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Postcopulatory Behavior of Tephritid Flies

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Abstract

Mating produces profound changes in the behavior of female flies, such as an increase in oviposition, reduction in sexual receptivity, increase in feeding, and even excretion. Many of these changes are produced by copulation, sperm, and accessory gland products that males transfer to females during mating. Our knowledge on the function of the male ejaculate and its effect on female insects is still incipient. In this article, we review peri- and postcopulatory behaviors in tephritid flies. We address the effects of male copulatory behavior; copula duration; and the male ejaculate, sperm, and accessory gland products on female remating behavior. Many species from these families are pests of economic importance; thus, understanding male mating effects on female behavior contributes to both developing more effective environmentally friendly control methods and furthering our understanding of evolutionary implications of intersexual competition and sexual conflict.

INTRODUCTION

Ejaculate: sperm plus seminal fluid produced by the male and transferred to the female

SFPs: seminal fluid proteins; produced in tissues of the male reproductive tract

MAGs: male accessory glands

SIT: sterile insect technique

Copulation includes several steps, such as intromission and ejaculate transfer, and may be accompanied by bouts of copulatory courtship. There is now wide consensus that opportunities for sexual selection do not end with mating, but instead continue during copulation and beyond. During copulation, male insects transfer sperm and seminal fluid proteins (SFPs), which are composed of accessory gland proteins from the male accessory glands (MAGs), as well as other molecules, including small quantities of carbohydrates; some lipids; uric acid, prostaglandins, and juvenile hormones; and peptides from the male apodeme and the ejaculatory bulb (111) (**Figure 1**). Female insects store sperm and receive SFPs, which can be found throughout the female body after copulation (114). Mechanical stimuli during copulation, SFPs, and/or sperm can induce postcopulatory changes in female phenotypes (111) (**Figure 1**). The roles of copulation, sperm, and SFPs in influencing female behavior and reproduction are particularly interesting within the Tephritidae family, as many species within this family are pests of worldwide economic importance. They threaten food security across developed and developing countries, hindering fruit exports and commercialization.

Investigating sexual behavior and reproduction, including postcopulatory processes, is key to understanding pest species and controlling them through environmentally friendly methods. Tephritid flies are particularly relevant because of the development of the sterile insect technique (SIT) for their control. The SIT consists of the mass production, sterilization, and release of the target insect into infested areas, where the sterile males mate with wild females, transferring dominant lethal mutations in the sperm and thus reducing population levels. The SIT is an
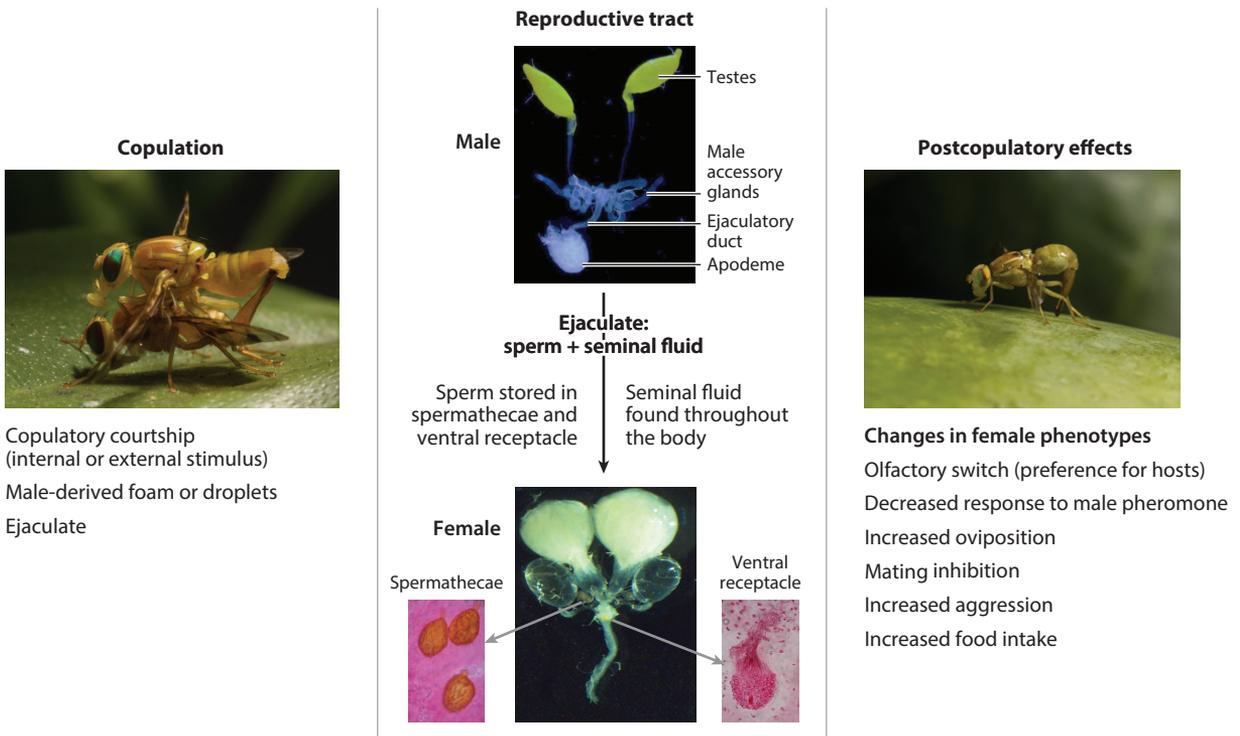

Figure 1

Stimuli or substances received during copulation and postcopulatory changes in female tephritids.

environmentally friendly means of control that has been used successfully to control and even eradicate pests (97). One of the disadvantages of the SIT is that mass-reared sterile males have lower mating success than wild males (108). However, most of the studies in this field have traditionally focused on precopulatory mating behavior, and it is only now that a significant amount of literature is available on sexual selection processes during and after mating (for a thorough review of techniques that disrupt mating, see 150).

In tephritids, mating is associated with an increase in female food intake, an increase in oviposition, decreased aggression, and increased immune response (24, 40, 59, 81, 92, 100, 119, 123), while receiving SFPs has been associated specifically with changes in female olfactory preferences and an inhibition of female remating (1, 74, 76, 116). In this article, we critically review the literature on the postcopulatory behaviors of tephritid flies. We address topics such as pericopulatory behavior, copula duration, female remating, and the male ejaculate and its components. We end with practical implications of these studies and suggestions for future research.

COPULATORY BEHAVIOR

During copulation, tephritid males maintain internal and external contact with the females, which has been interpreted as copulatory courtship. In sharp contrast to very detailed descriptions of precopulatory courtship (e.g., 32), there have been relatively few studies on copulatory courtship. Natural history descriptions of the mating behavior of many species stop at mating; however, a variety of behaviors continue during mating. For example, in a bamboo fly, *Anoplomus rufipes*, adults can fly and feed during copulation (78), and in the antlered fly, *Phytalmia mouldsi*, males contact guard females by repelling intruders, clasping the bases of the female wing in a wing lock, and remaining mounted as the female lays eggs (44). Males can provide stimuli during copulation, such as wing buzzing, body rocking, surstyli nipping, aculeus raising, hind tarsi rubbing, and palpitation or tapping the vertex of the female's head and dorsal anterior thorax with the proboscis. Males' foretarsi, mesotarsi, and metatarsi can touch and tap various portions of the female body. There can be thrusting of the aedeagus, as well as rubbing and rapid wing movements (18, 28, 45, 78). In *Anastrepha ludens* (42) and *Anastrepha obliqua* (see 18), males regurgitate a drop that is deposited on the female dorsum during mating. Female movement of legs up to their dorsum suggests that they are able to detect and possibly even transfer some of the components of the drop into their labella. In *Rioxa pornia*, males also produce a regurgitated foam; however, in this case, the male produces the foam and deposits it in the substrate, and the female feeds on it during mating (113). Sounds are usually emitted during the complex process of coupling, making individuals more vulnerable to predation (70, 144). We do not yet know if there are ecological or phylogenetic differences or resource patterns that can determine differences in the complex of pericopulatory behavior among species.

Internally, males perform a series of movements that include insertion of the genital rod into the vaginal sac and, in some species, inflation and frequent folding and unfolding of the sac of the distiphallus (28, 46, 48). The mechanical stimulus generated by the male during mating increases oviposition and levels of the immune enzyme phenoloxidase in females, even if the female does not receive an ejaculate (59, 119). This means that the female receives considerable internal and external stimuli that promote postcopulatory female responses beyond the effect of receiving sperm or SFPs. The male aedeagus and the female genital tract are long; thus, the process of intromission can be complex (28). The male aedeagus ranges from 2 mm to 6.1 mm in length depending on species (43, 73). Nevertheless, the aedeagus cannot directly reach the spermathecae and thus deposits sperm into the ventral receptacle and the anterior oviduct (54, 90, 148, 155). Thus, for sperm storage to occur, sperm needs to migrate to the spermathecae via peristaltic contractions of the female (54, 148).

Copula Duration and Sperm Transfer

There is a great diversity in copula durations in tephritid flies (**Supplemental Table 1**). During copulation, sperm are transferred and stored by females. One common misconception is that copulas last as long as males are transferring an ejaculate. However, only sperm storage during the copula has usually been quantified, and we lack information on the rate of transfer of other components of the ejaculate, such as SFPs. Depending on strain, species, and context, a longer copula duration may indicate a more numerous sperm transfer, but no consistent pattern has been found (**Supplemental Table 2**). There are conflicting results among species and even among studies; in many studies of tephritids, no linear relationship between copula duration and sperm transfer has been found (e.g., 5, 37, 68, 104, 107, 153), strengthening the hypothesis that longer copulations do not necessarily translate into more sperm transferred and, indirectly, higher reproductive success. Nevertheless, in other studies, a positive correlation has been found (e.g., 5, 33, 30, 52, 98, 105, 134, 155). Differences in results among species and methodologies suggest that no generalization may be valid.

Furthermore, regular dissecting techniques can underestimate the number of sperm found due to sperm clumping (138), and thus, molecular assays such as the use of real-time qPCR will give us a more accurate picture of the association between time in copula and sperm quantity (30). Copula duration may have a positive relationship with sperm transfer during the beginning of the copula and, after a certain point, could be regulated by other factors such as mate guarding (63). One thing is clear: Time spent in copula is not solely used for sperm transfer. For example, in *Anastrepha fraterculus*, longer copulations are associated with an increase in female sexual refractory period, and this has been potentially attributed to the reception of MAG homogenates (5). Therefore, copula duration needs to be understood in terms of mechanical stimulation, receiving sperm and SFPs, and sometimes mate guarding (96, 160). Techniques that allow the detection of and determine the rate of transfer of SFPs will allow a more precise prediction of the relationship among copula duration, ejaculate transfer, and internal and external stimuli and the function of this relationship on postcopulatory effects.

Copula duration is highly variable, condition specific, does not always correlate with sperm transfer, and is probably under female control (see the sidebar titled Who Controls Copula Duration?). Of the many factors that affect copula duration, age seems to be the most consistent, as all studies have found copulations to be longer in older flies (**Supplemental Table 2**). Do higher-quality males have longer copulations? Quality has been measured in terms of diet, size, mating history, or irradiation. There are conflicting results, with longer copulations observed in

WHO CONTROLS COPULA DURATION?

There is increasing evidence that seems to suggest that copula duration is controlled by the female (41, 91). When females have been incapacitated by decapitation, copulas are longer (53, 109); in *Bactrocera tryoni*, these longer copulas extend throughout the night until the early morning, whereas a normal mating lasts up to 7 hours (109). There is also evidence that the female diet or size, rather than male diet or size, determines copula length (50, 153). In addition, longer copulations have been found for older, bigger, and unmated females, often with no effect of male condition (**Supplemental Table 2**). Furthermore, when two morphotypes from the *A. fraterculus* cryptic complex mated (crosses between males and females of Argentinean and Peruvian morphotypes), copula duration was shorter when females were from the Peruvian morphotype, regardless of male morphotype (11). Thus, utilizing copula duration as a measure of male mating success, condition, or laboratory adaptation (e.g., 49) is probably not ideal, as it likely reflects the female rather than the male condition.

males fed yeast hydrolysate, in wild males, or in fertile males only in some cases (**Supplemental Table 2**). Do longer copulations necessarily imply more sperm transfer, a gain in paternity, and a decrease in female receptivity? The simple answer seems to be that it depends on the species; for example, in only 55% of studies (five of nine) was copula duration found to be correlated with female remating (**Table 1**).

Sperm Storage Patterns—Evidence for Possible Female Cryptic Choice?

Females can store a large quantity of sperm; for example, *Bactrocera tryoni* females can store approximately 8,000 sperm (137, 138). There is variation in the morphology and the number of the spermathecae according to species. For example, *Rhagoletis completa*, *Rhagoletis pomonella*, and *Anastrepha* spp. have three spermathecae (43). Two spermathecae are joined by a single spermathecal tube (doublet), and the other spermathecae is a singlet on a different spermathecal tube. Species in the Trypetinae, Dacinae, Tephritinae, Terelliinae, Oedasiinae, Ceratitis, and Bactrocera genera, as well as *Rhagoletis solanophaga* and *Haywardina cuculi*, have only two spermathecae (43, 126, 127).

Aside from the spermathecae, females also store sperm in the ventral receptacle (VR), which is the fertilization chamber, a specialized organ composed of numerous alveoli-like structures (51, 52, 148, 159). Sperm storage in these structures ranges from 50% in *A. obliqua* to 3% in *B. tryoni* (**Figure 2**). The spermathecae are thought to be the long-term sperm storage organs, and the VR is thought to be the short-term sperm storage organ where sperm are utilized for fertilization (159). Given that females could have up to four different sperm storage organs (from two to three spermathecae and the VR), this gives them ample opportunity to bias paternity through female cryptic choice. One possibility is that females influence sperm storage by sperm dumping; for example, even after mating, some females, ranging from 57.9% to 2.9%, depending on the species, are spermless (108 and references within). Alternatively, the findings of aspermia could be due to misidentification of an empty storage organ if the VR was not examined (52); aspermia could also be due to male infertility.

Asymmetry in Sperm Storage

Most tephritids studied have shown differential sperm storage between storage organs, but no consistent correlation with male or female phenotypes is evident, although in *Ceratitis cosyra*, male age was found to influence asymmetry in sperm storage (124) (**Supplementary Table 3**). Differential sperm storage after female remating has been demonstrated in *Anastrepha suspensa*, a species with a singlet and a doublet spermathecae (41). In that species, sperm from the first male is more often stored in the doublet spermathecae, while the singlet spermathecae is more likely to have sperm from the second male. Further evidence of sperm storage asymmetry is evident in the fact that the VR is more likely to store sperm from both males than are the spermathecae. In *B. tryoni*, females store more sperm from the second mate in the spermathecae where fewer sperm from the first mate have been stored (137). In *Ceratitis capitata*, sperm storage asymmetry was 23.6% on average between the two spermathecae and was negatively related to the total number of sperm stored (153). In *A. ludens*, storage asymmetry has been found in wild-caught females: All mated females had sperm in the VR, but 23.3% of those females only had sperm in one of the spermathecae (155).

By differentially storing sperm, females can potentially bias paternity through cryptic choice, and there are still important questions as to how females can dump, absorb, or use sperm. Dhakal et al. (41) argue that sperm competition does not take place at all; instead, second male paternity is the result of sperm stratification and usage of the second male sperm in the VR, with replenishment from the spermathecae, which also favors storage of the second male sperm (105, 107). Similarly, in *C. capitata*, sperm from both males are initially stratified in the VR but eventually do mix over time (133); thus, sperm competition could occur. Cryptic female choice

VR: ventral receptacle; sperm storage organ in females, also known as the fertilization chamber

Table 1 Effects of copula duration, irradiation, and MAGs on female receptivity

Factors affecting female receptivity	Ceratitis capitata	Bactrocera dorsalis	Bactrocera tryoni	Anastrepha ludens	Anastrepha fraterculus	Zeugodacus cucurbitae	Anastrepha serpentina	Anastrepha suspensa	Anastrepha obliqua
Is copulation duration positively associated with renewal of female receptivity?	No (96)[a], Yes (130)	ND	No (68)	No (7)	Yes (5, 7)	Yes (80)	No (85)	Yes (41)	ND
Does aspermia or sperm quantity cause females to remate?	Yes (96), No (77)	ND	No (68, 115)	No (7)	No (5, 7)	No (80)	No (85)	ND	ND
Does irradiation cause sperm depletion in males?	Yes (134, 151)[b], No (4, 29)	ND	Yes (68)	No (7)	No (8, 16)	Yes (80)	ND	ND	ND
Are MAGs involved in remating inhibition?	Yes (75)	ND	Yes (116)	No (10)	Yes (1)	Yes (80)	ND	No (87)	Possibly (104)
Do irradiated males have lower capacity to inhibit female remating?	Yes (96, 57)[a], (79), No (4, 75, 160)	Yes (140)	No (68, 115)	No (20, 33)	No (8)	No (66, 80, 154) Yes (139)	Yes (85)	ND	No (56)
Do irradiated males induce shorter refractory periods?	Yes (160)	No (140)	No (68)	No (20) Yes (128)	No (8)	No (80, 139)	ND	ND	ND

[a] Studies used sterile mass-reared versus wild males; that is, they evaluated the effects of irradiation plus mass rearing.

[b] Compare to Reference 152.

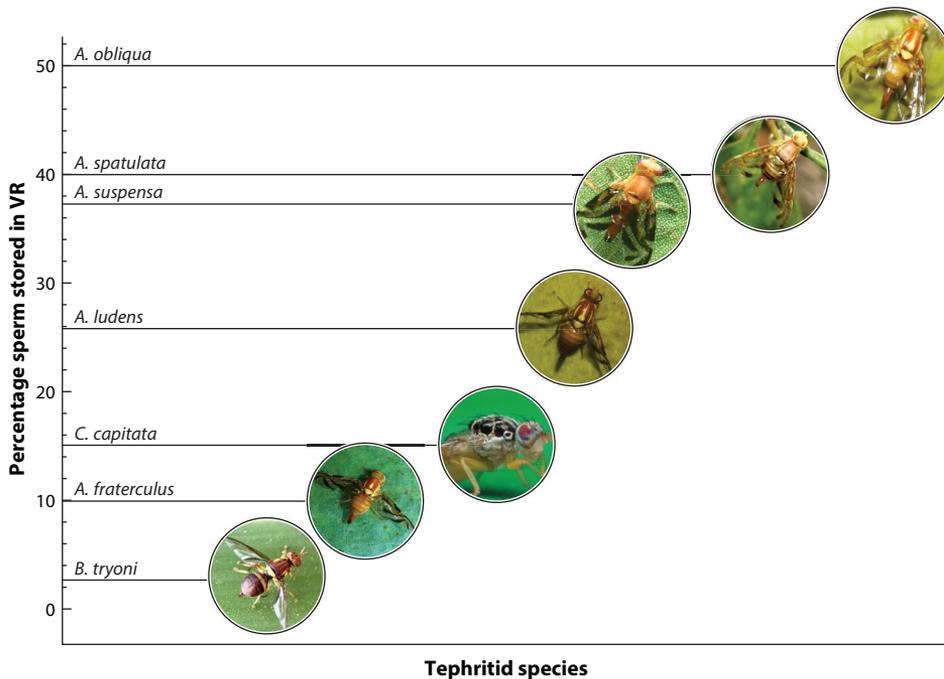Abbreviations: MAG, male accessory gland; ND, no data.

Figure 2

Percentage of sperm stored in the ventral receptacle (VR) for tephritid flies. Photo of *Bactrocera tryoni* (107)
© Tony and Jenny Dominelli (CC-BY-NC 4.0). Photo of *Anastrepha fraterculus* (5) © Guillermo Menéndez
(CC-BY-NC 4.0). Photo of *Anastrepha ludens* (105) © Juan Cruzado Cortés (CC-BY-SA 4.0). Photo of
Anastrepha suspensa (52) courtesy of Florida Department of Agriculture (CC-BY-SA 4.0). Photo of *Ceratitis
capitata* (159) courtesy of Santiago Murillo Dasso. Photos of *Anastrepha spatulata* (105) and *Anastrepha obliqua*
(105) courtesy of Maurilio López-Ortega.

could also still take place, with females quickly using sperm from the VR and selectively storing
sperm long term in certain spermathecae.

For the tephritids studied to date, there seems to be differential degrees of sperm mixing
when a female remates, with initial stratification, then mixing, leading to predominance of the
first male in *B. tryoni* and predominance of the second male in *R. pomonella*, *A. suspensa*, *C. capitata*,
and *A. ludens* (41, 99, 133, 136, 161). In contrast to *C. capitata*, in *A. ludens*, predominance of the
second male does not decrease with time (133, 161). One conflicting result for *C. capitata*, where
sperm precedence was not so clear to interpret, was possibly due to differences in the quantity
and quality of the ejaculate from the males evaluated (transgenic versus wild males) (118).

REMATING BEHAVIOR: FEMALE RECEPTIVITY IS A CENTRAL POINT OF REPRODUCTION

While considerable, detailed information has been made available on the life history feeding and
oviposition behaviors of pest tephritid flies since 1865 (112), the copulatory or remating behavior
of females has often been overlooked (112, 135). As far as we know, one of the first papers that cited
postcopulatory behaviors in tephritids was written by Boyce (27), who reported frequent female
remating in *R. completa*, as well as copulation immediately after or even during oviposition. An
early study in *B. tryoni* observed females repelling other males after mating (21). The importance
of postcopulatory behaviors was not realized within the broader field of sexual selection until

much later (e.g., 47); even so, it is somewhat surprising that, given the considerable efforts that went into the description of and development of early control methods for these pests in those early contributions, the act of mating, and thus reproduction itself, was either not easily observed or not deemed important enough to warrant a description. A more thorough investigation of female remating and sperm precedence in tephritids began with notable early studies by Katiyar & Ramirez (77), Cavalloro & Delrio (31), Tsiropoulos & Tzanakakis (158), Zouros & Krimbas (168), and Shoukry (143) in the early 1970s, but a more thorough study of remating and second male sperm precedence was not conducted until the work of Teruya & Isobe (154), Itô & Yamagashi (72), Kuba & Soemori (81), and others appeared in the 1980s.

It is important also to distinguish between rematings with the same male (multiple matings) and remating with different males (polyandry). In some species, multiple mating with the same male has been observed in field conditions (71). For example, in *Campiglossa genalis*, the male remains mounted on the female and repeats matings from two to seven times in a single event; in contrast, in *Dioxyna sororcula*, while there are three to four matings with the same male per mating episode, females lay one to two eggs in between matings (69).

There are two variables to be considered in studying female postcopulatory remating behavior: first, the propensity of a female to remate with a male, and second, the time taken to remate, that is, the sexual refractory period. Female decisions on these two variables may differ. That is, females subjected to a particular type of male may not readily remate, yet given enough time, oviposition opportunities, and access to males, she may decide to remate (facultative polyandry). Thus, it is important to consider which aspect of the remating behavior we are studying. Additionally, as pointed out by Shelly (139), most remating experiments are short and may not reveal wild female refractory periods, which have been shown to be longer than those of laboratory-reared females (93). Furthermore, we should strive to distinguish between a male's ability to inhibit females from remating and female choice to remate. It may be difficult to discern between these two processes, and they may be correlated, but ideally, we would need to distinguish between female choice to remain polyandrous or not, accepting or rejecting an additional mating, and male ability to inhibit female remating either through transfer of the ejaculate or through copulatory courtship. Experimentally, if we vary male phenotype, keep female phenotype constant, and observe whether a female remates, then we could interpret the observation as either a male's ability to inhibit female remating or a female's likelihood to remate. Describing remating behaviors through one or the other lens may shape our understanding of female sexual receptivity.

Polyandry

Polyandry refers to a female mating with more than one male; however, within polyandrous species, there is a wide range of behaviors, from mating twice or thrice in an individual's life-time (many *Anastrepha* and *Bactrocera* species) to mating several times within the same day (some *Ceratitis* and *Rhagoletis* species) (**Supplemental Figure 1**), and female refractory period can range from a few minutes to 63 days (**Supplemental Figure 2**). Despite the different fitness conse-quences that there may be for a female mating twice as opposed to many times, all of these species are categorized as polyandrous. Most of our knowledge on remating and paternity by multiple males comes from females that only mate twice. Furthermore, the percentage of females remating and the length of the refractory period depend on numerous factors, such as age; diet; irradiation; host availability; origin; and treatment with substances such as juvenile hormone, methoprene, cue lure, ginger root oil, or methyl eugenol (**Table 2**). This variation can be further confounded by experimental factors such as whether females were kept continuously with males and the length of the observation of the refractory period. Thus, some remating rates may not reflect actual rates found in nature, especially if females are unlikely to face continuous pressure to mate with other

Table 2 Factors influencing female remating, sperm quantity, and MAGs.

Factor or male condition	Remating propensity	Refractory period	Sperm quantity	MAGs
Laboratory or mass-rearing	Increase (91, 93, 96, 129)[a] No effect (66)	Decrease (6)	Decrease (33, 71)	ND
Domestication (number of generations)	Increase (14, 102)	ND	Increase (102)	ND
Irradiation	See **Table 1**	See **Table 1**	Decrease (33, 115) No effect (4, 64, 80)	Decreased effectiveness of MAGs in inhibiting female receptivity (1) Increased effectiveness of MAGs in inhibiting female remating (4) No effect on ectodermal MAG size; increase in mesodermal MAG size (100 Gy dose) (4)
Access to oviposition substrate	Increase (3, 86, 146) No effect (85)	No effect (3)	ND	ND
Consecutive matings	No effect (9) Increase (104)	Increase (20)	Prudent allocation (104, 115) Decrease (7)[b], (58)[c], (9, 137) Increase followed by decrease (29)	Decreased size (9)
Protein-fed male	Decrease (5, 23, 57, 67, 106, 167)	Increase (5, 19, 167)	Increase (5, 106) Decrease (23) No general agreement (63)	Increased effectiveness of MAGs in inhibiting female receptivity (1) Increased size (13, 110, 122, 120, 162, 163)
Male age	Decrease with increasing male age (142)[d] Decrease and then increase (57)[e] Increase (121)[f] No effect (37)[g], (106)[h], (2)[i]	ND	Decrease (106)[h], (151)[j], Increase (37)[g] No effect (101)[k], (4)[l]	Increase in size (121)[f], (162)[m], (163)[n], (110)[o]
Female age	Decrease (2)[i]	ND	NA	NA
Ginger root oil–exposed males	Decrease (95) No effect (141)	No effect (95)	ND	ND
Methoprene-fed males	Increase (8) No effect (12, 67, 120)	Decrease (8)	Increase (120)	Decreased effectivity of MAGs to inhibit female receptivity (8) Increase in size (120) No effect on size (13)
Methyl eugenol–fed males	ND	ND	Decrease (122)	No effect on size (122)
Raspberry ketone–fed males	No effect (15)	ND	ND	ND
Cue lure–fed males	Decrease (82)	ND	ND	ND
Males treated with *Bauveria bassiana*	ND	ND	Decrease (98)	ND

[a]Result evident only in the second generation of lab males.

[b]Decrease occurred in the third copulation.

[c]The study did not report whether differences were significant.

[d]3–4-days-old versus 5–10-days-old males.

[e]4, 11, or 18 days old.

[f]6, 9, 21, and 57 days old.

[g]4 to 20 days old.

[h]8 to 18 days.

[i]14–27 days versus 64–84 days old.

[j]3 to 11 days old.

[k]10 to 70 days old.

[l]3,4, and 5 days old.

[m]1 to 21 days old.

[n]1 to 15 days old.

[o]2 to 28 days old.

Male condition (e.g., protein-fed, age, ginger root oil) refers to the first male mating with females. Abbreviation: MAG, male accessory gland; NA, not applicable; ND, No data.

males. Molecular studies on remating using females captured in the wild are probably more accurate (22, 25, 26, 149), although they rely on small sample sizes, there can be variability between populations, and they may depend on the selectivity of traps favoring mated females.

From an applied perspective, the factors causing a female to remate are particularly relevant, as they could increase the reproductive potential of the pest and impact control measures. The one factor that consistently is reported across species as reducing female propensity to remate is mating with a male that has fed on a protein source (**Table 2**); thus, post-teneral treatments for sterile males including protein in the diet should be considered. For species that are controlled through the SIT, female remating behavior has implications for control measures such as release ratios. It is often assumed that females will be more likely to remate when they mate first with a sterile male, presumably as a result of sperm depletion. However, not all species suffer from aspermia as a result of irradiation, and aspermia or low sperm numbers are not correlated with a higher likelihood of female remating in all species (**Table 1**). While in *C. capitata* females mating with irradiated males are more likely to remate and have shorter refractory periods (57), this is not the case for other species (**Table 1**). Sterile males have been found to have lower capacity to inhibit female remating in only 37% of studies (6 of 16) and failure to inhibit female refractory period in only 25% of studies (2 of 8). Importantly, in *Bactrocera latifrons*, when females were artificially selected across 12 generations to mate with sterile males, this did not result in higher remating rates (84). Nevertheless, domestication, such as that which occurs for colonies in the SIT, does increase remating, but this depends more on female rather than male genotypes (14, 93).

Male Accessory Glands—Manipulation of Female Postcopulatory Behavior?

Following studies on other insects (e.g., 88), Cavalloro & Delrio (31) found that aspermic *Bactrocera oelae* males could inhibit female remating, thus suggesting that there are other products transferred during mating that could affect female receptivity (31). Indeed, other material aside from sperm was found to be transferred from the male to the female (147). Our understanding of the effect of the male ejaculate on females has improved considerably since these early studies. Some behavioral changes that differentiate a mated from an unmated tephritid female can be attributed to MAG homogenates. In *C. capitata*, *A. fraterculus*, and *B. tryoni*, females can display reduced sexual receptivity or a change in olfactory response from preferring male sexual pheromone to preferring host fruit volatiles (1, 74, 76, 116). In *A. suspensa* and *A. ludens*, MAG homogenates have not been found to influence sexual receptivity, oviposition, or olfactory response (10, 35, 87, 119, 122), and the whole ejaculate is needed to change the female's phenotypes. In the absence of mutant lines available for tephritids, and with the exception of a few studies using RNA interference (RNAi) (55), most studies on the effects of MAGs in tephritids use methodologies such as sperm depletion, aedeagal ablation, castration through removal of testes, injections, or sperm counting immediately in receptive females prevented from remating (68, 74, 80, 94, 96) (**Tables 1** and **2**). This highlights the opportunity and need for further molecular assays. The effects of origin, diet, age, post-teneral treatments, and consecutive matings on MAGs have been analyzed through the effects of MAGs on female behavior, protein content, or MAG size. The only consistent factor influencing female remating and MAGs across species seems to be inclusion of protein into the male diet, with all studies finding that it results in lower remating propensity, longer refractory periods, increased MAG size, and increased ability to inhibit female remating (**Table 2**).

Transcriptomic and proteomic characterizations of the male reproductive tissues and or ejaculate have revealed important genes and proteins in the MAGs and testes (34, 39, 62, 131, 145). However, only a few studies have determined whether these molecules are transferred to females. In MAGs, genes involved in multiple metabolic and cellular processes and ecdysteroid-related genes have been found, as well as genes expressing juvenile hormone–binding proteins (39, 131,

166). In *Bactrocera dorsalis*, the gene encoding the juvenile hormone–binding protein *Bdtakeout2* is highly expressed in the MAGs, and suppression of this gene decreases male mating success and decreases fertility of females (165). Genes involved in immune response have also been found in MAGs (39, 131, 145, 156, 166).

It would be useful to know which peptides are produced in the male reproductive tract and transferred to the female so they can be targeted using RNAi to control tephritid flies (89). For example, Gregoriou & Mathiopolous (61) silenced the sex peptide receptor in *Bactrocera oleae*, an homolog of Acp70 in *Drosophila*, and observed a decrease in oviposition in females fed with bacteria that produced double-stranded RNA for the sex peptide receptor gene, suggesting that this receptor plays a similar role in *B. oleae* as in *Drosophila*. RNAi techniques have also been used to knockdown genes involved in spermatogenesis, producing sterility (38). Other genes expressed in the MAGs, such as *yellow-g* and *troponin c* are also involved in oviposition (62), while genes regulated by MAG injections have been predicted to be involved in translational control, sugar regulation, diet detoxification, and lifespan determination (145). Orthologs to *Drosophila melanogaster* proteins associated with olfactory memory and odorant-binding proteins have been found in MAGs (34, 131) and mated females (60, 83). However, the extent to which MAG proteins can change female behavior in tephritids is still unclear, and we have little understanding of the evolutionary and fitness consequences of manipulating these proteins.

Male Accessory Gland Depletion

Decrease in the size of MAGs as an indicator of depletion of their contents after mating has been shown in *B. tryoni* (117), *A. obliqua*, and *A. ludens* (35). In *A. fraterculus*, a significant decrease in MAG size is not evident until a male has mated consecutively five times (9), indicating ejaculate partitioning or replenishment between matings. In contrast, in *B. dorsalis*, MAGs increased significantly in length and area immediately after copulation, suggesting that replenishment occurs during copulation (164), whereas in *B. tryoni*, replenishment occurs between 5 and 11 hours after mating (117), despite both of these species mating for more than 7 hours (**Supplemental Table 1**). More research is needed to understand MAG replenishment, cost of SFP production, and rate of MAG transfer to females.

Supplemental Material >

PRACTICAL APPLICATIONS AND FUTURE PERSPECTIVES

Why does a deeper understanding of mating matter? From the context of the SIT, a mating is important not only to induce sterility, but also to change female behavior and to potentially transmit pathogens or molecules that may aid in control. For example, sterile insects could be infected with entomopathogenic fungi or *Wolbachia* (150), which would then be transmitted through horizontal transmission during mating (157). Furthermore, response to traps based on fruit or protein odors may change according to female physiological condition (36); thus, knowledge of how mating affects olfactory response in pest females may aid in the development of more efficient traps.

Ideally release ratios should take into account female polyandry (**Supplemental Figure 2**) and sperm precedence, as they vary with species (see above). A female mating first with a sterile male and then potentially with a wild male may be more of a concern for species where the predominance of the second male appears to be maintained, such as for *A. ludens*, in contrast to *C. capitata*. Thus, release ratios could be fine-tuned depending on the specific species.

Despite the fact that researchers have studied female tephritid remating rates, sperm stores, and female fertility since at least the 1970s (31), we still know surprisingly little about the mechanism of how female sexual receptivity is inhibited or restored in tephritid flies. Information on most tephritids is lacking, even for those of economic importance. Some of the opportunities for

research can be seen in the absence of studies on various subjects (**Table 1**) and species (**Table 2**). Some of the topics still to be addressed are discussed below.

1. A functional analysis is needed of how both internal and external copulatory courtship influence female postmating behavior.
2. The use of metabolomics will aid our understanding of the interactions between male and female reproductive molecules, the relative contribution from each male and female organ to these interactions, and how reproductive molecules transferred from males are used by females (e.g., 132). Further biochemical and physical analysis of the male ejaculate as a whole and its interactions with the female reproductive system are needed.
3. There are very few studies on the function of the female accessory glands. Antibacterial peptides and ceratotoxins have been found in the female accessory gland (148), and they have been suggested to be involved in sperm storage and egg viability (125). However, these secretions should be further studied in terms of their biochemistry and their interactions with male-derived molecules.
4. Studies identifying the functions of individual SFPs using gene knockdown or knockout (e.g., through RNAi or CRISPR) are needed. A better understanding of neuropeptides, such as NTL peptides, implicated in modulating mating behavior could allow us to use these peptides as potential targets of control (65). Further studies utilizing RNAi for sterilization targeting spermatogenesis genes are promising (38, 89). Transcriptional studies on postmating responses have shown differences in gene expression of odorant-binding proteins and in immune response. Further insights are necessary into how plant- and/or host-mediated processes impact genes expressed as a result of mating (e.g., 83).
5. The effect of heat on sperm storage, heat shock proteins, sperm oxidative stress, and desiccation resistance (e.g., 103, 167) have hardly been studied in tephritids. How do the interactive effects of climate change affect male ejaculates and female postcopulatory behavior?

A full understanding of which postcopulatory phenotypes are induced through copulation, sperm, and/or SFPs is key to developing or improving control measures. Furthermore, in a field dominated by studies on *Drosophila* flies, the tephritid perspective can illuminate our understanding of female cryptic choice and contribute to sexual conflict theory.

SUMMARY POINTS

1. Females receive multiple stimuli during and after mating through the ejaculate or mechanical stimulation.
2. Copula length in tephritids seems to be under female control.
3. Female tephritids store sperm in spermathecae and the ventral receptacle with ample opportunity for biasing paternity.
4. Sperm-depleted males can still inhibit female remating.
5. Female remating decreases when first mating with males that have fed on a protein source.
6. Male accessory glands (MAGs) in certain, but not all, tephritid species are involved in inhibiting female remating and in changing olfactory responses from a preference for male pheromone to a preference for host volatiles.
7. Irradiation of males does not always lead to an increase in female remating.
8. Knowledge of the function of MAGs is still incipient in tephritids.

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