crease in oviposition time appears to arise from the search for safe sites that are close to new growth without exposing eggs to cannibalism. Although egg-like structures do not completely prevent oviposition, they do reduce oviposition frequency.

Heliconius can have a severe impact on its host plants. Females of several species frequently oviposit on seedlings and small vines in their forest understory habitats (*10*); single larva can totally defoliate individual plants. We routinely observe suppression of flowering and increased incidence of root disease in greenhouse plants exposed to heliconiine defoliation (*15*).

Although we have stressed coevolution, the plant mimicry of this example has also received attention (*16*). The existence of nonmimetic populations of *Passiflora* within some widespread species suggests that the evolution of egg mimicry is an ongoing process (*17*). However, every population in which we observed mimicry was monomorphic for the trait and was used by at least one *Heliconius* species. Under conditions in which a nonmimetic population containing a few mutant individuals is used by one or more cannibalistic *Heliconius* species, the egg-mimic genotypes might eventually replace all nonmimetic individuals on the basis of relative (rather than absolute) protection.

In a complex community containing ten or more species each of *Passiflora* and *Heliconius*, the microevolution of egg mimics by one *Passiflora* could all but eliminate that species from the host range of several coexisting *Heliconius* (*18*). At the same time, a new niche is then available to specialists able to utilize the mimetic species (*19*). *Passiflora* with egg mimics should also excel at expanding into new habitats already infested with *Heliconius* since fake eggs would provide some protection during the vulnerable establishment phase. Thus it appears that egg mimicry represents one way that a coevolutionary step might promote local diversity within a food web (*20*).

We have demonstrated that (i) *Heliconius* females respond to the presence of eggs; (ii) this response has a strong visual basis (*8*), although chemical cues are not altogether excluded; and (iii) the response to egglike structures of *Passiflora* and to real eggs both reduces the probability that eggs will be laid after host discovery and increases the time required to oviposit. Whereas other visual aspects of *Passiflora* that may be related to the defense of *Heliconius* are directed toward making the potential host plants less apparent or less recognizable to the butterflies (*21*), egg mimicry deters oviposition after the host has been discovered (*22*). Our study supports the egg mimicry hypothesis and is an instance of a plant structural trait resulting from coevolution with an insect herbivore.

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9. Original *H. cydno* stocks were collected by L. E. Gilbert at the Organization for Tropical Studies, La Selva station, Costa Rica. *Passiflora oerstedii* and *P. cyanea* stocks originated from populations at La Selva and Arima Pass, Trinidad, respectively. *Heliconius* and *Passiflora* species are maintained in temperature-controlled Lord and Burnham glass houses (5 by 8 m) at the University of Texas.
10. J. Smiley, thesis, University of Texas, Austin (1978).
11. M. D. Rausher has recently described search image formation for leaf shape by the pipe vine swallowtail, *Battus philenor* [*Science* **200**, 1071 (1978)].
12. Tarsal chemoreceptors are present in many other insects [W. H. Calvert, *Ann. Entomol. Soc. Am.* **67**, 853 (1974); C. J. C. Rees, *Entomol. Exp. Appl.* **12**, 565 (1969)].
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14. Time was measured from the butterfly's initial inspection until her abdomen curled and oviposition began. A *t*-test of the difference between two means was used (*ibid.*, pp. 220–223).
15. This statement is based on 10 years' experience by L.E.G. in growing *Heliconius* with *Passiflora*.
16. D. Wiens, in *Evolutionary Biology*, M. K. Hecht, W. C. Steere, B. Wallace, Eds. (Plenum, New York, 1978), vol. 11, p. 365.
17. *Passiflora ariculata* has well-developed egg mimics in Trinidad (modified petiolar nectar glands) but *P. ariculata* in Costa Rica is not mimetic.
18. Selection of oviposition hosts by *Heliconius* may reduce the number of potentially suitable *Passiflora* species used as larval hosts [J. T. Smiley, *Science* **201**, 745 (1978)].
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22. In our trials, butterflies were forced to choose between two potential oviposition hosts, since *Heliconius* butterflies rarely encounter patches of *Passiflora* in nature. M. Rothschild and L. M. Schoonvoven [*Nature (London)* **266**, 352 (1977)] reported that *Pieris brassicae* chose between two hosts with discriminatory behavior similar to that of *Heliconius*. With more potential hosts available, *Pieris* butterflies might spread their eggs less discriminantly throughout the plant population, spreading the risks of overcrowding, cannibalism, predation, and parasitism as P. M. Ives [*Aust. J. Ecol.* **3**, 261 (1978)] proposes.
23. Facilities necessary for this work were provided by grants from the University Research Institute, University of Texas, and NSF grant GB 4074-P to L.E.G. We thank many friends and colleagues for discussion of this work.
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Internal Fertilization in an Oviparous Frog

Abstract. Eleutherodactylus coqui, *an oviparous frog, undergoes internal fertilization. If this mode of fertilization occurs in other species of anurans, interpretations of anuran reproductive strategies based on the assumption of external fertilization must be reviewed.*

Mode of fertilization is a fundamental factor in the evolution of reproductive strategies, in part because it constrains the nature of the parental investment by each sex (*1*). In species with internal fertilization, the lower certainty of genetic relatedness of the male to the offspring (compared to that of the female) has often resulted in the evolution of maternal care systems, along with various mechanisms to ensure paternity. Parental care by males alone has been reported primarily in species with external fertilization (*2*).

Biologists have long assumed that most frogs employ external fertilization, and anuran reproductive strategies have been interpreted in light of this assumption (*3*). The only frog demonstrated to have internal fertilization is *Ascaphus truei*, a unique species in which adult males have a tail used as an intromittent organ. Internal fertilization has been inferred for the few known live-bearing frog species (*4*, *5*) but has never been demonstrated for an oviparous frog. We present evidence that the oviparous frog *Eleutherodactylus coqui* Thomas has internal fertilization.

Eleutherodactylus coqui is a nocturnal, terrestrial-breeding frog of Puerto Rico (*6*). Males call from elevated perches and exhibit parental care by attending eggs from oviposition to hatching. Eggs undergo direct development and hatch as tiny froglets. Males may remain with the froglets for up to 5 days after hatching. Courtship may occur at any time of night. A gravid female approaches a calling male and makes contact. The male

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then leads the female to one or more potential nest sites, usually curled leaves on or off the ground. The female initiates amplexus by backing under the male. Oviposition starts 7 to 10 hours after amplexus begins. During the first 1 to 2 hours of amplexus, the male exhibits heavy abdominal pulsations, while the female remains quiet. After the pulsations cease, both frogs are relatively quiet for 4 to 5 hours. They then assume a second amplectic position, with the female's hind legs on top of the male's hind legs. This pins the male's posterior end firmly against that of the female and against the substrate. Shortly thereafter, the female begins abdominal pulsations which regularly culminate in major body spasms. These continue for up to 2 hours before oviposition begins and probably correspond to ovulation (*7*). Oviposition begins without any visible interruption of the pulsation spasms and continues for 3 to 5 hours.

Seven females were removed from amplexus in natural nest sites from 21 June to 14 July 1980. One had completed ovulation and the other six had laid some eggs (Table 1). Four females were washed thoroughly (to remove any possibility of sperm on the skin), pithed, washed again, and dissected. The ovisac, an enlarged junction of the oviducts just anterior to the cloaca, which holds the ovulated eggs (*5*), was tied above the cloaca and removed intact to a separate area where it was cut open and the eggs were removed. Eggs were placed in a clean container for development. The other three females were washed and placed in clean plastic bags where they laid their remaining eggs. These also were put in clean containers for development. Eggs laid by females before capture were collected and allowed to develop as controls.

Most of the experimental eggs from all seven females developed and hatched normally, as did most of the control eggs (Table 1). In five instances, all experimental eggs developed. In another, four eggs from the most anterior part of the ovisac failed to develop. In another, the female laid 20 eggs and immediately ate them. A syringe stomach pump (*8*) was used to pump out the eggs, and nine subsequently developed. Thus, all seven experimental females had unlaid eggs that were viable.

There are two potential explanations for our results: internal fertilization and gynogenesis. Because sperm could initiate cleavage without actually fertilizing eggs, finding sperm in the ovisac would not determine which explanation is correct. However, a means of inferring the potential for gynogenesis exists, through use of the distinctive pattern polymorphism that the species exhibits.

Table 1. Fate of eggs obtained from seven *E. coqui* females removed from amplexus in the field. Eggs were obtained by oviducal dissection or by placing females in bags where they laid the eggs. Eggs that had been laid before capture were collected and allowed to develop as controls. For each value the denominator is the number of eggs obtained, and the numerator is the number that hatched.

Clutch	Dissected	Laid in bag	Laid before capture (control)
273	25/29		
275		22/22	6/7
4195	13/13		11/11
Exp 6	23/23		8/8
A48		5/5	13/13
A49		9/20	10/10
276	11/11		16/16

Eleutherodactylus coqui displays at least five basic dorsal pattern morphs, each occurring in an all-or-none fashion, as has been described for several species of Jamaican *Eleutherodactylus* (*9*). Morphs can be distinguished through the transparent egg membrane 1 to 2 days before froglets hatch, and are not subject to ontogenetic change (*10*). In 28 instances between January and July 1980, we determined the morphs of both parent frogs, observed the eggs through hatching, and obtained the data on hatchling morphs. In 17 pairs, the parental morphs were alike, and the hatchlings displayed only those parental morphs. In the other 11 pairs, the parental morphs were different. In all 11 cases, both parental morphs and only those morphs were represented in the hatchlings. Therefore, it appears that the male makes a genetic contribution to the offspring, and we tentatively reject the gynogenesis hypothesis.

The occurrence of internal fertilization in *E. coqui* has evolutionary and ecological implications. Except for the atypical *Ascaphus truei, E. coqui* is the first frog for which internal fertilization has been experimentally demonstrated. It is a significant exception to the rule of external fertilization in the order Anura. If other frogs have internal fertilization, the evolution of anuran reproductive strategies must be reexamined, especially for species that exhibit parental care.

One of the 15 species of *Eleutherodactylus* in Puerto Rico, *E. jasperi*, is ovoviviparous (*5, 11*). Internal fertilization in an oviparous frog is an obvious preadaptation for ovoviviparity.

Internal fertilization may be an adaptation for terrestriality, ensuring effective fertilization in a nonaquatic environment. *Eleutherodactylus* is a large neotropical genus (family Leptodactylidae) characterized by terrestrial breeding and direct development. Direct development presumably has been a major factor in the success of the genus as a terrestrial group (*12*). Internal fertilization could be a second such adaptation.

External fertilization can no longer be assumed for terrestrial-breeding anuran species. Mode of fertilization may cut across ecological boundaries and remain consistent within taxa as a fixed ancestral condition, or it may be responsive to environmental selection pressures and vary within phylogenetic lines. Significant patterns may emerge in tropical anurans, which exhibit great taxonomic diversity and a wide spectrum of reproductive modes (*13*).

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Intestinal M Cells: A Pathway for Entry of Reovirus into the Host

Abstract. *Thirty minutes after inoculation of reovirus type 1 into the intestinal lumen of the mouse, viruses were found adhering to the surface of intestinal M cells but not other epithelial cells. Within 1 hour, viruses were seen in the M cell cytoplasm and were associated with mononuclear cells in the intercellular space adjacent to the M cell. These findings suggest that M cells are the site where reovirus penetrates the intestinal epithelium.*

After their ingestion, enteric viruses move rapidly through the upper alimentary tract and into the lumen of the small intestine. Certain viruses, such as the human rotaviruses (the major cause of infantile viral gastroenteritis), infect intestinal epithelial cells and cause local inflammation and diarrhea (*1*). Many other viruses, including enteroviruses and mammalian reoviruses, enter the systemic circulation through the gastrointestinal tract during the early stages of infection but cause few if any local symptoms (*2, 3*). The factors that determine whether a particular virus will cause local infection or will simply "pass through" the gastrointestinal tract are not known. We have been using mammalian reoviruses to study the pathogenesis of systemic viral infections (*4, 5*). Reoviruses are icosahedral viruses consisting of segmented double-stranded RNA surrounded by a double capsid (shell) containing eight polypeptides (*6–8*).

We recently focused on the primary events determining entry of reoviruses from the gastrointestinal tract into the systemic circulation of mice (*5*). In examining the early stages of infection with type 1 reovirus after peroral inoculation, we noted a striking enlargement of Peyer's patches. This was accompanied by the rapid appearance of infectious virus in mesenteric lymph nodes, suggesting transport to this site (*9*). For these reasons we studied the anatomic localization of reovirus early in infection after its introduction directly into the gastrointestinal tract.

We now report that reovirus type 1 spares intestinal epithelial cells with the exception of the microfold or "M" cells, a population of specialized epithelial cells that overlie Peyer's patches (*10, 11*). M cells have been shown to transport macromolecules, such as horseradish peroxidase and ferritin, from the intestinal lumen across the epithelial barrier to the intercellular space, permitting their uptake by mucosal mononuclear cells (*11, 12*). We now present evidence that entry of reovirus type 1 into the intestinal lymphatics and ultimately the systemic circulation is similarly determined by a specific interaction between reovirus and M cells.

We inoculated reovirus type 1 into closed ileal loops of live suckling mice, which develop the most severe disease in response to virus and which were used in our prior studies of viral entry and spread (*5*). The closed-loop model was selected so that the earliest events of viral attachment and entry could be examined and to achieve a high concentration of virus in the lumen (*13*). The experiments were performed prior to closure of the intestine to macromolecular transport, which occurs at about 18 days of age (*14*).

Closed loops of ileum (1 to 2 cm in length) were constructed in ether-anesthetized BALB/c mice (7 to 10 days old) by suture ligation. We placed the distal ligature 1 to 1.5 cm proximal to the ileocecal valve, taking care to leave the mesenteric blood supply intact. Reovirus type 1 was grown in mouse L cells and purified (*7*). In order to facilitate visualization of virus, a large inoculum (5.6×10^8 plaque-forming units in a volume of 0.02 ml) was injected into the loop with a 26-gauge needle. The loops were removed 30 minutes or 1 hour after virus inoculation and processed for electron microscopy (*15*).

Absorptive cells were the predominant lining cell and were easily seen in all sections. Although an exhaustive search was performed, no viruses were detected adhering to or within absorptive cells lining Peyer's patches or absorptive and goblet cells lining adjacent villi. A few free viruses were occasionally found in the lumen, adjacent to microvilli. In contrast, many viruses were readily seen

Fig. 1. Events occurring in a closed ileal loop 30 minutes after inoculation with reovirus type 1. The electron micrograph shows an M cell (*M*) with reovirus type 1 (arrow) adhering to its luminal surface. The M cell is bordered by two absorptive cells (*A*) without adherent viruses and envelops a mononuclear cell (*N*) (×13,400). The intestinal lumen (*L*) is indicated. Inset: higher magnification of M cell surface with adherent virus particles (×38,800).

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