IS SPERMATOPHORE NUMBER A GOOD MEASURE OF MATING FREQUENCY IN FEMALE CALLOPHrys XAMI (LYCAENIDAE)?

Additional key words: copulation, female remating, mating system.

Recent developments in sexual selection theory suggest that the role of females in shaping the evolution of mating systems has been underestimated (Eberhard 1996). In particular, female mating frequency is considered a variable that affects the potential for, and the strength of, sperm competition (Drummond 1984) and cryptic female choice (Eberhard 1985, 1996). In Lepidoptera, spermophore counts have been used to determine the number of times a female has mated (Burns 1968, Drummond 1984, Eberhard 1985). However, the validity of spermophore number as a measure of female mating frequency is based upon a number of assumptions which need to be verified before inferences about mating systems are made (Burns 1968, Lederhouse et al. 1986, Brady 1986). Here, I report the number of spermophores found in a field sample of females of the lycaenid butterfly Callophys xami (Reakirt); and, based upon previous information on the mating behavior and spermophore production patterns in this butterfly (Cordero 1993, 1998), I discuss possible biases incurred when using such a measure as an estimate of female mating frequency.

I sampled females during eight sunny days, between 28 December 1989, and 23 January 1990 (this multivoltine species reaches its highest density between October and January (Soberón et al. 1988)) in the Pedregal de San Angel ecological reserve, located in the south of Mexico City (description of the area in Soberón et al. 1988). All females observed during these days were collected and frozen until dissection. I measured the length of the right forewing of each female with a calliper. I used this length as a measure of body size, considering that there was a positive correlation between wing length and body weight in a laboratory-reared sample of females ($r = 0.8, p < .001, n = 27$). I also determined the degree of female wing wear using the following scale: (1) similar to a recently emerged adult (wings mostly green on the ventral side with intact margins), (2) very worn female (wings mostly brown on the ventral side with worn margins), and (3) all individuals intermediate between (1) and (3). I evaluated the frequency of "successful" copulations by females (i.e., copulations that resulted in spermophore transfer) by counting the number of spermophores and spermophore remains in the corpus bursae.

The number of spermophores in the 28 females I collected ranged from 0 to 3 (Fig. 1). The percentage of females without spermophores was 32.1%, with one spermophore was 46.4%, and with more than one spermophore was 21.4%. The mean ± SD number of spermophores found in females with at least one spermophore was 1.37 ± 0.6. I looked for a relation between female wing length and spermophore number with Spearman correlation since wing length was not normally distributed. This correlation was not significant ($r_s = -0.07, p > 0.05, n = 27$). Since all females collected were in wing wear conditions 1 or 2, I compared the number of spermophores of females in each condition with a Mann-Whitney U test without finding significant differences ($U = 54, p > 0.05, \text{Fig. 1}$).

Since the work of Drummond (1984) is the only extensive summary presenting data from Lycaenidae, I use the data in that paper as a reference. Considering average number of spermophores per mated female, maximum number of spermophores, and proportion of females multiply mated, female Lycaenidae relative to other lepidopterans show the lowest degree of polyandry, comparable only with the Satyrinae (Drummond 1984). However, C. xami shows some differences when compared with the four lycaenid species included in Drummond (1984). The average copulation frequency estimated for mated females (1.37 ± 0.6), the maximum number of spermophores (3), and the proportion of females multiply mated (21.4%) in C. xami is higher than in the other four lycaenids (ranges of average copulation frequency values: 1.05–1.17; maximum number of spermophores: 2 for the four species; and proportion of females multiply mated: 3.7–12.7%). Furthermore, average number of copulations, maximum number of spermophores, and proportion of females multiply mated could be underestimated in C. xami, since no females in the very worn ("old") wing wear condition were collected (Fig. 1). A sampling bias could exist if "old" females were more difficult to detect or to capture. However, our research group has been studying this species in the field for more than 10 years, and we have no evidence of any greater difficulty in observing and catching "old" females. It is possible that most females do not live long enough to become very worn and, therefore, are rare; in this case our estimates of copulation frequency would be unbiased. On the other hand, since outside the sampling period we have observed very worn C. xami females in the field, it is also possible that the abundance of "old" females varies in time as a result of, for example, varying predation pressure or weather conditions. Under these conditions, average and maximum number of spermophores could vary with time depending on the age structure of females.

The method used to evaluate female copulation frequency in the field is based on three assumptions (modified from Drummond 1984):

Copulation always results in spermophore transfer. In C. xami this is not true because there are some copulations of very short duration that do not result in the transfer of a spermophore (Cordero 1993, 1998). However, these "interrupted" copulations are not common in the field (0/18 copulations observed in 1983–1985 and 2/27 copulations observed in 1989–1990; Cordero 1993). On the other hand, although the existence of interrupted copulations prevented the estimation of the total number of copulations performed by females, the figures obtained could be good estimates of the number of copulations resulting in spermophore transfer.

Males transfer only one spermophore per copulation. In C. xami this is not true since in laboratory experiments we observed three copulations in which different males transferred two spermophores during one copulation (Cordero 1998). Violation of this assumption results in an overestimation of copulation frequency.

---

**Fig. 1.** Distribution of females with different number of spermophores and the relationship between number of spermophores and wing wear category.
However, if the frequency of copulations resulting in the transfer of two spermatophores in the laboratory is a good estimate of their frequency in the field (3/199 copulations observed in the laboratory), its quantitative effect should be small.

Spermatophores always leave recognizable remains within the corpus bursae of the female. This is not true in C. xani since in the laboratory it was not always possible to observe clear spermatophore remains in very old females that had laid most of their eggs (pers. obs.). However, judging from wing wear, no female in this condition was sampled (see paragraph four above).

In conclusion, the possible violation of the first and the last assumptions, and the fact that some of the females may have mated again had they not been collected, results in an underestimation of the frequency of copulations in females; whereas the fact that some males transfer more than one spermatophore in one copulation results in an overestimation of the number of copulations. However, judging from the low frequency of "interrupted" copulations (4.4%), very few females in the field (at least during the sampling period), and copulations resulting in the transfer of two spermatophores (1.5%), I conclude that spermatophore counts are a reasonably good estimate of female copulation frequency in C. xani.

ACKNOWLEDGMENTS
I thank Gabriela Jiménez and Dr. Rogelio Macías for their valuable technical help, and Dr. J. M. Burns and an anonymous reviewer for their comments. This research was supported by a Consejo Nacional de Ciencia y Tecnología (Mexico) scholarship.

LITERATURE CITED


CARLOS CORDEIRO. Instituto de Ecología, Universidad Nacional Autónoma de México, Apdo. Post. 70-275, C.P. 04510 D.F., and Centro de Investigaciones Fisiológicas, Universidad Autónoma de Tlaxcuaca, Apdo. Post. 262, C.P. 90070 Tlaxcuaca, Tlaxcuaca, MÉXICO (Address for correspondence)

Received for publication 5 April 1999; revised and accepted 16 December 1999.

Journal of the Lepidopterists’ Society
53(4), 1999, 170–172

ADDITIONAL NOTES ON PROSERPINUS CLARKIAE AND ARCTONOTUS LUCIDUS (SPHINGIDAE)
LIFE HISTORIES FROM THE PACIFIC COAST OF NORTH AMERICA

Host associations for Proserpinus clarkiae (Boisduval) and Arctonotus lucidus (Boisduval) have recently been documented. Proserpinus clarkiae was found using Clarkia unguiculate (Lindley) in nature (Osborne 1995). Here, I compare results of my life history work on P. clarkiae with other results (Hardy 1959) on this species. The life history of A. lucidus is also known (Comstock & Henne 1942). However, the first natural host associations for A. lucidus were made by photographs and collections from Clarksia species in California, and are presented here along with observations on captive rearing of this moth. The immature stages of these related sphingid species have been confused in the field by some, possibly due to their sympatry, common use of Clarkia hosts, and superficial resemblance. Thus, I will also discuss morphological differences among these and other sympatric Clarkia feeding sphingids.

In presenting the biology of P. clarkiae (Osborne 1995), I repeated the assertion made by Hodges (1971) that its life history was unknown. Since that time, Dr. Frederick Rindge (American Museum of Natural History) has drawn my attention to a life history of P. clarkiae that predates both works. Larvae and a pupa reared from Vancouver Island (Hardy 1950) were described by Hardy (1959), and match the immatures of P. clarkiae from California. Hardy obtained seven ova by confining females over potted Galium aparine (Lewis & Szweykowski) (Rubiaceae). He reared at least one individual to pupation on that plant, but a field host was not given. The single fifth instar larva of P. clarkiae from Vancouver Island had the lateral dark blotches contiguous in an undulating line, a trait consistent with some (<5%) of the California material I reared (most California larvae had oblique blotches disjunct) (Osborne 1995). This dark form may be typical of cool, wet, north coastal localities, where darker maculation may impart local selective advantages, or may be an artifact of captive rearing.

Dr. Robert Raguso, who studied sphingid pollination of Clarkia species in central California (see Raguso & Pichersky 1995, Raguso et al. 1996, Raguso & Light 1998), sent me several suspected Proserpinus larvae, a reared pupa, and a photograph (Fig. 1) of a fifth instar larva in nature on Clarkia brevirostris (A. Gray) E. Greene. These specimens were all collected from C. brevirostris and Clarkia modesta (Jepson) at Del Puerto Canyon, Stanislaus Co., California in May, 1991. However, instead of P. clarkiae, all were determined (by KHO) to be Arctonotus lucidus, a closely related species from a monotypic genus. Early instar A. lucidus larvae may be separated from P. clarkiae by the presence of a black anal horn which is absent in P. clarkiae. Fifth instar A. lucidus lose the anal horn, but have dorsal and lateral markings of olive green (but briefly black just after molt [Comstock & Henne [1942]], not black or gray as in P. clarkiae. In addition, A. lucidus can be distinguished from P. clarkiae on the basis of dorsal, transverse intersegmental lines of tan or cream breaking the olive green field, and ventral whitish or gray. The ground color in fifth instar A. lucidus larvae is variable (Comstock & Henne 1942), ranging from black to olivaceous green to light green, to pink (Comstock & Henne 1942; D. Rubinoff pers. comm.; K. H. Osborne unpubl. obs.).