Content of the genital tracts inspected in mated females of the Silkworm (Lepidoptera Bombycidae)

Note 19 (Lepidoptera Ditrysia), released by Luigi De Marzo on May 2013 – Do spermatozoa transfer from the copulatory pouch to the spermatheca in this moth? l.demarzo@alice.it www.luigidemarzo.eu

SUBJECTS

• Starting from the Malpighi's study on the Silkworm in the 17th Century, several workers paid their interest to the functional anatomy of the reproductive system of the Ditrysia
• and provided the wide mass of information, which we can find today to be minutely reported and discussed by Khalifa (1950), Bourgogne (1951), Davey (1965) and Ferro & Akre (1975).
• Referring to the insemination, these reviews state that most workers agree in regarding this process as inclusive of two subsequent phases, according to the scheme reported in Fig. 1:

FIRST, male pulls sperm into the copulatory pouch and builds the structure reported as "spermatophore";
SECOND, mechanical breaking and/or chemical dissolving of this spermatophore cause releasing of the spermatozoa, which go to accumulate into the spermatheca.

• Anyhow, this "classical view" of the insemination process has been regarded as doubtful referring to the actual occurrence of the above second phase in a recent study on the Grape-vine tortricid, *Lobesia botrana* (Denis & Schiffermüller) (De Marzo, 2009).
• A discussion of this matter is opened again in the present note.

MATERIAL AND METHODS

• Cocoons of *Bombyx mori* (Linnaeus) were obtained through usual breeding on mulberry leaves and stored in separate jars as long as they formed.
• Breeding started from eggs in the first year and from full-grown larvae in the second year.
• Material was courteously supplied by Dr. Silvia Cappellozza (Section Bachicoltura, Exper. Institute of Agrarian Zoology in Padua).
• At each morning of the emergence period, males and females emerged in the night were put together in a vessel to obtain pairing.

• Pairs were anaesthetized by chloroform at established times from the copulation beginning.
• Females of each pair were dissected in salt solution (NaCl 0,9%) within 30 minutes and examined for the content of their genital tracts.
• Dislocation of the spermatozoa was appreciated on slides with a 40x phase-contrast lens.
• Examined females (Table A) included also some virgins and some mated females engaged in releasing eggs.
• Morphological details are termed in agreement with De Marzo (2010).

Tab. A – Number of Silkworm's females examined for each tested condition in the years 2010 and 2011.

female tested conditions	1st year	2nd year
virgins	8	0
10-15 min. from copulation beginning	4	16
about 40 min. from copulation beginning	15	11
about 90 min. from copulation beginning	10	15
copulation just concluded (24-48 hours after)	12	9
ovipositing mated females	10	8

RESULTS

----- ANATOMICAL OUTLINES

• Genitalia of examined Silkworm's females (Fig. 2) fully agree with the Malpighi's drawing reported by Berlese (1909) (Fig. 3.A);
• when compared with the general scheme assigned to Ditrysia (Fig. 3.B), they show the lack of two parts: the ***bulla*** and the ***signum***.
• Silkworm's ***copulatory pouch*** (Fig. 4.A) includes the larger part known as ***corpus bursae***, which is made with thin and folded cuticle,
• and the heavily sclerotized ***copulatory channel***.
• According to the rule in Ditrysia, the duct known as ***seminal channel*** does connect the copulatory channel with the vagina and is provided with a thick muscular sheath.
• The ***spermatheca*** of the Silkworm (Fig. 4.B) includes the regions generally occurring in Ditrysia: (I) the ***receptacle***, (II) the ***duct***, (III) the ***gland***;
• except for the gland, it is lined by a powerful muscular sheath, which causes it to contract vigorously in the salt solution.
• The receptacle exhibits a **main sector** and a **lateral sector**, which are both engaged in storing when the mass of sperm is somewhat abundant.

• The duct is provided with the **helical sclerite**, which supports the muscular sheath as in other Ditrysia.
• The gland is of tubular-type with its duct about 15 microns-large in diameter and variably branches according to specimen (Fig. 6).

----- CONTENT OF THE COPULATORY POUCH
• Copulatory pouch was found in collapsed condition only in the virgins (Fig. 5.A);
• otherwise, it was in swollen condition in all mated females (Fig. 5.B-E).
• These include both females sacrificed 10-15 minutes after copulation beginning and those engaged in releasing eggs.
• Swollen copulatory pouches contained small masses of spermatozoa scattered within an exceeding amount of amorphous material.
• Although content of the copulatory pouch was sometimes subdivided (Fig. 5.E), no enclosing structure embodying the "spermatophore" of other Ditrysia was recognized throughout.

----- CONTENT OF THE SPERMATHECA
• According to specimen, sperm occupied either both sectors or only the main sector of the receptacle;
• sometimes, it filled also the spermathecal duct and/or stuck into a proximal segment of the glandular duct, as reported in Fig. 4.B.
• in any case, it constituted a very dense mass.
• Within the females sacrificed at 10-15 minutes after copulation beginning, only 2 of them (out to 20 examined throughout) bore sperm in their spermatheca.
• Within the females sacrificed at 40 minutes after copulation beginning, the rate 1:1 was recorded between filled/empty conditions of the spermatheca as reported in following Table B and in Fig. 7 as well.
• Spermatheca filled with sperm was always found in females sacrificed later, including those engaged in releasing eggs.

Tab. B – Presence/absence of sperm in the spermatheca in mated Silkworm's females for each tested condition.

mated females: tested conditions	sperm absent	sperm present
10-15 min. from copulation beginning	18	2
about 40 min. from copulation beginning	13	13
about 90 min. from copulation beginning	0	25
copulation just concluded (24-48 hours after)	0	21
ovipositing mated females	0	18

DISCUSSION

• OBSERVATIONS ON THE COPULATORY POUCH OF THE SILKWORM SUGGEST THAT:
----- filling of the pouch completes a short time after copulation has begun;
----- no loss of turgescence occurs in mated females, even when these are engaged in discharging eggs;
----- power of the muscular sheath is unsufficient to cause pouch content to be pulled aside;
----- nor mechanical rupture neither chemical dissolution of the spermatophore do occur, as this structure is not built in the case of the Silkworm.

• OBSERVATIONS ON THE SPERMATHECA SUGGEST THAT:
----- although filling of the spermatheca is variably delayed according to pairs, it can sometimes occur precociously, i.e., even at 10-15 minutes from copulation beginning;
----- because content of the spermatheca always included a conspicuous mass of sperm, no symptom can be recognized for the progressive inlet of the spermatozoa into the spermatheca.

CONCLUDING REMARKS

• Seemingly, insemination process of the Silkworm doesn't include the passage of sperm from the copulatory pouch to the spermatheca.
• Possibly, its second phase (filling of the spermatheca) includes the direct introduction of one or more masses of spermatozoa into the spermatheca as long as copulation goes forward.
• Opposite to the classical view, this "alternative insemination mode" supposes for the Silkworm the following phases:

FIRST, the copulating male fills the copulatory pouch with heterogeneous material, including only a poor quantity of spermatozoa;
SECOND, the same male pulls its sperm directly into the spermatheca,

• according to the reported scheme (Fig. 8).

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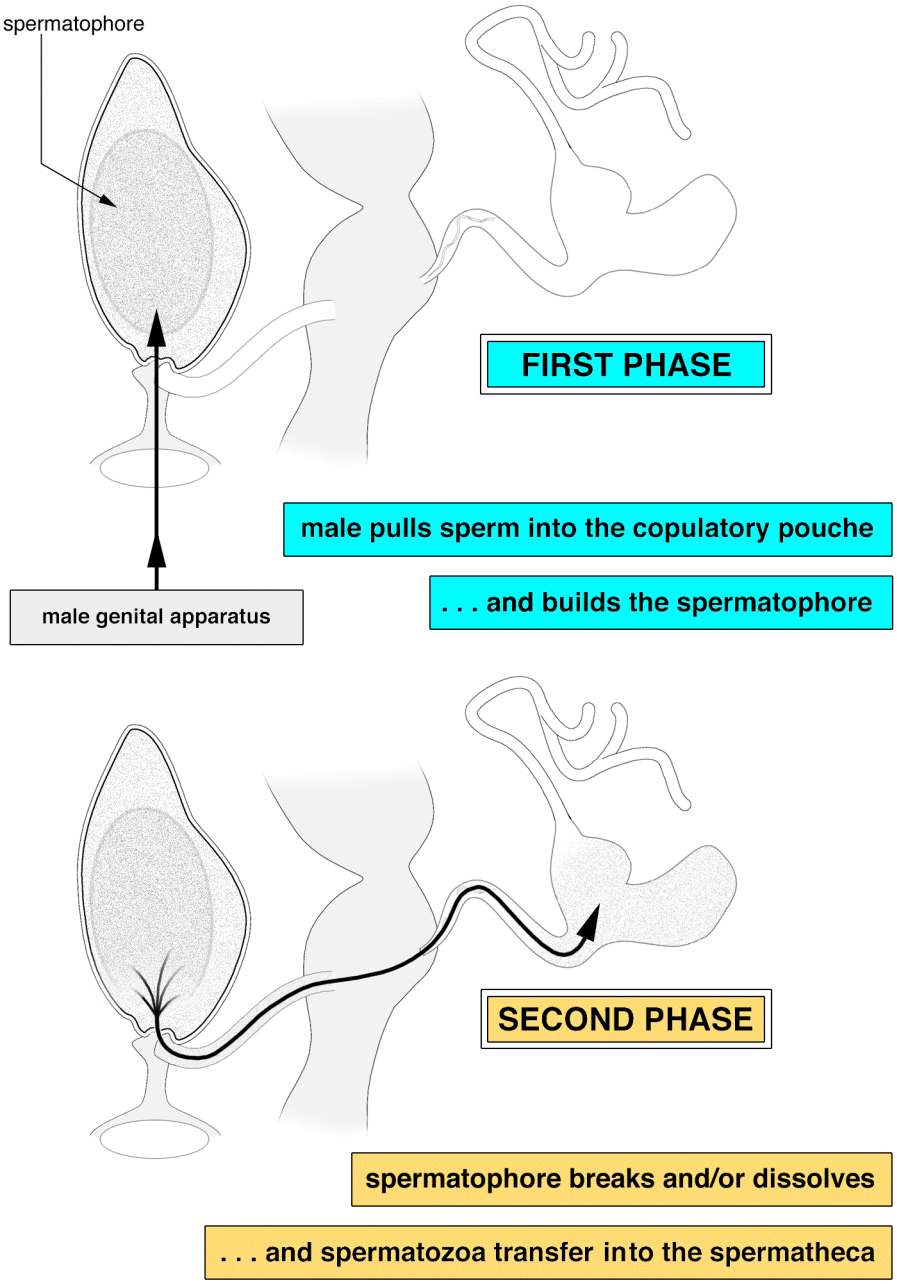

Fig. 1 – Scheme of the insemination process in Ditrysia according to the classical view.

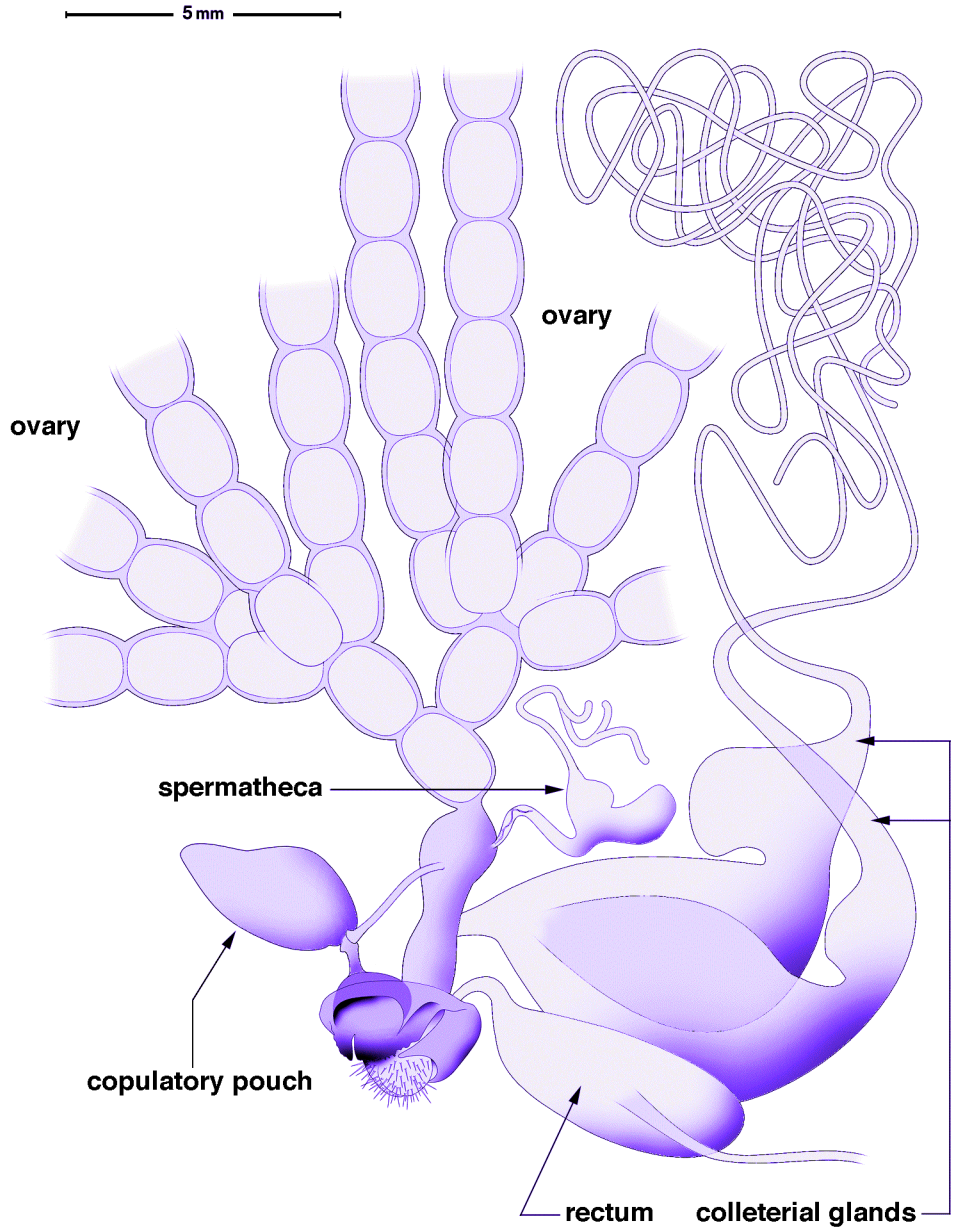

Fig. 2 - *Bombyx mori* (Linnaeus): anatomical outlines of the female genitalia.

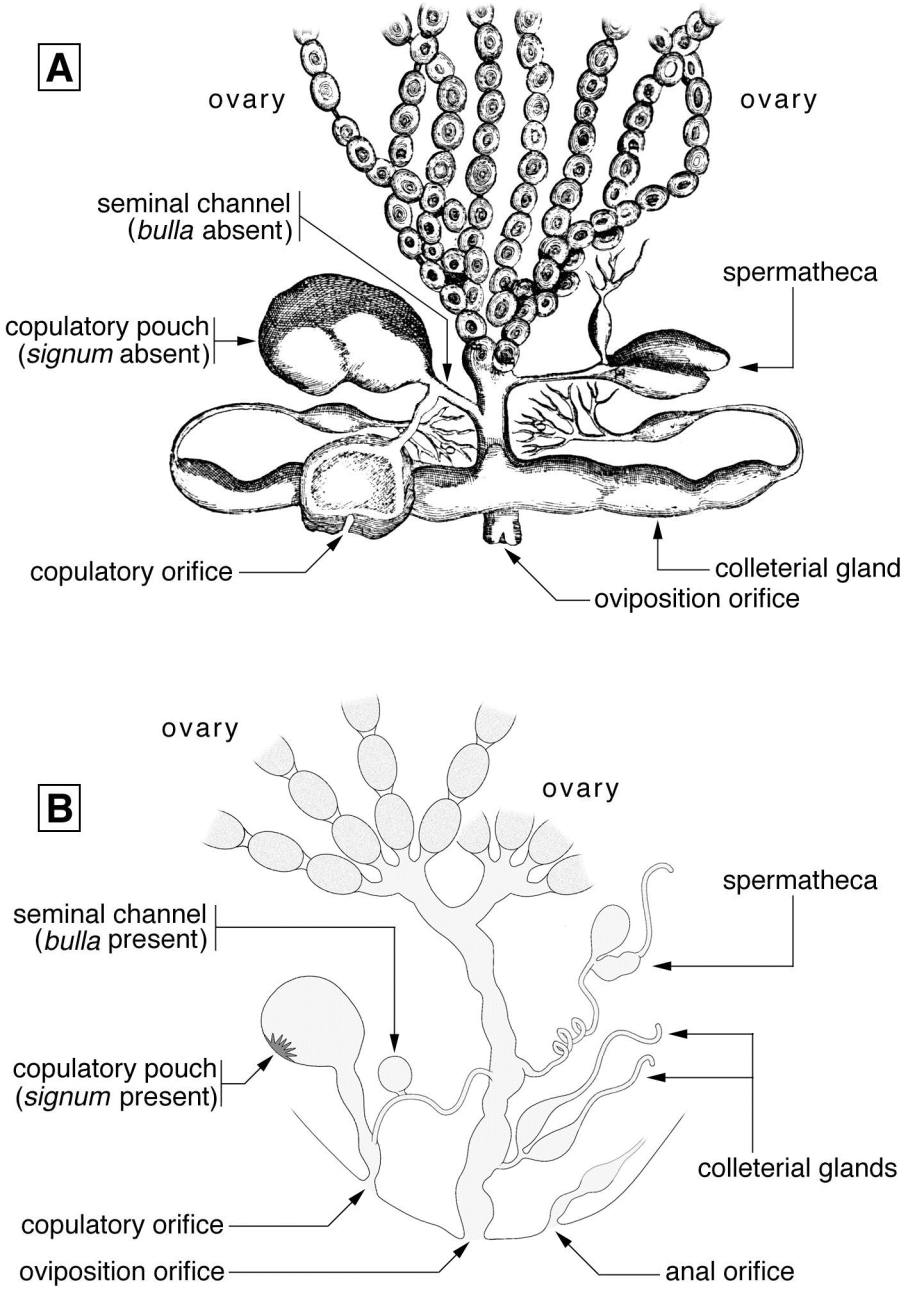

Fig. 3 – Anatomical outlines of the female genitalia of Ditrysia: A, classical figure of the Silkworm reported by Berlese (1909) on the basis of a Malpighi's study in the 17th Century; B, general scheme according to Bourgogne (1951).

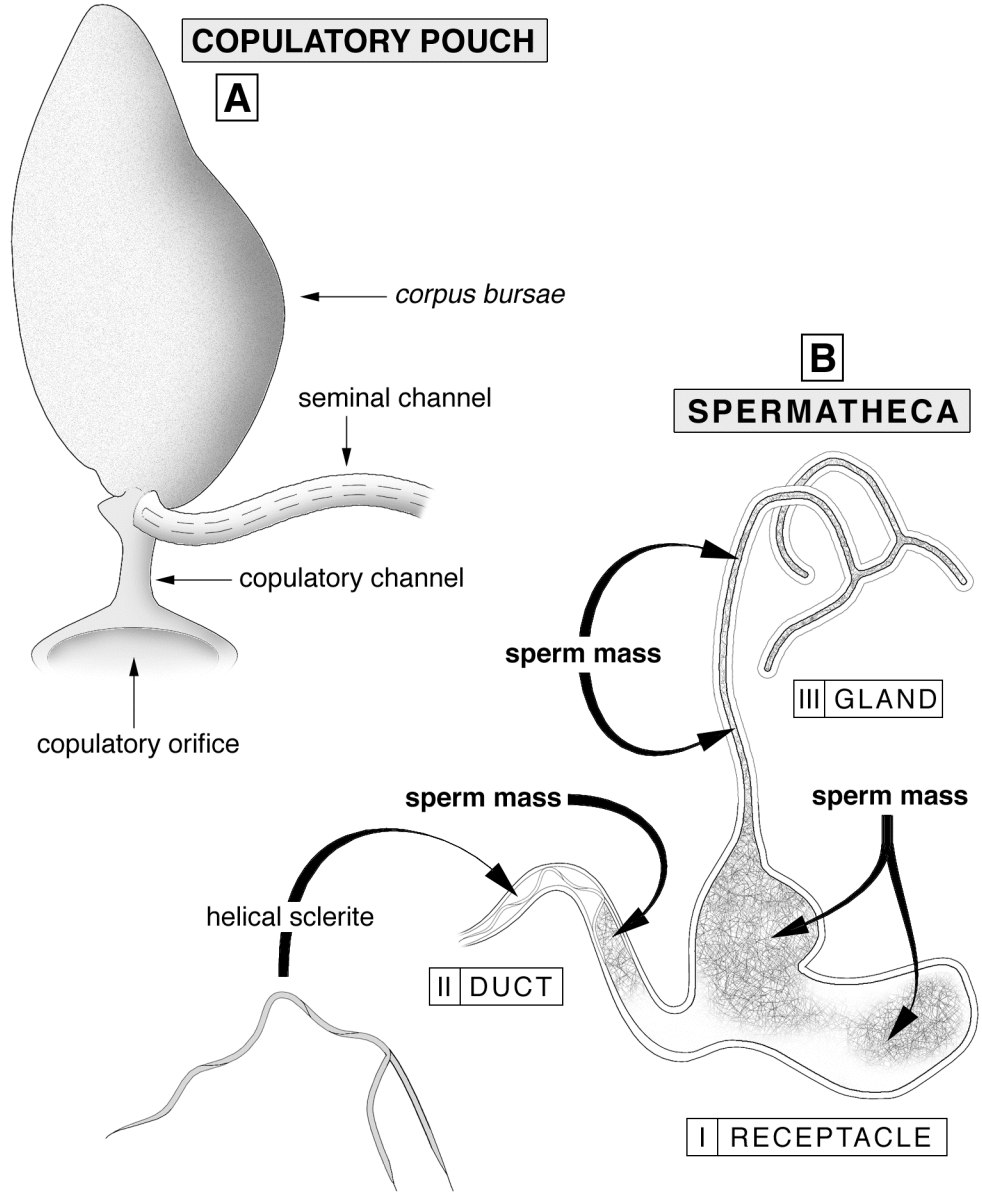

Fig. 4 - *Bombyx mori* (Linnaeus): terms referring to the copulatory pouch and the spermatheca. Arrows indicate the spermathecal regions where spermatozoa can accumulate.

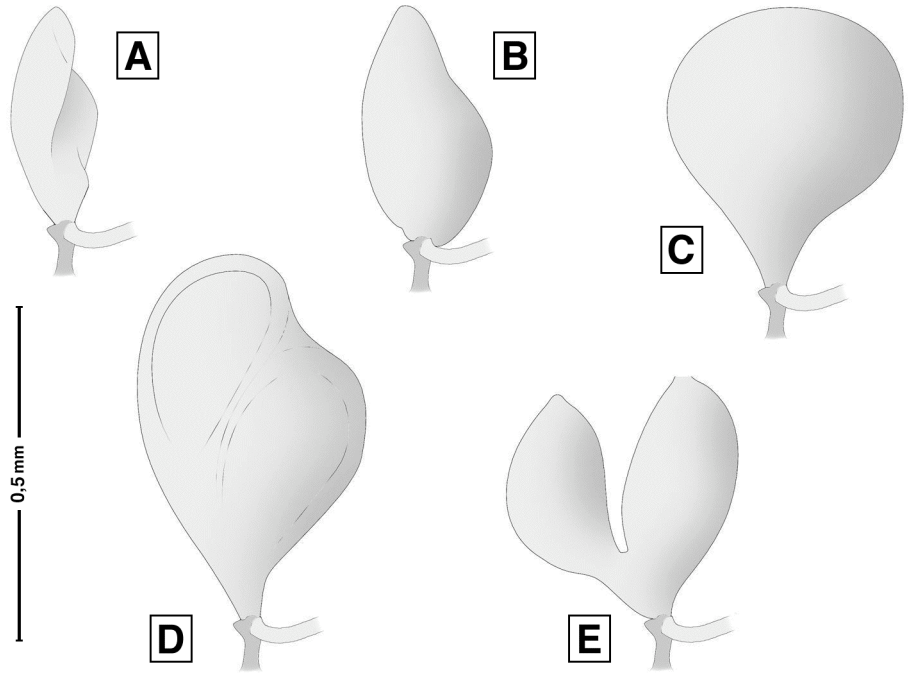

Fig. 5 - Conditions of the copulatory pouch of *Bombyx mori* (Linnaeus): A, collapsed pouch in a virgin; B-D, different swelling degrees recorded in mated females.

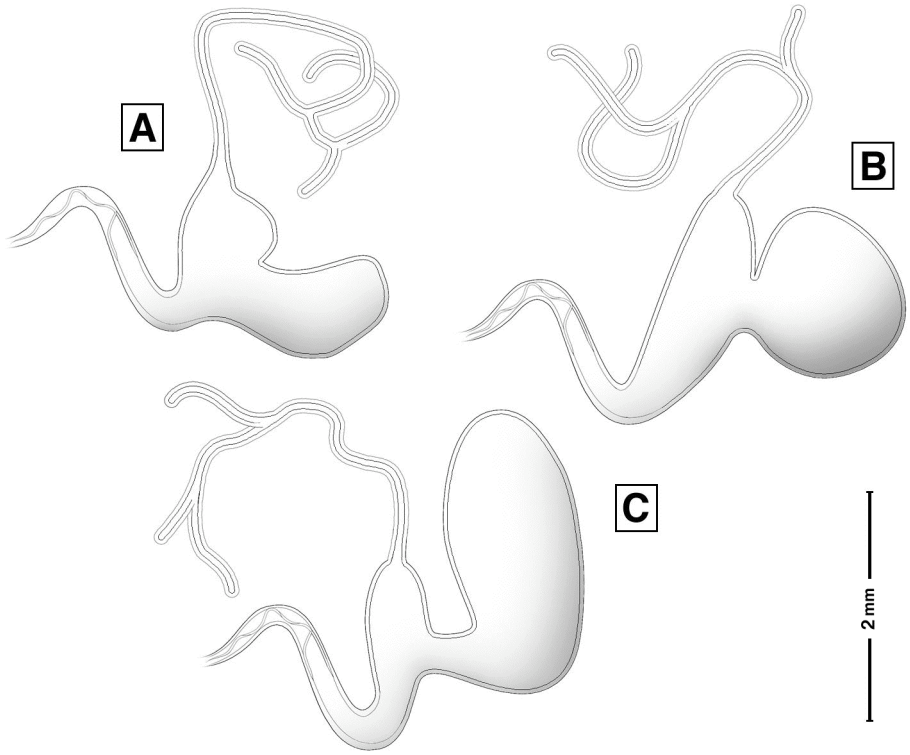

Fig. 6 - *Bombyx mori* (Linnaeus): different shape of the spermatheca observed in mated females.

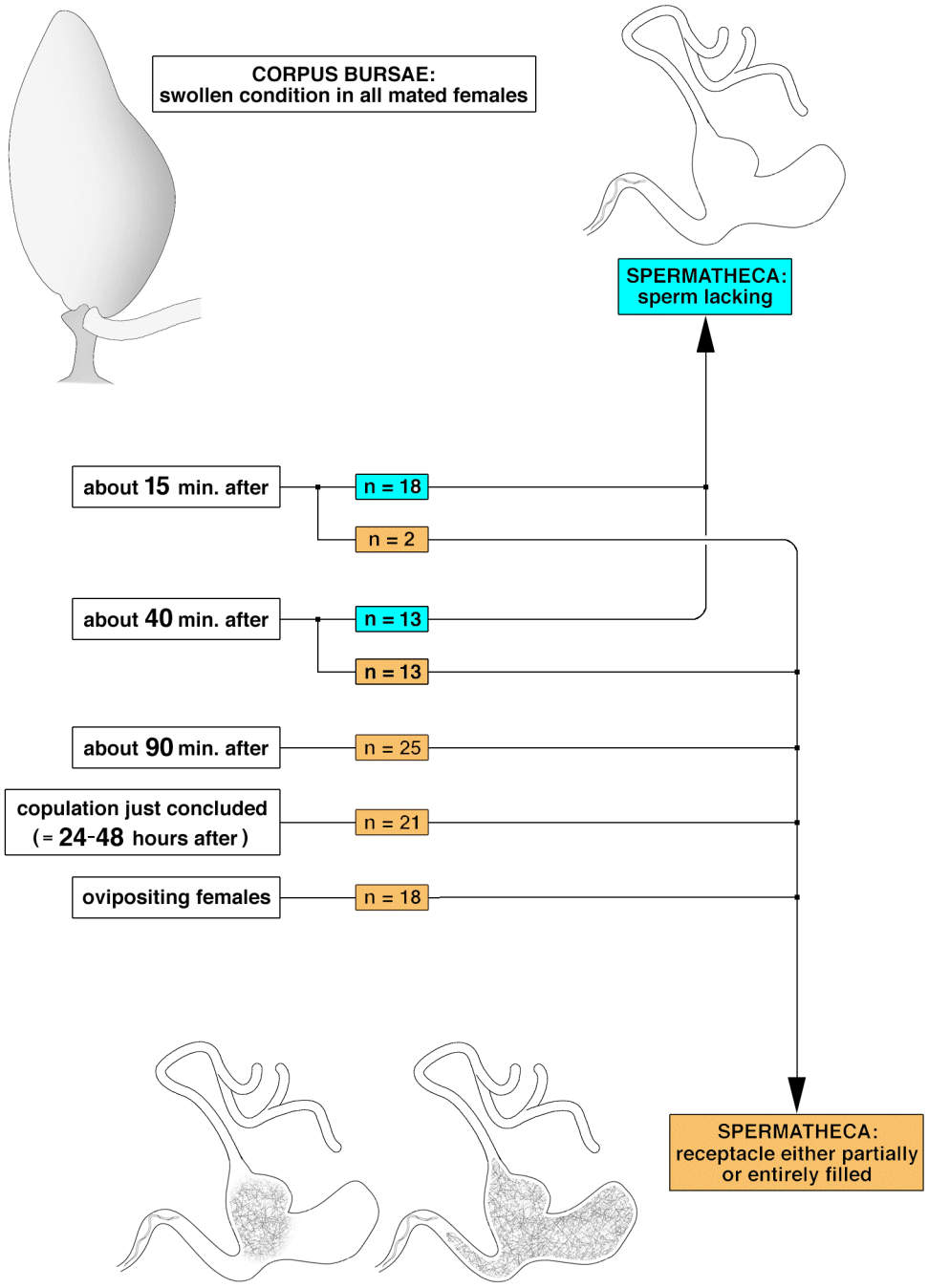

Fig. 7 – Results of the observations on Silkworm's mated females reported in a diagrammatic form, including number of females sacrificed at different time from copulation beginning and presence/absence of spermatozoa in their spermatheca.

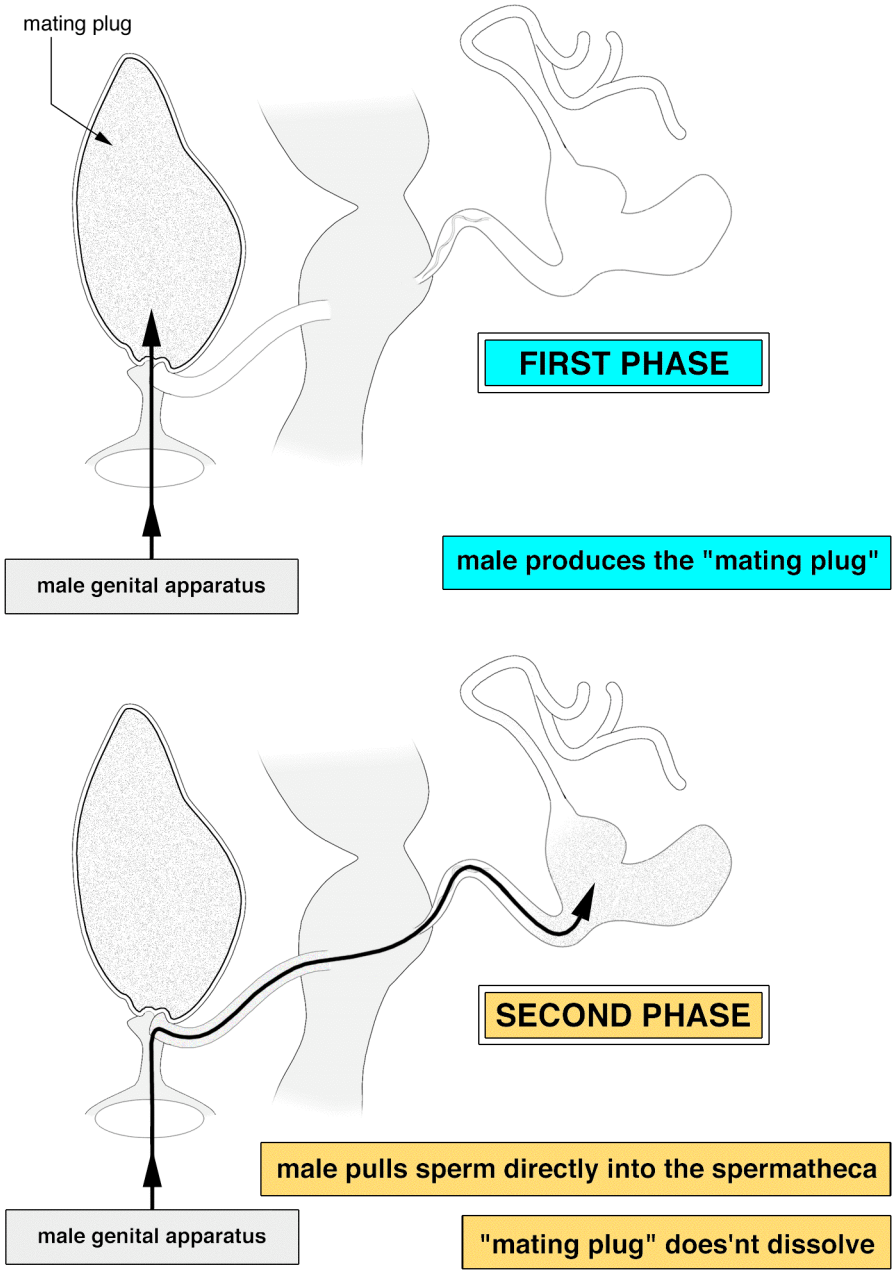

Fig. 8 – Alternative mode of the insemination process supposed for the Silkworm.