Ovarian Dynamics in Heliconiine Butterflies: Programmed Senescence versus Eternal Youth

Abstract. New oocytes are generated throughout long lives in butterflies of the genus Heliconius, which as adults feed on amino acids from pollen. In Dryas julia, a related heliconiine that feeds only on nectar and is relatively short-lived, the original oocyte supply is eventually depleted. Such divergent ovarian dynamics in closely related organisms are significant in terms of both their evolutionary basis and their physiological controls.

Reproduction and longevity in Lepidoptera are generally thought to be limited by nitrogenous reserves accumulated during larval feeding and stored in the fat body (1, 2). Gametes, which are largely proteinaceous (2), form at the expense of these reserves, and in female Lepidoptera the fat body is usually depleted as eggs mature (1, 3, 4). Although carbohydrates (and water) are often essential for complete utilization of fat body reserves and full realization of potential fecundity (3, 4), they cannot replace nitrogenous compounds consumed in egg formation. Therefore maximum egg production should correlate with the amount of stored nitrogenous nutrient available at eclosion. In fact, fecundity in several moths is proportional to pupal weight, which is a measure of larval feeding (2). Further, in one butterfly (Euphydryas editha) the maximum number of eggs laid is close to the average number of both immature and mature oocytes present at emergence (5), and hence is evidently determined by then. Older female Lepidoptera of a variety of species, allowed to oviposit throughout life, die with very few potential oocytes left (6–8). Limited nutritional supply may therefore dictate a limited oocyte supply, with both being determined at adult eclosion and simultaneously depleted during adult life.

Adults of the neotropical butterfly genus Heliconius are, however, not constrained by larval reserves, but extract amino acids from pollen, incorporate them rapidly into eggs, and live up to 6 months in nature (9, 10). Because these butterflies exploit a continual and theoretically unlimited source of dietary nitrogen, their oocyte supply may not necessarily be limited or determined by larval feeding. In fact, Gilbert (9) demonstrated that adult intake of amino acids has affected the reproductive pattern in Heliconius, since old females (4 to 6 months) do not differ from much younger individuals in either oviposition or the number of mature eggs present in dissected ovaries. We have extended these initial observations by monitoring both oviposition and ovarian composition as a function of age in individual Heliconius, primarily H. charitonius, and by comparing these parameters with those of individual Dryas julia, a related but short-lived heliconiine that does not collect pollen and hence may be more typical of the order (11). We show that pollen-fed Heliconius continuously produce new oocytes throughout lives of several months while both Dryas and H. charitonius, fed only carbohydrates, mature few if any more oocytes than are present at emergence and die at about 1 month of age with only residual ovaries. Heliconius offers the first case of long-term open-ended oogenesis reported for any lepidopteran and represents a convergence with ovarian dynamics of other insects whose reproduction depends on adult feeding (1, 2, 12).

Females were selected as pupae or as newly eclosed adults from populations originally collected in Costa Rica and Mexico and maintained in greenhouses (11). These were mated and reared individually, in 1.8 by 0.8 by 1 m net cages, with appropriate oviposition plants (vines of the family Passifloraceae) and either with or without flowering pollen plants [Anguria umbrosa (Cucurbitaceae)]. All were provided with 20 percent sucrose solution as artificial nectar (13). At least once a day, eggs were counted and removed, and the approximate amount of pollen collected was recorded. Butterflies were dissected at various ages or at death, the size and condition of the fat body was noted, and ovaries were removed and prepared as Feulgen-stained whole mounts (14).

Oviposition in Heliconius and Dryas begins 3 to 7 days after eclosion (15), and eggs are laid each day. In both genera, daily production of eggs fluctuates con-
siderably and is greatly affected by weather (16). However, the overall pattern differs strikingly between Heliconius and Dryas (Fig. 1). In individual Heliconius provided continuously with pollen, daily egg production fluctuates around a mean (18), which is maintained for at least 2½ months (Fig. 1a). One female H. charithonius laid 450 eggs during her last month of life and was still laying 11 to 12 eggs per day when dissected at 75 days. Another laid more than 700 eggs, having maintained an average of about ten eggs per day until death at 81 days (19). Similar lifetime records have been obtained for individual H. hecale. Since H. charithonius can live more than 3 months in our greenhouses, and up to 4½ months in nature (20), potential lifetime fecundity may regularly approach 1000 eggs per female. This is comparable to estimates made earlier for H. ethilla (10).

Two major oviposition patterns are observed in Dryas, probably reflecting variation in both weather conditions and quality of the host plant. Some females show an early peak of 30 to 50 eggs per day and then decline, while others maintain a rate of 15 to 20 eggs per day for 1 to 2 weeks before tapering off (Fig. 1c). In all, however, production decreases by 3 to 4 weeks, and cessation of oviposition is followed in a day or two by death. Dryas have lived from 15 to 40 days in our cages, laying totals of 150 to 391 eggs. Although less pronounced, this reproductive pattern and abbreviated lifespan resemble those reported for other "typical" Lepidoptera (2–7). Further, similar patterns are produced by both H. charithonius deprived of pollen from emergence and those that collect little or no pollen even though it is available (Fig. 1b). Pollen-deprived H. charithonius have lived from 21 to 38 days, laying from 70 to 330 eggs. Although they lose vigor at around 20 days and soon stop laying eggs, unlike Dryas they can live 5 to 10 days after oviposition ends. Some resume normal oviposition if given pollen at 2 to 3 weeks, but others will not collect it. Hence, in H. charithonius, pollen is essential for both continued oviposition and viability beyond about 1 month (21).

Fat body tissue, representing reserves stored from larval feeding and the site of yolk protein synthesis (1, 2, 22), fills the abdominal cavity of all newly emerged heliconiines. It is progressively depleted in 3 to 4 weeks in Dryas and in H. charithonius deprived of pollen, but is built up again in Heliconius that recover from pollen deprivation. However, in Heliconius provided continuously with pollen, the fat body does not diminish, and in older females collected in nature it is frequently even more extensive than in younger animals.

Ovaries of both Heliconius and Dryas (Fig. 2) conform to details of both ova-
day-old female that has laid only ten eggs (Fig. 2, b and c). Further, ovaries of one
*H. cydno* at 1 month and another at 4½ months are indistinguishable. In *Dryas*,
however, ovaries change dramatically with age. At 2 weeks, after laying some
170 eggs, one *Dryas* had about 280 oocytes remaining in her ovaries (about 45
per ovariole). Another, at 4 weeks and after laying 380 eggs, had only 75 left
(about nine per ovariole) (Fig. 2f) (28). Also, the germaria in *Dryas* ovaries be-
come smaller with age, and mitotic and pregrowth stages, numerous in even the
oldest *Heliconius* ovaries, are rare or absent. Hence generation of new oocytes
in older *Dryas* either slows considerably or stops entirely (29). The ovariole
sheaths become empty and collapsed as each ovariole shortens (Fig. 2f). We have
observed similar ovarian depletion in another non-pollen-feeding heliconiine,
*Agaristis vanilla*, collected in the Austin area, and comparable changes with age
have been described for a variety of other "typical" Lepidoptera (6–8). Finally
parallel though less dramatic change occur in ovaries of *H. charitonius* de-
prived of pollen from emergence (30). Unlike *Dryas*, however, mature eggs ac-
cumulate in the oviducts as oviposition declines, and some of these and many vi-
tellogenic oocytes are resorbed. Ovarian depletion is a normal event in *Dryas*, but
is not in *Heliconius*, and, in the continued absence of pollen, the latter evi-
dently conserves its resources by curtailing oviposition and resorbing oocytes
(16, 17). This correlates with their survival beyond cessation of oviposition
(Fig. 1b).

The single reproductive burst in *Dryas*
is then reflected in a large but finite sup-
ply of both oocytes and fat body re-
erves, while in *Heliconius* continued re-
production is based on an initially smaller
but continuously replenished supply of
both oocytes and nitrogenous nutrient.
However, immediate controls over oogenesis need not differ significantly
between the two species. Since, in *Hel-
iconius*, ovarian input (mitosis and initi-
ation of oocyte growth) evidently bal-
cances with output (terminal vitello-
genesis and ovulation), both could be si-
multaneously controlled by a hemo-
lymph factor such as juvenile hormone
or yolk protein (vitellogenin) (31), which
in turn could be regulated by available
nutrient. In older *Dryas*, depletion of nu-
trient reserves could lead to a decrease
both in the number of new oocytes gen-
erated and in the number of older oocytes
simultaneously accumulating vitelloge-
nin from the hemolymph, which may con-
tain less vitellogenin because of de-
creased synthesis by the fat body. *Hel-
iconius* may be merely extending condi-
tions present in young *Dryas*, and con-
tinual input of amino acids might not
only maintain oogonial mitosis and vitel-
logenesis in the ovaries, and the size and
activity of the fat body, but could also
contribute directly to vigor and longevi-
ty. Since *Heliconius* deprived of pollen
are to a certain extent "phenocopies" of
aging *Dryas*, enriching the diet of the lat-
ter with amino acids might increase their
fecundity and longevity (32). Ovarian de-
cline in *Dryas* then may not be evidence
of programmed tissue senescence but
may merely reflect programmed starva-
tion. In fact, heliconines could well be
an interesting system for studying physi-
ological controls over certain types of
tissue senescence and renewal.

In adults of typical Lepidoptera such
as *Dryas*, which do not exploit a usable
and adequate source of dietary nitrogen,
one would expect natural selection to
lead simultaneously to production of only
as many oocytes as can be matured and
deposited, and to minimization of
any residual postreproductive reserves
(33). Adult longevity would be selected
against, somatic senescence would tend
to parallel reproductive senescence, and
life spans would be determined by the
amount of larval reserve and by optimal
spatial and temporal distribution of off-
spring. However, once heliconines be-
gan collecting pollen, selection would
to favor a return to virtually unlimited
ovarian dynamics, along with an in-
creased life-span (34), since females can
sequester additional resources during
each day of adult life (35). We then pro-

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**Fig. 2.** Feulgen-stained whole mounts of ovaries of heliconiine butterflies. (a to c) *Heliconius charitonius*. (a) At emergence. Only previtellogenic oocytes are present. (b) At 7 days, after laying ten eggs. The spermatophore, the large body below the ovary and to the left, is removed in most preparations. (c) At 75 days, after laying more than 500 eggs. This individual has six ovarioles; such abnormal numbers are rarely but regularly encountered in *Heliconius*. (d to f) *Dryas julia*. (d) At 1 day after eclosion. Vitellogenesis has begun in the largest oocytes. (e) At 5 days, after oviposition has begun. (f) At 28 days, after laying 380 eggs. Thread-like empty ovariole sheaths in this "spent" ovary are barely visible. (The two halves of the ovary were separated in dissection.) Chorionated egg shrink in these preparations, and are especially shriveled in (c), where they appear as darkish masses in the oviducts. All the same magnification; bar in (f) indicates 1 mm.
pose, on the basis of both selective and physiological considerations, that evolution among heliconiine butterflies of the sensory, structural, and behavioral prerequisites for pollen collecting (9) might have quite readily led to the Heliconius pattern of living to a ripe old age (19) while maintaining fully functional "immortal" ovaries.

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References and Notes
1. V. B. Wigglesworth, The Principles of Insect Physiology (Chapman and Hall, London, ed. 7, 1972). Adult Lepidoptera depend on liquid food, and the few species tested lack gut proteases; this lack is related to their predominantly carbolydase diet from nectar or fruit. Some moths have neither functional mouthparts nor gut enzymes.
6. H. E. Ehrlich, Z. Angew. Entomol. 78, 1 (1929); ibid., 18, 57 (1930).
9. L. E. Gilbert, Proc. Natl. Acad. Sci. U.S.A. 69, 1403 (1972). Heliconius adults collect pollen from blossoms of the cucurbit vines Anguria and Garaflas, which bloom year-round in tropical rain forests. They then mix the pollen with liquid (probably nectar), inducing it to pregerminate and release amino acids into solution, which can then be ingested.
11. Unlike most Lepidoptera, Heliconius feeds and reproduces well in greenhouses, and Drays was chosen for comparison because it is similarly adaptable. However, Eueides, another heliconian that does not feed on pollen, would be a more typical lepidopteran for comparison in that it lays more eggs than Drays in a shorter life-span.
13. Nectars exploited by butterflies contain some amino acids [W. B. Watt, P. C. Hoch, S. G. Mills, Oecologia (Berlin) 14, 353 (1974); H. G. Baker and I. Baker, in Coevolution of Animals and Plants, L. E. Gilbert and P. H. Raven, Eds. (Univ. of Texas Press, Austin, 1975), p. 100] and sucrose solution may be an inadequate substitute. However, so far we have noticed no significant difference in longevity or fecundity between Drays reared with sucrose solution alone and those reared with continual access to Anguria or Lantana nectar.
15. Mating normally occurs during emergence in H. charitonia, and is probably limited to the few days of adult life in Drays. Unmated Heliconius lay many fewer eggs than mated females, but whether unmated Drays will oviposit is unknown.
16. Dissections both early and late in the day reveal that ovarioles containing all chorionated eggs each day, unless oviposition is inhibited by overcast or cold weather or lack of oviposition sites. If adverse conditions exist for more than several days, resorption begins in oocytes undergoing vitellogenesis and also occurs in a few stored ovulated eggs (17).
17. Egg resorption in insects is reviewed by W. J. Bell and M. K. Bohm (Biol. Rev. 39, 373 (1955)).
18. Means vary among H. charitonius females from 9 to 18 eggs per day, in direct proportion to adult size (forewing length). Slightly lower rates characterize other Heliconius, as for example: three to five per day in H. erato, five to seven in H. eritha (10), eight to 15 in H. hecale, but among species they are not necessarily correlated with average adult size. We have not yet studied ovipositions of species other than H. charitonius.
19. The cause of death in Heliconius is not known. Many greenhouse butterflies are killed by smoke or by carbon monoxide; some older Heliconius fail to progress properly, although in general the ability to collect pollen increases with age. In nature, their unprotected ovarioles have lower vitellogenesis than their vitellogenic ovarioles; in nature. Young, fit Heliconius females have sound ovary; but sometimes they may have them, which is still not known.
20. In all Heliconius thus far studied, pollen is not essential for egg formation although the onset of oviposition appears to be delayed at least 1 day in H. charitonia without pollen (Fig. 1b). Heliconius charitonia that regularly collect more pollen do not necessarily lay more eggs, and our data are inadequate to determine whether they live longer. However, egg production in this species increases up to approximately that predicted on the basis of forewing length (10) in pollen-deprived females that subsequently are given and collect pollen. Experiments performed earlier showed that Gilbert (11) that in H. eritha and H. erato, both of which encounter pollen more dependably in their natural habitats (deep rain forests), either pollen or added dietary amino acids cause an increase in daily egg production.
23. Divisions would include both those of stem-line oogonia (which are well defined in Diptera but have not been identified with certainty in any Lepidoptera) giving rise to definitive oogonia, and the three subsequent mitoses in incomplete cytokinesis generating each eight-celled oocyte-nurse cell complex (12); also W. H. Telfer, Adv. Insect Physiol. 11, 223 (1975).
24. Other heliconians that do not feed on pollen include Agraulis vanillae with 90 to 100 oocytes per ovariole at emergence and Eueides isabella with 70 to 80. This number may in fact be correlated predictably with the quality of normal adult nutrition, a fact noted by Michener and Styer (13) of about the same size as Heliconius. All Heliconius species examined emerge with fewer than 250 oocytes per ovariole. For example, in Pteronymia, an ithome that feeds on nitrogenous compounds in bird droppings and lives up to 8 months, the ovarioles are initially present in each ovariole. Most butterflies emerge with 45 to 100 or more oocytes per ovariole. The life span of Heliconius is usually not known.
28. Regardless of adult size or age, the sum of eggs laid per day is not constant. The largest of Heliconius and Doris females, ranging from 450 to 500 eggs, which is very close to the average number of oocytes at emergence (480).
29. Oogonial mitosis in young females of Drays and other species (26) may function to replace resorbed eggs (17). Mitosis may also continue in some species if adults obtain optimal nutrition from certain nectars (13, 25), causing egg production to exceed the original number of potential oocytes.
32. M. Norris fed Ephestia albumen without causing increased fecundity or longevity, but since the moths lack gut proteases they could not utilize the protein (13).
33. A postreproductive period has been proposed in warblingly colored and distasteful moths, in which older individuals presumably educate predators to avoid kin [A. D. Blate, Nature (London) 197, 1183 (1963)].
34. However, even Heliconius must eventually become senescent and die (19); see also, for example, P. B. Medawar, The Uniqueness of the Individual (Methuen, London, 1957).
35. Many Lepidoptera can exploit lower quality and less predictable nitrogenous sources than pollen [for example, certain nectars (13), dung and rotting fruit (9)]. Hence in environments such as tropical rain forests that select for reproductive longevity, ovariang dynamics approaching those of Heliconius may be common.
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