Male Contribution to Egg Production in Butterflies: Evidence for Transfer of Nutrients at Mating

Abstract. *Radiotracer studies on three butterfly species showed that nutrients contributed by males through mating are used by females for egg production and possibly for somatic maintenance.*

Nutrient investment in eggs constitutes a major expenditure of reproductive effort for female insects, with the nutrients involved being derived in various degrees from larval or adult feeding. Little attention has been paid, however, to the idea that female adult nutrition may include substances representing male investment in egg production (*1*). Such investment must, through sexual selection, affect the evolution of differences between the sexes in behavior, ecology, and so forth. Paternal investment includes prey provided to the female by the male in *Bittacus* (Mecoptera) (*2*), male secretions in *Panorpa* (Mecoptera) (*3*), and extruded spermatophores in Orthoptera (*4*). Further, females of several other insect orders, including Neuroptera and Lepidoptera, have long been hypothesized to absorb spermatophores internally, using the lipoprotein structure for egg production and general maintenance (*5*). However, the only published research (*6*) demonstrating the use in vitellogenesis of material transferred from the male to the female at copulation is that of Friedel and Gillot (*7*) for *Melanoplus sanguinippes* (Orthoptera) and of Goss (*8*) for *Lymire edwardsii* (Lepidoptera).

We now report that nutrients provided by the male (probably from the spermatophore) have been incorporated into eggs in three butterfly species with different mating and adult feeding habits. *Danaus plexippus*, *Heliconius hecale*, and *Heliconius erato* (Lepidoptera : Nymphalidae) were studied in radiotracer experiments aimed at determining whether male-derived substances are incorporated into eggs. As far as we know, *D. plexippus* feeds primarily on nectar as an adult, and the females mate as many as eight times (*9*). Adults of both *Heliconius* species collect pollen as well as nectar (*10*). Female *H. hecale* mate about three times on the average (*11*); *H. erato* females are usually mated at emergence (*12*) and average close to one mating.

Slightly different procedures were followed for *D. plexippus* and *H. hecale* as distinct from *H. erato*. In experiments with the first two, only male-derived investment in egg production and female soma was examined with radiotracers, whereas in *H. erato* both male- and female-derived investment was followed, but in egg production only.

The *D. plexippus* and *H. hecale* males were fed a mixture of sucrose with an algal protein hydrolysate (Amersham/Searle) uniformly labeled with ^{14}C, and then mated to virgin females. Eggs were collected at intervals from individual females and assayed for radioactivity (*13*).

One *H. hecale* female (No. 2) was dissected after 27 eggs had been laid, representing 5 days' oviposition. Individual body parts were analyzed for radioactivity.

As fifth-instar larvae, *H. erato* males and females were injected with amino acids (Amersham/Searle) labeled with either ^{14}C or ^{3}H; as adults, they were fed a 1:1 mixture of 20 percent sugar water and radioactive amino acids. All females mated only once. Eggs were collected at intervals from individual females and assayed for radioactivity (*14*).

In all species examined, the first eggs laid by the female contained the radioactive label administered to the male, which indicates rapid incorporation of substances contributed by the male (Fig. 1). Such radioactivity was not due primarily to fertilization by labeled sperm, as dissection of *H. hecale* female No. 2 showed that unfertilized eggs were roughly as radioactive as fertilized eggs (Table 1).

The dissection of *H. hecale* No. 2 further showed that substances from males were distributed throughout the female's body within a week of mating (Table 1). This finding suggests that such substances may contribute not only to vitellogenesis, but also to the female's somatic maintenance, with a possible effect on longevity.

Table 1. Summary of radioactivity in *H. hecale* No. 2.

Body part	Count per minute
Head	25,586
Wings	8,940
Thorax	123,522
Small drop of hemolymph	5,362
Gut and surrounding fat body	29,213
Abdomen walls	101,810
Washed fat body	11,629
Spermatheca and surrounding fat body	19,629
Spermatophore and bursa	111,880
Unfertilized eggs (N = 14)	33,946
Value per egg	2,424
Ovarioles and remaining eggs	7,503
Eggs laid before dissection (N = 13)	37,174
Value per egg	2,860

Second matings by *D. plexippus* and *H. hecale* resulted in a second peak of radiotracer incorporation into eggs, with a form similar to that of the first peak. This result suggests that the dynamics of incorporation do not vary with sequential matings by females.

The experiments with *H. erato* indicated that substances derived from female larval reserves, female adult pollen feeding, and nutrients provided by males all contributed to egg production (Fig. 1, C and D). The data hint at possible trade-offs through time in the rates at which the various nutrient sources available to the female are used. For example, in the case of *H. erato* No. 24, female adult feeding coincided with a surge in radioactivity from the adult female label and a depression in that from male sources. Such trade-offs would make energetic sense, as utilization of newly obtained nutrients saves having to convert them to a form suitable for storage and delays the expense of mobilizing other reserves.

In both *D. plexippus* and *H. hecale*, sequential mating by the same male resulted in a decrease in the amount of radioactivity transferred (Fig. 1). Such a decrease may be due to any combination of a decrease in the male contribution, siphoning off of label into male somatic metabolism, or use of different metabolite pools by the male in sequential matings.

The actual form of this male-derived nutrition is problematic. Observed changes in spermatophore weight and nitrogen content (*15*) do not account for the initial high activity followed by sharp decline observed in many species (Fig. 1). Nutritional inputs other than the spermatophore are thus likely to be involved. The constant increase in activity during the first 2 weeks in *H. erato* (Fig. 1D), however, may reflect the gradual breakdown and use of the spermatophore since it degenerates over a similar period in related *Heliconius* species (*16*).

Thus, material provided by the male, including the spermatophore, can be a major item in the nutrient and energy budgets of each sex within a species. In particular, male-derived nutrients may be more important in those species with little or no adult feeding (or with feeding on substances low in nitrogenous com-

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pounds), which must rely on larval reserves for both reproduction and somatic maintenance. In such species, mating patterns may interact with life history by influencing the timing and amount of nutrients and energy available to each sex. For example, within the genus *Euxoa* (Lepidoptera : Noctuidae), species that emerge with all oocytes mature and live shorter lives as adults have a lower incidence of multiple mating, whereas species that undergo vitellogenesis as adults and live longer have a higher incidence of multiple mating (*17*). Likewise, females of butterflies such as Danaines and Ithomiines with long reproductive lifespans (*18*), but without the quality of adult resources of *Heliconius* (*10*), mate repeatedly (*19*). In both groups, males attract females with pheromones (*20*). Could it be that males are a limited resource for females?

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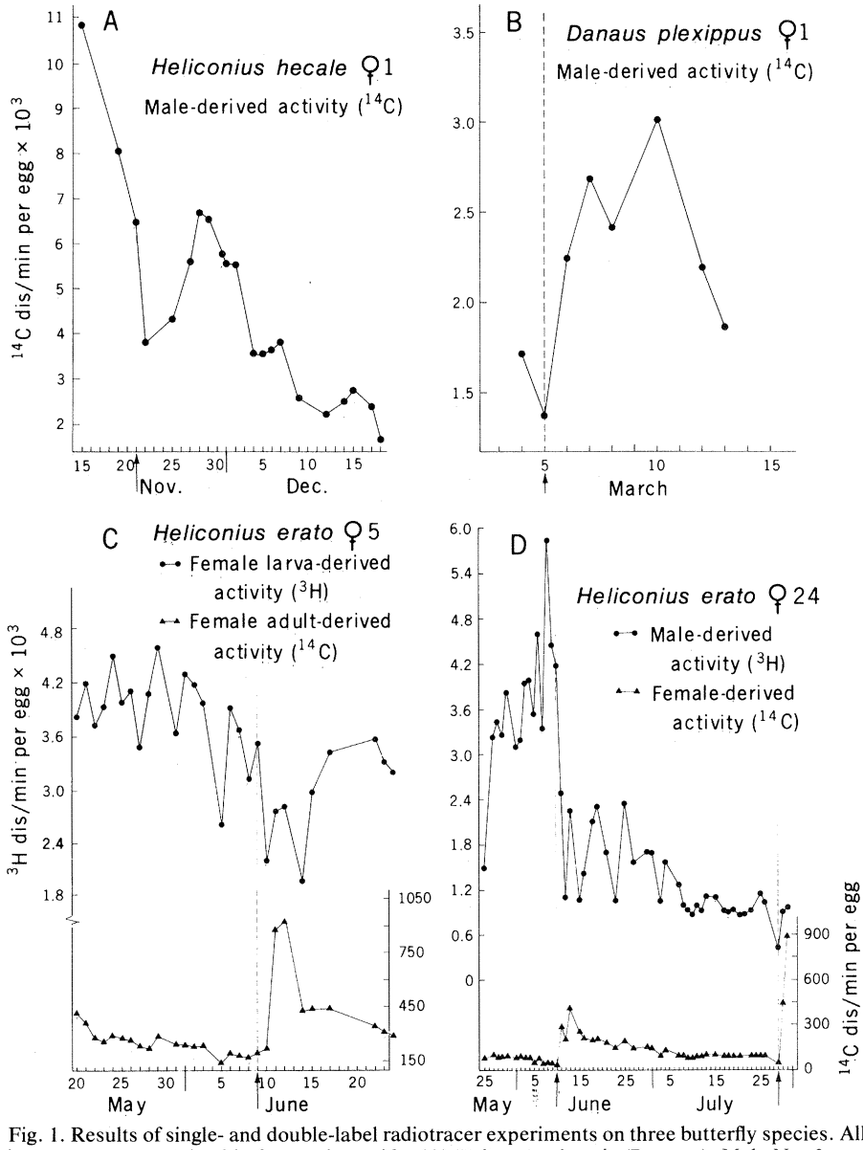

Fig. 1. Results of single- and double-label radiotracer experiments on three butterfly species. All isotopes were contained in free amino acids. (A) *Heliconius hecale* (Panama). Male No. 3 was fed 590 μCi of ^{14}C during the interval between 6 October and 7 November 1973 and was mated with female No. 1 on 12 and 21 November 1973. The peak of activity after the second mating was well below that after the first mating. (B) *Danaus plexippus* (Texas). Male No. 4 was fed 295 μCi of ^{14}C during the interval between 1 January and 8 February 1974 and was mated with female No. 1 on 2 March 1974. Male No. 5 was fed 390 μCi of ^{14}C during the same interval and was mated with female No. 1 on 5 March 1974. The rate of decline in activity (for example, 20 percent per day after first mating) may be attributable to the large number of eggs laid per day (30 to 65) by this species. Activity after the second mating increased rapidly. (C) *Heliconius erato* (Costa Rica). Female No. 5 was injected with 8.5 μCi of ^{3}H as a larva, emerged as an adult on 14 May 1975, and was fed 0.2 μCi of ^{14}C on 9 June 1975. She was mated with male No. 40 (cold) on 16 May 1975. The pulse of ^{14}C activity after the second feeding of ^{14}C to female No. 5 appears to have temporarily depressed larval-derived ^{3}H activity. (D) *Heliconius erato* (Costa Rica). Female No. 24 was injected with 0.075 μCi of ^{14}C as a larva, emerged as an adult on 20 May 1975, and was fed 0.05 μCi of ^{14}C on 9 June 1975. She was mated on 22 May 1975 with male No. 4. Male No. 4 had been injected with 10 μCi of ^{3}H as a larva, emerged as an adult on 14 May 1975, and was fed 20 μCi of ^{3}H on 16 May 1975. Male-derived radioactivity dropped abruptly after the female was fed.

References and Notes

1. Reviewed by R. Thornhill, *Am. Nat.* **110**, 153 (1976).
2. ———, *ibid.*, p. 529.
3. Cited by Thornhill (*1*).
4. R. D. Alexander and D. Otte, *Misc. Publ. Mus. Zool. Univ. Mich.* **133**, 1 (1967).
5. A. Khalifa, *Trans. R. Entomol. Soc. London* **110**, 449 (1949); G. E. Gregory, *ibid.* **117**, 33 (1965); A. Khalifa, *Proc. R. Entomol. Soc. London Ser. A Gen. Entomol.* **25**, 33 (1950); V. Landa, *Acta Soc. Entomol. Cech.* **57**, 297 (1960); O. R. Taylor, Jr., *Ann. Entomol. Soc. Am.* **60**, 583 (1967); R. W. Pease, Jr., *J. Lepid. Soc.* **22**, 197 (1968).
6. Unpublished preliminary work by L. E. Gilbert and S. Sumners, included in this paper, has been described as the first demonstration of male contribution to egg production during mating [(*1*); J. R. Byers, *Can. Entomol.* **110**, 193 (1978)].
7. T. Friedel and C. Gillot, *J. Insect Physiol.* **23**, 145 (1977).
8. G. Goss, thesis, University of Miami (1977).
9. T. Pliske, *Ann Entomol. Soc. Am.* **66**, 164 (1973).
10. L. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1403 (1972); H. Dunlap-Pianka, C. Boggs, L. Gilbert, *Science* **197**, 487 (1977).
11. Unpublished insectary observations by L. E. G.
12. L. Gilbert, *Science* **193**, 419 (1976).
13. The females were housed in 6.5-m by 4-m greenhouses and provided with a 20 percent sugar and water solution, as well as nectar and pollen sources. *H. hecale* No. 1 had access only to sugar water until the 21st day of oviposition, when a pollen and nectar source was introduced. Eggs were crushed manually in NCS tissue solubilizer (Amersham/Searle), allowed to sit overnight before being neutralized with HCl and addition of a toluene-based scintillation fluid. Vials were counted in a Beckman scintillation counter (Beckman LS-250) three times for 10 minutes each. The resulting data (count per minute) were converted to disintegrations per minute per egg according to the known additions method.
14. Arginine-HCl, leucine, proline, and valine were used. Adult butterflies were maintained in 0.8-m by 1-m by 1.8-m net cages within a larger greenhouse. *Lantana* spp. flowers and 20 percent sugar water solution were provided. Eggs were dissolved in 100 μl of tissue solubilizer for 24 hours and then neutralized with 50 μl of glacial acetic acid and addition of a toluene-based scintillation fluid. Vials were then counted as for *D. plexippus* and *H. hecale*, except that conversion to disintegrations per minute was done through the use of a toluene standard efficiency curve.
15. J. D. Rainbolt and C. Boggs, unpublished data.
16. C. Boggs, thesis, University of Texas at Austin (1979).
17. J. R. Byers, *Can. Entomol.* **110**, 193 (1978).
18. Ithomiines and Danaiines live longer than 6 months [L. Gilbert, unpublished data; F. Urquhart and N. Urquhart, *Can. Entomol.* **111**, 15 (1979)].
19. A. Ehrlich and P. Ehrlich, *J. Kans. Entomol. Soc.* **51**, 666 (1978).
20. R. Silberglied, in *How Animals Communicate*, T. A. Sebeok, Ed. (Indiana Univ. Press, Bloomington, 1977), p. 362.
21. We thank S. Sumners, J. Skipper, and H. Clinton for help with the radiotracer work; T. H. Hamilton for the use of facilities and radioisotopes; W. Lee for help with egg collection; and A. Ehrlich for access to unpublished data. W. Watt and two anonymous reviewers provided helpful comments on the manuscript. Supported in part by the University of Texas at Austin grant BSSG RR-07091-06 and NSF grant GB 4074-P to L.E.G. C.L.B. was supported by an NSF predoctoral fellowship.
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