Temporal Overlap of Flowering Species with the Same Pollinating Agent Class: The Importance of Habitats and Life Forms

Nelson Ramírez
Universidad Central de Venezuela, Fac. Ciencias, Instituto de Biología Experimental, Centro de Botánica Tropical. Aptdo. 48312, Caracas 1041 A, Venezuela

Abstract: One hundred and fifty five plant species were evaluated monthly in relation to flowering phenology, habitats and life forms in the Venezuelan Central Plain. Beetle and wind-pollinated species shown the largest levels of pollination overlapping and the lowest was found in bat-pollinated species. Intermediate values were associated to pollinating bees, flies, butterflies, moths, birds and wasps, respectively. The average values of pollination overlapping among the same or similar life form was larger than values among different life forms. The a-posteriori tests were significant among all average values of pollinating agent class overlap. For example, pollination overlapping within tree species was significantly lower than those values within herbaceous species. The average values of pollination overlap increased significantly from forest to disturbed area. The values of pollination overlapping within and among habitat exhibited the following tendency: overlapping values between forest and each one of the other habitats were the lowest ones. With the exception of forest, pollination overlapping between habitats and within each one of the habitats showed an increasing patterns from different to similar pair of habitats. Therefore, pollination overlapping could be attenuate by life form and habitat seasonality, in such way that plant species from the same life form in the same habitat have the highest values of pollination overlapping.

Key words: Community, flowering phenology, habitat, life form, pollination, pollinating agent, pollination overlap, savanna, Venezuela

INTRODUCTION

Temporal distribution of flowering phenology is one of the best known mechanisms that conevolve to avoid pollination overlap[2-8]. In spite of many studies of pollination biology, there is no comparative analysis of overlapping of pollinating agents at the community level. Flowering phenology of plant species with the same pollinating agent may be separated in time or overlap during certain periods of time. Pollinating agents sparsely distributed throughout the year may occur as a consequence of empty niches or competitive interaction if pollinators are available over all the year. In contrast, pollinating agents concentrated during certain periods of the year may promote large pollination overlap. In this context, the opportunity for less interference among plant species pollinated by the same pollinating agent could occur due to a better reproductive success during certain periods of time[7]. Under such circumstances, flowering segregation of plant species with the same pollinating agent class could be recognized as the basic mechanism avoiding interference.

On the other hand, the peak of flowering period might coincide with the highest frequency of plant species with pollination overlapped. Alternatively, during peak flowering periods selection might be expected to favor increased diversity of strategies and pollen-vector types to minimize interference. In addition, the level of pollination overlap may be related with the habitat type and plant life-form. Generalist and unspecialized pollination systems are expected in poorly structured vegetation type[6-12]. Lack of tight plant insect associations has been found in herbaceous Mediterranean plant communities[11]. Generalization would be expected in short-lived species such as annual herbs[12,13] and trees are often pollinated by a variety of generalist insects[14].

This study examines pollination overlap of flowering species with the same pollinating agent class. The aim of this study was to evaluate and compare the levels of pollination overlap for plant species with different pollinating agent classes. In addition, given that life-forms and habitats exhibit different patterns of flowering phenology[2,3] and the pollination agent classes are statistically associated with habitats and life forms[10], this research examines whether life forms and habitat may also act, in addition to flowering phenology, reducing the level of pollination overlap. In this sense, the following questions are formulated: does the level of pollination
overlap vary for different pollination agent classes? Do life forms and habitats affect the levels of overlap of pollinating agents?

MATERIALS AND METHODS

Study site: The fieldwork was conducted in Trachypogon savannas at the Estación Biológica de los LLanos, Sociedad Venezolana de Ciencias Naturales, located approximately 12 km south-west of Calabozo, Estado Guarico, Venezuela (8°36' N, 67°25' W). According to Santamaría and Bonazzi[11,12], the main operational factor in the savanna is the presence of an excessively dry soil and an indurated ironstone horizon close to the surface. The climate is markedly seasonal[13,14]. The temperature is isothermic with an annual average between 26 and 29°C. The rainy season occurs from May to November and from April to June. The annual precipitation varies between 720 and 1839 mm and the average annual temperature is 27.6°C. In this study, June and November are considered as the rainy-dry transition period and April and May are considered as the dry-rainy transition period.

The 250-ha site includes at least four vegetation types. Differences in vegetation structure are caused by varieties of soil types or by anthropogenic activity. The area has been protected from fire and cattle grazing since 1961. If protected from fire, human disturbance, cattle breeding and logging, savanna vegetation gradually changes into a denser tree community, even if the ironstone outcrop is faulted with cracks[15]. Four vegetation types were distinguished on the basis of the structure and floristic composition: (1) forest vegetation or groves, called locally Matas, composed of discontinuous patches with several trees and a typical understory layer of herbs and shrubs[16,17], (2) ecotone vegetation, transitional between forest and savanna, (3) savanna vegetation, consisting of scattered trees on a continuous grass stratum dominated by Trachypogon and Axonopus spp. and (4) secondary vegetation, herbaceous vegetation on disturbed areas, dominated by pioneer plant species. The main forms of disturbance in these areas were fire, grazing and plow. Habitat preference or specialization of the plant species has been determined in a previous study[18]. Based on this characterization, I assigned each plant species to one or more habitats. The number of plant species studied in each habitat was 47 in forest, 98 in forest-savanna transition, 46 in savanna and 34 in disturbed area. Plant species were classified as trees (N = 26 spp.), shrubs (N = 24 spp.), lianas (N = 28 spp.), perennial herbs (N = 36 spp.), annual herbs (N = 38 spp.) and epiphytes (N = 3 spp.).

Flowering phenology: Plant species were monitored at one month intervals and the censuses included 5-10 reproductively mature individuals of each species[19]. Using data of a previous phenological study over a three year period from 1983 to 1986[19], pollinating agent classes were evaluated in relation to flowering phenology.

Pollinating agent classes: Information on pollination system was recorded from Ramirez[20]. The pollination censuses included reproductively mature individuals of each plant species in each habitat. Censuses were conducted every one-month during ten uninterrupted years. The activity of visitors was monitored daily during the period of floral activity, from 10 to 20 h per plant species. As many visitors as possible were captured monthly, including the period of maximum blooming, in different years and different habitats, when plant species occurred in more than one habitat. Pollinators were distinguished from floral visitors using five criteria according Ramirez[20]: (1) Presence and estimated abundance of pollen of the visited plant, comparing pollen grains transported and samples of preserved anthers. The abundance was estimated qualitatively as abundant, scarce, or absent. (2) Part of the body where pollen was located and its relationship with the position or orientation of the sexual organs in the blossom during the pollination process. (3) Whether pollen on the body of a vector could potentially be transferred to a receptive stigma (i.e. the pollen load made contact with the stigma during a visit). (4) Relationship between blossom (Pollination unit: flower, inflorescence or flower-inflorescence) size or a specific area of the blossom and visitor size or specific part of the visitor body. (5) Relative abundance of each visiting species (number of visits per unit time). Each visitor species was evaluated separately and later grouped in pollinating agent classes (see below). Flower visitors were considered as pollinators when the first three criteria were covered, the second two criteria were used as complementary for those visiting agent classes which transported scarce pollen load[20]. To confirm the wind as pollinator, plant species suspected to belong to this syndrome were tested by enclosing emasculated flowers or inflorescences in nylon mesh bags with 1 X 1 mm opening, which excluded most insects but allowed the passage of airborne pollen.

Nine pollinating agent classes were distinguished: (1) wind, (2) beetles, (3) flies, (4) bees, (5) wasps, (6) butterflies (diurnal butterflies), (7) moths (nocturnal butterflies or moths), (8) birds and (9) bats. Using the criteria mentioned above, each plant species might be associated to one or more than one pollinating agent class. Therefore, each plant species may enter more than
once in the calculation, as many times as they had different pollinating agent classes.

**Phylogenetic effect:** Phylogenetic effects were minimized by increasing the number of different families, genera and species evaluated. Plant species were selected in such a way that the largest number of plant families and species were included in the study (50 families out 65 in the flora of the area and 164 out 285 plant species in the area). In those largest families, Caesalpiniaceae (N = 12; 7.7%), Fabaceae (N = 25; 16.1%) and Poaceae (N = 14; 9.0%), plant species with the most different pollinating agents were included in the study. However, Poaceae species were all wind pollinated. The phylogenetic effect was tested prior to the analysis. The three large families (Fabaceae, Caesalpiniaceae and Poaceae) do not have a phylogenetic effect on life form (df = 8, \( \chi^2 = 10.72, p = 0.2178 \)), habitat (df = 6, \( \chi^2 = 12.50, p = 0.06166 \)) and flowering time (df = 6, \( \chi^2 = 7.57, p = 0.2707 \)). However, the phylogenetic effect on the pollinating agent class was not statistically tested because the two-way contingency table created more than 20% of the cells with low or zero counts\[23\]. Under such preliminary results, the analyses of variance were performed under unrestricted phylogenetic effect, except for the relation between pollination and phylogeny, where wind pollination is dominated by Poaceae.

**Temporal overlap of flowering species with the same pollinating agent class:** An approximation to find overlap of pollinating agent class was calculated with the percentage of similarity of S\textsuperscript{2}\textsubscript{Orens\[24\]}\textsuperscript{2}, following a similar procedure used by Arroyo et al.\[22\]. The similarity was determined between all possible pairs of plant species with the same pollinating agent class. 

\[ S_i = 2a/b+c \]

Where, a is the number of months in which each plant species with the same pollinating agent was overlapped and b and c are the total number of months in which each plant species was flowering. Average values of the pairwise overlap were then computed separately within pollinating agent classes and within and among habitats and life forms respectively, using one way ANOVA\[20\]. Values of similarity were transformed by arcsine of their square root before analysis. For all cases, Bartlett’s test for homogeneity of variance was not significant. To test the statistical significance between particular groups, an unplanned post-hoc test was employed.

**RESULTS**

The average values of overlap for pollinating agent classes were significantly different (F\textsubscript{2,666} = 11.83; p = 0). The largest overlap occurred among species pollinated by beetles and wind, respectively. The lowest level of overlapping was found among bat pollinated species (Table 1). Intermediate values were associated to pollinating bees, flies, butterflies, moths, birds and wasps, respectively.

The average values of pollinating agent overlap within the same and among different life forms were different (F\textsubscript{20,660} = 63.36; p = 0). The a-posteriori tests were significant among all average values of pollinating agent class overlap. Perennial and annual herbaceous species revealed the largest values of pollination overlapping, respectively. There was a trend to decrease the level of pollination overlapping from similar life forms to different life forms (Table 2). This trend was similar for tree species, except that the overlap value between trees and shrubs was larger. In this case, pollination overlapping within tree species was significantly lower than those values within herbaceous species. Pollination overlapping was different for shrubs, lianas and epiphytes (Table 2). The average value within shrubs was similar to the overlap between shrubs and trees, but the former was significantly larger than the overlap values of shrubs with lianas and herbaceous species. The overlap value of pollinating agent within lianas was significantly higher.

---

**Table 1: Mean±SD of pollination overlapping (S) and results of the a posteriori test**

<table>
<thead>
<tr>
<th>Pollination mode</th>
<th>Overlap (S)</th>
<th>Posteriori test (p&lt;0.04)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee (BE)</td>
<td>0.33±0.32</td>
<td>BA&lt;BE&lt;BT, WI</td>
</tr>
<tr>
<td>Wasp (WA)</td>
<td>0.34±0.31</td>
<td>BA&lt;WA&lt;BT, WI</td>
</tr>
<tr>
<td>Fly (FL)</td>
<td>0.34±0.33</td>
<td>BA&lt;FL&lt;BT, WI</td>
</tr>
<tr>
<td>Butterfly (BU)</td>
<td>0.37±0.31</td>
<td>BA, FL&lt;BE&lt;BT, WI</td>
</tr>
<tr>
<td>Moth (MO)</td>
<td>0.39±0.32</td>
<td>BA, BT&lt;MO&lt;BT, WI</td>
</tr>
<tr>
<td>Beetle (BT)</td>
<td>0.52±0.26</td>
<td>BA, BI, MO, BU, WA, FL, BE&lt;BT&lt;WI</td>
</tr>
<tr>
<td>Bird (BI)</td>
<td>0.31±0.24</td>
<td>BA=BI&lt;MO, BT, WI</td>
</tr>
<tr>
<td>Bat (BA)</td>
<td>0.10±0.23</td>
<td>BA&lt;BE, WA, FL, BU, MO, BT, BI, WI</td>
</tr>
<tr>
<td>Wind (WI)</td>
<td>0.58±0.27</td>
<td>WI&lt;BE, WA, FL, BU, MO, BI, BT, BA</td>
</tr>
<tr>
<td>Total</td>
<td>0.38±0.32</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Mean±SD of pollination overlapping (S) among life form pairwise comparison

<table>
<thead>
<tr>
<th>Life Form</th>
<th>Trees</th>
<th>Shrubs</th>
<th>Lianas</th>
<th>Perennial herbs</th>
<th>Annual herbs</th>
<th>Epiphytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trees</td>
<td>0.29±0.54</td>
<td>0.39±0.29</td>
<td>0.30±0.34</td>
<td>0.57±0.26</td>
<td>0.60±0.24</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Shrubs</td>
<td>0.37±0.31</td>
<td>0.26±0.29</td>
<td>0.37±0.34</td>
<td>0.55±0.26</td>
<td>0.29±0.29</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Lianas</td>
<td>0.23±0.32</td>
<td>0.32±0.29</td>
<td>0.39±0.32</td>
<td>0.38±0.30</td>
<td>0.29±0.29</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Perennial herbs</td>
<td>0.24±0.25</td>
<td>0.35±0.29</td>
<td>0.37±0.34</td>
<td>0.57±0.26</td>
<td>0.60±0.24</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Annual herbs</td>
<td>0.18±0.24</td>
<td>0.28±0.30</td>
<td>0.39±0.32</td>
<td>0.38±0.30</td>
<td>0.29±0.29</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Epiphytes</td>
<td>0.38±0.27</td>
<td>0.45±0.27</td>
<td>0.32±0.29</td>
<td>0.38±0.30</td>
<td>0.29±0.29</td>
<td>0.37±0.05</td>
</tr>
</tbody>
</table>

All posthoc comparisons were statistically significant at p<0.05

Table 3: Mean±SD of pollination overlapping (S) among habitat pairwise comparison

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Forest</th>
<th>Ecotone</th>
<th>Savanna</th>
<th>Disturbed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>0.29±0.54</td>
<td>0.33±0.31</td>
<td>0.37±0.34</td>
<td>0.57±0.26</td>
</tr>
<tr>
<td>Ecotone</td>
<td>0.30±0.32</td>
<td>0.33±0.31</td>
<td>0.42±0.30</td>
<td>0.57±0.26</td>
</tr>
<tr>
<td>Savanna</td>
<td>0.28±0.29</td>
<td>0.37±0.31</td>
<td>0.39±0.32</td>
<td>0.38±0.30</td>
</tr>
<tr>
<td>Disturbed area</td>
<td>0.27±0.29*</td>
<td>0.40±0.31</td>
<td>0.50±0.29</td>
<td>0.63±0.24</td>
</tr>
</tbody>
</table>

All posthoc comparisons were statistically significant at p<0.05, except those marked with asterisk

than the value between lianas and trees and significantly lower than the average value between lianas and perennial herbs and between lianas and annual herbs (Table 2). The average values of pollination overlapping within habitats increased significantly from forest to disturbed area (Table 3). In addition, the values within forest were not statistically different from the values between forest and other habitats. The values of pollination overlapping within and among habitat exhibited the following tendency: overlapping values between forest and each one of the other habitats were the lowest ones (Table 3). With the exception of forest, pollination overlapping between habitats and within each one of the habitats showed an increasing patterns from different to similar pair of habitats. The pollination overlapping values were lower between contiguous more structured habitats than between herbaceous habitats (Table 3). For example, considering savanna, pollination overlapping values between savanna and forest, between savanna and forest-savanna transition, in savanna and between savanna and disturbed area, increased in the same order. In this case, the value within savanna was lower than the value between savanna and disturbed area. The values for forest-savanna transition and disturbed area exhibited the same trend (Table 3).

**DISCUSSION**

In spite of many studies on pollination biology, there is no comparative analysis among overlapping of pollination. Three aspects may affect the level of overlapping in different pollination modes: (1) the number of plant species in each pollination group, (2) the diversity of pollination agents and (3) the characteristics of the pollination mode. The similarity of overlapping found for bee, fly, moth, bird and wasp pollinated species, indicate that flowering phenologies are similarly overlapped in these species irrespectively of their abundances in the community. Flowering phenology during the dry and rainy season for pollinating bees, butterflies, flies and wasps results in a broader distribution of these pollinating agents and promote similar and intermediate values of pollination overlapping irrespective of their abundance in the community. Bee pollinated species, the best represented pollination mode, exhibits a similar level of overlapping as other pollination modes, which are less abundant. Species pollinated by bees seem to have segregated theirs flowering time so, that bees are always the most abundant group of pollinators in the Venezuelan Central Plain, with the highest diversity of pollinating species and most diverse pollination strategies. For example, flowers pollinated by bees show diverse sizes and forms. Among intermediate values of pollination modes overlapping, the lowest value was found for hummingbird-pollinated species. The presence of hummingbird-pollinated species along the year could enhance intermediate values of pollination overlapping because bird-pollinated species occur in different plant life forms, which allow a flowering phenology segregate along the year. In addition, the low number of plant and bird species interacting could reduce the level of pollination overlapping.

The lowest value of pollination overlapping was found in bat-pollinated species. In a seasonal forest, bat species share floral resources to a large degree in a seasonal forest. The interaction of the lowest pollinator species diversity, only two bat species have been recorded in the study site (Ramirez unpublished data), together with the low number of plant species and the highly demanding energy requirement of bats, seems to be associated with the low overlap in bat-pollinated species in the Venezuelan Central Plain. Segregation of the flowering time appears as a consequence of plant species competition for bat pollinators, which adjust evolutionarily their flowering periods in the Venezuelan Central Plain. Non-seasonal flowering pattern among plant species with the same pollinating agent may be associated with competition. Many bat-pollinated species are woody species, therefore may be selected to flower at any time of the year because this life form is not constrained structurally to flower during any time. In contrast, pollinating bats occurred for perennial herbs...
during the rainy season and therefore different flowering time associated to different life forms seem to allow a better sequential segregation for pollinating bats and consequently promote low pollination overlapping pattern in bat-pollinated species.

The highest values of overlapping in wind and beetle pollination are associated with the flowering time concentrated during the rainy season, respectively. However, the number of plant species is much larger in wind-pollinated species than in beetle-pollinated species and the interactions may be reduced by different ways. The high overlap in flowering and pollinator use can be maintained because current competition for pollination is rare or mild. In pollinating beetles interaction may be reduced by beetle diversity, which comprises different groups and different pollination strategies. For example, Curculionidae and Scarabaeidae pollinated flowers with different adaptations. In addition, beetle pollination occurs in different life forms, which could reduce the interference among these species by spatial isolation. On the contrary, wind pollination depends only on the wind for pollination. The large pollination overlapping may be reduced in wind pollination species by different height of plants, different habitats, life forms, another opening time and time of pollination activity. Alternatively, evidences suggest that competitive avoidance is achieved by such characteristics as long-lived flowers and self-pollination.

The average values of pollination overlapping among the same or similar life forms were frequently larger than the average values among very different life forms. Plant species from the same or similar life forms represent groups of plant species sharing many characteristics. Grubb recognized the life form niche, including phenology. In this context, plant species of the same or similar life forms commonly use the same resources because their relationship of size and space and therefore their pollination overlapping level is larger. More seasonal flowering phenology promotes higher values of pollinating agents overlap (e.g. annual herbs) than less seasonal flowering phenology, segregated along the year (e.g. trees). The low values of pollination overlapping among contrasting life forms could be explained because flowering peak of different life forms occur at different time in the Venezuelan Central Plain.

Spatial segregation may reduce the frequency of interspecific visitation. In the Venezuelan Central Plain, spatial isolation seems to reduce pollination overlapping only if isolation is accompanied by a differentiation in the structure of vegetation, mainly in woody habitats. Pollination overlapping tends to decrease from adjacent habitats, within or among herbaceous habitats, to distant habitats, within or among herbaceous and woody habitats. Combination of life forms per habitat could enhance a better distribution of flowering time among plant species and therefore low levels of pollinating agents overlap. In addition, pollination overlapping values within habitats tend to increase from forest to disturbed areas in the Venezuelan Central Plain. This result indicates that more structured habitats have a major organization in pollination if compared with early successional habitats. Species of the late successional community showed more niche specialization than species of early successional communities and a continuous relationship between plant population and pollinator population rarely occurs, hence, species in both groups remain unspecialized in disturbed sites. These trends agree with the results recorded in the Venezuelan Central Plain, where pollination overlap seems to be smaller in forest compared to disturbed area.

Finally, the seasonality in pollinating agents was associated with the pollination overlapping level: (1) Seasonality in pollinating agents was associated with synchronization of flowering phenology during the rainy season and consequently high values of pollination overlapping and (2) non-seasonality in pollinating agents was associated with alternate flowering peaks of different life forms and low values of pollination overlapping. Therefore, life form may be considered as proximate elements reducing interference in the pollination process, together with spatio-temporal heterogeneity of the community.

ACKNOWLEDGMENTS

The author thanks A. Dafni, J. Nassar and J. Ollerton for comments on the first draft of the manuscript. Special thanks to H. Briceno, M. Lopez, M. Cuberos, O. Hokche, Y. Brito, G. Leal, G. Rodriguez, J. L. Perez, I. Bastidas and D. Vasquez for field and laboratory assistance. Many people made possible to complete this research, for all they many thanks. This research was supported by Proyecto Consejo de Desarrollo Cientifico y Humanistico, Universidad Central de Venezuela CDCH-03.10.2483.91 and Proyecto CONICIT SI-96001695. I wish to express my gratitude to the Sociedad Venezolana de Ciencias Naturales for letting make use of the headquarter at the Estacion Biologica de los Llanos, Venezuela. I am grateful to L. Cardenas, L. Aristigueta, A. Krupovickas, R. Liesner, R.S. Cowan, J. Steyermark, M.M. Arbo, L. Anderson, G. Morillo, C. Benitez, R.M. Harley, V.M. Badillo, R. Barney, H. Moldenke, C.L. Cristobal, F. Ayala, G. Pedrulli, F. Gonzalez, G. Davidsae, G. France, G. Agostini, P. Berry, P. Ravena, B. Holst, M.J. Huft.
REFERENCES


