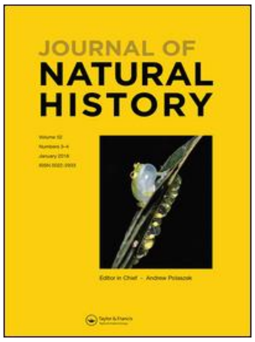

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Expulsion and consumption of male ejaculates by promiscous female *Euxesta eluta* and *Euxesta mazorca* (Diptera: Ulidiidae)

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While expulsion of male ejaculates by the females after copulation has been reported for various animal groups, expulsion followed by consumption of the expelled ejaculate is a rare behaviour outside spermatophylax-producing orthopterans. Among Diptera, this behaviour has been reported for a few species of Piophilidae, Empididae and Ulidiidae. Here we report on its occurrence among *Euxesta eluta* and *Euxesta mazorca* (Diptera: Ulidiidae). We also attempt to characterize the mating system of *E. eluta* in order to facilitate future hypothesis testing to understand the behavioural factors leading to the evolution of this peculiar behaviour. For this, courtship sequences, copulation duration, frequency of ejaculate expulsion and subsequent consumption, and latency to ejaculate expulsion for both *E. eluta* and *E. mazorca* were recorded. The time of sexual maturation, the time window of sexual receptivity during the day, and the mating frequency and variance in mating success for males and females (degree of polygamy) were determined for *E. eluta*. Both *E. eluta* and *E. mazorca* males engaged in elaborate courtship sequences involving visual and tactile displays before copulation. Females of both species almost invariably expelled and consumed ejaculates after copulation. Female *E. eluta*, required a 6- to 9-day period feeding on protein and sugar before becoming sexually receptive. Reproductive activity occurred continuously over the day with an early morning and late afternoon peak. Both males and females could mate multiply, with multiple partners over a 2-h observational period. Sexual network analysis revealed that some males and females had greater mating success than others. Ejaculate consumption appears to be widespread in the genus *Euxesta*. It is possible that females obtain nutrients from this behaviour while exerting some control over egg fertilization.

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Introduction

While expulsion of male ejaculates has been documented for several animal species (Eberhard 1996; Holman and Snook 2006), consumption of expelled ejaculates by females is an extremely rare behaviour (Bonduriansky et al. 2005). Several species of Orthopteran males produce an edible spermatophylax, attached to the sperm-containing spermatophore upon which females feed, providing them with nutrients for reproduction and/or survival (Gwynne 1997). Outside katydids and crickets, consumption of part or all of the male's ejaculate has been reported for a few molluscs, cephalopods and a few fly species (Hanlon and Messenger 1999; Bonduriansky 2003; Bonduriansky et al. 2005).

Among Diptera, ejaculate feeding occurs in several species of Piophilidae (Bonduriansky and Brooks 1998; Bonduriansky 2003). Some minutes after the end of copulation, once their two small spermathecae are filled with sperm, piophilid females usually expel the remaining contents of their bursa copulatrix from the ovipositor in the form of droplets, and immediately ingest them (Bonduriansky et al. 2005). Besides Piophilidae, expulsion and consumption of male ejaculates by females has been recorded for a species of Empididae and one species of Ulidiidae in the genus *Otites* (Morgulis 2013). *Empis trigramma* females expel and consume a liquid drop a few minutes after copulation, consumption of this drop appears to promote ovarian development (Preston-Mafham 1999). Similarly, 15–20 minutes after copulation, *Otites grata* females expel a droplet at the tip of the aculeus that is deposited on the substrate and consumed (Morgulis 2013). For most of these dipteran species, this behaviour has been interpreted as an early form in the evolution of nuptial gifts.

Among several species in five genera of Diopsidae, males produce and transfer spermatophores to females during copulation; spermatophore size and copulation duration are positively correlated, the remains of the spermatophore are expelled by the female but are not subsequently consumed (Kotrba 1996). In the case of several species of tephritid flies, males produce and offer froth masses to females (Friedberg 1981; Dohm et al. 2008, 2014), this behaviour has been termed mating trophallaxis and has been defined as 'trophallaxis between mates, connected to copulation and taking place, shortly before, during or after copulation' (Freidberg 1982). It has been hypothesized that these froth masses are either odorous, tasty or nutritious but these hypotheses have not been tested. In *Metasphenisca negeviana* (Diptera: Tephritidae) males and females contact their probosces before and after copulation, with the male transferring a droplet of translucent liquid to the female (Freidberg 1997). Freidberg (1997) argued that post-copulatory trophallaxis could function to ensure that the sperm deposited in the female reproductive system would fertilize the eggs. In general, nuptial feeding can encompass any form of nutrient transfer from the male to the female directly before, during, or directly after courtship and/or copulation. Nuptial gifts may take the form of food captured by males, parts, or even the whole of the male's body, glandular products such as salivary secretions, external glandular secretions, spermatophores and substances in the ejaculate (Markow and Ankney 1984; Vahed 1998).

The genus *Euxesta* (Diptera: Ulidiidae), composed of 69 primarily Neotropical species, has been mostly studied because several species [*Euxesta annonae* (Fabricius), *Euxesta stigmatias* (Loew), *Euxesta mazorca* (Steyskal), *Euxesta eluta* (Loew)], are major pests of corn in the Americas (Steyskal 1974; Arce de Hamity 1986; Nuessly and Capinera 2006;

Goyal et al. 2011). Recent studies on the natural history and sexual behaviour of *Euxesta bilimeki*, a species of little economic importance associated with agaves in the highlands of Mexico, led to the discovery that this species exhibits ejaculate expulsion followed by consumption (Brunel and Rull 2010). A large proportion of copulations observed in nature resulted in the expulsion and consumption of spermatozoa and seminal fluids by females (Brunel and Rull 2010). Under laboratory conditions, experiments revealed that by consuming the ejaculate females obtain water (Rodríguez et al. 2013). Ejaculate consumption increased longevity by approximately 48 hours in females deprived of food and water compared with females that were not allowed to consume the ejaculate (Rodríguez et al. 2013). Nevertheless, ejaculate consumption did not result in increased egg production in *E. bilimeki* females. In contrast to Piophilidae, Empididae, and *Otites*, ejaculate expulsion in *E. bilimeki* occurs a few seconds after copulation (Rodríguez et al. 2013). Female *E. bilimeki* can expel the entire ejaculate without storing any sperm (Rodríguez et al. 2013). Field and laboratory observations established that *E. bilimeki* is a multivoltine species; adults reach sexual maturity at 15 days; require consumption of both carbohydrates and proteins for maintenance and oogenesis; males and females mate multiply (up to three times in an hour); and males continuously harass females, both in nature and under laboratory conditions (Brunel and Rull 2010; Rodríguez et al. 2013). Harassment consists of continuous chases, repeated wing displays, abdominal tapping and mounting attempts by one or several males (simultaneously) during intervals that can last for more than 1 hour (Brunel and Rull 2010). The testes in male *E. bilimeki* occupy approximately 70% of the abdominal cavity (Brunel and Rull 2010). Consumption of ejaculates in *E. bilimeki* suggests that females may be able to manipulate paternity assignment through ejaculate expulsion and consumption, possibly to the detriment of males, and that selection imposed on males by the need to supply numerous ejaculates to ensure fertilization has resulted in large testis size, which may be the reason why the testes in male *E. bilimeki* occupy so much of the abdominal cavity.

Except for the studies on *E. bilimeki* mentioned above, there is only a brief description of courtship and mating of *E. stigmatias* on corn tassels (Seal and Jansson 1989), nevertheless, sperm expulsion and consumption were not reported. What little is known about *E. eluta* and *E. mazorca* is that copulations occur at dawn and dusk (Cruz et al. 2011). There is no other knowledge on pre- and post-copulatory behaviour of males and females of these species. Preliminary observations on *E. eluta* and *E. mazorca* revealed that females of both species engage in expulsion and consumption of male ejaculates. If sperm expulsion and consumption is widespread among *Euxesta*, flies in this genus could be a good model for testing hypotheses on sexual conflict over nuptial gifts and post-copulatory sexual selection (Gwynne 2008; Peretti and Aisenberg 2015). Additionally, given the increased economic importance of some *Euxesta* species for corn production (Bertolaccini et al. 2010), detailed studies on reproductive behaviour are necessary for the development of bio-rational pest management strategies. The objective of the present study was to describe the key aspects of the sexual behaviour of two species in the genus *Euxesta* in order to characterize their mating system, and construct the basis for informed hypothesis testing. We therefore set out to (i) describe the sexual behaviour (courtship sequences, copulation duration, and frequency of sperm expulsion and subsequent consumption, and latency to sperm expulsion) of both *E. eluta* and *E. mazorca*; (ii) determine the time of sexual maturation; (iii) determine the time window of sexual receptivity during the day, and (iv) determine the mating frequency and variance of mating success for males and females (degree of polygamy), using a sexual network interaction model for *E. eluta*. Network analysis is an approach to characterize how local interactions between individuals provide a quantitative tool to capture sexual patterns and dynamics (McDonald et al. 2013).

Materials and methods

Collecting and rearing

Corn cobs were collected between January and April of 2014, 2015 and 2016 at several corn-growing localities in Argentina (see Table 1). Collection and rearing data are shown in Table 1.

Sampling was carried out, by collecting cobs damaged by *Euxesta*. Corn cobs were placed in 20-l cylindrical plastic buckets (34-cm height, 27-cm diameter) covered by a voile cloth and labelled with collection date and place. Cobs were taken to the laboratory and kept at 22–25°C, 60% relative humidity. When flies emerged, in all cases, they were sorted to species (according to wing pattern) and sex (to ensure virginity until observations), and placed in 500-ml plastic containers covered with voile cloth in groups of a maximum of 25–30 flies per container (to prevent wing damage), with *ad libitum* access to water and food. Flies were fed with adult diet normally used to rear tephritid flies, consisting of sugar (57.9%) (Ledesma S.A., Jujuy, Argentina), hydrolysed yeast (14.5%) (Yeast Hydrolysed Enzymatic; MP Biomedicals), hydrolysed corn (27.3%) (Santa Ana, CA, USA), (Gluten Meal; ARCOR, Tucumán, Argentina), and vitamin E (0.3%) (Parafarm, Buenos Aires, Argentina) (w/w) (Jaldo et al. 2001). Such a diet has been found to promote egg maturation and fly survival for other species in the genus (CR and JR unpublished results).

Due to the limited number of *E. eluta* and *E. mazorca* reared/emerged, only the description of sexual behaviour could be carried out for both species. The remaining assays were conducted exclusively on *E. eluta*.

Description of sexual behaviour was based on observations on adult individuals of the F0 generation reared in the laboratory from the field-collected corn cobs. After observation on field-collected flies in 2016, adults of both sexes were placed in 14-l rectangular rearing cages (32 × 26 × 20 cm) with water and food, and allowed to interact freely. Fresh cobs were placed inside the cages to allow females to oviposit. Approximately one cob per 25 females was introduced; after a week, cobs were removed and conditioned in 500-cm^3 plastic containers covered with voile cloth. All other trials were carried out with the F1 or F2 generation recovered from flies reared on cobs in the laboratory. In our experience, courtship and mating behaviour of artificially reared flies does not differ from that of field-collected individuals during the first five generations.

Observations were conducted at the Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales, (LIEMEN-PROIMI), Tucumán, Argentina.

Description of sexual behaviour

Observations were carried out on 20- to 30-day-old F0 adults. Virgin flies were placed in pairs (one male and one female per container), in 8 × 8 × 8-cm acrylic transparent containers at approximately 09:30 h and observed for 1 hour. If copulation occurred, the

Table 1. Date, location, number of collected corn ears, and date, species and sex of emerged flies used for behavioural observations and experiments.

Year	Collection date	Province	Locality	No. of corn collected	Emergence date	No. of *E. eluta* emerged	No. of *E. mazorca* emerged
2014	26 January	Corrientes	Goya	~ 10	11–14 February	26 (13 ♀;13 ♂)	0
	26 January	Corrientes	Los Sauces	~ 10	8–11 February	18 (9 ♀; 9 ♂)	0
	21 January	Misiones	S 27o 34′ 36′′ O 55o 22′ 49,3′′	~ 10	14–26 February	2 (1 ♀; 1 ♂)	8 (4♀; 4♂)
2015	20 January	Tucumán	??	~ 14	10–20 February	12 (7 ♀; 5 ♂)	0
2016	10 February	Tucumán	Cañete	6	1–3 March	0	15 (7♀; 8♂)
	20 March	Tucumán	Las Cejas	14	11–19 April	38 (19 ♀; 19 ♂)	0
	22 April	Tucumán	Monte Redondo	14	17-may	220 (107 ♀; 113 ♂)	0

duration of copulation, the latency to expulsion of ejaculate (time between the end of copulation and the moment the female laid the ejaculate) and the occurrence of consumption behaviour were recorded. Altogether, 50 pairs of *E. eluta* and 11 pairs of *E. mazorca* were observed. Observations were therefore carried out on four pairs per day.

Age of sexual maturity

To establish the time required to reach sexual maturity with *ad libitum* access to a protein and carbohydrate diet (as described above), virgin F1 *E. eluta* adults were placed in pairs (male + female) in inverted 50-ml transparent plastic cups and observed for an hour from 10:00 to 11:00 h (an interval within the maximum period of mating activity). Flies were observed on a daily basis from emergence until the first copulation was recorded. After observation, flies were discarded. This assay was repeated twice (Figure 3).

Sexual receptivity throughout the day

For these assays, 31- to 40-day-old, F2 generation adults were used. Virgin flies were placed in pairs (one male and one female per cup) in 50-ml plastic cups and observed for 1 hour. Observations were carried out five times a day: at 09:00, 11:00, 13:00, 15:00 and 17:00 h. The mating and remating percentages were calculated after each observation interval by summing the number of pairs that mated and those that remated (within the observation interval) divided by the total number of pairs (typically 10) on each observational bout, and multiplied by 100. After each observation, all flies were discarded and in the next observation naive virgin flies were used. In total, 57 pairs were observed at 09:00 h, 44 pairs at 11:00 h, 20 pairs at 13:00 h, 30 pairs at 15:00 h and 34 pairs at 17:00 h.

Sexual network assay

One day before the observations, five pairs of virgin, 15- to 30-day-old, F1 *E. eluta* individuals were marked on the scutum with a dot of paint (Politec®) (Politec, Rodin, Mexico DF, Mexico) of one of four different colours: red, pink, blue, or green. An additional group of individuals was left without mark (wm) but were subjected to the same treatment as the marked individuals. Adults were individually aspirated by aid of a plastic tube (aspirator) and introduced into a small voile cloth bag, kept immobile for 10 seconds, aspirated and placed with marked insects.

Five marked pairs (five females and five males) were released together in a 30 × 20 × 20-cm glass cage at 09:30 h and observed until 11:30 h. Eight replicates were carried out (labelled R1, R2, …R8) but only seven were included in the analysis because during one replicate (R2) only one copulation was recorded. Male and female identity (colour), duration of copulation, latency to expel the ejaculate and occurrence of consumption behaviour were recorded.

Data analysis

Network analysis was performed using observational data for eight independent groups of five females and five males. R Studio Version 0.99.903 was used for network data analysis with bipartite software. Network diagrams were drawn using NetDraw 2.160 software. Each observed individual (male or female) is considered a node and the connections (copulations) between individuals are termed links. Connectance values represent the number of observed over potential connections an individual could have, whereas linkage density is the ratio of existing links to the total number of possible links.

Results

Description of sexual behaviour (E. eluta and E. mazorca)

Of 50 *E. eluta* pairs observed, 24 copulation sequences were recorded. Of these, 20 females expelled the ejaculate after mating. Copulation lasted 11 ± 1.74 seconds (mean ± SD), (*n* = 13), and latency to expulsion was 88.3 ± 17.31 seconds, (*n* = 9). The typical behavioural sequence preceding copulations that ended with ejaculate expulsion and consumption is depicted in Figure 1.

Of the 11 *E. mazorca* pairs observed, eight copulations were recorded. All the females expelled the ejaculate after mating and consumed it. Copulation duration was 26 ± 3.58 seconds, (*n* = 8), and latency to expulsion was 14.4 ± 2.46 seconds, (*n* = 5). In one case, not used for calculations, a female was continuously harassed by the male and expelled the ejaculate after 1020 seconds. The typical behavioural sequence preceding copulations which ended with ejaculate expulsion and consumption is depicted in Figure 2.

Time of sexual maturity (E. eluta)

The earliest copulations for *E. eluta* adults were recorded 6 days after emergence (two of 19 couples). Following that day, the proportion of pairs observed mating gradually increased until reaching c. 60%, 9 days after emergence. The proportion of flies mating at different ages (days) is shown in Figure 3.

Sexual receptivity throughout the day (E. eluta)

Numerically greater proportions of observed adults mated and remated during the morning (09:00–10:00; 11:00–12:00) and during late afternoon (17:00–18:00) than during the middle of the day (Figure 4).

Sexual network (E. eluta)

Sixty-two copulations were recorded over the seven replicates included in the analysis. Of these, in 58 cases females expelled the ejaculate, in three cases females did not expel ejaculates within the observation period and in one case it was not possible to record whether or not expulsion occurred. All females that expelled the ejaculate, consumed it (*n* = 58). Copulation duration was 13.32 seconds (± 0.55 SD, *n* = 57), and latency to expulsion was 115.87 seconds (± 10 SD, *n* = 54).

Both males and females mated with different mates during the 2 hours of observation (Figure 5). The frequencies of the number of mates per male/female are shown in Figure 6.

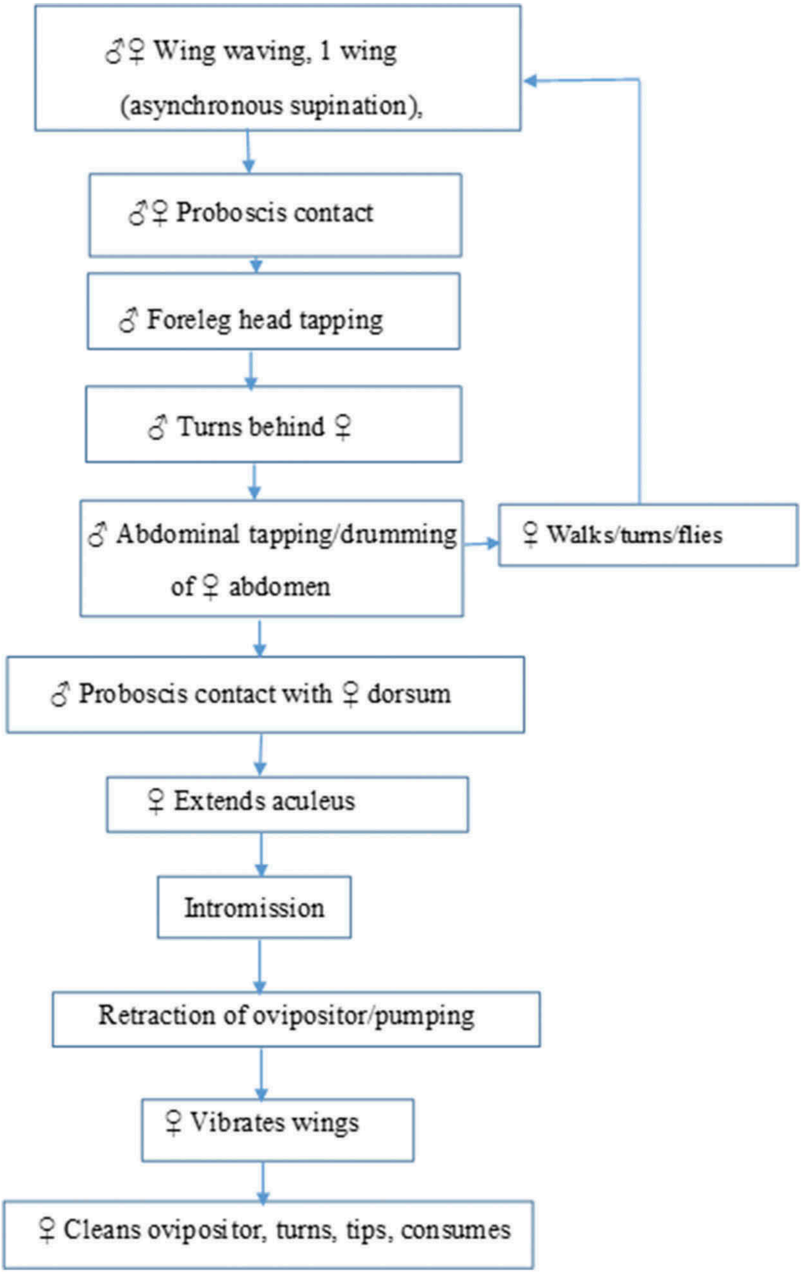

Figure 1. Ethogram depicting a typical mating sequence of *Euxesta eluta*. The ethogram was constructed after 1-hour observations of 50 sexually mature pairs. The ethogram was reconstructed based on records of partial and complete records of mating sequences.

During replicates R1, R4, R5 and R8, 80% of males mated at least once, during R3, R6 and R7 60% of males mated at least once. In the case of females, 80% in replicate R5 mated on at least one occasion, for replicates R4, R6 and R7, 60% of the females mated at least once, 40% of females in replicates R3 and R8 mated at least once, and only one (20%) female mated in replicate R1.

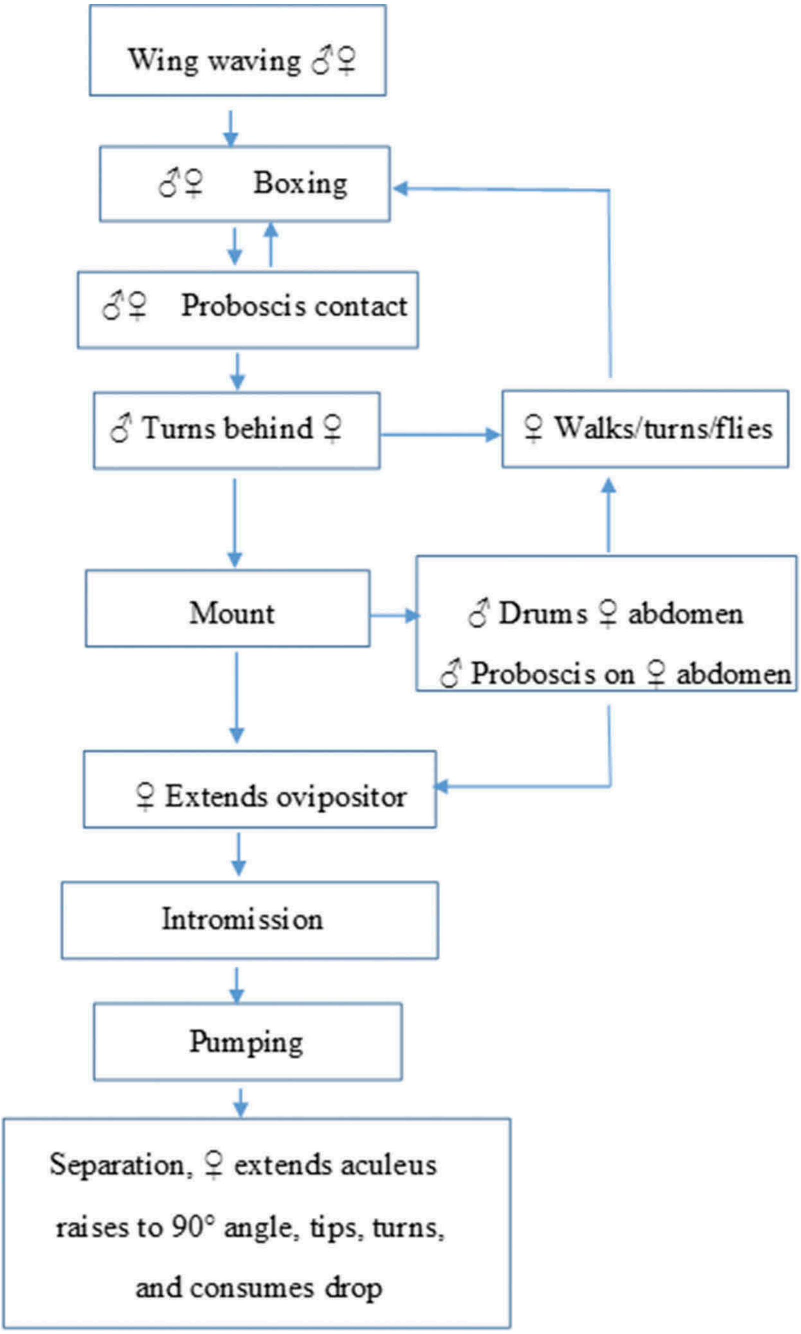

Figure 2. Ethogram depicting a typical mating sequence of *Euxesta mazorca*. The ethogram was constructed after 1-hour observations of 11 sexually mature pairs. The ethogram was reconstructed based on records of partial and complete records of mating sequences.

The networks of *E. eluta* groups (replicates) exhibited different types of structures. The highest connectance value was recorded for R1, where all possible interactions for that network were observed, while the lowest values were obtained for three groups (R6, R7 and

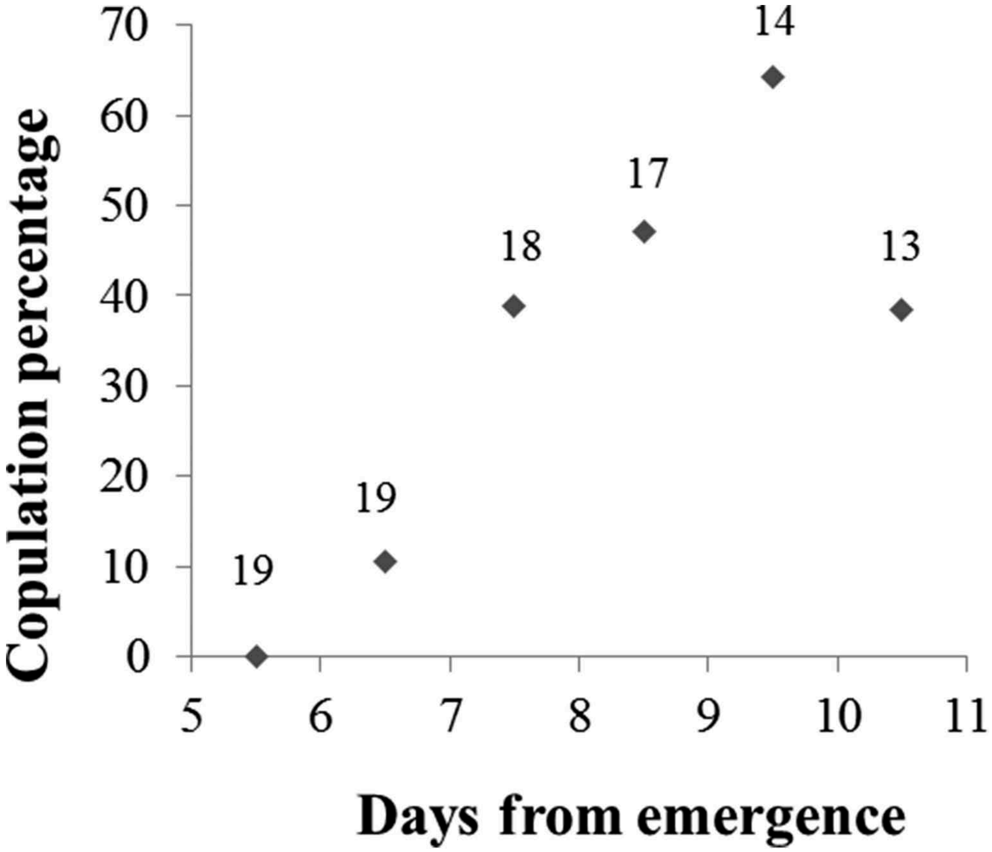

Figure 3. Per cent of *Euxesta eluta* copulating pairs, according to days after adult eclosion. Numbers above the diamonds are the sample sizes of pairs (mated and non-mated) according to age category (days after adult eclosion).

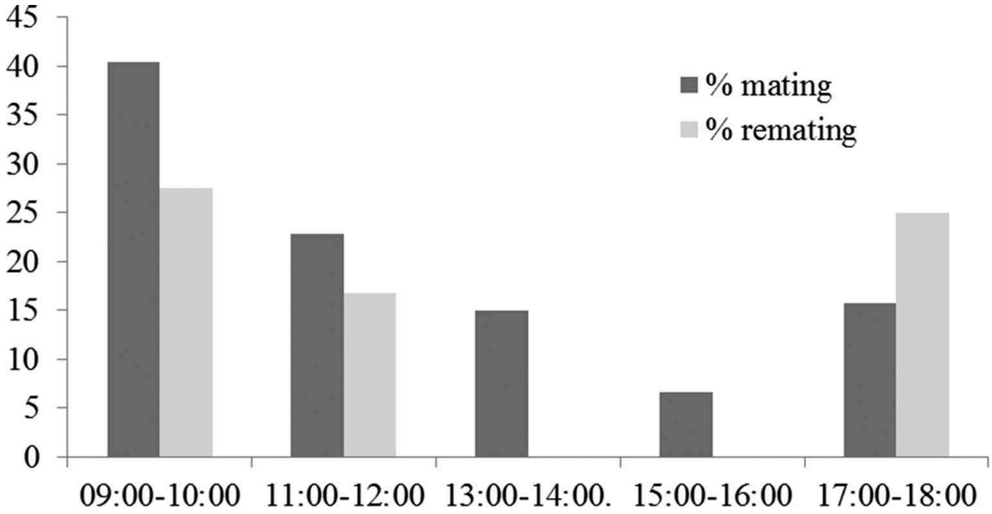

Figure 4. Proportion of observed mating and remating pairs of sexually mature *Euxesta eluta* according to 1-hour intervals over the day.

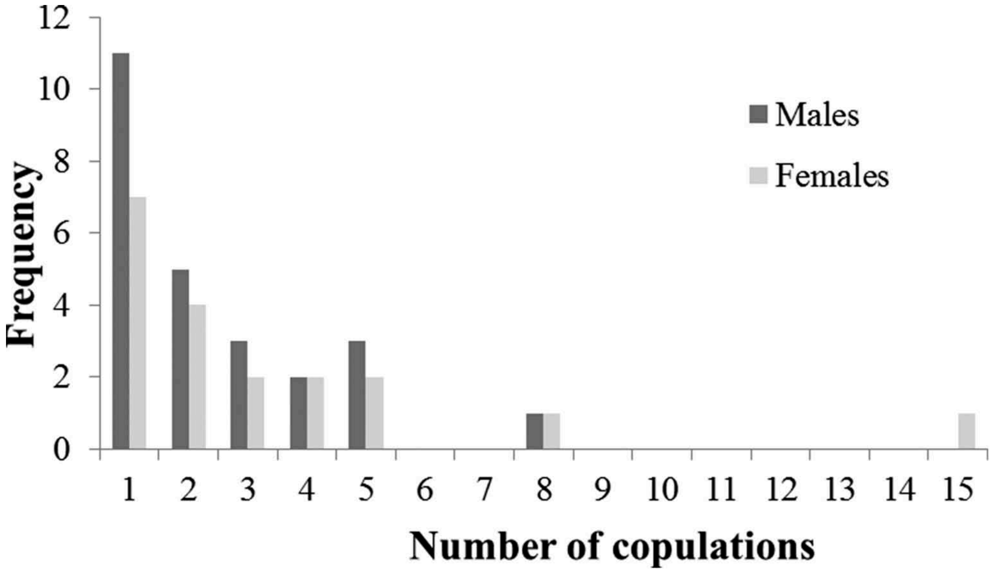

Figure 5. Number of copulations per male and female for seven groups (replicates) of five pairs of *Euxesta eluta* flies, observed in the laboratory for a period of 2 hours.

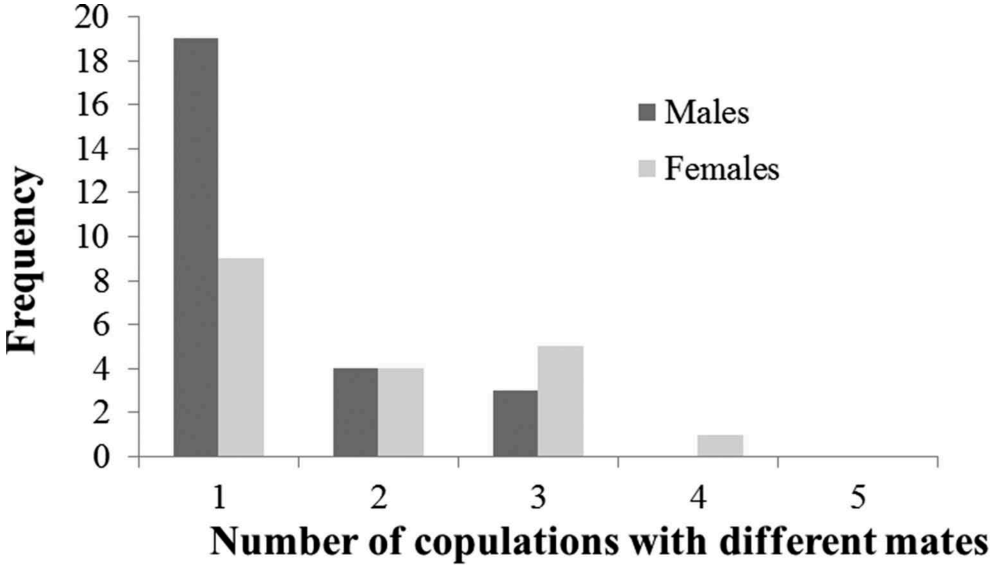

Figure 6. Number of mates per male and female in seven groups of five pairs of *Euxesta eluta* flies, observed in the laboratory for a period of 2 hours.

R8) (Table 2, Figure 7). Similar patterns were obtained in the case of linkage density, but in this case, values for R4 and R5 were greater than those obtained for R3. Both males and females who mated more than once and with different individuals were recorded for all networks. In replicates R1 and R3 each female in its interaction network mated with four and three males, respectively. Such females represented the nucleus for their network. For R1,

Table 2. Descriptive data for eight mating networks resulting from 2 hours of observation of five pairs of individually marked, sexually mature, male and female *Euxesta eluta*.

Group (males: females)	R1	R3	R4	R5	R6	R7	R8
Mated males	4	3	4	4	3	3	4
Mated females	1	2	3	4	3	3	2
Observed mating	16	5	13	13	5	6	4
Nodes	5	5	7	8	6	6	6
Links	4	4	7	8	4	4	4
Connectance	1	0.666	0.583	0.500	0.444	0.444	0.489
Linkage density	2.272	1.931	2.027	2.102	1.466	1.555	1.621
Male nucleus	NaN	1.154	1.305	0.866	1.154	1.154	NaN
Female nucleus	NaN	0.707	0.577	1.224	1.154	1.154	0.707

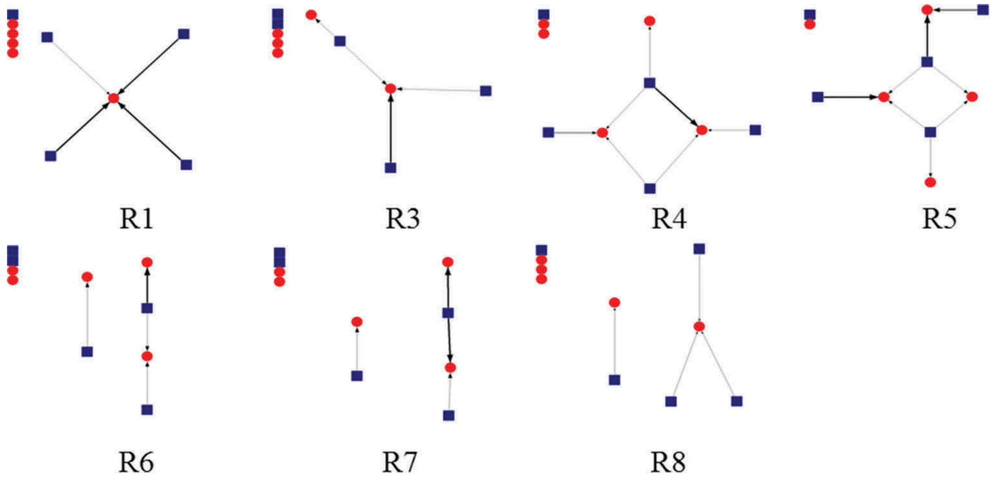

Figure 7. Mating networks of male and female groups of *Euxesta eluta* observed in the laboratory. Blue squares represent the males and the red circles represent the females. Arrows correspond to an observed copulation. Thicker arrows correspond to remating between pairs. Unconnected nodes represent individuals that did not mate.

the nucleus value could not be calculated because it was the only female mating in the network, while for R3 a nucleus female and a peripheral female could be observed. For R4, the network centrality was shared by two females that mated both with three males and one of which remated with one of the males. In R5, one female mated with two males and one with three, this was the network with greatest centrality. For replicates 6, 7 and 8 two groups were structured in each network; in all cases one group corresponded to one pair.

Discussion

Males of both *E. eluta* and *E. mazorca* engaged in courtship sequences involving visual displays (wing waving), physical contact (tapping) and proboscis contact. These sequences would presumably be the result of pre-ejaculatory sexual selection (Parker 2014) where females select male traits that indicate genetic quality or confer direct reproductive benefits (Kirkpatrick 1982). Females of both species could interrupt the courtship sequence at two specific stages. Females facing courting males exhibiting wing displays could avoid copulation by rotating or fleeing when males attempted to surround them to mount them from behind. Once females are mounted they invariably extended the ovipositor from its sheath before intromission, without extension intromission does not occur. These instances suggest that females should be able to reject courting males. Nevertheless, females are typically under continuous and intense male harassment. In the case of *E. eluta* and *E. mazorca* in our study it could be argued that intense male harassment was an experimental artefact of confined conditions; however, Brunel and Rull (2010) also observed numerous *E. bilimeki* males in nature following females for long periods of time (hours), constantly attempting copulation. Seal and Jansson (1989) in turn, also report large numbers of *E. stigmatias* courting and copulating on corn tassels. In this case, an individual male was observed copulating four times with the same female during a 5-minute interval. If resisting male harassment is costly, it might be beneficial for females to accept mating (Gerber and Kokko 2016). If this was the case it could be expected that courtship displays and male traits are under weaker selection pressure than male–male combat or scramble competition. Ejaculate expulsion may therefore be beneficial for females forced into copulation. Indeed, female *E. bilimeki* can expel part or all of the male's ejaculate (Rodríguez et al. 2013). Hence, if females have some control over the amount of expelled ejaculate, pre-copulatory and copulatory courtship could influence female cryptic choice and paternity assignment.

It is worth noting that both species engaged in intersexual proboscis contact before turning and mounting, and mounted males could contact the female dorsum with their proboscis during courtship. Coupled with physical contact during tapping, boxing and mounting, these behaviours could result in chemical exchange of information on the physiological state of a potential mate. Cuticular hydrocarbons are known to function for species and gender recognition (Howard and Blomquist 2005). In *Drosophila* tapping may result in detection of hydrocarbons via taste hairs on the legs, while licking of the female genital region is often the immediate precursor to attempted copulation by the male (van Naters 2014); such contact conveys information on the status of potential mates. Mating trophallaxis has been documented for several species of Tephritidae (Freidberg 1981). In such cases trophallaxis involves the exchange of froth masses or fluid droplets produced by the male and consumed by the female before, during and/or after copulation (Freidberg 1981, 1982, 1997; Dohm et al. 2014). However, in the case of *E. eluta* there was no evident exchange of material during male–female proboscis contact, which was also very brief.

Newly emerged adults of *E. eluta*, engaged in mating activity after a short period of sexual maturation. *Euxesta bilimeki*, another species in the genus, requires feeding on protein sources as an adult for ovarian and testis development (Rodriguez and Rull, unpublished data). Such a condition can be termed anautogeny (Attardo et al. 2005). Our results seem to indicate that *E. eluta* is also an anautogenous species. Adult *E. stigmatias*, were able to survive in the laboratory for 70–120 days (Hentz and Nuessly 2004), and *E. eluta* adults can survive for up to 4 months (Abraham et al. unpublished results); therefore, these species can have a long period of sexual activity.

Two mating activity peaks were recorded, during early morning and late afternoon, during observations of sexual behaviour at different periods of the day. Such a daily pattern seems to correspond to patterns reported in nature for other species in the genus (Seal and Jansson 1989; Brunel and Rull 2010), and are thought to occur because of elevated temperatures during the middle of the day when flies seek refuge and exhibit an overall reduction of activity.

Considering the number of copulations during observational periods (2 hours) and the network connectance values recorded, we can categorize *E. eluta* as a promiscuous species where both males and females mate and remate with more than one individual during a single reproductive cycle (Jennions 1997). In three of the networks analysed some individuals did not group with the rest of the network. This outcome could be an experimental artefact, produced by the short duration of observations (2 hours) in relationship to lifetime sexual activity. It is therefore likely that the number of interactions and their complexity would substantially increase with longer periods of observation. Additionally, the number of pairs that could be simultaneously observed was low. Nevertheless, some networks produced a high interaction density and high connectivity among potential pairs. The existence of nucleus males and females in some networks also indicates differential mating success, probably linked to female–male preferences, on which we have no information.

In sum, it appears that the mating system of ulidiid flies in the genus *Euxesta* is promiscuous (*sensu* Clutton-Brock 1989) and that ejaculate expulsion and consumption by females may be a common behaviour in the genus. The testes of *E. bilimeki* males are extraordinarily large (Brunel and Rull 2010) suggesting that they face intense sperm competition and produce large numbers of sperm. As both sexes engage in multiple copulations with multiple partners, it would be interesting to establish the number of copulations and mating partners that females require to optimize egg fertilization and other measures of fitness. Additionally, it is probable that female *E. eluta* and *E. mazorca* as *E. bilimeki* derive nutritional benefits from ejaculate consumption. The fact that despite promiscuity, the four studied species of *Euxesta* retain and express elaborate courtship sequences, as reported by Seal and Jansson (1989) for *E. stigmatias*, by Brunel and Rull (2010) for *E. bilimeki*, and for *E. eluta* and *E. mazorca* in this study, could imply that female decision to expel or retain all or part of the ejaculate could constitute a form of cryptic female choice. Ejaculate expulsion and consumption could have evolved in females to cope with male harassment and sexual conflict over mating rates; furthermore, they could be benefiting by deriving nutrients from this behaviour. Finally, corn-infesting flies in the genus *Euxesta* can be obtained and reared in the laboratory and are therefore good models to test predictions about the evolution of sexual conflict and female cryptic choice.

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