the banding patterns of the membrane-carcinogen complexes.

Our experiments show that (i) CsCl equilibrium density gradient centrifugation is useful for the study of membrane–nucleic acid and membrane-carcinogen interactions, (ii) the chemical carcinogens studied so far interact with the membrane to change its density and, in certain cases, fractionate the membrane into two or more distinct classes, (iii) both the degree and the pattern of binding of nucleic acids to microsomal membrane may be influenced by the carcinogen, and (iv) the changes described in (ii) and (iii) are different for each of the compounds studied.

It is not clear whether multiple membrane zones observed after incubation with AAF and its derivatives are due to certain existing differences within the liver microsomal membrane population or whether the membrane was modified to varying extents during the incubation with the carcinogen. The first possibility suggests that there exist in rat liver two or more classes of membranes each binding preferentially either a particular carcinogen or its metabolite to form a complex with a new density in CsCl. The second scheme suggests that all members of the population of membranes have equal chance to react with either the carcinogen or its hydrolysis or metabolic products. We favor the first scheme since it appears that preexisting receptors for the two nucleic acids are separated and distributed at new densities in the gradient in the presence of AAF and its derivatives. This is suggested by the quantitatively similar binding of the nucleic acids in the control experiment and in the presence of AAF and N-OH-AAF.

Several other substances such as antibiotics, pesticides, and metal ions also changed the density of the microsomal membrane in CsCl and altered the pattern of the nucleic acid binding. Each compound changes these patterns in its own specific and unique way, although, as found in other experiments, substances related chemically tend to have similar activities. The available data suggest that several biologically active molecules, including chemical carcinogens, act by interfering with the chemistry and the biological activity of cellular membranes. Binding of chemical carcinogens to endoplasmic reticulum was first reported more than a decade ago (*9*). Whether the observed reactions between the carcinogens and the membrane may be involved in the process of tumor induction or in the perpetuation of the malignant state (or both) is not clear. Although additional experiments are required to evaluate this possibility further, alterations of the cellular surfaces observed during malignant transformation (*10*) make this suggestion plausible.

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2. H. Kubinski and C. B. Kasper, *Abstracts* (International Cancer Congress 10th, Houston, Texas, 1970), p. 29; ———, in preparation.
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4. Abbreviations used: poly A, poly C, poly G, poly U, homopolymers of riboadenylic, -cytidylic, -guanylic, and -uridylic acids, respectively. Poly C · poly G denotes the complex between the complementary homopolymers formed by simple mixing of equimolar quantities of each under the conditions of ionic concentration and temperature favoring the formation of such complexes. AAF, N-Ac-AAF, and N-OH-AAF: 2-acetyl aminofluorene and its *N*-acetoxy and *N*-hydroxy derivatives, respectively; BPL, β-propiolactone; MTT, 3-methyl-1-*p*-tolyltriazene.
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12. We thank P. Gibbs, M. Heise, and D. Dowd for technical assistance; and Drs. E. Miller and J. Miller for guidance in choosing the chemical carcinogens. Supported by PHS grants CA 08959 and CA 07175.

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Euglossine Bees as Long-Distance Pollinators of Tropical Plants

Abstract. *Euglossine bees may return to a nest from as far away as 23 kilometers in a tropical rain forest. These bees apparently forage long distances and visit the same plants repeatedly along a feeding route. They probably promote outcrossing among tropical plants with low population density; therefore, they may permit the existence of plant species whose densities have been forced very low by such things as competition and predators on seeds and seedlings.*

Euglossine bees (*Euplusia, Eulaema, Euglossa*) are important pollinators of lowland neotropical woody plants (*1*, *2*). There have been many studies of their nesting and social behavior (*2*) and on the obligatory mutualistic interaction between euglossine males and the orchids they pollinate (*3*). The spectacular pollination activities of the males are probably of much less importance to tropical community structure than are those of the females, whose foraging behavior is almost unknown. I here report some details of this foraging behavior in hopes of encouraging field ecologists to study these bees before they become extinct through human destruction of neotropical vegetation (*4*).

Three lines of indirect evidence strongly suggest that euglossine females fly a much longer distance from nest to host, and between hosts, than is generally expected of bees (*5*).

Female *Euplusia surinamensis* were captured while sleeping in their nests in lowland Costa Rican rain forest, marked by wing notching, and stored in individual containers until the following day. They were transported in a dark box and two bees were released at 1, 2, 3, 4, 5, and 6 km from their nests at about 11:15 a.m. on a clear day. By 3:00 p.m. all had returned and resumed nest construction. A year later, this experiment was repeated, but 12 bees were released between 6:36 a.m. and 6:58 a.m. at 14, 17, 20, and 23 km from the nest, three bees at each distance. From these distances, two, one, three, and one had returned by nightfall. The fastest bees were from 20 km (65 minutes) and 14 km (47 minutes). These two bees returned with full pollen loads but the other five appeared not to have foraged for nesting materials. Of the five that did not return, three were just beginning cell construction and therefore may have had little experience at foraging away from the nest site. The speedy and accurate return of *E. surinamensis* females strongly suggests that these bees were released in an area known to them and therefore probably within their foraging range.

I have observed female *Euplusia* or *Eulaema* flying across Gatun Lake in the Canal Zone (*6*) and the lake behind Presa Aleman, in eastern Oaxaca, Mexico (*7*). These bees were crossing 1 to 5 km of open water; behind Presa Aleman two *Eulaema polychroma* with pollen loads were collected over water 2.5 km from the nearest land. These bees were flying in different directions but in straight lines. When released, they flew off in the same direction they were originally headed. These bees were probably foraging on one side of the lake to provision cells on the other. If these bees will cross this much water to forage, they are certainly at least capable of foraging this far over forest, and probably do.

In contrast to many wild bees that forage on trees with many flowers, or in large patches of flowering herbaceous plants, euglossine bees get both pollen and nectar from large plants that usually occur as widely scattered individuals in forest undisturbed by European types of agriculture (for example, Apocynaceae—*Stemmadenia, Urechites, Thevetia, Mandavilla*; Leguminosae—*Canavalia, Dioclea, Clittoria, Cassia, Swartzia*; Convolvulaceae; Bignoniaceae —*Cydista, Bignonia, Amphilophium*; Rubiaceae—*Sabicea, Pentagonia, Cephaelis*; Solanaceae; Lecithidaceae; Bixaceae; Melastomataceae—*Blakia, Conostegia*; Guttiferae—*Clusia*; Heliconiaceae; Marantaceae; Costaceae).

These observations should not be construed as indicating that female euglossine bees are cosmopolitan in host preference. Any given species at any given locality at any specific time of year usually has one or two primary pollen hosts and perhaps twice as many nectar hosts (*1*). It is doubtful whether her feeding route ever includes more than five to ten species of plants (*8*).

Each of these plants bears few flowers at one time, although it normally bears them for many consecutive months. A female bee can gather only a small fraction of the pollen or nectar she needs from one plant. For example, a *Euplusia surinamensis* returns to the nest (*9*) with an amount of pollen approximately equal to the daily pollen production of 60 *Cassia* or *Solanum* flowers. If, on the average, each flower she visits has been visited by at least two other bees, this means she has to visit 180 flowers to obtain one pollen load. A representative plant has five new flowers to visit per day so she has to visit at least 36 plants per round trip. If the plants are 100 m apart, on the average (*10*), she has to fly at least 3.6 km during the hour. When provisioning a cell, she makes up to six trips, each averaging about an hour, during a day. This means a maximum of at least 21.6 km of flying between host plants, to say nothing of how far she flies to get to her foraging area. I have timed *E. surinamensis* females flying 20 km per hour in direct flight away from the nest (*9*). A female spends about a minute on a plant with five flowers and therefore needs at least 46.8 minutes to collect a pollen load. Since she may spend up to 2 hours on a single trip, she may be flying for as much as 24.4 km (73.2 minutes) to get from the nest to the foraging area and return.

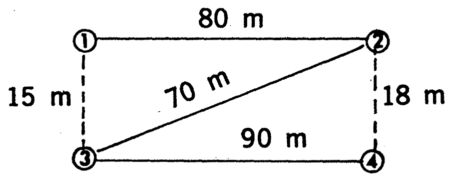

Fig. 1. Flight path (solid line) of female *Eulaema cingulata* between four *Heliconia* plants. She flew 80, 70, and 90 m rather than the more economical 15, 70, and 18 m (dashed line) because she was visiting the plants in the original order that they were incorporated into her feeding route.

Circumstantial evidence, discussed below, indicates that a female bee visits the same set of plants each day, and probably in the same order. For example, when the one to two new flowers of an apocynaceous vine (*Urechites andrieuxii*) were removed each of 3 consecutive days before they could open (*11*), the plant continued to be visited each day by the same three individual *Euglossa* female bees (*12*) seeking nectar; one bee regularly came twice in the first 2 hours after dawn, and the other two visited once only (between 9 and 11 a.m.). When a small patch of *Calathea insignis* (Marantaceae) was cut at dawn (*13*), thereby removing its seven inflorescences which normally bore two to five new flowers each morning, there were 37 visits made by *Euglossa, Eulaema,* and *Euplusia* males and females in search of nectar during that and the following morning (*14*). When a *Solanum lanceifolium* (Solanaceae) vine growing on the edge of primary forest (*15*) was cut down and removed, females of *Eulaema meriana, E. speciosa,* and *E. luteola* were occasionally seen hovering and flying back and forth through the vine's previous location during that day and the following one.

When three or four host plants are growing such that they can be watched from the same vantage point, it is common to observe a large euglossine bee visit them in the same order each day at about the same time. For example, four *Heliconia imbricata* plants were visited for nectar in the same order by the same female *Eulaema cingulata* between 7:00 and 7:15 a.m. on five different mornings (*16*). Along the bee's route, the plants were 80, 70, and 90 m apart. Her flight path (Fig. 1) was in the form of a flattened "Z" rather than a flattened backwards "N" (which would have given distances of 15, 70, and 18 m, respectively, between plants), which indicates that she visited the plants in the original order of incorporation into her feeding route, rather than opportunistically relocating the last three plants after finding the first (*17*).

When one collects euglossine bees, it is commonplace to collect three to six bees at one host plant on one day (assuming all visitors are caught), and then on each subsequent day to catch none to two bees. For example, a common pollen host for large euglossine bees, *Cassia bacillaris (18)*, yielded two *Eulaema luteola*, two *E. cingulata,* and one *E. speciosa* on the first morning of collection, and during the next 5 days, a total of two *E. cingulata*, one *E. speciosa*, and one *E. meriana* (seen but not collected). I interpret this to mean that the initial collecting takes those bees that visit the plants on a daily basis, and subsequent collections represent a combination of females that are searching for new plants to add to their feeding route and those bees that were engaged in activities such as nest construction on the first day of collection.

Nectar hosts yield similar results. A single bush of *Pentagonia wendlandi* (Rubiaceae) near slightly disturbed rain forest (*19*) yielded 46, 42, 12, 3, 5, and 7 *Euglossa* male and female bees of 12 species on 6 consecutive days. The first bees arrived between 7:10 and 7:31 a.m. and the last shortly before 11:30 a.m. The first day's collection was not begun until 8:30 a.m., however, and therefore the second day's collection probably contains a number of bees missed on the first day. This plant produced 8 to 12 new flowers each day on a total of eight major stems, and had been flowering for at least 2 months and probably much longer. Since 38 male and female *Euglossa* of the same species were collected, and many more seen, at other nectar hosts within a half mile (0.8 km) of the *P. wendlani* bush, it is certain that the total *Euglossa* population had not been decimated but that on the first 2 days those bees were collected that included this individual bush in their regular feeding route.

Euglossine bees behave as if they are very familiar with the immediate surroundings of the plants they visit, as well as if they know where the plants are. Their approach to a host plant is very fast ("zooming out of nowhere"), after which there is a short period of hovering in front of the flower. While it hovers, the bee turns and appears to be looking in different directions. If a stationary object (clipboard, stake, camera) is placed within 50 to 100 cm of the flower, before the bee arrives, the bee usually leaves directly or hovers at a distance of several meters from the flower and then leaves.

Thus, it appears that euglossine females fly long distances from nest site to host plant and between host plants, and visit the same individual plants on a daily basis rather than finding them anew each day. These two traits are of great significance to the host plants for the following reasons:

1) Effective outcrossing may occur at the very low densities that characterize many tropical plant populations.

2) To attain the small number of bee visits per flower that are necessary for pollination, large amounts of energy need not be expended on an excess of synchronized flowers that attract large numbers of bees with less regular visitation behavior.

3) Small amounts of reproductive energy may be used directly each day for flower production, rather than stored for a synchronized flower crop.

4) As is frequently true with other highly host-specific interactions of flowers and bees, floral morphology may become specialized to exclude all other types of bees (*20*), thereby alleviating the necessity of a large energy expenditure to feed many bees in order to attract a few that may be carrying pollen from other plants of the same species.

5) Since only a small number of flowers need be produced per unit time to yield effective outcrossing, a woody plant may reproduce at a much smaller size or in a less nutrient- and energy-rich habitat (such as heavily shaded rain forest understory) than is possible for a plant pollinated by bees of some other type.

6) Floral visibility over long distances is relatively unimportant in attracting pollinators, and therefore pollination may be assured even in heavily shaded forest understory.

In addition to euglossine bees, my cursory observations while collecting large Central American bees (*1*) indicate that *Xylocopa, Ptiloglossa* (*21*), *Centris*, and *Bombus* (and probably all other large lowland tropical bees) exhibit behavior like that described above for Euglossinae. Some Central American hummingbirds appear to behave similarly, although distances from nest to flower are probably not so dramatic as those postulated for the larger Euglossinae (*22*). Tropical forest sphinx moths also appear to behave in this manner. The hosts of both hummingbirds and sphinx moths often have characteristics similar to those of the bees listed above (a few new flowers open each day over a long period, long distances between individual plants, poor floral visibility at a distance, almost every flower produces a fruit, floral morphology is adapted to a long tongue).

A major trait of tropical plant populations is the ability to survive at the low densities to which the population may be reduced by seed and seedling predators (*23*). This means that many more plant species can be present than would be expected if each species' density were set by competitive interactions alone. The predictability of the physical environment is clearly one major factor in the population's survival at low density. A second factor may well be the existence of the complex of large bees, hummingbirds, and sphinx moths which may provide reliable outcrossing over distances far greater than would be expected if one were only to consider the pollinators typical of temperate zones, such as wind and small bees.

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References and Notes

1. Based on extensive field experience collecting these and other large bees in Central America from 1963 to 1970.
2. See the review by R. Zucchi, S. F. Sakagami, J. M. F. Camargo [*J. Fac. Sci. Hokkaido Univ. Ser. 6* **17**, 271 (1969)].
3. See the review by C. H. Dodson, R. L. Dressler, H. G. Hills, R. A. Adams, N. H. Williams [*Science* **164**, 1243 (1969)].
4. It has been my experience that during the first 1 to 5 years after agricultural exploitation of a lowland neotropical site, the large solitary bees disappear or become extremely rare, with the exception of the few species that can nest in dwellings (for example, *Xylocopa, Euplusia surinamensis*) and forage on crop plants and roadside weeds. In much of lowland Central America, it is currently impossible to study the plant-insect interactions described here, owing to habitat destruction.
5. It is generally considered that solitary and social bees usually forage distances up to 1 to 3 miles (up to 5 km) from the nest, and much shorter distances between individual host plants.
6. 5 July 1967.
7. October 1963 to July 1964.
8. A study is currently under way to analyze the species composition of pollen stores in euglossine nest cells at (*9*).
9. 2 March 1967 and 7 March 1968; the nests were under eaves and flooring of the buildings above the airstrip in the rain forest at Rincon, Osa Peninsula, Puntarenas Province, Costa Rica; the female bees sleep in their partly completed cells and are easily captured by prodding them until they fall out into a bag.
10. Estimates of population density of euglossine hosts are very difficult to obtain but my general impression is that in undisturbed or lightly disturbed forest, the female bees average at least 100 m in flights between hosts, and often much more; in badly disturbed forest, most of the nectar and pollen hosts are extinct or nonproductive, but those that remain (for example, *Cassia, Solanum, Bixa*) are often common and clumped; here the bee will have to fly much less between hosts, but is probably in trouble during the seasons when none of the few host species are in flower.
11. 8 June 1966; 1 mile (1.6 km) north of Cardenas, Tabasco, Mexico; vine growing in 3-year-old fallow milpa.
12. Recognition of individual bees was achieved through marking (rarely since it badly disturbs the bee) or fortuitous encounters with bees with distinctively worn wing margins or body coloration.
13. 3 March 1967; in lightly disturbed rain forest upstream on the River Agua Buena near the airstrip at (*9*).
14. The first morning 24 bees came, which suggests that some bees did not return to the site after failing to find nectar the first morning. The bees that failed to return could have (i) had short memories, (ii) been extremely perceptive and observed the absence of an inflorescence, or (iii) had many alternate hosts to visit.
15. 4 August 1969; east boundary of Finca La Selva, near Puerto Viejo, Sarapiqui District, Heredia Province, Costa Rica.
16. 1 to 7 March 1967 at (*13*).
17. H. Kennedy tells me she has also observed this regular foraging pattern with *Eulaema speciosa* when visiting *Calathea altissima* near San Vito de Java, Puntarenas Province, Costa Rica, June 1969.
18. 6 to 11 August 1965; along a small creek north of Puerto Viejo at (*15*).
19. 14 to 17 August 1965; 3 miles (5 km) west of Palmar Norte along the north bank of the Rio Terraba, Puntarenas Province, Costa Rica; I thank J. Sarukhan for identifying this plant.
20. Zucchi *et al.* (*2*) point out that most flowers regularly visited for nectar by euglossine bees have tubular corollas and the bee needs an exceedingly long tongue (15 mm plus) to reach the nectar; in my experience, these and other large bees are often the only visitors of these large flowers.
21. D. H. Janzen, *Behavior* **32**, 33 (1968).
22. L. L. Wolf, F. G. Stiles, H. G. Baker, personal communication.
23. D. H. Janzen, *Amer. Natur.*, in press.
24. In preparing this paper I have benefitted from discussion with faculty and students in the courses taught under the auspices of the Organization for Tropical Studies, and much of the research was done while teaching for them. Supported by NSF grants GB-5206, GB-7805, and GB-7819. The following people have offered useful comments on the topics in the manuscript: H. G. Baker, G. Frankie, P. D. Hurd, D. A. Levin, E. G. Linsley, M. Lloyd, A. E. Michelbacher, C. D. Michener, N. J. Scott, R. W. Thorp, L. L. Wolf. I thank R. L. Dressler for identifying the bees.

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