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**LETTER**

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# Spatiotemporal variation in the role of floral traits in shaping tropical plant-pollinator interactions

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**Abstract**

The pollination syndrome hypothesis predicts that plants pollinated by the same pollinator group bear convergent combinations of specific floral functional traits. Nevertheless, some studies have shown that these combinations predict pollinators with relatively low accuracy. This discrepancy may be caused by changes in the importance of specific floral traits for different pollinator groups and under different environmental conditions. To explore this, we studied pollination systems and floral traits along an elevational gradient on Mount Cameroon during wet and dry seasons. Using Random Forest (Machine Learning) models, allowing the ranking of traits by their relative importance, we demonstrated that some floral traits are more important than others for pollinators. However, the distribution and importance of traits vary under different environmental conditions. Our results imply the need to improve our trait-based understanding of plant-pollinator interactions to better inform the debate surrounding the pollination syndrome hypothesis.

**KEYWORDS**
Afrotropics, foraging behaviour, Mount Cameroon National Park, pollination syndrome, pollination systems, pollinator predictability

## INTRODUCTION

The importance of floral traits for plant-pollinator interactions has been apparent since the 18th century (e.g. Sprengel, 1793). Darwin placed the origin of floral traits into the modern evolutionary framework (Darwin, 1859, 1862). Following suggestions of floral trait classifications according to their adaptive relationships to particular pollinators resulted in an influential ecological and evolutionary hypothesis, the pollination syndrome hypothesis (Faegri & van der Pijl, 1979; Fenster et al., 2004; Vogel, 1954). It defines that specific sets of convergent floral traits (e.g. colour, shape, odour or production and display of floral rewards) presumably evolved to attract particular groups of pollinators (Faegri & van der Pijl, 1979).

Nevertheless, the pollination syndrome hypothesis has been recently questioned. Community-wide studies exploring complex plant-pollinator networks demonstrated a higher generalisation in pollination systems and a lower predictability of pollinators based on floral traits than expected (Ollerton et al., 2009; Waser et al., 1996). This discrepancy may be related to considering all plants in the community, compared with individual case studies frequently targeting highly specialised and 'attractive' pollination systems (Dellinger, 2020; Waser

et al., 1996). Additionally, the degree of specialisation can differ among regions depending on their evolutionary history and stability (Johnson & Steiner, 2000). Recent empirical efforts have shown that the predictability of pollination syndromes in individual pollination systems varies from relatively high (e.g. Dellinger, Chartier, et al., 2019; Fenster et al., 2015; Hargreaves et al., 2004; Muchhala, 2006; Rosas-Guerrero et al., 2014; Vandelook et al., 2019) to low (e.g. Ollerton et al., 2009; Paudel et al., 2019; Rocha et al., 2020; Wang et al., 2020). Researchers also demonstrated that pollination systems can show parallel adaptations to multiple pollinator groups (Dellinger, Scheer, et al., 2019; Muchhala et al., 2009), and suggested reclassification of some pollination syndromes (Dellinger, Chartier, et al., 2019), or proposed that the pollination syndrome theory can be improved by other concepts such as evolutionary stable strategy (Pyke, 2016). Additionally, Abrahamczyk et al. (2017) highlighted the importance of using quantitative data, besides the common categorical traits, when testing pollination syndromes.

Simultaneously, detailed studies on particular traits from pollination syndromes have shown our insufficient understanding of their functions and importance (Dellinger, 2020). For instance foraging from long-spurred flowers do not always correspond to long-proboscid visitors (Vlašánková et al., 2017), while hummingbird visits are driven rather by nectar reward than other floral traits (Maruyama et al., 2013; Waser et al., 2018). The traditional pollination syndrome hypothesis, as a generalising concept, also largely overlooks some traits specific for individual plant lineages. For example a pollen expulsion mechanism representing an adaptation to buzz-pollination by bees (De Luca & Vallejo-Marín, 2013; Dellinger, Chartier, et al., 2019), or various corolla constrictions limiting access of visitors (Clark et al., 2015). Furthermore, the observed trait configuration is not necessarily an adaptation to pollinators only. Floral antagonists exert negative selection on floral traits, like size or amount of flowers, counteracting pollinator-mediated selection (Gélvez-Zúñiga et al., 2018). It is questionable to what extent red flowers are an adaptation to bird vision, or defence against nectar-thieving bees (Chittka & Waser, 1997; Rodríguez-Gironés & Santamaría, 2004; Wester et al., 2020), and if this role differs spatially (Chen et al., 2020). Moreover, some traits such as floral colour could have an additional role in interspecific plant competition for pollinators (Muchhala et al., 2014). Altogether, if individual floral traits largely differ in their role in plant-pollinator interactions (e.g. Maruyama et al., 2013; Schmid et al., 2015) and their synergistic effects are important (Fenster et al., 2015), the original category-based ordinations and classifications of individual traits are probably not the best expression of the real situation in nature (Abrahamczyk et al., 2017). Hence, new approaches are needed to reflect more complex interactions among floral traits and

to assess their importance for pollinators (Cutler et al., 2007; Dellinger, Chartier, et al., 2019; Pichler et al., 2020).

The role of individual floral traits and the associated selection can also vary in space and time (Abrahamczyk et al., 2011; Albrecht et al., 2018; Chen et al., 2020; Hawkins & Devries, 2009), which makes relying on traits to predict pollinators even more challenging. Consequently, the concept of pollination syndromes as a simple classification system represented by the categorical table of individual floral traits for particular pollinator groups (reviewed in Waser, 2006) should change into a flexible system considering different importance of individual traits for particular pollinators (i.e. Figure 1a), and/or under different environments (i.e. Figure 1b,c).

Elevational gradients in seasonal ecosystems feature high adaptive trait differentiation. This makes them an opportunity to study the spatiotemporal variability in the importance of individual floral traits in plant-pollinator interactions. Elevational gradients allow us to observe substantial changes in abiotic and biotic features (McCain & Grytnes, 2010), including taxonomical and functional diversities of plants and pollinators (Albrecht et al., 2018; Janeček et al., 2015), as well as their interactions (Mertens et al., 2021; Olesen & Jordano, 2002; Ramos-Jiliberto et al., 2010). Seasonal differences in communities and interactions are also known (Abrahamczyk et al., 2011; Maicher et al., 2018; Mertens et al., 2021). Distribution of individual floral traits have also been reported along elevation or among seasons (Chen et al., 2020), their role, however, remains unclear.

The importance of individual floral traits can be related to pollinator requirements and pollinator-community organisation. Spatiotemporal variation in the role, importance and diversity of pollinators has been observed (Abrahamczyk et al., 2011; Fenster & Dudash, 2001; Mertens et al., 2021), although little is known on the related variation in floral traits, except for floral resource availability. Pollinators have greater energetic requirements at higher elevations due to lower temperatures, or lower air pressure which hinders flight (Feinsinger et al., 1979), or during the wet season in humid tropical forests (Janeček et al., 2015; Maicher et al., 2018). Consequently, such conditions can increase the importance of floral traits related to energetic rewards (e.g. nectar production or concentrations), causing a higher specialisation of endothermic bird pollinators for floral resources during wet season (Janeček, Chmel, Mlíkovský, et al., 2021). The unfavourable conditions might also cause the increasing prevalence of larger pollinators during wet seasons, such as nectarivorous birds whose flight is less affected by rainy conditions compared with insects (Cruden, 1972; Janeček et al., 2015; Maicher et al., 2018). Nevertheless, some relationships remain stable, independent of seasonal changes, as was confirmed for the relationship between corolla tube length and bill length in hummingbird-plant interactions (Weinstein & Graham, 2017).
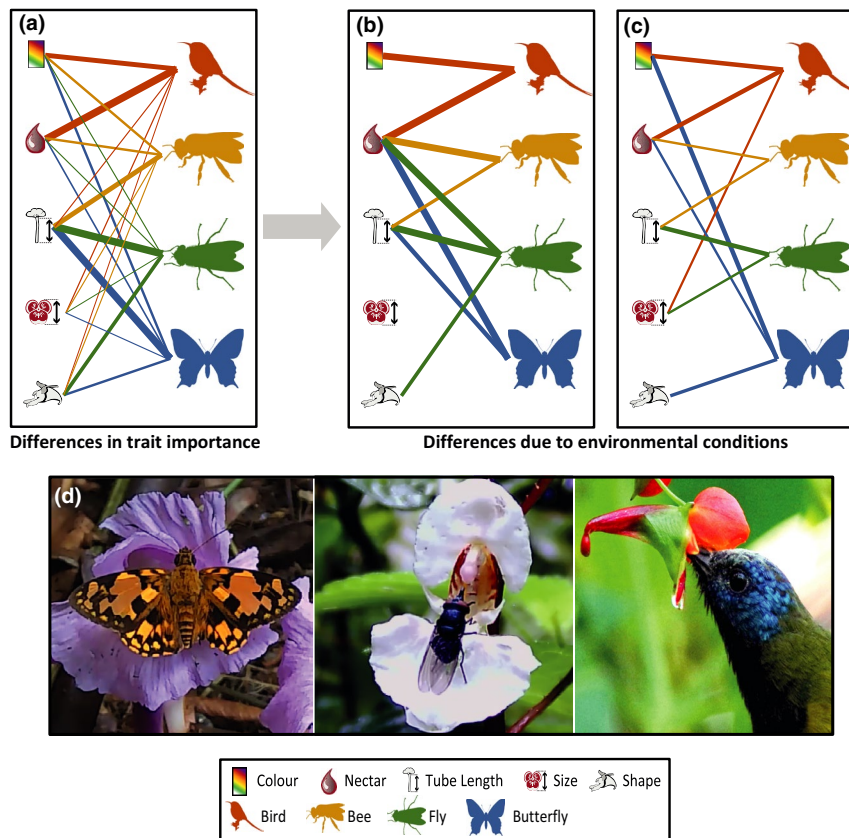
**FIGURE 1** Conceptual figure. (a) Compared to the 'traditional' pollination syndromes, floral traits differ in their relative importance for individual pollinator groups. (b, c) The relative importance of individual floral traits differs under different environmental conditions, for example along an elevational gradient and between seasons. (d) Examples of flower visitors found on Mount Cameroon: *Apallaga meditrina* on *Aframomum* sp., *Rhingia* sp. on *Impatiens burtonii* and *Cyanomitra oritis* on *Impatiens sakeriana* (first two pictures are screenshots from the video recordings, the third picture was taken by Š. Janeček). Note that the relationships shown here do not reflect the situation in the field, they are purely meant to conceptualise our study

We aimed to reveal how trait importance differs for different pollinator groups and under contrasting environmental conditions (Figure 1). We mainly focused on the role of floral traits for primary pollinators to challenge the pollination syndrome hypothesis. We recorded plant-pollinator interactions and floral traits at the community level at four sites along an elevational gradient on Mount Cameroon. We sampled in distinct seasons, with extreme rains in the wet season and almost no rain during the dry season (Maicher et al., 2020). We challenged the following hypotheses: (1) The relative importance of individual floral traits differs among particular groups of primary pollinators (Figure 1a); (2) There are environmentally (i.e. seasonal and elevational) driven changes in floral trait distribution and their relative importance for pollinators (Figure 1b,c).

## MATERIALS AND METHODS

### Study sites

This study was carried out on Mount Cameroon, Southwest Region, Cameroon (4.203°N and 9.170°E), the highest mountain in Western and Central Africa (4095 m

a.s.l.; Cable & Cheek, 1998). It represents an important biodiversity and endemism hotspot due to its location in the Cameroon Volcanic Line on the border of the Congo and Guinean bioregions (Sosef et al., 2017; Ustjuzhanin et al., 2018).

We sampled along the continuous elevational gradient of pristine rainforests, from lowland (650 m a.s.l.), to upland (1100 and 1450 m a.s.l.) and montane (2200 m a.s.l.) forests at the natural timberline on the southwestern slope of the mountain (Hořák et al., 2019; Maicher et al., 2020). The region experiences strong seasonality, with annual precipitation exceeding 12,000 mm at the lower elevations with monthly precipitation of over 2000 mm during the wet season (June to September), and almost no rainfall during the dry season (mid-November to February; Maicher et al., 2018, 2020). At each studied elevation, six transects (200 m × 10 m; at least 100 m apart) were established to characterise the local forest heterogeneity. We sampled along these transects; however, the search area also included the surrounding vegetation in case of insufficient replicates for particular plant species. All samplings were repeated in the dry and wet seasons of 2017 (1450 and 2200 m a.s.l.) and 2018 (650 and 1100 m a.s.l.).

## Floral trait measurements

We selected floral traits commonly included in the pollination syndromes definitions (Ollerton et al., 2009; Ollerton & Watts, 2000; Table S1). Morphometric traits (size, tube length/width) were measured with an electronic calliper, whilst visual (shape, symmetry, colour, flower/anther position, nectar guides) and olfactory traits (odour strength) were recorded by the observer. We simplified the classification of odour to weak-no, moderate and strong odour. Observers were trained together to minimise the individual bias for estimations of odour strength. The traits were measured for up to five individuals of the focal plant species.

Quantification of nectar sugar production was done by covering flowers with mesh bags for 24 h. Nectar from individual flowers with high nectar production was extracted following Bartoš et al. (2012) using capillary tubes. The nectar concentration was measured using a Pal-1 (Atago co.) pocket refractometer, after which we calculated the amount of sugar per μl of nectar following Galetto and Bernardello (2005). This was converted to the amount of sugar per flower by multiplying it with the measured volume in capillary tubes. For low-nectar producing flowers, we washed the flowers using a Hamilton syringe with filtered water, added ethanol to the samples and boiled it for 15 min to deactivate enzymes (Chlumská et al., 2014; Janeček, Chmel, Ewome, et al., 2021). The nectar samples were dried in the laboratory, where they were transferred into constant volumes. The concentrations of individual sugars were measured with high-performance liquid chromatography (HPLC) using the ICS-3000 system (Dionex) with an electrochemical detector and CarboPac PA 1 column (Janeček, Chmel, Ewome, et al., 2021).

## Visitor recording

The flowering plant species along the studied transects were recorded for 24 h using security cameras (VIVOTEK IB8367T with IR night vision; for more information on the methodology see Mertens et al., 2018, 2020; Klomberg et al., 2019) to detect their visitors. We recorded flowers at all vegetation strata from understorey to canopies (reached using tree climbing methods).

The functional groups were defined following the common pollination syndrome groups (birds, flies, bees, wasps, beetles, butterflies, moths, non-flying-mammals; Willmer, 2011), with the addition of cockroaches (Mertens et al., 2018; Vlasáková et al., 2019).

Only visitors observed touching anthers or stigmas were considered potential pollinators (referred to as pollinators in our paper) and included in our analyses (Padyšáková et al., 2013). Although pollination effectiveness of each flower visitor should combine visitation

frequency and pollen transfer per visit (Mayfield et al., 2001; Rosas-Guerrero et al., 2014), the scale of this study did not allow to include pollen transfer, and we relied on contact with reproductive organs as a proxy of pollination (similar to e.g. Biella et al., 2019).

## Statistical analyses

Following Dellinger, Chartier, et al. (2019), we used Random Forest models (abbreviated as RF; Breiman, 2001) to identify the most important floral traits for the primary pollinators (used as the categorical response variable, consistently with the pollination syndrome hypothesis). Here, we emphasise that 'importance' refers explicitly to model testing as we have not performed any ethological experiments that query the animals directly. We distinguished plants according to their most primary pollinators based on the visitation frequency, expressed as number of visits standardised by the recording time and recorded flowers, that is visitation frequency per flower and minute of recording.

RF is a classifier tool capable of evaluating the importance of both categorical and continuous predictor variables to classify given objects into particular groups of interest, in our case, plants with different pollination syndromes. This approach is based on selection among a high number of individual decision trees created by machine learning (i.e. a model automatically conditioned using a training data set to classify certain types of objects). Its branching algorithm is composed of nodes in which the objects (e.g. plants in our case) can be classified according to some rule into one of two groups (e.g. plants with smaller or larger flowers than 5 cm), branches leading from one node to another and terminal nodes. First, each decision tree is constructed using a randomly selected training subset of samples from our data set (e.g. a subset of plant species). Consequently, only a few randomly selected variables are evaluated for each node split, that is it is determined how well they split the objects of different properties (e.g. pollinator groups) and the best is then chosen for this particular node. The samples which were not used to build a given tree ('out of bag' samples in the RF terminology, abbreviated as OOB) serve for an estimation of the accuracy of the individual variables' classification in the given tree. In our case, the accuracy with which the plants from the used subset of data are correctly classified into primary pollinator groups according to their floral traits. The importance of each variable is measured by a decrease in the classification accuracy before and after random permutation of the variable values in the OOB samples. The results from individual trees are then pooled and the final decision on the importance (e.g. mean decrease in the accuracy) of each variable is made. Values ≤0 depict variables which did not improve the model's prediction accuracy. For more details on RF and its application

in ecology see Cutler et al. (2007), Johnson (2013), or Dellinger, Chartier, et al. (2019).

To reveal general patterns in floral trait importance, we firstly performed a single aggregated RF model for all floral traits and all four elevations and seasons. To test the most predictive model we afterwards removed the floral traits not improving the whole model accuracy (Table S3). These traits increase the model noise and decrease its total accuracy, because whenever only the unimportant traits are selected in a particular node of a decision tree of RF, the plants are affiliated into dichotomously created 'false' groups and such tree consequently reduce the model ability to classify the plants correctly. In both whole and reduced models, the primary pollinator was the most frequent visitor of each plant species without considering where and when the plants were recorded. Additionally, seasonal and elevational differences in the trait importance were explored by: (1) eight RF models separately for each season x elevation combination, (2) two RF models separately for wet versus dry season and (3) four RF models separately for each elevation. In these cases, the primary pollinator was the most frequent visitor of each plant species separately for individual elevations and/or seasons. We ran RFs comprising 300, 500, 1000 or 2000 trees each, and one to eight variables tested at each split (called *mtry*), our decision on the settings was made during the model tuning in *caret* package (Kuhn, 2008). For the individual elevations and seasons, the most important trait for each primary pollinator group was extracted from these analyses (based on the mean decrease in accuracy; MDA). Per trait, we listed the most common trait value found in plants visited by the specific pollinator groups in our data set. For the analyses, we used the *randomForest* package (Liaw & Wiener, 2002) in R 3.6.1 (R Core Team, 2019). To test the RFs' significance, we compared model *OOB error* against *random OOB errors* after the response variable (primary pollinators) randomisation. The null distribution of random OOB errors was performed by 199 permutations using *rf.significance* function in *rfUtilities* package (Evans & Murphy, 2018). To explore the accuracy of RF models for plants visited by particular primary pollinators, we calculated *sensitivity* (i.e. proportion of plants visited by the primary pollinator correctly predicted by the RF model) and *specificity* (i.e. proportion of plants correctly predicted as unvisited by the primary pollinator).

To study differences in the distribution of individual floral traits among elevations and seasons, we used Canonical Correspondence Analysis (CCA), a unimodal constrained multivariate method based on fitting the explanatory variables into the ordination space defined by the response variables (Ter Braak, 1986). We considered only those traits found to be the most important for at least one primary pollinator in at least one season x elevation combination (Table 3). Occurrence of plant species in individual seasons and elevations served as explanatory variables, whilst floral traits' values for individual plant species were used as response variables. We applied Monte Carlo permutation tests to evaluate the effect of elevation and season on the distribution of individual floral traits (Šmilauer & Lepš, 2014).

Additionally, we performed eight (i.e. separately for individual elevations and seasons) step-wise forward-selection CCAs to find the five most important (according to their contribution to variability explained by particular models) traits for the composition of pollinator communities in each elevation and season. In these analyses, the floral traits' values represented explanatory variables, whilst the visitation frequencies of individual pollinator groups to individual plant species served as response variables. All CCAs were performed using Canoco 5 (ter Braak & Šmilauer, 2012).

## RESULTS

Based on the 24-h video recordings of flowers of 117 plant species with a complete set of floral traits, we observed 13,024 individual interactions with potential pollinators, defined as contacting the plants' reproductive organs (Table S2). Bees were the most common primary pollinators (for 44 plant species), followed by flies (for 38 plant species), moths (for 14 plant species), birds (for 7 plant species), butterflies (for 7 plant species), beetles (for 4 plant species) and wasps (for 3 plant species), while cockroaches and non-flying mammals were primary pollinators of no plant species. Nevertheless, four and three plant species with beetles and wasps as the primary pollinators, respectively, were excluded from the RF analyses, because they were too rare for this method. Using our extensive floral trait database for all studied co-occurring flowering species at our focal sites, we were able to identify the importance of each trait for the primary pollinators across elevation and seasons. Most of the floral traits had small (if any) importance, and in consequence the complex RF model was not significantly predicting the pollinators (OOB error = 0.600; random OOB error = 0.650; $p = 0.09$; Table S3). Nevertheless, the simplified model including only three traits considerably improved the model mean accuracy (colour, sugar per flower and floral size), significantly predicting the plant pollinators (OOB error = 0.518; random OOB error = 0.645; $p < 0.001$; Table 1), indicating these three traits to be crucial in the studied plant-pollinator systems. For individual primary pollinators, our model showed the importance of colour for bees and moths, sugar per flower for birds and flies and floral size for butterflies (Table 1). However, the model sensitivity and specificity for individual pollinator groups largely differed. Whereas plants pollinated primarily by bees or flies were relatively well predicted, the model predictions based on the combinations of the floral traits were relatively unreliable for the other primary pollinators,

**TABLE 1** Floral traits' importance (expressed as the Mean Decrease in Accuracy), sensitivity and specificity for individual primary pollinator groups and for the whole RF models with the three most important traits

| Floral trait | Primary pollinator | | | | | Whole model |
| --- | --- | --- | --- | --- | --- | --- |
| | Bees | Birds | Butterflies | Flies | Moths | |
| Colour | **0.0442** | 0.0337 | 0.0272 | 0.048 | **0.0091** | 0.0396 |
| Sugar per flower | 0.0338 | **0.1488** | 0.0031 | **0.0898** | — | **0.0447** |
| Size | 0.0087 | — | **0.0908** | 0.0283 | — | 0.0113 |
| Sensitivity | 0.7045 | 0.1429 | 0.1429 | 0.5263 | 0 | |
| Specificity | 0.5 | 1 | 0.9905 | 0.7222 | 0.9688 | |

*Note:* In bold, the most important trait for each pollinator group and for the whole model are highlighted. '—' means no importance of the trait for a particular primary pollinator (i.e. Mean Decrease in Accuracy ≤ 0).

expressing the highly variable importance of the tested floral traits for different pollinator groups (Table 1).

We revealed spatiotemporal differences in the traits' importance in individual elevations and seasons (Table 2; see also detailed results of the RF models separately for individual seasons and elevations in Table S4), as well as in the traits' importance for the particular primary pollinators in individual elevations and seasons (Table 3; Table S5). The most conspicuous patterns were the common importance of sugar per flower in wet season, and of floral size at lower elevations. Various other traits were important in particular situations, such as anther position as the most important trait in the dry season at the highest elevations, and tube length in plant-pollinator communities around 1450 m a.s.l. (Table 2; Tables S4 and S5). Colour seemed to be important during the dry season at lower and middle elevations (Table 2; Tables S4 and S5). Looking at the particular primary pollinators, we identified the importance of high amounts of nectar sugar per flower for birds at two elevations in the wet season, whereas low amounts of nectar sugar were most important for flies at two elevations (Table 3). The only important floral traits for butterflies were floral size and shape. The traits important for bees and moths were highly variable. Nevertheless, the traits typical for the mellitophilous (bee-pollinated) and phalaenophilous and sphingophilous (moth-pollinated) pollination syndromes (e.g. nectar guides for bees, and strong odour for moths) were revealed as most important in particular elevations and seasons, similarly to the above-mentioned traits most important for the other primary pollinators (Table 3).

Elevation and season marginally significantly influenced the distribution of most important traits for pollinator composition, as listed in Table 3 (Figure 2a; $F = 1.2$, $p = 0.07$, 17.2% explained variability). The first and second axes correlated with season and elevation respectively. The CCA showed higher amounts of nectar sugar per flower at the highest elevation (Figure 2a), whereas some other traits, for example longer tubes or larger flowers, were affiliated to the lower elevations. Closed zygomorphic and opened actinomorphic flowers occurred mainly in wet and dry season respectively.

The partial CCA models identifying the role of floral traits in particular seasons and elevations revealed an increase in the variability explained by the traits towards the higher elevations and from dry to wet season (Figure 2b; for details on individual traits' importance see Table S6). These analyses confirmed the importance of floral traits for pollinators in conditions with scarcer resource availability (flowering plant diversity and amount of nectar resources; i.e. higher elevations and wet season). Additionally, we found that floral colours were often among the significant traits explaining the composition of pollinator communities at lower elevations and dry season, and that the high sugar production increased in its importance at higher elevations and during the wet season (Figure 2b).

## DISCUSSION

In the tropical forests of Mount Cameroon, our study showed that: (1) individual elevations and seasons hosted flowering plants with specific floral traits, (2) individual floral traits differed in their importance for particular groups of primary pollinators and under differing environmental conditions and (3) the floral traits were not only associated with the primary pollinators, but also with the composition of all pollinators. These, together with the low number of floral traits predicting the primary pollinators in our complex models and the low specificity and sensitivity of these predictions for some pollinator groups (Table 1), questioned validity of the 'traditional' definition of pollination syndromes. Nevertheless, such a low predictability does not mean that some plants are not pollinated according to the traditionally defined syndromes. On Mount Cameroon, the validity of pollination syndromes was already approved by case studies of several individual pollination systems (Klomberg et al., 2019; Mertens et al., 2018, 2020). Nevertheless, we have shown that individual floral traits play different roles for particular primary pollinators, which is getting even more complicated with differing environmental conditions. This is congruent with butterflies dominating in pollination of psychophilous (i.e. butterfly-pollinated) *Scadoxus cannabarinus* at lower elevations only (Mertens et al., 2020), or with nectarivorous sunbirds specialising in ornithophilous

**TABLE 2** Importance of floral traits (expressed as Mean Decrease in Accuracy) for primary pollinators in separate RF models for the four elevations and two seasons

| Floral trait / Elevation | 2200 m Dry | 2200 m Wet | 1450 m Dry | 1450 m Wet | 1100 m Dry | 1100 m Wet | 650 m Dry | 650 m Wet |
|---|---|---|---|---|---|---|---|---|
| Colour | — | 0.0024 | — | 0.0082 | 0.0299 | — | **0.0315** | 0.0017 |
| Sugar per flower | 0.0714 | **0.0100** | — | — | — | **0.0168** | 0.0258 | 0.0058 |
| Size | | — | 0.006 | 0.0059 | **0.0587** | 0.0083 | 0.0300 | **0.0079** |
| Shape | 0.003 | — | — | 0.0068 | 0.0068 | — | 0.0079 | — |
| Symmetry | — | — | 0.0068 | 0.0003 | 0.0039 | — | — | — |
| Tube length | — | — | **0.0375** | **0.0099** | 0.0030 | 0.0081 | 0.0115 | — |
| Odour strength | 0.0045 | — | 0.0008 | 0.0095 | — | — | — | — |
| Anther position | **0.0736** | 0.0032 | — | 0.0033 | — | — | 0.0001 | — |
| Nectar guides | 0.0181 | — | — | 0.0048 | 0.0086 | — | — | — |
| Flower position | — | — | — | — | — | — | — | 0.003 |
| Model OOB error | 0.2222 | 0.2105 | 0.5238 | 0.3200 | 0.4418 | 0.4737 | 0.4444 | 0.2105 |
| Random OOB error | 0.5556 | 0.2632 | 0.7143 | 0.3600 | 0.5882 | 0.4737 | 0.5556 | 0.2352 |
| p-value | **0.010** | **0.010** | 0.075 | 0.116 | **0.010** | 0.365 | 0.350 | **0.005** |

*Note:* In bold, the most important trait for the each elevation × season model is highlighted. '—' means no importance of the trait for a particular primary pollinator (i.e. Mean Decrease in Accuracy ≤ 0).

plants during the wet season (Janeček et al., 2015) whilst being more generalised during the dry season (Janeček, Chmel, Ewome, et al., 2021). Additionally, the seemingly low predictability of our models for some primary pollinators could also be related to the complexity of plant-pollinator interactions, whilst our analyses focused on a few selected floral traits. For instance the low ability of complex models to predict plants visited (or unvisited) by birds is congruent with a recent study on the asymmetric nature of sunbird-plant interactions (Chmel et al., 2021). Its authors demonstrated that while few plants with bird-pollination syndromes were frequently visited by birds, they also visited a wide spectrum of plants with other traits.

Seasonality affected the distribution of floral traits and their importance for the individual primary pollinator groups. The prevalence of closed flowers in the wet season (in contrast to open flowers in the dry season, Figure 2a) might be explained by their role in limiting the dilution of nectar (Aizen, 2003; Dafni, 1996; Pacini & Nepi, 2007), washing and/or damaging of pollen (Huang et al., 2002; Pacini & Franchi, 1984) or as a shelter for visitors. Rainfall increases the energy costs of flight in birds and bats (Aizen, 2003; Voigt et al., 2011), and especially for smaller insects whose activity or even abundance can be reduced by direct damage from rain (Kishimoto-Yamada & Itioka, 2015; Maicher et al., 2018; Struck, 1994). Accordingly, the nectar production was a good predictor of pollinators at the highest (2200 m) and middle (1100 m) elevations and during the wet season. Nectar sugar production was especially important for birds, the most energy-demanding pollinator group (Aizen, 2003), during the wet season. Since birds are more capable of dealing with rainfall (Aizen, 2003), other floral traits

associated with birds are expected to stand out as well. According to the individual RF analyses (Table 3), nectar sugar production was indeed more important for bird pollination. It has been already suggested that mainly food rewards determine pollinators' floral choice (Chmel et al., 2021; Janeček, Chmel, Ewome, et al., 2021; Schmid et al., 2016; Waser et al., 2018). Additionally, red and orange flower colours, associated with the bird pollination syndrome (Faegri & van der Pijl, 1979) and proven by numerous studies (Rodríguez-Gironés & Santamaría, 2004; Wester et al., 2020, although see Waser et al., 2018), were confirmed important to determine the composition of the pollinators (Figure 2b).

The particular floral traits' role for pollinating flies has been repeatedly studied, for example floral shape and symmetry were revealed as important (Lázaro et al., 2008). Comparing to bees, flies and vertebrates are more common pollinators at the higher elevations (Dellinger et al., 2021; Willmer, 2011). Fly-related traits (e.g. open flowers) were more common (Figure 2a) and generally more important for pollinator community composition at our highest elevation (Figure 2). This is contrary to a sharp decrease of floral traits associated with fly or hawkmoth pollination in orchid species along elevation in Réunion (Jacquemyn et al., 2005). In our study, the tube length and odour were more important traits for moth pollination consistent with Martins and Johnson (2013), Johnson et al. (2017) and Mertens et al. (2021).

We showed floral colour as the most important trait for bees (Table 1), although various traits were important in particular elevations and seasons (Table 3). This can be related to the high diversity in bees' morphology and reward preferences (e.g. pollen, nectar, resin) allowing the group to exploit a wide range of floral designs (Ollerton,

**TABLE 3** The most important floral trait (expressed as Mean Decrease in Accuracy) for individual primary pollinators according to the separate RF models per season and elevation

| Elevation/Season | Bees | Flies | Butterflies | Moths | Birds |
| --- | --- | --- | --- | --- | --- |
| 2200 m/Dry | Anther position (*exposed*) | Sugar per flower (*low*) | NA | NA | NA |
| 2200 m/Wet | NA | Sugar per flower (*low*) | NA | Colour (*green*) | Sugar per flower (*high*) |
| 1450 m/Dry | Tube length (*short*) | Tube length (*short*) | NA | Symmetry (*actinomorphic*) | — |
| 1450 m/Wet | Colour (*pink/white/yellow*) | Tube length (*short*) | NA | Odour strength (*strong*) | — |
| 1100 m/Dry | Size (*small*) | Size (*small*) | Shape (*tube/closed*) | Colour (*purple/yellow/white*) | — |
| 1100 m/Wet | Nectar guides (*present*) | Sugar per flower (*high*) | — | NA | Sugar per flower (*high*) |
| 650 m/Dry | Sugar per flower (*low*) | Shape (*open*) | Size (*large*) | Colour (*white/yellow/orange*) | NA |
| 650 m/Wet | Size (*small*) | — | — | Tube length (*long*) | — |

*Note:* The detailed trait values (written in italics) denote the primary pollinator preferences based on partial dependencies in particular RF models. 'NA' means there was no visit by the particular primary pollinator recorded in the specified combination of elevation and season. '—' means there was no trait with the positive importance values (Mean Decrease in Accuracy > 0) in the specified combination of elevation and season.

2017; Willmer, 2011). Therefore, a single syndrome for such a diverse group can be problematic, and some alternative views, such as the buzz-pollination syndrome, have been recently considered (De Luca & Vallejo-Marín, 2013; Dellinger, 2020). In a global analysis of pollination syndromes (Ollerton et al., 2009), bee and fly pollinated plants were predicted more accurately than the other syndromes, while other studies did not show such patterns (e.g. Johnson, 2013). This is confirmed by our local study where these groups were correctly associated as primary pollinators of most plants predicted by their floral traits (Table 1). Besides both groups are key pollinators in many ecosystems, they were the most common floral visitors and primary pollinators in our study (Table S2), which should substantially increase the efficiency of the applied machine-learning methods. However, we are aware that the single most frequent visitor may not accurately depict true pollination (Mayfield et al., 2001), especially when visitation is clouded by abundant generalists (Dellinger, 2020; Padyšáková et al., 2013). Moreover, the less frequent visitors also generate selection (Aigner, 2001). Plants with morphologically generalised flowers are rather prone to this mismatch (Bartoš et al., 2015) and are thought to form a generalist syndrome (Waser et al., 1996; Willmer, 2011).

Flower size was shown as the most important trait for butterflies (Table 1), together with closed and tubular flower shape (Table 3). This partly follows the definitions of traditional pollination syndrome which highlighted their preference for large, brightly coloured flowers with longer floral tubes and ample amounts of nectar (Faegri & van der Pijl, 1979; Willmer, 2011). Nevertheless, floral trait importance was shown to differ among butterfly families (e.g. Mertens et al., 2021).

As we have shown, not all floral traits are equally important for individual pollinator groups (Tables 1 and 3), as has also been confirmed by others (Dellinger, Chartier, et al., 2019; Johnson, 2013). However, such complex view has not been implemented in recent syndrome or classification-related studies (Dellinger, 2020). From a methodological standpoint, machine learning approaches (such as RF) offer an avenue for dealing with such shifts in individual trait importance, although strong data sets are needed (in our study, the most common primary pollinators showed the most robust results). However, the necessity of complete and non-collinear traits could be limiting (Dellinger, Chartier, et al., 2019; Pichler et al., 2020). Regardless, even without a priori floral trait selection, the robustness of RF seems to allow for an ecologically realistic inference of pollinator predictability (Pichler et al., 2020).

## CONCLUSION

Our results showed the importance of individual floral traits for plant-pollinator interactions in the
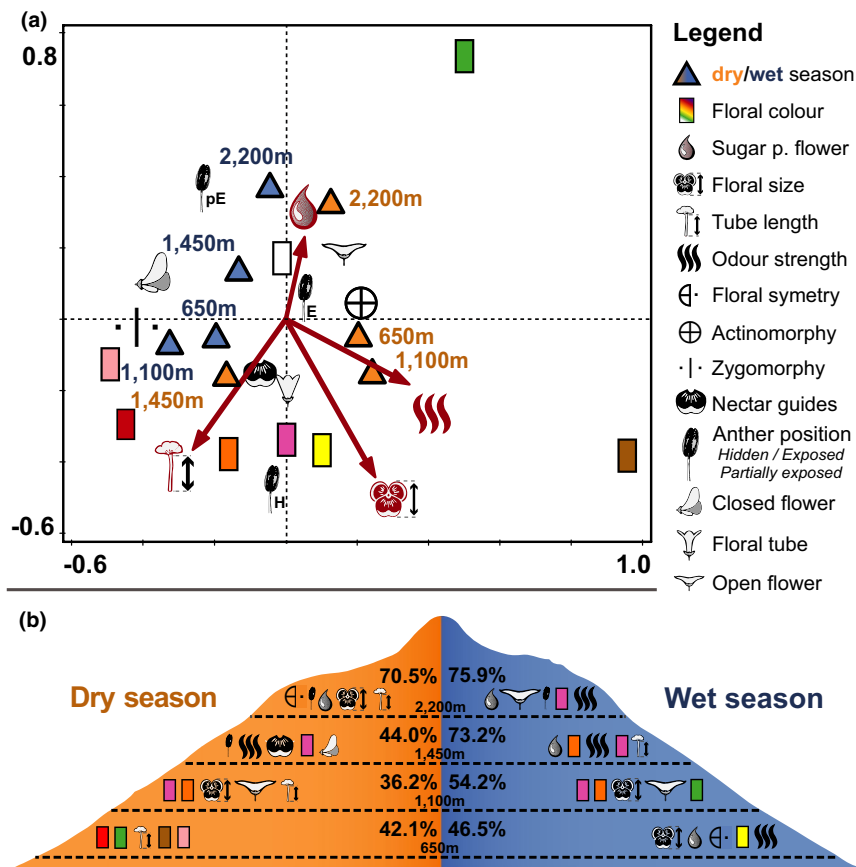
**FIGURE 2** (a) CCA ordination diagram visualising the distribution of individual floral traits between seasons and along elevation (m a.s.l.). (b) Summarised outcomes of individual CCAs of floral trait importance per elevation and season. Visualising the five most important floral trait characteristics for pollinator community composition based on step-wise forward selection. The traits are sorted from left to right according to their decreasing importance. Percentages in the middle states the variation explained by each model. See Table S6 for detailed statistics on the importance of individual floral traits

understudied Afrotropics, with the traits being more important for pollinator composition towards higher elevations and wet seasons. These shifts in floral trait dependence among elevations and seasons showed the importance of including spatiotemporal factors within pollination studies. Consequently, the pollination syndromes varied in their ability to accurately predict primary pollinators under different conditions and for particular pollinators. Additionally, variation in the importance of specific discriminative traits for individual pollinator groups, together with spatiotemporal differences within these groups, suggested that following a complex predetermined list of equally important traits can be problematic for predicting potential pollinators. This spatiotemporal and pollinator-specific variation has convinced us to step back from the rigid definition of pollinator syndrome and rather improve our trait-based understanding of plant-pollinator interactions at a community level under different environmental conditions in the future studies.

## AUTHOR CONTRIBUTIONS

## DATA AVAILABILITY STATEMENT

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.