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RESEARCH ARTICLE

Evolutionary Consequences of Desiccation Resistance in the Male Ejaculate

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Abstract Avoiding water loss for insects is critical for survival. Selection for reduced water loss will depend on trade-offs between resources allocated for reproduction and those allocated for resisting desiccation. However, we lack knowledge on how selection for desiccation resistance can affect the male ejaculate. Furthermore, as male ejaculate composition is complex, desiccation resistant females could evolve traits that enable them to derive longevity benefits from mating. Here, we assessed how selection for desiccation resistance impacts male testes and accessory gland size, protein content of these organs, female sperm storage and male ability to inhibit female remating behavior, in the Mexican fruit fly *Anastrepha ludens*. Additionally, we tested if mating increased longevity and fecundity in desiccation resistant females. Males selected for resistance to desiccation stress had smaller accessory glands and seminal vesicles and females mating with these males stored less sperm compared to control males. Females mating with resistant males had lower fecundity compared to females mating with control males. Desiccation resistant females lived longer than control females, yet this was irrespective of mating. Rapid evolutionary responses to hydric stress can have correlated effects in reproductive capabilities, which are not restricted to pre-copulatory traits. Trade-offs between resistance to desiccation stress are reflected in decreased allocation of resources to reproductive organs. Thus, production of the ejaculate may be costly for *A. ludens* males. Knowledge on the evolution of ejaculate traits and reproductive organ size in response to directional selection for desiccation resistance, will aid our understanding of differential sex-specific responses to environmental stress.

Keywords Seminal fluid · Tephritidae · Diptera · Reproductive trade-off · Sperm · Accessory glands · Remating

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Introduction

Among the most important factors that can affect survival of organisms in the field are relative humidity and water availability (Cloudsley-Thompson 1975). Insects are especially susceptible to desiccation conditions because smaller organisms have a relatively bigger surface area for water loss (Price 1997). Accordingly, it is expected that insects evolve traits that increase their desiccation resistance, such as increasing water storage and decreasing water loss (Chown et al. 2011). Resistance to desiccation usually involves food and water deprivation (Matzkin et al. 2009), and both can severely affect the physiology of an insect in terms of lipid, glycogen, weight or water content (Rose 1984; Service et al. 1985; Hoffmann and Harshman 1999). Furthermore, the reproductive ability of an insect subjected to desiccation stress can be diminished if resources are allocated towards lifespan instead of reproduction (e.g. Rion and Kawecki 2007; Zera and Harshman 2001; Huestis and Lehmann 2014). The cost of reproduction and desiccation stress have focused on pre-copulatory behaviours such as mating, changes in cuticular hydrocarbons or reproductive consequences of females in terms of fecundity (Gefen and Gibbs 2009; Kwan et al. 2008; Gefen and Brendzel 2011; Kwan and Rundle 2010; Stinziano et al. 2015), while it is not known what evolutionary trade-offs there are between desiccation resistance, investment in the ejaculate or gonads and other female post-copulatory responses such as remating.

Physiological costs of mating have usually been examined in terms of sperm and egg production (reviewed in Flatt 2011), and correlated responses to stress have been assessed on sperm traits (e.g. Rohmer et al. 2004; Liao et al. 2014; for sperm traits under high temperatures or heat shock; Singh et al. 2016 for sperm attributes in cold shock selected lines; Reinhardt et al. 2016 for a review on environmental effects on sperm phenotype, not including desiccation stress). However, the composition of the male ejaculate in insects is complex. Seminal fluid is composed of accessory gland proteins, sperm and also contains water, salts, sugars, fats and additional molecules (Perry et al. 2013), can be costly to produce (Dewsbury 1982; Olsson et al. 1997; Wedell et al. 2002) and could require considerable resources for insects subjected to hydric stress. Proteins produced in the male accessory glands in insects are responsible for many physiological changes in females after copulation, such as an increase in egg-laying and a decrease in sexual receptivity (Gillott 2003; Avila et al. 2011). Males have been shown to tailor their investment in specific seminal proteins depending on the mating status of females or developmental environment (Sirot et al. 2011; Wigby et al. 2016). However, it is unclear how ejaculate components and male reproductive organs are affected by stress such as desiccation.

Selection pressures can result in differential responses between males and females to hydric stress (Kwan et al. 2008; Sassi and Hasson 2013). For example in the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae), females are more susceptible to desiccation than males, independent of sexual size dimorphism (Tejeda et al. 2014). Also when exposed to stress, males consumed significantly more water than females, however, both sexes utilized similar quantities of lipids (Tejeda et al. 2014). Females could also evolve adaptations for increased desiccation tolerance by deriving water and/or nutrients from the male ejaculate. For example, in the desert *Drosophila* species, *D. mojavensis* and *D. arizonae*, mated females had higher resistance to desiccation conditions compared to virgin females across all populations of both species (Knowles et al. 2004, 2005). In *Drosophila melanogaster* mated females have higher starvation resistance than virgin females (Rush et al. 2007; Goenaga et al. 2012). In seed beetles, females derive water from the male ejaculate, and remate more often when they have no access to water (Edvardsson and Canal 2006; Ursprung et al. 2009; Harano 2012). Another instance where females apparently derive water from their mates is in the Ulidiid maguey fly *Euxesta bilimeki* adapted to semi-arid zones in the central Mexican highlands, where females expel the ejaculate after mating and then consume them (Brunel and Rull 2010). When female *E. bilimeki*, were housed with males under starvation and desiccation conditions they lived longer than females with no access to males (Rodriguez-Enriquez et al. 2013).

In this study, we assessed if resistance to desiccation may impact male investment in the quantity or quality of the ejaculate. Specifically, we measured the accessory gland and testes sizes, protein content of accessory glands and testes, and sperm storage of a desiccation resistant strain of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae). Furthermore, we tested male ability from the resistant strain to inhibit wild female remating. Given the divergent sex-specific response to stress, we also examined if desiccation resistant females under stress derived fitness benefits from mating. We predicted that resistant males would have diminished ejaculates and reproductive organs from trade-offs associated with desiccation resistance. For desiccation resistant females subjected to hydric stress we predicted that mating would increase fecundity and longevity.

Wild *A. ludens* are broadly distributed from the semi-arid south of Texas to the tropical forests of Costa Rica (Stone 1942; Ruiz-Arce et al. 2015). This fly has great plasticity and capacity to adapt to different humidity conditions (Celedonio-Hurtado et al. 1995; Thomas 2003). A desiccation resistant strain was developed from a mass-rearing strain using directional selection, which affected certain life-history traits. This strain has higher longevity, bigger body size, increased body lipids and water, and longer pupal

stages; females have delayed sexual maturation, decreased daily fecundity but overall equal fecundity compared to non-selected females (Tejeda et al. 2016). No apparent pre-copulatory costs were detected, as selected males were just as likely to mate with wild flies than non-selected males. The selection protocol is outlined below and in Tejeda et al. (2016).

Materials and Methods

Anastrepha ludens

Origin of Selected and Control Flies

Anastrepha ludens flies were obtained as pupae from a mass-reared strain produced at the MoscaFrut biofactory at Metapa de Domínguez, Chiapas, Mexico. Approximately 300 million individuals of this fly strain are produced per week. From this original population, 10 experimental populations of 400 flies each were obtained: five selected for desiccation resistance (hereafter named as "resistant") and five unselected, control populations (Tejeda et al. 2016). Males and females were placed in separate cages (200 females and 200 males per replicate) to prevent mating before selection. Resistant populations were obtained submitting flies to hydric stress and starved for food [desiccation treatment: without access to water or food and very low relative humidity (~22%)] and crossing individuals that survived. Desiccation conditions were achieved by placing three plastic containers containing silica gel (Sigma-Aldrich, PubChem Substance ID: 24899758) in each cage. The containers were covered with a nylon mesh to avoid direct contact (Tejeda et al. 2014). Cages were then sealed with self-adhesive plastic film. Observations took place every 8 h or less until approximately 12% of the population remained alive. Survivors were transferred to cages with water and food in the form of sugar and hydrolysed yeast (ICN Biochemicals, Aurora, OH) provided in a 3:1 ratio. At least 25 pairs of each of the 10 populations were used to reproduce the following generation. An additional 25 pairs were used for selected populations if 25 pairs were not obtained in the first cohort (group of individuals that emerged on the same day). In the control populations, random matings (random crosses) were conducted without applying hydric or food stress (Tejeda et al. 2016). Pairs were sampled randomly from control populations. Resistant and control populations were reared at low densities for 31 generations. A pool of the 10 experimental populations (resistant and control), were sent as pupae by air transportation to Xalapa, Veracruz, Mexico. Experiments were carried out at the Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Xalapa, Veracruz, Mexico.

Sperm Storage

Control and resistant flies were tested 23 days after adult emergence. Crosses between control females and control males (n = 25) or resistant males (n = 26) were obtained as above. To decrease female mediated effects on sperm transfer and storage, dissections of spermathecae were done immediately after the end of copulations. Females were dissected under a dissecting microscope (Leica S8AP0) following Taylor et al. (2000). Reproductive tracts were removed and placed over a slide with a 50 µl drop of saline solution (NaCl 0.9%, PISA®). Spermathecae were dissected and placed together on slides with 10 µl of saline solution containing 0.1% of soap (Triton®). Spermathecae were broken with fine forceps and the drop was stirred quickly with entomological pins for 1 min. An 18 × 18 mm coverslip was then placed on top of the storage organs and secured with transparent nail polish. Spermatozoids were counted under a phase contrast microscope (Leica CME) at ×200 magnification. The whole slide was covered by counting all spermatozoids in 50 randomly selected fields, which corresponds to 12.11% of the total area. To obtain the total number of sperm stored, a conversion factor of 8.25 was applied to the sperm counted. When no sperm was counted in 50 fields, a coverslip screening was carried out to ensure that there was no sperm in the storage organs of the female.

Protein Content in Accessory Glands and Testes

Protein content of accessory glands and testes were assessed. Virgin control and resistant males of 12–13, 17–18 and 30–31 days-old were used, spanning the ages when males have the highest mating and insemination success (Harwood et al. 2015; Reyes-Hernández and Pérez-Staples 2017). Males were dissected in cold saline solution (NaCl 0.9%, PISA®) and glands or testes were transferred to a 1.5 ml centrifuge tube with saline and protease inhibitors (Roche® Complete Protease inhibitor cocktail) and gently crushed with a micro-size tissue grinder for 1 min to release the content. 35 accessory glands (i.e. 35 males) of each male category and strain were pooled in 35 µl of saline with protease inhibitors. For testes, 20 pairs (i.e. 40 testes = 20 males) were used in 40 µl of saline with protease inhibitors. This aqueous extract was centrifuged at 12,000 rpm at 4 °C for 1 min (Hermle Z 300K centrifuge). The resulting supernatant was placed in crushed ice and the pellet was discarded. Protein quantification was carried out with the Bradford reagent (Bio-Rad, USA) at 595 nm in an ELISA spectrophotometer (GENESYS, model Genesys 10, Rochester, N.Y., USA) (Standard: bovine serum albumin, Bio-Rad, USA), following Bradford (1976). Each sample was measured three to five times. Three different batches of flies were used in seven replicates (N = 66).

Size of Reproductive Organs

Virgin control (n = 51) and resistant (n = 51) males of 18–20 day-old were used. Males were anesthetized in ethyl acetate and dissected between 15 and 18 h when *A. ludens* exhibits sexual displays and seminal vesicles have been shown to be fuller than during periods with no sexual displays (Reyes-Hernández and Pérez-Staples 2017). The long and short arms of the male accessory glands, seminal vesicles, area with sperm in the seminal vesicles, testes, ejaculatory bulb and thorax (as a proxy for male overall size), were photographed with a camera (Jenoptik, optical systems GmbH ProgRes® C3, Jena, Germany) attached to a stereoscopic microscope (Olympus, SZX7 zoom, Japan). Organs were measured using Image J software (ver. 1.48).

Female Fitness

Anastrepha ludens virgin control and resistant flies were tested 16–17 days after adult emergence. Matings between control and resistant flies were obtained by placing pairs in plastic 300 ml cups covered with a mesh during the time of sexual activity. Four combinations were obtained: control ♀ × control ♂ (N = 30); control ♀ × resistant ♂ (N = 28); resistant ♀ × control ♂ (N = 28); and resistant ♀ × resistant ♂ (N = 29). As an additional control, resistant (N = 30) and control (N = 30) virgin females were placed individually in the same type of cups. The latency to mate (time elapsed between being placed together and the start of mating), copulation duration and number of matings were recorded. After copulation, males were removed and females were kept individually without water or food for the desiccation treatment. To decrease relative humidity inside the cups, a small plastic bottle of 15 ml was added with 7 grams of silica gel (Sigma-Aldrich), covered with a nylon web lid to prevent the flies from direct exposure (Tejeda et al. 2014). To register fecundity, an artificial oviposition substrate was added, consisting of a petri dish (5 cm in diameter) filled with water and covered with Parafilm® placed upside down on top of the cup. The oviposition substrate was changed every day until females died. From the following morning, female mortality was registered three times a day, at 9:00 am, 15:00 pm and 21:00 pm hours until the death of all females. Fecundity was registered once a day at 9:00 am until all females had died.

Female Remating Behaviour

For remating experiments wild females were used since mass-reared control *A. ludens* females are not consistently inhibited from remating (Abraham et al. 2014; Meza et al. 2014). Wild flies were recovered from infested oranges collected at Tuzamapam, Veracruz, Mexico. Fruits were taken to the laboratory and placed in 30 × 50 × 15 cm plastic trays with soil. Larvae migrated from the fruit to the soil where they pupated. After 7–10 days, the sand was sieved and recovered pupae were placed in 27 L cages at 26 ± 2 °C and 80 ± 10 RH until adult emergence. On the day of emergence, laboratory and wild flies were sorted by sex and were transferred to 27 L cages in groups of approximately 100 adults, with water and food provided ad libitum. Flies were fed with adult diet consisting of sugar and hydrolyzed yeast (Yeast Hydrolyzed Enzymatic, MP Biomedicals®) in a 3:1 ratio.

Control (n = 108) or resistant (n = 108) males of 20–30 day-old and wild females of 30–35 days were placed in 300 ml plastic containers at 16:00, one pair per container. Mating frequency was registered. Once copulation ended, males were discarded and females were kept individually with water and food *ad libitum*. Two days later, one wild male of 30–35 day-old was offered to each female and the frequency of remating females was registered. Two replicates were done with different batches of flies.

Statistical Analysis

Number of sperm stored in the three spermathecae of the females mated with control or resistant males was analyzed with a GLM with Poisson distribution and log link function.

Amount of protein produced in male accessory glands and testes of control and resistant males were analyzed with a Generalized Linear Mixed Model (GLMM) with a normal error distribution and identity as a link function, with micrograms of proteins per accessory gland or per testicle as the response variable and male strain as predictor. Additionally, male age nested in batch (fixed), batch (fixed) and replica (random) were included as error terms.

The size of long and short arms of accessory glands, seminal vesicles, area with sperm in seminal vesicles, ejaculatory bulbs and testes of control and resistant males were compared with ANCOVAs, using the size of the different organs as the dependent variable, male treatment (control or resistant) as the classification variable, thorax length as a covariable and the interaction term between them to account for differences in size interactions across males.

Female survivorship and fecundity were analyzed with general linear models (GLM) with Poisson distribution and log link function. Female–male combinations and female type (virgin control or virgin resistant) was included as a fixed factor. Post-hoc comparisons were performed using contrasts. The relationship between fecundity and longevity according to treatment was analyzed by linear regression. Fecundity was log transformed, only females that mated and laid eggs were included. Latency to mate and copulation duration was analyzed with a one-way ANOVA for female-male combinations. Analyses were performed on JMP version 7 and 9 (SAS, Institute Inc.). The number of remating

females was analyzed with a Fisher´s exact test using R statistical package version 3.2 R Core Team (2016).

Results

Sperm Storage

The number of sperm stored was lower in females mated with resistant males compared with females mated with control males ($\chi^2_1 = 154.55$, $N = 51$, $p < 0.0001$) (Fig. 1).

Protein Amount in Accessory Glands and Testes

Accessory glands from desiccation resistant males had (LS means ± Std. error) 1.68 ± 0.09 µg and control males had 1.92 ± 0.09 µg of protein per gland. However, there was no significant effect of male strain on protein quantity in the accessory glands (GLMM) ($N = 66$, $F_{1,\ 6.202} = 0.417$, $P = 0.086$). For error terms there were significant differences for male age nested in batch ($F_{4,5.98} = 5.348$, $P = 0.035$) and batch ($F_{2,6.78} = 41.684$, $P = 0.0002$). The random effect of the replica accounted for 36.97% of the total variation.

For testes, resistant males had (LS means ± Std. error) 1.58 ± 0.13 µg and control males had 1.56 ± 0.13 µg of protein per pair of testes. There was no significant difference between male strains in the protein content of testes ($N = 66$, $F_{1,\ 5.829} = 0.038$, $P = 0.852$). For error terms, age nested in batch ($F_{4,5.47} = 2.58$, $P = 0.148$) was also not significant, while there were significant differences between batches ($F_{2,6.01} = 13.494$, $P = 0.006$). The random effect of replica accounted for 69.33% of the total variation, while the residual accounted for 30.67%.

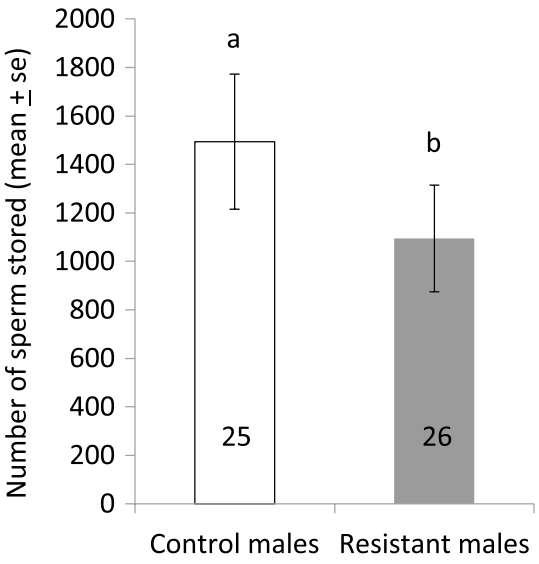

Fig. 1 Mean (±s.e.) number of sperm stored by females mated to either desiccation resistant or control males. Numbers inside bars represent sample sizes. Different letters indicate significant differences ($p < 0.05$)

Size of Reproductive Organs

Thorax length of resistant males was significantly larger than that of control males (3.08 ± 0.03 and 2.99 ± 0.03 for resistant and control males, respectively; $N = 51$, t (two-tailed) = 2.15, $P = 0.03$). Taking into account this difference in body size, we found that resistant males have significantly smaller accessory glands (both for the short and long arms) than control males ($F_{1,90} = 17.14$, $P < 0.0001$; and $F_{1,89} = 28.2$, $P < 0.0001$; for long and short arms of accessory glands, respectively) (Fig. 2). Similarly, resistant males have significantly smaller seminal vesicles ($F_{1,90} = 13.8$; $P < 0.001$) (Fig. 2), but the area with sperm in the seminal vesicles was not significantly different between resistant and control males ($F_{1,55} = 0.24$; $P = 0.62$). There were no differences in the size of the ejaculatory bulb or testes between resistant and control males ($F_{1,98} = 1.7$, $P = 0.19$ and $F_{1,98} = 28.2$, $P = 0.67$; for ejaculatory bulb and testes size, respectively). Variation of thorax length was not correlated with observed variation on reproductive organ size ($F_{1,55-98} < 2.6$, $P > 0.1$ for all models) and this pattern was maintained within control and resistant males ($F_{1,55-98} < 1.4$, $P > 0.23$, for the interaction term of all models).

Female Fitness

When females were exposed to desiccation stress, there was no significant difference in the survival of mated or virgin resistant females, regardless of which type of male (control or resistant) they had mated with (Log Rank $X^2 = 2.8$, $df = 2$, $P = 0.242$). However, resistant females lived longer than control females, irrespective of their mating status or if they had mated with control or resistant males ($X^2 = 272.6$, $df = 5$, $P < 0.001$) (Fig. 3).

There was a significant effect of female strain and mating condition on fecundity ($X^2 = 551.1$, $df = 5$, $P < 0.001$) (Fig. 4). Post-hoc contrast comparisons revealed that resistant females had lower fecundity compared to control females. For resistant females, mating with a resistant or control male had no effect on fecundity. However, contrasts revealed control females mated to control males had significantly higher fecundity than control females mated to resistant males. Mating per se did increase fecundity for both resistant and control females, as virgin females had lower fecundity (Fig. 4).

There was no significant effect of male or female strain on latency to mate or copulation duration ($F_{111,3} = 0.12$, $P = 0.945$; $F_{111,3} = 1.67$, $P = 0.176$ for mating latency and copulation duration, respectively) (Table 1).

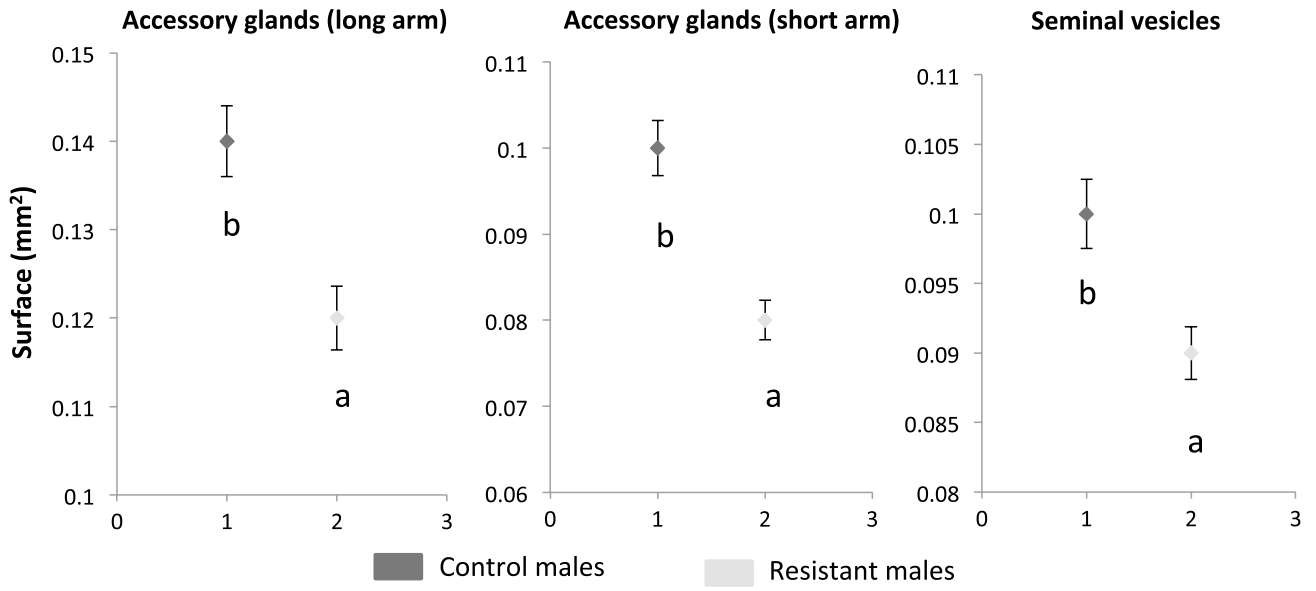

Fig. 2 Mean (±s.e.) size (mm^2) of the long and short arm of accessory glands and seminal vesicles from desiccation resistant and control males. Different letters indicate significant differences ($p < 0.05$)

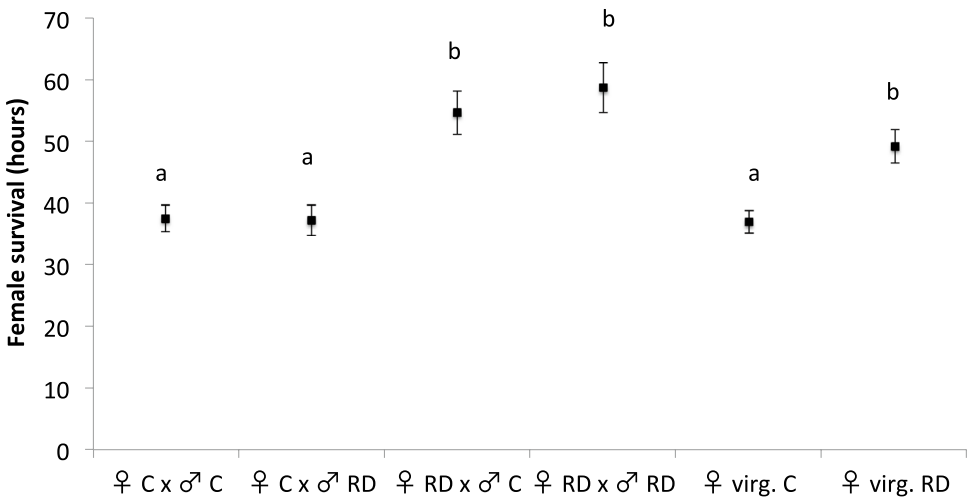

Fig. 3 Mean (±s.e.) female survival (hours) after mating with either C (Control) males or RD (resistant to desiccation) males and then subjected to hydric stress. Virgin resistant and virgin control females were used as control. Different letters indicate significant differences ($p < 0.05$)

There was no significant relationship between log transformed fecundity (only females that laid eggs) and longevity (hours) for mated females ($R^2 = 0.244$, $F_{1,6} = 1.944$, $P = 0.213$ control females mated to control males; $R^2 = 0.034$, $F_{1,3} = 0.070$, $P = 0.816$ control females mated to resistant males; $R^2 = 0.013$, $F_{1,5} = 0.067$, $P = 0.805$ resistant females mated to resistant males). Only resistant females mated to control males had a significant but negative relationship between fecundity and longevity ($R^2 = 0.711$, $F_{1,5} = 12.322$, $P = 0.017$).

Female Remating Behaviour

Control and resistant males had similar mating frequencies with wild females. Wild females were just as likely to remate after mating with a resistant or control male (Fisher's exact test, $p = 0.28$) (Fig. 5).

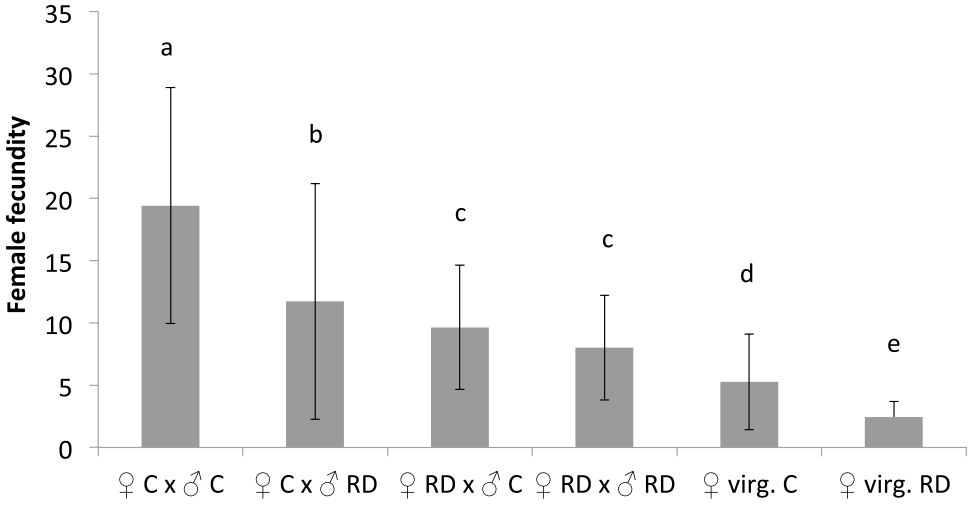

Fig. 4 Mean (±s.e.) female fecundity (number of eggs laid) after mating with either C (Control) males or RD (resistant to desiccation) males and then subjected to hydric stress. Virgin resistant and virgin control females were used as control. Different letters indicate significant differences ($p < 0.05$)

Table 1 Average ± std. error latency to mate and copula duration of desiccation resistant or control females mated with control or resistant males and subsequently subjected to hydric stress

Treatment	Latency to mate (min)	Copulation duration (min)
Control ♀ × Control ♂	79.6 ± 5.3 (30)	83.2 ± 7 (30)
Resistant ♀ × Resistant ♂	77.3 ± 5.3 (29)	68.0 ± 6.6 (29)
Resistant ♀ × Control ♂	76.5 ± 4.2 (28)	76.2 ± 6.0 (28)
Control ♀ × Resistant ♂	80.1 ± 4.8 (28)	89.9 ± 8.9 (28)

Sample sizes are in parenthesis. There were no significant differences for either behavior (see text)

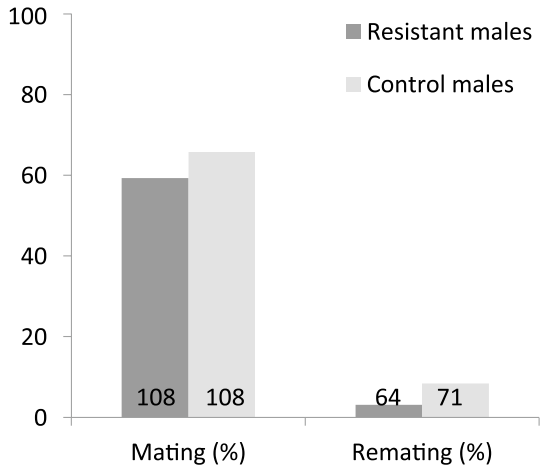

Fig. 5 Percentage of wild females mating or remating when first mated with either a control or desiccation resistant male. Numbers inside bars represent sample sizes

Discussion

Responses to desiccation resistance may be varied, yet the evolutionary trade-off between specific components of the male ejaculate and tolerance to hydric stress is unknown. Here, we investigated the possible trade-off between resistance to desiccation and quality of the male ejaculate, reproductive organ size and ability to suppress female remating. For resistant females we studied potential fitness benefits from ejaculate use after mating. We demonstrate that only certain components of the male ejaculate or reproductive organs were compromised by increased tolerance to hydric stress, while mating condition did not affect female *A. ludens* capacity to resist desiccation. As far as we know, this is the first report of selection for desiccation resistance on male reproductive organ size and seminal fluid components. Due to climate change and increasing temperatures worldwide, there is likely to be natural selection for stress resistance in insects. Our study demonstrates how different components of the ejaculate can respond to stress.

We found that males selected to resist desiccation had smaller accessory glands and seminal vesicles. Furthermore, this lower ejaculate quality could have consequences for female fecundity, as control females mated to resistant males had lower fecundity than females mated to control males. These results are consistent with resistant males having potential trade-offs between traits favored by artificial selection for desiccation resistance and traits favored by reproduction. However, no differences in protein quantity in either accessory glands or testes were observed, nor in their ability to inhibit females from remating. Although this

strain has not lost its reproductive capability in terms of pre-copulatory mating success (Tejeda et al. 2016), it remains to be seen if further post-copulatory trade-offs for resistant males translate into decreased offspring production or female fertility, which may be the case as females mating with resistant males stored less sperm than females mating with control males. In contrast to this hypothesis, in *D. melanogaster* selected for cold shock resistance, males have more fertile sperm and their sperm have higher offence ability during sperm competition (Singh et al. 2016).

Given the potential trade-offs between reproductive traits and those conferring a longer life-span, we expected resistant males to have a lower quality ejaculate than non-resistant males, and thus be less able to prevent females from remating. However, no such effect was found, which may be related to the fact that protein content for the reproductive organs was similar between strains despite the fact that accessory glands were smaller in resistant males. This suggests, resistant males are investing in the production and maintenance of accessory gland proteins on a par with control males, yet suffer a reduction in sperm production. Differences in the size of the accessory glands may be related to other components in these organs that were not measured, such as water or lipids. At the moment, for *A. ludens* there is no concrete evidence as to which component of the ejaculate transferred by the males is the responsible to female sexual inhibition, however the synergic effect of sperm and accessory gland products seem to be necessary to decrease female sexual receptivity (Abraham et al. 2016).

Trade-off theory states that if two life history traits share a common resource pool, and internal resources are limited and insufficient for somatic maintenance, then there will be an increment of resources allocated to one trait in detriment of another trait (Zera and Harshman 2001). For example, in *D. melanogaster* resistant to starvation, there is a negative correlation between body lipids and lipids stored in ovaries, and in wing polymorphic crickets (various *Gryllus species*), variation in internal allocation have been observed in enhancement of ovarian growth and reduction of somatic triglyceride that serves as flight-fuel, thus leading to trade-offs between early fecundity and dispersal capability (Zera and Harshman 2001; Karla and Parkash 2014). In our case, we found that males that were selected for desiccation resistance suffered from a decrease in size of accessory glands and seminal vesicles (where mature sperm are stored, Martínez and Hernández-Ortíz 1997), while females that mated with resistant males stored fewer sperm. Starvation and desiccation resistance are associated with an increase of lipids and glycogen content (Burke and Rose 2009; Tejeda et al. 2014), and these resources may be necessary for males to build and maintain reproductive organs such as accessory glands, seminal vesicles and produce sperm. Given these diminished ejaculate resources, desiccation resistant males could perhaps suffer from more rapid ejaculate depletion compared to control males. However, there is no evident negative functional interaction between desiccation resistance and the rest of the measured ejaculate traits.

Female *A. ludens* are less resistant to desiccation and starvation stress than males, with males resisting stress for approximately ten more hours than females of similar size (Tejeda et al. 2014), an effect which has also been found in other tephritids (Weldon et al. 2013, 2016). In *A. ludens*, females emerge with fewer lipids, yet consume less water compared to males when stressed (Tejeda et al. 2014). Thus, we expected a strong selective pressure for females subjected to hydric stress, to utilize all possible sources of water and/or nutrients, such as those found in the male ejaculate. However, female longevity was correlated to female strain but did not depend on mating. Desiccation resistant females had higher survival than control females as expected, yet this was irrespective of mating with either a control or selected male, indeed virgin selected females had comparable longevity to mated selected females. This suggests that increased desiccation resistance is not dependent on male ejaculate traits or on female ability to exploit these ejaculates. Contrary to our case, in *D. mojavensis* and *D. arizonae*, mating significantly increased female resistance to desiccation stress, augmenting female survival for as much as 20 h (an increase of 62% compared to virgin females) (Knowles et al. 2004). In *D. melanogaster*, mating increases female survival more than 15 h under starvation (Goenaga et al. 2012), and in *E. bilimeki*, female survival under desiccation and starvation conditions is increased for approximately 2 days when they consume ejaculates (Rodriguez-Enriquez et al. 2013). In these cases, males have evolved larger ejaculates and testes (Pitnick et al. 1997, 1999; Rodriguez-Enriquez et al. 2013).

A previous study with this same strain, found resistant and control lines to have the same overall fecundity (Tejeda et al. 2016). Here, we predicted that when females would be exposed to desiccation stress, resistant females would be under more selective pressure to use the male ejaculate, and that this in turn would result in higher fecundity. However, after being exposed to stress, resistant females did not have higher fecundity regardless of the male they mated with. On the contrary, control females mating with resistant males laid significantly fewer eggs in the hours they survived, suggesting they received a lower quality ejaculate. Mating per se did increase egg-laying as both resistant and virgin females laid fewer eggs. This may be due to seminal proteins transferred in the ejaculate such as ovulin (peptide 26Aa) which in *D. melanogaster* induce oogenesis, and together with sperm stimulate oviposition (Chapman et al. 2001; Heifetz et al. 2001; Xu and Wang 2011).

Conclusions

Despite many studies on the cost of reproduction under stress, very few have examined correlated responses in the male ejaculate. Seminal fluid proteins evolve faster than proteins from other non-reproductive tissues (Haerty et al. 2007), and here, rapid artificial selection for desiccation resistance in *A. ludens* also affected certain components of the ejaculate and reproductive organs. Selected males had smaller accessory glands, seminal vesicles than control males, suggesting a reproductive cost of desiccation resistance in males. This cost could be associated with a lower fecundity for females, as control females mating with resistant males had lower fecundity and stored less sperm than females mating with control males. Studying how reproductive interactions shape divergent responses of the sexes to hydric stress should increase our incipient understanding of ejaculate complexity and its diverse effects on female post-copulatory behaviour.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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