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THE EVOLUTIONARY AND BIOLOGICAL SIGNIFICANCE OF MULTIPLE PAIRING IN LEPIDOPTERA

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The frequency of pairing in Lepidoptera may be related to population structure (Labine, 1964), to courtship behavior (Marshall, 1901) or to the balance of a polymorphism in a mimetic population (Burns, 1966). In this paper a sample of female *Utetheisa ornatrix bella* (L.) (Arctiidae) from the polymorphic Florida population is analyzed by phentotpye and pairing frequency for evidence of sexual selection; data taken from the literature are summarized; and the importance of mating frequency for the evolution of behavior and of population structure is discussed.

Studies of copulation have shown that in many Lepidoptera the male constructs a sperm containing bag (spermatophore) in the genitalia of the female (Callahan and Cascio, 1963: Callahan and Chapin, 1960; Khalifa, 1950; Norris, 1932; Weidner, 1934). Mating refers to copulation; successful mating requires transfer of at least one spermatophore. Since spermatophores remain in the bursa copulatrix, the number of successful pairings can be ascertained by dissecting the bursa and counting spermatophores. The spermatophore is reported to disintegrate as soon as it is formed in certain Microlepidoptera (Callahan and Casico, 1964: 554). While spermatophore remnants are difficult to count, the hardened duct (collum) through which sperm pass from the spermatophore to the ductus seminalis is retained more or less complete in Utetheisa ornatrix, and the number of colli is an index of pairing frequency. At most one spermatophore is produced during a single copulation of this species. Of twelve virgin females mating once in controlled breeding experiments in 1961, all had one and only one spermatophore in the bursa copulatrix.

In the pink bollworm, *Pectinophora gossypiella* (Saunders) (Gelechiidae), spermatophores were formed in 199 of 219 laboratory matings of virgin females (Ouye, *et al.*, 1965, experiment 1). Seventeen of the

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20 pairs which did not form spermatophores remained in copulo no longer than 30 minutes. Thus, a certain minimum time is required for spermatophore construction and no more than one spermatophore is formed during a single copulation.

The distribution of spermatophore frequencies in a sample of 89 females of *U. o. bella* collected in Florida is tabulated in Table 1. One bursa was lost in the process of dissection. Fifty-three males were collected with the females. The mean number of spermatophores was 3.489 with standard deviation 2.656. One individual had copulated at least 11 times, and eight had not mated successfully.

Before dissection, the condition (fresh vs. worn) of each specimen was estimated on the basis of external appearance and graded A, B, C, or D. Not unexpectedly, the mean number of spermatophores increases from 1.455 for very fresh (A) individuals to 6.250 for very worn (D) individuals (Table 2). Moths reared under constant conditions (80° F, 12–16 hours light per day) usually mated at night although a few males collected in the field mated in the afternoon by artificial light. Diurnal mating may be an adaptation to the Florida winter season with its cold nights and warm days. A refractory period may follow copulation during which time the male will not mate successfully (cf. Khalifa, 1950: 39, *Galleria mellonella* (L.) (Pyralidae); Ouye, *et al.*, 1965 experiment 2, *Pectinophora gossypiella*).

The sample was tabulated separately for five wing pattern characters and tested for differences in mean spermatophore number between categories (Table 3). The characters were subdivided into categories as follows: (1) forewing ground color—*yellow* or *orange*, *red orange* and *orange red*, or *red*; (2) the distribution of forewing ground color—*colored* or *streaked*, *intermediate* and *white*; (3) black spotting on forewing *spotted* or *unspotted*; (4) hindwing black markings—*wide*, *semi* or *narrow*; (5) hindwing ground color—*red*, *pink* and *flush* versus *white* (Pease, 1968).

When the t-test was applied independently to each of the five characters in Table 3, only the difference between the mean number of individuals with spotted *versus* unspotted forewings was statistically significant with probability less than .02 of a difference between the means as large or larger. However, the probability is about ¹/₄ that one or more of five independent tests is significant at the .05 level, $(1 - .95^5)$. Since the statistical hypothesis was formulated after looking at the data, the result does not favor a hypothesis that wing pattern is a factor determining mating frequency. This is consistent with the observation that this species mates at night and that the bright pigmentation serves as a warning stimulus (aposematic coloration) to potential diurnal predators. Toxic substances have been found in the haemolymph by M. Rothschild. The species exudes a frothy bubble at the tegulae when seized suddenly (reflex bleeding).

The average number of spermatophores in the tiger swallowtail (*Papilio glaucus* L.), a species with dimorphic females, was greater for yellow females than for dark females in samples from Mountain Lake, Virginia and Baltimore, Maryland (Burns, 1966). The resemblance of dark females to the unpalatable blue swallowtail (*Battus philenor* (L.)) is believed to confer protection from predators whose vision enables them to distinguish the two forms. The population frequencies of dark and yellow females are affected by two antagonistic forces of natural selection (an example of disruputive selection). An extra-specific environmental factor, mimicry, favors the mimetic dark female; an intra-specific factor, sexual selection, favors the yellow female.

The preference of the male tiger swallowtail, which is always yellow, for the yellow female is relatively independent of the frequencies of dark and yellow females. At Baltimore the frequency of dark females is .4483 and the mean number of spermatophores was .3366 greater in yellow than in dark females. At Mountain Lake, Virginia where the frequency of dark females is almost doubled (.8571) the difference in mean spermatophore number between yellow and dark females is nearly the same (.3889) (Burns, 1966 Tables 1 and 2).

The difference in means for spermatophores in dark and yellow forms is significant at the 0.2 level for the sample from Mountain Lake, Virginia and is significant between the 0.2 and 0.3 level for the sample from Baltimore County, Maryland (Appendix 1). The "true" difference between the means is important to the theory of polymorphic populations. The experimental biologist can determine the correct sample size to prove or disprove the theory by using the data given below.

Population parameters for the frequency of dark and yellow forms of the tiger swallowtail and the mean number of spermatophores for each follow (Burns, 1966):

| | Мои | untain Lake, V | irginia | Baltimore County, Maryland | | | | | | | |
|----------------|-----------------------------|--|--------------------------------|-----------------------------|--|--------------------------------|--|--|--|--|--|
| | Frequency of Female Type | Mean number of Spermatophores Per Female | f Standard Deviation-(s) | Frequency of Female Type | Mean number of Spermatophores Per Female | f Standard Deviation-(s) | | | | | |
| Dark | .857 | 1.694 | .781 | .448 | 1.538 | .776 | | | | | |
| Yellow | .143 | 2.083 | 1.379 | .552 | 1.875 | .619 | | | | | |
| Combin Data | ed – | 1.750 | .890 | _ | 1.724 | .702 | | | | | |

For an assessment of how many specimens should be collected from each population so that the estimated difference in the average number

TABLE 1. DISTRIBUTION OF SPERMATOPHORES IN FIELD SAMPLES OF VARIOUS LEPIDOPTERA AND EXPERIMENTS ON MATING BEHAVIOR

| N—sample size; \overline{X} —mean α of the sample standard dev | or av iatio | erage n. | e; s ² - | —sa | mp | le v | /ar | ian | ice | , 1 | me | an | sq | uare or | square |
|---|----------------|-------------|---------------------|-----|-----|------|----------|-----|-----|-----|-----|------|-----|-------------------------|--------|
| Number of spermatophores | N | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | $\overline{\mathbf{X}}$ | s^2 |
| <i>Utetheisa ornatrix bella</i> (L.) (Arctiidae)—Archbold Biol. Station, Lake Placid, Florida (net collection) | 88 | 8 | 12 | 15 | 20 | 11 | 5 | 4 | 4 | 2 | 3 | 3 | 1 | 3.4886 | 7.0573 |
| Pseudaletia unipuncta (Haw.) (Noctuidae)—Louisiana Callahan and Chapin (1960) Table 1—p. 779 (light trap) | 417 | 182 | 107 | 76 | 36 | 14 | 2 | - | _ | - | | _ | - | 1.0384 | 1.3447 |
| Peridroma saucia Hbn. (= margaritosa Haw.) (Noctuidae)—Louisiana Callahan and Chapin (1960) Table 2—p. 780 (light trap?) | 239 | 203 | 16 | 5 | 8 | 6 | 1 | - | - | - | - | - | _ | .3305 | .8525 |
| Heliothis zea (Boddie) 1 (Noctuidae)—Louisiana Callahan (1958) Table 6—p. 427 (light trap) | 295 | 519 | 455 2 | 227 | 77 | 16 | 1 | - | | - | | - | - | .9336 | .9338 |
| | N | les | s tha | n 2 | | | | 2 | 01 | r n | 101 | e | | | |
| Euphydryas editha (Bdv.) (Nymphalidae)—California Labine (1964) | 23 | | 14 | | | | | | | 9 | | | | | |
| | N | 0 | 1 | 2 | 2 | 3 | 4 | 4 | 5 | 5 | | Ż | ζ | s^2 | |
| Battus philenor (L.) (Papilionidae)—Mtn. Lake Biol. Sta., Virginia Burns, 1966—table 2. | 33 | 0 | 17 | 11 | L | 3 | | 1 |] | L | | 1.72 | 273 | .95 | 45 |
| <i>Papilio glaucus</i> L. (Papilionidae) Burns, 1966—table 2. | | | | | | | | | | | | | | | |
| Mtn. Lake Biol. Station, V | irgini | ia | | | | | | | | | | | | | |
| dark females | 72 | 0 | 33 | 30 |) | 8 | - | - |] | L | ļ | 1.69 | 944 | .60 | 95 |
| yellow females | 12 | 0 | 6 | 2 | 2 | 2 | | 1 |] | L | 4 | 2.08 | 333 | 1.90 | 15 |
| Total | 84 | 0 | 39 | 32 | 2] | 10 | | 1 | 2 | 2 | | 1.78 | 500 | .79 | 22 |
| Baltimore County, Maryla | nd | | | | | | | | | | | | | | |
| dark females | 13 | 0 | 8 | 3 | 3 | 2 | - | - | - | - | Ì | 1.53 | 385 | .60 | 26 |
| yellow females | 16 | 0 | 4 | 10 |) | 2 | - | - | - | - | | 1.87 | 750 | .38 | 33 |
| Total | 29 | 0 | 12 | 13 | 3 | 4 | - | - | - | - | | 1.72 | 241 | .49 | 26 |

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| TABLE | 1 | Continued |
|-------|---|-----------|
| | | |

Pectinophora gossypiella—(Saunders)

1. Number of copulations (spermatophores not counted) during the lifetime of individual pairs maintained in laboratory population cages.

| No. of copulations No. of | of ma | oths | N | 0 | 1 | 2 | 3 | 4 | $\overline{\mathbf{X}}$ | S^2 |
|---------------------------|-------|------|-----|----|----|----|---|---|-------------------------|-------|
| in Po | pulat | ion | | | | | | | | |
| Cage | : 8 | Ŷ | | | | | | | | |
| Lukefahr and Griffin | 1 | 1 | 100 | 72 | 16 | 3 | 3 | 1 | .3500 | .5934 |
| (1957, 1967) | | | | | | | | | | |
| Ouye, et al. (1964) | 1 | 1 | 94 | 24 | 50 | 14 | 5 | - | .9894 | .6343 |
| Ouye, (in litt. 1967) | | | | | | | | | | |

2. Number of spermatophores produced during lifetime exposure to moths of opposite sex under laboratory conditions (Ouye, *et al.*, 1965, experiment 4—table 3; experiment 5—table 4, and Ouye, *in litt.* 1967).

| No. of | N | o. of | : | | | | | | | | | | | | | |
|----------------|------|----------|---------------|-----|-------|-----|-------|------|----------|------|------|-----|-----|-----|-------------------------|--------|
| spermatophores | M | oths | 5 | | | | | | | | | | | | | |
| formed | | in | | | | | | | | | | | | | | |
| during | Pop | Ca_{i} | $\mathrm{g}e$ | | | | | | | | | | | | | |
| lifetime | 8 | Ŷ | N | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\overline{\mathbf{X}}$ | s^2 |
| Males | 3 | 1 | 206* | 10 | 19 | 20 | 25 | 41 | 29 | 29 | 16 | 9 | 5 | 3 | 4.2524 | 5.3116 |
| Females | | | | | | | | | | | | | | | | |
| (A) Crowded | 1 75 | 25 | 196 | 3 | 49 | 74 | 45 | 22 | 2 | 1 | - | _ | _ | - | 2.2245 | 1.1186 |
| (B) Uncrowd | ed 1 | 6 | 266 | 8 | 64 | 83 | 69 | 34 | 6 | 1 | - | 1 | _ | _ | 2.3195 | 1.4334 |
| Combined (A | +B) | | 426 | 11 | 113 | 157 | 114 | 56 | 8 | 2 | - | 1 | _ | _ | 2.2792 | 1.2993 |
| * Am | ends | N in | expe | rim | ent 4 | and | l tal | ole, | Ou | iye. | , et | al. | , 1 | 965 | | |

Spermatophore formation during the first 24 hours after eclosion of females 3. (Ouye, et al., 1965, experiment 3 table 2, Ouye, in litt. 1967). $\overline{\mathbf{X}}$ s^2 No. of spermatophores N 0 1 2 3 Time exposed to males in population cages 15-21 hours 373 222 44 16 2 .9812 .5776 89 9-15 hours 118 65 42 7 4 .5763.5711_ 1 1 .2500 .6000 3-9 hours 16 14

4. Distribution of spermatophore number in a sample of females collected at light traps in the vicinity of Brownsville, Texas (Graham, et al., 1965; and Ouye, in litt. 1967). 6 $\overline{\mathbf{X}}$ s^2 No. of spermatophores N 0 1 2 3 4 5 No. of moths 2570 284 1872 332 65 13 3 1 1.0911.4067

of spermatophores lies within .05 of the "true" value 95% of the time, see Appendix 2.

Disruptive selection in the tiger swallowtail may be an example of evolutionary homeostasis at the population level; that is, constant intraspecific factors of selection counterbalance variable extra-specific environmental factors and tend to restore primitive population conditions.

| Condition | # Individuals | Mean Number of Spermatophores |
|-----------|---------------|-------------------------------|
| A | 22 | 1.455 |
| В | 32 | 2.781 |
| С | 26 | 5.231 |
| D | 8 | 6.250 |

TABLE 2. Utetheisa ornatrix bella in each of four grades of condition with mean number of spermatophores per female

If this is true, when the environment ceases to favor the evolutionary novelty (the dark female), the force of sexual selection will restore a uniformly yellow population. This hypothesis is consistent with the observed correlation between the distribution of the blue swallowtail and a high frequency of the dark female form of the tiger swallowtail.

Data on spermatophore frequency in Lepidoptera are summarized in Table 1. The maximum number of spermatophores counted in a female was 11 (*Utetheisa ornatrix*). The maximum number of spermatophores formed by a male was 11 (*Pectinophora gossypiella*—Ouye, *in litt.* 1967).

The mating habits of the pink bollworm moth (*Pectinophora gossy-piella*) have been thoroughly studied (Lukefahr and Griffin, 1957; Ouye, *et al.*, 1964; Ouye, *et al.*, 1965; Graham, *et al.*, 1965; Ouye, *in litt.* 1967). Data are summarized in Table 1. Pairs of moths copulated an average of .350 times during their lifetime in the experiments of Lukefahr and Griffin and .989 times in the experiments of Ouye and his workers (spermatophores were not counted). The two means are different (Appendix 3).

Females mate successfully as many as four times during the first 24 hours after eclosion; males produce no more than one spermatophore in a 24 hour period. The average number of spermatophores formed during the life of a male is 4.252 under laboratory conditions. This is almost double the lifetime average of 2.279 for the combined data of females in crowded (A) and uncrowded (B) population cages. (Table 1, experiments 2 and 3) (Appendix 4).

Thus, while the female pink bollworm moth mates successfully more often in one day, the male can mate successfully almost twice as many times as the female in the moths' lifetimes.

The greater number of spermatophores formed by the male is consistent with a hypothesis that natural selection acts more strongly on the male than on the female. Two factors reduce the male's average under the competitive conditions in the field. Males may compete more actively for females than females compete for males (intra-specific sexual selection). Extra-specific factors of natural selection sometimes

TABLE 3. NUMBER OF INDIVIDUALS AND MEAN NUMBER OF SPERMATOPHORES IN EACH CATEGORY OF THE FIVE CHARACTERS FOR WING PATTERN AND PIGMENTATION IN Utetheisa ornatrix bella from Florida

| Character | Phenotype | Number of Individuals | Mean Number of Spermatophores |
|-----------|-------------------------------------|-----------------------|----------------------------------|
| 1 | Colored/streaked | 36 | 3.750 |
| | Intermediate | 22 | 3.136 |
| | White | 30 | 3.433 |
| 2 | Yellow/orange | 76 | 3.526 |
| | Red/orange red/red oran | ge 10 | 3.100 |
| | ("Redless" Aberration) ¹ | 2 | 4.000 |
| 3 | Spotted | 69 | 3.145 |
| | Unspotted | 19 | 4.737 |
| 4 | Wide | 55 | 3.618 |
| | Semi | 30 | 3.367 |
| | Narrow | 3 | 2.333 |
| 5 | Red/pink/flush | 82 | 3.304 |
| | White | 4 | 2.000 |
| | ("Redless" Aberration) ¹ | 2 | 4.000 |

 $^1\operatorname{Specimens}$ in which the red pigment is missing on both the upper and under surface of the wings.

favor survival of the female at the expense of the male, as for example, when the female is protectively colored. Thus, at the population level, the male's greater reproductive potential compensates for individual competition among males and for the greater risk involved in being a male.

It seems intuitive that the competition for mates affects the distribution of spermatophores formed by the two sexes, perhaps, by increasing the variation in the number of spermatophores formed by the males. However, the design of a practical experiment to collect data, and a method of analysis are a challenge to the ingenuity of the experimental biologist.

In samples of Lepidoptera collected at light traps, the average number of spermatophores varies from 1.0911 (*Pectinophora gossypiella*) to .3305 (*Peridroma saucia* Hbn. (Noctuidae)). The average number of spermatophores in a collection made with a net was 3.4886 for *Utetheisa ornatrix*. These data are not comparable to laboratory data because of the mixed age distribution in feral populations.

Some calculations (by Graham, *et al.*, 1965) suggest that the first mating of the pink bollworm is density dependent, but that multiple paring is density independent. This hypothesis is based on a correlation (or absence of) between the log of the number of moths collected in light traps and (a) the proportion of moths which had mated, (b) the

TABLE 4. SPERMATOPHORE NUMBER IN FALL-WINTER VERSUS THE OVERWINTERING SPRING POPULATIONS OF *D. plexippus* in California (data from Table 16, Williams, *et al.*, 1942)

| Date | Location | N | 0 | 1 | 2 | 3 | 4 | 5 | $\overline{\mathbf{X}}$ | s ² |
|---------------------|--|----|----|----|---|---|---|---|-------------------------|----------------|
| Oct. 1938—Feb. 1939 | Pacific Grove San Diego El Carrito | 38 | 17 | 19 | 2 | 0 | 0 | 0 | .605 | .353 |
| April 1939 | San Francisco | 5 | 0 | 0 | 2 | 0 | 1 | 2 | 3.600 | 2.300 |

proportion of once mated or multiply mated moths, (c) the mean number of spermatophores for mated and unmated moths combined, and (d) the mean number of spermatophores for mated moths.

Observations on the sex ratio, migration, spermatophore number, adult activity and mating behavior of the winter population of the monarch butterfly (*Danaus plexippus* (L.) (Nymphalidae)) in California are reported by J. A. Downes (Williams *et al.*, 1942: 160–165). No more than two spermatophores were found in any female until after February (Table 4). Although both sexes migrate, the estimated male: female ratio in the population remaining at Pacific Grove in winter quarters was 1000 to 1 by the second week in May. Females apparently migrate first. Some mating occurs in the hibernating population at Pacific Grove (no reports from November to January, however) even though egg follicles remain unripe until the last week in March.

Species in which both sexes pair several times contrast with those in which the female is prevented from multiple insemination by a sphragis or plug which the male constructs in the genitalia (*Acraea* : Marshall, 1901, 1902, and Eltringham, 1912; *Parnassius* : Eltringham, 1925; *Bombyx mori* L. : Omura, 1938). Marshall hypothesized that differences in courtship behavior separate sphragis building genera (e.g., *Acraea* and *Parnassius*) from many other butterflies, "marriage by capture" (Poulton, 1911) *versus* "marriage by courtship." In other words, sphragis construction complements a behavior pattern in which the male "grapples" with the female after a rudimentary display; in species with more complex behavior patterns, courtship may be terminated at any of several stages (e.g., Brower, *et al.*, 1965).

Eltringham (1912) suggests that the sphragis may block the release of a chemical attractant which serves to assemble males. If this hypothesis is correct, the "capture" system may represent only the final stages of courtship.

In terms of evolutionary potential, no matter how effective "no" signals

are, the rare male which succeeds in breaking through the defense mechanisms of an already mated female will leave more offspring than the male which does not mate under such circumstances. Unless there is a selective advantage to multiple insemination such as a short life span of sperm or a prolonged oviposition period, the balance between multiple versus single copulation should occur when the effort required to copulate with an already mated female will reduce, first, the probability of insemination of unmated females and, second, the males contribution to the gene pool of the next generation. For multiple insemination to persist under such circumstances, the number of fertile offspring left by the more versatile male should outnumber those left by the virgin inseminator.

If this were not so, the evolution of more effective mechanisms against multiple pairing would be expected.

A relationship between mating frequency and interpopulation gene flow has been suggested (Labine, 1964—Euphydryas editha (Bdy.) (Nymphalidae)). Populations in which the female mates before migration and only once can be contrasted with those in which the female mates several times and especially with endemic males after migration. In the first instance, a migrating female produces offspring with a gene complement derived exclusively from the parent population while all offspring produced by migrant males mated to endemic females receive half their genes from the parent population and half from the other. In contrast, if both sexes mate after migration, essentially, all offspring are interpopulation hybrids. Thus, multiple pairing may increase the proportion of hybrid offspring among progeny of migrants and hasten the breakdown of introduced gene complexes. On the one hand multiple pairing may increase variation through recombination between the two gene pools and thereby influence the speed of adaptive change. On the other hand, if the crossing of the two gene pools proves deleterious and non-adaptive, F1 offspring of migrants will be at a competitive disadvantage even though offspring homozygous for genes from the parent population compete successfully in the new habitat. Thus, hybridization is an effective strategy on the part of the endemic population for increasing the probability of beneficial combinations, and at the same time, serves to reduce the competitive advantage of a closely related invading species provided that the reproductive potential of the endemic population is great enough to prevent swamping.

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Appendix

Statistical Notes (Anderson and Bancroft, 1952: Fraser, 1958)

1. Tests for equality of the average number of spermatophores in samples of dark and yellow females of *Papilio glaucus* L. (Burns, 1966).

Hypothesis Test Degrees of Value of Value of Tabulated Significance Conclusion Freedom Experimental Statistic Level Statistic

Mountain Lake Biological Station, Virginia

 $\mu_{\rm D} = \mu \gamma \qquad \begin{array}{c} \mu_{\rm D} = \mu \gamma \\ {\rm t'} \quad 71 \quad 11 \quad .13496 \quad 1.980 < {\rm t'} < 2.201 \quad .05 \quad {\rm means \ equal} \\ {\rm (Cockran \ and \ Cox)} \end{array}$

(Note: a value of the experimental statistic as large or larger is expected on the basis of chance alone about .8 of the time).

Baltimore County, Maryland

 $\mu_{\rm D} = \mu \gamma \qquad \begin{array}{ccc} {}^{Dark \ Yellow} \\ {\rm t'} \ 12 \ 15 \ 1.090 \qquad 2.131 < {\rm t'} < 2.179 \quad .05 \quad {\rm means \ equal} \\ {\rm (Note: \ a \ value \ of \ the \ experimental \ statistic \ as \ large \ or} \end{array}$

larger is expected on the basis of chance alone between .7 and .8 of the time).

2. An approximate solution can be found using the following expression for the confidence limits for the difference of two means (Fraser, 1958: 281):

$$\overline{X}_{d} - \overline{X}_{y} \pm t_{.05} \bigg(\frac{1}{DN} + \frac{1}{YN} \bigg)^{\frac{1}{2}} s$$

- $t_{.05}$ —is the t statistic with N 2 degrees of freedom but evaluated here for an infinite number of degrees of freedom since the sample size is large but unknown.
- N -Sample size.
- D -- frequency of dark females in sample.
- Y —frequency of yellow females in sample.
- s —square root of the variance. Since the variance for spermatophore numbers may differ in the two types of females, an approximate solution is obtained by using the population variance calculated from the combined data.

The solution is obtained by choosing N such that

$$t_{.05} \left(\frac{1}{DN} + \frac{1}{YN} \right)^{\frac{1}{3}} s = .05$$

or
 $N = 400 s^2 (1.96)^2 \left(\frac{1}{D} + \frac{1}{Y} \right).$

The answer depends on the relative frequencies of dark and yellow females in the sample. The smallest adequate sample is one in which the frequencies of dark and yellow females are equal (*i.e.*, D = Y = .5). When equal numbers of both types of female are collected, 3028 females comprise an adequate sample in Maryland and 4870 in Virginia.

3. Means differ at the .02 level of significance, but variances are equal for the two sets of data.

| Lukefa | ahr and | d Griffin | , 1957 | $N_L = 100$ | $\overline{\mathbf{X}} =$ | .350 | $s_{L}^{2} = .593$ |
|--|---------|-----------------------|-----------------------------------|--|---------------------------|------------------|--------------------|
| Ouye, | et al., | 1964 | | $N_o = 94$ | $\overline{\mathbf{X}} =$ | .989 | $s_{0}^{2} = .635$ |
| Hypothesis | Test | Degrees of Freedom | Value of Experime Statistic | Value of ental Tabulated Statistic | Sig Le | nificance vel | Conclusion |
| $\sigma_{ m L}{}^2 {\equiv} \sigma_{ m o}{}^2$ | F-test | 93, 99 | 1.070 | $1.59 < F_{.02} <$ | 1.87 | .02 va | ariances equal |
| $\mu_{ m L} \equiv \mu_{ m o}$ | t-test | 192 | 7.256 | $2.33 < t_{.02} <$ | 2.36 | .02 m | eans not equal |

4. Data for females in crowded (A) versus uncrowded (B) population cages were tested for equality of means and variances. Combined data for females under both conditions were compared with the data from males for equality of means and variances.

| Crowd | ed fem | ales (A) | | $N_A = 196$ | $X_A =$ | 2.22 | 4 $s_{A}^{2} = 1.146$ |
|--|---------|-----------------------|----------------------------------|--|--|------------------|-----------------------------|
| Uncrow | wded fe | emales (] | B) | $N_B = 266$ | $\overline{X}_{B} =$ | 2.32 | $0 	 s^2{}_{\rm B} = 1.433$ |
| Combi | ned da | ta for fer | males | $N_{\rm C}=462$ | $X_{\rm C} =$ | 2.27 | 9 $s_{\rm C}^2 = 1.299$ |
| Males | | | | $N_M = 206$ | $\overline{\mathbf{X}}_{\mathbf{M}} =$ | 4.25 | $s_{M}^{2} = 5.315$ |
| Hypothesis | Test | Degrees of Freedom | Value oj Experim Statistic | f Value of ental Tabulated Statistic | Si L | ignifica evel | nce Conclusion |
| $\sigma_{\rm A}{}^2 \equiv \sigma_{\rm B}{}^2$ | F-test | 265,195 | 1.251 | $1.28 < F_{.02}$ | < 1.59 | .02 | variances equal |
| $\mu_{\rm A} = \mu_{\rm B}$ | t-test | 460 | .770 | $2.33 < t_{.02}$ | < 2.36 | .02 | means equal |
| $\sigma_{\rm C}^2 \equiv \sigma_{\rm M}^2$ | F-test | 205,461 | 4.091 | $1.00 < F_{.02}$ | $<\!1.48$ | .02 | variances unequal |
| $\mu_{\mathrm{C}} = \mu_{\mathrm{M}}$ | ť | 205,461 | 11.664 | $2.33 < t'_{.02}$ | $<\!2.35$ | .02 | means unequal |
| | (Cock | ran and | Cox) | | | | |

LEONARD STEVENS PHILLIPS (1908–1968)

Leonard Stevens Phillips was born December 4, 1908 at Le Claire, Iowa. He died suddenly in Chicago, Illinois, February 13, 1968. He was the son of Clyde and Winifred Phillips. His marriage to Merle Olive Garton took place May 26, 1937. She survives him; there were no children.

He attended the public schools of Le Claire and received his B.A. degree from the State University of Iowa in 1932. He did graduate work there and at the Iowa State University of Agriculture, and received his teacher's certificate from the Iowa State Teachers' College in 1938.

He engaged in private business from 1936 to 1946, then became a laboratory assistant in the Stritch School of Medicine, Loyola University, Chicago, a position which he held until 1950. Following a period as laboratory technician with Swift and Company in Chicago, he became Assistant Biologist at the Illinois Institute of Technology Research Institute. Here he was in charge of the animal room and worked on many projects involving the use of small animals in behavioral studies and biochemical research. In 1965 he joined the Loop City College of Chicago as a laboratory assistant, and in 1967 returned to private business, in which he was engaged at the time of his death.

Leonard was an active and enthusiastic collector of Lepidoptera. He collected personally in every state of the continental United States and

maintained an active correspondence and exchange with fellow lepidopterists throughout the world. He contributed several papers to the pages of this *Journal*; a bibliography is given below. His collection of some 5,600 specimens of worldwide Lepidoptera is being retained by his widow for the present, but will be presented to Buena Vista College, Storm Lake, Iowa.

Among his other interests were collections of pressed plants, minerals, and stamps; woodworking, and amateur art. He was active in Boy Scout, boys' club, and church work. He was a member of the Chicago Entomological Society, and had been a member of the Lepidopterists' Society since 1948.

Leonard's many friends and correspondents will miss his friendly, outgoing personality, his enthusiasm for his avocation, and his willingness to be of service to others.

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