# Population Structure of Pierid Butterflies

## IV. Genetic and Physiological Investment in Offspring by Male *Colias*

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**Summary.** Population structure encompasses all the rules by which a population's gametes come together, including genetic and physiological investment in offspring. We document female use of nutrients donated by males at mating, and complete sperm precedence, in *Colias eurytheme* Boisduval. The effect of these phenomena on the population structure of this species is discussed.

## Introduction

The Wrightian concept of population structure (Wright 1946, 1951) includes all aspects of the rules by which a population's gametes come together. The rules are determined in part by the reproductive strategies of each sex, including the relative timing of mating and dispersal, temporal and spatial patterns of egg laying, degree of sperm precedence, etc. Aspects of these strategies will, of course, coevolve between the sexes.

Each sex "invests" genetically in offspring, by its contribution of gametes. In addition, females obviously invest physiological resources in offspring through the maturation of relatively massive eggs. It is now clear in several insect groups that males also invest physiologically in their offspring, *via* nutrient transfer to females at mating (Goss 1977; Friedel and Gillot 1977; Boggs and Gilbert 1979; Boggs 1981). These basic facts constitute a framework for the coevolution of the sexes' reproductive strategies, which can give rise to diverse effects on population structure. Such effects include:

a) Adult survivorship impacts of nutrient transfer: Males may decrease their own survival prospects, hence prospects of further mating, by transferring nutrients to their female mates, whereas the female recipients' longevity will increase if these nutrients are available for their own maintenance;

b) Adult fecundity impacts of nutrient transfer: Females may produce more eggs, and/or more viable eggs, using male-derived nutrients, and this will increase the fitness of both parents.

The matter is further complicated by the routine occurrence of multiple mating by *both* sexes in wild *Colias* populations (Rothschild 1974; Graham et al. 1980; Rothschild et al., MS in prep.), and the resulting question as to sperm precedence: does it occur at all, partially, or completely? The nature of the answer to this question in any particular system will set off a series of possible coevolutionary-strategic issues: when is it to a female's advantage to mate again? If possible, should males prevent or delay additional matings by their mates, and by what mechanisms might this evolve? and so forth. These may in turn feed back onto the evolution of male nutrient invest-

ment patterns. And, the overall results will affect population structure not only as to the "simple" effects, noted above, on survivorship and fecundity schedules, but also with respect to the reproductive effectiveness of dispersal: what is the remaining reproductive value (Fisher 1958) of a male or female in a new population reached through dispersal? what is the resulting effective pattern of *gamete* dispersal? and so forth.

Here we report on nutrient transfer at mating and sperm precedence in the pierid butterfly *Colias eurytheme* Boisduval. Previous work on this genus has characterized the spatial and temporal distribution, numbers, dispersal behavior, etc., of adults of several species (Watt et al. 1977; Watt et al. 1979; Tabashnik 1980). Aspects of reproductive strategy have also been studied in a variety of species (Rothschild 1974; Graham et al. 1980; Rothschild et al. MS in prep.). The general reproductive strategy of *Colias*, from these sources, is as follows: Courtship behavior occurs throughout adult life. Dispersal history does not affect courtship behavior or oviposition. Females mate more than once, as shown by spermatophore counts from dissected females. Finally, females lay one egg per oviposition bout, and may lay several hundred eggs over their lifespans. The present work further extends our understanding of the population structure in this genus, which is being used as a model system for study of evolutionary mechanisms (eg., Remington 1954; Burns and Johnson 1967; Watt et al. 1974; Watt 1977; Graham et al. 1980).

## Materials and Methods

### Animals

Individual *Colias eurytheme* used in these experiments were obtained from an ongoing laboratory colony at Stanford University. The colony is maintained on vetch (*Vicia* spp.) according to standard procedure and was originally established from insects collected near Tracy, California. Individuals used came from generations 27 through 52 of one sub-colony, and generations 1 through 5 of another sub-colony.

### Sperm Precedence Procedure

Phosphoglucose isomerase (PGI) alleles were used as markers in the sperm precedence study. PGI genotypes of all larvae and their parents were determined using discontinuous polyacrylamide gel electrophoresis run in the manner of Watt (1977), except that BioRad slab gel apparatus was used. PGI pedigrees were known for all colony insects, allowing planned combinations of matings.

Newly emerged females were first mated to several day old males in controlled fashion. Females were then allowed to lay eggs on vetch for two to five days. Second matings of females were done using males whose parents had no PGI alleles in common with the parents of the first male. Females were returned to individual cages containing

vetch and allowed to lay eggs until death. In four cases, the first one to ten eggs laid by a female after a second mating were collected, and, where fertile, raised individually. In all cases, vetch with eggs was removed daily and fresh vetch plants provided to females. Females were fed 25% honey water solution twice daily; all animals were kept on a 16:8 L:D cycle at 25° C. The resulting larvae were raised on vetch to fourth instar, then genotyped. The few deaths occurring between hatch and fourth instar were attributable to bacterial disease, and were random with respect to PGI genotype.

*Nutrient Transfer Procedure*

Male pupae were anaesthetized with $CO_2$ and injected in the thorax with 3 μCi of $^3H$ arginine or leucine. The resulting adults were fed a 25% honey water solution, allowed to age several days and hand paired with virgin non-radioactive females. In some cases, individual males were used in up to three matings; three females were mated a second time with non-radioactive males.

Females were placed in individual cages with vetch as an oviposition plant. This was replaced daily, and eggs were counted, gently pried off the leaves, and, in some cases, weighed. Females were fed a 25% honey water solution twice a day and kept on a 16:8 L:D cycle at 25° C.

To verify broad distribution of male-derived nutrients in females, five females were killed by freezing after one to five days' egg laying. Head, thorax, wings, spermatophore plus bursa plus accessory sac, several chorionated unfertilized eggs, the rest of the ovary and the rest of the abdomen were analyzed separately in the manner described below.

Males, females, female body parts and eggs were all examined for radioactivity using liquid scintillation counting. The object to be analyzed was crushed in a 12 × 75 mm test tube in 0.1 ml of a buffer containing 0.01 M $NH_4Cl$, 0.01 M Tris and 0.02% sodium azide, and added to 10 ml Bray's solution (Bray 1960) in a polyethylene vial. The test tube was rinsed with another 0.1 ml of buffer which was also added to the counting vial. Vials were counted for 10 min each in a Packard model 2450 scintillation counter. Resulting counts per minute were corrected for background radioactivity and for quenching using a standard quench curve.

## Results

*Sperm Precedence*

In all five females examined, sperm precedence was complete (Table 1). That is, the first male did not father any offspring after a second mating by the female.

The first eggs collected immediately after a second mating were infertile for three of four females (Table 1). The one exception involved a female which was mated in the afternoon and instead of being placed on vetch at 25° C immediately, was placed in a 20° C incubator overnight and placed on vetch at 25° C
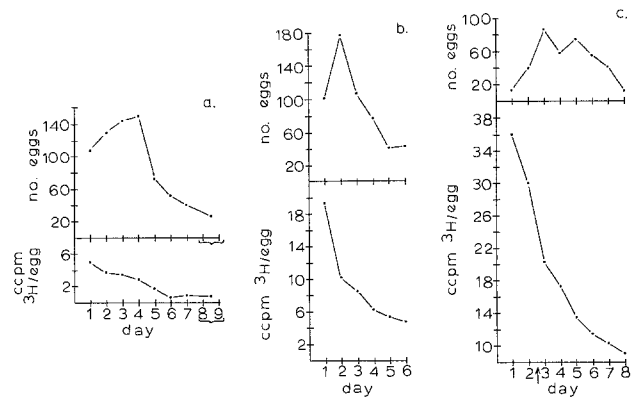


**Fig. 1a–c.** Corrected counts per minute per egg and number eggs laid per day versus time for female *Colias eurytheme* mated to males injected as pupae with labelled amino acid: **a** female # 25, mated when five days old to male # 16, injected with $^3H$ leucine; **b** female # 68, mated when one day old to male # 64, injected with $^3H$ arginine; **c** female # 58, mated on eclosion day to male # 53, injected with $^3H$ arginine. The arrow indicates time of second mating by the female to an unlabelled male

the next morning. In this case, the first egg laid was already red, indicating that it had been fertilized 12 to 24 h previous to oviposition. The second and third eggs laid were pale cream colored as usual for newly fertilized eggs, and were fathered by the second male. The first egg failed to hatch; however, given the fact that it was so far advanced developmentally when laid, it must have been fertilized by the first male's sperm prior to the second mating. The second and third, as well as subsequent, eggs matured and hatched normally.

*Transfer of Nutrients*

Radiotracer analysis of twenty-one females and their eggs showed that male derived compounds are used in egg production and are distributed throughout the female's body, apparently aiding in somatic maintenance (Fig. 1; Table 2). Chorionated unfertilized eggs dissected out of ovaries in five females were as radioactive as oviposited eggs (Table 2), showing that the radioactivity did *not* derive primarily from sperm, or from "contamination" with male accessory gland fluids which might be residually present near the spermatheca.

In general corrected counts per minute per egg fell with time, as did total corrected counts per minute for eggs laid each day (Fig. 1). However, some fluctuation was present, which may have been due to temporary nutritional stress on females.

**Table 1.** Documentation of sperm precedence in *Colias eurytheme* using phosphoglucose isomerase as a parental marker. In each case, the first male to mate with the female had fathered eggs laid prior to the second mating

| PGI genotype | | | # days eggs laid before 2nd mating | # days eggs laid after 2nd mating | Offspring PGI genotypes post 2nd mating | Comments |
|---|---|---|---|---|---|---|
| ♀ | 1st ♂ | 2nd ♂ | | | | |
| 5/5 | 5/5 | 4/4 | 3 | 3 | 5 4/5 | |
| 4/5 | 4/4 | 3/3 | 2 | 3 | 52 3/5:46 3/4 | 1st egg laid after 2nd mating red; hatch failure |
| 5/5 | 3/3 | 5/5 | 3 | 4 | 28 5/5 | 1st egg laid after 2nd mating infertile |
| 4/4 | 4/4 | 5/5 | 3 | 7 | 91 4/5 | 1st 11 eggs laid after 2nd mating infertile |
| 4/4 | 4/4 | 3/5 | 2 | 5 | 48 4/5:32 3/4 | 1st 6 eggs laid after 2nd mating infertile |

**Table 2.** Corrected counts per minute for $^3H$ transferred to female *Colias eurytheme* at mating by males which had received $^3H$-leucine or -arginine as pupae

| # | Head | Wings | Thorax | Spermatophore plus bursa | Rest of abdomen | Oviposited eggs (ccpm/egg) (N) | Unfertilized eggs (ccpm/egg) (N) | Male label |
|---|---|---|---|---|---|---|---|---|
| 43 | 68 | 19 | 351 | 2,447 | 521 | 4.8 (485) | 5.6 (10) | leucine |
| 44 | 113 | 21 | 1,131 | 1,531 | 1,276 | 5.1 (62) | 4.8 (25) | leucine |
| 45 | 50 | 28 | 355 | 727 | 593 | 1.5 (355) | 1.9 (20) | leucine |
| 46 | 336 | 83 | 3,076 | 7,698 | 4,380 | 10.0 (43) | 16.7 (20) | arginine |
| 48 | 190 | 81 | 1,863 | 5,015 | 1,262 | 10.5 (320) | 22.0 (5) | arginine |
| 18 | 12 | 0 | 291 | 12 | 654 | 2.8 (472) | | leucine |
| 19 | 0 | 102 | 103 | 1,121 | 314 | 3.2 (568) | | leucine |
| 20 | 69 | 26 | 749 | 1,781 | 1,652 | 2.9 (261) | | leucine |
| 21 | 25 | 0 | 153 | 1,438 | 357 | 3.6 (774) | | leucine |
| 22 | 36 | 64 | 546 | 1,943 | 1,199 | 2.8 (664) | | leucine |
| 23 | 22 | 0 | 203 | 1,257 | 615 | 3.7 (644) | | leucine |
| 24 | 14 | 43 | 428 | 369 | 497 | 1.2 (66) | | leucine |
| 25 | 0 | 0 | 109 | 2,340 | 284 | 3.1 (726) | | leucine |
| 49 | 33 | 0 | 179 | 1,217 | 329 | 1.0 (137) | | leucine |
| 58 | 333 | 33 | 1,783 | 17,412 | 2,543 | 17.5 (382) | | arginine |
| 66 | 210 | 87 | 1,342 | 11,541 | 2,489 | 16.1 (281) | | arginine |
| 67 | 115 | 42 | 964 | 12,605 | 1,294 | 24.1 (810) | | arginine |
| 68 | 102 | 11 | 357 | 8,790 | 1,333 | 10.3 (552) | | arginine |
| 69 | 374 | 27 | 586 | 1,552 | 697 | 5.1 (667) | | arginine |
| 70 | 79 | 58 | 476 | 3,257 | 976 | 6.2 (468) | | arginine |
| 71 | 62 | 27 | 435 | 2,487 | 757 | 7.0 (638) | | arginine |
| 72 | 113 | 17 | 502 | 3,870 | 1,940 | 7.7 (248) | | arginine |
| 73 | 63 | 25 | 237 | 2,954 | 877 | 7.4 (526) | | arginine |

**Table 3.** Total corrected counts per minute $^3H$ transferred to the female by males mating more than once

| Male # | Total ccpm transferred to female | | |
|---|---|---|---|
| | 1st mating | 2nd mating | 3rd mating |
| 38 | 16,339 | 11,889 | |
| 62 | 20,041 | 6,386 | |
| 63 | 34,297 | 8,106 | 8,345 |
| 64 | 16,231 | 7,653 | 7,928 |

Similarly, number of eggs laid per day by a given female rose to a peak on about the second or third day after onset of oviposition and then fell irregularly with time (Fig. 1). However, there was no significant correlation between number of eggs laid per day and corrected counts per minute per egg in a given female. There was also no significant correlation between daily egg weight and time or corrected counts per minute per egg in a given female.

Second mating by females had no observed effect on the pattern of incorporation into eggs of radioactivity derived from the first mate (Fig. 1). Males with more than one mating, however, showed a two to four fold decrease between the first and subsequent matings in total radioactivity transferred to females (Table 3).

Finally, label derived from arginine treated males was transferred more readily than label derived from leucine treated males. Females mated with males injected with $^3H$ arginine showed two to ten times higher corrected counts per minute per egg than females mated with males injected with $^3H$ leucine.

Analysis of radioactivity present in females at death (Table 2) showed the same pattern, indicating that the difference was due to a differential transfer from the male rather than a differential incorporation into eggs.

## Discussion

Although the data presented here are from laboratory colonies of *Colias*, our results are directly relevant to wild populations. First, the time course of oviposition and numbers of eggs produced in our study correspond to reproductive patterns found in the wild (Rothschild 1974; Rothschild et al. MS in prep.). Thus, our observations on nutrient incorporation into eggs were made in a natural egg production context. Second, as pointed out earlier, multiple mating occurs routinely in the field (Graham et al. 1980; Rothschild 1974; Rothschild et al. MS in prep.), such that issues related to sperm precedence are of real significance to the animals' biology.

Our analysis of female use of male-derived compounds has concentrated on amino acid/protein resources because of the particular methods used. Marshall (pers. commun., MS) has shown that other compounds besides proteins, most notably hydrocarbons, vanish from the spermatophore with time in *Colias eurytheme* (and *C. philodice* Latreille). Presumably these compounds are also utilized by females. However, nitrogenous resources may be of critical importance to *Colias*, since only low levels of such compounds are available in the adult diet (Watt et al. 1974). In this context, the result that arginine treated males transferred more label to females than did leucine treated males gains added interest: arginine is much more nitrogen rich than leucine, suggesting that males may be using relatively "expensive" resources in the manufacture of the spermatophore. This result further suggests that the spermatophore may contain compounds of "higher than average" value to the female. We also note that Stern and Smith (1960) report normal or near-normal oviposition by females mated once, then fed only sugar solutions thereafter – suggesting that at least the majority of nitrogen invested in eggs derives either from male-supplied materials or the female's larval-derived storage reserves.

Transfer of nutrients from males to females, as found in

this study, has very different implications for male as opposed to female survivorship, and hence population structure. Males with large numbers of matings should have depleted reserves compared to relatively inexperienced males, particularly since each spermatophore represents several per cent of a male's total weight on a wet weight basis (Marshall, pers. commun.). In times of nutritional stress, such as decreasing nectar resource availability due to drought, increasing butterfly population size, termination of the flowering season, etc., survivorship of males already having large numbers of matings should be lower than of males with fewer matings. Conversely, since mating represents nutritional intake for females, females with a large number of matings should be expected to have a higher survivorship, particularly in periods of adult nutritional stress, than females with only one mating.

Similarly, transfer of nutrients from males to females may affect fecundity, especially if normal adult food resources are restricted. Some female Lepidoptera are known to resorb eggs during periods of nutritional stress (Bell and Bohm 1975; Dunlap-Pianka et al. 1977). During periods of restricted nectar supply, male derived nutrients may be especially critical in maintaining normal egg production levels, affecting both male and female fitness. In such a case, under adult nutritional stress, females with a large number of matings would be expected to show fewer signs of egg resorption in their ovaries and higher egg outputs than their counterparts with a smaller number of matings.

Such effects of male nutrient donation on survivorship and fecundity should be most readily discernable under extreme environmental conditions. Such conditions are not uncommon on an evolutionary time scale. For example, as a result of a drought in 1971, *all* nectar resources for *Colias* in the areas around the Rocky Mountain Biological Laboratory dried up during the adult flight season (Watt et al. 1974, 1977). Such episodes of catastrophic selection may be as important as steady selection pressure in developing and maintaining male nutrient donation at mating.

Male nutrient donations to females may also interact with female nutrient budgets to affect fecundity under normal environmental conditions. For example, most species of *Colias* exhibit a sex-limited polymorphism in wing color controlled by a single locus with two alleles (Gerould 1911, 1923; Lorkovicz and Herman 1961; Remington 1954). Females carrying the dominant allele *A* ("alba" phenotype) have white wings, whereas homzygous recessive *aa* females exhibit species-specific yellow, orange or red wings like conspecific males. "Alba"'s wing color results from a shift of pteridine wing pigment precursors to other uses, such that only colorless pigments occur in the wings (Descimon 1966; Watt 1973). Graham et al. (1980) found that reallocation of resources by "alba" females is associated with more rapid pupal development, larger fat bodies at adult eclosion, and, under cool conditions, more rapid initial egg maturation. However, "alba" females are also less attractive to males, and contain significantly fewer spermatophores at any given age, compared to *aa* females. The more frequent male contribution of nutrients to *aa* females may play an important role in "balancing" this wing color polymorphism, by compensating in part for the greater level of nutrients carried over by "alba" females from their own larval feeding.

Donation of nutrients by male *Colias* at mating will interact with the occurrence of complete sperm precedence to affect mating strategies of both sexes. Females' re-mating strategies may be affected in different ways. Females should be receptive to males when levels of compounds derived from male donated nutrients fall. In such cases, females may be most receptive to males likely to donate large amounts of resources. Some pierid females may be able to discriminate among males in this respect. Rutowski (1979) has shown in *Pieris protodice* Boisduval and Leconte that males' courtship persistence is directly related to the size of spermatophore they are capable of giving to the female at any given mating. That is, males who have just completed a mating donate smaller spermatophores than males who have not mated recently. The latter group of males showed more persistent courtship than the former.

Females may in some cases incur a disadvantage from re-mating. The results of this study suggest that if egg laying resumes immediately after a mating, the first few eggs laid by a female are infertile. However, if the female is sufficiently nutritionally stressed, the nutritional advantages of an immediate mating may outweigh the disadvantage of a few infertile eggs.

The interaction between complete sperm precedence and nutritional aspects of the spermatophore poses different pressures for males. In general, it is to the male's advantage to donate large amounts of nutrients to his mate, increasing both their fitnesses. However, with complete sperm precedence, a subsequent mating by the female would result in the first male's nutrient investment being spent on offspring fathered by another male. Thus, it should be to a male's advantage to prevent subsequent matings by his mate while eggs using "his" nutrients are being manufactured.

Previous studies on Lepidoptera (Labine 1964; Boggs 1981) have suggested that the spermatophore has a role in preventing or delaying future mating by a female in some species. In particular, the bursa may contain a stretch receptor measuring the mass of spermatophore(s) present; female receptivity to mating is thus inhibited by a large mass in the bursa. If such is true in *Colias*, it would be to the male's advantage to make a large spermatophore. Our findings that the amount of label transferred to females declines with subsequent matings suggests that male *Colias* make smaller spermatophores on subsequent matings, as they do in *Papilio zelicaon* Lucas (Papilionidae) (Sims 1979) and *Dryas julia* Fabr. (Nymphalidae) (Boggs 1981). Possible consequences of this require further study.

Both degree of sperm precedence and transfer of nutrients at mating interact with dispersal patterns to affect population structure. Since courtship occurs throughout the adult flight season, a dispersing male has a finite likelihood of achieving additional matings. Complete sperm precedence means that these later matings will result in effective male gamete dispersal. Likewise, a dispersing female may re-mate, and her subsequent eggs will be fathered by a male of her new locality.

Further, multiple mating by individuals of either sex may increase their reproductive value, through sperm precedence effects for males and nutrient acquisition for females. Such increases should occur even for individuals dispersing late in the flight season, although numbers of eggs left to be laid by females are smaller and spermatophore sizes are smaller.

These results emphasize the necessary interconnections of bioenergetic ecology with demographic, formal properties features of population structure. Such a view has also been stressed by Pianka (1976). Further study of *Colias* and other insect systems will yield fuller understanding of these interconnections.

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