at least two locations in the eastern United States (McRobert and Tompkins, 1986*b*, 1986*c*) and since males of these species have been observed to court each other at these feeding sites (McRobert and Tompkins, 1986*a*), it seems reasonable to assume that this phenomenon may occur in the wild.

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INSEMINATION REACTION IN *DROSOPHILA*: FOUND IN SPECIES WHOSE MALES CONTRIBUTE MATERIAL TO OOCYTES BEFORE FERTILIZATION

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Patterson (1946) was the first to describe the opaque mass that fills the uterus in certain *Drosophila* species immediately after mating. This insemination reaction results from a massive secretion by the vaginal wall (Patterson, 1946) in response to a nonsperm component of the ejaculate (Lee, 1950). Subsequently, Wheeler (1947) screened a large number of species for the occurrence of this insemination reaction, and the findings were used to assign *Drosophila* species to three categories, depending upon the size and duration of the mass. Class I contains species in which no mass is observed. Species exhibiting a slight uterine reaction following insemination were grouped in Class II. Those species showing a strong reaction and mass of long duration were assigned to Class III. Samples of the species in each group are shown in Table 1.

A number of interpretations have been offered as to the significance of the reaction mass. Because interspecific matings were observed to result in masses of greater size and duration than those produced following intraspecific matings, Patterson (1946) and Patterson and Stone (1952) suggested that the insemination reaction functioned as a postcopulatory reinforcement of reproductive isolation between related species. These same investigators offered the alternative explanations that the reaction is somehow preparatory to fertilization or egg production or that it acts as a copulatory plug. These explanations, however, fail to account for

why intraspecific matings result in a mass that lasts for 10 hours in some species, while in other species no reaction mass is ever observed after mating. Grant (1983) reported that the reaction mass occurs independently of copulation duration, leaving the question of the interspecific variability in the reaction unresolved.

The present study was undertaken to test the hypothesis that the interspecific variability in the occurrence of the insemination reaction is related to interspecific variability in the appearance of ejaculate-derived substances in female soma and eggs. Markow and Ankney (1984) compared two *Drosophila* species for the occurrence of male material contributions to females and offspring. Male *D. melanogaster* were not observed to provide a significant material contribution to females. However, in *D. mojavensis,* substances derived from the male ejaculate were detected in large quantities in female somatic tissue and ovarian oocytes. Inspection of Table 1 reveals that *D. melanogaster* is a Class I species, while *D. mojavensis* is in Class III. If the mass is related to the presence of male-derived material in females and progeny, other species of Class III are expected to show male-derived label in female somatic tissues and oocytes, while Class I species should not. To examine this expected relationship, we examined 19 species in the three different classes for the presence of male material contribution.

Materials and Methods

Five species from Class I, six from Class II, and eight from Class III were examined for the size and duration of the insemination reaction. All but three of these had been classified earlier by Patterson and Stone (1952). The remaining species (*D. grimshawi, D. mettleri,* and *D. nigrospiracula*) were examined by us prior to testing for the transfer of label, and assignment to classes was made according to Patterson and Stone (1952). The species and their classifications are shown in Table 1.

Cultures of each species were obtained from the *Drosophila* Species Stock Center at Bowling Green State University. Fly strains were cultured in uncrowded conditions on banana medium at 24° ± 1°C on a 12L: 12D photoperiodic cycle. The only exception to this procedure was the rearing of *D. grimshawi* on Wheeler-Clayton medium and subsequent transfer to sand jars during later larval instars.

Males were labeled by collecting 50 eggs from females of each species. All 50 eggs were placed in a vial containing 1.5 g of culture medium prepared with 70 μCi of a mixture of ^{14}C labeled amino acids (ICN 10147). Virgin males were separated upon eclosion and stored under low densities (five per vial) until they were mated to virgin unlabeled females.

Reproductive maturity is reached at different ages in different species, and in some species males become reproductively mature later than females. For each species, matings were performed at ages when flies of both sexes achieve reproductive maturity (previously determined by Markow [1982, 1985]). Labeled males were aspirated individually into shell vials containing single females. Copulating pairs were undisturbed. After males had dismounted, they were removed, and females were transferred to new vials to await dissection either immediately or six hours after copulation.

Females were dissected in insect Ringer's solution. Immediately after mating, reproductive tracts (minus ovaries) from three females of each species were collected. Other females were dissected six hours after mating, and the following parts were separated: reproductive tracts minus ovaries, ovarian oocytes, and remaining somatic tissues. These parts were washed in a second dish of Ringer's solution. For each of the three separated structures, parts from three females were pooled and placed in a scintillation vial containing 100 ml of Scintigest tissue solubilizer, and crushed with glass rods prior to digestion for 24 hr at 50°C. Glacial acetic acid (2.5 ml) was added to neutralize the solutions, and then, 5 ml of Scintiverse I scintillation fluid was added. Each vial was allowed to sit for an additional 24 hr at 24°C prior to counting. For each species, three replications were performed and counted. Control tissues from females that had been mated to unlabeled males were obtained and prepared for counting by identical procedures.

Results

The levels of radioactivity detected in female body parts are shown in Table 1. The unlabeled control samples for each species showed virtually identical amounts of background radioactivity. Table 1 presents the control values for *D. melanogaster.* Females of five species (*D. grimshawi, D. simulans, D. hydei, D. nigrospiracula,* and *D. melanogaster*) did not differ significantly from controls for the amount of label detected in their somatic tissue or ovarian eggs. *D. prosaltans* females did not differ from control females with respect to radiolabel in their oocytes but showed a significant amount of label in their somatic tissues. While *D. pseudoobscura* females showed the presence of label in oocytes, no significant amount was found in their somatic tissues. For the remaining species, the amounts of label detected in oocytes and somatic tissues were strikingly high, even after only six hours. For those species in which little or no label appeared, additional flies were sampled for label up to 48 hours after mating, in order to detect any slow uptake and metabolism of label. No differences were found beyond those reported in Table 1. If flies did not show labeling after six hours, no significant label was found later. Both the mean disintegrations per minute for ovarian eggs (oocytes) and the mean disintegrations for body parts from different species were compared by the Duncan multiple-range test at $\alpha = 0.05$, both before and after arcsin transformation of the data. Species assigned to different subsets show significantly different amounts of label (Table 1). All Class I species were assigned to a single subset. There was more variation among Class II and Class III species, but all Class III species differed significantly (showed more label) than Class I species. Class II species generally showed amounts of label intermediate to Classes I and III.

Discussion

The subset groupings support the suggestion that the reaction mass is associated with the transfer of labeled material from ejaculate into female tissues. In Class I species, male label was limited to the reproductive tract in females. Class III female tissues incorporated the largest amount of male-donated label.

D. robusta was reported by Wheeler (1947) to show no reaction mass following copulation and was there-

TABLE 1. ^{14}C radiolabel (disintegrations per minute ± SE) in reproductive tracts immediately after copulation and in reproductive tracts, oocytes, and body parts six hours later in various *Drosophila* species, classified according to insemination reaction (see text). Counts per minute were converted to disintegrations per minute following a standard quench curve. Statistics were performed on untransformed values and after arcsin transformation, with similar results; differences between species were examined with a Duncan multiple-range test at $\alpha = 0.05$ for each variable. Subset membership by this test is indicated by the letter following the mean radioactivity for that variable.

Species	Class	Reproductive tracts (0 hr)	Reproductive tracts (6 hr)	Oocytes	Subset	Body parts	Subset
D. melanogaster	I	1,112 ± 163	130 ± 16	46 ± 8	a	54 ± 8	a
D. simulans	I	965 ± 59	72 ± 9	44 ± 7	a	43 ± 7	a
D. nigrospiracula	I	385 ± 29	165 ± 18	51 ± 5	a	46 ± 8	a
D. hydei	I	313 ± 25	91 ± 13	46 ± 7	a	53 ± 7	a
D. grimshawi	I	312 ± 30	151 ± 19	43 ± 4	a	45 ± 6	a
D. affinis	II	320 ± 19	300 ± 22	58 ± 5*	a	72 ± 8*	b
D. prosaltans	II	627 ± 44	201 ± 20	43 ± 6	a	69 ± 7*	b
D. pseudoobscura	II	640 ± 32	611 ± 20	71 ± 8*	b	54 ± 6	a, b
D. persimilis	II	618 ± 52	598 ± 52	85 ± 12**	b, c	80 ± 13*	b
D. robusta	II	1,380 ± 179	998 ± 100	101 ± 17**	c	123 ± 19**	c
D. mettleri	II	745 ± 51	399 ± 24	180 ± 20**	c	119 ± 16**	c
D. arizonensis	III	1,991 ± 140	613 ± 79	92 ± 12**	c	142 ± 19**	c
D. immigrans	III	2,034 ± 198	1,380 ± 142	132 ± 27**	c	162 ± 29**	c
D. texana	III	646 ± 82	298 ± 60	220 ± 49**	d	189 ± 60**	c
D. americana	III	359 ± 45	280 ± 31	61 ± 7*	b	82 ± 9**	b, c
D. virilis	III	4,082 ± 269	750 ± 86	200 ± 19**	c, d	178 ± 21**	c
D. funebris	III	1,002 ± 145	414 ± 60	79 ± 13*	b, c	91 ± 17**	c
D. mojavensis	III	2,401 ± 217	411 ± 58	150 ± 17**	c	238 ± 33**	d
D. giberosa	III	398 ± 62	241 ± 55	65 ± 9*	b	63 ± 5*	b
Cold control (*D. melanogaster*)	—	—	48 ± 6	46 ± 7		52 ± 5	

* $P < 0.05$ (different from cold control).
** $P < 0.01$ (different from cold control).

fore assigned to Class I. Yet, in our study, females of this species showed male-derived label in the tissues. We reexamined *D. robusta* for the presence of the insemination reaction, with an interesting result. Ten females were mated and then dissected. Three of the females showed a mild insemination reaction characteristic of Class II species and had motile sperm in their storage organs. However, seven females showed no reaction mass or sperm, suggesting a high frequency of pseudocopulation in laboratory strains of this species. The strain of *D. robusta* we employed is clearly more similar to Class II species, and we reassigned it to that group.

How can the interspecific variability in the reaction mass and the correlated male-derived material be explained? It could be argued that the reaction mass is a mating plug originating as a male adaptation to avoid sperm competition in those species with multiple mating by females. Markow (1982, 1985) has surveyed remating frequencies and average progeny production per mating across a large number of species. *Drosophila* species vary greatly with respect to the frequency of female remating. Even in Class I, females of some species, such as *D. hydei* and *D. nigrospiracula,* will remate several times in one morning, so that several sires may be represented by progeny in a single brood (Markow, 1982, 1985). Females of other Class I species, such as *D. melanogaster,* show remating intervals of several days (Fowler, 1973). In *D. mojavensis,* a Class III species, the great majority of females will not remate until about 18 hours after the disappearance of the mass (Markow, 1982). Thus, interspecific differences in frequency of remating and sperm utilization are not tightly associated with the occurrence of the insemination reaction. Also, it has been documented that some *Drosophila* species use other means to impede female remating, such as the production and transfer of antiaphrodisiac pheromones in *D. melanogaster* (Scott, 1986).

Attempts to explain the reaction mass by correlating it with other components of mating have been unsuccessful. For example, although *Drosophila* species vary tremendously in copulation duration (Spieth, 1952), no relationship appears to exist between copulation duration and the subsequent appearance of the uterine mass (Grant, 1983). Additionally, perusal of Table 1 reveals that the female uptake of the male donation is independent of ejaculate size. Male *D. melanogaster,* for example, deliver more labeled material to female tracts than do *D. texana* males, but the latter show a tremendous contribution to oocytes and female soma, while the former show none. It should also be noted that species with relatively large body sizes, such as *D. nigrospiracula, D. grimshawi, D. robusta,* and *D. giberosa,* occur in all three classes. There is also no evidence that species in any one group produce more offspring per mating relative to another (Markow et al., 1978; Markow, 1982, 1985). Finally, species exhibiting

the uterine mass are scattered throughout several subgenera (*Drosophila, Hirtodrosophila,* and *Sophophora*) of *Drosophila.*

Theoretical work by Trivers (1972) suggests the importance of relative parental investment in shaping variability in mating systems. One prediction is that when males provide some material benefit to females, in addition to their genetic material, selection should favor some sort of adaptation to assure their paternity, and indeed, the relative reproductive investment made by each sex has been empirically demonstrated to be associated with certain behavioral and physiological reproductive adaptations in numerous species (Thornhill and Alcock, 1983). One such adaptation frequently seen in insects involves a copulatory plug formed in the female reproductive tract after mating, which prevents immediate remating by the female. The finding that the reaction mass is associated with the appearance of male-derived substances is what would be predicted if Patterson and Stone's (1952) suggestion that the mass acts as a mating plug is correct.

The single factor consistently associated with the mass is a significant material donation by males. This is not to imply that the mass is only comprised of male ejaculate substances. On the contrary, Lee (1950) was able to induce a slight reaction mass by injecting Ringer's solution into female tracts. The materials transferred to female tissue have not yet been characterized, nor have any potential female contributions to the mass. It is unclear whether the labeled substances detected in females are identical to those in the male ejaculate or whether they have been modified in some way. Many proteins are manufactured in the *Drosophila* male accessory glands, and these proteins show notable electrophoretic variability within and among species (Whalen and Wilson, 1986; Chen, 1976). Any relationship between this variability and the incorporation of male-derived materials into female tissues remains to be established.

In *D. melanogaster,* male accessory-gland secretions have been shown to stimulate oviposition and apparently migrate from the female reproductive tract in order to do so (Chen and Buehler, 1970; Leahy and Lowe, 1967). If this is the case, these substances should be detectable in female tissues. Using a different label, ^{35}S, we have been able to detect low levels of a male-derived substance in *D. melanogaster* females (Markow, unpubl.), which may represent the oviposition-stimulating sex peptide of Leahy and Lowe (1967). While the amount of this ^{35}S-labeled material is far less than the amount transferred in those species showing a reaction mass, the observation suggests a mechanism to explain the occurrence of male donation in those species in which it is evident. Male substances may have originally been selectively transported into female somatic tissues to induce oviposition, thereby increasing fitness of the males. Subsequently, in some species, females may have evolved means of exploiting that transport mechanism in order to obtain an additional source of nutrients. In order to avoid exploitation in species in which females remate, male stimuli evoking production of the mass may have then evolved as a male adaptation. It could be argued that, if female attractiveness is temporarily reduced by the presence of the reaction mass, mated females may save energy associated with the avoidance of courting males. While no direct tests have been made of the ability of the reaction mass to hinder mating, we have never observed females of Class III species remating immediately, while females in several Class I species have been shown to do so (Markow, 1982, 1985; Fuerst et al., 1973). In this case then, if the reaction mass delays remating, it could be argued to be a female adaptation: if female attractiveness is temporarily reduced by its presence, mated females may save more energy by avoiding courting males than they might gain from further incorporation of material donated in a second mating. However, it is difficult to see how a larger gain in nutrients (Class III) would place a greater penalty on rapid remating. Hence, the transferred materials must be presumed to lack a nutritive role if the reaction mass is both a female adaptation and a copulatory plug. Those species that are classified as having a more moderate plug appear to be intermediate with respect to the amount of male-derived label that appears in females after mating, suggesting that these adaptations may be still evolving in these species.

A correlation between the reaction mass and male donation suggests the possibility of a relationship between the donation and male and female fitness. Some evidence exists that male donations increase female fitness. For example, Turner and Anderson (1983) reported that, under food-stress conditions, *D. pseudoobscura* females that were allowed to mate repeatedly showed higher fertility, even after allowance was made for their decreased longevity relative to females whose matings were restricted; *D. pseudoobscura* oocytes incorporate male material. Finally if the donation is costly for males to produce, male discrimination prior to mating would be expected in Class III species. We are currently testing this prediction.

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FEMALE MATE PREFERENCE AND THE EVOLUTION OF FEMALE-LIMITED BATESIAN MIMICRY

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The difficulty in developing a general theory for the evolution of Batesian mimicry is that mimetic species differ in the distribution of mimetic and nonmimetic forms between the sexes and may also be monomorphic or polymorphic. In butterflies, both sexes may be mimetic and monomorphic (e.g., *Limenitis arthemis astyanax*) or polymorphic (e.g., *Eurytides lysithous* [West, 1985]), or mimicry may be limited to females, again either monomorphic (e.g., *Speyeria diana*) or polymorphic (e.g., *Papilio dardanus* [Ford, 1936]). The latter species has nonmimetic females in some populations. Certain situations are not found, however: male-limited mimicry (although Vane-Wright [1984] suggests the possibility in *Euthalia monina* [Fleming, 1975 pl. 47] and male mimetic/nonmimetic polymorphism.

The two-step model of Fisher (1958), coupled with a negative frequency-dependent advantage of a new mimetic form, suffices to explain the evolution of monomorphic and polymorphic Batesian situations with no sex-limitation. It does not, however, explain female-limited mimicry or the absence of male-limited mimicry.

Belt (1874 pp. 384–385) first suggested that female-limited mimicry evolves because females prefer males of the "primordial" color. An incipient mimetic male form would therefore be opposed by sexual selection, and modifiers that suppress the mimetic phenotype in males would be selected (Turner, 1978). Since males are less discriminating in courtship and will approach any optical stimulus that appears to be sexually positive (Magnus, 1963), the female phenotype is not so constrained and can evolve mimicry. For a detailed review of the dynamics and evolution of mimicry, see Turner (1977, 1978, 1984).

Belt's theory has one serious weakness in the lack of evidence for female mate choice. Silberglied and Taylor (1978) showed that *Colias eurytheme* females preferred males that reflected ultraviolet light, and Turner (1978) and Rutowski (1984) believed that sexual selection by females is most likely responsible for the evolution of female-limited mimicry in butterflies. In

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