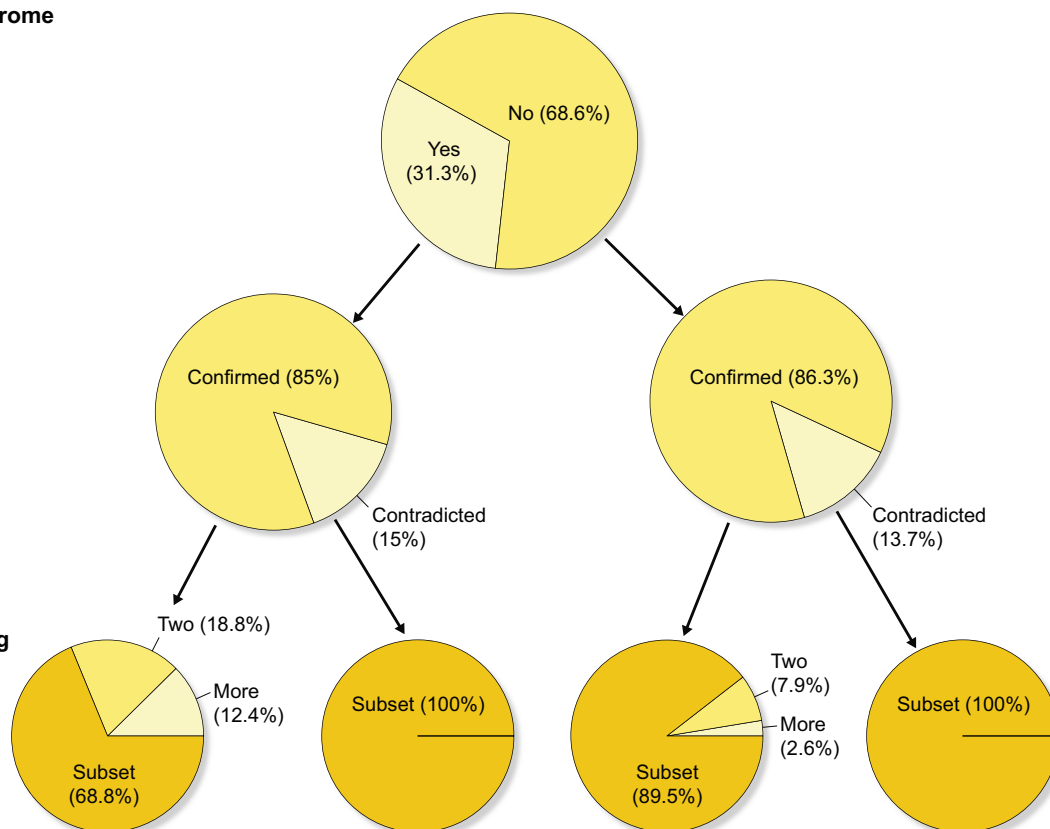


Fig. 3 Pollination syndromes could be used to correctly predict pollinators in *c.* 85% of studies. (a) Studies expressing doubt on clear-cut syndromes due to unusual trait combinations or no doubt due to matching of traditional syndrome traits. (b) Studies confirmed or contradicted syndromes equally often. (c) Pollinator generalisation or specialization did not impact the predictive potential of syndromes.

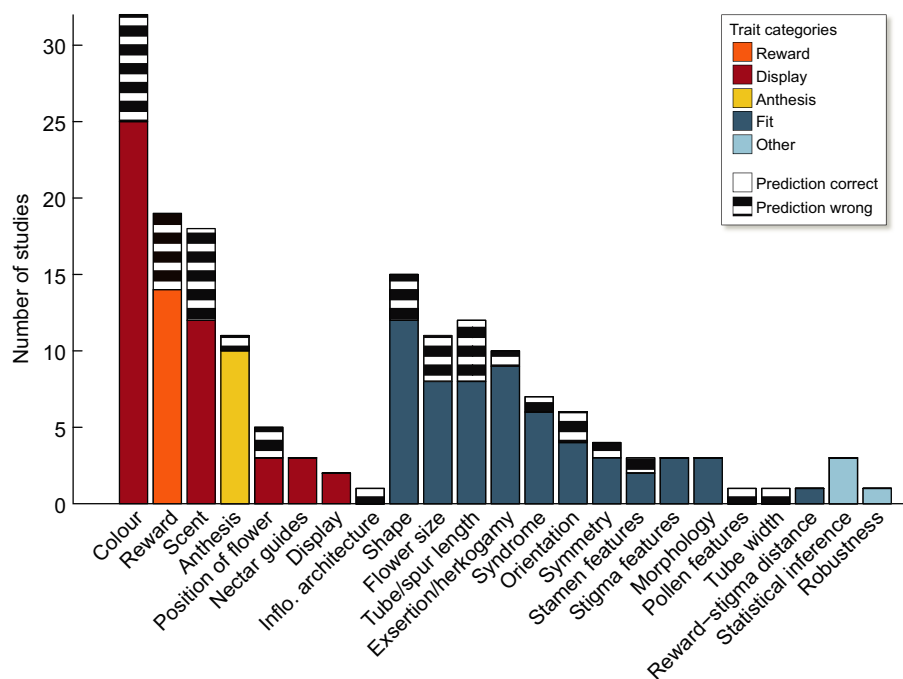


Fig. 4 Floral traits named to substantiate syndrome prediction. Colour, reward, scent and flower shape were used most often to justify syndrome expectations; erroneous predictions are indicated by black bars.

Table 1 Methods for data acquisition – compilation of floral traits which may be studied under a pollination biological perspective to expand our understanding of pollination syndromes.

Trait	Parameters to measure	Possible methods for data acquisition	Type of floral material needed	Examples for functional significance in pollination
Anthesis	Start time, duration	Documentation in the field	Fresh flowers	Filter on activity pattern of pollinator, traditional syndrome character
Flower exposure	Relative position to foliage	Documentation in the field, from photos or herbarium specimens	Fresh flowers, photos or herbarium vouchers	Advertisement (Fenster <i>et al.</i> , 2015; Diniz <i>et al.</i> , 2019), accessibility filter, traditional syndrome character
Flower orientation	Quantitative or qualitative (e.g. horizontal, vertical; Fenster <i>et al.</i> , 2009)	Documentation in the field, from photos or herbarium specimens	Fresh flowers, photos or herbarium specimens	Advertisement, efficiency (Fenster <i>et al.</i> , 2009b; Wang <i>et al.</i> , 2015), response to abiotic conditions, traditional syndrome character
Flower colour	Reflectance/absorption spectra, UV-patterns, colour contrasts within different parts of the flower (e.g. stamens, pollen), qualitative classification	UV-sensitive cameras, spectrophotometers (Johnson, 2013; van der Kooi <i>et al.</i> , 2019), qualitative classification	Fresh flowers, photos	Advertisement, deterrence (reviewed in van der Kooi <i>et al.</i> , 2019), traditional syndrome character
Surface structures of floral organs	Measurements or classification of cell shapes	Scanning electron microscopy or light microscopy (Papiorek <i>et al.</i> , 2014; Dellinger <i>et al.</i> , 2019a)	Ethanol-preserved flower material	Advertisement, deterrence (Comba <i>et al.</i> , 2000), modulating the grip for small, alighting pollinators (Whitney <i>et al.</i> , 2009)
Flower scent	Characterisation and quantification of scent bouquets, qualitative classification	Dynamic headspace techniques, gas chromatography-mass spectrometry (Burkle & Runyon, 2017), qualitative classification	Fresh flowers	Advertisement, deterrence (Schiestl & Dötterl, 2012; Burkle & Runyon, 2017)
Reward	Reward type	Qualitative classification	Fresh flowers or ethanol-preserved material	Advertisement, traditional syndrome character
	Amount/volume	e.g. nectar quantification by microcapillaries (Vandeloock <i>et al.</i> , 2019)	Fresh flowers	Advertisement (Katzner <i>et al.</i> , 2019; Vandeloock <i>et al.</i> , 2019)
	Availability (e.g. timing of nectar secretion)	Documentation in the field	Fresh flowers	Advertisement (Maruyama <i>et al.</i> , 2013)
	Nutritive value, reward composition	e.g. calorimetric analyses (Dellinger <i>et al.</i> , 2014), High performance liquid chromatography for nectar sugar composition (Krömer <i>et al.</i> , 2008)	Collection of reward (e.g. preservation in ethanol)	Advertisement (Vandeloock <i>et al.</i> , 2019; Dellinger <i>et al.</i> , 2019b)
Perianth (corolla)	Exact location in flower	Documentation in the field, flower sections and light microscopy (Dellinger <i>et al.</i> , 2019b)	Fresh or ethanol-preserved flower material	Efficiency (Armbruster <i>et al.</i> , 2009)
	Size (diameter, petal length)	Measurements, e.g. with callipers	Fresh or ethanol-preserved flower material, herbarium specimens	Advertisement, traditional syndrome character
	Fusion (e.g. tubes), constrictions (width : length)	Measurements, e.g. with callipers	Fresh or ethanol-preserved flower material, herbarium specimens	Advertisement, deterrence (Zung <i>et al.</i> , 2015), efficiency (Muchhala, 2006)
	Shape	Qualitative classification; quantification through length measurements or geometric morphometrics (van der Niet <i>et al.</i> , 2010; Wang <i>et al.</i> , 2015; Strelin <i>et al.</i> , 2017)	Fresh or ethanol-preserved flower material, (herbarium specimens)	Advertisement, deterrence, efficiency (Muchhala, 2007; Gómez <i>et al.</i> , 2016; Smith & Kriebel, 2018; Benítez-Vieyra <i>et al.</i> , 2019), traditional syndrome character

Table 1 (Continued)

Trait	Parameters to measure	Possible methods for data acquisition	Type of floral material needed	Examples for functional significance in pollination
Androecium	Heteranthery	Qualitative classification	FRESH or ethanol-preserved flower material, herbarium specimens	advertisement, efficiency (Velloso <i>et al.</i> , 2018)
	Position in flower	Qualitative classification or quantification through (geometric) morphometric measurements	Fresh or ethanol-preserved flower material, herbarium specimens	Advertisement (Sapir <i>et al.</i> , 2017), efficiency (Armbruster <i>et al.</i> , 2009; Xiao <i>et al.</i> , 2017; Dellinger <i>et al.</i> , 2019c)
	Size (length and width of anthers, filaments etc.)	Length measurements	Fresh or ethanol-preserved flower material, herbarium specimens	Advertisement (Sapir <i>et al.</i> , 2017), efficiency (Kudo, 2003)
	Structure/texture of anthers	Qualitative classification	Fresh or ethanol-preserved flower material	Efficiency (Falcão <i>et al.</i> , 2016; Dellinger <i>et al.</i> , 2019a)
	Presence of appendages/trichomes/glands	Qualitative classification or measurements	Fresh or ethanol-preserved flower material, herbarium specimens	Advertisement, efficiency (Dellinger <i>et al.</i> , 2014)
Pollen	Timing of anther dehiscence, pollen dispensing schedules	Qualitative classification, documentation in the field	Fresh flowers	Advertisement, efficiency (Castellanos <i>et al.</i> , 2004; Xiao <i>et al.</i> , 2017)
	Mechanism of pollen transfer	Qualitative classification	Pollinator observations, artificial pollination experiments	Efficiency (Wester & Classen-Bockhoff, 2007; Dellinger <i>et al.</i> , 2014, 2019a)
	Function ('pollen-dilemma'), is pollen also the reward?	Qualitative classification	Pollinator observations	Advertisement (Dellinger <i>et al.</i> , 2019a)
	Amount	Manual counting or use of a particle counter (e.g. Castellanos <i>et al.</i> , 2004)	Fresh or ethanol-preserved flower material	Efficiency (Cruden, 2000, but also see Lozada-Gobilard <i>et al.</i> , 2018)
	Quality (viability, protein content)	Germination experiments (e.g. Carr <i>et al.</i> , 2015)	Anthers preserved in lactophenol with aniline blue	Advertisement (Carr <i>et al.</i> , 2015)
Gynoecium	Pollen grain size and ornamentation	Scanning electron microscopy or light microscopy (e.g. Konzmann <i>et al.</i> , 2019)	Ethanol-preserved flower material	Efficiency (collectability of pollen e.g. Lunau <i>et al.</i> , 2015; Konzmann <i>et al.</i> , 2019)
	Pollen kit, viscin threads	e.g. Sudan dyes (Pacini & Hesse, 2005)	Ethanol-preserved flower material	Multifunctional (UV or water loss protection, pollen packaging; reviewed in Pacini & Hesse, 2005)
	Timing of stigma receptivity	Documentation in the field	Fresh flowers	Efficiency (Armbruster <i>et al.</i> , 2009)
	Position of stigma(s) relative to stamens (site of pollen release) or reward	Qualitative classification or length measurements or quantification through geometric morphometrics	Fresh or ethanol-preserved flower material	Efficiency (Cruden, 2000; Dellinger <i>et al.</i> , 2019a)
	Stigma size or shape	Qualitative classification or measurements, scanning electron microscopy	Fresh or ethanol-preserved flower material, herbarium specimens	Efficiency (Cruden, 2000, but also see Lozada-Gobilard <i>et al.</i> , 2018)
Herkogamy	Number of ovules	Quantification through microscope or HRX-CT (Staedler <i>et al.</i> , 2018)	Fresh or ethanol-preserved flower material	Efficiency (outcross pollen transfer, Opedal, 2018)
	Spatial separation between anthers and stigma	Measurements or morphometrics	Fresh or ethanol-preserved flower material	

Methods, type of floral material needed, and information on comparative or experimental evidence of the functional significance of certain traits in the pollination process are given. Where possible, objective (ideally quantitative) measurements on traits should be prioritised over qualitative categorisation. Information on methodological approaches is by no means exhaustive but represents examples of recent applications, more comprehensive methodology may be found in, for example, Kearns & Inouye, 1993 or the trait-specific literature.

These results confirmed that pollination-syndrome studies are often limited to narrow, qualitative, rather than quantitative, sets of traits. While pollinators could obviously be successfully predicted even from only a few (qualitative) traits, I argue that pollination syndromes should not be limited to pollinator prediction. By definition, pollination syndromes are multivariate in nature and (may) carry information on complex, multitrait phenotypes (Junker & Parachnowitsch, 2015). The long-lasting focus on the same narrow set of traits prevents us from exploring new areas of phenotypic evolution and gaining a more profound understanding of how flowers function. Adding new traits to studies pays off, however. For example, the relatively recent addition of quantitative assessments of floral scent through headspace techniques and gas chromatography-mass spectrometry analyses has opened up a new 'unseen' world (Schiestl & Dötterl, 2012). As studies in different systems are compiled, we start to see patterns of scent compounds that are potentially relevant in the communication with different functional pollinator groups and floral antagonists (Junker & Parachnowitsch, 2015). Another fruitful potential addition to pollination syndromes could be petal cell shape. Several authors have reported conical cells associated with bee pollination and flat cells found in bird-pollinated plants (Papiorek *et al.*, 2014; Dellinger *et al.*, 2019a). Such findings merit further investigation to evaluate whether these patterns are system specific or possibly applicable across broad taxonomic scales.

The choice of appropriate methods and metrics for the collection of phenotypic data is crucial in all biological disciplines and often limited by time constraints, equipment or available (floral) material (Table 1). Coding for traits qualitatively, as is common in pollination-syndrome research, and is fast and informative particularly when analysing large numbers of disparate species. The discretisation of biological traits always bears risks, however (Wilson *et al.*, 2007; Abrahamczyk *et al.*, 2017). First, discretisation may be highly subjective and circular in that categories are established for patterns (e.g. syndromes) one expects to find. Second, while character coding may be accurate when performed by the same observer, it may be difficult to repeat for a different observer. And third, trait categorisation misses the recording of within-taxon variation, which is essential to understand the adaptive potential of populations or direction/strength of selection. Thus, authors should critically evaluate whether and which traits they can record quantitatively in order to arrive with objective, comparable and repeatable datasets. Quantitative trait measurements are nowadays increasingly used for advertisement traits (Table 1; Fig. 1b) but still relatively uncommon when studying flower–pollinator fit traits. Morphometrics provides a useful approach to quantifying fit traits (Pérez *et al.*, 2007; Kaczorowski *et al.*, 2012; Pérez-Barrales *et al.*, 2014; Strelin *et al.*, 2017; Wilson *et al.*, 2017; Smith & Kriebel, 2018). To date, most authors have considered flowers in only two dimensions and focused on corolla shape only (e.g. Kaczorowski *et al.*, 2012; Gómez *et al.*, 2016; Smith & Kriebel, 2018; Benítez-Vieyra *et al.*, 2019). It is high time, however, that we start studying flowers (and their pollinators) as what they are: highly three-dimensional structures. The relative position of the corolla and the reproductive organs is decisive in mediating fit with the pollinators and assuring pollen transfer.

Recent advances in obtaining detailed 3D-flower models through high-resolution X-ray computed tomography (HRXCT) now provide the necessary basis for studying flower shape variation through landmark-based geometric morphometrics without losing information on the spatial arrangement of floral organs (van der Niet *et al.*, 2010; Sedeek *et al.*, 2014; Wang *et al.*, 2015; Wilson *et al.*, 2017; Dellinger *et al.*, 2019c; Kellenberger *et al.*, 2019; Reich *et al.*, 2020). A wide array of methods is available and constantly being expanded, allowing for statistical analyses of multivariate quantitative trait datasets (Table 2). If, finally, authors choose to rather work with qualitative traits, they need to establish objective criteria for trait discretisation which may also be employed by future researchers working on the same system (Wilson *et al.*, 2017; Dellinger *et al.*, 2019a). To this end, categorisation may outperform quantification when working with morphologically or functionally complex composite traits which cannot be expressed by simple measurements (e.g. pollen-expulsion mechanism, Dellinger *et al.*, 2019a).

8. Not all floral traits are equally informative

Not all floral traits are affected equally by (pollinator-mediated) selection (Pérez *et al.*, 2007; Pérez-Barrales *et al.*, 2014; Fenster *et al.*, 2015; Caruso *et al.*, 2018), and not all traits are equally important in each pollination syndrome (Johnson, 2013; Zung *et al.*, 2015; Dellinger *et al.*, 2019a). Despite this knowledge, traditional pollination syndromes often lump together all traits without further analysing differences in specificity and function. Are there (traditional syndrome) traits that are commonly recorded but bear little predictive power?

In total, 77 of the 97 studies estimating pollinator efficiency recorded floral traits. The traits most commonly assessed were (mostly nectar) reward (62.3%), timing or duration of anthesis/sexual phase (48.1%), scent (35.0%), corolla length (32.5%), corolla width (29.9%) and colour (29.8%; Fig. 5a; Table S3). Interestingly, 56 of these studies reported some traits as being particularly informative in characterising the specific pollination syndrome or in discriminating visitors from pollinators (Fig. 5b; Table S4). Nectar (39.3%), colour (30.4%), scent (26.8%), flower size (19.6%) and anthesis patterns (16.1%) were reported as important traits most often. In addition, 25 studies stated which traits were uninformative or misleading in syndrome classification (Fig. 5c; Table S5), with colour (52.0%) being most uninformative/confounding (i.e. contradicting traditional syndrome expectations), followed by scent (32.0%), shape (24.0%) and reward (16.0%).

Clearly, these results have to be treated with care, as traits have not been recorded/analysed the same way. However, it is noteworthy that nectar rewards are assessed most often and reported as highly informative, while colour is assessed only half as often but reported as most uninformative/misleading (Fig. 5d). Flower colour undoubtedly is highly important in many systems (Dudash *et al.*, 2011; Johnson, 2013; Rodrigues *et al.*, 2018), but not in all. Selection studies have shown, for example, that flower colour is not selected by pollinators, while display size and plant height are (*Silene*, Brothers & Atwell, 2014; Fenster *et al.*, 2015).

Table 2 Methods for data analyses.

Purpose	Questions	Methods and application	References
Characterising pollination syndromes	Which floral traits are most powerful in separating different pollination syndromes? Which traits vary independently from pollination syndromes? Which pollination syndromes can be separated reliably with the given floral characters?	Discriminant analyses: identification of floral traits which are most powerful in separating flowers pollinated by different functional pollinator groups Random Forest analyses: examination of the contribution and behaviour of floral traits (predictor variables) in separating predefined groups (i.e. functional pollinator groups)	Serrano-Serrano <i>et al.</i> (2017) Johnson (2013), Dellinger <i>et al.</i> (2019a)
Disparity of pollination syndromes or floral modules	Do pollination syndromes differ in their morphological diversity, that is do certain functional pollinator groups select for more constrained (less disparate) phenotypes? Are some syndromes morphologically more constrained? Are certain parts of the flower (e.g. 'efficiency traits') more constrained than others?	Calculating dissimilarity per syndrome or per floral module, testing for significant differences between syndromes or modules using PERMANOVA (nonparametric analyses of variance)	Chartier <i>et al.</i> (2017), Dellinger <i>et al.</i> (2019a), Vasconcelos <i>et al.</i> (2019)
Visualising pollination syndromes	Do species pollinated by the same functional pollinator group occupy a similar area of multivariate trait space? Do species pollinated by different functional pollinator groups occupy different areas of trait space?	Principal component analysis: for traits recorded at interval scale (e.g. morphometric measurements) Non-metric multidimensional scaling, principal coordinates analysis: traits recorded at other scales (interval, ordinal, categorical), calculation of distance matrix (i.e. euclidean distances, Bray–Curtis)	Strelin <i>et al.</i> (2016) Wilson <i>et al.</i> (2007), Chartier <i>et al.</i> (2017), Dellinger <i>et al.</i> (2019a)
Summarising interaction networks	Is there a relation between pollination syndrome and connectivity or nestedness? Which floral traits contribute to specialization and/or network modularity in different plant communities? How does (human-induced) environmental change affect interaction networks?	Network analyses (calculate e.g. modularity, specialization, connectivity, nestedness), bi-partite networks of flower and pollinator traits, Machine Learning algorithms	Olesen <i>et al.</i> (2007), Watts <i>et al.</i> (2016), Pichler <i>et al.</i> (2020)
Predicting pollinators	What is the most likely pollinator of a certain plant species?	Discriminant analyses: predicting pollinators through the most discriminant floral traits of species with known pollinators (see above) Cluster analyses, Machine Learning (e.g. Random Forest analyses: classification through Machine Learning, Random Forest is trained on floral trait data for species with known pollinators, then pollinators are predicted for species without pollinator information) Expert coding by a single trait (or multiple traditional syndrome traits)	Martén-Rodríguez <i>et al.</i> (2009), Lagomarsino <i>et al.</i> (2017), Serrano-Serrano <i>et al.</i> (2017) Johnson (2013), Abrahamszyk <i>et al.</i> (2017), Dellinger <i>et al.</i> (2019a), Pichler <i>et al.</i> (2020) Lagomarsino <i>et al.</i> (2017), Fragoso-Martínez <i>et al.</i> (2018)
Testing/ validating pollination syndromes	Do floral traits and pollinator assemblages correlate?	Correlation analyses (e.g. Mantel test) on distance matrices or bi-partite networks of flower and pollinator traits and Machine Learning algorithms	Smith <i>et al.</i> (2008), Martén-Rodríguez <i>et al.</i> (2009), Johnson (2013), Pichler <i>et al.</i> (2020)
	Does prediction by different floral trait sets yield the same result?	Validate classification through the construction of a morphospace based on traits not used for classification (i.e. if syndromes classified on colour, exclude colour from validation)	Lagomarsino <i>et al.</i> (2017)
	Are pollination syndromes reliable tools to predicting pollinators?	Validate syndrome prediction through empirical observations in the field	Armbruster <i>et al.</i> (2011)

Table 2 (Continued)

Purpose	Questions	Methods and application	References
Evolution of pollination syndromes	What is the ancestral pollination syndrome? How often have shifts in pollination syndrome occurred? Is there a recurring shift directionality? Are there any reversals? Are some syndromes more likely to shift than others?	Ancestral state reconstruction of selected floral traits or pollination syndromes on phylogeny; when reconstructing deep nodes, heritable floral traits may be more reliable than evolutionarily labile pollination syndromes (van der Niet <i>et al.</i> , 2014)	Lagomarsino <i>et al.</i> (2017), Serrano-Serrano <i>et al.</i> (2017), Dellinger <i>et al.</i> (2019a)
	Do floral traits respond to pollinator-mediated selection? Are pollination syndromes associated with different evolutionary optima? Do flowers pollinated by the same functional pollinator group converge in the same optimum? Do flowers with different secondary pollinators evolve towards different optima?	Phylogenetic comparative methods (e.g. Brownian motion, Ornstein–Uhlenbeck models)	Smith <i>et al.</i> (2008), Strelin <i>et al.</i> (2016), Lagomarsino <i>et al.</i> (2017), Smith & Kriebel (2018)
	What is the impact of pollinator shifts on plant diversification? Do certain functional pollinator groups increase speciation or extinction rates? Are certain (combinations of) pollinators evolutionarily more successful? Do different floral trait clusters evolve at different rates? What is the relative importance of other biotic interactions (e.g. competition for pollinators) and abiotic conditions in clade diversification?	Modelling speciation/extinction/diversification in relation to pollination syndromes	Muchhala <i>et al.</i> (2014), Lagomarsino <i>et al.</i> (2016), Serrano-Serrano <i>et al.</i> (2017), Kriebel <i>et al.</i> (2019), Wessinger <i>et al.</i> (2019)
	How do flowers adapt to different pollinator selection regimes? Are certain floral parts/modules more evolvable and evolve at different rates? Can pollinator shifts disrupt within-flower correlation/covariance structures? Are some syndromes more evolvable than others?	Geometric morphometrics of flower shape and multivariate phylogenetic comparative analyses	Dellinger <i>et al.</i> (2019c), Reich <i>et al.</i> (2020)

Overview of questions which can be addressed using pollination syndromes and a nonexhaustive compilation of methods for trait and syndrome data analyses and recent applications. Note that when testing the evolution of syndromes and traits, robust phylogenies are a prerequisite; difficulties of inferring phylogenies (i.e. due to incomplete lineage sorting or ancient hybridisation) are not dealt with here.

Indeed, as an advertisement trait, colour may also underlie selection by antagonists (avoidance of herbivores, Camargo *et al.*, 2018) or pollinator competition (Muchhala *et al.*, 2014). It would be too early (and most likely wrong) to dismiss any trait from pollination syndromes. Instead, I argue that we should focus on traits which do and do not match syndrome expectations alike. Trait mismatch may come from complex biotic interactions, but also be a mere consequence of a species' phylogenetic background (developmental/genetic constraints) and the limited number of trait combinations that could evolve (O'Meara *et al.*, 2016). By systematically evaluating the relative importance of a large number of traits and quantifying (mis)match with traditional syndromes, we may better pinpoint the traits important in adaptation to pollinators in each study system and also understand how adaptation can be achieved through new trait combinations (Dellinger *et al.*, 2014, Pichler *et al.*, 2020).

9. Analysing floral evolution and diversity in the context of pollination syndromes

To date, our understanding of how flowers evolve and adapt to pollinators remains fragmentary. From a functional perspective, flowers are highly synorganised (integrated) structures in which different organs co-function to achieve flower protection,

pollinator attraction and rewarding as well as pollen transfer, fertilisation and seed development (Armbruster, 2014; Diggle, 2014). Despite this overall (functional) integration, the above-mentioned processes may influence different parts of the flower differently, potentially leading to different (functional) modules *within* the flower (Armbruster, 2014; Esteve-Altava, 2017). Recent macroevolutionary analyses have indeed demonstrated that such functional modules may evolve independently of each other, that is along different trajectories and at different rates, in response to pollinator-mediated selection (Opedal, 2019; Dellinger *et al.*, 2019c; Reich *et al.*, 2020). This relative evolutionary independence of functional modules has been proposed to facilitate (and hence explain the frequent occurrence of) shifts between pollination syndromes in closely related plant species (Armbruster, 2014). Several authors have suggested that a single mutation of large effect in advertisement traits (i.e. colour or scent) suffices to induce a pollinator shift, as it may lead to the attraction of a different pollinator (Bradshaw & Schemske, 2003; Wessinger *et al.*, 2014; Wessinger & Rausher, 2015; Sheehan *et al.*, 2016 there were only two studies that calculated disparity). Alternatively, scenarios have been proposed in which strong (extrinsic) alterations in the pollinator community (i.e. environmental perturbation, colonisation of new habitat) render a new pollinator more efficient and flowers gradually adapt to this new pollinator, that is through

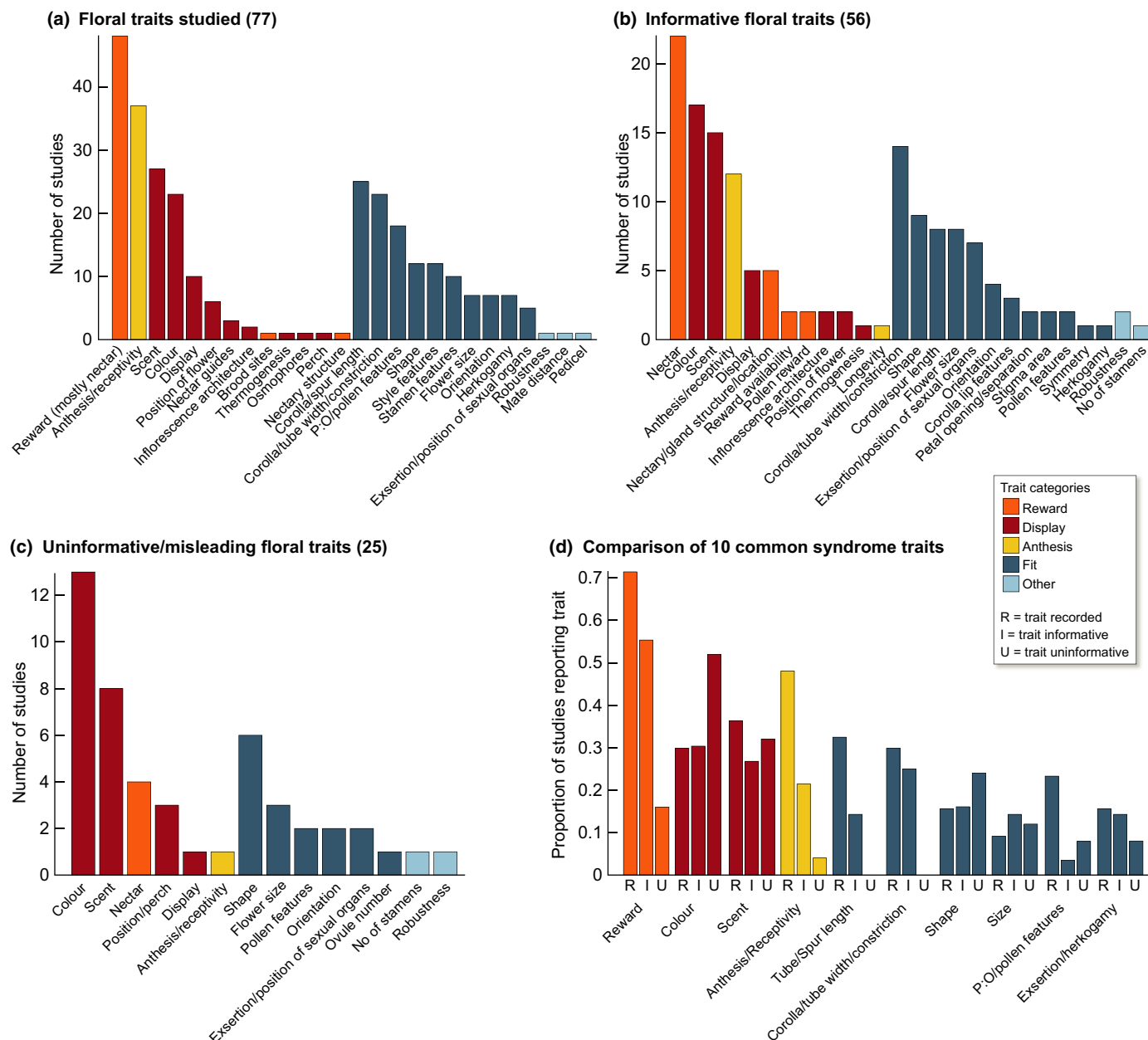


Fig. 5 Floral traits studied and reported as informative or uninformative/misleading in differentiating/characterising syndromes; advertisement traits appear in red colours, efficiency traits in blue colours. (a) Reward, anthesis, scent, colour, corolla length, corolla tube width and pollen : ovule ratios were studied most often. (b) Reward (nectar), colour, scent and corolla tube width were reported as most relevant in characterising syndromes while (c) colour, scent and flower shape were reported as misleading/indicating other pollinators than observed. (d) Proportional comparison of (a–c).

optimisation of efficiency traits (Thomson & Wilson, 2008; Kay *et al.*, 2019). What is the likelihood of either of these scenarios? Do advertisement traits consistently evolve faster than and change before efficiency traits? How important are changes in advertisement traits in mediating reproductive isolation in sympatry compared to allopatry? And to what extent are such trait changes independent (modular) or integrated?

Molecular developmental studies, experimental manipulations, selection studies and QTL screening have the capacity to get to the core of these questions (Bolstad *et al.*, 2014; Wessinger *et al.*, 2014). In recent years, particular focus has been placed on studying the

advertisement traits colour and scent (each trait included in 33% of studies in the literature review, but also see Kostyun *et al.*, 2019). In several pollination-syndrome model systems (e.g. *Antirrhinum*, *Mimulus*, *Penstemon*, *Petunia*), these traits are controlled by few genes and trait modification have had large effects on pollinator attraction. Through the creation of floral phenotypes intermediate between bee and moth pollination, for example, Dell'Olivo & Kuhlemeier (2013) could show that colour change significantly increased pollination by the new pollinator, supporting the idea that single mutations of large effect could trigger pollinator shifts. Furthermore, changes in colour have also brought about a change in

other functional traits such as nectar guides (affecting the correct positioning of pollinators) or petal cell shapes (reviewed by Yuan *et al.*, 2014). Such observed correlations have been attributed to pleiotropy (e.g. reward and corolla shape in *Penstemon*, Wessinger *et al.*, 2014; flower size and nectar in *Jaltomata*, Kostyun *et al.*, 2019) or to linked, but independent, gene clusters (e.g. colour, scent and reproductive organ length in *Petunia*, Hermann *et al.*, 2013; Kostyun *et al.*, 2019). Both processes allow the rapid adaptation of multiple traits to changing pollinator selection regimes.

The above-mentioned studies linking phenotypic to genotypic variation are restricted to a limited number of model systems that can be propagated and crossed in great numbers (Smith *et al.*, 2020). The vast majority of biological systems and plant–pollinator interactions is not part of this group, however. Phylogenetic comparative methods offer a powerful tool to analysing tempo, mode and mechanisms of floral trait evolution in these systems (Smith & Kriebel, 2018; Wessinger *et al.*, 2019; Dellinger *et al.*, 2019c). In recent years, authors have often tested the importance of a single or a few isolated traits in explaining diversification (Lagomarsino *et al.*, 2016). For understanding mechanisms and patterns of flower evolution and diversification in relation to pollination, however, multivariate trait datasets are needed. (Geometric) morphometrics are particularly promising for testing different hypotheses of shape evolution in flowers. While such tests have a long tradition in anthropology and zoology (Esteve-Altava, 2017), this is not the case in plants. This is somewhat surprising as monophyletic plant lineages with repeated independent shifts into different pollination syndromes offer the optimal set-up to testing the role of developmental constraints and functional adaptation under divergent (pollinator) selection regimes. How do pollinator shifts affect correlation structures within the flower? Is there developmental, functional or evolutionary linkage between floral organs/modules? Are certain intrafloral modules more integrated than others and (how) do they differ in their rate of evolution? Are these patterns different between different pollination syndromes? Although testing of these questions at macroevolutionary scales in plants is in its infancy, first patterns start arising. First, efficiency traits involved in the direct physical interaction with pollinators show particularly strong signals of selection and correlated (integrated) evolution (Opedal, 2018, 2019; Benítez-Vieyra *et al.*, 2019; Dellinger *et al.*, 2019c; Reich *et al.*, 2020; compared with Caruso *et al.*, 2018, also see Fig. 5d). Second, pollinator shifts could disrupt both type and strength of correlation structures within flowers and change functional modules (Dellinger *et al.*, 2019c; Reich *et al.*, 2020). And third, the modularity of specialised flowers was found to be structured by function, while modularity of generalised flowers was structured by developmental affinities of floral organs (Reich *et al.*, 2020). It will be interesting to relate these results to advertisement traits to understand more deeply their interplay during pollinator shifts and, potentially, speciation.

Another concept that ties in here is the concept of evolvability. The standing genetic variation of a trait under selection determines its potential to adapt, and hence to constrain or facilitate shifts in pollination syndrome (Opedal, 2019). Rarely have pollination biologists aimed at quantifying this variation and associated

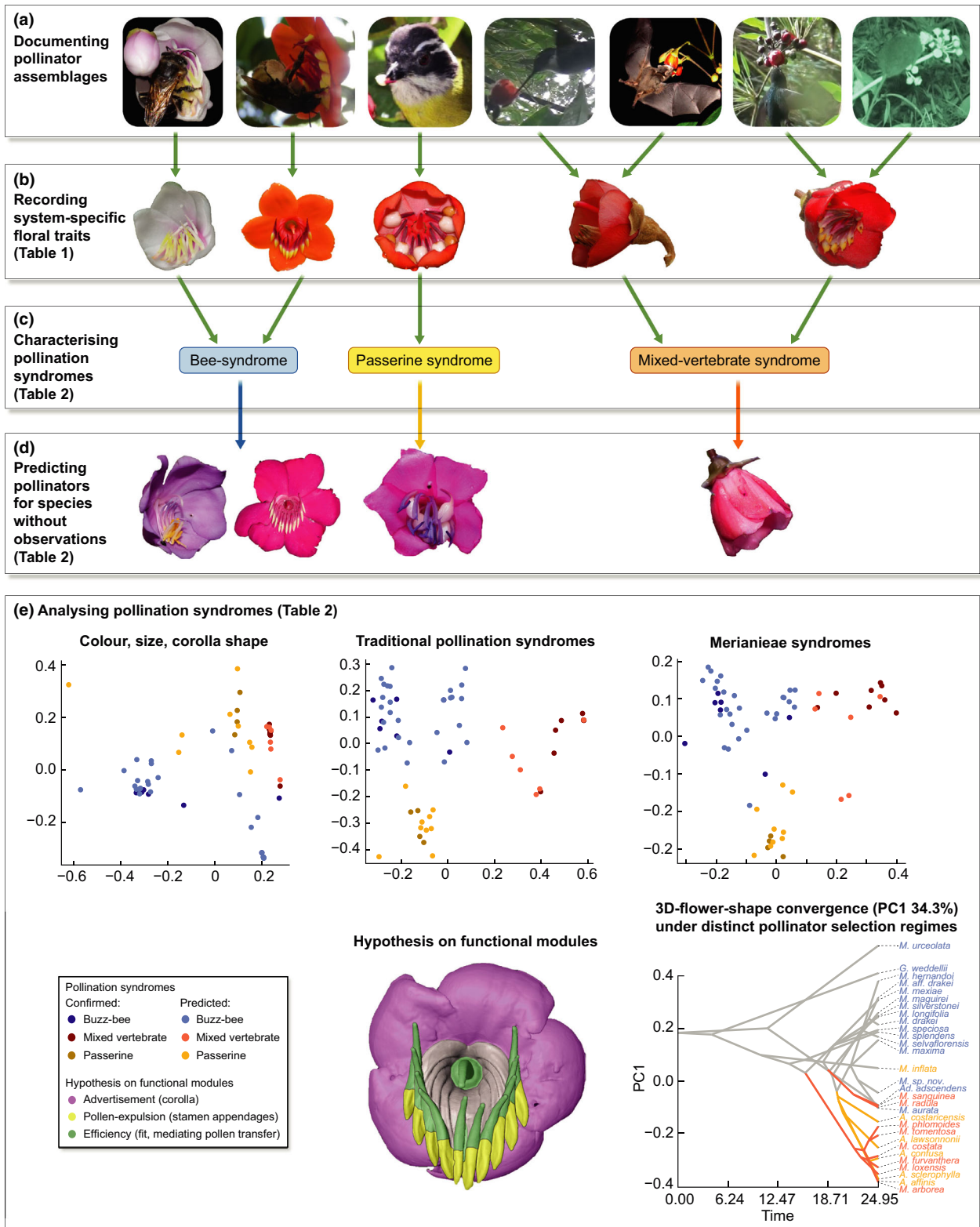
evolutionary potential (Armbruster, 2014). What is the relationship between floral phenotypic variation/diversity (flower disparity, Chartier *et al.*, 2017), evolvability and functional pollinator groups? Are flowers adapted to certain functional pollinator groups more disparate than flowers adapted to other groups? Are ancestral pollination syndromes (estimated from extant taxa) more disparate than shifted syndromes? And is disparity (as a measure of evolvability) in the ancestral pollination syndrome a prerequisite for pollinator shifts (Opedal, 2019)? Although disparity could be calculated and analysed easily from multivariate trait datasets (31.1% of studies in the literature reviewed contained multivariate analyses), there were only two studies that calculated disparity (Dellinger *et al.*, 2019a; Vasconcelos *et al.*, 2019). These studies found high disparity in two large bee-pollinated clades (Melastomataceae, Myrtaceae), but this led to pollinator shifts in only one clade (Dellinger *et al.*, 2019a). Disparity of the shifted syndromes was significantly lower (Dellinger *et al.*, 2019a).

Earlier this year, the extension of comparative methods to genotype-to-phenotype mapping approaches through the development of new software tools has been proposed (Smith *et al.*, 2020). This extension holds tremendous and exciting potential for pollination syndromes to more deeply investigate the questions and patterns outlined above in nonmodel organisms (Smith *et al.*, 2020).

III. Merianieae as a case study – pollination syndromes and flower evolution

In our study on floral evolution in Merianieae (Dellinger *et al.*, 2019a), we aimed to describe pollination syndromes at the tribal level (Fig. 6). Hence, we expanded trait coding beyond traditional syndrome traits and included 61 floral traits that were potentially relevant for pollination in the group. By contrast with many other studies (see section II.6 ‘Prediction of pollinators’), syndrome predictions were inaccurate when using only a subset (colour, size, shape) of traditional syndrome traits (Fig. 6e; Tables S6, S7). When including the full set of characteristics used in traditional syndromes, the same three syndromes (‘buzz bee’, ‘passerine’, ‘mixed-vertebrate’) could be distinguished, as with the 61 traits (Fig. 5e). Analysis of the 3D flower shape space through geometric morphometrics in Merianieae yielded the same three syndromes (Dellinger *et al.*, 2019c).

Through Machine Learning approaches, we found that several traits that were not included in traditional syndromes but specific to Merianieae, such as the pollen-expulsion mechanism or structural stamen properties, were most important in differentiating syndromes (Table 1; Fig. 6e). Specifically, buzz pollination with associated tubular anthers is ancestral in Merianieae and pollen can only be released through vibrations produced by bees (Dellinger *et al.*, 2019a). Although vertebrates cannot vibrate flowers, the tubular anther structure typical for buzz syndrome was retained during shifts to vertebrate pollination (Dellinger *et al.*, 2019a). This mismatch between trait and syndrome expectation is highly interesting as it possibly reflects a developmental constraint and led to the evolution of complex alternative pollen-expulsion mechanisms (Dellinger *et al.*, 2014).



The availability of a large trait dataset and a (dated) phylogeny made it possible to analyse flower evolution in Merianieae more deeply. First, we tested one basic assumption of the pollination-syndrome concept that has been tested surprisingly infrequently: that flowers pollinated by different functional groups underlie

divergent selection regimes. Indeed, OU models, explaining adaptation under different selection regimes, fitted the data best (Fig. 6e; Dellinger *et al.*, 2019c). Second, we tested different hypotheses on developmental or functional modularity of Merianieae flowers (Fig. 6e). These analyses revealed that all flowers are

Fig. 6 Exemplary workflow for establishing and studying pollination syndromes at low taxonomic scales (tribe Merianieae, Melastomataceae). (a) Documenting pollinator (and visitor) assemblages throughout anthesis, ideally quantitatively (Supporting Information Notes S1), should be the first step when establishing a new study system. (b) Recording a large set of system-specific, potentially pollination-relevant floral traits (Table 1). Advantages of objective quantitative trait coding have been demonstrated (Abrahamczyk *et al.*, 2017). (c) Characterising and describing pollination syndromes for species with known pollinators using objective methods (i.e. Machine Learning approaches, Pichler *et al.*, 2020). These methods may also be used to validate syndrome delimitation by testing the predictive power on species with known pollinators (Tables 2, S7). (d) Based on syndromes characterised in step (c), predictions of pollinators may be made for species where no pollinator observations are available or feasible, that is to study pollination syndromes across speciose clades (Table 2). (e) Further analyses of pollination syndromes may include calculation of disparity (morphological diversity), testing different hypotheses of floral modularity or studying floral evolution and angiosperm diversification (Table 2). Here, three different morphospaces have been calculated to exemplify how differences in the number and type of traits studied affect syndrome delimitation. While flower colour, size and corolla shape are not sufficient in differentiating syndromes, grouping into syndromes became clear when using 14 traditional pollination syndrome characters and 61 Merianieae specific characters (Tables S6, S8). Pollination syndromes may further be used to analyse flower evolution more deeply: here, a hypothesis of floral modularity, partitioning the flower into three functional modules, is presented as well as the results of modelling 3D-flower-shape evolution under an Ornstein–Uhlenbeck process to test for convergence under distinct pollinator selection regimes. All presented data, syndrome classification, morphospace calculation and traitgram are derived from Dellinger *et al.* (2019a,c). Pictures of flowers stem from Dellinger *et al.* (2019a), pictures of pollinators have been taken by Dellinger with the exception of the bat pollination picture © Nathan Muchhala, used with permission. Coloration in morphospaces is based on syndrome predictions from Dellinger *et al.* (2019a).

structured by functional adaptations to their specific pollinators and that different organ types can make up a functional module. The ‘buzz bee’ syndrome was the most modular (particularly in corolla shape). Modularity was significantly weaker in the two vertebrate syndromes in which corolla shape has acquired a new pollinator fit function (also see Table S8). Finally, analysis of the rate of evolution between advertisement and efficiency module showed that advertisement traits evolve around six times faster than efficiency traits (Fig. 6e; Dellinger *et al.*, 2019c).

IV. Concluding remarks

Much discussion in the last decades has lingered on the same problems regarding the validity and predictive potential of pollination syndromes. My review demonstrates that some of these problems (i.e. predictive inaccuracy, visitation by multiple functional groups) have been solved by refining techniques, while others (i.e. few traits studied) prevail. Overall, I argue that pollination syndromes are a robust concept to accurately circumscribe plant–pollinator relationships both at large and small scales, as long as the longstanding caveats are acknowledged. I emphasise that each study system has its specific discriminant traits, which the researcher has to identify before employing any categorisation or syndrome prediction. Hence, instead of focusing our discussion on whether a flower corresponds to a specific traditional syndrome, we may rather want to quantify the reason, extent and way by which it deviates from a traditional syndrome. Sophisticated statistical tools help us to approach these questions and provide remedy against typological classification (Table 2). In any case, an essential prerequisite is the detailed and objective (ideally quantitative) assessment of flower and pollinator data.

Furthermore, I emphasise that pollination syndromes, as a multivariate concept, should not be limited to pollinator prediction. Particularly the combination of broad trait datasets with quantitative pollinator data and molecular/phylogenetic comparative methods holds tremendous potential for more deeply understanding mechanisms of floral evolution through selection, developmental/genetic constraints and trade-offs. Studies on flower trait functioning have shown that traits involved in

pollinator attraction are evolutionarily labile and underlie weaker selection compared with traits mediating physical interaction with pollinators. In this respect, pollination syndromes will be important to extrapolate findings, that is whether certain functional trait clusters are particularly strongly selected by certain functional groups (or generalist assemblages) but not by others, and whether these findings are consistent in diverse plant lineages and communities in different parts of the world. Finally, pollination syndromes are also an important tool in conservation and may serve, for example, for large-scale analyses of the effect of environmental perturbations on functional diversity.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Methods S1 Literature review – explanations on the criteria used for data categorisation from the literature.

Methods S2 List of literature reviewed.

Methods S3 Literature review – data analyses.

Notes S1 Methods for data acquisition – pollinator data.

Table S1 Number of studies in different plant families.

Table S2 Traits used to substantiate syndrome expectations.

Table S3 Traits recorded in syndrome studies.

Table S4 Number of studies that found specific traits informative.

Table S5 Number of studies that reported specific traits as uninformative or misleading.

Table S6 List of traits considered as ‘traditional syndrome traits’ in empirical case study.

Table S7 Probability of correct pollinator predictions using Random Forest analyses.

Table S8 Disparity (morphological diversity) of the three different organ types in the different pollination syndromes in Merianieae.

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