Dynamics of Sperm Transfer, Mixing, and Fertilization in *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) in Kenya

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ABSTRACT Two specialized structures affect sperm transfer, sperm mixing, and fertilization in mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant. One is a large muscle block attached to the ejaculatory duct sheath of the male beetle. It propels sperm into the spermatophore, driving it into the vagina. The muscular action further forces the sperm into the spermatheca, simultaneously inflating an air pump of the spermatheca. The spermatheca receives sperm from 3 to 4 males at a time, and the sperm are mixed rapidly and thoroughly by the air pump before fertilization. The other structure is a bell-shaped sac that surrounds the base of the seminal duct. It helps connect the spermatophore and the seminal duct during sperm transfer; it also receives mixed sperm from the spermatheca and leads them to the micropyles of the egg. Only 1 egg is fertilized at a time. Frequent and multiple matings during oviposition keep *C. montrouzieri* genetically diversified.

KEY WORDS mealybug destroyer, muscle block, spermatophore, fertilization sac, sperm mixing

FOR THE PAST 12 yr or so, I have studied the sperm priority or nonpriority in beetles. During this period, I found 3 beetles (2 carabids, 1 heterocerid) in which the sperm of a few different males (3–5) are thoroughly mixed in the females' spermathecae before fertilization (Kaufmann 1986, 1988, 1993). These beetles are all active and gregarious, living in precarious habitats such as the flood plains of braided rivers and along the margins of soda lakes.

The lady beetle *Cryptolaemus montrouzieri* Mulsant inhabits the trees of pricklypear cactus, *Opuntia Ficus indica* Mill, infested with its main prey, the mealybug, *Dactylopius opuntiae* Cockerell, in Kenya. *C. montrouzieri* is an active predator, but is neither gregarious nor an inhabitant of a precarious environment. Does this beetle practice sperm mixing? This study amply demonstrates that *C. montrouzieri* is not only a sperm mixer, but also has developed highly sophisticated organ systems for this purpose. Possible evolution of such a nonpriority system is discussed.

Materials and Methods

For the biological study, the eggs of *C. montrouzieri* laid in captivity were raised to adults in clear plastic boxes (20 by 15 by 10 cm) provided with pieces of pricklypear cactus leaves naturally infested by *D. opuntiae*. Adults were transferred to a wire-gauze cage (30 cm diameter, 45 cm height) placed on a basin containing soil in which infested cactus leaves were placed vertically.

Behavioral observations as well as specimen collection were made in a private plot in Nakuru where a few large pricklypear cacti (≈2 m tall) were planted as a part of the fence.

The collected specimens were dissected immediately under a stereoscope microscope to extract gonads, which were then examined with a compound microscope. Actual movements of sperm in spermatophore, spermatheca, and vagina were thus observed at first hand. The total number examined was 906 (580 males, 326 females).

Results

General Biology. *C. montrouzieri* eggs are, unlike those of other coccinellids, laid singly, a few (3–4) at a time and are hidden among the cottony cushions of *D. opuntiae*, the prey species of the lady beetle. The incubation period is 6–7 d in the laboratory at 20–23°C. The larvae molt 4 times, and the larval period lasts 34 d (1st stadium, 6–7; 2nd, 7–8; 3rd, 7–9; 4th, 10–13 d). They are camouflaged by fluffy white waxen coats, and feed not only on all stages of *D. opuntiae*, but also on their own eggs, larvae, pupae, and even newly emerged adults. The last instars, therefore, often move away from other still-feeding larvae and aggregate in small groups of 3 or 4 to a dozen on the undersides of noninfested cactus leaves. The pupal period is 10–13 d.

Table 1. Sex ratio of *C. montrouzieri* in different months

Year	Months	Males, %	Females, %	No. examined
1994	May	58	42	57
	June	68	32	85
	July	80	20	112
	Aug.	86	14	85
	Sept.	63	37	86
	Oct.	63	37	82
	Nov.	49	51	80
	Dec.	64	36	56
1995	Jan.	61	39	74
	Feb.	46	54	65
	March	56	44	62
	April	43	57	62

Newly emerged adults are sexually immature in both sexes. The sex ratio differs in different months (Table 1), but overall ratio was 64% males and 36% females. Sexual dimorphism is slight; the simplest distinction is that the prothoracic legs are orange in males, black in females. The beetles do not aggregate as adults, but individuals meet frequently, because they are very active during the sunny hours, and their movement is limited to a single large cactus or a bunch of smaller ones close together. *Opuntia Ficus indica* is not abundant in Nakuru. Adults live 2–3 mo and there are 5 generations per year.

Male Reproductive System and Sperm Transfer. Testes are originally paired but usually occur in a single bunch consisting of 18–26 circular follicles. Vasa deferentia and 2 accessory glands are paired (Fig. 1A). At emergence, testes are immature and composed of spermatogonia and sper-

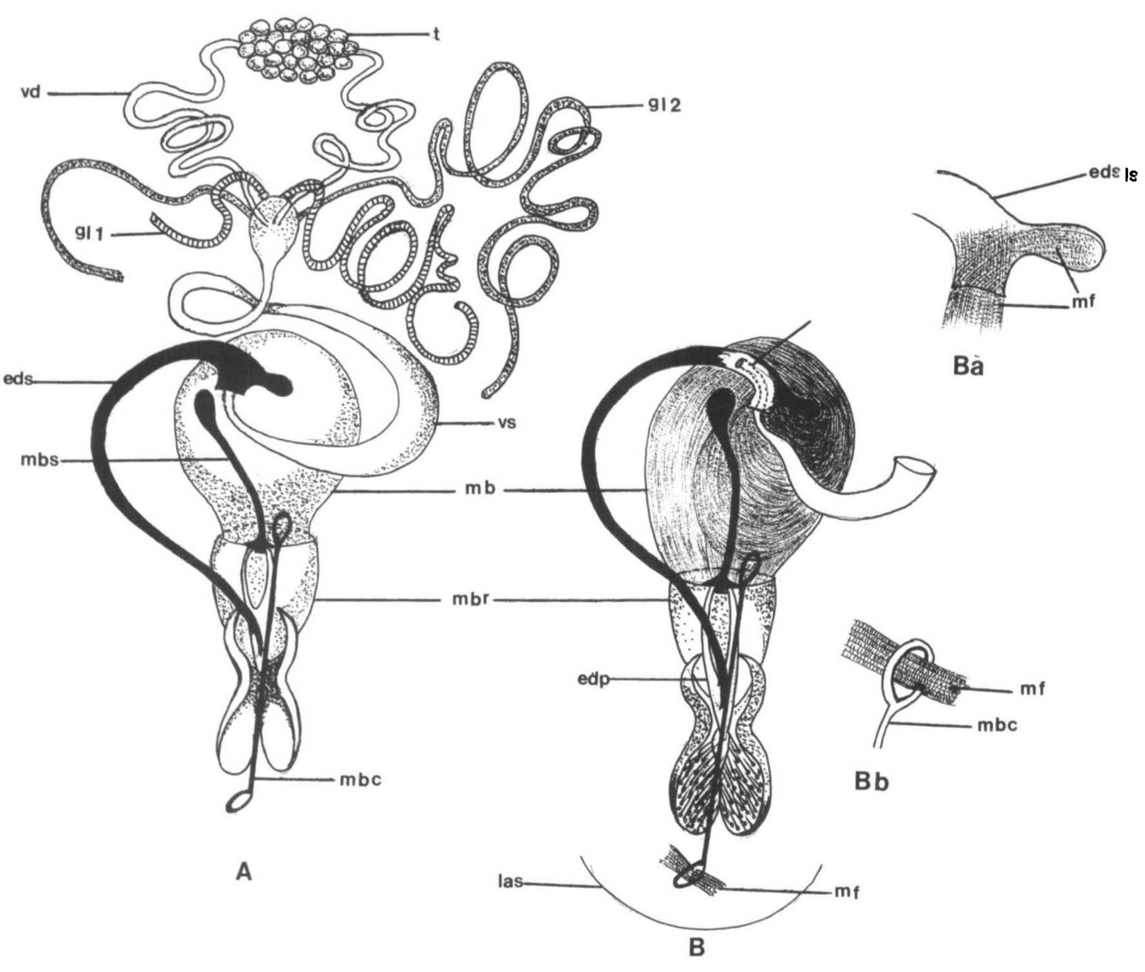

Fig. 1. Male reproductive system. (A) Entire system. t, Testes; vd, vas deferens; gl 1, accessory gland 1; gl 2, accessory gland 2; eds, ejaculatory duct sheath; vs, vesiculum seminis; mbs, muscle block support; mb, muscle block; mbr, muscle block receptacle; mbc, muscle block control. (B) Muscle block showing radiation of muscle fibers from the mouth of ejaculatory duct sheath. ed, ejaculatory duct; edp, ejaculatory duct pocket; las, last abdominal segment; mf, muscle fiber. (Ba) Muscle attachment to ejaculatory duct sheath. (Bb) Muscle attachment to upper end of muscle block control.

matocytes. In 7–10 d, however, spermatid bundles develop, enlarge, and break up into spermatozoa. The vasa deferentia and 2 accessory glands converge at the tip of a single stout duct leading to the vesiculum seminis. Presumably, sperm mix with the secretions of these glands at this point. The ejaculatory duct is enveloped totally in ejaculatory duct sheath (Fig. 1A), a semicircular chitinous loop, the end of which rests in the ejaculatory duct pocket (Fig. 1B). Attached to and radiating from the wide mouth of the ejaculatory duct sheath are numerous muscle fibers that form a large powerful muscle block (Fig. 1B). Two chitinous structures are attached to the muscle block: muscle block support lying across the surface of the block, and muscle block control which regulates the movement of the block (Fig. 1B). The upper end of the muscle block control is fastened by muscle fibers to a lower part of the muscle block (Fig. 1B, Bb), and the lower end is similarly bound by muscle fibers to the last abdominal segment (Fig. 1B). The bottom part of the muscle block enters the muscle block receptacle (Fig. 1B).

When mating, the male bends his last abdominal segment downward and forward, the action of which pulls the muscle block control, outstretching the muscle block and releasing the ejaculatory duct sheath from its pocket (Fig. 1B). The abdomen of the beetle then returns to its normal position; the block contracts, being pulled back by the muscle block control. The contraction of the muscle block now propels sperm into the spermatophore, which is driven into the vagina of the female. In a newly emerged male, it takes 3 wk or more before the muscle block becomes fully operational. Therefore, although testes mature in a week or so, no sperm can be transferred without the muscular action of the muscle block. Consequently, *C. montrouzieri* males begin mating 20–30 d after emergence.

The spermatophore of *C. montrouzieri*, when filled with sperm, resembles a swimming seal, consisting of head, trunk, and tail (Fig. 2, 1). It is a colorless, elongated, and weakly chitinized sac with an open end through which sperm are driven in. When just placed inside the vagina, the sperm in the spermatophore look as if immersed in vigorously bubbling liquid, forming a swirling whirlpool (Fig. 2, 1). The sperm then flow swiftly toward the nozzle, or the head region of the sac whose tip is connected to the seminal duct of the spermatheca (Fig. 2, 1). In the nozzle, the speed of the flowing sperm is further accelerated and before entering the seminal duct, another whirlpool may be created (Fig. 1, 2).

The living spermatophore occupies the entire length of the vagina and is enveloped by the fertilization sac (Fig. 2, 1) while sperm transfer is taking place. From entrance of the spermatophore into the vagina to completion of transfer takes ≈30 min. Immediately after the spermatophore detaches itself from the seminal duct, a small quantity of

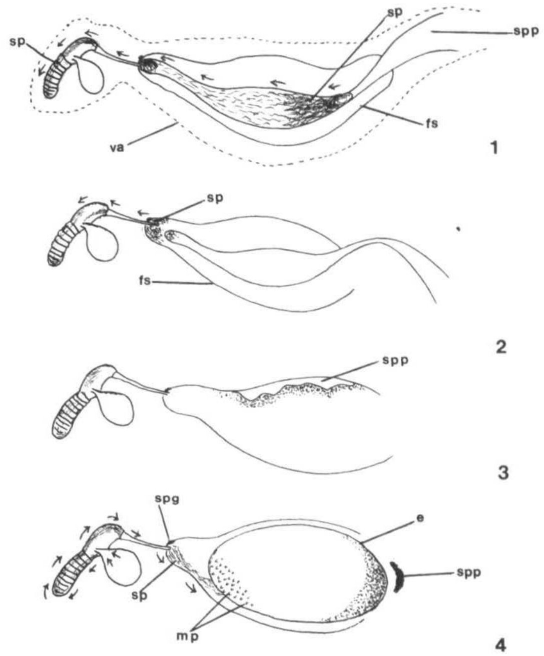

Fig. 2. Sperm transfer. (1) Sperm in spermatophore entering spermatheca. sp, Sperm; spp, spermatophore; fs, fertilization sac; va, vagina. (2) Remnant of sperm entering spermatheca. (3) Empty spermatophore. (4) Fertilization of egg. mp, Micropyles; e, egg. Arrows show movement of sperm.

remaining sperm in the nozzle is poured out into the pocket of the fertilization sac (Fig. 2, 2) so that these remnant sperm continue to flow into the spermatheca for the additional 3–4 min. The now empty spermatophore loses its elegant form and sinks to either side of the fertilization sac as a shapeless, crumpled sac (Fig. 2, 3). Eventually, the sac leaves the vagina together with a fertilized egg (Fig. 2, 4). The posterior part of the sac, which has been enveloping the spermatophore, opens at the end of sperm transfer (Fig. 2, 3).

Female Reproductive System and Sperm Mixing. The female reproductive system is composed of a pair of ovaries, each with 10–13 ovarioles; a pair of lateral oviducts that join just above where the oviducts open into vagina; a vagina with a spermatheca; and a short common oviduct (Fig. 3A). The spermatheca is a small, golden yellow, well chitinized sac, consisting of nonsegmented anterior and segmented posterior chambers (Fig. 3D). The vagina is sac-like and contains within it another sac, a bell-shaped fertilization sac (Fig. 3A and B), the anterior end of which surrounds the base of the seminal duct and the open posterior part ends where the lateral oviducts enter the vagina (Fig. 3A). There is a tiny piece of chitin where seminal duct meets the fertilization sac; it is a sperm plug (Fig. 3B), which directs sperm flow at fertilization.

In a newly emerged female, the ovaries are tiny and wrapped heavily in fat and tracheoles. None-

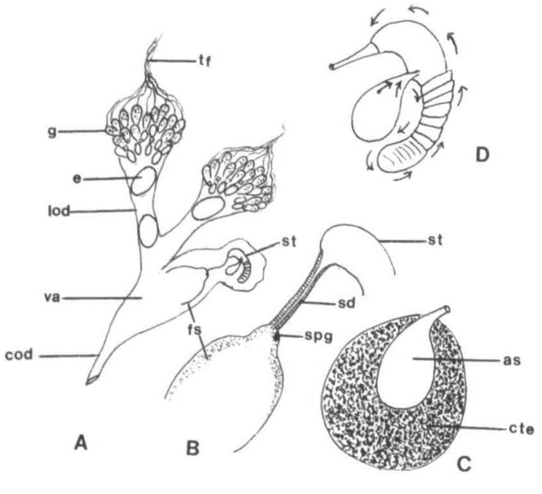

Fig. 3. Female reproductive system. (A) Entire system. tf, Terminal filament; g, germarium; e, mature egg; lod, lateral oviduct; va, vagina; cod, common oviduct; st, spermatheca; fs, fertilization sac. (B) Seminal duct and fertilization sac. sd, seminal duct; spg, sperm plug. (C) Air sac. as, Air sac; cte, connective tissue envelope. (D) Sperm mixing. Arrows show the direction of sperm movement.

theless, the female mates already at this time. The 1st lot of sperm occupies 1 side of the segmented area of the spermatheca as a bundle, the spermatozoa lying parallel to each other. The 2nd lot settles in the opposite side of the same area. The 3rd then rests in the anterior chamber. Thus, there are usually 3 sperm bundles at a time; that is, 3 spermatophores from 3 different males. Occasionally, 1 more may be added.

The spermathecal air pump is surrounded by a thick connective tissue envelope (Fig. 3C). It is inflated already 60–80% at emergence, and each time sperm are driven into the spermatheca, the pump is inflated further so that when the spermatheca is filled with sperm, the pump is ready for action. It blows out air, starting to circulate the sperm toward the posterior end, then forward toward the seminal duct, and finally back to where the circulation started (Fig. 3D). If fresh sperm enter the spermatheca at this time, they are swept up by the circulating current. All the sperm move fast and vigorously round and round the spermatheca until the pump becomes deflated to ≈40%. The pump has never been observed completely deflated; the level of inflation ranged from 40 to 100%. When the spermatheca is full and the air current is strong—for instance, when the pump is 90–100% inflated—a whirlpool is frequently formed around the mouth of the pump (Fig. 3D). Thus, the sperm from 3–4 males are thoroughly mixed before fertilization.

Fertilization. From 7 to 10 d after emergence, eggs begin to develop, and mature in ≈3 wk. The total number of eggs per female during 2 periods of oviposition is 40–50. The eggs in the ovaries do not develop simultaneously; 2–4 mature at a time and descend the lateral oviduct one by one. The egg enters the fertilization sac instead of vagina directly. The sac houses only 1 egg at a time. Of the ovipositing females examined, 1.5% (n = 141) contained 2 eggs in the fertilization sac. This happens when mature eggs do not descend the oviduct in time but accumulate in the ovaries for some reason. When this occurs, the eggs not only disfigure the fertilization sac, but also are so misplaced in the sac that normal fertilization is not possible. When an egg comes to rest in front of the anterior pocket of the fertilization sac (Fig. 2, 4), the air inside the seminal duct is compressed toward the spermatheca and clears the passage for sperm to appear and temporarily accumulate in the pocket immediately above the micropyles (Fig. 2, 4). The micropyles are at the top of the egg and number 60–80. The emerging sperm are guided by the sperm plug to flow in 1 direction only (Fig. 2, 4) preventing them spreading out in all directions. The fertilized egg now descends the common oviduct to be laid. Thus, only 1 egg is fertilized at a time by the mixed sperm from 3–4 males. To fertilize the 40–50 eggs that a female produces in her adult life, frequent matings during oviposition are essential. As stated, males constitute 64% of the *C. montrouzieri* population (n = 906), a ratio that favors multiple matings. Frequent and multiple matings should contribute to the genetic diversity of this species.

Discussion

Sperm mixing by powerful muscles also occurs in the carabid, *Bembidion confusum* Hayward (Kaufmann 1986). In this beetle, however, the spermatheca itself is surrounded by muscles, the contraction of which mixes the sperm. The mixed sperm then fertilize 18–24 eggs in the vagina. In comparison, in *C. montrouzieri*, the large muscle block acts as a "powerhouse" to drive sperm into spermatophore, vagina, and spermatheca, simultaneously inflating the air pump attached to the spermatheca. In other words, the direct driving force for sperm mixing is air from the pump, but the pump is filled with air by the muscular action of the muscle block. The large streamlined spermatophore is well suited for propelling sperm, which are still immobile at this stage. In the female, the fertilization sac envelops and affixes the spermatophore to the seminal duct; it also receives the emerging mixed sperm from the spermatheca and guides them to the micropyles of a single egg. Thus, in *C. montrouzieri* the system is more complicated but highly specialized and efficient and is, therefore, more advanced than that of *B. confusum*.

My studies of beetles—including carabids, tenebrionids, heterocerids, crysomelids, and coccinellids—show that frequency in mating encounters seems to be the prime factor in determining the size of the spermatheca. Active beetles with frequent mating opportunities need only a small spermatheca in which to keep a limited quantity of sperm from a few males to fertilize a few eggs at a time. Frequent matings, in turn, necessitate a rapid evacuation of sperm from the spermatheca. This situation further induces the development of a mechanism by which sperm can be moved about: by the movement of the sperm themselves (Kaufmann 1988), muscular action (Kaufmann 1986), capillary action (Kaufmann 1993), and muscular action and air current as in *C. montrouzieri.*

More sedentary beetles with less frequent mating opportunities such as many tenebrionid species (Kaufmann 1955) and the heterocerid *Centriatus auromicans* Kiesenwetter (Kaufmann 1988), however, have developed a large, sac-like spermatheca where sperm from each mate can accumulate one after another and are stored for a much longer period. Under such circumstances, the last-in, first-out mode of fertilization, or male priority, is the general rule (Taber 1955, Schlager 1960, Kaufmann 1988).

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