

## Differences in lifetime reproductive output and mating frequency of two female morphs of the sulfur butterfly, *Colias erate* (Lepidoptera: Pieridae)

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**Abstract.** Both female morphs of the sulfur butterfly *Colias erate* were captured in the field and dissected to investigate whether differences of reproductive output are affected by mating frequency between them. Lifetime mating frequency of the yellow morph was significantly lower than that of alba. In every female the immature egg load decreased with age. During their life span the monandrous yellow morph laid about 550 eggs, with the monandrous alba producing about 400 eggs. Polyandrous alba laid more eggs than either the monandrous or polyandrous yellow morphs. Thus, multiple mating is important for alba to increase its reproductive output, supporting the field observation that alba effectively attracts males.

### INTRODUCTION

Some butterfly species show wing color polymorphism, such as *Papilio polytes* (Watanabe 1979, Uesugi 1992) and *Lycaena phlaeas* (Brakefield & Shreeve 1992). Komai and Ae (1953) reported that the Japanese sulfur butterfly, *Colias erate*, exhibits a sex-limited wing color dimorphism in females with yellow (ancestral) and white (alba) morphs. Both morphs occur sympatrically although the yellow morph is never more abundant than alba (e.g., Watanabe & Nakanishi 1996), unlike most American *Colias* species. Gilchrist and Rutowski (1986) explained the adaptive significance of alba from the viewpoint of reproductive success.

Emmel (1972) hypothesized that female-limited dimorphism is balanced by differential mate selection by males. Ley and Watt (1989) studied female limited dimorphism and concluded that the dimorphism is balanced by differential predation on the morphs. The persistence of the two morphs in time, however, suggests that they are equally fit. Other studies on *C. erate* (e.g., Watanabe et al. 1997) demonstrated a higher frequency of mate attraction by the alba morph which would give them a fitness advantage. The latter observation predicts that the yellow morph must have a compensatory advantage beyond mating that equalizes their lifetime fitness. Mating behavior of female morphs has been reported for some *Colias* species in America (e.g., Gilchrist & Rutowski 1986, Graham et al. 1980). Our study focuses on the lifetime reproductive success of the two morphs.

Recent studies have shown that female butterflies may engage in polyan-



dry to obtain sperm and/or nutrients that are ejaculated from males (e.g., Boggs & Gilbert 1979). When a mated female accepts subsequent males the last male's sperm has precedence (e.g., Watanabe 1988). In *C. erate* Watanabe & Nakanishi (1996) pointed out that females are polyandrous. However, there is no report on the lifetime reproductive success of the two morphs of this species from the viewpoint of female polyandry. In this paper, we examine how frequently females in the field mated during their life span, and then estimated the fecundity for each morph.

## MATERIALS AND METHODS

The data were obtained mainly from the summer generations of *C. erate* in Shirouma of Nagano Prefecture, which is located in a cool temperate zone of Japan. The details of the study area have been described elsewhere (e.g., Watanabe & Nakanishi 1996). The habitat consisted of rice fields and five ski slopes where nectar sources and larval food plants were abundant.

We collected females engaged in various activities including feeding, roosting, flying, copulating and ovipositing on calm sunny days from late July through mid-August of 1989 to 1994 ( $n=34$  days). When females were captured, their abdomens were amputated and immersed in 50% ethyl alcohol. Forewing length of each was also recorded. The age of each female was estimated mainly by wing wear condition, and rated among 1 to 5 age classes (Watanabe & Nakanishi 1996).

Thirty seven *C. erate* larvae (mainly 3rd to 5th instar) were collected on a ski slope during late June 1993. They were reared on clover at 25 °C in the laboratory (16L/8D). All pupated and eclosed; 22 out of 37 were female. Immediately after emergence, their abdomens were amputated and immersed in 50% ethyl alcohol. All abdomens were dissected and examined for male spermatophores in the bursa copulatrix. Eggs in the ovaries were also counted and classified into three groups (mature, submature and immature), as has been done with the other pierid butterflies (Ando & Watanabe 1992, 1993, Watanabe & Ando 1993, 1994). Most statistical comparisons were done with a Mann-Whitney U-test, except for the Kendall test on mating frequency in relation to age.

## RESULTS

### Females immediately after emergence

No diseased individuals or parasitic wasps were noted in the 37 field captured larvae. Among the 22 females of the 37 emergences, the size was not significantly different for the two morphs (Table 1). Females of both morphs carried more than 750 immature eggs and 40 submature eggs. No mature eggs were found in either morph. Therefore, if no more immature eggs were added during her life span, as in *Pieris rapae* (Watanabe & Ando 1993), the fecundity of *C. erate* females would be about 800 for either morphs. Since adult size and fecundity are largely dependent upon the quality and quantity of food during the larval stage, we assume that each larva had consumed similar quantities of food.

Of 474 albas captured in the field, 5 were virgin, while we found no virgin yellow morphs. Such virgin albas were considered freshly eclosed. Table



Table 1. Fecundity and size for two morphs of female *C. erate* at emergence reared in the laboratory, comparing those of field-captured alba having no spermatophore ( $\pm$ SD).

	Yellow	Alba	Field-captured virgin alba
Number of females	6	16	5
Forewing length (mm)	29.8 $\pm$ 1.52	30.0 $\pm$ 1.34	29.4 $\pm$ 1.33
Number of immature eggs	794.2 $\pm$ 93.57	766.8 $\pm$ 41.87	688.8 $\pm$ 188.82
Number of submature eggs	40.3 $\pm$ 6.11	37.8 $\pm$ 8.66	99.2 $\pm$ 78.32
Number of mature eggs	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	3.8 $\pm$ 5.02
Total number of eggs	821.0 $\pm$ 209.22	795.6 $\pm$ 178.55	791.8 $\pm$ 253.11

Table 2. Forewing length for two morphs of female *C. erate* captured in the field (mm  $\pm$  SD).

	Age class	Alba	Yellow	Mann-Whitney U-test
Monandrous	I	29.5 $\pm$ 0.97(139)	29.4 $\pm$ 1.16(43)	U=96.5, n.s.
	II	29.7 $\pm$ 1.59(79)	29.1 $\pm$ 1.31(25)	U=86.0, n.s.
	III	29.4 $\pm$ 1.94(48)	29.0 $\pm$ 1.69(19)	U=16.0, n.s.
	IV	30.0 $\pm$ 1.35(9)	29.1 $\pm$ 2.86(4)	U=7.0, n.s.
	V	30.2 (1)	28.8 $\pm$ 0.77(3)	U=11.0, n.s.
Polyandrous	I	29.6 $\pm$ 1.81(19)	30.3 $\pm$ 1.44(5)	U=9.0, n.s.
	II	29.5 $\pm$ 1.38(31)	29.7 $\pm$ 0.96(12)	U=43.5, n.s.
	III	30.5 $\pm$ 1.31(55)	29.9 $\pm$ 1.49(30)	U=142.0, n.s.
	IV	29.6 $\pm$ 1.51(48)	30.3 $\pm$ 0.48(14)	U=18.0, n.s.
	V	30.2 $\pm$ 1.12(17)	29.6 $\pm$ 0.10(5)	U=4.0, n.s.

( ):Sample size



Table 3. Frequency distributions of the number of spermatophores in the bursa copulatrix of females in two morphs of *C. erate* captured in the field.

Morph	Number of Spermatophores	Age class				
		I	II	III	IV	V
Alba	0	5	0	0	0	0
	1	143	79	48	10	1
	2	22	28	51	34	13
	3	0	3	6	15	2
	4	0	0	0	3	2
	Total	170	110	105	62	18
Yellow	Mean	1.13	1.30	1.60	2.05 <sup>a</sup>	2.27 <sup>b</sup>
	0	0	0	0	0	0
	1	43	28	21	5	3
	2	5	12	29	13	7
	3	0	0	1	2	0
	4	0	0	0	0	0
	Total	48	37	49	21	10
	Mean	1.10	1.32	1.63	1.80 <sup>a</sup>	1.70 <sup>b</sup>

Mann-Whitney U-test, a:  $U=818.0$ ,  $P=0.05$ ; b:  $U=126.0$ ,  $P=0.03$

1 gives their fecundity. The number of immature and submature eggs per female was not significantly different from reared females, although wild females carried a few mature eggs.

Table 2 gives forewing length of field captured specimens. The difference between morphs was not significant in each age class and body size was not correlated with their age class. Watanabe and Nakanishi (1996) showed that the population structure of this species was similar for each year in the same study area.

### Spermatophores in the bursa copulatrix of field-captured females

We dissected 474 alba and 166 yellow morphs in this study. No seasonal effect on mating frequency was found, as in *P. rapae* (Watanabe & Ando 1993). Table 3 shows that the youngest alba (age class I) had a single spermatophore in the bursa copulatrix, while 5 were virgin, and 14 had been polyandrous. The average number of matings was 1.1 for age class 1. The number of matings for alba increased with age (Kendall Test,  $\tau=1.000$ ,  $P<0.01$ ). The average number of matings in alba exceeded 2. Although the number of matings for the yellow morph increased with age (Kendall Test,  $\tau=0.800$ ,  $P<0.05$ ), their mating frequency was significantly lower than for alba by age class 4 ( $U=818.0$ ,  $P=0.05$ ) and age class 5 ( $U=126.0$ ,  $P=0.03$ ). Therefore, the yellow morph mated less than alba over their life (less than 2).

Figure 1 shows the change in the number of immature eggs with mating frequency. Every female carried a decreased load of immature eggs within



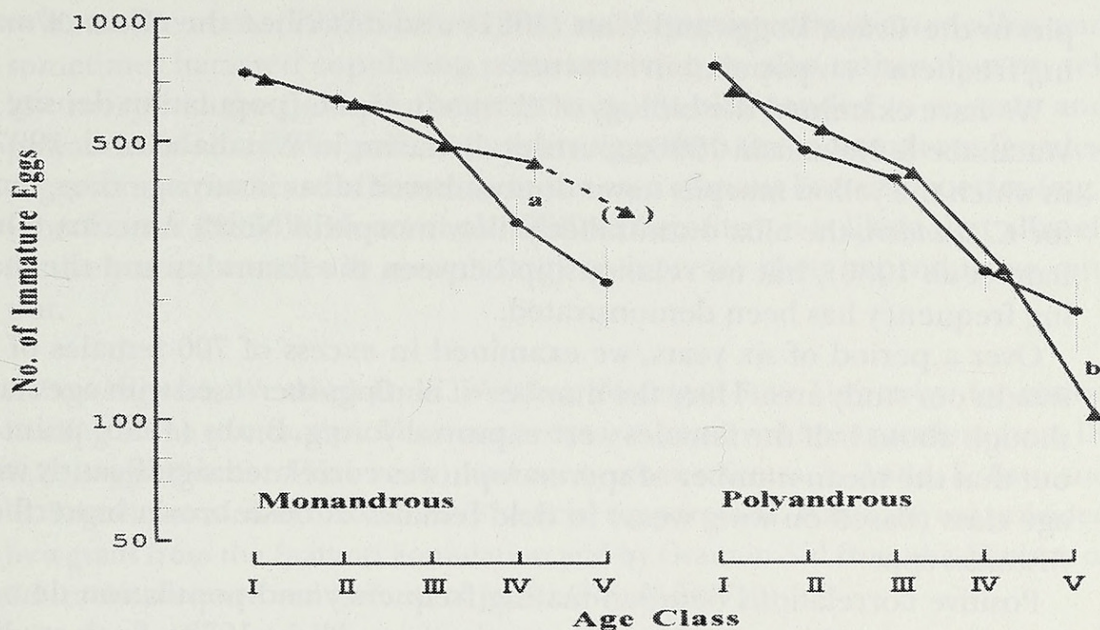


Fig. 1 Changes in the number of immature eggs of respective age (I, II, III, IV, and V) in relation to mating frequency in wild females of *C. erate*. Circles and triangles indicate the data for yellow morph and alba, respectively. Each bar represents SE. a and b are the results from Mann-Whitney U-test for  $P=0.04$  ( $U=10.0$ ) and  $P=0.03$  ( $U=58.0$ ) respectively. Parentheses show one sample.

its age class. We never observed fused eggs in the ovaries, suggesting that eggs were not consumed for somatic maintenance. If no immature eggs were added during adult stage, the decreasing number of immature eggs assumed due to oviposition.

For monandrous females, there was a significant difference in number of immature eggs carried by age class 4 among alba and yellow morphs ( $U=10.0$ ,  $P=0.04$ ). Because virgin females revealed about 750 immature eggs, about 550 eggs were laid by yellow morph and about 400 eggs by alba during their life span. Thus when monandrous, a yellow morph female lays more eggs than an alba morph.

In polyandrous females, alba lays more eggs than the yellow morph ( $U=58.0$ ,  $P=0.03$ , in the age class 5), with alba laying about 650 eggs and the yellow morph laying about 550 eggs, a similar number to the monandrous yellow morph.

## DISCUSSION

*Colias* species are widely used for studies of butterfly biology (e.g., fecundity in Stern & Smith 1960; thermoregulation in Watt 1968, 1973), including reports showing that pteridine or nitrogen pigments on the wings are important for their reproductive success. Watt et al. (1989) showed that population structure of *Colias* species co-existing with another pierid butterfly, *Pieris napi*, influenced the frequency distribution of wing color mor-



phs in the *Colias*. Boggs and Watt (1981) also described the effect of mating frequency on population structure.

We have examined the biology of *C. erate* in Japan (population density in Watanabe & Nakanishi 1996; courtship behavior in Watanabe et al. 1997), in which the yellow morphs never outnumbered albas in any age class. Only for *C. scudderi*, the alba outnumber yellow morph in North America (Graham et al. 1980), but no relationship between the fecundity and the mating frequency has been demonstrated.

Over a period of six years, we examined in excess of 700 females of *C. erate* in our study area. Here the number of matings increased with age class, though about half the females were captured young. Braby (1996) pointed out that the mean number of spermatophores correlated significantly with age class (based on wing wear) in field females of bush brown butterflies, *Mycalesis* spp.

Positive correlations between mating frequency and population density have been noted in some butterfly species (e.g., Pliske 1973). Such studies suggest that at high density competition among males for females becomes more intense and the number of matings increases. Although Watanabe and Ando (1993) showed for *P. rapae* that the number of active males searching for mates differed between years, male density did not affect the number of matings by females. Thus females must exhibit mate choice (Rutowski 1978). For *C. erate* alba, the increasing tendency in the number of matings with age class was similar for *P. rapae*, while the yellow morphs were apt not to re-mate at older ages (age class 3, 4, and 5) than albas.

There were a relatively few mature eggs and a small number of submature eggs in the ovaries of young virgin alba, in which fecundity was estimated at about 800. Although we have no data on the fecundity of virgin yellow morphs from the field, laboratory populations indicated that females of both morphs have similar fecundities. The immature eggs loaded in virgin females of *C. erate* was the highest among other pierid butterflies inhabiting the study area, *P. rapae* (Watanabe & Ando 1993) and *P. melete* (Ando & Watanabe 1993).

Watanabe and Ando (1994) pointed out that monandrous females of *P. rapae* laid fewer eggs than polyandrous females. Multiple spermatophores have been shown to increase female reproductive output in some butterfly species (e.g., Watanabe 1988, Wiklund et al. 1993). However, in the yellow morph of *C. erate* females, mating frequency did not correlate with the number of eggs laid during their life span. In other words, the yellow morph does not need the extra spermatophores for oviposition, suggesting that one mating provides a female with enough sperm to fertilize all of the eggs (e.g., Suzuki 1978), and that spermatophores are not available energy for egg production (e.g., Svård & Wiklund 1988). In fact, we observed small but intact shaped single spermatophores in older yellow morphs. Since the yellow morph might be mimetic to males to avoid further matings (unpublished data), they may have evolved an increased reproductive output without multiple matings.



Watanabe et al. (1997) showed that males persistently courted albas and sometimes harassed copulating pairs involving an alba rather than an yellow morph in the field. In the present study, alba tended to re-mate and polyandrous alba laid significantly more eggs than the monandrous females, suggesting an increase of fitness as in the case of many butterfly species (e.g., Oberhauser 1989, Wiklund et al. 1993). Therefore, it is likely that alba effectively attract males in morphology to increase their reproductive output.

*Acknowledgments.* We thank Dr W. Wehling (Michigan State University) for critical reading of the manuscript. The manuscript was improved by suggestions from Dr C.L. Boggs (Stanford University) and anonymous referees. S. Ando, K. Sato and M. Taguchi assisted in field survey. Financial support to M. Watanabe was provided by a grant from the Inamori Foundation and by Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 05640710).

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Nakanishi, Yasuyuki, Watanabe, Mamoru, and Ito, Takahiko. 2000.  
"Differences in lifetime reproductive output and mating frequency of two female morphs of the sulfur butterfly, *Colias erate* (Lepidoptera: Pieridae)." *The Journal of Research on the Lepidoptera* 35, 1–8.  
<https://doi.org/10.5962/p.266567>.

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