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Pollen Feeding and Reproductive Biology of *Heliconius* Butterflies

(fat body/nitrogenous compounds/reproductive strategy)

LAWRENCE E. GILBERT

Department of Zoology, University of Texas, Austin, Texas 78712

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ABSTRACT **Butterflies of the neotropical Genus *Heliconius* feed on pollen. This is the first known instance in butterflies of a habit that is well known for other insects. The butterflies remove amino acids and proteins from pollen; this feeding innovation plays a role in the reproductive and population biology of these insects. It is suggested that other animals may use pollen in a similar fashion.**

It is generally thought that adult lepidopterans are unable to assimilate amino acids and protein, and therefore that the nitrogenous compounds of their eggs are derived only from reserves (fat body) laid down as a result of larval feeding (1, 2). Eggs are, therefore, produced at the expense of fat body, and when it disappears no further eggs are produced. Recently, however, a Malayan moth has been described that takes blood meals much as mosquitoes do (3). The present report discusses the evidence for an analogous development in certain members of the neotropical butterfly genus *Heliconius* (Nymphalinae), in which the source of amino acids and proteins for the adult butterfly is pollen rather than blood. Studies performed in the lab, in insectaries, and in the field indicate how the nitrogenous compounds of pollen are removed by heliconius and how they influence the reproductive strategy of these butterflies.

Study areas

Most field observations were made in lower montane wet forest [610 m (2000 ft) elevation] near Arima Pass, Trinidad and in two lowland rain-forest sites in Central America [0–122 m (0–400 ft) elevation]: Barro Colorado Island, Canal Zone, and Osa Peninsula, Costa Rica. Further observation and most experimental work was performed on butterflies living either in outdoor screened insectaries (at the New York Zoological Society's Tropical Research Station at Simla, Arima Valley, Trinidad) or in 6.4 × 4 m (21 × 13 ft) glass greenhouses at Stanford University and The University of Texas, Austin. The greenhouse populations have been maintained as continuously breeding populations for over 2 years, and adult behavior in such enclosures is in most aspects identical to that of wild individuals.

Evidence for pollen feeding

Numerous lines of evidence indicate that pollen is collected for its nutritive value and not picked up as an indirect result of nectar visits.

(1) There is a distinctive pollen-collecting behavior. *Heliconius erato* gathering pollen in the insectary have been observed to remain at a single floret of *Lantana camara* for as long as 10 min, whereas nectar visits typically last no more than 3 sec. During pollen visits, the butterfly repeatedly scrapes its proboscis tip over the anthers with short jerky thrusts and rarely thrusts deep into the corolla for nectar.

(2) Other species of butterfly do not accumulate pollen loads even when visiting those flowers that provide pollen for a number of *Heliconius* species. For example, during a study (December 1969–December 1970) at Arima Pass, Trinidad, W.I., *Heliconius ethilla* frequently collected easily visible pollen loads from *Palicorea crocea* (Rubiaceae), whereas *Parides* spp., the only other common butterfly visitors to these flowers, did not collect pollen. In an insectary at The University of Texas at Austin, *Dryas julia* and *Eueides isabella* (both nonpollen feeding heliconiines), and *Heliconius erato* all visit *Lantana camara* but only *H. erato* builds pollen loads.

Pollen loads similar to that in Fig. 1 have been observed in the following species of *Heliconius: erato, melpomene, charitonia, clysonimus, sara, hecale, ethilla, doris, ismenius, sapho, wallacei, cydno, pachinus,* and *hewitsoni. Eueides,* a genus often lumped with *Heliconius,* does not contain pollen-gathering species. The same is true for the remaining six genera of heliconiines and all other New World groups examined.

(3) There exists an elaborate pollen processing behavior that begins with the formation of a dry mass on the ventral side of the proboscis near the head. Next, a clear liquid (probably nectar) is exuded from the proboscis tip and is mixed with the pollen. Subsequently, the wet pollen load is agitated for several hours through a coiling and uncoiling of the proboscis.

(4) Morphological features of the proboscis correlate with the functions described above. Papillae on the proboscis tip (Fig. 2*A*) probably act as chemo–mechano receptors in addition to functioning together as a pollen brush. Some insects that do not feed on pollen such as *Papilio* and *Danaus* lack these papillae entirely (Fig. 2*C*), while in others (such as numerous nymphaline genera) papillae are oriented differently and are grouped differently so as to serve a different function (L. Gilbert, unpublished observations). Large mechano-receptor hairs near the head may provide feedback concerning the pollen load shape and size (Fig. 2*B*). These hairs are less developed in all species examined that do not feed on pollen. Blood-feeding moths have evolved similar morphological features that correlate with the feeding habit (4).

(5) Experiments with artificial flowers in an insectary at

FIG. 1. *Heliconius ethilla* bearing large (no. 3) pollen load collected in less than 3 min from a single male flower of *Guarania spinulosa* near Arima Pass, Trinidad.

Stanford show that *Heliconius erato* has equal preference for pollen and pollen-sized glass beads. In a choice experiment, *Heliconius* in an insectary were given an array of 25 artificial flowers in sets of 5 containing dry pollen, glass beads, a sucrose extract of pollen, 15% sucrose, and water. A time-lapse video tape recorder allowed 20,000 sec of visit time to be analyzed. On the first day, the length of an average visit in seconds was 40, 40, 21, 34, and 9, while the percentage of total visiting time was 32, 21, 17, 17, and 3 for pollen, glass beads, pollen extract, sucrose, and water, respectively. On subsequent days the butterflies seemed to differentiate between pollen and glass beads, but the latter still received a greater percentage of total visiting time than did the other "flowers" that do not possess pollen. Moreover, loads of glass beads are collected and treated exactly as if they were pollen loads. This eliminates the possibility that butterflies involuntarily pick up pollen because of some chemical mimicry of nectar. Details of these experiments will appear elsewhere.

(6) Experiments by Linskens and coworkers have shown that certain pollens will actively release most of their proteins and free amino acids within minutes of being incubated in sucrose solution (5, 6). During the first minute, about 50% of the free amino acids have been released from living pollen grains when incubated at 25° in 10% sucrose solution. Pollen with a *total* amino-acid pregermination concentration of 2.56 μmol/mg of pollen, released 560 nmol of amino acids into the sucrose solution after 2 hr of incubation. During germination there is a net synthesis of amino acids by the pollen.

Because of this feature of pollen physiology, ingestion and/or enzymatic digestion are not required for the butterfly to be able to remove these compounds. Indeed, when wet pollen loads were removed from insectary *Heliconius* and their proteins were analyzed by electrophoresis, it was found that they contained pollen enzymes only; no butterfly gut enzymes were detected. [Pollen loads were immediately suspended in chilled 15% sucrose solution and centrifuged. Proteins of supernatant were separated by electrophoresis on discontinuous acrylamide gels (EC Apparatus Corp., Philadelphia, Pa., Technical Bulletin no. 140), which were stained for leucine amino peptidase (EC 3.4.1.1) and nonspecific esterases. Likewise crop and gut content of the butterflies were assayed for the same enzymes.]

(7) To see if amino acids ingested by adult *Heliconius* were directly involved in egg production, I ran the following experiment: Each day for 8 days, 15 *H. erato* females were fed a sufficient amount of [^{14}C]aminoacids to approximate the intake of those amino acids in maximum daily pollen loads [under field conditions (1.5 mg)]; assuming a 2-hr incubation period. After 2 hr of incubation, 1 mg of germinating pollen will have released 3.3, 3.3, 4.4, and 6.0 nmol of arginine, leucine, lysine, and valine, respectively (6).

Eggs were collected for 6 days before, and 8 days after the 8-day period during which label was administered. Each

day's egg production was treated with weak chlorox solution to remove accessory gland protein and chlorion, homogenized in scintillation fluid (Aquasol, New England Nuclear Corp.), and counted. The results plotted as average counts per min per egg against days, indicate that uptake of free amino acids into developing eggs is rapid and striking (Fig. 3). This is not unexpected in insects, such as many *Heliconius* species, which produce mature eggs from tiny oocytes in just a few days. For instance, at any given time, each ovariole of *H. erato* contains 6–7 visible oocytes, the largest of which will mature into a large 0.50- to 0.65-mg egg within a day, the smallest of which is about 80 μm in diameter.

It is of interest to point out here that lepidopterans are known to transfer injected foreign protein unchanged from the hemolymph into developing eggs by pinocytosis (7), suggesting that free amino acids would be removed in proportion to their concentration in the hemolymph.

(**8**) In an experiment run in an outdoor insectary in Trinidad, daily egg production by five *Heliconius ethilla* females fed a diet of nectar and pollen from their emergence day (Group I) was compared with that of five females fed nectar only (Group II). Group I averaged 6.25 eggs per day during the first 5 days of egg production. During the first 5 days after ceasing pollen feeding their individual egg productions were 28, 32, 24, 23, and 21 for a total of 128 eggs. Group II (5 insects) was at the same time in another cage without pollen; the egg production during the first 5 days was 3, 5, 4, 4, and 7 for a total of 23 eggs. Thus, the pollen-fed group averaged about 5 times as many eggs per day both during, and for 5 days after, the pollen feeding period than were produced during the first 5 days of egg production by the group that never received pollen.

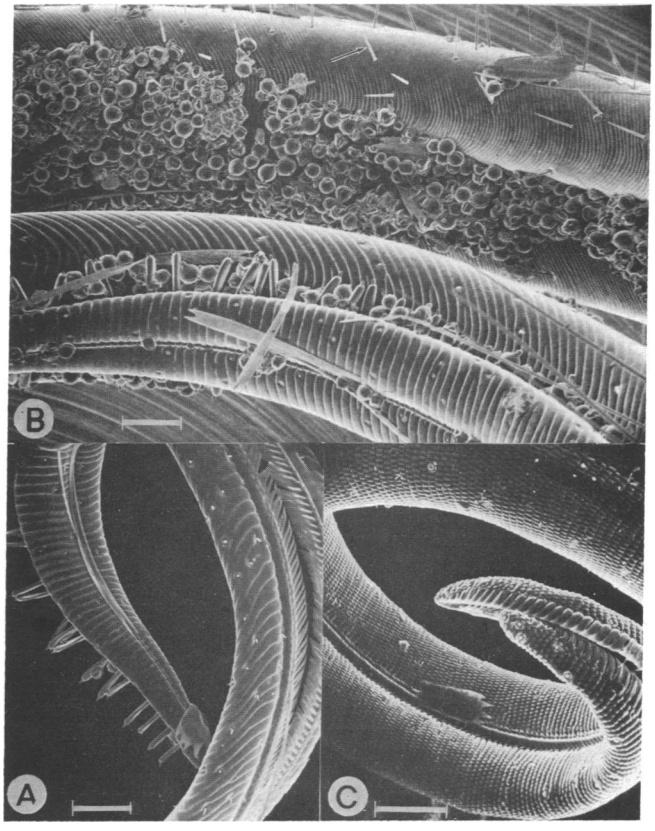

FIG. 2. (*A*) Proboscis tip of *Heliconius ethilla* showing terminal papillae. Scale: 0.1 mm: × 160. (*B*) Pollen load on proboscis of *H. melpomene*. Mechano-receptor hairs are visible near the top of the photograph (*arrow*). Butterfly's head is just off the upper right of the picture. Scale: 0.1 mm: × 165. (*C*) Proboscis tip of *Danaus plexippus* showing absence of papillae. Scale: 0.1 mm: × 200.

Pollen feeding and biology of *Heliconius*

Various aspects of the life history and reproductive biology of pollen-collecting *Heliconius* are consistent with the hypothesis that the nutrients provided by pollen are of major importance to adult maintenance as well as reproductive activity. Evidence from three species indicates that these may possess the longest active adult life spans for butterflies, with individuals often living 6 months both in insectaries (Gilbert, unpublished) and in the wild (ref. 8, Ehrlich and Gilbert, unpublished). The problem of obtaining a regular source of energy for flight may be partly solved first, by abundance of proline, which constitutes about 50% of the free amino acids in pollen (6) [proline is an important energy-storage compound in insect hemolymph and flight muscle (9, 10)], and second, by availability of pollen, which is supplied at a steady rate through the life-span of a *Heliconius* due to special features of the plants that provide the pollen (8).

Pollen feeding also helps to explain the pattern of reproduction seen in many *Heliconius* species. Throughout its long life, the daily egg production of a *Heliconius* female continues at a relatively constant, low rate, and varies from 1–4 eggs (*H. erato, melpomene*) to 7–10 eggs (*H. ethilla*). For example, the number of mature eggs found in a series of insectary *H. erato* was 0, 0, 4, 1, 4, 3, 5, 4, and 4, for females aged 0, 1, 14, 39, 53, 74, 84, 100, and 182 days, respectively. *H. ethilla* females collected from the field that are known (by marking) to be over 4 months old, lay eggs at a rate equal to that of laboratory females less than 20 days old. This pattern is in striking contrast to that for species such as the nymphaline *Euphydryas editha*, which lays several hundred eggs during the first days of adult life and lives no more than 3 weeks in the wild (11). Because of pollen feeding, the total mass of eggs produced by *Heliconius* probably equals and may ex-

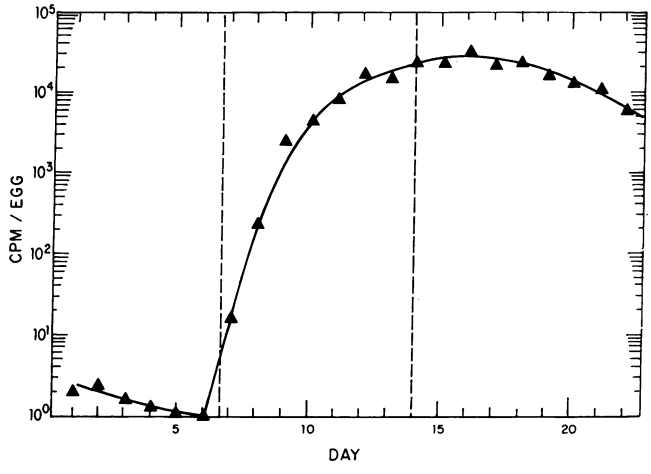

FIG. 3. Incorporation of free [^{14}C]aminoacids into *Heliconius* eggs; cpm per egg are plotted against days. Labeled compounds were administered from late in day 6 to day 14 (*dotted lines*).

ceed that of *Euphydryas*. This is counter to Labine's estimates (11) that were based on inadequate data.

That males, like females, carry on reproductive activity throughout life is evidenced by the successful mating of a wild male *H. ethilla* (no. 438) to a tethered virgin female 120 days after its first capture in the Arima Pass population. On the other hand, the low recruitment rate of virgin females in *Heliconius* populations (8) would not require that a male's daily spermatophore production be the energetic equivalent of a female's egg production for equal Darwinian fitness. This suggestion is supported by the fact that females consistently carry the largest pollen loads (see Fig. 1) in the field, while both sexes gather large loads when enclosed with an abundance of pollen. Data collected over a year (1970) at the Arima Pass *H. ethilla* population show that 95% (N = 43) of all no. 3 loads (in a scale of 0–3) observed were borne by females, while only 34% (N = 794) of the captured insects were females.

DISCUSSION

The pattern of oviposition allowed by pollen feeding maximizes dispersion of offspring in time. This is one important method of escape from pre-imaginal parasites and predators, which are without doubt the major mortality factors for most butterflies, especially tropical species. Moreover, the allocation to adult *Heliconius* of what for most butterflies is typically larval foraging activity (i.e., that fraction of feeding devoted to egg production) would tend to shorten feeding time and lessen larval exposure to predation. This is known to be the case in mosquitoes such as *Aedes atropalpus*. Strains of *A. atropalpus* for which a blood meal is required for egg production spend less time in the larval stage than strains in which adults do not require a blood meal yet lay an equal number of eggs (12, 13). At the same time, however, this extra adult foraging activity would tend to increase adult exposure to predation. Interestingly, for highly distasteful insects like *Heliconius* butterflies (14), conspicuous adult activity is part of the warning signal to vertebrate predators.

Other tropical and temperate butterflies may have increased adult longevity and the adult fraction of total reproductive effort by exploiting other sources of nitrogenous compounds. For example, ithomiine butterflies feed on fresh bird droppings in addition to nectar (15), and marking studies in Trinidad indicate that at least two species (*Ithomia pellucida* and *Hypothyris euclea*) live at least 4 months (L. Gilbert, unpublished). Charaxine and nymphaline species such as *Charaxes*, *Anaea*, *Historis*, *Prepona*, *Adelpha*, *Marpesia*, *Asterocampa*, *Caligo*, etc. are known to feed on rotting fruit, fermenting sap, urine, and dung. It may also be possible that under special circumstances nectar itself contains amino acids, although a systematic check for nitrogenous compounds in nectar has not been performed (16). While these sources of nitrogen in the adult diet might supplement the compounds stored by the larval stage, none of them are likely to supply the highly concentrated source of the 10 amino acids required by insects that are provided by pollen (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). For example, the percentage of free amino acids in bananas (17) is about 1/120 of that found in pollen (6), and numerous important amino acids are missing from the bananas.

The ease with which *Heliconius* can extract nitrogenous compounds from pollen provides a reason for reexamining the means of pollen digestion by other insects such as honey bees and syrphid flies that are known to require pollen in the adult diet for fat body (18) and egg production (19), respectively. Indeed, other nectar-feeding animals such as nectar bats and hummingbirds, which obtain nitrogen through occasional insect feeding, are fully equipped to use pollen much as the butterflies do. This possibility is strengthened by these facts: at least some hummingbirds are known to ingest pollen (R. K. Colwell*, personal communication), and nectar bats are often taken with guts stuffed with pollen.

Because a resource collected by the mobile adult is important in extending the life span as well as sustaining optimal rates of reproduction by *Heliconius*, the amount and distribution of this resource in space and time is an important key to understanding the structure and dynamics of *Heliconius* populations (8) as well as certain aspects of heliconiine community structure and evolutionary diversification. Judging from the observation that pollen feeding is restricted to the single genus *Heliconius*, and from the apparent ubiquity of the habit in the genus, this innovation in adult feeding may have been the decisive step in the divergence of this group from primitive heliconiine stock.

Moreover, the development of pollen feeding in an insect unable to chew or ingest pollen increases the probability that the plants involved will be able to take the first coevolutionary step leading to mutualistic relations with the insect. Some of the plants known to be involved in such relationships with *Heliconius* (L. Gilbert, unpublished), are summarized here: *Anguria triphylla*, *A. umbrosa*, *Gurania spinulosa*, (all Cucurbitaceae), *Palicourea crocea*, *Cephaelis tormentosa*, (both Rubiaceae), Trinidad; *A. warcewicziii*, *A. sp.*, *Gurania levyana*, *Cephaelis tormentosa*, Costa Rica; *A. warcewiczii*, Panama; and numerous related plant species throughout the tropics of the New World. These plants, all of which provide pollen at a limited but steady rate throughout the year, are important factors in making *Heliconius* a year around feature of most neotropical forests; and as these plants increase the predictability and stability of the habitat for the butterfly, the butterfly as pollinating agent, becomes an important and predictable resource for the plants. Details of evidence for mutualism between *Heliconius* and their pollen plants will be reported elsewhere.

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* Department of Zoology, University of California, Berkeley Calif.

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