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Reproductive Behavior of *Echinothrips americanus* (Thysanoptera: Thripidae)

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Abstract

Most Thysanoptera possess a haplo-diploid reproductive mode and reproduce via arrhenotoky. Females can mature eggs successively throughout almost their entire life, but in most terebrantian thrips spermiogenesis is complete by adult male eclosion, and testes contain only mature spermatids. In parasitoid wasps this phenomenon of preadult spermiogenesis is described as prospermatogeny. It is unclear if prospermatogeny and this pre-determined sperm quantity have implications for mating strategy and fitness. In this study, we give a detailed description of mating behavior of the thripine species *Echinothrips americanus*, which largely corresponds with the only available data of another species of this family, *Frankliniella occidentalis* (Thysanoptera: Thripidae). With investigations using light microscopy, we describe for the first time the chronological sequence of internal processes during copulation. The release of male accessory gland material followed subsequently by spermatozoa indicates production of a female-determined type 1 spermatophore. Despite prospermatogeny, males are able to inseminate 10 females with an equal amount of spermatozoa. It is only the quantity of glandular material that decreases with the number of previous copulations. Based on these new findings, the reproductive strategy of this species is discussed.

Key words: sperm transfer, copulation, ejaculate volume, progenesis, prospermatogeny

Detailed knowledge of reproduction is critical to managing those species that compete with us in the field, forest and greenhouse (Heming 1995). Each year, Thysanoptera cause millions of U.S. dollars of crop loss (Lewis 1997a). By piercing and sucking the cells of leaves, flowers, and fruits, thrips species, mainly from the suborder Terebrantia, produce significant damage. Additionally, some of the small species (1–2 mm) are able to transmit plant viruses (Tospoviruses, Machlomovirus, Ilarvirus, Carmovirus, and Sobemovirus), bacteria, and fungi (Ullman et al. 1997, Gitaitis et al. 2003, Jones 2005).

The natural process of thrips dispersal by wind has been supplemented, as in many other insects, by human-mediated global trading activities (Lewis 1997b). One example is the Poinsettia-Thrips, *Echinothrips americanus*, a species that is native to the eastern United States (Stannard 1968). As a greenhouse pest, it has spread over Canada, Europe, Asia, and Northern Australia within the last 30 years (Vierbergen et al. 2006, Hoddle et al. 2012, Krueger et al. 2015). Its broad host range and feeding damage (Oetting and Beshear 1993, Oetting et al. 1993, Vierbergen 1998, Trdan et al. 2003) make it a potential major pest. But as in most species of Thysanoptera knowledge of reproduction is still anecdotal (Moritz 2006).

Most Thysanoptera possess a haplo-diploid reproductive mode and reproduce via arrhenotoky. Haploid males originate from unfertilized eggs, whereas fertilized eggs develop into diploid females. Although females can mature eggs successively throughout almost their entire life, spermiogenesis in most terebrantian males is completed at adult eclosion (Bournier 1956, Heming 1970, Bode 1983). In parasitoid wasps, this phenomenon of sperm progenesis completed prior to emergence is described as prospermatogeny (Boivin et al. 2005). In contrast, synspermatogenetic species are able to produce spermatozoa throughout their whole lifetime. Whether prospermatogeny in Thysanoptera will result in sperm limitation and consequent implication for their mating strategy, behavior, and fitness, is unclear. Detailed description of mating behavior within the Terebrantia is available only for *Frankliniella occidentalis* (Terry and Schneider 1993), *F. schultzei* (Milne et al. 2007) and *Scirtothrips aurantii* (Rafter and Walter 2013). In *F. occidentalis*, males attempt to mate with as many virgin females as possible. In contrast, females reject mating attempts for many days after an initial copulation. Strong competition occurs among males to find virgin females (Terry and Schneider 1993). After a very brief precopulatory period, the male mounts a female, clasps her pterothorax with his legs and twists the tip of his abdomen underneath hers. Insertion of the

aedeagus follows. For successful copulation, antennal contact between individuals, as well as stroking the females' back with the mesothoracic legs of the male is necessary. Later, several periods appear, where no leg or head movement occur (Terry and Schneider 1993). On average, copulation lasts nearly 4 min (238.6 s, Terry and Schneider 1993). The other reported species, *F. schultzei* and *S. aurantii*, show similar behavioral patterns (Milne et al. 2007, Rafter and Walter 2013) and similar mating position. This is also known for several other Terebrantia (Franssen and Mantel 1964, Heming 1995) although complete knowledge of the mating behavior is lacking.

Improving the knowledge of mating behavior and reproductive strategies was the aim of our study because understanding the biology is essential for efficient pest control. Here, we give a precise description of mating behavior, mating frequency, duration, and chronological sequence of copulation. Additionally, we quantified the transfer of spermatozoa to get indications of possible effect of prospermatogeny.

Materials and Methods

Animal Breeding and Keeping
E. americanus laboratory culture was maintained at the University of Halle, Germany. In acrylic cages ($50 \times 50 \times 50$ cm), they were reared on *Phaseolus vulgaris* and *Gossypium sp.* as host plants. The culture was kept at $23 \pm 1\,°C$, $60 \pm 10\%$ RH with a photoperiod of 16:8 (L:D) h at 5000 Lux during the photophase.

To get virgin and naive males and females, females were allowed to lay eggs in wells of 12-well microtiter plates (Greiner, Cat.No: 665 180, Sigma-Aldrich, Munich, Germany). Each well (Ø 2.2 cm) was filled with 1.5 ml of 1.4% (w/v) agar and topped with a leaf disc (Ø 1.6 cm) of *P. vulgaris*. Hatched larvae were reared separately and sexed after eclosion. The plates were kept in a climate chamber under the same rearing conditions as the lab culture.

Mating Behavior and Chronological Sequence
Virgin females of an age of 2–5 d post emergence, males with an age of either 1–3 d (later referred as "young males") or 10–12 d ("old males") were used to analyze mating behavior. Classification was adjusted after the reported mean lifespan of 10–14 d (Krueger et al. 2015).

Prepared wells of 12-well microtiter plate (same as described under rearing conditions) acted as observation arenas. Three females and one male were recorded for 1 h with a HD Webcam C525 (Logitech, Lausanne, Switzerland).

To make our behavioral analysis comparable to other studies of Terebrantia, we recorded the same behavioral traits as Terry and Schneider (1993) (Table 1). Video playbacks have been used for investigation and analysis of duration and number of behaviors. Due to the small size of the insects, we were unable to analyze antennation and stroking via video playbacks. Therefore, data were supplemented by direct observations. Direct observations were conducted in the same observation arenas under a stereomicroscope (Leica S8 APO, Leica, Wetzlar, Germany).

Internal processes during mating and chronological sequences of copulation were analyzed by using histological methods. Couples were frozen in supercooled carnoy's fluid ($-80\,°C$) (abs. ethanol:chloroform:glacial acetic acid 6:3:1) at different time intervals after insertion of the phallus (1, 3, 5, 7 min, control: recently finished copulation). Afterward, specimens were prepared for light or electron microscopy. Specimens for light microscopy were dehydrated in a graded ethanol series, embedded in Surgipath Paraplast (Leica, Prod. No. 39601006, Leica), cut into 5-µm sections (Leica SM 2000R microtome, Leica) and stained with hematoxylin-eosin. Slides were observed with a

Table 1. Observed mating behaviors with their description in *E. americanus*

Behavior observed	Description
Test start to male mounting	Time between start of video recording and male mounting
Number of prior matings of male	Number of previous matings of male before recorded copulation
Female rejects male prior mating	Number of rejections of female prior copulation (same individuals)
Initial contact to male mounting	Time between initial contact (antennal contact) and male mounting
Contact position	Position of male and female during initial contact (head to head, head to abdomen, undetermined)
Antennation	Antennal contact of male and female
Stroking	Male palpate females back with his mesothoracic legs
Duration of mating	Time between insertion of aedeagus and separating
Calm period during mating	Period during copulation without any observable movement of both sexes
Female walking during mating	Period of female walking with male on her back during copulation

Leitz DMBRE (Leica) fitted with a Leica DFC 450C. Electron microscopy samples were fixed, dehydrated, and embedded as samples for light microscopy. Specimens were cut into half by consecutive removing of 5-µm sections (Leica SM 2000R microtome, Leica). Afterward, specimens were carefully washed in xylene and 90% EtOH to remove paraplast. Hexamethyldisilazane was used to finish the samples. Later, these were mounted on aluminium stubs with double-sided adhesive pads. Gold coating was performed with a Balzers SCD 004 sputter coater (Bingen, Germany) for 200 s at 20 mA. Samples were visualized with a scanning electron microscope Hitachi SEM S-2400 (Tokyo, Japan) at 18 kV and documented on ILFORD FP 4 (Knutsford, Great Britain) roll film. Images of both microscopic techniques were adjusted for contrast and brightness. Furthermore, males were restained on the scanned electron-microscopy images with the help of Photoshop CS5 (Adobe, San Jose, CA).

Remating Frequency of Females
Remating frequency was observed for copulations with the same male, as well as for copulations with new virgin males. For initial mating, couples were placed in an observation arena for 24 h. After initial mating, couples were separated. Every 24 h either the same male (as initial mating) ($n = 27$) or a different male ($n = 18$) was presented again to each female. Within 30 min, number of mating attempts and conducted matings were counted visually under a stereo microscope (Leica S8 APO, Leica). After the 30-min test interval, the individuals were separated and kept in plates as described before (animal breeding). The test runs until natural death of individuals (females' death in remating trial with different male, males' death in remating trial with the same male).

Quantification of Sperm Transfer
In order to quantify transfer of spermatozoa to the female and sperm limitation in the males, one male and two females were placed in a well of a 12-well Greiner-plate, prepared as described above and kept in a climate chamber. Every 2 d, the male was transferred to two new virgin females. The procedure was stopped after 0, 5, or 10 consecutive

matings of the male. To check for age-dependent effects, virgin males were treated similarly but without females. Afterward, all individuals were processed for histological analysis as illustrated in section Chronological Sequence. After DAPI staining (0.2 µg/ml Dapi in Aqua dest., time: 15 min, washing 3× Aqua dest.), samples were mounted in Anti-Fade-Medium (recipe after Jackson ImmunoResearch, www. jacksonimmuno.com/technical/anti-fade.asp). Slides were examined with a light microscope Leitz DMBRE (Leica), fitted with a Leica DFC 450C, and processed with ImageJ software (version 2.0.0, Wayne Rasband, http://imagej.nih.gov/ij/). Dapi intercalates with DNA; therefore, the brightness of the coloration indicates the amount of DNA present (e.g., Darzynkiewicz 2010) and thus can be used to estimate spermatozoan quantity. Inverted 8-bit gray scale images (0= black, 255= white) were used to measure area, and mean gray values of the sperm ball within the spermatheca or of the spermatozoa remaining within the testes (see Supp Appendices S1–S4 [online only]). To ensure comparable results, staining procedure, microscope setting, and room conditions were kept the same for all measurements. Further, mean gray value of testes or sperm ball was taken in relation to background (minimal coloration produced) and nucleus of somatic tissue (maximal coloration produced) to limit staining artefacts.

The index of the gray values of testes or sperm ball was calculated as follows:

$$\frac{(mean\ gray\ value\ of\ testes\ or\ sperm\ ball - mean\ gray\ value\ background) * 100}{(mean\ gray\ value\ nucleus - mean\ gray\ value\ background)}$$

Mean gray value of background was measured in a 20 × 20 µm area outside of tissue. Maximal produced staining was measured on nuclei of the gut (Supp Appendices S1–S4 [online only], measurement of background not shown).

Statistical Analysis

Data analysis was performed with WinStat for Excel (Fitch Software, Bad Krozingen, Germany) and SPSS Statistics 22 (IBM Corp., Armonk, NY). Prior to analysis, data were checked for normal distribution using Shapiro–Wilk test ($P > 0.05$). Because of data and residual distribution, data on mating behavior were analyzed by using nonparametric tests, whereas data on sperm quantification were handled with parametric tests.

Mating Behavior

Times of test start to mounting, initial contact to male mounting, duration of mating, calm period, and walking of female during copulation were analyzed with Mann–Whitney U test to distinguish between young and old males ($P < 0.05$). Frequency of contact position was analyzed with 2 × 3 Fishers exact test, $P < 0.05$.

Frequency

Frequency of mating attempts and mating in both remating trials were analyzed with 2 × 2 Fishers exact test, $P < 0.05$.

Sperm Quantification

Area and index of gray values of testis and sperm ball within female were analysed with analysis of variance (ANOVA), means were separated by LSD post hoc test ($P < 0.05$). Data of both testes of one individual showed no difference (paired t-test, area: $t = -0.811$, $P > 0.05$; index of gray value: $t = -0.336$, $P > 0.05$) and were therefore pooled.

Results

Mating Behavior and Chronological Sequence

Copulation involves three phases: precopulation, copulation, and postcopulation (Table 2). The precopulatory phase starts with an initial contact of both sexes. Generally, the male contacts the female with his antenna either in a head-to-head position (50%) or head to female abdomen/thorax position (27.7%). Then a receptive female lowers her abdomen and thorax, whereas the male mounts onto the female's back. Unreceptive females throw off males by raising their abdomen. Males repeatedly attempt to mate with a female, even when she is not receptive. In the following copulation period, the male twists his abdomen underneath that of the female and tries to insert his aedeagus into

Table 2. Mating behaviors of *E. americanus* analyzed from video observations, different letters indicate for significant differences

Behavior	n	Duration in s ± SD	Frequency in %	Range in s
Precopulation				
Test start to male mounting				
Young males	10	633 ± 533.8a		165–1641
Old males	9	1113.2 ± 860.6a		105–2304
Initial contact to male mounting				
Young males	10	8 ± 2.8a		5–13
Old males	9	9.7 ± 5.1a		4–20
Contact position				
Head to head	18		50	
Head to female abdomen	18		27.7	
Undetermined	18		27.7	
Copulation				
Duration				
Young males	10	403.2 ± 31.28a		342–444
Old males	8	610.66 ± 202.2b		389–995
Calm period				
Young males	10	297.3 ± 128.2a		91–830
Old males	8	373.22 ± 271.1a		88–423
Female walking				
Young males	10	11.1 ± 35.1a		0–131
Old males	8	82.8 ± 145.4a		0–111

the female genitalia. Meanwhile, he palpates her antennae with his antennae, as well as stroking her back with his mesothoracic legs. Successful insertion is followed by a calm period, which can vary greatly in duration. This phase is characterized by no movement of any part of the body. Prior to separation of the couple, the female sometimes starts to walk around with the male on her back (Table 2).

Mating behavior was analyzed to compare male age at mating. Younger males had a significantly shorter duration of mating

(U-test, $Z = -2.711$, $P = 0.006$), than older males. All other recorded behaviors did not differ significantly.

During copulation, both parameres and the primitive aedeagus were completely inserted within the females' vagina (Fig. 1A–E). Endotheca and phallotheca were evaginated from the beginning of copulation (Fig. 1D), and the paraphyses were located near the opening of the spermathecal duct (Fig. 1D and E). One minute after insertion, the endotheca seems to be filled mainly with accessory
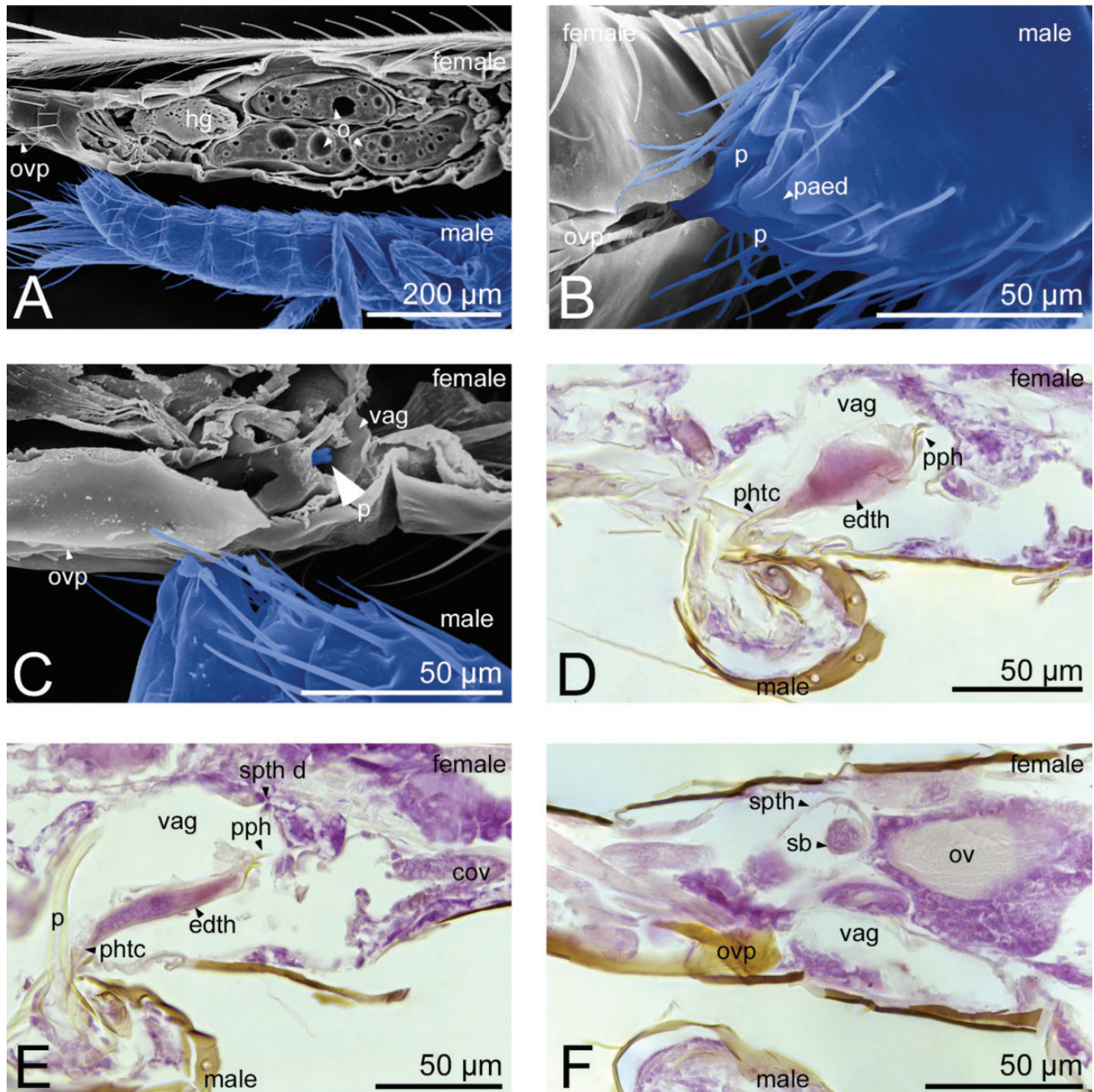
Fig. 1. Copulation of *E. americanus*. (A–C) Male in blue color, SEM, light microscope, hemalaum-eosin stained (D–F). (A) Sagittal view of male and female in copula, note in life male is on the back of the female, clasps her pterothorax with his legs and twists the tip of his abdomen underneath that of the female. (B) Male with genitalia fully inserted into female, cranio-dorsal view of couple. (C) Detailed view of inserted male genitalia (blue), note the parameres within the females' vagina (big arrowhead). (D) Couple fixed 1 min after insertion of aedeagus, note the magenta colored male accessory gland secretion within endotheca (sagittal section). (E) Couple fixed 3 min after insertion of aedeagus, note the magenta colored male accessory gland secretion and few sperm (lilac) within endotheca (sagittal section). (F) Couple fixed 7 min after insertion of aedeagus, note the filled spermatheca of the female (sagittal section). cov, common oviduct; edth, endotheca; hg, hindgut; o, oocyte; ovp, ovipositor; p, paramere; paed, primitive aedeagus; phtc, phallotheca; pph, paraphyses; sb, sperm ball; spth, spermatheca; spth d, spermathecal duct; vag, vagina.

gland secretion (magenta-stained) and only few spermatozoa (Fig. 1D). After 3 min, more spermatozoa are visible within the endotheca (Fig. 1E). With increased time after the start of copulation, the number of visible spermatozoa within the endotheca increases. But spermatozoa were first observed in the spermatheca at 7 min after start of copulation (Fig. 1F). After successful copulation, a sperm ball of twisted spermatozoa was located within the spermatheca (Fig. 1F).

Remating Frequency of Females

In both conditions (remating with the same or a different male), only one female mated again with the same male 3 d after the initial copulation. But no significant differences in mating attempts or frequency of mating were observed between both remating trials (mating attempts, Fishers exact test, $P = 0.766$; matings, Fishers exact test, $P = 1$) (Table 3).

Quantification of Sperm Transfer

The area of sperm content within the testes differs between the conditions (ANOVA, df1 = 4, df2 = 74, F = 23.673, $P < 0.001$). We observed a decrease in sperm content over time, regardless of the number of previous matings: the area of sperm within the testes of

Table 3. Frequency of mating attempts and matings in remating trials with different male and with the same male after initial copulation

	Remating trial with different male	Remating trial with the same male
n (number of tested females)	18	27
Mating attempts	9	11
Matings	0	1

10-d-old virgin males were significantly smaller than in 5-d-old virgin males or in freshly emerged males (0-d virgin) (posthoc LSD, 10-d virgin vs. 5-d virgin, $P = 0.001$; 10-d virgin vs. 0-d virgin, $P < 0.001$). Additionally, the area of sperm content decreased with the number of repeated copulations (Posthoc LSD, 5× mating vs. 10× mating $P < 0.001$) (Fig. 2).

The index of gray value of sperm within the testes differs significantly between the conditions (ANOVA, df1 = 4, df2 = 74, F = 6.211, $P < 0.001$). Age of the males had a significant effect on index of grey value. Freshly hatched adult males (0-d virgin) have significant lower index than 5-d virgin males (posthoc LSD, $P = 0.003$). Index differ also between virgin and mated individuals (posthoc LSD, 5-d virgin vs. 5× mating, $P = 0.041$; 10-d virgin vs. 10× mating $P = 0.001$). Furthermore, the sperm number decrease with consecutive matings (posthoc LSD, 5× mating vs. 10× mating $P = 0.03$) (Fig. 3).

The sperm ball size within the spermatheca decreased after copulation with males with increased number of previous matings (ANOVA, df1 = 5, df2 = 50, F = 5.504, $P = 0.001$) (Fig. 4). Interestingly, the index of gray value and therefore brightness of sperm ball did not differ with number of previous matings by the male (ANOVA, df1 = 5, df2 = 50, F = 1.239, $P = 0.306$) (Table 4).

Discussion

Mating Behavior

Mating behavior was analyzed with video playbacks, as well as direct observations. Behavior in *E. americanus* corresponded largely to that of *F. occidentalis* described by Terry and Schneider (1993), *F. schultzei* (Milne et al. 2007), and *S. aurantii* (Rafter and Walter 2013). After the phase of initial contact, which was much shorter in
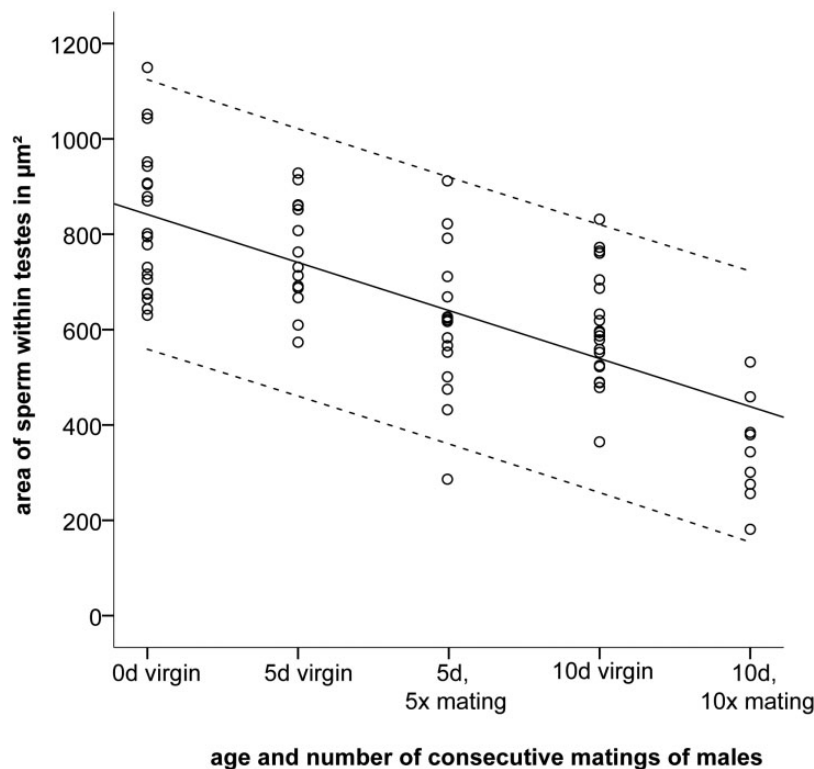
Fig. 2. Area of sperm within testes of male *E. americanus* depending on age and number of previous matings. Solid line indicates the regression line, dashed line the 95% confidence interval.
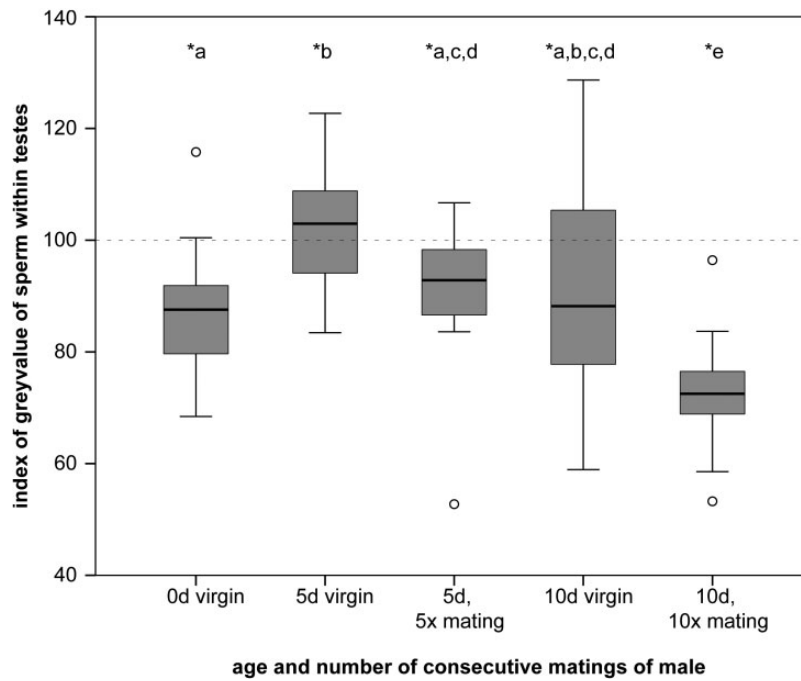
Fig. 3. Box-plot of the index of grey value of sperm within testes of male *E. americanus* depending on age and number of previous matings calculated by the formula: Index = (mean gray value of testes − mean gray value background) × 100/(mean gray value nucleus − mean gray value background), bold line indicates the median, different letters indicate for significant differences.
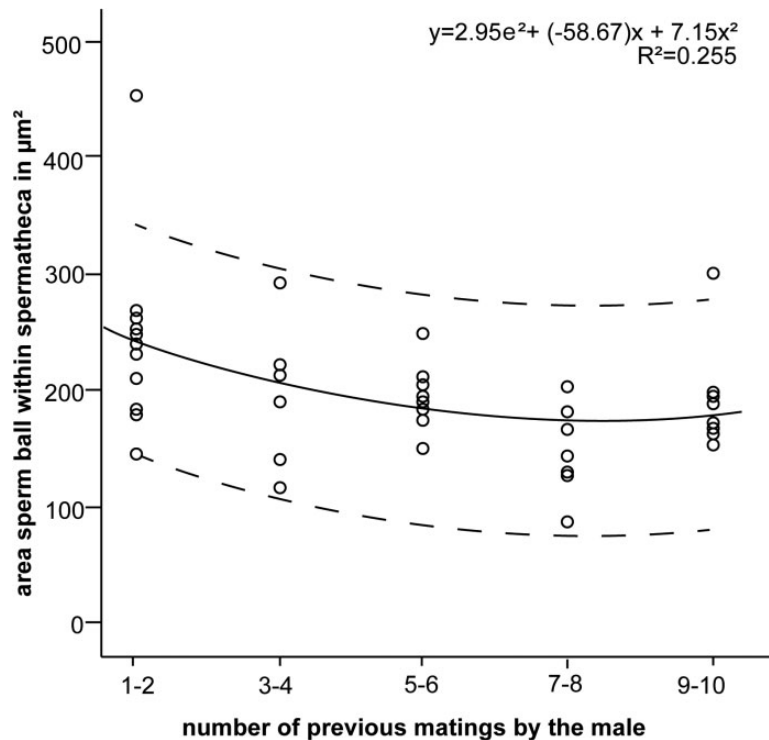
Fig. 4. Area of sperm ball within the spermatheca after a single mating by a female with males that had mated previously with other females in *E. americanus*. The number of previous matings by the male ranged from x1 to x10. Solid line indicates the regression line, dashed line the 95% confidence interval.

E. americanus (about 8.61 s in *E. americanus*, 19 s in *F. occidentalis*), mounting occurred. The male twisted his abdomen underneath that of the female and tried to insert his aedeagus into the female genitalia. The duration of copulation varied with male age. Older males copulated significantly longer than younger males, a phenomenon known also in *Tenebrio molitor* (Carazo et al. 2011). Presumably longer copulation is necessary to ensure transfer of enough sperm to the female, because we demonstrated a decrease in

Table 4. Index of gray value of sperm ball within spermatheca after a single mating by a female with males that had mated previously with other female *E. americanus*

Number of previous matings by the male	1–2	3–4	5–6	7–8	9–10
N	15	6	14	8	13
Index of gray value ± SD	95.59 ± 8.7	111.50 ± 11.9	97.0 ± 22.37	93.02 ± 24.15	98.69 ± 13.04

The number of previous matings by the male ranged from × 1 to × 10

spermatozoa quantity within the testes (see section below: sperm quantification), or older males are less effective in sperm transfer (Carazo et al. 2011).

However, copulation duration in *E. americanus* was much longer than in *F. occidentalis* (Terry and Schneider 1993), but seems typical for terebrantian species (*Caliothrips fasciatus* 1–10 min, Lewis 1973; *Thrips major* and *Thrips fuscipennis*, 6 min, Kirk 1985; *F. schultzei* 9 min, Milne et al. 2007; *S. aurantii*, 13 min, Rafter and Walter 2013).

Chronological Sequence

Copulation starts with insertion of the phallus into the vagina. Phallotheca and endotheca are everted, presumably by hydrostatic pressure (Heming 1970, Pitkin 1972). The vagina is heavily stretched, compared to virgin females. The placement of the gonopore near the mouth of the spermathecal duct has also been described in *F. fusca* (Heming 1970). Sensilla on the outside of the parameres presumably assist in positioning the phallus within the female.

First, accessory gland material is transferred into the female, whereas spermatozoa transfer occurs 3 min after phallus insertion. Since no glandular material or spermatozoa were found inside the vagina, and the paraphyses were located close to the spermathecal duct, it can be assumed that the ejaculate migrates directly to the duct and to the spermatheca. However, this process seems to take some time to complete, as filling of the spermatheca could be observed 7 min after insertion. After copulation, a sperm ball of twisted spermatozoa is observable within the spermatheca. This sperm ball consists of a spherical capsule of densely packed secretion outside and less electron dense material and spermatozoa inside (Teuber 2011). The encapsuled sperm ball is similar to that of other species within the Thripidae (Heming 1995).

Bournier (1956) supposed this type of sperm ball to be a spermatophore. He uses the term spermatophore in a stricter sense as an ampulla or capsule created by males and transferred to the female. Teuber (2011) argued that the unequal distribution of secretion within the sperm ball suggested a spermatophore rather than a coagulation within the female reproductive tract. But the gradual release of glandular secretion, and delayed transfer of spermatozoa shown in this study, might be reasons for the former reported distribution patterns of spermatozoa and glandular material. Therefore, the time-delayed transfer of secretion and spermatozoa, as well as the delivery near the spermathecal duct shown here, support Bode's (1975) assumption of coagulation of the secretion within the spermatheca.

The sperm ball seems to be a special form of "female-determined type 1 spermatophore" according to Gerber (1970), which is characterized by an ejection of secretion in a definite sequence before or after the spermatozoa and a form of coagulated material, determined by the female genital tract. This was already suspected by Heming (1995). A similar "spermathecal spermatophore formation" is known only in the psocids *Leptinotus patruelis* (Finlayson 1949) and *Trogium pulsatorium* (Klier 1956). Within the tubuliferan

species only *Tiarothrips subramanii* is known to produce spermatophore-like objects (Ananthakrishnan 1990).

Frequency

Remating frequency was extremely low in *E. americanus*. Although males mate multiple times, females reject mating after initial copulation, also noted by Li et al. (2014). Females of *F. occidentalis* refuse matings over a period of 15 d (Terry and Schneider 1993) and *Thrips tabaci* is the only known species within the Terebrantia with repeated matings (Li et al. 2015). Frequent matings are also typical within the Tubulifera (Crespi 1986a,b, 1988a,b).

In *E. americanus*, a single copulation is enough to provide sufficient sperm to fertilise eggs throughout female's life time (Krueger et al. 2015). But monandry (female mate with one male during lifetime) is rare in insects (Arnqvist and Nilsson 2000, Hosken et al. 2009).

Quantification of Sperm Transfer

As expected, males show a significant decrease in area of spermatozoa within the testes in relation to the number of consecutive copulations. Additionally, the lifespan of males also has an impact on spermatozoa area quantity. Possibly muscle contraction in the testis wall, suppression by other internal organs, or consumption of possible secretions within the testes are reasons for this observation. Brightness of sperm within testes, and therefore the amount of contained sperm, decreases with the number of matings. But after 10 copulations, the testes still contain 70% of spermatozoa compared to 5-d-old virgin males.

Freshly emerged virgin males had a lower index of gray value, than 5- or 10-d-old virgin males. Possibly, maturation of sperm is not entirely completed at this point. Afterward (5- or 10-d-old males), the age of virgin males had no impact on index of gray value of spermatozoa within testes; therefore, no decrease in amount of spermatozoa with the age is detected. However, we did not test fertility of aged sperm, which is known to decrease with progressive age of sperm cells (Reinhardt 2007).

In females, brightness of sperm ball within the spermatheca did not differ. In our study, females seem always to receive a similar amount of spermatozoa, even from copulation with a tenfold mated male. But area of sperm ball significantly decreased with number of copulations. Probably the amount of transferred male accessory gland material decreases over time. In histological sections, flattened and emptied accessory glands were conspicuous in ×10 mated males (see Supp Appendices S5 and S6 [online only]).

Nonetheless, sperm limitation of males, prospermatogeny is of minor importance. Males of *E. americanus* are able to successful inseminate up to 10 females. Males of *Trichogramma evanescens* transfer relatively constant numbers of sperm to the first 10 females, but subsequently fewer (Damiens and Boivin 2005). Transfer of accessory gland material might influence insemination success. Male *Drosophila melanogaster* run out of gland material after 4–5 consecutive matings and fail to transfer sperm (Lefevre and Jonsson 1962).

A similar phenomenon is described in *Drosophila silvestris* after two matings (Schwartz 1991) and in bedbugs (Reinhardt et al. 2011). Gland substances are linked not only to sperm transfer and sperm protection but also to the induction of refractoriness in females, reduction of attractiveness, sperm competition, regulation of egg development, and induction of ovulation and oviposition (see Chen 1984; Gillot 1988, 2003).

The different mating strategies of polygynous males and monandrous females within this species lead to a high risk of sexual competition. It is not clear if females control the opportunity to mate with their limited receptivity, or if the monandry is male determined. In addition to the resistance of the female to continuous male mating attempts, males are able to manipulate female receptivity either by their accessory gland products (see above) or by transferring of anti-aphrodisiacs (Hosken et al. 2009). Such a pheromonal substance is detected for *E. americanus* (Krueger et al. 2016), but such substances have only a transient effect and serve as bridging until the female is able to produce her own pheromone (Simmons 2001). Further investigations are needed to distinguish the role of both sexes in this mating strategy.

Nevertheless, prospermatogeny in *E. americanus* does not lead to a restriction of mating success and seems to be an adaptation to their lifestyle. The short lifespan (10–14 d, Krueger et al. 2015), the brief opportunity to mate (low remating frequency of females), and the small bodysize (because of the relation of energy costs for maintaining gamete production and testes tissue in proportion to size) favors the development of prospermatogeny (see Boivin et al. 2005). Therefore, as in many flower-living Thripidae, *E. americanus* seems to be closely adapted to rapid reproduction and dispersal.

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Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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