

Biologically Active Compounds in Orchid Fragrances

Function of natural plant products in orchid flower odors
and the attraction of specific pollinators are described.

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Species-specific attraction of pollinators is characteristic of many of the more highly evolved species of orchids (1). In those orchids the pollinating agents isolate and prevent hybridization between compatible populations. The development of numerous species in the orchid family (more than 10 percent of all species of flowering plants are orchids) can probably be attributed in large part to the attraction of particular kinds of pollinators. Some of the most bizarre pollination systems known function in attraction of specific pollinators; among them are food and prey imitation, sexual deception, and pseudoantagonism (1). Generally, the pollinators are attracted by means of brightly colored flowers or strong fragrances associated with the production of nectar. Bees, butterflies, moths, flies, and birds are the most common kinds of orchid pollinators; bees predominate (1). Much of the floral variation of orchids is a result of morphological rearrangements necessary to accommodate the physical characteristics of such diverse pollinating organisms.

Fragrance is often the dominant means of attraction, particularly in moth-pollinated flowers, which are searched out and visited at night. Many bee- and fly-pollinated species of orchids depend upon fragrance as an attractant and then reinforce that stimulus with flower colors and elaborate structural arrangements. Fragrances may attract a broad spectrum of pol-

linators; in these cases, structural modifications, which exclude all but one or a few kinds of pollinators, become critical. This is particularly true where such modifications are necessary to maintain the integrity of closely related and interfertile species which occur together.

Euglossine Bees as Orchid Pollinators

The evolution of certain orchids in tropical America is related to the presence of euglossine bees (tribe Euglosini, subfamily Bombinae—closely allied to the honey bees and especially the bumblebees). Male bees are specifically attracted to certain fragrances. This phenomenon has been partially elucidated during the past 10 years. The visits of euglossine bees to orchids were first reported by Cruger (2) and discussed by Darwin (3) in his classic treatise on the modifications of the orchid flower. In succeeding years there were additional reports of euglossine bees visiting orchids, but in all cases the observers assumed that the bees were visiting the flowers to obtain some kind of food. The flowers involved do not contain nectaries, and it was thus assumed that the bees gnaw on the flowers. Observers may have been misled by flowers that had been partly eaten by crickets, cockroaches, or other chewing insects. Ducke (4) made many observations of euglossine bees and noted that only male bees were visiting most orchid flowers, but he also believed, as did Porsch (5) and Allen (6), that the bees were eating the flower tissues. Dodson and Frymire (7) were the first to note that the bees do not chew on the flowers, but scratch or brush on the surface, frequently stop-

ping to hover nearby. They observed that the bees became less wary after visiting a flower for a few minutes, and seemed to become "drunk" or intoxicated in some manner.

Dodson and Frymire suggested that bees were detecting and absorbing some substance by the "brushes" which are found on the front legs of euglossine males. Vogel (8) made similar observations and presented evidence that the bees might be depositing some substance in their inflated hind tibiae. He did not attach much significance to this and suggested that the flowers were mimicking the sexual odors of the female bees and that the flowers were morphologically simulating the bee's nest cells. He suggested that the males arrived in search of females and scratched on the surface of the flower in frustration when no female could be found; however, the systematic and continued brushing of the bees at specific places on the flower does not lend itself to the idea of "scratching in frustration." Similar observations on flowers of other plant families which produce the same fragrance components as orchid flowers also weaken this idea. For example, *Spathiphyllum cannaefolium* (Dryand.) Schott, *Anthurium* spp. (both in the Araceae), *Gloxinia perennis* (L.) Fritsch and *Drymonia turrialvae* Hanstein. (both in the Gesneriaceae) elicit the brushing behavior by the bees (9). After further observations Vogel was convinced (as were we) that the bees were gathering some substance which they placed in their hind tibiae, he called this *Duftstoff* or "odor substance" (8) and suggested that the males were utilizing it as a borrowed pheromone to assist in marking their territories or to attract females by adding it to their own tibial secretions. Our observations suggest odor marking, but the flowers never attract female bees. If the odor substances are used unmodified, as a borrowed pheromone, it must be for a different purpose than sexual attraction of female bees.

Natural History of Euglossine Bees

Euglossine bees (more than 200 species) are restricted to the tropical regions of the Western Hemisphere. They are divided into six genera: *Eulaema*, *Euglossa*, *Euplusia*, *Eufriesea*, *Exaerete*, and *Aglae*. *Eulaema*, *Euglossa*, and *Euplusia* are most frequently associated with orchid flowers; the other three genera rarely visit

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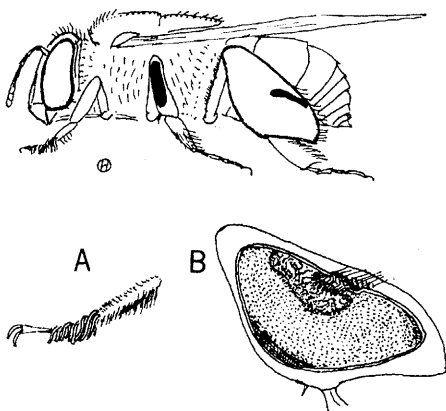


Fig. 1. Male euglossine bee. (A) Tarsal brushes; (B) section showing the glandular interior of the posterior tibia.

orchids. *Eulaema* consists of about 17 species of large hairy bees, and *Euplusia* has about 40 species which are superficially similar to species of *Eulaema*. *Euglossa* consists of over 100 species (more than twice the number of species that were recognized when we began our study in 1959) of small to medium-sized, brilliantly metallic blue, green, or golden bees that are sparsely hairy. All of these bees are rapid fliers and tend to be very wary. The males are characterized by brushes on the front tarsi and by greatly inflated hind tibiae which bear "scars" [openings densely covered by hairs, through which liquids may pass (Fig. 1)]. The mid-leg also shows a "velvet" area on the tibia, but its function is unknown. The brushes and scars are not as well developed in *Exaerete* and *Aglae* as in the other genera.

The feeding behavior of *Euglossa*, *Eulaema*, *Euplusia*, and *Eufriesia* is essentially similar although nesting behavior is characteristic for each genus (10). *Exaerete* and *Aglae* are parasitic

bees that lay their eggs in the nests of other euglossine bees. The females of the nonparasitic genera forage for materials for nest construction and food for storage in the nest cells. In most species there appears to be little division of labor, and each female constructs her own nests and cells. In some species of *Eulaema* and *Euglossa* several females may occupy a single large nest and apparently cooperate in its construction and provisioning (10); however, there is no evidence of caste formation.

Males leave the nest immediately upon emerging and do not return. They live a vagabond life, feeding on nectar-producing flowers and visiting orchid flowers (and other flowers that produce the same odors as orchids). The males are known to live as long as 6 months (10), which is considered to be very long for male bees.

The male establishes a display site which he defends and patrols in a manner typical of his species. Some choose a limb or a small tree trunk, land on it for a moment, and then fly a prescribed pattern before returning and landing again. Others fly a zigzag pattern, buzzing as they fly over or near the site without landing. This behavior may continue for hours and frequently occurs on several succeeding days at the same spot. Other males of the same species frequently may be attracted by the activity of the first bee and hover nearby. Apparently a fragrance is left at the spot where landing takes place, for if one male is captured a new male frequently takes its place at the same spot the following day. While the bee is on the tree, he buzzes loudly every few seconds, and a fine mist can be seen to emanate from the posterior portion of the bee (it has not been

possible to determine if the emanation is from the abdomen or the legs). The female is attracted either by the loud buzzing, by the flight pattern, or by the odor produced by the male. Copulation takes place near the site (10).

Visits to Flowers

It has been established that euglossine bees are attracted to odors (5, 10). Most of the flowers visited by euglossine bees produce a strong fragrance and, even if the flowers are hidden from view, the bees will seek them out. Adams (11) placed flowers of *Catasetum maculatum* L. C. Rich ex Kunth inside a clear plastic insect trap, left the flowers for a few minutes, and then removed them. Bees were attracted to the residual odor in the trap.

The bees characteristically approach the flowers from downwind, stopping to examine any object in their path as they search for the source of the odor. When the bees arrive at the flower they are generally very wary, hovering and darting away only to return and hover again as they test the fragrance. Immediately after landing on the flower, the bees begin to rub the lip of the flower with their tarsal brushes. While the bees are brushing, their antennae are extended and depressed so that the bees seem to be continually sampling the odors near the surface where they are brushing. After 30 to 60 seconds of brushing, the bees resume flight and hover downwind in front of the flower, rubbing the tarsal brushes against the scars on the swollen tibiae. They then return to the flower, resume brushing, and repeat the process.

Chromatographic analysis of the contents of the tibiae indicate that the odor substance is transferred from the tarsal brushes to the hind tibiae, where it is evidently absorbed through the scars. The tibiae contain the same odor substances as those of the fragrance produced by the flowers visited (8, 11).

Once a bee has landed and brushed, it becomes less wary and can often be caught by hand. Formerly we believed that the bees became "intoxicated" by the materials obtained by rubbing on the flowers (7, 10), but recent observations indicate that female euglossine bees react in much the same manner when collecting resin for nest construction. Once they begin collecting the resin and transferring it to the pollen baskets, they are much less wary. It is unlikely that the resin "intoxicates" the female bee; therefore, it

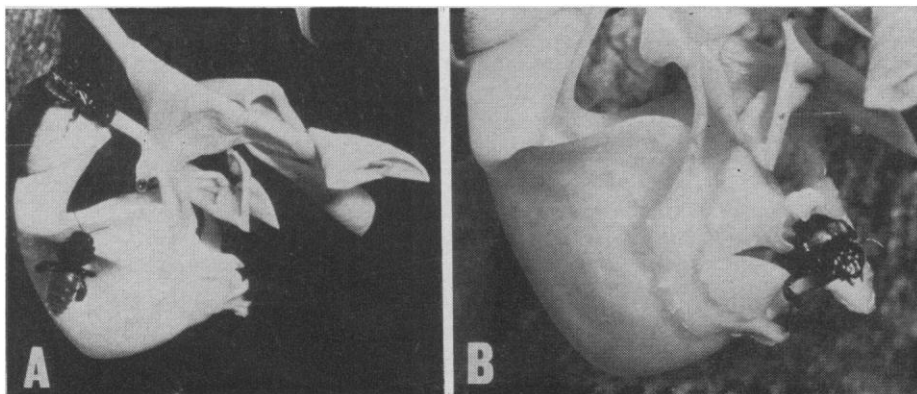


Fig. 2. (A) Male bees of *Euplusia superba* visiting the flowers of *Coryanthes rodriguezii* at Iquitos, Peru. The bees rub on the upper portion of the lip and fall into the bucket-shaped lower portion which is filled with water from glands on either side of the column. (B) One of the bees crawling out of the bucket under the stigma and anther of the flower. As the bee crawls out the pollen masses adhere to the dorsal abdomen.

seems more likely that the "drugged" aspect of the behavior of both male and female bees results from "concentration" rather than from a physiological reaction to substances produced by the plants.

While visiting orchids, the bees, whether drugged or not, often slip and fall. The flower surfaces may be very smooth, which increases the frequency of slipping, and the structure of the flower may be such that the bee falls in an awkward position, or finds its wing movements hampered by projections of the flower, so that it must fall a few centimeters before it can right itself and continue flight. Falling is especially frequent when the bee releases the flower to hover and transfer the fragrance material to the tibial organs. In a number of species this falling is an essential part of the pollination mechanism (Figs. 2 and 3).

Some orchids pollinated by euglossine bees are relatively simple morphologically (Fig. 4) and are visited and pollinated in the same manner as many other orchids. Others have quite bizarre structures and pollination mechanisms. The pollinaria (pollen masses and associated structures which attach the pollinia to the pollinator) are attached to the bee in specific places and are transported to the stigma of another flower of the same species. Because of variation of the point of attachment of the pollinarium, (behind the head, behind the thorax, on top of the thorax, on the front of the head, under the abdomen, on the legs, and so forth (9), and because of the varying positions of the stigma in relation to the posture of the entering bee, it is possible for several sympatric species of orchid to be pollinated by the same bee species without hybridization occurring (9, 10). The more complex flowers, such as *Gongora* spp. and *Stanhopea* spp., have correspondingly more complicated pollination systems. In these genera pollination is accomplished by a bee falling through the flower and picking up or depositing the pollinarium as the bee passes the end of the column (Fig. 3). In the genus *Coryanthes* the apical portion of the lip forms a "bucket" into which water drips from glands on the side of the column (Fig. 2). The bee falls into the water in the bucket and must crawl under the stigma and the anther to escape from the flower.

Flowers which are pollinated by euglossine bees [euglossine orchids (9)] usually have highly specific odors which attract only one or only a few species even in areas where many spe-

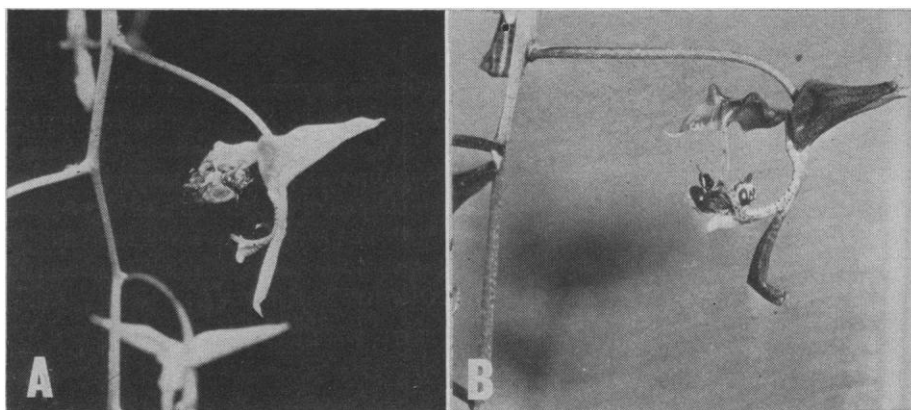


Fig. 3. (A) Male *Euglossa gorgonensis* rubbing on the lip of *Gongora* aff. *quinquenervis* (Guapiles 3). (B) Male *Euglossa cyanura* falling and sliding off the column of *Gongora tricolor*. When attempting to fly from the flower to transfer the compounds from the tarsal brushes to the tibial organs, the bee falls. When it passes the end of the column, the pollen masses adhere to the thorax. The pollen masses are inserted into the stigma of a succeeding flower in the same manner.

cies of bees occur. In Panama, *Gongora tricolor* (Lindl.) Reichb. f. is visited only by *Euglossa cyanura* Cockrell, while in the same area *Gongora quinquenervis* Ruiz & Pavon is visited by five other species of *Euglossa*. There are other species of euglossine orchids which also attract several species of bees, but generally any species of orchid will be pollinated by only one or very few species of bee. In some instances where an orchid species attracts several species of bee, some of the bees are accessory visitors (species which are of the wrong size or behavior to effect pollination). At Cerro Campana in Panama, *Stanhopea* aff. *inodora* Lodd. ex Lindl. is pollinated by a large bee, *Euplusia ornata* (Moc-sary). The flower is also visited by several species of *Euglossa* which are too small to effect pollination. These bees rarely enter the lip of the flower, where the normal pollinator would brush, but tend to brush on the sepals and petals. This behavior is a frequent phenomenon in euglossine orchids and permits pollination by a single species of bee, even when the chemical attraction is not specific.

In other euglossine orchids dimensions of the flower may serve as isolating mechanisms through adaptation to large or small bees; however, morphological isolating mechanisms alone are rarely sufficient to assure specific pollination. In central Panama there are 60 known species of euglossine bees and probably 40 of these species could be found in any favorable orchid habitat in the area. Adaptation to size of pollinator and placement of pollinaria are not sufficient to provide the specificity necessary to prevent hybridization between many interfertile species.

Strong attraction of euglossine bees to specific fragrances produced by the flowers provides the necessary reproductive isolation to maintain the integrity of interfertile species.

Components of Floral Fragrances

Orchid floral fragrances are diverse in their composition. Gas chromatographic analysis of the fragrances of 150 species from 25 genera (largely euglossine-pollinated species) indicate approximately 50 different compounds in orchid flower fragrances (10). Most species produce from seven to ten compounds, but some produce as many as 18 or as few as three. Of the 50 compounds present, 16 have been identified by relative retention times on two columns, enrichment, smell (most of



Fig. 4. Male *Eulaema polychroma* visiting the morphologically simple flower of *Pescatoria wallisii*. While the bee rubs the lip, the pollen masses adhere behind the head.

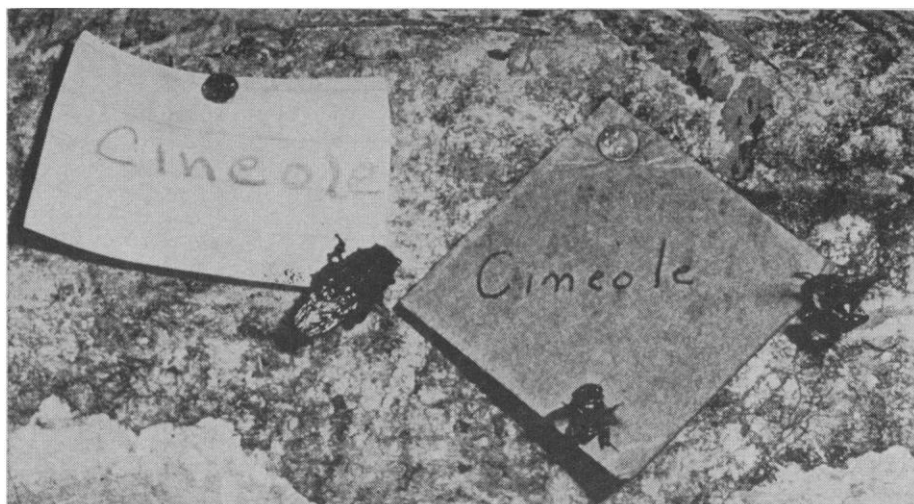


Fig. 5. Male bees attracted to blotter paper saturated with 1,8-cineole. (Left) *Eulaema nigrita*; (middle) *Euglossa dodsoni*; (right) *Euglossa asarophora*.

the compounds are common perfume or flavor ingredients), and biological activity (10–13). Ten additional compounds have been tentatively identified and have proven to attract euglossine bees.

Not all compounds are produced with equal frequency. Almost 60 percent of the species sampled produce 1,8-cineole, but less than 5 percent produce methyl cinnamate. The amount of a compound in a fragrance also varies; for example, 1,8-cineole forms about 90 percent of the odor of *Stanhopea cirrhata* Lindl., but only about 7 percent of the fragrance of *Catasetum maculatum* L. C. Rich. ex Kunth. The percentage of a compound in a fragrance may not determine its overall smell to humans; for example, methyl cinnamate forms only 1 percent of the total fragrance of *Catasetum roseum* (Lindl.) Reichb. f., yet the floral fragrance of that species is reminiscent of cinnamon.

Field Tests of Compounds

To determine the effects of the identified compounds on the bees, field tests were carried out in tropical America. The first series of tests, in central Panama in early February 1968, were to determine the kinds of bees attracted to pure compounds and to mixtures of compounds. A second series of tests designed to survey the attraction potential of the compounds and to determine geographical distributions of the bees, was made from February to August 1968 in Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Venezuela, and Trinidad.

The first series of tests took place in the central portion of Panama on both sides of the Canal Zone. Rainfall and vegetation of the three sites—Cerro Jefe east of Panama City, Santa Rita Ridge east of Colon, and Cerro Campana west of Panama City—are similar, and all three sites were known to have numerous euglossine bees.

Blotter papers (5 by 5 centimeters) were saturated with the compounds to be tested and tacked 2 to 5 meters apart on logs or tree trunks in forest areas (Fig. 5). The tests were made between 7 a.m. and 1 p.m.—when euglossine bees are most active. All bees attracted were captured and killed for species determinations and body counts. Blotter papers were replenished when a compound appeared to have evaporated—some of the compounds are very volatile while others last for several days.

During a 5-day test period in central Panama, 42 of the 60 species known from that area were attracted. Of the other 18 species, some were present at the time of the tests but were not attracted. Some of the unattracted species are seasonal or extremely rare, and they may not have been present. Subsequently, 12 of the 18 species have been attracted to the original or to additional compounds. Six species remain unattracted.

During the Panama tests, pure compounds and mixtures of pure compounds were assayed. In pure form 1,8-cineole, methyl salicylate, and benzyl acetate acted as general attractants. Two compounds, α -pinene and β -pinene, failed to attract.

During the 5-day test period, 1,8-cineole attracted 433 male euglossine bees (no other kinds of bees were at-

tracted) representing 35 species; methyl salicylate attracted 113 individuals of 11 species; and benzyl acetate, 36 individuals of 6 species. When one part 1,8-cineole and 39 parts benzyl acetate were mixed (the approximate proportions of these compounds in the fragrance of *Stanhopea tricornis* Lindl.) only 49 individuals from 8 species were attracted. When α -pinene was added to the mixture of 1,8-cineole and benzyl acetate in the same proportion found in the same orchid, only six individuals of two species were attracted. The species were *Eulaema meriana* (Olivier), the known pollinator for *Stanhopea tricornis* in its native habitat in Colombia and Ecuador, and *Euglossa dodsoni* Moure, a bee much too small to effectively pollinate *S. tricornis*.

In the second series of tests pure compounds were tried at numerous sites in tropical America. Additional compounds not tried in the first series were eugenol, methyl cinnamate, bornyl acetate, α -phellandrene, and myrcene. Piperonal and indole were tried only in Panama, whereas vanillin was tested in Panama and Ecuador. All of these compounds attracted bees. Vanillin and piperonal have not been positively identified as components of orchid fragrances, but the odor of vanillin is reminiscent of the fragrance of *Stanhopea oculata* (Lodd.) Lodd. ex Lindl., *Catasetum russellianum* Hook, and *Mormodes unciunculus* Reichb. f. Bees attracted to these orchids were also attracted to vanillin.

The most effective general attractant has been 1,8-cineole except in western Mexico where eugenol was as effective in general, and more effective with particular kinds of bees (Table 1). Methyl cinnamate was more effective in Mexico than in Ecuador, Colombia, or Venezuela. The other compounds tested attracted fewer species and were effective only in certain areas. In Mexico and Panama, *d*-carvone attracted several species of *Euplusia*. Bornyl acetate, anethole, geraniol, pulegone, linalyl acetate, and α -phellandrene each attracted a few species but only in Panama and Costa Rica. Myrcene attracted six species in Panama, Colombia, and Ecuador and was the only compound to attract females (two were seen to hover near the blotter).

The absolute concentration of the compounds seemed of little consequence in most cases. Totally saturated pads were most effective in attraction except those saturated with β -ionone and anethole. β -Ionone was most effective in cases where only a single

drop was placed on the blotter whereas blotters saturated with anethole attracted bees only after 2 to 3 days of exposure. In all cases the bees behaved in the same manner at the liquid-soaked blotters as at flowers. They hovered in front of the pad testing the fragrance, landed and rubbed the blotters, launched into flight and rubbed the tarsal brushes on the tibial scars, and became less wary.

The general attractants are the most frequently produced odor compounds in orchid flowers. Over 70 percent of the attracted species of euglossine bees are drawn to 1,8-cineole, which is produced by 60 percent of the orchid species sampled (14). Benzyl acetate occurs in about 25 percent of the orchid species sampled and attracts 10 percent of the bee species, while methyl salicylate occurs in 4 percent of the orchids and attracts 20 percent of the bee species. Many species of bees were attracted to several compounds, but some were attracted to only one compound (14). *Eulaema cingulata* (Fabricius) was attracted to nine of the compounds tested, but was not attracted to 1,8-cineole. This is one of the most frequent pollinators of orchids and can be very selectively attracted to certain orchid fragrances (10).

The combination of two attractants modifies the attraction potential of the total fragrance and often fails to draw in species that would visit one of the attractants in pure form. The addition of a third compound (such as α -pinene) may further reduce the numbers of species attracted. This selectivity of mixtures is very significant, and some combinations of compounds will attract only one or very few bee species, even though some of the ingredients would,

by themselves, attract many species. The differential production of fragrance components by different orchid species may thus limit the number of bee species attracted when numerous species are present. Once the number of species attracted has been reduced, mechanical isolation can play a more effective role in pollinator specificity.

Conclusions

Many orchids pollinated by male euglossine bees produce compounds in their fragrances which attract particular species of bees. No food is provided by the flowers. The bees collect the fragrance components which they store in their hind tibiae. Why do the bees collect these materials? We have little documented evidence at present to answer that question, but we offer three possibilities.

1) Male euglossine bees live longer than is normal for other male bees and some evidence exists (10) that the bees die if deprived of the compounds. The bees may metabolize the compounds to cover some natural deficiency.

2) Male euglossine bees may convert the compounds into sex attractants. Female bees are not attracted to the pure compounds, but they are somehow attracted to the territorial displays of the males, and they copulate at the site (10).

3) Males may use the compounds to attract other males of the same species, so that several males are present at the mating site. This occurs in other arthropods (15) and probably tends to insure fertilization of the occasional female which passes by. The female euglossine bees may be attracted by the

loud buzzing of the males rather than by chemical sex attractants.

In an atmosphere saturated with certain of the compounds, the bees tend to die quickly. The presence of the collecting and storage organs at distal portions of the legs rather than in the body cavity may protect the organisms from the compounds. If the compounds are deleterious to the bees when taken internally, then the bees could not collect them with the tongue and store them in the body. A male *Euglossa* sp. (an unnamed species designated as "RD 725") which emerged from a nest in our laboratory at Miami was allowed to collect pure cineole for 3 days and was then dissected and examined for the presence of cineole in various parts of its body. Cineole was found only in the tibial organ.

Whatever the reason for the collection of the fragrance compounds, the bees have had a great effect on the evolution of the orchids which produce the compounds. Speciation in these orchids could occur by minor genetic changes affecting the production of fragrance components (16). The production of additional components or the failure to produce a component could change the attraction potential of the fragrance of a genotype. If a different species of bee is attracted and the pollinator of the parental form is not attracted, a new reproductively isolated population could develop. For example, the members of the population of *Stanhopea tricornis* at Santo Domingo, Ecuador, have a slightly different combination of odor components from the population at Turbo in Colombia (Fig. 6). Both are probably pollinated by the same species of bee

Table 1. Numbers of male euglossine bees collected while visiting some of the primary attractants at various localities in tropical America. (A) 1,8-Cineole; (B) benzyl acetate; (C) eugenol; (D) methyl salicylate; and (E) methyl cinnamate.

| Locality | Total (No.) | Average/day (No.) | Species (No.) | Visitors at attractant | | | | |
|---------------------|----------------|----------------------|------------------|------------------------|-----|-----|-----|----|
| | | | | A | B | C | D | E |
| Eastern Mexico | 203 | 29 | 9 | 167 | 3 | 12 | 1 | 20 |
| Western Mexico | 119 | 39 | 5 | 40 | 4 | 74 | | 1 |
| Southern Mexico | 605 | 201 | 9 | 530 | 1 | | 1 | 6 |
| Guatemala | 68 | 11 | 6 | 47 | 1 | 15 | 1 | 3 |
| El Salvador | 31 | 10 | 6 | 10 | 2 | 16 | | 3 |
| Honduras | 28 | 28 | 8 | 20 | 1 | 2 | 5 | |
| Nicaragua | 73 | 18 | 4 | 61 | 1 | 1 | 10 | |
| Northern Costa Rica | 88 | 22 | 17 | 56 | 6 | 11 | 5 | |
| Southern Costa Rica | 93 | 31 | 20 | 87 | 1 | | 5 | |
| Panama | 927 | 46 | 48 | 685 | 38 | 28 | 153 | 23 |
| Eastern Colombia | 160 | 16 | 42 | 133 | | 3 | 24 | |
| Western Colombia | 29 | 14 | 6 | 16 | | | 8 | 5 |
| Eastern Ecuador | 427 | 42 | 18 | 157 | 24 | 11 | 229 | 6 |
| Western Ecuador | 121 | 30 | 13 | 117 | 1 | | 2 | 1 |
| Eastern Venezuela | 89 | 44 | 18 | 70 | 1 | | 12 | 4 |
| Western Venezuela | 173 | 43 | 15 | 152 | 14 | | 6 | 1 |
| Guianas | 107 | 21 | 4 | 107 | | | | |
| Trinidad | 244 | 81 | 13 | 114 | 69 | 15 | 41 | 5 |
| Totals | 3585 | 38 | 85 | 2569 | 167 | 188 | 502 | 78 |

[*Eulaema meriana* (Olivier) pollinates this species in Ecuador]. Further fragrance changes could attract different species of bees and reproductively isolate the two populations.

This system would permit sympatric speciation, which we believe is occurring in the genus *Gongora* in Central America. Extensive populations of *Gongora* aff. *quinquenervis* Ruiz & Pavon occur in Costa Rica and Panama. The plants are essentially the same in all external characters, but the flowers tend to be variable in color within a given population, and in some cases morphological characters of the lip of the flower may vary. Early taxonomic treatment had separated several forms on the basis of color, but recent taxonomists have reduced all gongoras which resemble *G. quinquenervis* to that species. At Guapiles, in Costa Rica, a large population of *Gongora* produces flowers which are structurally quite similar but which diverge in color, fragrance spectra, and pollinators. One form, *G. unicolor* Schltr., produces flesh-pink flowers and a sweet fragrance which we are unable to analyze with the gas chromatograph. It is recognizable only by the color of the flowers and the fragrance it produces, yet it is pollinated by a bee, *Euglossa purpurea* Friese, which does not visit any of the other members of the group. The other members of the group produce flowers which are nearly indistinguishable from one another in form, but vary widely

Table 2. Pollination spectrum of the Orchidaceae indicating the percentage of orchid species pollinated by various agents. [Taken from van der Pijl and Dodson (1)]

| Orchid species (%) pollinated by: | | | | |
|-----------------------------------|----|--------------|--|----|
| Hymenoptera | | Other agents | | |
| Wasps | 5 | Moths | | 8 |
| Lower bees | 16 | Butterflies | | 3 |
| Carpenter bees | 11 | Birds | | 3 |
| Euglossine bees | 10 | Flies | | 15 |
| Social bees | 8 | Mixed agents | | 8 |
| Mixed bees | 10 | Apomictic | | 3 |

in color from white flecked with red to completely wine red. Two fragrance types (Fig. 7) associated with pollinators, were designated "Guapiles 3 and 4." The plants designated "Guapiles 3" attracted *Euglossa gorgonensis* Cheesman while "Guapiles 4" attracted *E. bursigera* Moure. Only one of the three types can be distinguished (*G. unicolor*—by color of the flower) when pollinator data and fragrance spectra are ignored. The indication is that adaptation radiation to different pollinators is taking place. One form, *G. unicolor*, has reached a point of color differentiation. In time the other two forms might develop morphological differences easily recognizable by taxonomists.

We have discussed orchid speciation based on attraction of specific kinds of euglossine bees. These orchids constitute about 10 percent of the species in the family. How did the other 90 percent accomplish comparable speciation? We know that most of the members of the

genera *Brassavola*, *Epidendrum*, *Habenaria*, and *Campylocentrum* in the neotropics and *Angraecum*, *Aerangis* and *Platanthera* in the Old World tropics are adapted to pollination by night-flying moths. Orchids adapted to moth pollination are characterized by white or light green flowers, strong fragrances at night, and long nectar tubes. Because of the difficulties in observing night pollination, few data have been accumulated concerning pollinator specificity in these orchids. These genera contain intrageneric interfertile species, many of which are sympatric, but few reports of natural hybrids exist. Preliminary studies with gas chromatography of species of *Brassavola* and *Angraecum* indicate that species-specific fragrances are produced.

Similar situations appear to exist with such fly-pollinated genera as *Pleurothallis* in the neotropics and *Bulbophyllum* in the Old World tropics. Both genera are very large (near 1000 species) and have allied genera with numerous species. Scattered observations indicate that the species are isolated by pollinator specificity (10, 17). Further study may demonstrate that differential fragrance spectra are involved in pollinator specificity.

About 33 percent of the species of orchids are covered by the euglossine bee-, moth-, and fly-pollinated classes (Table 2). Obviously specific attraction by fragrance is not the only factor in the evolution of the family; however,

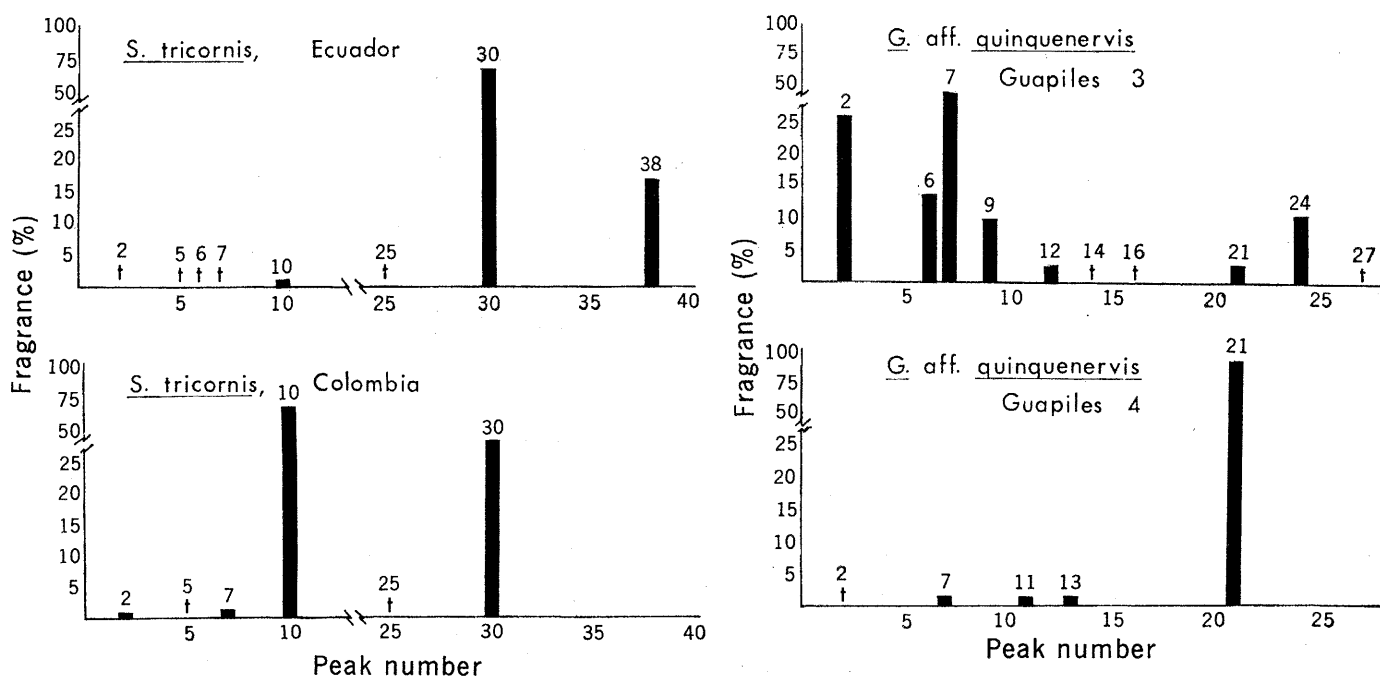


Fig. 6 (left). Compounds present in the fragrance of *Stanhopea tricornis* from populations in Colombia and Ecuador (calculated from gas chromatographs of the floral fragrances). Fig. 7 (right). Compounds present in the fragrance of two kinds of *Gongora* closely related to *Gongora quinquenervis* Ruiz & Pavon which occur together in a large population at Guapiles, Costa Rica.

the majority of the large genera are covered by this system. An exception is *Dendrobium* (estimated to consist of 1600 species) which occurs throughout tropical Asia and Australia. Here spatial isolation on islands, flower color, and periodicity in flowering (1) may have played a more important role. In *Dendrobium* social bees, which are not instinctively associated with attraction by specific fragrances, join with flies, birds, and butterflies as pollinators.

Summary

Certain orchids in tropical America have become adapted to pollination by male euglossine bees. The bees are attracted by floral fragrances, and the chemical composition of the fragrances determines which species are attracted. The male bees collect the fragrance materials directly from the flower by rubbing the surface of the flower with special tarsal brushes. The bees launch into flight, transfer the fragrance materials, and store them in swollen glandular tibiae of the rear legs. The contents of the tibiae after floral visits were

analyzed by gas chromatography; they were the same as the floral fragrance.

Approximately 50 compounds are present in euglossine orchid fragrances, and some species may produce as many as 18 of the compounds. Other species produce fewer compounds. Certain of the compounds, when presented to the bees in tropical America, proved to attract many species of euglossine bees. Other compounds attracted only a few species although in some cases no bees were attracted. Combinations of the compounds attracted markedly fewer species than pure compounds. Appropriate combinations of compounds, in the proportions found in orchid fragrances, attract the same bees which are attracted by the flowers. Speciation and reproductive isolation in euglossine bee-pollinated orchids appears to be based on specific attraction of pollinators to odors produced by the orchid flowers; the substances are believed to play a role in the life cycle of the bees.

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Time-Zone Effects

Disruption of circadian rhythms poses a stress on the long-distance air traveler.

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The main celestial events within our planetary system occur at certain intervals, are repetitive and periodic, and follow predictable time patterns. Since the earth, moon, sun, and other celestial bodies are connected by such basic and ubiquitous phenomena as gravity and radiation, it seems plausible to assume that the variations associated with geophysical and diurnal cycles affect the course of life processes. Well-known examples of periodicities in our daily life are the day-night cycle, the wake-sleep cycle, the work-rest cycle, and the menstrual cycle. Certain physiological cycles appear to depend, there-

fore, on an "internal clock," a more or less accurate biological mechanism.

Halberg in 1959 introduced the term *circadian* (derived from the Latin *circa dies*) for a time period which approximates 24 hours; thus *circadian* means about the same thing as *diurnal* (*diurnal* is defined as "daily"). Although these circadian periodicities had been known for several centuries, only recently has research on the temporal dependencies of biological systems become systematic and "mission-oriented" (1).

The adverse effects of unusual schedules on the individual's subjective and physical state is noted in the Declara-

tion of Independence and constituted one of the colonists' grievances against King George III. This passage reads: "He has called together legislative bodies at places unusual, uncomfortable, and distant . . . for the sole purpose of fatiguing them. . . ." People traditionally have been very sensitive to, and resistant to, alterations in their cycles of sleep and wakefulness. Rural people have been inclined to maintain these cycles in phase with sunset and sunrise. They tend to arise earlier in the summer, when sunrise is earlier, than they do in the winter. The first published suggestion that great economies could be effected by instituting what was later to be called "daylight saving time" appeared in 1784 in the *Journal of Paris*; the author was Benjamin Franklin. From the fact that the suggestion was not followed until the 20th century, we see how the human being clings to his customary sleep-wake cycle (2).

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