

Attraction of Male Euglossine Bees to Orchid Fragrances in Primary and Secondary Lowland Forests of Costa Rica

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Abstract. Male euglossine (orchid) bees forage through tropical forests in search of floral fragrances for the attraction of females (Tropical Nature, 1984). By attracting male euglossine bees with methyl salicylate, eugenol, and vanilla in Costa Rican rain forest during the rainy season, I found no significant change in the numbers of attractable bee species in primary versus secondary forest. However, within both forest types, male euglossine bees were significantly more attracted to the artificial flowers during morning hours rather than afternoon hours. This data strongly suggest that male orchid bees mainly forage in the morning hours to possibly avoid environmental factors, such as increased temperatures and high wind speeds or where reproductive success rates with females would be the highest. Yellow and orange rather than green artificial flowers with eugenol as the floral bait had the highest rates of visitation. Also, 6.25 in. artificial flowers had the highest rates of visitation when combined with all other factors. I hypothesized that the euglossine bees would be attracted to the combinations of odor, color, and size of artificial flowers that best mimic those found in natural tropical primary and secondary forests, and their visitation will be based on these combinations as well as time of day rather than random chance. I used chi square analysis to compare the frequencies of visits by the male euglossine bees to the artificial flowers in relation to the odor, color, size variations of each artificial flower, and time of day.

Introduction

Male euglossine bees (Apidae: Euglossini) are a group of brightly colored bees that are known for their role in the pollination of a large number of species in the orchid family (Orchidaceae) (Armbruster and McCormick, 1990). There are six genera of bees of which the three *Eulaema*, *Euglossa*, and *Euplusia* are thought of as orchid bees (Dodson C.H et al, 1969). These genera are currently recognized and found on mainland tropical America and the western hemisphere (Dressler, 1982, Dodson C. H. et al, 1969). The orchid bees can also be found from sea level to 1600 m in elevation (Dressler, 1982). The euglossine bees visit these flowers not to

gather food or nutrients, but rather to gather the chemicals produced by the flowers. The bees then store the gathered chemicals in their hind legs in order to attract females (Forsyth, A. and Miyata, K. 1984, Janzen et al 1982, Dressler R.L, 1982, Williams and Dodson, 1972). It is also thought that the diel (daily) pattern of euglossine bees is influenced by the flowers activities. There are also other factors that could influence the daily visitation patterns of the male bees to the chemical sources. Such sources include temperature, cloud cover, and wind speed. Therefore, in order to attract male euglossine bees I used undiluted methyl salicylate, eugenol, and vanilla as chemical baits to census the male euglossine bees to compare visitation rates to time of day,

color, and size of artificial flowers in primary and secondary forests. I hypothesize that the male euglossine bees will be attracted to the artificial flowers due to the factors above rather than from random chance.

Materials and Methods

Baits and Set-up

Methyl salicylate, eugenol, and vanilla were chosen as baits because these chemicals attract substantial numbers of male euglossine bees. The chemicals were obtained from Eastern Connecticut State University biology department, and were stored in bottles which were then placed in a zip-lock bag until used.

Artificial flowers were made out of circular colored disks that were then laminated before use. The circular disks were 2.5 in., 4 in., and 6.25 in. in diameter and were made using three different random colors, yellow, orange, and green. Three disks of each color and size were made so as to have 3 sets of nine artificial flowers for each scent to use. Small half inch cubes made out of sponge were made to use for the chemical scents. The cubes were placed in the center of the artificial flower and tacked with clear tacks to random trees in order to keep the flower and scent all together. The sponges were then saturated with one of the three chemicals scents for each group.

Areas of primary and secondary forest were chosen on the basis of being able to group each scent together, but also having each group far enough apart to allow for a separation of scents. Each group of artificial flowers was placed randomly on tree trunks and branches in semi-shaded areas in each location.

Locations and Time of Day

The experimentation was conducted in primary and secondary forest locations around Selva Verde Lodge, Costa Rica. Data was collected during 6 trials each for roughly 2 hour increments, in the morning and afternoon on three randomized days. Due to weather restrictions, two trials one in the morning and the other in the afternoon were only one hour in duration. Morning and afternoon data collection were rotated each day in primary and secondary forest; starting in primary forest in the morning on the first day, and then in the afternoon on the second day. GPS coordinates and elevations were also recorded at each experimentation location. The primary forest locations were found at N 10° 26.839' W 084° 04.031' at an elevation of 366 ft above sea level, N 10° 26.842' W 084° 04.033' at an elevation of 380 ft above sea level, and N 10° 26.794' W 084° 04.016' at an elevation of 483 ft above sea level. Secondary forest locations were N 10° 26.911' W 084° 04.189', at an elevation of 353 ft above sea level, N 10° 26.918' W 084° 04.189' at an elevation at 210ft, and N 10° 26.989' W 084° 04.201' at an elevation of 373 ft above sea level.

Bee Attraction

The three chemical baits were used in order to attract and census as many orchid bees as possible for each two hour trial. All bees were recorded as visiting a flower if they came very close to it or landed on it, and the bee color and approximate size was also recorded. All data for bee attraction to the artificial flowers were pooled to calculate results for primary versus secondary forests,

morning versus afternoon visitation, and differences between scents, color, and flower sizes.

Results

The results of 10 hours of observation were pooled in various groups for interpretation on the frequencies of bee visitation to scent, color, size of artificial flower, and time of day. There were a total of 137 bee visitors to all artificial flowers. The frequencies of visitation in primary versus secondary forest was more or less evenly distributed (56.9% and 43.1% respectively) and therefore were not significant ($p > 0.05$). Green, blue and bronze metallic colored bees made up the majority of visitors to the artificial flowers in this study, although none of the species were ever identified specifically. These species are most likely examples from the largest genus *Euglossa*, which has almost 60 described species and 40 species that are awaiting description (Dressler, 1982). In addition, Dressler (1982) notes that bronze is the predominant color for orchid bees in Costa Rica, where some of these species only occur in Costa Rica. Contrarily, my data indicate that green was the predominant color in these areas of study.

Chemical Scent

The chemical fragrance eugenol attracted the most visitations by the male euglossine bees with 70 visits (51.1% of all visitations). Methyl salicylate visitation trailed closely with 60 visits (43.8% of all visitations). Lastly, vanilla was the poorest attractant for male euglossine bees with only 7 visits (5.1% of all visitations) to all artificial flowers. Chi-square analysis (Table 1) shows no

significant differences between each of the fragrances in primary or secondary forest. $X^2 = .85$ and $p = 0.70-0.50$.

Color of Artificial Flowers

The color orange was seen to attract the highest percentage (44.5%) of male euglossine visitors in primary and secondary forest with a total of 61 bee visits. The yellow flowers were visited 49 times which was 35.8% of the time, and green flowers had the least amount of visitation with only 27 visits or 19.7% of total visitation. Green artificial flowers therefore, were visited less than half as much as the orange flowers. Through chi-square analysis (Table 2), color variation of artificial flowers is not a significant factor between primary and secondary forest of Costa Rica. $X^2 = 3.38$ and $p = 0.20-0.10$.

Size of Artificial Flowers

The 6.25 in. artificial flowers attracted the greatest number of orchid bees followed by 4 in., then 2.5 in. artificial flowers. There were 57 visits (41.6% of all floral visitations) to the 6.25 in. flowers, 43 visits (31.4% of all floral visitations) to the 4 in. flowers, and only 37 visits (27% of all floral visitations) to the 2.5 in. flowers. Chi-square analysis (Table 3) shows a significant difference between each of the sizes in primary versus secondary forest. $X^2 = 9.83$ where $p = 0.01$.

Time of day

Of both times of day, 91.2% of orchid bee visitation was seen in the morning hours versus only 8.8% visitation in the afternoon hours. There were 125 male

euglossine bee visits in the morning, and only 12 bee visits in the afternoon. Through chi-square analysis (Table 4) there was a significant difference between the visitation in the morning versus the afternoon between primary and secondary forest. $X^2 = 4.0$ where the $p < 0.05$.

Discussion

Bee/Orchid Relationship

All factors were compared in primary versus secondary forests. Male euglossine bees can be baited to certain fragrant chemical scents in both primary and secondary forests because these chemicals are structurally similar to the compounds found in natural orchid floral fragrances in both locations (Williams and Dodson, 1971). The daily visitation of these male euglossine bees to each site could impact the plants for which they pollinate in that the time of day that the flowers open and the release of fragrances should correspond with the peak time in which the bees are collecting their fragrances (Armbruster and McCormick, 1990). In general, male euglossine bees forage for fragrances in the morning hours between 9:30 am and 11:30 am. Foraging then slows and almost completely stops during afternoon hours between 2:00 pm and 4:00 pm. Even through randomized placement of the artificial flowers, the male euglossine bees were the most highly attracted to the 6.25" artificial flowers. These data suggest that the size of flowers in nature plays a role in their attractiveness to the bees. The bigger the flowers are, the greater the chances of pollination by a broad range of male euglossine bee species. In nature, some orchids are only pollinated by a specific

species, although they are visited by smaller species that do not have any affect on the pollination of the flower (Dodson, C.H. et al, 1969). The smaller male euglossine bees are able to capture the chemical scents from the flowers, but the flowers get no benefit from that particular bee species. Therefore, it is seen in this data that many species were visiting the large artificial flowers simply because they could all benefit from the flower size independently of the size of the bee.

Temperature constraints in the morning versus afternoon can also indicate a pattern of early foraging by the male euglossine bees where peak foraging activity is reached around 11:00 am (Armbruster and McCormick, 1990). By the afternoon, the resources from each flowering orchid could be exhausted, proving a wasted attempt for the bee to forage in the afternoon.

Eugenol was the fragrance that best attracted the bees in this lowland tropical forest of Costa Rica. This attraction by the bees may suggest that eugenol is included as one of the most abundant natural fragrances in this region of Costa Rica, or that the bees collect it the most as a female attracting pheromone (Armbruster and McCormick, 1990). It can also be noted that not all species of orchid bees were even attracted to any of the chemical scents. The data indicate that vanilla scented artificial flowers were visited the least often, which could indicate that either vanilla is not a popular natural scent found in the lowland tropics of Costa Rica or male euglossine bees do not use it for the attraction of females. In addition, the colors of the artificial flowers had no significant differences between them for the attraction of male euglossine bees. These species of bees

may not be able to decipher between certain colors.

Conclusion

The foraging rates of male euglossine bees in the lowland tropics of Costa Rica occur mainly during the morning hours before the temperature and other factors can work against foraging. The only other variable to significantly affect the frequency of bee visitation to the artificial flowers was the size of the flower. The bigger the flower, the more visitations were seen by the male euglossine bees. Many species of orchid bees were seen foraging, but a metallic medium-sized green species seemed to dominate. This species was always the first and last seen at the artificial flowers, and seen defending the flowers from other visitors. The data suggest that through many combinations of factors the orchid bees choose which flowers to go to rather than random chance.

A concern about the validity of the data was that at any given site the chemical scents could have been too close together. The mixing of chemical scents could alter the perception of the orchid bee to increase or decrease visitation rates. It would then mean that the bees were not attracted to the individual scent of each flower, but rather the combination of chemical scents.

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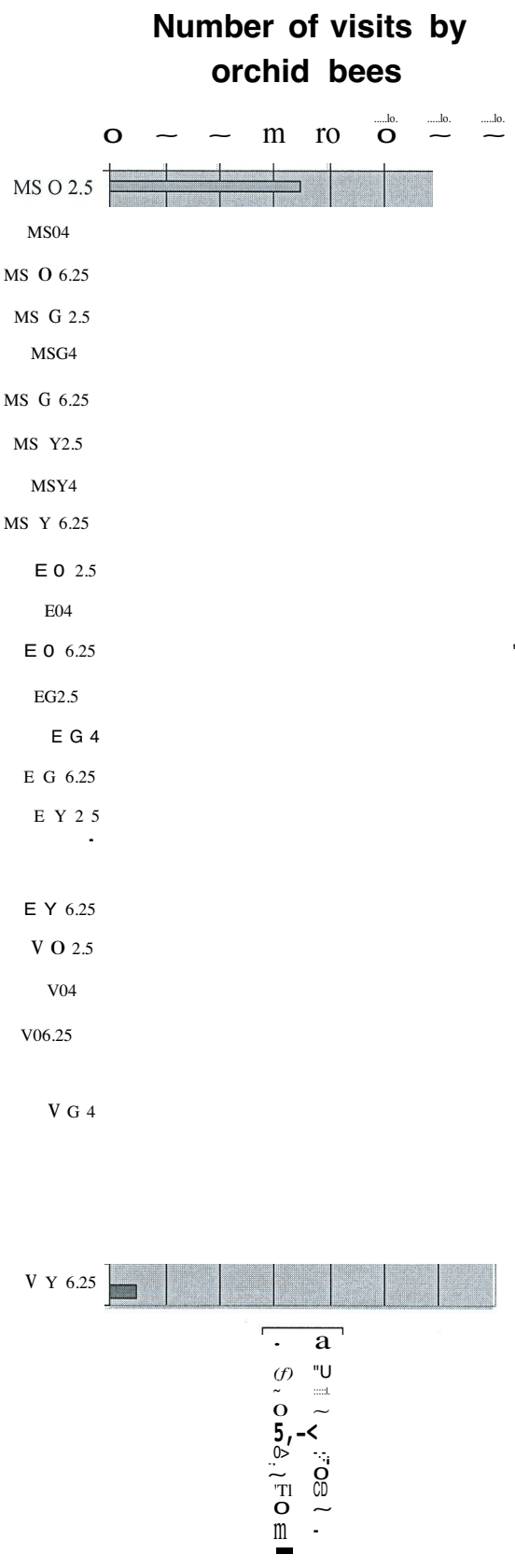
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V. STATION 2010-2011 NUMBER OF VISITS BY ORCHID BEES



*MS O 2.5, MS O 6.25, MS G 2.5, MS G 6.25, MS Y2.5, MSY4, MS Y 6.25, E O 2.5, E04, E O 6.25, EG2.5, E G 4, E G 6.25, E Y 2.5, E Y 6.25, V O 2.5, V04, V06.25, V G 4, V Y 6.25

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Visitation of Orchid Bees to Artificial Flowers in the Morning Vs. Afternoon

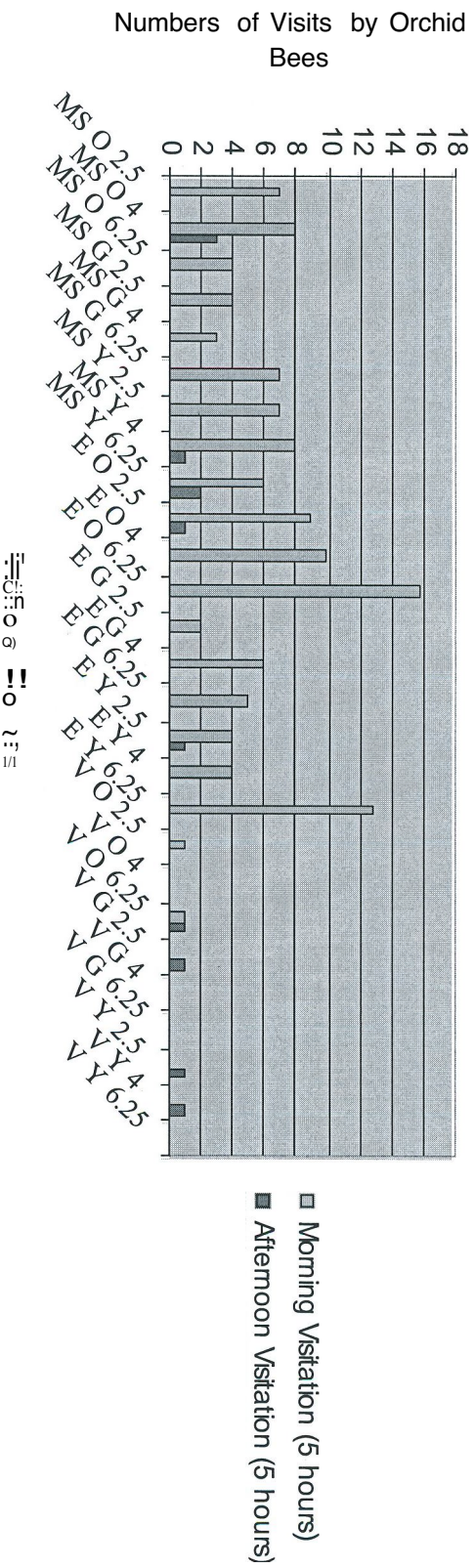


Figure 2. Male euglossine bee visitation to artificial flowers in the morning versus afternoon for six trials over a three day period in both primary and secondary forest. Trial times were summed for morning and afternoon visitation rates.

* For both figures 1 and 2 an additional key is needed as follows

- MS = methyl salicylate
- E = eugenol
- V = vanilla
- O = orange
- G = Green
- Y = yellow
- 2.5, 4, and 6.26 = diameters in inches of artificial flowers

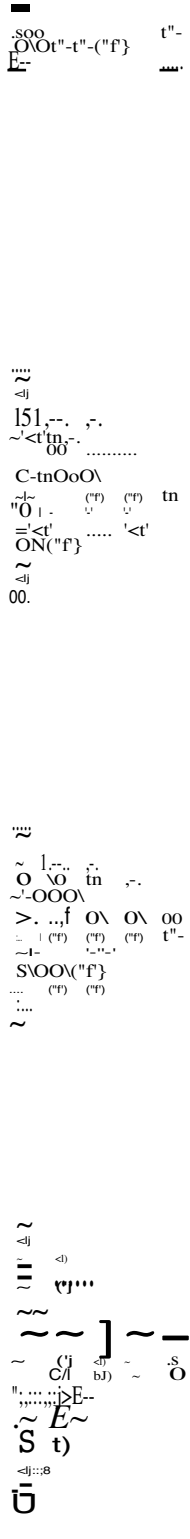


Table 1. Chi-square analysis of visitation rates by male euglossine bees to three chemical fragrances in lowland forests of Costa Rica. The expected values are in parenthesis followed by the experimental (field) results.

Colors of Artificial Flowers	Primary Forest	Secondary Forest	Total
Orange	37 (34.72)	24 (26.27)	61
Green	18 (15.37)	9 (11.62)	27
Yellow	23 (27.89)	26 (21.10)	49
Total	78	59	137

Table 2. Chi-square analysis of visitation rates by male euglossine bees to three artificial flower colors in lowland forests of Costa Rica. The expected values are in parenthesis followed by the experimental (field) results.

Colors of Artificial Flowers	Primary Forest	Secondary Forest	Total
Orange	37 (34.72)	24 (26.27)	61
Green	18 (15.37)	9 (11.62)	27
Yellow	23 (27.89)	26 (21.10)	49
Total	78	59	137

Table 3. Chi-square analysis of visitation rates by male euglossine bees to three artificial flower size variations in lowland forests of Costa Rica. The expected values are in parenthesis followed by the experimental (field) results.

Colors of Artificial Flowers	Primary Forest	Secondary Forest	Total
Orange	37 (34.72)	24 (26.27)	61
Green	18 (15.37)	9 (11.62)	27
Yellow	23 (27.89)	26 (21.10)	49
Total	78	59	137

Table 4. Chi-square analysis of visitation rates by male euglossine bees to time of day in lowland forests of Costa Rica. The expected values are in parenthesis followed by the experimental (field) results.