

THE EFFECT OF FEMALE MATING FREQUENCY ON EGG FERTILITY IN THE BLACK SWALLOWTAIL, *PAPILIO POLYXENES ASTERIUS* (PAPILIONIDAE)

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ABSTRACT. Most black swallowtail females mate more than once to replace a sperm supply that has deteriorated with time, although some do so to replace a deficient spermatophore. Fecundities and oviposition rates correlated significantly with weights of females at emergence. Courtship flights were brief (\bar{x} = 42.4 sec) and copulations averaged 44.8 min. Average mating frequency increased as females became older, with an overall mean of 1.30. Although there was no relationship between the number of eggs oviposited and the fertility of those eggs, the average fertility of eggs decreased significantly 10 days after the female mated. Older monogamous females had lower fertilities than young monogamous or older multiple-mated females. The short lifespan of most butterflies results in a mating mode of one.

Multiple mating has been detected in all subfamilies of butterflies and in several skippers (Burns, 1968; Pliske, 1973; Ehrlich & Ehrlich, 1978) and appears to be the norm in many species. Sperm precedence, reviewed by Parker (1970), clearly indicates the advantage of multiple mating for males. In most Lepidoptera, the last male to mate with a female sires the majority of her subsequent offspring (Clarke & Shepard, 1962; Ae, 1962; Labine, 1966). The adaptive advantage of multiple mating for females that absorb the spermatophore (Taylor, 1967) may be the acquisition of nutrients such as nitrogen (Boggs & Gilbert, 1979). However, an advantage for females where the spermatophore persists other than nutrient acquisition (Boggs & Watt, 1981) has not been demonstrated.

This study was designed to evaluate three of the most common hypotheses for multiple mating in female butterflies. Females may mate more often than they need to rather than refuse or evade a persistent male (Alcock et al., 1977). There may be too few spermatozoa in one insemination to fertilize all of the eggs that a female will lay (Ehrlich & Ehrlich, 1978). Sperm may deteriorate with age and must be replaced with a fresh supply (Labine, 1966). Females of the black swallowtail, *Papilio polyxenes asterius* Stoll were studied, since preliminary dissections indicated that spermatophores persisted throughout the lives of females, and a portion of field-collected females carried more than one spermatophore. In evaluating these hypotheses, I determined the ability of females to reject copulation attempts and estimated the costs involved in additional copulations. The fertility

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of eggs was determined relative to the ovipositional sequence and to the period since mating. I also compared the hatchabilities of eggs from fresh and worn females that had mated once or more than once.

MATERIALS AND METHODS

Field observations of courtships and copulations were made near Ithaca, New York, often using marked individuals. Females dissected to determine mating frequency were collected from field sites at different times during each brood. Most females were rated for wing condition when collected (Lederhouse, 1978). The females in the experimental groups were reared in the laboratory from eggs laid by field-collected females. Newly-emerged females were hand-paired with males that were at least two days old (Clarke & Sheppard, 1956). Females were paired once with males copulating for the first time. Some males were paired on successive days to additional females not in the experimental group. All pairing was done at 22°C in a controlled chamber.

To determine fecundity and fertility, females were placed in individual cloth-covered cages (0.3 m × 0.3 m × 0.6 m) with potted cultivated carrot plants. The cages were in a bioclimatic chamber with a 16 h light and 8 h dark photoperiod. Daytime temperature was 22°C and the night time temperature was 15°C. Each female was fed twice daily with a 50% honey-water solution, and her eggs were counted daily. When about 50 eggs had accumulated on a plant, it was removed from the cage and replaced with a fresh plant. The plants were kept in the bioclimatic chamber until all eggs had hatched or until 10 days had elapsed since the last egg had hatched. Larvae were counted and collected daily. Ten days after the last larva hatched, unhatched eggs were counted, and their stage of development was recorded. The experiment continued for each female until she died. Each female was dissected to determine the presence of a spermatophore. Females that lacked a spermatophore, oviposited for less than 5 days, or laid fewer than 50 eggs were excluded from the analysis of fecundity.

In *Papilio polyxenes*, a brown ring appears in the upper half of the yellow egg as development proceeds (Ae, 1979). When the embryo is fully formed, the egg turns black. Thus, eggs that showed no signs of development were classified as infertile. Eggs that developed partially but failed to hatch were classified as inviable. Egg fertility and hatchability were calculated only for those females that laid 25 or more eggs of which some hatched.

RESULTS

The total number of eggs laid (fecundity) correlated significantly with the initial weight of black swallowtail females after emergence

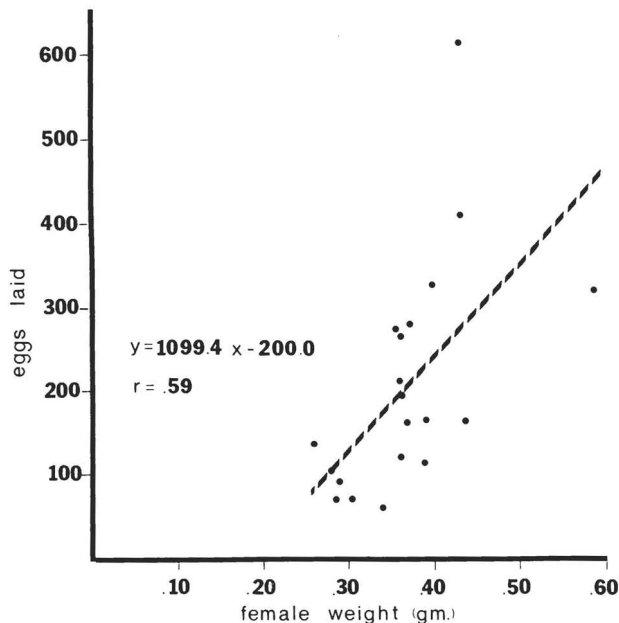


FIG. 1. The relationship between female weight and egg production under laboratory conditions.

(Fig. 1). The mean period between mating and the start of oviposition for 33 females was 3.5 ± 2.5 days. Freshly emerged females had few mature ova (12.9 ± 3.4 , $n = 7$) when dissected during the first day after emergence. Females that were not paired until their second or third adult day had a shorter period between mating and oviposition. The mean number of eggs laid by females meeting the sampling criteria was 205.9 ± 136.9 under the chamber conditions. The oviposition rate correlated significantly with female weight (Fig. 2). The total number of eggs also correlated significantly with the length of the oviposition period (Fig. 3). However, the relationship between the length of the oviposition period and female weight was not significant ($r = 0.25$, $P > 0.05$).

Courtship flights that terminated in copulation were brief (Table 1). The female flew a short distance (19.8 ± 18.4 m, $n = 16$) after the male started to court. If receptive, she would land on a perch, and the male would land nearby and initiate copulation. The female had to land for copulation to take place. However, if the female was not receptive, she would attempt to evade the courting male. This resulting in mating-refusal flights of significantly longer duration (Table 1, Mann-Whitney U Test, $P < 0.001$). Usually the female would fly

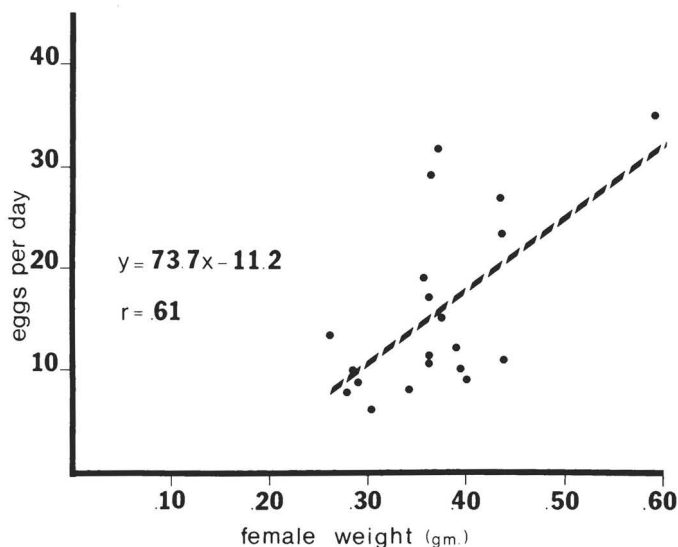


FIG. 2. The relationship between female weight and oviposition rate under laboratory conditions.

high into the air (>10 m) and dive rapidly if followed. This pattern was repeated until the male gave up or was evaded.

The mean duration for copulations in the field was significantly shorter than the mean of hand-paired copulations in the laboratory (Table 1, Mann-Whitney U Test, $P < 0.01$). Males that mated frequently had much longer copulation durations. The second pairings within 24 h of laboratory males were significantly longer (Mann-Whitney U Test, $P < 0.04$). Likewise, the second copulations of field males that mated twice on the same day were longer than the average duration of field copulations (Mann-Whitney U Test, $P < 0.01$).

The reliability of spermatophore counts in estimating frequency of mating of black swallowtail females was confirmed by dissecting 84 females that had been hand-paired a single time. Of these, 79 contained one spermatophore, and the remaining five had none. Single matings never resulted in the passage of more than one spermatophore (Sims, 1979). Females that lived longer under laboratory conditions (>35 days) than field females could be expected to live contained spermatophores that were easily detected. The spermatophore shells were still thick and tough, yet the spermatophore itself was usually collapsed. Although some pairings did not result in the passage of a spermatophore, counts did accurately measure the frequency

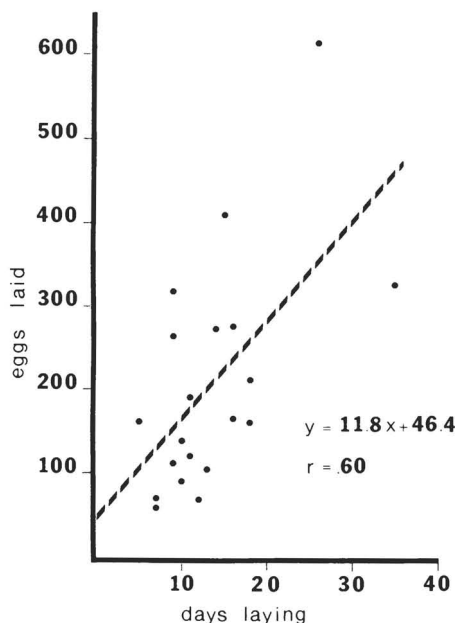


FIG. 3. The relationship between laying period and egg production under laboratory conditions.

of insemination. Although no pair-bond is formed, females that have mated once have been termed monogamous (Wiklund, 1977b). Females that mate more than once would therefore be polyandrous.

The average width for 26 *Papilio polyxenes* spermatophores from single hand-pairings in this study was 1.92 ± 0.32 mm, which is almost identical to values reported by Sims (1979) for the closely related *P. zelicaon* Lucas. Using Sims' method of calculation and assuming the same sperm density, an average value of 800,000 sperm per copulation was calculated for 2–5 day old males. Even if storage is low (20%, Lefevre & Jonsson, 1962), there would be about 160,000 sperm to fertilize no more than 600 to 800 eggs or about 200 to 1. However, the first copulation of some females results in few or no viable sperm being transferred. Some females containing small spermatophores laid no fertile eggs.

Females that had been collected from the field for a variety of purposes were pooled to determine an average mating frequency. The mean number of spermatophores contained by 171 females was 1.30 ± 0.54 . Only 2.3% of the females were virgin, and three individ-

TABLE 1. The durations of courtships and copulations.

Interaction	n	Mean	S.D.
Courtship leading to copulation	33	42.4 sec	40.5
Mating refusal flight	22	99.8 sec	74.4
Copulation (field)	21	44.8 min	12.3
Copulation (laboratory)	22	51.3 min	8.6
Second copulation on same day (field)	3	256.2 min	93.1
Second copulation within 24 h (laboratory)	7	78.5 min	30.0

uals had mated three times (Table 2). First and second brood females yielded nearly identical mean frequencies (Table 3). The mean of third brood females was somewhat lower but not significantly different from those of the first and second broods. Means of yearly samples from the second brood were quite consistent for three years (Table 3).

Females in condition classes showing greater wear had significantly higher mean numbers of spermatophores (Table 2). When fresh or slightly worn, females contained two spermatophores, usually one or both were smaller than average. In most cases, the position of the smaller spermatophore indicated that it had been deposited first.

To determine whether older females mated again to prevent a drop in fertility related to the number of eggs already laid or to the period of time since the last mating, the influence of the expected correlation between these two factors had to be removed. Since the rate of oviposition was positively correlated with adult weight, these alternative factors were distinguished by determining the fertility and hatchability of eggs laid by females of different initial weights that had been hand-paired once. Sequential samples of about 50 eggs were ranked according to fertility and hatchability for each female. The results for 10 females, each with four samples, and for five females with five samples were nearly random (Friedman two-way analysis of

TABLE 2. Female mating frequencies related to wing condition. *z* and *P* values are for a one-tailed Mann-Whitney *U* Test.

Condition class	Number of spermatophores					<i>z</i>	<i>P</i>
	0	1	2	3	Mean		
Fresh	4	36	1	0	0.93	3.63	<0.001
Slightly worn	0	37	13	0	1.26		
Intermediate	0	16	14	0	1.47		
Very worn	0	6	12	3	1.86		
Undetermined	0	20	9	0	1.31	2.16	<0.016
Total	4	115	49	3	1.30		

TABLE 3. Female mating frequencies related to brood and year.

Brood	Year	Percent of females in each spermatophore class					Mean
		n	0	1	2	3	
First		41	2.4	68.3	24.4	4.9	1.32
Second		109	2.8	63.3	33.0	0.9	1.32
Third		21	0.0	85.7	14.3	0.0	1.14
Second	1974	22	4.5	59.1	36.4	0.0	1.32
Second	1975	42	2.4	61.9	35.7	0.0	1.33
Second	1976	45	2.2	66.7	28.9	2.2	1.31

variance, Siegel, 1956). Fertility and hatchability were independent of the number of eggs that had been laid previously.

Although the fertility and egg hatchability of singly-paired females did not decrease with the number of eggs they laid, they did decrease with the time since mating. The egg hatchabilities of 16 of 18 individuals (88.9%) were fairly constant through the first 10 days after mating. Samples from 11 females that had oviposited until at least 15 days after mating were divided into those laid less than 8 days, those laid 8 to 14 days, and those laid more than 14 days after mating. When these categories were ranked by fertility and hatchability, a significant decrease was related to the length of the period since mating in both (Table 4, Friedman two-way analysis of variance, χ^2_r for fertility = 7.8, df = 2, $P < 0.05$, χ^2_r for hatchability = 8.9, $P < 0.05$). However, the percent of fertile but inviable eggs showed no relationship with the duration since mating. One female was hand-paired a second time 16 days after her first mating. She showed an increase in hatchability of her eggs and a decrease in the percent of infertile eggs.

The relationship between the period between mating and oviposition and the fertility of eggs was further investigated with field-

TABLE 4. The fertility and viability of eggs laid during the first, second, and third weeks after mating by females hand-paired once. The top value is the mean and the lower value is the standard deviation (n = 11).

Egg class	Days laid after mating		
	1-7	8-14	15+
Infertile	8.4	17.3	31.8
	8.3	16.5	32.8
Inviable	10.7	12.9	9.5
	8.5	8.6	9.5
Hatched	80.9	69.8	58.7
	11.8	19.7	32.1

collected females. Since the exact period between mating and oviposition could not be determined, it was estimated from the condition of the female at the time of capture. The hatchability of eggs laid by 23 females was recorded. The females were grouped by condition and the number of spermatophores they contained. Slightly worn and intermediate classes were pooled. Fresh monogamous females had significantly higher egg hatchabilities than slightly worn and intermediate females that had mated once ($98.8 \pm 1.0\%$ vs. $81.7 \pm 23.9\%$, $U = 8$, $P < 0.05$). Worn females that had mated more than once had significantly higher egg hatchabilities ($98.1 \pm 1.8\%$) than monogamous females of slightly worn and intermediate condition ($U = 13$, $P < 0.05$). However, fresh monogamous females did not differ from worn polyandrous females. The egg hatchabilities of all field-captured females with one spermatophore did not differ from singly-paired laboratory females (Mann-Whitney U Test, $z = 1.41$, $P > 0.15$). The egg hatchabilities of all field females combined were significantly higher than those of laboratory females mated a single time ($z = 2.31$, $P < 0.05$). Mating an additional time restored the hatchability of eggs of old females to the level of a fresh, newly-mated female.

DISCUSSION

Females of many butterfly species can reject male advances by signals (Wago, 1977; Wiklund, 1977b), postures (Shapiro, 1970; Scott, 1973; Suzuki et al., 1977), and evasive flights (Stride, 1958; Rutowski, 1978). The evasive flights of black swallowtail females seem quite effective in this regard. Since the duration of a mating-refusal flight is very short compared to that of a copulation, it is unlikely that a female would mate rather than refuse or evade a courting male. This conclusion is reinforced by the increase in copulation duration of males that have mated frequently. Thus in a species with effective mating-refusal behaviors such as *P. polyxenes*, a female controls how frequently she mates, as long as males are available.

P. polyxenes females mate for the first time soon after emergence. Virgin females were rarely captured as in other species (Shields, 1967; Burns, 1968). Each was collected on the mating territory of a male (Lederhouse, 1982). Released virgin females flew preferentially to male territories as reported for *P. zelicaon* (Shields, 1967). It is likely that most females mate by the end of their first adult day.

The mean mating frequency of *P. polyxenes* was very consistent from brood to brood and from year to year (Table 3, Sims, 1979). This consistency contrasts with considerable variation in the density of males on mating areas (Lederhouse, 1978). The male density during the second brood of 1975 was more than twice that of both first and

second broods of the other years. Such uniformity in mating frequency would not be expected if females were mating more than they needed to. Burns (1968) reported an inverse relationship between population density and mating frequency for several species of skippers. However, positive correlations have been reported for a pyralid moth (Goodwin & Madsen, 1964) and a gelechiid (Graham et al., 1965). In a hilltop territorial mating system, the availability of male mates may be relatively independent of the absolute species density. Females would have similar opportunities to mate as long as the main territories were occupied. The trend of a lower mean mating frequency in third brood females may result from shorter average longevities of the females, rather than a scarcity of males since no virgins were collected. Thus, mating frequency may be independent of density in a hilltopping species.

A normal first copulation provides ample sperm to fertilize all the eggs that a female is likely to lay (Labine, 1966; Suzuki, 1978; Sims, 1979). Therefore, it is not surprising that there is no decline in fertility related to the number of eggs a female has laid previously. However, spermatophores with a low sperm count may result from young males or males that have mated frequently prior to the mating in question (Sims, 1979). These spermatophores are considerably smaller than average. Sugawara (1979) has demonstrated the importance of spermatophore size in controlling female receptivity. In *Pieris rapae crucivora* Boisduval, a mated female remained receptive unless her bursa copulatrix was stretched by a volume one half the size of the average spermatophore or greater. This agrees with the observation that fresh or slightly worn females that have mated twice frequently have a small first spermatophore. Thus a portion of polyandrous females mate again to replace a deficient initial spermatophore.

An increase in mean mating frequency with female age is well documented (Pliske, 1973; Ehrlich & Ehrlich, 1978; Sims, 1979; Suzuki, 1979). David & Gardiner (1961) report a refractory period between effective matings for *Pieris brassicae* (L.) females of six to nine days under laboratory conditions. *Pieris rapae crucivora* females mate for a second time at about 8 days of age under field conditions (Suzuki, 1979). Nearly three-quarters of the very worn females in this study had mated more than once. Although one normal copulation transfers abundant sperm, the sperm of the black swallowtail deteriorates with time. Thus, a high percentage of eggs laid late in a female's life may fail to develop if she has mated only once. An additional mating restores fertility levels, probably as a result of sperm precedence. Although female butterflies may not be able to determine directly the quality of stored sperm, the collapse of the spermatophore with time

may serve as an indirect measure. Once it has collapsed sufficiently, the sexual receptivity of the mated female is restored (Sugawara, 1979).

The cost to a female that mates an additional time may be quite low. The actual copulation consumes less than one hour. The cost only becomes considerable if the female must move a great distance from oviposition habitat to a mating area and back again (Wiklund, 1977a). Although there is a delay between mating and oviposition in newly emerged females, there is none for females after an additional mating. The benefit of mating again can be substantial. The mean egg hatchability of older condition class females that had mated once was 17.1% lower than fresh monogamous females. Their mean egg hatchability was also 16.4% lower than females in similar condition that had mated more than once. Similarly, the mean egg hatchability of all females in the laboratory dropped 15.8% between the first 10 days and after the fifteenth day. Since several of these females dropped to below 50% fertility, the benefit of an additional mating must surely outweigh its cost.

The prevalence of monogamous females in studies that have counted spermatophores has been interpreted as indicating that monogamy is the most adaptive female mating strategy (Wiklund, 1977b). The rationale is that the time saved by mating only once can be used for locating host plants and ovipositing, thus maximizing reproductive output. However, this is true only if high fertility is maintained throughout the lifetime of the female. A mating mode of one is a result of the short lifespan of most butterflies (Scott, 1974). The majority of females in most species die before a second mating becomes advantageous. If the mortality rate of *P. polyxenes* females is similar to that of males (Rawlins & Lederhouse, 1978), less than 35% of the females would be expected to live more than the 10 days of uniform fertility. Actually 30.4% of the sampled females had mated more than once. Evidently, most females that live long enough to make an additional mating advantageous, in fact do mate again.

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