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Atypical functioning of female genitalia explains monandry in a butterfly

David Xochipiltecatl [1], **Joaquín Baixeras** [2], **Carlos R Cordero** [Corresp. 3]

[1] Posgrado en Ciencias Biológicas, Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City, Mexico City, México

[2] Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Paterna, Valencia, Spain

[3] Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de México, Mexico City, Mexico City, México

Corresponding Author: Carlos R Cordero
Email address: cordero@ecologia.unam.mx

Monandrous species are rare in nature, especially in animals where males transfer nutrients to females in the ejaculate. The proximate mechanisms responsible for monandry are poorly studied. In butterflies and moths, the male transfers a nutritious spermatophore into the corpus bursae (CB) of the female. The CB is a multifunctional organ that digests the spermatophore and has partial control of the post-mating sexual receptivity of the female. The spermatophore distends the CB and the post-mating sexual receptivity of the female is inversely proportional to the degree of distension. The CB of many butterfly species has a muscular sheath whose contractions mechanically contribute to digest the spermatophore. As the contents of the CB are absorbed, the degree of distension decreases and the female recovers receptivity. We studied the monandrous butterfly *Leptophobia aripa* (Boisduval, 1836) (Pieridae) and found that females do not digest the spermatophores. We investigated the structure of the CB and found that a muscular sheath is absent, indicating that in this butterfly females lack the necessary "apparatus" for the mechanical digestion of the spermatophore. We propose that female monandry in this species is result of its incapability to mechanically digest the spermatophore, which results in a constant degree of CB distension after mating and, thus, in the maintenance of the sexually unreceptive state of females. Hypotheses on the evolution of this mechanism are discussed.

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David Xochipiltecatl [1], Joaquín Baixeras [2], Carlos Cordero [3]

[1] Posgrado en Ciencias Biológicas, Instituto de Ecología, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México

[2] Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de Valencia, Paterna, ES-46980 Valencia, Spain

[3]Departamento de Ecología Evolutiva, Instituto de Ecología, Circuito exterior s/n, Ciudad Universitaria, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México

Corresponding author:

Carlos Cordero

E-mail address: cordero@ecologia.unam.mx

ABSTRACT

Monandrous species are rare in nature, especially in animals where males transfer nutrients to females in the ejaculate. The proximate mechanisms responsible for monandry are poorly studied. In butterflies and moths, the male transfers a nutritious spermatophore into the corpus bursae (CB) of the female. The CB is a multifunctional organ that digests the spermatophore and has partial control of the post-mating sexual receptivity of the female. The spermatophore distends the CB and the post-mating sexual receptivity of the female is inversely proportional to the degree of distension. The CB of many butterfly species has a muscular sheath whose contractions mechanically contribute to digest the spermatophore. As the contents of the CB are absorbed, the degree of distension decreases and the female recovers receptivity. We studied the monandrous butterfly *Leptophobia aripa* (Boisduval, 1836) (Pieridae) and found that females do not digest the spermatophores. We investigated the structure of the CB and found that a muscular sheath is absent, indicating that in this butterfly females lack the necessary "apparatus" for the mechanical digestion of the spermatophore. We propose that female monandry in this species is result of its incapability to mechanically digest the spermatophore, which results in a constant degree of CB distension after mating and, thus, in the maintenance of the sexually unreceptive state of females. Hypotheses on the evolution of this mechanism are discussed.

INTRODUCTION

Monandrous species, in which most females copulate just with one male, are rare in most animal groups (*Pizzari & Wedell, 2013; Taylor, Price & Wedell, 2014*). There are two general hypotheses to explain the existence of monandry. First, monandry could be selected for when females maximize their fitness with just one mating, which could happen if, for example, polyandry imposes high costs on females (*Arnqvist & Andrés, 2006; Jiggins, 2017*). Second, if polyandry increases female fitness, sperm competition could favour male adaptations that impose monandry and, in consequence, fitness costs on females (*Hosken et al., 2009*). Different female adaptations are expected to evolve in each case. For example, if monandry is adaptive for females, they could evolve structures that facilitate the deposition and storage of male-derived anti-aphrodisiacs, as in *Heliconius* butterflies (*Jiggins, 2017*). On the other hand, if males impose monandry selection could favour the evolution of counter-adaptations, female traits that prevent or reduce male manipulation, as the anti-antiaphrodisiacs of the plant bug *Lygus hesperus* (*Brent, Byers & Levi-Zada, 2017*). These examples show that understanding the proximate mechanisms preventing female remating sheds light on the ultimate causes of monandry.

During copulation, male lepidopterans transfer ejaculates, mostly contained within a spermatophore, into a bag-shaped organ of the female reproductive tract known as *corpus bursae* (CB hereafter) (*Drummond 1984; Watanabe & Sato, 1993; Watanabe, 2016; Meslin et al., 2017*). In most butterflies and moths studied to date, the ejaculates are rich in nutrients (*Boggs & Gilbert, 1979; Marshall, 1985; Pivnick & McNeil, 1987; Boggs, 1990; Lai-Fook, 1991; Watanabe & Sato, 1993; Bissoondath & Wiklund, 1995, 1996a, 1996b; Karlsson, 1998; Molleman et al., 2005; Watanabe, 2016; Meslin et al., 2017; Cannon, 2020*) and other chemical compounds (*Dussourd et al., 1988, 1989; Eisner & Meinwald, 1995; Smedley & Eisner, 1996;*

Cardoso, Roper & Gilbert, 2009; Watanabe, 2016) that enhance female fitness (*Vahed, 1998; Arnqvist & Nilsson, 2000; Oberhauser, 1989; Eisner & Meinwald, 1995; González et al., 1999; Torres-Vila, Rodríguez-Molina & Jennions, 2004; Torres-Vila & Jennions, 2005; Watanabe, 2016; Meslin et al., 2017; Cannon, 2020*). The fact that most of these components are unavailable in the adult diet adds to their importance for female fitness and explains, in some extent, the ubiquity of polyandry in this group (*Drummond, 1984; Eberhard, 1985; Simmons, 2001; Sánchez, Hernández-Baños & Cordero, 2011; Cannon, 2020*). However, intriguingly, in Lepidoptera there are some monandrous species (*Drummond, 1984; Eberhard, 1985; Walters et al., 2012; Caballero-Mendieta & Cordero, 2013; Konagaya, Idogawa & Watanabe, 2020*).

After mating, female butterflies of polyandrous species become sexually unreceptive for a period of time (*Sugawara, 1979; Drummond, 1984; Oberhauser, 1989, 1992; Kaitala & Wiklund, 1995*). During this refractory period, the sperm is transferred from the spermatophore to the spermatheca, its final storage place within the female. The resource-rich spermatophore is gradually digested within the CB (*Drummond, 1984; Oberhauser, 1992; Galicia, Sánchez & Cordero, 2008; Walters et al., 2012; Meslin et al., 2015; Plakke et al., 2015; Watanabe, 2016*). At the proximate level, female sexual receptivity and mating frequency are controlled by multiple factors (*Sugawara, 1979; Drummond, 1984; Wedell, 2005*). One important factor is the mechanical stimulation resulting from distension of the CB by the spermatophore (*Labine, 1964; Sugawara, 1979; Oberhauser, 1992*). Sugawara (1979) clearly demonstrated that reception of a spermatophore in the butterfly *Pieris rapae* (Pieridae) induces females to display mate rejection behaviour when courted and that stretch receptors on the surface of the CB are involved in this behavioural change. Sugawara (1979) showed that the frequency of afferent nervous impulses from the stretch receptors increase tenfold after reception of a spermatophore, however, females

remain sexually receptive if the CB is filled with less than half the volume of an average spermatophore (recently and multiply mated males produce smaller spermatophores). In polyandrous species, female receptivity is gradually recovered as the amount of spermatophore remaining in the corpus bursa decreases (*Oberhauser, 1989,1992*) due to its digestion and absorption (*Boggs & Gilbert, 1979; Lai-Fook, 1986; Galicia, Sánchez & Cordero, 2008; Meslin et al., 2015; Plakke et al., 2015*).

Besides its effect on female receptivity, the presence of a spermatophore in the CB triggers the periodical contraction of the muscles surrounding the CB (*Sugawara, 1979*), resulting in tearing of the spermatophore envelope with the sclerotized structures located in the inner wall of the CB (called *signa*) and the mechanical digestion of the spermatophore contents (*Sugawara, 1979; Rogers & Wells, 1984; Tschudi-Rein & Benz, 1990; Galicia, Sánchez & Cordero, 200*8). The frequency of contractions of the CB muscles is directly correlated with the volume of the spermatophore (*Sugawara, 1979*). In species lacking signa, such as *Calpodes ethlius* (Hesperiidae), mechanical tearing and digestion of the spermatophore is also achieved via the "relatively violent" contractions of the muscles surrounding the CB (*Lai-Fook, 1986*: p. 556). The ubiquity of the mechanical digestion function of the CB is supported by transcriptomic studies showing highly expressed genes whose products are biased towards muscle organization and activity in the CB of *P. rapae* (*Meslin et al., 2015*) and the moth *Ostrinia nubilalis* (Crambidae) (*Al-Wathiqui, Lewis & Dopman, 2014*). More general, the presence and layout of well-developed muscles surrounding the CB, and their common association with the signa, is consistent with a mechanical digestion function of the CB in Lepidoptera (*Sugawara, 1979; Drummond, 1984; Rogers & Wells, 1984; Kristensen, 2003; Lincango, Fernández & Baixeras,*

101 *2013*). However, a muscle sheath is absent in some species lacking signa (J. Baixeras, personal
102 observations).

103 A third function of the CB is the absorption and transport of substances contained in the
104 spermatophore. In his general review of lepidopteran genitalia, Kristensen (2003: p. 438)
105 mentions: "The bursa is obviously capable of absorbing breakdown compounds from the
106 spermatophore". This function was demonstrated in *C. ethlius* (*Lai-Fook, 1991*) and is consistent
107 with radiotracer studies in three nymphalid species *(Boggs & Gilbert, 1979*), transcriptomic
108 studies in *P. rapae* identifying highly expressed genes associated to the transport function
109 (*Meslin et al., 2015*), the presence of pores and the structure of epithelial cells in the monarch
110 butterfly (*Rogers & Wells, 1984*), and observations of pores on the inner surface of the CB of
111 several species of moths in the family Tortricidae (*Lincango, Fernández & Baixeras, 2013*;
112 although these authors suggest pores could be involved in secretion of substances to the interior
113 of the CB).

114 Summarizing, in recently mated female lepidopterans, the distension of the CB by the
115 spermatophore turns-off sexual receptivity and triggers contractions of the muscles surrounding
116 the CB that result in the piercing or tearing of the spermatophore envelope and the mechanical
117 digestion of its contents (*Sugawara, 1979; Lai-Fook, 1991*). Female receptivity is recovered as
118 the amount of spermatophore remaining in the corpus bursae decreases (*Sugawara, 1979;*
119 *Oberhauser, 1989,1992*) due to digestion and absorption in the CB (*Boggs & Gilbert, 1979; Lai-*
120 *Fook, 1991; Galicia, Sánchez & Cordero, 2008; Meslin et al., 2015; Plakke et al., 2015;*
121 *Watanabe, 2016*). Thus, the CB plays a fundamental role in the control of sexual receptivity
122 (although it is not the only factor; see *Wedell, 2005*) and mating frequency in female Lepidoptera

and, therefore, it is an obvious place to look for genital adaptations to monandry in Lepidoptera (*Cordero & Baixeras, 2015*).

Here, we report observations regarding the possible mechanism responsible for female monandry in the butterfly *Leptophobia aripa* (Boisduval, 1836) (Pieridae, Pierinae). In the field, females of this species mate on average (SD) 1.19 (0.4) times, as judged from spermatophores counts in mated females (*Caballero-Mendieta & Cordero, 2013*). We studied the fate of the spermatophore within the CB with the aim of measuring its rate of digestion and, surprisingly, found that the spermatophore does not show any sign of being digested. To shed light on why the spermatophore is not digested, we studied the musculature of the CB, as well as the fine structure of its inner surface. We found that *L. aripa* lacks the necessary "apparatus" for the mechanical digestion of the spermatophore.

Materials and Methods

Butterflies studied and laboratory rearing

L. aripa is the most abundant butterfly in Mexico City, flying all year (*Díaz-Batres & Llorente-Bousquets, 2011*). Their caterpillars feed on a variety of plant species and are considered a pest of cabbage, broccoli and cauliflower crops in México and Central America (*CATIE/MIP, 1990*). The butterflies used in our experiments and in most observations were the offspring of females collected in the Ciudad Universitaria campus of the Universidad Nacional Autónoma de México (CU-UNAM), located in southern Mexico City. Individual females were fed ad libitum every morning a 10% sugar solution and allowed to lay eggs in plastic containers with fresh leaves of *Tropaeolum majus* (Tropaeolaceae), the main food plant in our study location. To stimulate

oviposition, the containers, covered with mesh cloth, were located under (about 20 cm) an incandescent white light bulb for 90 minutes, although females frequently lay eggs even in the absence of these bulbs. The larvae were reared individually in small plastic containers (10 cm diameter, 4 cm height) with *T. majus* fresh leaves. Upon emergence, adults were individually marked on the wings with a permanent marker (Sharpie™) and kept individually in the same plastic containers in which they were reared.

Experiment on the fate of the spermatophore within the corpus bursae

This experiment was originally designed to determine the pattern of digestion of the spermatophore within the CB. Virgin females were mated with virgin males and euthanized by freezing at different times after the end of copulation (0, 8, 16, 24, 48, 72 and 96 hours). Matings were obtained by placing males and females in cylindrical cages made of mesh cloth and metal wire (~60 cm height and ~25 cm diameter) in the gardens of the Instituto de Ecología, located in CU-UNAM, between 10AM and 15PM (Mexico City time). With exception of the females frozen immediately after finishing copulating (0h), all experimental females were allowed to lay eggs daily as explained above. All females laid eggs and in most cases these were numerous, although they were not counted.

The frozen females were thawed at ambient temperature and their abdomens separated from the body, opened and cleaned with forceps. Then, the CB and the spermatophores were carefully dissected out, examined and photographed under a stereomicroscope (Olympus™ BX 51). A total of 49 females were studied: $N_{0h} = 7$ females, $N_{8h} = 6$, $N_{16h} = 7$, $N_{24h} = 8$, $N_{48h} = 9$, $N_{72h} = 5$, and $N_{96h} = 7$. Females under laboratory conditions lay most of their eggs within four or five days after mating, and few live more than a week, despite being fed daily (D. Xochipiltecatl, personal observations).

Preparation of samples for microscopic observation

Observations and photographs of the CB, the ductus bursae (the duct connecting the CB with the copulatory pore known as ostium; Fig. 1) and the spermatophore, of dry and fixed specimens (see below), were made with stereomicroscopes (Olympus™ BX 51 and Leica™ MZ8) and a scanning electron microscope (SEM; Hitachi™ S4800).

For observation of the muscles associated to the CB and ductus bursae (DB hereafter) we used two methods. First, three laboratory-reared virgin females were introduced in a freezer at -20 °C for about 3 minutes and then gently injected Karnovsky's fixative (paraformaldehyde 2% / glutaraldehyde 2.5%) in the body cavity through the thorax and the abdomen. Then the abdomen was separated from the rest of the body and submerged in the same fixative until dissection. For SEM observation, the abdomens were transferred to centrifuge tubes with phosphate buffer 0.1M and rinsed during several minutes in a shaker (MRS-Mini Rocket Shaker, Biosan™). Then, the abdomens were carefully removed and cleaned with forceps, and the CB and DB were dissected out, stained with 2% osmium tetroxide during 20 minutes followed by thoroughly washing with water, placed in microporous specimen capsules (30 μm pore size, Ted Pella Inc., product number 4619) and dehydrated in increasing grade ethanol. The CB and DB were then dried to critical point in an Autosamdry 814™ (Tousimis), positioned on SEM stubs using silver conducting paint and sputtered with Au-Pd.

We also observed the muscles associated to the CB and DB in three field collected mated females (captured while laying eggs) that were brought to the laboratory and allowed continuing laying eggs (one female two days and two females three days), before being euthanized by freezing at -70 °C and then their abdomens were separated from the body and preserved in 100% ethylic alcohol. The abdomens were carefully opened and the CB and DB dissected out and

carefully cleaned, with micro-scissors, fine forceps and fine brushes, in glass embryo dishes under the stereomicroscope. The spermatophores contained in these females were also used to confirm that they are not digested in the CB (see Results).

For observation of the inner surface of the CB in the SEM, we used three laboratory-reared virgin females, two of them preserved dry and one fixed (with paraformaldehyde 2% / glutaraldehyde 2.5%) as explained above. The abdomen of the fixed specimen was rinsed in phosphate buffer 0.1M, as explained above. The abdomens were separated from the rest of the body and digested in 10% KOH at 90 °C for about 90 minutes. Then, the abdomens were stained for about 30 seconds in chlorazol black (0.1% in ethanol 70°) and the CB was removed, carefully cleaned and cut longitudinally. Subsequent digestions with KOH were performed when needed. Fragments were processed in a similar way to the treatment of complete CB and DB.

Results

The spermatophore is not digested in the corpus bursae

The forty-nine virgin females mated with virgin males and frozen at different times after the end of copulation had their spermatophores carefully dissected and were examined under the dissection microscope. Despite the fact that most females laid eggs before being dissected (the exception being the females frozen immediately after mating), the spermatophores contained in the CB of all females remained intact independently of the time elapsed after the end of copulation (Fig. 2).

The bulbous spermatophore occupies most of the CB (Fig. 1B), is bilobed (Fig. 3A) and has a tubular prolongation, called *collum*, which extends along the DB (Fig. 1B, 2 and 3A),

blocking it almost completely (Fig. 1B). Only the area of the spermatophore that is in contact with the signum looked somewhat deformed but not broken (Fig. 3A).

These observations were corroborated with detailed SEM observations of the spermatophores obtained from the three females collected in the field while laying eggs. As observed in the previous experiment, the external envelopes of these spermatophores were also intact although somewhat compressed near the signum (Fig. 3A). No deflation, perforations or tearing were observed in any of them (Fig. 3A). Thus, our observations indicate that the spermatophore is not digested at least up to four days after mating, which is enough time for females to lay most of their eggs under laboratory conditions (D. Xochipiltécatl, personal observations).

The corpus bursa lacks the necessary "apparatus" for the mechanical digestion of the spermatophore

Observations of virgin and mated females fixed either with formaldehyde/glutaraldehyde (N = 3) or with absolute ethylic alcohol (N = 3) showed that there were no muscles enveloping the CB in *L. aripa* (Fig. 3B,C), thus indicating that mechanical digestion of the spermatophore is not possible in this species. The ductus bursa, on the contrary, is covered with muscle fibers extending from a ventromedial line and forming a series of rings (Fig. 3C), some of these fibers are inserted in the junction with the CB (the area known as *cervix bursae*), at the base of the signum and they could help this structure to exert pressure and deform the spermatophore without breaking it (Fig. 3A).

Detailed observations in the SEM of the inner surface of the CB showed a complete absence of pores (Fig. 3D).

235

236 **Discussion**

237 In this paper, we show that in the butterfly *L. aripa* the spermatophore remains intact within the
238 CB at least up to four days after mating, the period during which females lay most of their eggs
239 in captivity (D. Xochipiltecatl, personal observations). These observations indicate that in this
240 butterfly females do not digest the spermatophores, in contrast with most lepidopterans studied
241 (*Drummond, 1984; Oberhauser, 1992; Galicia, Sánchez & Cordero, 2008; Walters et al., 2012;*
242 *Meslin et al., 2015; Plakke et al., 2015; Watanabe, 2016*). We also show that there are no
243 muscles enveloping the CB. Since in other lepidopterans the spermatophore is mechanically
244 digested due to the contractions of the muscular sheath of the CB (*Sugawara, 1979; Rogers &*
245 *Wells, 1984; Lai-Fook, 1986*; *Al-Wathiqui, Lewis & Dopman, 2014; Meslin et al., 2015*), we
246 propose that the absence of a muscular envelope prevents the CB from digesting mechanically
247 the spermatophore. In other words, females of this species lack the "apparatus" required for the
248 mechanical digestion of spermatophores. Furthermore, judging from the intact condition of the
249 spermatophores several days after mating in females that laid eggs during several days,
250 enzymatic digestion seems to be absent. The observed absence of pores on the inner surface of
251 the CB is also consistent with this idea because pores could be involved both in the absorption of
252 products from digestion of spermatophores (*Lai-Fook, 1986*), and in the secretion of molecules
253 used for the chemical digestion of spermatophores within the CB, as suggested for Tortricidae
254 (*Lincango, Fernández & Baixeras, 2013*).

255 In the introduction, we reviewed evidence indicating that in many Lepidoptera female
256 sexual receptivity is at least partially controlled by the mechanical stimulation (distension) of the
257 CB by the spermatophore. The degree of distension of the CB is inversely related to the sexual

receptivity of females, and the receptivity is recovered as the ejaculate is digested and the CB deflates (*Labine, 1964; Sugawara, 1979; Oberhauser, 1992*). *L. aripa* females tend to be monandrous (*Caballero-Mendieta & Cordero, 2013*) and we propose that female monandry in this species is result of its incapability to mechanically digest the spermatophore, which results in a constant degree of CB distension after mating and, thus, in the maintenance of the sexually unreceptive state of females. Thus, we propose that the absence of muscles enveloping the CB explains monandry in *L. aripa*. A possible explanation for the rare cases of twice-mated females in this species (*Caballero-Mendieta & Cordero, 2013*) is that their first mate was recently mated and/or small, since both male conditions are known to result in the transfer of relatively small spermatophores (*Caballero-Mendieta & Cordero, 2013*). According to Drummond, "In some short-lived temperate zone butterflies, the spermatophore is known to persist intact in laboratory-held females for longer than the life expectancy of a female in the wild" (*Drummond, 1984*: p. 303). We predict that these species are monandrous and possibly have a CB devoid of a muscular sheath. It will be interesting to study if the CB of known monandrous butterflies and moths (*Drummond, 1984; Sánchez, Hernández-Baños & Cordero, 2011; Konagaya, Idogawa & Watanabe, 2020*) also lack a muscular sheath.

There are two general hypotheses to explain the evolutionary origin and maintenance of monandry in insects (*Arnqvist & Nilsson, 2000; Arnqvist & Andrés, 2006; Hosken et al., 2009*): either monandry is selected for in females when they maximize their fitness with just one mating, or sperm competition favours male adaptations that impose monandry on females that, otherwise, could obtain benefits from multiple mating. We suggest that the absence of a key adaptation required for the mechanical digestion of spermatophores sheds light on the selective pressures that favoured monandry in *L. aripa*. The muscular sheath of the CB is generally associated to the

presence of signa (*Sugawara, 1979; Drummond, 1984; Rogers & Wells, 1984; Lai-Fook, 1986; Kristensen, 2003; Lincango, Fernández & Baixeras, 2013; Al-Wathiqui, Lewis & Dopman, 2014; Meslin et al., 2015*) and signa appears to be a general feature of Lepidoptera (*Sánchez, Hernández-Baños & Cordero, 2011*). Thus, although a proper phylogenetic study is required, we hypothesize that the muscular sheath was lost in *L. aripa* and that this loss is a female adaptation to monandry.

Why monandry could be adaptive for females in this species remains to be studied. One interesting hypothesis is that increases in the availability of nutrients in food plants have reduced the importance of spermatophore-derived nutrients for female reproduction and favoured monandrous mating in females, possibly because in this way females reduce copulation time costs and predation risk during courtship and copulation. A recent study proposed this idea and presented evidence that anthropogenic nutritional enrichment of food plants has an effect on female mating frequency (*Espeset et al.,* 2019). A comparison of an "agricultural population" (AP) of the butterfly *Pieris rapae*, where fertilizers, irrigation and low levels of pesticides resulted in increased availability of nitrogen in food plants (canola), with a non-agricultural population (NAP) showed that, as predicted, most females of the AP mated once whereas more than half of the females of the NAP mated two or three times (*Espeset et al., 2019*). In the case of *L. aripa* in our study site, the females lay eggs mostly in a non-cultivated plant (*T. majus*) that grows forming large patches in disturbed places like the side of roads, but also grows within the gardens of the University, where at least receives irrigation. On the other hand, in other parts of the city, this butterfly uses as host plants cultivated vegetables that can be fertilized and are irrigated (such as cabbage, broccoli and cauliflower; *CATIE/MIP, 1990*). A second hypothesis is that monandry evolved in response to male adaptation to sperm competition. For example, if

304 males evolved spermatophores that are difficult to digest to delay female remating, a point could
305 be reached in which spermatophore digestion becomes excessively expensive due to
306 physiological or ecological reasons, and favours females that avoid these costs by abandoning
307 polyandry.

308

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Figure 1

Figure1 Female genitalia of the butterfly *Leptophobia aripa*.

(A) Genitalia of a virgin female: note the empty corpus bursae (cb) and the signum (si) near the junction (cervix) with the ductus bursae (db). (B) Corpus bursae and ductus bursae of a mated female: note the spermatophore almost filling the corpus bursae and the collum of the spermatophore filling the ductus bursae. Scale bars A = 500 µm; B = 1000 µm.

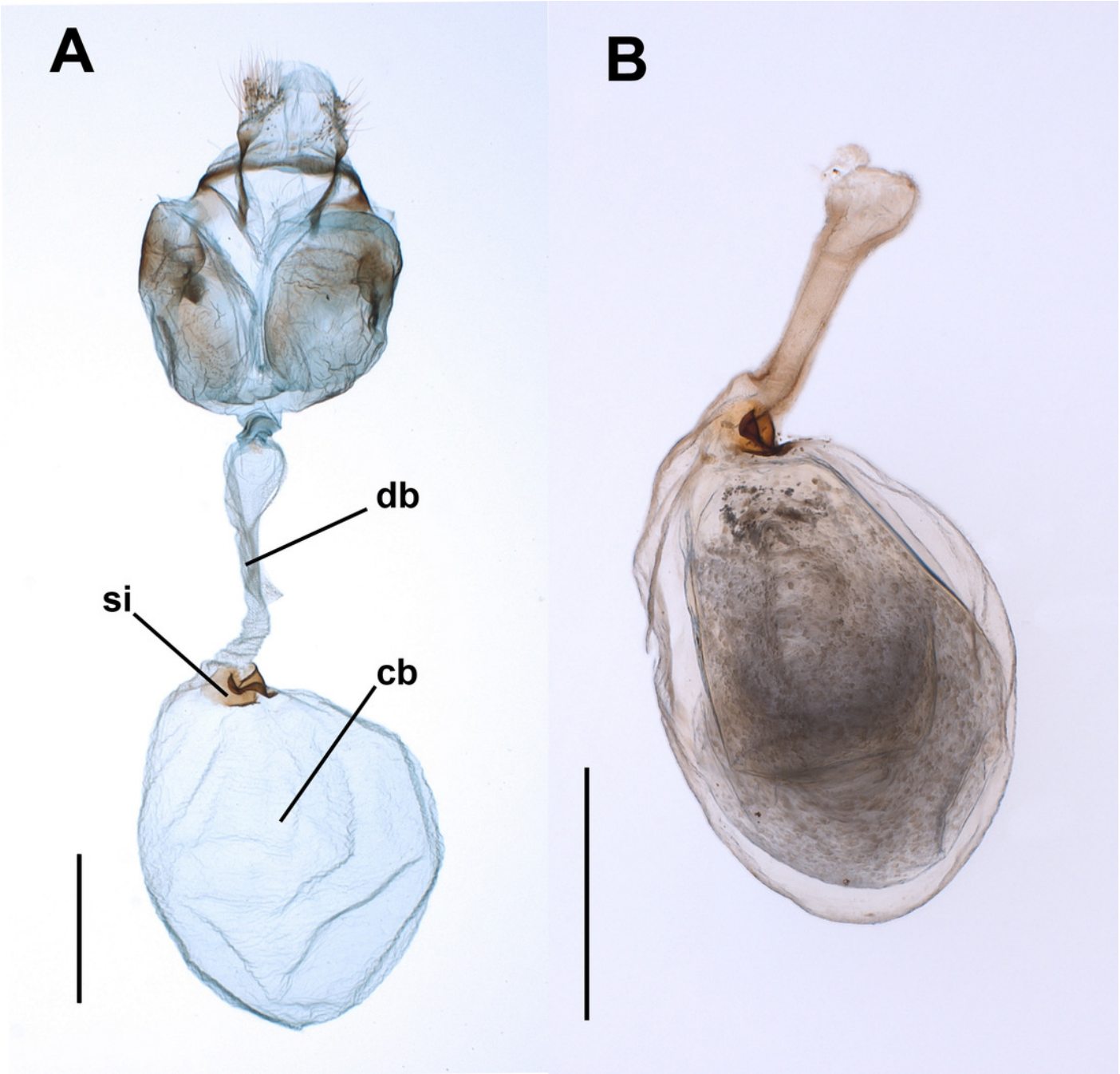

A
B
db
si
cb

Figure 2

Figure 2 The spermatophores are not digested within the corpus bursae of female *Leptophobia aripa* butterflies.

Typical examples of spermatophores showing that they remain intact in the CB independently of the time elapsed after the end of copulation (number of hours written besides each photograph) and of the fact that females laid eggs. Scale bar = 1 mm.

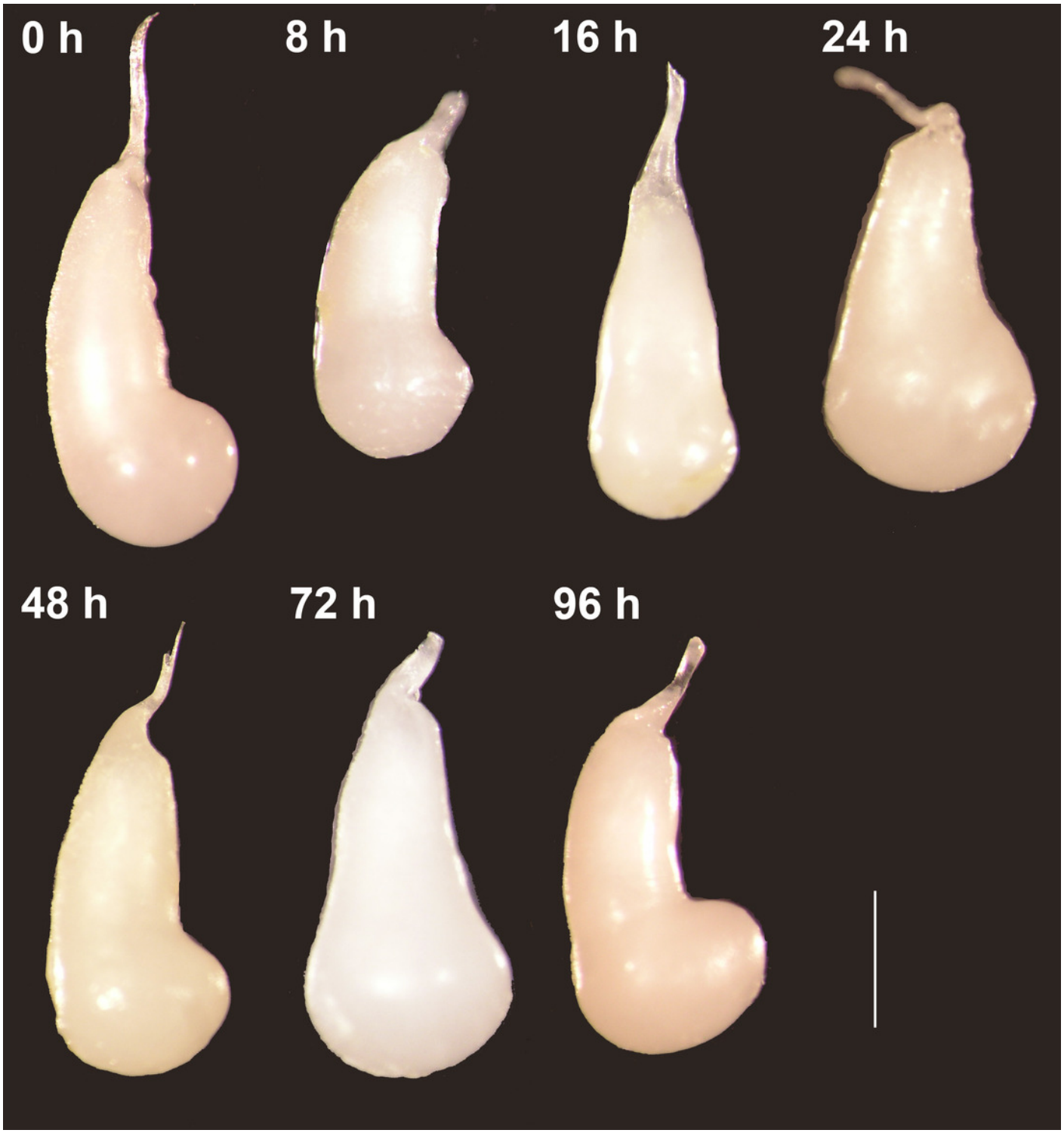
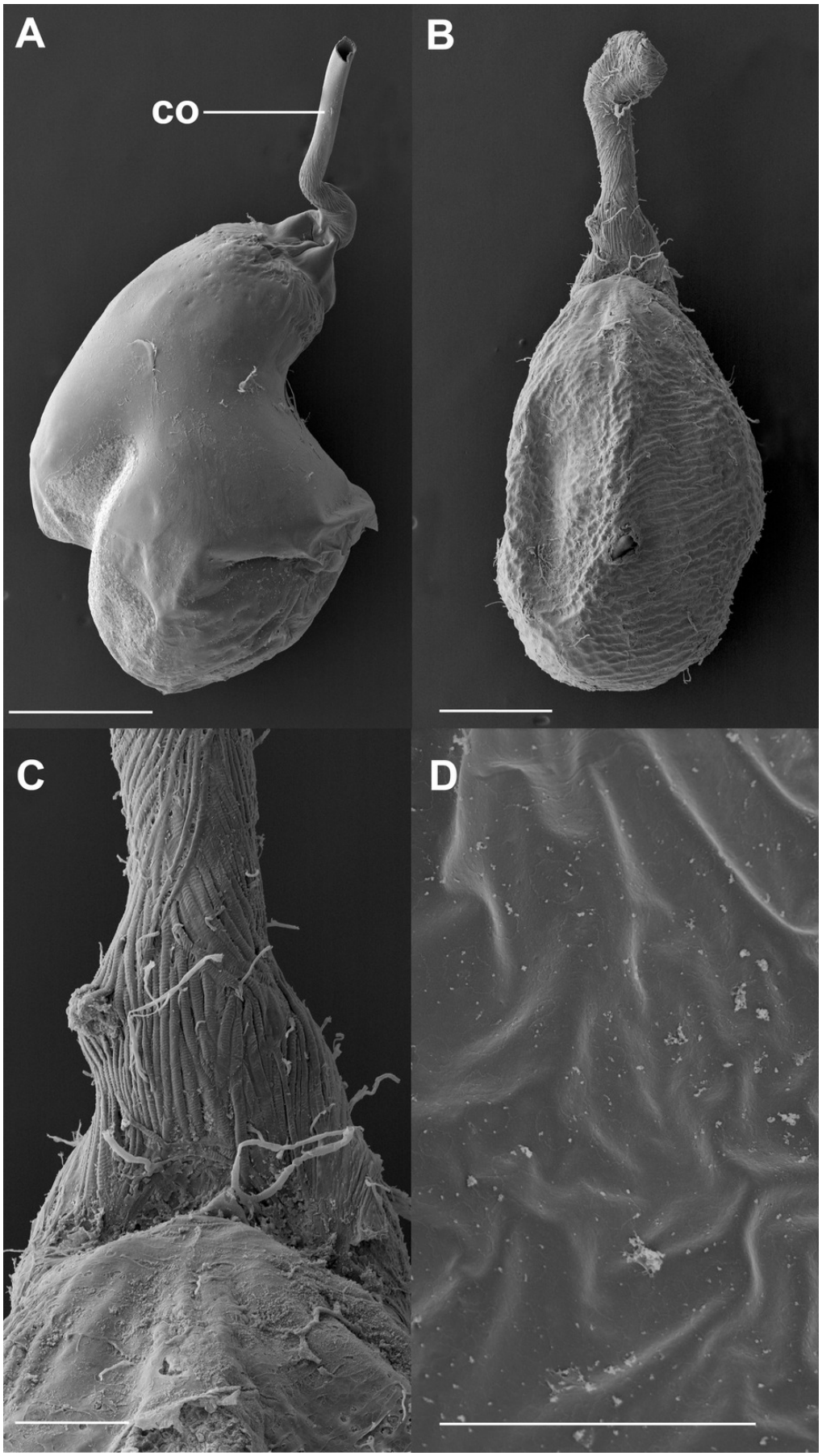

0 h
8 h
16 h
24 h
48 h
72 h
96 h

Figure 3

Figure 3 SEM images of the spermatophore, corpus bursae and ductus bursae, and its associated muscles, of the butterfly *Leptophobia aripa*.

(A) An intact spermatophore obtained from a female collected while laying eggs, taken to the laboratory and allowed to continue laying eggs for two more days; notice the tubular collum (co). (B) Image showing the absence of muscles enveloping the corpus bursae and the muscles that cover the ductus bursae. (C) Close-up of the “junction” area of the corpus bursae and the ductus bursae (the *cervix*) showing muscle fibers covering the ductus bursae and absent on the corpus bursae. (D) Vestiture of the inner surface of the corpus bursae showing a complete absence of pores. Scale bars A and B = 500 µm, C = 150 µm, D = 5 µm.


A
co
B
C
D