Life history and demographic traits of the marula fruit fly, *Ceratitis cosyra*: Potential consequences of host specialisation

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Abstract

Life history strategies are diverse both across and within species, but the factors shaping this diversity are not fully understood. Here, we investigate the life history strategies of the marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) and how they differ between the sexes. We measured lifespan and age-dependent reproductive effort in both sexes. In females, reproductive effort was measured as fecundity (egg counts) and for males, courtship behaviour, mating propensity and sperm transfer at ages 5, 15 and 25 days were assayed. The flies lived for an average of 104.6 ± 2.8 (± SE) days with females living on average 9 days longer than males. Total female fecundity and time until peak egg production both positively correlated with lifespan. The proportion of males courting and mating was similar at 5 and 15 days, but courtship activity increased while mating success decreased significantly by 25 days. The number of sperm transferred and sperm storage asymmetry were highest at 15 days, with 12173 ± 826 sperm stored per female after mating. We also compared life history traits in *C. cosyra* with other tephritids, to see how niche breadth might contribute to life history evolution. In comparison with other tephritids, *C. cosyra* has a long lifespan and relatively low lifetime fecundity, but males transfer particularly large numbers of sperm during copulation. These life history traits may be associated with the seasonal availability of marulas, which are its preferred native host.

Introduction

Life histories describe how organisms invest in traits such as growth, reproduction and survival throughout their lives (Braendle et al., 2011). The optimal life history strategy would be for an organism to mature quickly, and begin reproducing at a high rate indefinitely (Stearns, 1992). However, this ideal life history does not exist because life history strategies are constrained by trade-offs between traits, where elevated investment in one trait necessitates reduced investment in another (Stearns, 1992, Braendle et al., 2011). One of the most widespread trade-offs is between reproduction and lifespan (Stearns, 1992). Comparative analyses of mammals, birds and reptiles show that life history strategies often vary along a continuum, with species that develop rapidly having high reproductive output and short lifespan at one end, and species that develop more slowly having lower reproductive rates but living for longer at the other (Gaillard, 1989, Bauwens & Diaz-Uriate, 1997). Within species, high investment in reproduction is often correlated with either reduced lifespan or reduced future reproductive success. For example, male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), tend to live longer lives when housed in smaller cohorts with fewer mating opportunities (Papadopoulos et al., 2010). However, this trade-off between lifespan and reproduction is not universal: some high quality individuals can invest heavily in reproduction and still live longer than many of their counterparts (Carey et al., 1998a).

The sexes often invest very differently in survival and reproduction (Carvalho, 2007; Bonduriansky et al., 2008). Typically, females make few, very large, and costly gametes (eggs), whereas males produce high quantities of small, cheap gametes (sperm) (Trivers, 1972). This can affect how females and males schedule their investment in reproductive effort over age, and in turn, can drive sex differences in lifespan (Bonduriansky et al., 2008). For example, the high costs of producing gametes mean that females are likely to have a “slow and steady” strategy of reproductive effort, where they invest moderately but steadily in offspring production across their lifetime (Bonduriansky et al., 2008). In contrast,

reproductive effort in males may involve intense competition early in life to attract as many mates as possible or secure paternity, even if this results in reduced survival or reproductive success later in life (Kokko, 1997). This “live-fast, die-young” strategy may select for a shorter life in males relative to females. Alternatively, males may increase their reproductive success as they grow older. For instance, to attract or win mates they need to express a trait that takes time to attain (Pérez-Staples et al., 2010). If older males win more mates than younger males, then this should select for males to live longer lives than females (Carvalho, 2007). Clearly, to fully understand the life history of a species we need to study both sexes, but few studies quantify age-dependent investment and lifespan in reproductive effort in females and males (Bonduriansky et al., 2008).

Ecology may also shape sex-specific life histories and one factor that may be important, is niche-breadth (Donohue, 2005). Being a host specialist may shape life histories if the host species is not available for much of the year. For example, if a specialist has to survive between host fruiting periods, this may select for longer lifespans relative to polyphagous species, which have a wide variety of available hosts throughout the year (Levins & MacArthur, 1969). This rationale may also affect juvenile developmental times, as evident in host specialists like the apple maggot, *Rhagoletis pomonella* (Walsh) (Dambroski & Feder, 2007) and olive fly, *Bactrocera oleae* (Rossi) (Daane & Johnson, 2010), where adult emergence coincides with the availability of hosts. It is feasible that the effects of niche-breadth on life histories may differ across the sexes. For example, if females can store sperm it may be less important for males to invest heavily in lifespan to survive periods when hosts are unavailable, and instead, males may maximise their fitness by investing intensely to attract as many mates as possible and transferring large quantities of sperm early in life. However, very few studies attempt to relate niche breadth to sex-specific life history strategies. To begin to build this understanding, more data on female and male life history strategies need to be collected in species that have narrow and broad dietary niches.

The family Tephritidae (Diptera) consists of ca. 4300 species from >450 genera (Smith et al., 2002). Tephritids are found on a variety of host plants, where larvae feed on stems, flowers or fruit (Smith et al., 2002). Most frugivorous tephritid larvae live inside fruit, making it unmarketable (Aluja & Mangan, 2008, Jin et al., 2011, Benelli et al., 2014). A small number of these frugivores are highly polyphagous (Aluja & Mangan, 2008), and are destructive pests (Benelli et al., 2014). Accordingly, life history studies on tephritids to date have largely focused on a few generalist species within the genera *Bactrocera* Macquart (e.g. Vargas et al., 1984, Ekesi et al., 2006, Fanson et al., 2012), *Ceratitis* Macleay (e.g. Vargas et al., 1984, Papadopoulos et al., 2010, Carey, 2011) and *Anastrepha* Schiner (e.g. Pérez-Staples & Aluja, 2004, Carey et al., 2008). These studies have shown that adult lifespan in tephritids varies significantly among species. For instance, *C. capitata* live for around 34 days (Shoukry & Hafez, 1979) but with considerable variation having been detected [e.g. mean lifespans of 16.5 days (Chapman et al., 1998), 36.8 and 48.1 days (Gaskin et al. 2001) and 39.8-106.9 days (Papadopoulos et al. 2010)] while mean lifespan of the melon fly, *Zeugodacus cucurbitae* (Coquillett) is ~67 days (Huang & Chi, 2012).

To broaden our understanding of sex-specific life histories and how these may be affected by dietary niche breadth, we study a specialist tephritid, the marula fruit fly, *Ceratitis cosyra* (Walker). *Ceratitis cosyra* only infests ~10 host species within the Afrotropics, in contrast to at least 194 host species for *C. capitata* and 114 for the Natal fly, *Ceratitis rosa* (s.l.) Karsch, (Centre for Agriculture and Biosciences International, 2017). The preferred hosts of *C. cosyra* are marula [*Sclerocarya birrea* (A. Rich.) Hochst.] and mango (*Mangifera indica* L.) (Rwomushana & Tanga, 2016), both of which are in the family Anacardiaceae. Mangoes and marulas are only available in summer (Department of Agriculture Forestry and Fisheries, 2010) and because there is no evidence of larval or pupal diapause (Rwomushana & Tanga, 2016), adults must survive for a long period of time until fruit become available in the next season. This long adult lifespan might come at a cost of reduced or delayed reproduction, but as discussed, these effects may differ across the sexes. Our objectives were to compare

female and male lifespan and determine age-specific reproductive investment for females (fecundity) and males (courtship, copula latency, mating propensity and sperm transfer) of *C. cosyra*. We then compared our results with published estimates for other tephritids to begin to see if host breadth might drive differences between the host specialist studied here, and the generalist species more widely studied. All studies were done using a "close-to-wild" population because wild flies do not readily lay eggs under laboratory conditions.

Materials & methods

Flies

Wild *C. cosyra* were collected as larvae infesting mangoes from various locations in Mpumalanga province, South Africa, and introduced to a controlled environment room at the Hatfield Campus of the University of Pretoria. The controlled environment room was maintained at 23-27°C, 40-50% relative humidity, and a light cycle of LD 12:12 h. A one-hour artificial sunrise (0600-0630 hours GMT+2 light intensity: ~0.3 W/m^2; 0630-0700 hours: ~0.8 W/m^2) and sunset (1700-1730 hours: ~0.8 W/m^2; 1730-1800 hours: ~0.3 W/m^2) was simulated by fluorescent tubes turning on before and turning off after the main room lights (0700-1700 hours: ~1.8 W/m^2). Sunset was simulated to provide conditions required for mating by *C. cosyra*, which occurs at dusk (Manrakhan & Lux, 2009). The mangoes were placed on moist, washed sand into which third-instar "hopping" larvae could migrate and pupate. The sand was sifted to retrieve pupae, which were then transferred to a plastic Petri dish that was placed in a 5 L cage. The cage was a clear plastic container with one side replaced with insect screen, and the lid modified to include a sleeve of voile curtain fabric.

On adult emergence, male *C. cosyra* were transferred to another 5 L cage with water-soaked cotton wool, and separate Petri dishes containing white sugar and hydrolysed yeast (Yeast Extract Powder, Biolab, Merck) for food. After 15 days, female *C. cosyra* from a laboratory culture from Citrus Research International, Nelspruit, South Africa, and

maintained on the Hatfield Campus of the University of Pretoria were introduced to the cage containing wild males. Wild males were mated with females from a laboratory culture to introduce wild genetic material, while not compromising the tendency for females to mate under laboratory conditions. An attempt was made to cross wild females with laboratory males, but this was unsuccessful despite observations over several dusk mating periods.

Two days after mating, an oviposition substrate was introduced to the cage into which females could lay eggs. The oviposition substrate comprised a 125 ml plastic container with a wide opening (diameter = 63 mm) with the base lined with a single sheet of tissue paper that was soaked in water and 3 ml of guava concentrate. The plastic container of the oviposition substrate was then covered by a taught double layer of laboratory film (Parafilm 'M', Bemis, USA) that had been pierced with an entomological pin approximately 50 times. Eggs laid into the oviposition substrate were rinsed into a glass dish with distilled water where they settled to the bottom before being collected with a disposable plastic Pasteur pipette. The eggs were then transferred to a carrot-based artificial larval rearing medium (Citrus Research International, Nelspruit, South Africa) at a rate of approximately 2.5 eggs/ml of prepared diet. Containers of inoculated larval diet were placed on sand in ventilated plastic containers in the controlled environment room. Pupae were sieved from the sand after 15 days before being placed in 5 L cages as described above. Emerging adults were maintained as a mixed-sex culture with *ad libitum* access to water, sugar and hydrolysed yeast. This process was repeated for a further three generations without further outcrossing before obtaining experimental adults. In each generation, the population comprised no fewer than 300 adults. The age at which eggs were collected varied across 10 and 30 days after adult emergence to avoid unintended selection for age of reproduction. All experiments were first carried out in 2013 and then repeated in 2015 to increase sample size and account for potential cohort effects.

Lifespan

In both 2013 and 2015, 100 newly eclosed virgin females and 100 newly eclosed virgin males were transferred to individual cages. Each cage comprised two 125 ml plastic containers, one of which had the base removed and was nested within the other. The cages were covered with a square (approx. 10 mm × 10 mm) of insect screen that was secured by two rubber bands. Each fly was provided with sugar and hydrolysed yeast separately in lids of microcentrifuge tubes. Distilled water was provided from a filled 200 µl pipette tip that was loosely capped with a putty-like adhesive (Prestik, Bostik, South Africa). Water was checked daily and replaced as necessary. Mortality was recorded daily and dead flies were placed in individual microcentrifuge tubes and stored at -20˚C. Later, the head and right wing of each fly was removed and photographed with a digital camera (Dino-eye C-mount, AnMo Electronics Corporation, Taiwan) mounted to a dissection microscope (Olympus SZ61, Olympus Corporation, Japan). Originally both head width and wing length were measured as proxies for fly size using ImageJ software (v 1.50b, National Institutes of Health, USA). However, regression analysis, in Statistica (v 12, Statsoft), showed a significant correlation between head width and wing length (Head width = 0.139 + 0.501 x Wing length, $F_{1,34}$ = 16.878, $p < 0.001$). Therefore, only head width was used as proxy for size in statistical data analyses because older flies often had damaged wings.

Female age-specific fecundity

Female age-specific fecundity was recorded for 30 females in 2013, and 100 females in 2015. Newly emerged virgin females were transferred to individual cages as described above. Each female was also given an artificial egg laying dish. The artificial egg laying dish comprised a black screw-top lid (volume = 5 ml, diameter = 32 mm) containing a 1:10 orange essence-water solution that was covered with a taught, stretched double layer of laboratory film pierced 10 times with an entomological pin. As in other studies (e.g. Chapman et al., 1998, Carey et al., 2008, Fanson et al., 2009) virgin females were used because female *C. cosyra* lay eggs throughout their life regardless of having mated. Every 5

days until death, the artificial egg laying dish was removed, eggs were counted, and a fresh egg laying dish was placed in the cage. The base of the cage was also inspected for eggs, which were removed and added to the count. From these data we calculated four measures of reproductive effort: (1) total egg production was the number of eggs laid over the entire lifespan of a female; (2) egg production rate was the mean number of eggs laid per day (i.e., egg production rate = total egg production/lifespan), (3) maximum egg production was the highest number of eggs laid over a five-day period; and (4) time to peak egg production was the number of days to reach maximum egg production. At death, females were frozen as described above and their head width was measured.

Male age-specific reproductive effort

Male mating behaviour was observed at 5, 15, and 25 days after adult emergence. This involved pairing virgin males of each age with a virgin female from the laboratory culture approximately one hour before the beginning of simulated sunset. All females were 10-20 days of age to minimise the effect of female reproductive maturity on measurements of male reproductive effort. Paired females and males were placed in cylindrical plastic containers (height = 52 mm, diameter = 35 mm). The times when males began to call and when mating occurred were noted. Calling was observed as the male rapidly vibrating its wings. Due to most matings lasting for up to 12 hours (personal observation), the flies were left overnight. The sample sizes for this experiment were 20 pairs per age group in 2013 and 50 pairs per age group in 2015. Mated males were placed in individual microcentrifuge tubes and stored at -20˚C for later measurement of head width.

At 0900 hours on the morning after the male mating behaviour experiment, mated females were dissected under a stereo microscope to remove their spermathecae in order to determine the number of sperm that were transferred by males. This was done by freezing the female with a freeze spray (Freezer BR, Cramolin, South Africa) before placing it on a microscope slide. A cut was then made at the base of the ovipositor sheath with sharpened

forceps. The ovipositor, with female reproductive tract attached, was pulled from the abdomen and put in a drop of distilled water, after which the spermathecae were individually removed and transferred to two separate 15 µl drops of distilled water on another microscope slide. Each spermatheca was crushed with the head of an entomological pin attached to a thin wooden dowel. The crushed spermatheca was then spread by stirring the drop of water vigorously for 30 s before covering it with a 22 × 22 mm cover slip. The slides were allowed to dry before the corners of the coverslips were secured by small drops of clear nail varnish. Sperm were counted at 100× magnification using a phase contrast microscope (Olympus BX43, Olympus Corporation, Japan). Rather than counting all sperm, we followed Taylor *et al.* (2000) to estimate the total number of sperm based on counting a matrix of 5 × 5 fields of view (17.36% of the coverslip area). The number of sperm counted was then multiplied by 5.76 to estimate the total number of sperm per spermatheca. The possibility of sperm storage asymmetry was tested by calculating the absolute difference in the number of sperm between the two spermathecae for each female.

Statistical analysis

Lifespan analyses were performed using Cox proportional hazards regression using 'survival' and 'car' libraries in R (v 3.3.1, The R Foundation for Statistical Computing) running in R Studio (v 0.99.903, RStudio Inc.). The model included sex and head width as predictor variables, and replicate (2013 and 2015) as a random effect. The model was simplified through stepwise deletion of terms based on Akaike's information criterion using 'step'. An analysis of deviance table using type III sums of squares was generated to summarise significant effects in the final model.

Multivariate analysis of variance (MANOVA) was used in Statistica (v 12, Statsoft) to determine the effects of lifespan and replicate on measures of female reproductive effort (i.e., total egg production, egg production rate, maximum egg production, and time to peak egg production). Females that did not lay any eggs were excluded from this analysis. Total

egg production, egg production rate, and maximum egg production were not normally distributed, so were log-transformed. The minimal adequate model was determined by stepwise deletion of the least significant terms. To explore which response variables drove significant multivariate responses, separate univariate ANOVA were performed.

The effects of age, head width and replicate on the proportions of male calling and mating propensity were determined using logistic regressions run in Statistica. The effects of the same predictors on the onset of calling and copulation in relation to the commencement of artificial dusk lighting were determined using general linear models in Statistica. The number of sperm transferred during copulation was square root transformed, after which a general linear model was run in R Studio to determine the effects of age, replicate and head width on the number of sperm transferred. The minimum adequate model was again determined through stepwise deletion of terms based on Akaike's information criterion. To analyse sperm asymmetry, a general linear model was run in R Studio with age, total sperm count and replicate as predictors, and the minimal adequate model was again determined.

Results

Lifespan

The mean (± 1 S.E.) lifespan of adult *C. cosyra* was 104.6 ± 2.8 days (Fig. 1). Virgin males had a slightly shorter mean lifespan (99.6 ± 4.4 days) than virgin females (108.6 ± 3.5 days). However, the greatest maximum lifespan was observed in males (females = 209 days; males = 269 days). The Cox proportional hazards regression showed that females had a 1.37 ± 0.13 times higher mortality risk than males ($X^2 = 4.375$, df = 1, p = 0.036). The effect of replicate on survival was slightly non-significant and there was no significant effect of head width.

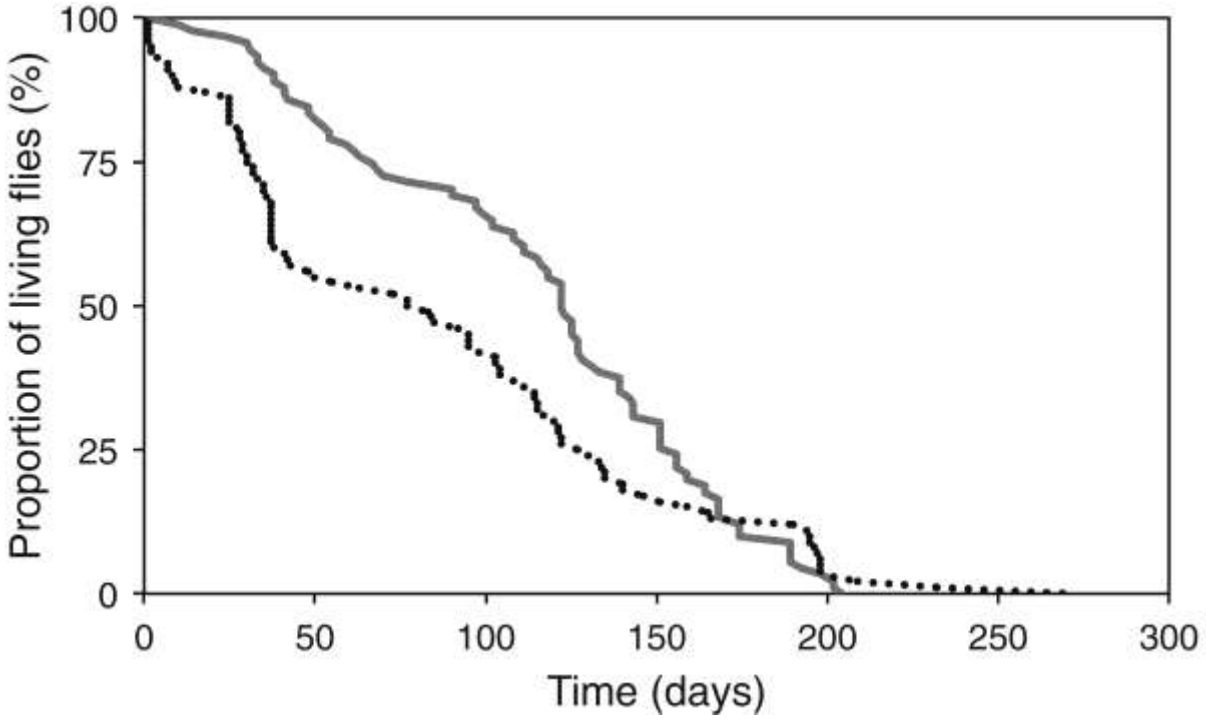

Fig. 1 Survival of female and male *C. cosyra*. Females are represented by the solid line and males by the dotted line.

Female fecundity

The number of eggs produced varied greatly among individual flies. Although total egg production averaged at 67.1 ± 6.4 (mean ± 1 S.E.) eggs per female, fecundity ranged from 0 to over 200 eggs over the lifespan or each female (Fig. 2). The average time until maximum fecundity was 44.84 ± 3.59 days. Measures of female reproductive effort were affected significantly by lifespan (MANOVA: Wilk's $\lambda = 0.050$, $F_{4,4} = 397.875$, $p < 0.001$). The effects of replicate and head width were not significant. The multivariate response was driven by significant positive relationships between lifespan and total egg production (Parameter estimate: log(Total egg production) = 0.005x + 0.955; $F_{1,86} = 13.290$, $p < 0.001$) and time to peak egg production (Parameter estimate: Time to peak egg production = 0.498x + 6.684; $F_{1,86} = 56.475$, $p < 0.001$). There was no significant effect of lifespan on egg production rate ($F_{1,86} = 0.153$, $p = 0.697$) or maximum egg production ($F_{1,86} = 2.117$, $p = 0.149$).

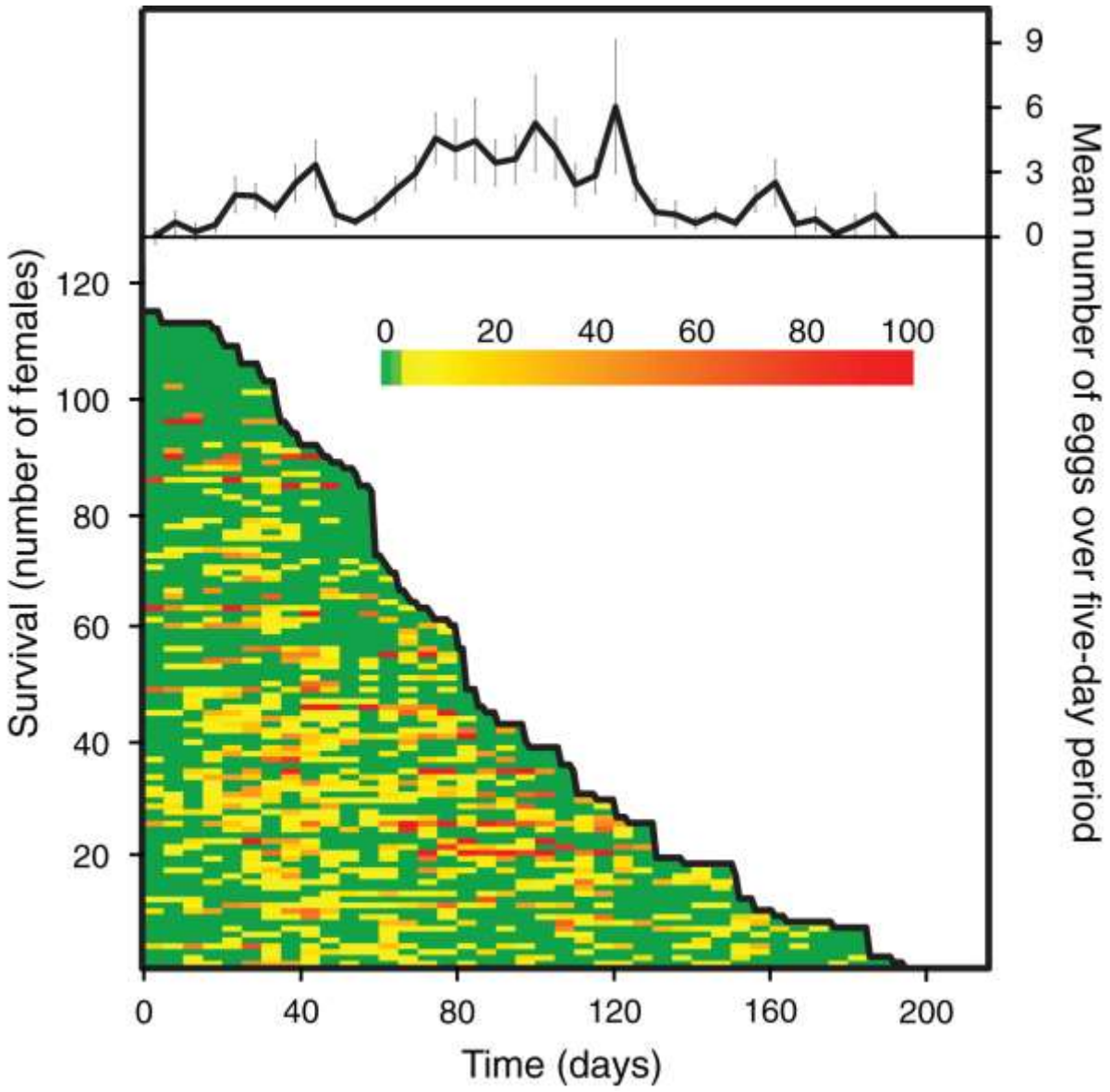

Fig. 2 Number of eggs laid (mean ± SE) over 5-day periods (above), with an event history chart (below) for virgin *Ceratitis cosyra* with mortality over time. The coloured area shows the fecundity over 5-day periods, with green showing no eggs laid, and yellow to red showing a range from 1 to 89 eggs laid.

Male mating propensity

Courtship in young flies was similar between replicates with proportions of 0.40 ± 0.11 and 0.42 ± 0.07 for the two replicates, respectively (Fig. 3a). Thereafter courtship increased as males aged. However, there was a significant interaction between age and replicate (χ^2 = 15.16, df = 2, $p < 0.001$), with 15 and 25 day old males in the 2013 replicate courting more

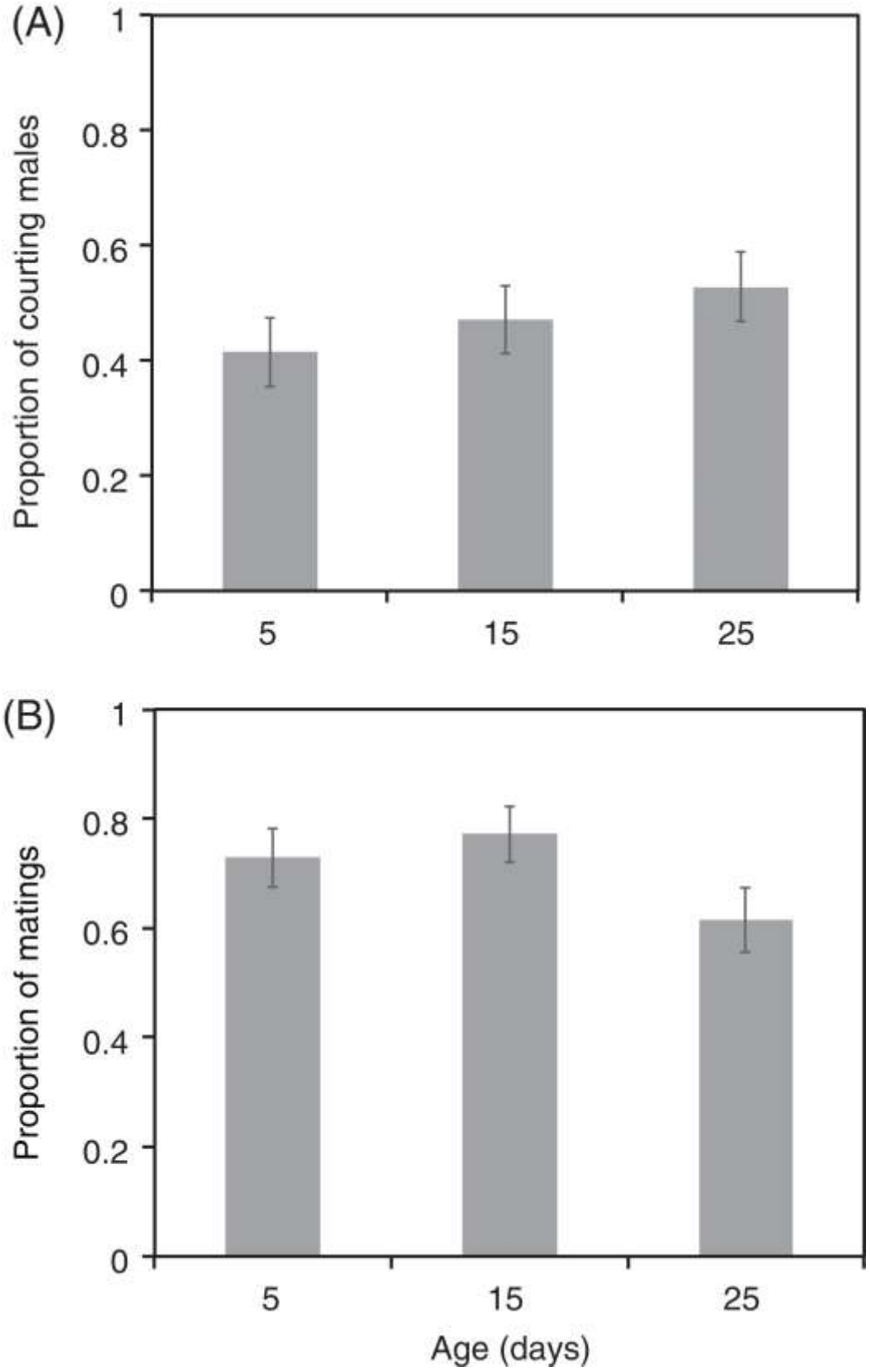

Fig. 3 Male mating behaviour of *C. cosyra* for first matings at 5, 15 and 25 days after eclosion (± SE) with a) the proportion of males showing courtship behaviour and b) the proportion of males mating regardless of whether courtship occurred.

than those of the 2015 replicate. The combined proportion of males mating, was similar for 5 and 15 day old flies (0.73 ± 0.05 and 0.77 ± 0.05), but decreased significantly in 25 day old flies (0.61 ± 0.06) (χ^2 = 7.66, df = 2, p = 0.021) (Fig. 3b). Replicate did not have an effect on mating propensity. The mean times to the onset of courtship (8.90 ± 2.59, 7.18 ± 2.34 and 12.32 ± 2.15 minutes after onset of experiment for ages 5, 15, and 25 days) were not affected by replicate and were similar for all three age groups ($F_{2,96}$ = 1.746, p = 0.180). However, males took significantly longer to mate at age 5 days (21.06 ± 4.20 minutes) than at ages 15 (4.04 ± 4.14) and 25 days (3.86 ± 4.15) ($F_{2,145}$ = 8.003, p < 0.001). Head width did not affect courtship or mating propensity during the experiment and was dropped from all minimal adequate models.

Sperm Storage

Sperm transfer (± S.E.) differed significantly between the age groups ($F_{2,125}$ = 7.452, p < 0.001) (Fig. 4a) with the most sperm transferred at the age of 15 days (12173 ± 826 sperm) and the lowest at the age of 5 days (6746 ± 467 sperm). Sperm storage asymmetry was found in all three age groups and differed significantly between ages 5 and 15 days as well as between 15 and 25 days ($F_{2,124}$ = 5.523, p = 0.005) (Fig. 4b). The magnitude of sperm asymmetry increased with total sperm transferred ($F_{1,124}$ = 74.857, p < 0.001), with the age of 15 days showing the greatest asymmetry with a mean difference of 39.6 ± 2.6% between the two spermathecae. Head width and replicate had no effect on sperm transfer and were removed from the minimum adequate modal.

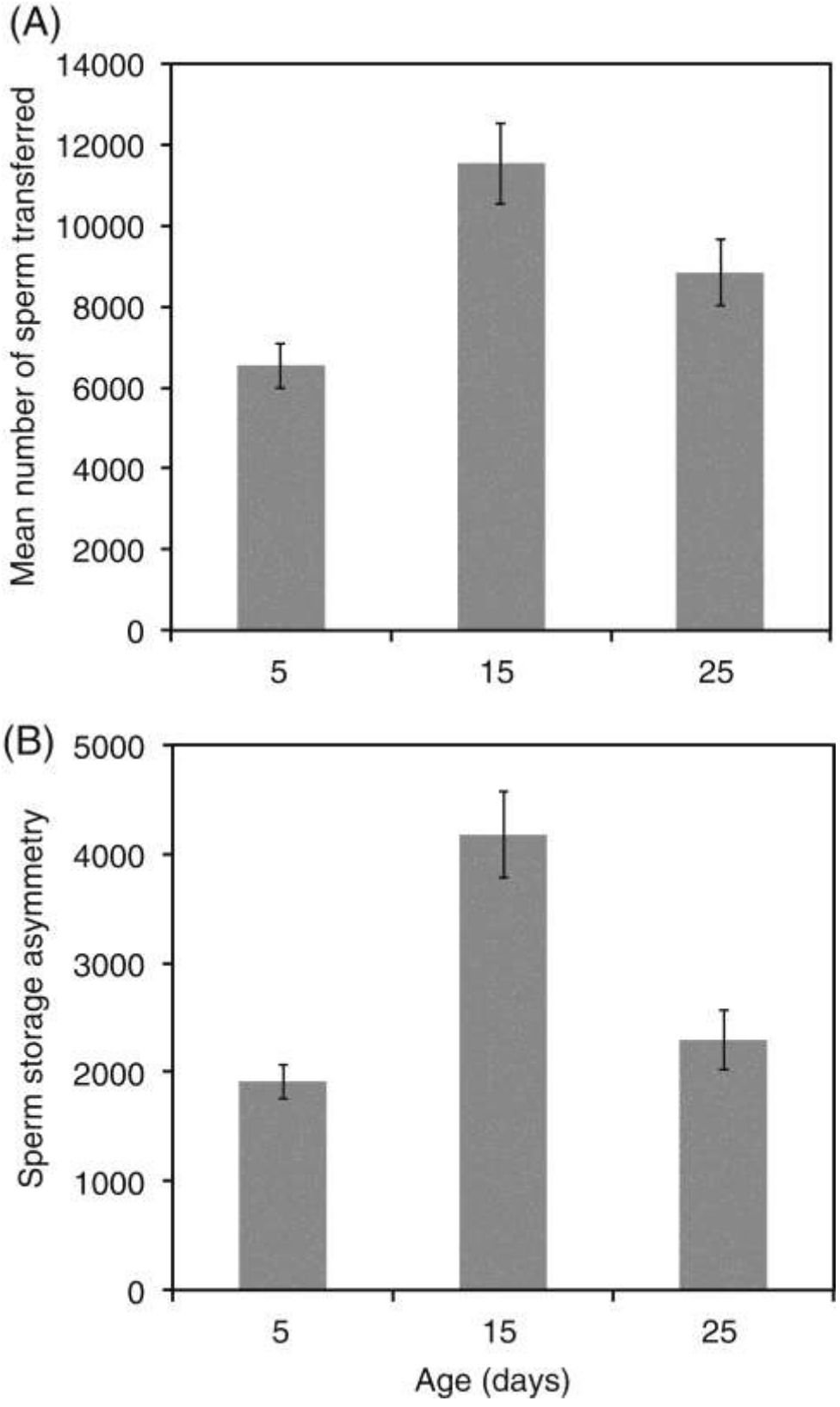

Fig. 4 Sperm stored in *C. cosyra* spermathecae after one mating with males of different ages (± SE) showing (a) the mean number of sperm transferred and (b) the mean difference in sperm stored between the two spermathecae.

Discussion

The main focus of the present study is to characterise survival and reproductive schedules in female and male *Ceratitis cosyra*, as well as to compare these traits with that of other tephritid species. When comparing sex differences in survival, average lifespan was greater in females than males, but overall females showed a higher mortality risk. This is reflected in the slightly male-biased sex-ratio from 140 days onward (females: 48 flies, males: 52 flies). Only 11 (4 females and 7 males) flies lived for longer than 200 days, with the oldest female living for 209 days and the oldest male for 269 days. This means that life expectancy is higher for newly eclosed females than males, but is reversed as the cohort ages. A similar, yet opposite result was found by Carey et al. (1995), where average lifespan of female *C. capitata* was shorter than that of males, but some females lived the longest. Carey and Liedo (1995) found that in large *C. capitata* populations mortality rates were higher in females up to 12 days old after which males had a higher mortality until 30 days old. After 30 days females and males showed similar aging patterns. Furthermore, Carey et al. (1998b) found that female mortality is low at the onset of egg laying, but increases at older ages when more eggs are produced. Our results may reflect longer-term energy expenditure in egg laying, which is higher than the short-term investment of courtship in high quality males. The higher mortality rate for young male *C. cosyra* in comparison with young females follows this trend, but is not supported in older individuals. We hypothesise that this is due to condition-dependent mortality in both females and males. It may be that low quality males die sooner due to the cost of sex-specific traits such as sexual signalling, while high quality males survive longer. This has been observed in *C. capitata*, where males that mated at a high rate, which relates to male quality, lived longer (Papadopoulos et al., 2010). In our study, although males were kept individually, they were observed to exhibit courtship behaviour throughout the longevity experiments.

Longer living female *C. cosyra* produced many more eggs than their shorter lived counterparts. Although not specifically mentioned in other studies, this seems to be the case for most tephritid species (see Carey et al., 1998a, Carey et al., 2008, Moraiti et al., 2012). Unlike in a few other studies (e.g. Carey et al., 1998a, Carey et al., 2008) where early reproductive outputs did not differ significantly among flies that lived to different ages, *C. cosyra* showed a trend in which flies that had a later peak egg production lived longer. This corresponds with the idea that early reproduction negatively affects survival later in life (Stearns, 1992). We recognise that egg-laying was only tested on virgin females and that the rate of egg-laying may differ from mated flies. However, there are many studies in which virgin flies have been used due to the tendency for tephritid females to lay eggs at intervals regardless of whether they have mated (e.g. Chapman et al., 1998, Carey et al., 2008, Fanson et al., 2009). From this experiment it is clear that female *C. cosyra* lay eggs throughout their lifespan. This might be the case even without the presence of an oviposition substrate as many of the eggs were laid on the plastic floor of the cage. Continuous egg-laying might be to produce viable offspring, or may just be a mechanism to release old eggs from the female regardless of whether they were fertilised. In their study on *C. capitata*, Carey et al. (1998a) suggested that this continual egg-laying phenomenon may be due to unlimited presence of food resources. By not limiting food intake, nutritional costs and the trade-off between somatic investment and reproduction that are present under field conditions are greatly reduced (Carey et al., 1998a).

Male mating propensity of *C. cosyra* decreased at an older age. Although some data suggest that older males are more attractive than younger males (Pérez-Staples et al., 2010), there have been numerous studies showing clear declines in male competitiveness, mating success and fertility with age (Jones & Elgar, 2004, Fricke & Maklakov, 2007). It is clear that age affects the number of sperm transferred by previously unmated males during mating. For *C. cosyra*, the highest number of sperm was transferred at 15 days after eclosion, with lower numbers at 5 and 25 days. This suggests that males reach a peak in

sexual fitness at around 15 days, although we recognise we did not assay the entire life-course of the study animals. As with other studies on *C. capitata* (Taylor et al., 2001) and *B. tryoni* (Pérez-Staples et al., 2007a), sperm storage asymmetry increased with the number of sperm transferred. However, unlike the findings of Pérez-Staples et al. (2007a), the relative sperm storage asymmetry (total asymmetry ÷ total sperm in both spermathecae) also increased with total sperm transferred. Although the reason for sperm storage asymmetry has not been tested in tephritids, it has been suggested by Pérez-Staples et al. (2007a) as a mechanism used by females to exert some level of sperm selection after multiple matings. As is found in other animals (Eberhard, 1985, Hellriegel & Ward, 1998), it may be possible that females direct sperm of a second mating with a different male to another spermatheca. Although it is untested, managing sperm storage by females may counteract the inhibiting effects of accessory gland proteins transferred by males (see Abraham et al., 2016).

Comparison of female and male reproductive effort reveals a difference in the age of optimal fitness. Males transferred the most sperm at 15 days, while most females only started laying high numbers of eggs at 25 days and reach maximum egg production even later. This is in accordance with a different study on *B. tryoni*, where males tended to mate at a younger age than females (Pérez-Staples et al., 2007b). The time to fertilisation after mating does not account for this difference, as larvae can hatch from eggs in less than five days after mating (personal observation). One possible explanation for this is the cost of gamete formation. Much less energy is needed in the production of large quantities of sperm than the development of eggs (Trivers, 1972). By producing eggs at a later age, females therefore have more time to acquire energy and nutrients from food (Rivero et al., 2001).

The lifespan of *C. cosyra* in the laboratory is long in comparison with other tephritids (Table 1). Although it is surpassed by *C. rosa* (a host generalist), *C. cosyra* lives more than twice as long as most other host generalist fruit flies when held individually in the laboratory. This strategy is in contrast with the only host specialist (and monophagous) fruit fly species for

Table 1. Life history traits, including mean adult female and male longevity, fecundity (number of eggs laid over lifespan) and sperm transfer, of *Ceratitis cosyra* compared with those of other tephritid species. The summarised values were obtained from studies that used a similar experimental design to the current study, including flies housed individually and a similar range of temperatures unless otherwise noted. Female fecundity was measured for virgin females unless annotated by an asterisk (*). Host range values were sourced from CABI (2017).

Species	Host range	Longevity (females)	Longevity (males)	Fecundity	Sperm transfer per mating	Notes	Reference
Ceratitis cosyra	10 species 6 families	109 ± 3.5	100 ± 4.4	67 ± 6.4	9087 ± 509	-	Current study
C. capitata	194 species 49 families	31a	36a	826*/248a	3212b	-	a Shoukry & Hafez., 1979; b Yuval et al., 1996
C. rosa	114 species 33 families	140	141	-	-	Generation F1	Duyck et al., 2010
Bactrocera dorsalis	305 species 60 families	75	86	1056*	-	Generation F1; 28°C	Ekesi et al., 2006
B. tryoni	132 species 37 families	58a	79b	262a	1066 (median)c	Long term mass reared cultures; a Nutrition experiment	a Fanson et al., 2009; b Dominiak et al., 2008; c Pérez-Staples et al., 2007a
B. oleae	1 species	29	26	409*	-	Nutrition experiment; exhibits diapause	Tsiropoulos, 1977
Zeugodacus cucurbitae	61 species 19 families	59a	75a	860*a	~3300b	a Generation F3	a Huang & Chi, 2012; b Kuba & Itô, 1993
Anastrepha ludens	36 species 19 families	50	47	335	-	Nutrition experiment	Carey et al., 2008
A. obliqua	29 species 13 families	~85a	~50a	-	2122 (median)b	Flies emerging from wild-collected pupae	a Joachim-Bravo et al 2003; b Pérez-Staples & Aluja 2006

which longevity data was collected in a similar way to ours, *B. oleae*, which has a relatively short adult lifespan that reflects its ability to enter developmental quiescence and time its emergence with host availability (Daane & Johnson, 2010). However, the lifetime fecundity of *C. cosyra* is far less than that of the other studied species assayed in the same way. This may provide evidence for a trade-off between lifespan and reproduction. Although it has been shown that lab-reared flies tend to produce more eggs than wild flies (Meats et al., 2004), it should not be relevant here as most of the data on other species are from studies on the first generation in the laboratory. In contrast with the number of eggs laid, many more sperm are transferred during *C. cosyra* matings than any of the other tephritid species that have been studied. A possible explanation for high levels of sperm transfer in *C. cosyra* is to provide sufficient sperm competition throughout their long lifespan. In *C. capitata* it is known that mating inhibition is at least partially driven by the number of sperm stored (Mossinson & Yuval, 2003), so it may be that male *C. cosyra* transfer more sperm to limit the chance of female remating and thus to enhance paternity. This hypothesis is yet to be tested. Prevention of remating in *C. cosyra* may be of particular relevance for males of this species due to the highly seasonal availability of fruiting hosts. Its indigenous host plant, the marula, is available for only a limited period of time during the Austral summer (Department of Agriculture Forestry and Fisheries, 2010), meaning that mated females may go many months without encountering suitable host plants for oviposition.

In conclusion, the mean lifespan of *C. cosyra* exceeds that of many other species that have been studied. Mean and total lifespan of females and males of the species differ, and there is some evidence for these patterns to be shaped by a trade-off with reproduction. These patterns may be shaped by the availability of preferred hosts, although a comparative analysis is needed to test this. This study is a foundation for further life history studies on both *C. cosyra* and other tephritids as it gives a comprehensive base for the lifespan and reproductive effort of both females and males. Further knowledge on this species may be

gained by observing the mated flies held individually or in groups, as well as providing fluctuating or semi-field conditions.

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