short-term effects on benthic organisms (*3*, *26*), oil stranded in marshlands contaminates the sediments and becomes incorporated in the tissues of marine organisms affecting the survival and recovery of marine populations for many years. Recovery from even this small spill of fuel oil at West Falmouth is still incomplete after 7 years.

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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 shorter-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-

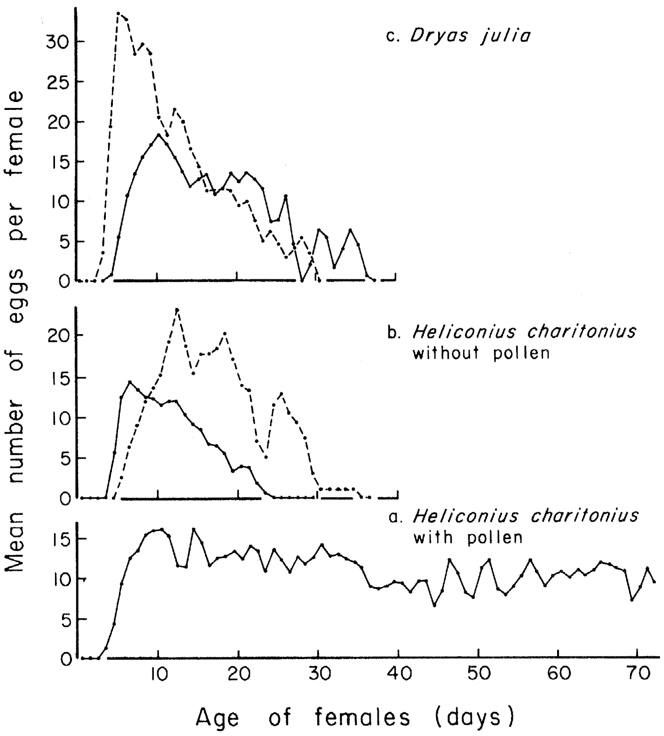

Fig. 1. Daily oviposition patterns in heliconiine butterflies. Each curve averages combined data from several females, with each point representing a 2-day moving average. (a) *Heliconius charitonius* provided with pollen. Records from eight butterflies are included here, but only one was monitored throughout the 74-day period (days 1 to 16, $N = 5$; days 17 to 29, $N = 6$; days 30 to 40, $N = 4$ to 5; day 41, $N = 3$; and days 42 to 74, $N = 2$; forewing lengths, 3.7 to 4.6 cm). (b) *Heliconius charitonius* reared without pollen (solid line: $N = 7$; forewing lengths, 3.9 to 4.8 cm) and means of two large individuals (broken line; forewing length, 4.5 cm) provided with pollen but who would not collect it. (c) *Dryas julia*. The broken line indicates average egg production for two individuals (forewing lengths, 3.9 and 4.0 cm) which showed an early peak in oviposition, while the solid line averages oviposition in seven females (forewing lengths, 4.0 to 4.1 cm) with flatter oviposition patterns.

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. charitonius* 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. charitonius* 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. charitonius* deprived of pollen from emergence and those that collect little or no pollen even though it is available (Fig. 1b). Pollen-deprived *H. charitonius* 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. charitonius*, 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. charitonius* 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 ovarian structure and morphological aspects of oogenesis described for other Lepidoptera (*2, 6, 8, 23*). Eight ovarioles are coiled up in the posterior fat body tissue. Each ovariole consists of a distal germarium in which new oocytes arise, followed proximally by a graded series of enlarging oocytes, all enclosed in a peritoneal sheath. Throughout most of oogenesis each oocyte connects syncytially with seven sibling nurse cells, and the entire oocyte–nurse cell complex is surrounded by a layer of somatic follicle cells. Pregrowth stages between mitosis (*24*) and initiation of growth are indistinct, and only progressively enlarging follicles in single file can be counted accurately. Oogenesis encompasses three major stages: (i) previtellogenesis, primarily a period of nurse cell development, (ii) vitellogenesis, entailing rapid oocyte growth through accumulation of yolk, and eventual nurse cell shrinkage, and (iii) chorion secretion by the follicle cells. Ripe eggs then ovulate from the follicular envelope into one of the eight branches of the oviduct. In any one individual, all ovarioles are similar in both total number of oocytes and in stages represented.

Although adult *H. charitonius* and *Dryas* are of similar size, and their mature eggs are of similar volume, at eclosion the ovaries of the former are notably smaller (Fig. 2, a and d). Each ovariole in a newly emerged *H. charitonius* contains from 13 to 35 oocytes, in rough proportion to adult size. In *Dryas*, however, 55 to 65 oocytes occur in each ovariole at emergence, and even larger numbers characterize emergent ovaries of other heliconiines that do not feed on pollen (*25*). As in many butterflies and some moths (*3–6, 8, 27*), ovaries initially contain only previtellogenic oocytes (Fig. 2, a and d). Vitellogenesis begins at eclosion or soon after, and, in ovipositing females of both species, 20 to 30 percent of all oocytes are vitellogenic or older (Fig. 2, b and e). The same span of stages is represented in both, but more intermediate-sized oocytes occur in *Dryas*. *Dryas* ovaries then start out with more oocytes, and at least for the first week process more simultaneously. There is no evidence yet that the time required for complete development of one oocyte differs significantly between *Dryas* and *Heliconius*.

With increasing age, as long as a female *Heliconius* collects and processes pollen (*9, 19*), her ovaries do not change in either size or composition. Hence there is no evidence of ovarian senescence. The ovaries of a 75-day-old *H. charitonius* that has laid over 500 eggs do not differ significantly from those of a 7-

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 become 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, *Agraulis vanillae*, 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 changes occur in ovaries of *H. charitonius* deprived of pollen from emergence (*30*). Unlike *Dryas*, however, mature eggs accumulate in the oviducts as oviposition declines, and some of these and many vitellogenic 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 evidently 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 supply of both oocytes and fat body reserves, while in *Heliconius* continued reproduction 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 *Heliconius*, ovarian input (mitosis and initiation of oocyte growth) evidently balances with output (terminal vitellogenesis and ovulation), both could be simultaneously controlled by a hemolymph factor such as juvenile hormone or yolk protein (vitellogenin) (*31*), which in turn could be regulated by available nutrient. In older *Dryas*, depletion of nutrient reserves could lead to a decrease both in the number of new oocytes generated and in the number of older oocytes simultaneously accumulating vitellogenin from the hemolymph, which may contain less vitellogenin because of decreased synthesis by the fat body. *Heliconius* may be merely extending conditions present in young *Dryas*, and continual input of amino acids might not only maintain oogonial mitosis and vitellogenesis in the ovaries, and the size and activity of the fat body, but could also contribute directly to vigor and longevity. Since *Heliconius* deprived of pollen are to a certain extent "phenocopies" of aging *Dryas*, enriching the diet of the latter with amino acids might increase their fecundity and longevity (*32*). Ovarian decline in *Dryas* then may not be evidence of programmed tissue senescence but may merely reflect programmed starvation. In fact, heliconiines could well be an interesting system for studying physiological 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 offspring. However, once heliconiines began collecting pollen, selection would then favor a return to virtually unlimited ovarian dynamics, along with an increased life-span (*34*), since females can sequester additional resources during each day of adult life (*35*). We then pro-

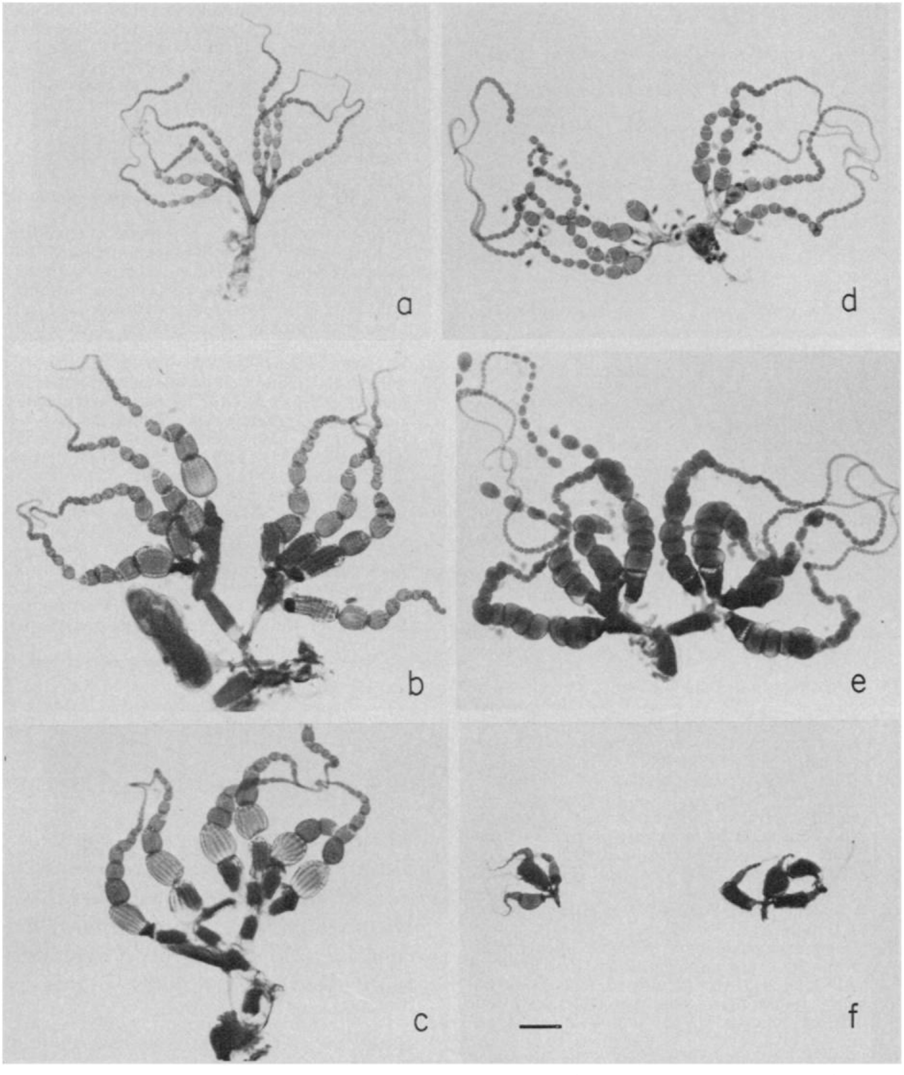

Fig. 2. Feulgen-stained whole mounts of ovaries of heliconiine butterflies. (a to c) *Heliconius charitonius*. (a) At emergence. Only previtellogenic oocytes are present. (b) At 7 days, after laying ten eggs. The spermatophore, the large body below the ovary and to the left, is removed in most preparations. (c) At 75 days, after laying more than 500 eggs. This individual has only 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. Threadlike 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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8. M. J. Norris. *Proc. Zool. Soc. London* **1932**, 595 (1932).
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 *Gurania*, 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.
10. P. R. Ehrlich and L. E. Gilbert, *Biotropica* **5**, 69 (1973).
11. Unlike most Lepidoptera, *Heliconius* feeds and reproduces well in greenhouses, and *Dryas* was chosen for comparison because it is similarly adaptable. However, *Eueides*, another heliconiine that does not feed on pollen, would be a more typical lepidopteran for comparison in that it lays more eggs than *Dryas* in a shorter lifespan.
12. R. C. King, *Ovarian Development in* Drosophila melanogaster (Academic Press, New York, 1970).
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 *Dryas* reared with sucrose solution alone and those reared with continual access to *Anguria* or *Lantana* nectar.
14. R. C. King, R. G. Burnett, N. A. Staley, *Growth* **21**, 230 (1957).
15. Mating normally occurs during emergence in *H. charitonius*, and during the first few days of adult life in *Dryas*. Unmated *Heliconius* lay many fewer eggs than mated females, but whether unmated *Dryas* will oviposit is unknown.
16. Dissections both early and late in the day reveal that *H. charitonius* normally lay all chorionated eggs each day, unless oviposition is inhibited by overcast or cold weather or lack of oviposition sites. If adverse conditions persist for more than several days, resorption begins mainly 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.* **50**, 373 (1975)].
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. ethilla* (*10*), eight to 15 in *H. hecale*, but among species they are not necessarily correlated with average adult size. We have not yet studied oviposition in *Dryas* of variable size.
19. The cause of death in *Heliconius* in nature is not known. Many greenhouse butterflies are killed by spiders. Some older *Heliconius* fail to process pollen properly, although in general the ability to collect pollen increases with age. In nature, their unpalatability probably eliminates vertebrate predation as a source of mortality [L. P. Brower, J. V. Z. Brower, C. T. Collins, *Zoologica (N.Y.)* **48**, 65 (1963); W. W. Benson, *Am. Nat.* **105**, 213 (1971)]. Older individuals have worn wings (*10*) and thinner cuticles, both involving nonrenewable tissues, and may be more susceptible to both invertebrate predation and various forms of lethal damage.

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21. 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. charitonius* without pollen (Fig. 1b). *Heliconius charitonius* 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 (*18*) in pollen-deprived females that subsequently are given and collect pollen. Experiments performed earlier by Gilbert (*9*) show that in *H. ethilla* 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.
22. M. L. Pan, W. J. Bell, W. H. Telfer, *Science* **165**, 393 (1969); W. W. Doane, in *Developmental Systems: Insects*, S. J. Counce and C. H. Waddington, Eds. (Academic Press, New York, 1973), vol. 2.
23. R. C. King and S. K. Aggarwal, *Growth* **29**, 17 (1965).
24. 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 with incomplete cytokinesis generating each eight-celled oocyte–nurse cell complex [(*12*); also W. H. Telfer, *Adv. Insect Physiol.* **11**, 223 (1975)].
25. Other heliconiines 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, at least among butterflies of about the same size as *Heliconius*. All *Heliconius* species examined emerge with fewer than 40 oocytes per ovariole. Furthermore, in *Pteronymia*, an ithomiine that feeds on nitrogenous compounds in bird droppings and lives up to 4 months in nature (*9*), only 30 oocytes are initially present in each ovariole. Most butterflies emerge with 45 to 100 or more oocytes per ovariole (*5, 6, 26*).
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27. M. L. Pan and G. R. Wyatt, *Dev. Biol.* **54**, 127 (1976); K. Endo, *Dev. Growth Differ.* **11**, 297 (1970).
28. Regardless of adult size or age, the sum of eggs laid plus those remaining in the ovary at dissection is remarkably consistent among *Dryas* 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 *Dryas* 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, 35*), causing egg production to exceed the original number of potential oocytes.
30. *Drosophila* females deprived of essential amino acids show similar ovarian changes [J. H. Sang and R. C. King, *J. Exp. Biol.* **38**, 793 (1961)].
31. Hormones can control mitosis [R. Turkington, in *Developmental Aspects of the Cell Cycle*, I. Cameron *et al.*, Eds. (Academic Press, New York, 1971)], and the control of mitosis in imaginal disks by hormones and vitellogenin has been proposed [W. J. Gehring and R. Nöthiger, in *Developmental Systems: Insects*, S. J. Counce and C. H. Waddington, Eds. (Academic Press, New York, 1973), vol. 2, pp. 260–261]. The regulation of vitellogenin synthesis or uptake (or both) by juvenile hormone in Lepidoptera and other insects is well established [(*22, 27*); P. Sroka and L. I. Gilbert, *J. Insect Physiol.* **17**, 2409 (1971); M. M. Nijhout and L. M. Riddiford, *Biol. Bull. (Woods Hole, Mass.)* **146**, 377 (1974)].
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 (*1, 3*).
33. A postreproductive period has been proposed in warningly colored and distasteful moths, in which older individuals presumably educate predators to avoid l→ [A. D. Blest, *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 nitrogen 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, ovarian dynamics approaching those of *Heliconius* may be common.
36. We thank O. P. Breland and A. G. Jacobson for use of laboratory space and microscopic and photographic facilities, F. Davidson for photographic assistance, M. C. Singer for many suggestions and continuing interest in the research, and R. H. Barth, G. Freeman, and especially E. R. Pianka for critical reviews of the manuscript. C.L.B. is supported by an NSF predoctoral fellowship. This research was funded in part by NSF grant GB 4074 X-P to L.E.G.

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Bag Cell Control of Egg Laying in Freely Behaving *Aplysia*

Abstract. *Neuroendocrine (bag cell) control of egg laying was studied in freely behaving* Aplysia. *Surgical lesions showed that bag cells are not necessary for egg laying, although they play a crucial role in its control, and that the pleurovisceral connectives are the afferent pathway to the bag cells. Recording in vivo showed that synchronous bag cell spikes progressively invade the network, leading to prolonged repetitive firing that initiates natural egg laying.*

Since the initial discovery that the bag cells of the marine gastropod *Aplysia* have the morphological characteristics of neurosecretory cells (*1, 2*) and contain a hormone capable of inducing egg laying (*3*), there have been many electrophysiological (*2, 4–9*), biochemical (*10, 11*), and behavioral (*3, 11–13*) studies of this model neuroendocrine system. Bilateral bag cell clusters (about 400 somata each) are located around the pleurovisceral connectives at the rostral margin of the abdominal ganglion, and their neurites extend for a short distance within the connective tissue sheath, which is believed to serve as a neurohemal organ (*1*). Bag cells are normally silent in the isolated ganglion. Brief stimulation of the distal portion of either connective can trigger a synchronous bag cell afterdischarge lasting tens of minutes (*14*), which led to the hypothesis