ANNALS OF
BOTANY
Founded 1887

A global test of the pollination syndrome hypothesis

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● *Background and Aims* 'Pollination syndromes' are suites of phenotypic traits hypothesized to reflect convergent adaptations of flowers for pollination by specific types of animals. They were first developed in the 1870s and honed during the mid 20th Century. In spite of this long history and their central role in organizing research on plant–pollinator interactions, the pollination syndromes have rarely been subjected to test. The syndromes were tested here by asking whether they successfully capture patterns of covariance of floral traits and predict the most common pollinators of flowers.
● *Methods* Flowers in six communities from three continents were scored for expression of floral traits used in published descriptions of the pollination syndromes, and simultaneously the pollinators of as many species as possible were characterized.
● *Key Results* Ordination of flowers in a multivariate 'phenotype space' defined by the syndromes showed that almost no plant species fall within the discrete syndrome clusters. Furthermore, in approximately two-thirds of plant species, the most common pollinator could not be successfully predicted by assuming that each plant species belongs to the syndrome closest to it in phenotype space.
● *Conclusions* The pollination syndrome hypothesis as usually articulated does not successfully describe the diversity of floral phenotypes or predict the pollinators of most plant species. Caution is suggested when using pollination syndromes for organizing floral diversity, or for inferring agents of floral adaptation. A fresh look at how traits of flowers and pollinators relate to visitation and pollen transfer is recommended, in order to determine whether axes can be identified that describe floral functional diversity more successfully than the traditional syndromes.

Key words: Convergent evolution, floral traits, global, montane meadow, multidimensional scaling, mutualism, phenotype space, pollination syndromes, temperate grassland, test, tropical forest, tropical mountains.

'[*Solanum dulcamara*] ist … ein gutes Beispiel … der Willkürlichkeit und Unnatürlichkeit, in die man unvermeidlich verfallen muss, wenn man die fast unendliche Mannigfaltigkeit der Blumenformen in eine gewisse Zahl scharf umgrenzter Grundformen (Typen) einzuzwängen versucht.'

('[*Solanum dulcamara*] is … a good example … of the arbitrariness and unnaturalness one must unavoidably fall into, if one tries to force the almost infinite diversity of floral form into a certain number of sharply delineated basic forms [types]).'

Hermann Müller (1882, p. 20)

'As accumulation of knowledge continues, we eventually find facts that will not fit properly into any established pigeon-hole. This should at once be the sign that possibly our original arrangement of pigeon-holes was insufficient and should lead us to a careful examination of our accumulated data.'

H. A. Gleason (1926, p. 7)

INTRODUCTION

Convergent evolution is a ubiquitous feature of the biosphere, as indicated by correlations between phenotype and ecology across distantly related taxa. Examples include the broad correlations between tooth traits and diet in mammals (e.g. Eisenberg, 1983) and between leaf traits and the physical environment in higher plants (e.g. Givnish, 1986). In pollination ecology, 'pollination syndromes' provide the prototypical example. These are suites of convergent floral traits hypothesized to adapt distantly related angiosperm species to particular types of pollen vectors. Distinct syndromes have been proposed for abiotic pollination by wind or water, as well as

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for pollination by broad animal groups such as butterflies, bees or birds. The pollination syndromes have roots in the writings of Federico Delpino (1873–1874), who proposed two distinct schemes for categorizing flowers according to traits such as shape, colour, scent and size. Delpino's schemes were discussed vigorously in the literature (not always favourably; the quote above from Hermann Müller is a critique), and eventually merged by Vogel (1954) and further modified by van der Pijl (1960) and Faegri and van der Pijl (1979). Waser (2006) provides a historical overview of developments that led to the current manifestation of these traditional pollination syndromes.

The pollination syndromes have been of service in three main ways. First, they have supplanted earlier, more phenomenological schemes for organizing the overwhelming phenotypic diversity of flowers. Indeed, their second use has been to provide a mechanistic explanation for floral diversity, i.e. convergent adaptation for specific types of pollinating agents (e.g. Fenster *et al.*, 2004). Finally, they have been and still are used to infer what pollinates plant species in the absence of direct observations (e.g. Pérez *et al.*, 2006; Whittall and Hodges, 2007). It is no surprise, then, that the syndromes have played a central role in the development of pollination biology, as evidenced by their prominent position in review volumes (e.g. Knuth, 1898; Faegri and van der Pijl, 1979; Proctor *et al.*, 1996), and by their continued use (e.g. Fenster *et al.*, 2004; Whittall and Hodges, 2007).

However, the pollination syndromes represent a specific hypothesis regarding the nature of floral variation and its ultimate causal roots. Surprisingly, little effort has been made to subject this important hypothesis to any form of rigorous test at a large (e.g. community) scale. Previous tests of the hypothesis have been theoretical (Ollerton and Watts, 2000) or have focused on single plant species (e.g. Consiglio and Bourne, 2001; Hargreaves *et al.*, 2004; Zhang *et al.*, 2005; Valdivia and Niemeyer, 2006), small clades (e.g. Kay and Schemske, 2003; Wilson *et al.*, 2004; Wolfe and Sowell, 2006) or specific floral traits (Herrera, 1996; Waser *et al.*, 1996; Perret *et al.*, 2001; Raguso *et al.*, 2003; Ramírez, 2003) – often a subset of the phenotypic traits by which the traditional pollination syndromes have been described, or novel traits specific to certain clades (e.g. Kay and Schemske, 2003; Wilson *et al.*, 2004; Pauw, 2006). As valuable as these approaches are, the only previous study which assessed the predictive value of traditional syndromes in diverse communities, using a wide range of floral traits and with no prior knowledge of pollinators, was that of Hingston and McQuillan (2000).

In this study we attempted a general test of the utility of the traditional animal pollination syndromes. To this end, we ask two questions, (1) Do most animal-pollinated plants fit into traditional pollination syndromes? (2) Do these syndromes successfully predict the most frequent pollinators of the flowers? Data were collected for the test in six plant communities around the world. Flowers in these communities were chosen in an unbiased way and scored in standardized fashion for phenotypic traits included in the most commonly cited statement of the animal pollination syndromes, that of Faegri and van der Pijl (1979), with secondary reference to Proctor *et al.* (1996) in a few instances. We began by ordinating the descriptions of these traditional syndromes to define a multivariate phenotype space that described the distribution of groups defined by the syndrome attributes. We next calculated scores for individual plant species in this multivariate space and asked whether they fell within the traditional syndrome clusters. Finally, we asked whether the most frequent pollinators of plant species fit those predicted by the nearest syndromes.

METHODS

Community surveys

Floral traits were surveyed in six communities from Africa, North America and South America, ranging from temperate grassland and sub-alpine meadows to tropical rainforest (Table 1, and Supplementary Data 1, available online). The surveys encompassed all of the animal-pollinated plants in bloom and accessible in each community during the survey period, between 25 and 90 % of all of the biotically pollinated plants in the community. The pollinators of as many plants as possible were also observed, as explained in Supplementary Data 1. A visitor was considered to be a pollinator only after five or more 'legitimate' visits (i.e. not nectar or pollen robbing) by that animal species to different individuals of a given plant species had been observed, and after evidence of contact between the animal and both male and female reproductive organs of flowers had been obtained. Pollinators were pooled into functional groups (*sensu* Fenster *et al.*, 2004; Ollerton *et al.*, 2006, 2007) of similar species (e.g. 'bees', 'birds', 'butterflies'). For plants visited by more than one functional group, the major pollinator group was identified on the basis of visit frequency.

Faegri and van der Pijl (1979) and Proctor and Yeo (1996) describe 11 syndromes for animal-pollinated plants: bat, bee,

TABLE 1. *Characteristics of the communities surveyed in this study*

Site	Latitude	Longitude	Elevation	Survey month	Survey year	Habitat type(s)	No. of plant species surveyed	% of plant species surveyed
Guyana	3°16′N	59°45′W	100 m	April	2000	Rainforest, savannah	92	Approx. 50 %
Venezuela	10°28′N	67°45′W	5 m	May	2000	Coastal scrub, rainforest	70	Approx. 30 %
South Africa	29°37′s	30°08′E	1200 m	December–January	2000–2001	Grassland	70	Approx. 25 %
Colorado	38°59′N	106°58′W	3400 m	June–August	2000, 2001	Dry montane meadow	55	Approx. 90 %
California	34°13′N	116°57′W	2316 m	June–August	2001, 2002, 2003	Dry montane meadow	66	Approx. 75 %
Perú	13°12′s	72°5′W	4000 m	January	2002	Cloud forest, open scrub	129	Approx. 60 %

beetle, bird, butterfly, carrion fly, fly, hawkmoth, moth, non-flying mammal and wasp. Each syndrome is characterized by timing of anthesis, the presence and qualities of floral scent and nectar, and aspects of flower colour, size and morphology. From the syndrome descriptions of these authors, 41 floral traits were identified in 13 broad categories (see Supplementary Data 2, available online), and these were used to score flowers in our six communities, as well as the idealized syndrome descriptions (see below), for the presence (scored as 1) or absence (scored as 0) of each trait manifestation. Thus flowers of each plant species were described by a vector of 41 ones and zeroes.

An earlier study by Ollerton and Watts (2000) generated a single floral trait vector for each idealized syndrome, but syndromes actually comprise multiple trait combinations. For example, the bee pollination syndrome comprises, amongst other traits, vividly coloured flowers with or without nectar guides, and thus is described by trait vectors scoring 1 on vivid colour and either 1 or 0 for presence of nectar guides. Therefore, multiple trait vectors consistent with each syndrome were generated, yielding 537 total vectors across ten syndromes (moth and hawkmoth syndromes were combined because preliminary analysis showed that they were indistinguishable using this approach). Supplementary Data 3 (available online) shows the idealized syndrome vectors.

Statistical analyses

Non-metric multidimensional scaling (NMDS) in PC-ORD 5·14 (McCune and Mefford, 2006) was used to ordinate the 537 vectors described above. Unlike other ordination techniques, such as principle components analyis (PCA) or detrended correspondence analysis (DCA), NMDS makes no assumptions as to the distribution of the variables (Minchin, 1987; McCune and Grace, 2002; McCune and Mefford, 2006). Instead, NMDS ordinates objects based on rank distances, thus preserving ordered relationships, so that similar objects are close to each other (Legendre and Legendre, 1998). Sorensen's index (Bray–Curtis), which expresses the proportion of the maximum distance possible, was used to establish distance relationships between the objects in our data set (McCune and Grace, 2002). Sorensen's index maintains its sensitivity with complex data and is recommended for binary data such as ours (Beals, 1984; Faith *et al.*, 1987; Boyce and Ellison, 2001; McCune and Grace, 2002; McCune and Mefford, 2006).

NMDS uses an iterative approach for arranging objects in X dimensions, while minimizing stress, or departure from the distance relationships in the original data (McCune and Mefford, 2006). By comparing the level of stress (0–100, with 0 = no stress) in relation to the dimensionality, it is possible to identify a smaller number of dimensions that still preserve the original distance relationships among the objects with minimum stress (Legendre and Legendre, 1998; McCune and Grace, 2002). The best dimensionality to represent the data was identified by calculating the average stress values for 1–6 dimensions for 100 runs of real data (see below). These were compared with a Monte Carlo test with 500 ordinations of randomized data. Mean stress declined

from one to three dimensions, but did not appreciably decline further for higher dimensionality. Therefore, a three-dimensional (3-D) space was chosen for analyses.

A single run of the NMDS analysis first assigned random starting coordinates for the 537 idealized trait combinations in an ordination space of three dimensions, calculated Euclidean distances among the coordinates and compared the resulting distance matrix with distance relationships in the original trait space. Then the coordinates were moved along gradients of decreasing stress within the 3-D space until a local minimum was achieved. This process was repeated for 500 sets of random starting coordinates to find the best global solution, rather than a solution representing only a local minimum. Finally, the best of the 500 solutions was used as a starting point for one final run, which yielded the final ordination of the ten idealized pollination syndromes as clouds of multiple alternative trait combinations in three dimensions.

Ordination of the idealized syndrome trait combinations yielded a 'phenotype space' into which real plant species could subsequently be assigned. Coordinates were calculated for the plant species from each of the six communities, using the NMDS Scores Prediction algorithm in PC-ORD 5·14. This algorithm is conceptually similar to regression techniques, in that new observations are presented to a model, which then produces the corresponding outputs without altering the model itself (McCune and Grace, 2002). This is achieved by a successive focused search for the best position in the NMDS space, i.e. the position that minimizes the overall stress (McCune and Grace, 2002). In the first step, each axis, plus 20 % of the margins on both ends, is divided into 28 segments. The section with the lowest stress value is chosen, and is sub-divided into ten smaller intervals. The interval with the lowest stress is again chosen and is sub-divided once more into ten intervals. In the last round of the search, the coordinates with the lowest stress are identified as the best position for the particular observation (McCune and Mefford, 2006).

After calculating the position for each plant species in a community, the species were assigned to idealized syndromes based on the shortest Euclidean distance between the coordinates of each flower in the NMDS space and the centroids of clusters of points representing idealized syndromes. Alternative methods, including discriminant function analysis (DFA) and hierarchical clustering, produce similar results (not shown). So as not to be overly conservative in the assignment of plants to syndromes, we considered not only the closest syndrome to each flower, but also the second closest syndrome, so long as this was within 10 % of the distance to the first closest. Next, to address whether pollination syndromes accurately predict actual pollinators, the expected pollinators to each plant species were compared with our field observations of the most frequent pollinators.

Data for each community will be deposited in the Interaction Web Database at the National Center for Ecological Analysis and Synthesis (http://www.nceas.ucsb.edu/interactionweb/). In Supplementary Data 4 (available online) we list all plant species from each community that were included in the analyses, together with predicted pollinator groups and observed major pollinators.

RESULTS

Idealized syndromes and real flowers in phenotype space

NMDS ordination of the idealized syndromes resulted in well-resolved discrimination in 3-D phenotype space that retained nearly 90 % of the variance of the original among-syndrome variation (stress = 12·78, axis 1 $R^2 = 0.28$, axis 2 $R^2 = 0.50$, axis 3 $R^2 = 0.10$, cumulative $R^2 = 0.88$). Correlations between particular floral traits and the three axes can be seen in Supplementary Data 2 (available online). In this analysis, the traditional syndromes therefore occupy discrete regions of the phenotypic multivariate space, with no overlap (Fig. 1A), although some syndromes fall closer together than others (e.g. bat and non-flying mammal, bee and butterfly).

If plants within a community conform to the floral trait combinations expected by traditional pollination syndromes, then they would be expected to fall within or near the idealized syndrome clusters. In Fig. 1B–G, each plant surveyed within our six communities has been scored in the same NMDS space defined by the traditional syndromes. In all the communities some of the plants cluster together on the basis of shared floral traits. Whether or not they form clusters, however, actual flowers rarely fall within clouds of points representing an idealized syndrome. Only three of 482 species (approx. 1·0 %) across the six communities fell within a syndrome cluster; two species in the butterfly syndrome and one in the wasp syndrome.

Predictive utility of traditional pollination syndromes

The proportion of plant species for which we empirically determined major pollinators varied across communities (compare 'Total plant species' and 'Total comparisons' rows in Table 2). In most communities, it was >35 % of the species in flower, except for the Guyana and Venezuela communities, in which low rates of flower visitation meant that the pollinators of fewer species could be identified. Not surprisingly, our success in predicting major pollinators of flowers based on the closest idealized syndromes increased when the second closest syndrome was included according to the rules described above. With or without this inclusion, however, the prediction of pollinators was successful for less than half of all plant species overall in all communities except South Africa (Table 2). The mean ($\pm$ s.d.) percentage of correctly predicted species across the six communities was 29·7 $\pm$ 14·1 % based on the closest syndrome alone; this rose to 35·0 $\pm$ 13·6 % based on a match to either the closest or second closest syndrome in NMDS space.

Some pollination syndromes had greater predictability than others (Table 3). Bee- and fly-pollinated plants, for example, were accurately predicted more frequently from this analysis than other syndromes, whereas beetle and moth pollination were least often predicted. Furthermore, the predictive utility of different syndromes varied across communities. There is no obvious geographical pattern to this variation, although higher elevation sites (>2000 m; California, Colorado and Perú) had more fly-pollinated plants accurately predicted. Tropical communities, for example, did not exhibit greater predictability (see also Table 2), as might have been expected given that they possess on average a greater number of functionally specialized pollination systems than temperate communities (Ollerton *et al.*, 2006), although a larger sample of communities would be necessary to explore any geographical patterns properly.

It was also expected that predictability might be better for some plant families and worse for others. For example, families such as Lamiaceae and Fabaceae with largely zygomorphic flowers might fall near the bee pollination syndrome, and those with usually radially symmetrical, tubular flowers such as Rubiaceae and Apocynaceae might fall near moth or bird syndromes, whereas those renowned for having a high proportion of generalized pollination systems, such as Asteraceae and Apiaceae, might fare poorly. Table 4 shows that some of these expectations were indeed met. Surprisingly, however, Asteraceae was represented among the successfully predicted families in four of the six communities and in some cases by multiple species. This may be because Asteraceae were widely represented overall in these communities and some taxa had quite functionally specialized pollination systems, such as hummingbird-pollinated *Barnadesia* species in Perú.

Some of the surveys included most of the growing season and thus most of the plant species in the community, whereas others did not. Could this sampling variance have biased our results? We think not. Whereas small samples can miss some plant–pollinator links, and hence bias conclusions about the degree of ecological specialization (Ollerton and Cranmer, 2002; Herrera, 2005; Alarcón *et al.*, 2008; Petanidou *et al.*, 2008), sampling effects on our results are less clear. Variation in sampling should not have affected the analysis of clustering of real floral phenotypes with idealized syndromes, because plant species were randomly sampled. Sampling could have a greater impact on the analysis of the degree to which major pollinators are successfully predicted from phenotypic proximity to the nearest idealized syndrome. The results could be biased if certain syndromes are associated with low visitation rates, and hence left out of the second analysis. It is also possible that undersampling could increase the error in predicting major pollinators; we think this unlikely, however, because undersampling differentially affects observations of rare pollinators, not common ones, and indeed we detected no significant correlation between survey length (in months) and the proportion of correctly predicted pollinators (Spearman's $r = 0.20$, $n = 6$, $P = 0.70$).

DISCUSSION

How does one 'test' the pollination syndromes? This is not obvious, which is probably one reason for the paucity of tests! As we see it, there are three steps. First, one must define what one means by the syndromes. Secondly, one must make the syndromes operational in order to test them quantitatively. Finally, one must decide what properties or predictions of the syndromes are the most important ones to scrutinize.

Numerous definitions seem possible, and, indeed, the syndromes have long had a quality of being something each worker understands but none exactly agrees upon. For example, a recent review (Fenster *et al.*, 2004) first defines syndromes (their p. 376) as evolutionarily convergent suites of
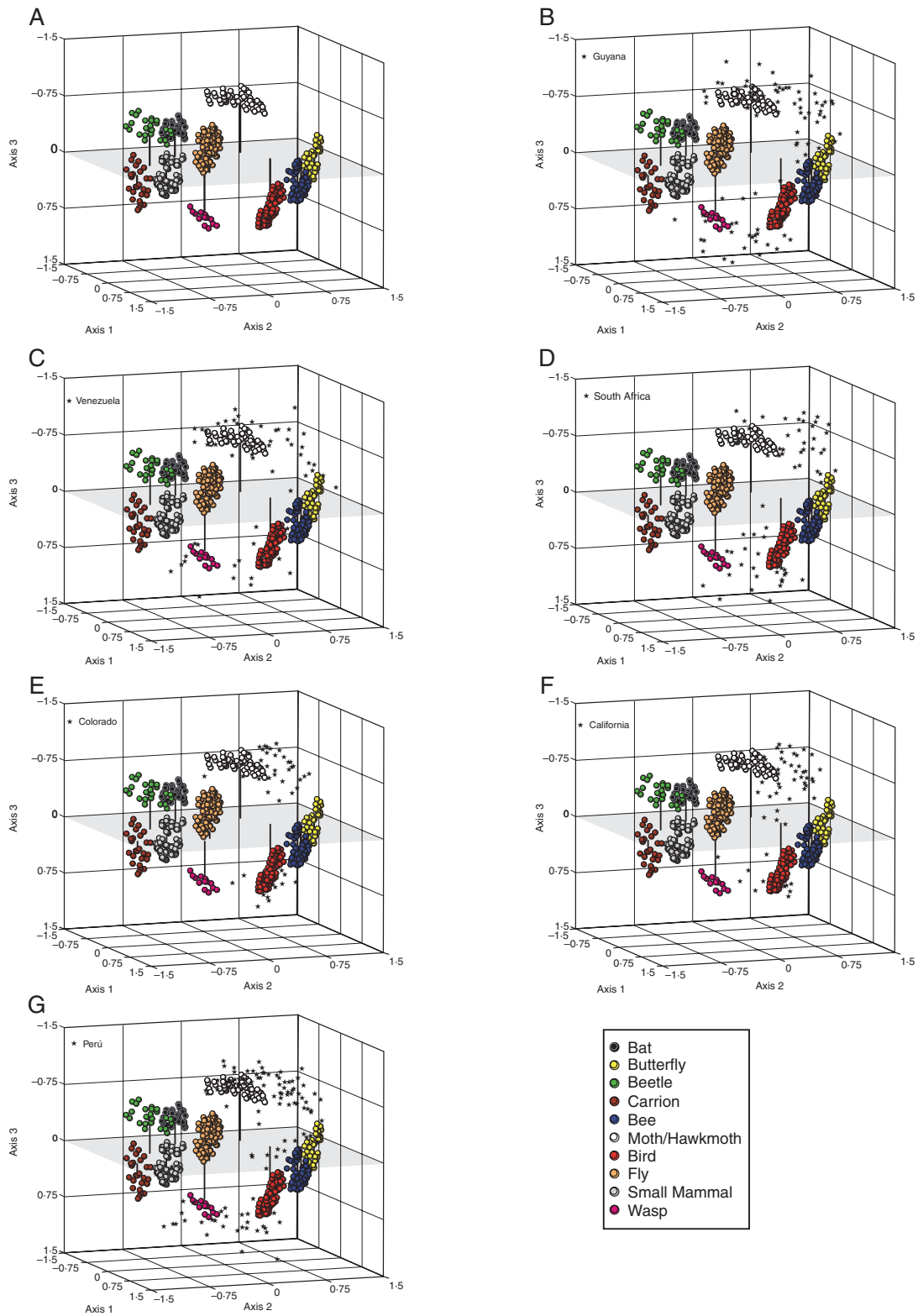
FIG. 1. (A) Non-metric multidimensional scaling (NMDS) ordination of 537 idealized pollination syndrome trait combinations as described in the literature. Each idealized syndrome consists of multiple alternative trait combinations and this is represented by multiple points. For explanation of the axes see text. (B) Ordination of the Kumu, Guyana, plant assemblage (black stars) in the idealized phenotype space. (C) Ordination of the Bahía de Petanamo, Venezuela, plant assemblage (black stars) in the idealized phenotype space. (D) Ordination of the Wahroonga, South Africa, plant assemblage (black stars) in the idealized phenotype space. (E) Ordination of the Virginia Basin, Colorado, plant assemblage (black stars) in the idealized phenotype space. (F) Ordination of the San Bernardino Mountains, California, plant assemblage (black stars) in the idealized phenotype space. (G) Ordination of the Mantanay, Perú, plant assemblage (black stars) in the idealized phenotype space.

TABLE 2. *Statistical details of the syndrome prediction analysis for the individual communities*

	Guyana	Venezuela	South Africa	Colorado	California	Perú
Total species	92	70	70	55	66	129
Total comparisons	23	14	24	27	44	59
Number of correct placements (closest syndrome)	6	1	12	10	12	18
% correct placements (closest syndrome)	26·1	7·1	50·0	27·0	27·3	30·5
Number of correct placements (first and second closest syndromes)	7	2	13	12	16	18
% correct placements (first and second closest syndromes combined)	30·4	14·3	54·2	44·4	36·4	30·5

'Total species' is the number of plant species in each survey; 'Total comparisons' is the number of plants for which major pollinators were determined; 'Number of correct placements' refers to the number of plants in 'Total comparisons' for which the pollinators were correctly identified using the Euclidean distance to the closest idealized syndrome(s) (see Methods).

TABLE 3. *Comparison of the predictive value of individual pollination syndromes for each community, and averaged across all communities*

Syndrome	Guyana	Venezuela	South Africa	Colorado	California	Perú	Mean % (s.d.)
Bee	14·3 (1/7)	50·0 (1/2)	23·1 (3/13)	16·7 (2/12)	31·3 (5/13)	0·0 (0/18)	22·6 (17·0)
Beetle	0·0 (0/7)	0·0 (0/2)	0·0 (0/13)	0·0 (0/12)	0·0 (0/13)	0·0 (0/18)	0·0 (0·0)
Bird	0·0 (0/7)	0·0 (0/2)	7·7 (1/13)	8·3 (1/12)	12·5 (2/13)	61·1 (11/18)	14·9 (23·2)
Butterfly	57·1 (4/7)	0·0 (0/2)	15·4 (2/13)	0·0 (0/12)	12·5 (2/13)	0·0 (0/18)	14·2 (22·1)
Fly	0·0 (0/7)	0·0 (0/2)	7·7 (1/13)	75·0 (9/12)	43·8 (7/13)	33·3 (6/18)	26·6 (29·9)
Moth	0·0 (0/7)	0·0 (0/2)	23·1 (3/13)	0·0 (0/12)	0·0 (0/13)	0·0 (0/18)	3·9 (9·4)
Wasp	28·6 (2/7)	50·0 (1/2)	23·1 (3/13)	0·0 (0/12)	0·0 (0/13)	5·6 (1/18)	17·9 (19·8)

Only the seven major types of pollinators which were known to be present in most of the communities surveyed were included. The values are the percentages of plant species which were correctly predicted to have particular major pollinators, organized by pollination syndrome. Figures in parentheses are the number of 'syndrome' matches/number of plant species correctly placed in that community. Note that for the sake of brevity only the results for the assignments to either the first or second closest syndromes have been included.

TABLE 4. *Family-level taxonomic identity of the plant species for which pollinators were correctly predicted on the basis of their pollination syndrome (either first or second closest syndrome)*

Guyana	Venezuela	South Africa	Colorado	California	Perú
Apocynaceae × 5	Apocynaceae	Apiaceae	Apiaceae × 2	Asteraceae × 4	Agavaceae
Asteraceae	Fabaceae	Apocynaceae × 4	Asteraceae	Caryophyllaceae	Alstroemeriaceae × 2
Rubiaceae		Fabaceae	Caryophyllaceae	Fabaceae × 3	Apocynaceae
		Gentianaceae	Fabaceae	Melanthiaceae	Asteraceae × 5
		Iridaceae × 2	Geraniaceae	Onagraceae × 2	Bromeliaceae
		Orchidaceae × 2	Orobanchaceae	Orobanchaceae	Caryophyllaceae
		Rubiaceae	Plantaginaceae	Plantaginaceae	Ericaceae
		Scrophulariaceae	Polemoniaceae	Portulacaceae	Onagraceae
			Rosaceae	Ranunculaceae	Passifloraceae × 2
			Valerianaceae × 2	Rosaceae	Solanaceae

Note that in some communities more than one species in a family was correctly predicted, as indicated, for example, by 'Apocynaceae × 5'. Families are arranged alphabetically within columns.

floral traits 'associated with the attraction and utilization' of specific 'functional groups' (p. 377ff.) of pollinators. Later (p. 388), a close reading suggests that the 'convergence' and 'trait suite' components of the definition can be relaxed to include any association of floral traits with functional pollinator groups within a specific lineage, whether or not the association adheres to a specific set of floral traits. Finally, these authors speak of adherence to 'traditional syndromes' (p. 395). We have attempted to test only the last of these three definitions, but this raises the question of what the 'traditional syndromes' are. Hence the next task is to choose a

source of syndrome descriptions. We chose Faegri and van der Pijl (1979), with occasional additional guidance from Proctor *et al.* (1996). These sources distinguish syndromes that will not accord with the intuition of all workers [as examples, the bee syndrome is not divided further into syndromes for small vs. large bees, as was done by Vogel (1954) and others; and some workers, such as Hess (1983), omit all aspects of flower shape from syndromes]. Furthermore, they give little or no guidance as to how different floral traits ought to be weighted, so that the default is equal weighting, as we have used. Finally, they imply that one set

of syndromes will apply across geographic regions and plant taxa (below alternatives to such a 'universalist' approach are discussed below). On the other hand, these two books are frequently cited in discussions of pollination syndromes, and provide a starting point for a test.

How do we next prepare the verbal descriptions of syndromes, derived from our source books, for analysis? Whereas it is straightforward to classify a given flower as white or yellow, some other trait descriptions are more difficult to interpret (e.g. 'vivid' colour, 'stiff' anthers), and it took considerable discussion and re-reading of the source texts in order to reach consensus. Acknowledging these difficulties, we now must subject verbal descriptions to quantitative scrutiny. This would be impossible without modern methods of multivariate analysis, which allow the conversion of words into trait vectors. The method used here is NMDS, which is recommended for ordination of binary ($+/-$) ecological data (Minchin, 1987; McCune and Grace, 2002). In contrast to NMDS, which makes no assumptions about the distribution of the variables, other techniques assume that variables are unimodally distributed [e.g. correspondence analysis (CA) and DCA], or assume linear relationships among variables (e.g. PCA and DFA), thus rendering them inappropriate for data such as ours (McCune and Grace, 2002).

Finally, what properties or predictions of the syndromes should we examine? We have examined their ability to describe actual trait combinations in flowers and to predict major pollinators. Is the latter reasonable? As explained in the Introduction, the pollination syndromes are an evolutionary concept (leaving aside that several strong proponents couched them in essentialist and teleological, rather than strictly Darwinian, terms; see Pancaldi, 1984; Vogel, 1954, 2006). The syndromes describe presumed adaptations to 'attract and utilize' pollinators, i.e. results of past (and potentially ongoing) natural selection on the floral phenotype (e.g. Faegri and van der Pijl, 1979). Therefore, we might expect to see the same types of pollinators at present as those that have formed the pollination (=selection) environment of the past. This argument supports successful prediction of pollinators as one criterion for evaluating the utility of syndromes. It is reasonable to argue, of course, that pollination environments observable at the present time will not always indicate past environments (Ollerton, 1996; Lamborn and Ollerton, 2000; Rivera-Marchand and Ackerman, 2006). We certainly agree that plant–pollinator interactions can be dramatically altered by such things as anthropogenic disruption (e.g. Kearns *et al.*, 1998). On the other hand, we are unaware of any evidence for recent modifications of pollination environments sufficiently widespread to render prediction of pollinators an inappropriate test of syndromes. Furthermore, arguing that current pollination does not reflect past pollination may lead to the conclusion that any observation is consistent with interpreting a given floral phenotype as 'the ghost of pollination past' – in other words, it is in danger of explaining everything, and therefore nothing.

How did the syndromes fare by our test? We found that each idealized syndrome forms a cluster of points in floral phenotype space, and that these clusters segregate reasonably well in the multivariate space. However, the regions of phenotype space that the syndromes define are largely unoccupied by real plant species. In other words, the combinations of floral traits of real plant species rarely conform exactly to the traditional pollination syndromes (we know, for example, that there are bird-pollinated flowers with blue, zygomorphic corollas, and beetle-pollinated flowers that are small, yellow and unscented, even though the traditional syndromes do not include such combinations). Furthermore, the primary pollinator was successfully predicted by the nearest syndrome for only about one-third of the plant species for which data on pollinator visitation frequencies as well as floral phenotype were obtained. What should we conclude? Most readers might agree that traditional syndromes (as defined above) fail to describe actual floral trait combinations accurately, but success in predicting major pollinators for one-third of all plant species is open to more individual interpretation. There is no disagreement that some fraction of angiosperms produces generalized flowers not strongly adapted to any particular type of pollinator (e.g. Delpino, 1874, p. 364; Proctor *et al.*, 1996, p. 173ff.). If one assumes that this fraction is small, then successful prediction in one-third of all cases is not very impressive, whereas if one assumes (say) that half of all plant species have generalized flowers, then successful prediction in one-third of all species might evoke the opposite reaction. However, in either case, prediction of pollinators from the traditional syndromes alone, as various recent workers have done (e.g. Grant, 1994; Bernardello *et al.*, 1999; Harrison *et al.*, 1999; Hansman, 2001; Perret *et al.*, 2001; Carpenter *et al.*, 2003), seems a risky business.

We stress that we do not take our results as evidence against convergent floral adaptation resulting from pollinator-mediated natural selection. In fact, we adhere strongly to the view that many floral traits reflect adaptive responses to selection by pollinators, and that the direction of selection is a function of properties of pollinator morphology and behaviour (e.g. Waser, 1983). However, we propose that thinking solely in terms of selection by a single 'most effective pollinator' (the most common functional group of visitor, or the one most effective in transferring pollen during a single visit, which are sometimes taken to be the same thing; Stebbins, 1970) fails to capture the range of logical possibilities. Floral adaptation might also be influenced by antagonistic floral visitors (e.g. Strauss and Armbruster, 1997; Strauss and Irwin, 2004), by mixtures of pollinators of different functional types (e.g. Hurlbert *et al.*, 1996; Waser, 1998) and, indeed, by pleiotropic effects on other plant traits (e.g. Rausher and Fry, 1993; Levin and Brack, 1995; Simms and Bucher, 1996). Observed floral phenotypes might even represent adaptations to 'minor' pollinators (Aigner, 2001, 2006), which certainly would contribute to mismatch between observed 'major' pollinators and putative syndromes! We argue for this broader set of perspectives as working hypotheses to explore empirically.

In the end, readers will draw their own conclusions about our test and its results, and it is sincerely hoped that some will devise and implement additional tests. Nonetheless, we would like to end by offering our own personal views on possible directions for future work on these questions. Of course we advocate a continued discussion of the classical syndromes, but our hope is that these will eventually be replaced with a conceptual view of plant–pollinator interactions that is less

classificatory in its aims and that relates directly to both pollinators and antagonists, and their ability to influence the evolution of the floral phenotype, with reference to the phylogenetic constraints or other influences that may also be important. We can think of three general ways to proceed toward this goal. First, we might adopt a 'bottom-up' mechanistic perspective, putting aside the traditional syndromes, starting fresh from simple assumptions about which traits matter most in determining which pollinators visit which flowers, which traits are the result of selection by antagonists and which are a result of the phylogenetic identity of the plant species in question. Such a 'minimalist' approach of identifying only those traits that are important may take us far toward explaining observed patterns of plant–pollinator interactions, and the (majority of) exceptions which do not seem to fit into the classical scheme. Several recent studies exemplify such a strategy. Stang *et al.* (2006) could predict most observed plant–pollinator links in a Spanish community in relation to accessibility of floral reward. Furthermore, such an approach successfully explained observed features of plant–pollinator interaction webs within single communities (Stang *et al.*, 2007) and across multiple communities (Santamaría and Rodríguez-Gironés, 2007). Secondly, we could take a 'top-down' pattern-analytic approach, using multivariate analysis to explore associations between floral traits and pollinator communities. We recognize the grave difficulties here of knowing which traits are relevant to pollinator attraction and use, and of measuring them in ways that reflect pollinator cognition (which varies even within taxa), rather than human cognition (the basis for traditional syndromes). Thirdly, we could use the approach of authors such as Armbruster (1993) and Castellanos *et al.* (2006), among many others, to map floral traits, pollinators and antagonists on to well resolved phylogenies in order to understand the association between particular flower phenotypes and the pollinating vector – a 'pollination systems' approach that requires a combination of rigorous field work and molecular laboratory skills. Currently some workers are using syndromes in this context, but in a more informed way than previously, with some supporting field evidence (e.g. Whittall and Hodges, 2007); however, the role of antagonists vs. pollinators has barely been explored in this regard (though see Armbruster, 1997).

It is not a foregone conclusion that any of these strategies (or others that future workers may devise) will uncover a universal or near-universal set of syndromes. Any syndromes that emerge may turn out to be idiosyncratic to geographic region or plant taxon (see also Ollerton *et al.*, 2003; Fenster *et al.*, 2004; Goldblatt and Manning, 2006). Region-specific traits are suggested by the difficulty of applying traditional syndromes developed in the Northern Hemisphere to the Gondwanan flora (Newstrom and Robertson, 2005), and by the poor predictive value of, for example, the butterfly syndrome in Tasmania (Hingston and McQuillan, 2000), in contrast to the Guyana community surveyed in this study. Taxon-specific traits are suggested by our results, with apparent differences across plant families in the predictive ability of traditional syndromes: Fabaceae, Apocynaceae and (surprisingly) Asteraceae fare better than other families. Indeed, some taxon-specific traits not included in the traditional

syndromes have been emphasized in recent literature, for example the green vs. red floral bracts and differing schedules of pollen presentation correlated with bee vs. hummingbird pollination in *Costus* and *Penstemon*, respectively (Thomson *et al.*, 2000; Kay and Schemske, 2003; Castellanos *et al.*, 2006), and the details of scent chemistry identified by Andersson *et al.* (2002) and Raguso *et al.* (2003). We view such idiosyncrasy, if it is confirmed, as no less interesting in suggesting mechanisms of floral evolution and patterns of floral ecology than universally recognizable end-points such as those proposed by the traditional syndromes.

The possibilities outlined above, and others we have not thought of, will provide exciting grist for the mill of future research, and should help in devising more profitable ways for reducing the dimensionality of floral variation and understanding the evolution of floral phenotypes. The traditional pollination syndromes have contributed a great deal to the development of pollination biology as a field, but our test across diverse communities suggests that the way forward lies in looking beyond them.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org/ and consist of the following files. (1) Descriptions of the six field sites in which surveys were conducted, and additional information on sampling protocols. (2) The list of 41 floral traits in 13 broad categories used to classify flowers into traditional pollination syndromes and their correlations with the axes from the non-metric multidimensional scaling ordination. (3) A table of the 41 floral traits × 537 idealized syndrome combinations used to generate the idealized syndrome phenotype space. (4) A list of all of the plant species from each community included in the analyses, together with the predicted pollinator.

ACKNOWLEDGEMENTS

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