ECOLOGY AND MANAGEMENT OF D. SUZUKII

Reproductive Biology of *Trichopria anastrephae* (Hymenoptera: Diapriidae), a Biological Control Agent of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae)

Alexandra P. Krüger[1] · Amanda M. Garcez[1] · Tiago Scheunemann[1] · Daniel Bernardi[1] · Dori E. Nava[2] · Flávio R. M. Garcia[1,3]

Abstract

Hymenopteran parasitoids, like any other insect, employ strategies to ensure their reproduction. Understanding these strategies is important for ecological purposes, but also to improve mass rearing of biological control agents. Here, we describe mating strategies used by the pupal parasitoid *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), a potential biocontrol agent, that has been considered for augmentative releases for management of the invasive pest species *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). We studied the emergence pattern of males and females of *T. anastrephae* and the effects of parental ae on offspring number and sex ratio. Polygamy was also studied and its effects on parasitism and offspring production were described. Adults from this species emerge in the first hours of photophase, and males emerge before females, demonstrating that *T. anastrephae* is a protandrous species. Parasitoid age when first mated influences the parasitism and sex ratio. Younger females result in a higher number of offspring (8.16 parasitoids/day), while older males result in a more female-biased sex ratio of offspring (64% females). Both males and females are polygamic, and the order in which a female is mated by the male affects parasitism, viability of parasitized pupae, and sex ratio of offspring, with the first female performing the highest parasitism and sex ratio (63.83 and 61% of females, respectively), but the lowest viability (92.92%). Females that are allowed to mate multiple times generate lower numbers of offspring (113.05 parasitoids) when compared to virgin or single-mated females (135.20 and 130.70 parasitoids, respectively), but the highest sex ratio (49% of females). Data present in this study and how it can be used to improve parasitoid rearing and field releases of *T. anastrephae*, in biological control programs for *D. suzukii* are discussed.

Keywords Protandry · Polygamy · Offspring · Sex ratio · Parasitism

Introduction

Hymenopteran wasps follow a sequence of events and adopt strategies, especially related to mating, that increase their genetic success and ensure their reproduction (Thornhill

✉ Alexandra P. Krüger
alexandra_kruger@hotmail.com

1 Crop Protection Department, Federal University of Pelotas (UFPel), Capão do Leão, RS, Brazil

2 Embrapa Temperate Agriculture, Pelotas, RS, Brazil

3 Ecology, Zoology and Genetics Department, UFPel, Capão do Leão, RS, Brazil

and Alcock [1983](#)). One strategy frequently observed in parasitoids is protandry, a phenomenon of males emerging hours or days before females (Teder et al. [2021](#)), which typically occurs in quasi-gregarious (solitary species that develop in hosts that are aggregated) and gregarious species (Bourdais and Hance [2019](#)). Protandry may allow males to maximize their mating opportunities and reduce the time between female emergence and mating event, increasing the chance of occurrence of mating before female dispersal (Bourdais and Hance [2019](#); Pompanon et al. [1995](#)).

Another reproductive strategy is based on the number of matings that both sexes perform in a given period (Thornhill and Alcock [1983](#)). In most parasitoid species, males mate more than once (polygyny) (Makatiani et al. [2013](#)). The experience gained after repeated matings, allow males of some hymenopteran wasps to respond and court more successfully

(Perez-Lachaud and Campan 1995; He and Wang 2008). However, females that mate with experienced males may receive fewer sperm and, then, produce a higher proportion of male offspring (Steiner et al. 2008; Kant et al. 2012). To avoid or minimize any negative consequences of mating, females of some species may mate with different males (polyandry) (Makatiani et al. 2013). Although monoandry is most common for parasitoid species (Ridley 1993), there is a tendency for gregarious and quasi-gregarious species to be polyandrous (Godfray 1994; Santolamazza-Carbone and Pestaña, 2010).

The success of mating strategies in parasitoids is often perceived in the sex ratio of the offspring since unsuccessful matings or insufficient sperm transferred during mating will result in male-biased progeny in arrhenotokous species. Thus, studying mating strategies used by parasitoids is important to improve laboratory mass rearing and increase the success of field releases (Makatiani et al. 2013) since female-biased offspring is favorable for biological control due to increased population growth rates and female contribution to pest mortality (Heimpel and Lundgren 2000, Ramadan and Wang 2021). Here, we determined mating strategies used by *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), a potential biological control agent against the invasive pest species *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) (Garcia et al. 2022).

Drosophila suzukii, also known as spotted-wing Drosophila, is native to Asia and expanded its range in recent years and became a major pest of small fruits causing an economic impact mainly in countries in Europe and North and South America (dos Santos et al. 2017; Knapp et al. 2021), but its presence was also recently recorded in Africa (Boughdad et al. 2020; Hassani et al. 2020). This pest is usually controlled with insecticides (Beers et al. 2011, Bruck et al. 2011, Gress and Zalom 2018, Van Timmeren et al. 2019), but due to risks of insecticide resistance and residue limits (Diepenbrock et al. 2016, Gress and Zalom 2018), alternative control methods, such as biological control, are encouraged.

Since its first association with *D. suzukii* in southern Brazil (Wollmann et al. 2016), the resident pupal parasitoid *T. anastrephae* has been more extensively studied and presented itself as an efficient parasitoid of *D. suzukii* in different laboratory conditions (Krüger et al. 2019; Vieira et al. 2019, 2020; Garcia 2020a; Tait, et al. 2021). Nowadays, *T. anastrephae* is one of the parasitoids in the Neotropical Region with the greatest potential to be used in future biological pest control programs (Garcia 2020b; Garcia et al. 2022). This parasitoid also shows the potential to search and find hosts located in attacked fruits (De la Vega et al. 2021), and successfully parasitize *D. suzukii* in the presence of another parasitoid (Oliveira et al. 2021) or other products such as insecticides used to control the fly (Geisler et al. 2019; Schlesener et al. 2019; Shawer 2020; Tait et al.

2021). However, information is lacking on the reproductive strategies employed by *T. anastrephae* and their relationship to parasitism ability and offspring sex ratio. Knowing that understanding factors influencing offspring production and sex ratio are linked to a biocontrol agent success—as stated before, female-biased offspring is favorable for biological control—and that *T. anastrephae* is a potential agent for augmentative releases in biological control programs against *D. suzukii*, we conducted a series of experiments aiming to describe the linkage between mating strategies and parasitism aspects, using *D. suzukii* as a host. Among the mating strategies, we verified the emergence pattern of *T. anastrephae* and the effects of male and female age at the time of mating on offspring. We also evaluated the occurrence of polygamy (polygyny and polyandry) and their effects on parasitism aspects. And we evaluated the effects of mating on female longevity and parasitism.

Material and methods

Maintenance rearing of *D. suzukii* and *T. anastrephae*

Rearing of *D. suzukii* and *T. anastrephae*, as well as all experiments here described, was conducted under controlled conditions (23 ± 2°C, 70 ± 10% r.h., and 12 h of light). Both colonies originated from infested fruits collected in nearby organic farms, and new insects were periodically introduced to maintain colony vigor. Flies were reared on a cornmeal-based diet (described by Schlesener et al. 2018), and adults were held in plastic cages ($262 \times 177 \times 147$ mm) (length $\times$ height $\times$ width), with two side openings (80×100 mm), and an opening in the lid (155×50 mm), covered with voile cloth. Parasitoids were reared on *D. suzukii* pupae, and adults were held in plastic cages, as described previously and fed pure honey (ad libitum).

Emergence pattern

Pupae of *D. suzukii* (less than 12 h old) were exposed to *T. anastrephae* females (15 pupae/female), for a total of 30 females. Groups of 15 pupae were placed in plastic lids (20-mm diameter), containing humid cotton balls, and exposed to parasitoid females for 6 h. Then, pupae were isolated in microtubes (1.5 mL), and after exposure to parasitism, the emergence of pupae was verified every 2 h during photophase (7h00 to 19h00). In each observation, the number and sex of parasitoids were verified. Observations occurred until there was no parasitoid emergence for 2 days.

To verify if the age of males and females influence parasitism and the sex ratio (proportion of female) of the offspring, we exposed *D. suzukii* pupae to *T. anastrephae*, as described before. To obtain enough parasitoids from different

ages, different batches of pupae were exposed to parasitoids for three consecutive days, and each batch was exposed for 24 h. After exposure, parasitoids were individually placed in microtubes (1.5 mL), and at the moment of emergence, they were coupled according to the treatments. Thus, parasitoids were virgin at the moment of the experiment setting, guaranteeing that only age was a source of variation, not experience with mating. The treatments were formed by 1-, 2-, or 3-day-old males coupled with 1-, 2-, or 3-day-old females, in every possible combination, resulting in 9 treatments. Couples, according to each treatment, were placed in plastic cups (200 mL), with a hole (15-mm diameter) on top, covered with voile fabric. Pure honey and water were provided in plastic lids (10-mm diameter) containing cotton.

During 14 days, 15 *D. suzukii* pupae were offered daily for each couple. After the exposure period, pupae were removed and placed in acrylic tubes (25 × 45 mm). After the emergence of flies and parasitoids, parasitoids were sexed and counted, and the pupae that remained intact were dissected, and the presence of flies or parasitoids to determine actual parasitism rates. Percentage of parasitized pupae [(number of emerged + non-emerged parasitoids)/15] per female, viability of parasitized pupae [(number of emerged parasitoids*100)/ number of parasitized pupae], number of emerged parasitoids, and sex ratio were evaluated. The experimental design was completely randomized with 20 replicates per treatment. All couples were set on a single day.

Polygyny

After emergence, virgin males (obtained as described before) were isolated in plastic cups (200 mL). A virgin female was added to each male, and the occurrence of mating was observed. After mating ended, the female was removed, and a new virgin female was added to each male until the male had mated with ten females. Matings were observed during the photophase (7h00–19h00). After removal, mated females were isolated in plastic cups (200 mL) and identified according to the male whom they mated and the order in which they were mated. Females were fed pure honey and water, and for 14 days, 15 *D. suzukii* pupae were offered daily. After exposure, pupae were removed and kept in acrylic tubes for the emergence of flies and parasitoids. In this experiment, we evaluated the number of females mated by each male, the percentage of parasitized pupae, the viability of parasitized pupae, and the number and sex ratio of emerged parasitoids. The experimental design was completely randomized with 20 replicates per treatment (order in which females were mated). All replicates were set on a single day.

Polyandry

Newly emerged virgin females were isolated in plastic cups (200 mL), and two virgin males were placed with each female. Occurrences of mating with one or both males were observed, during the first 4 h of photophase (7h00–11h00). After the observation period, both males were removed. Two virgin males were offered for each female every three days for 12 days, totaling 5 opportunities to mate. During the entire period, females were fed pure honey and water, and 15 *D. suzukii* pupae were offered daily. After exposure, pupae were placed in acrylic tubes for emergence, and the percentage of parasitized pupae, viability of parasitized pupae, and number and sex ratio of emerged parasitoids were evaluated. The experimental design was completely randomized with 20 replicates (females). All replicates were set on a single day.

Effects of mating on female longevity and parasitism

To obtain virgin parasitoids for mating experiments, *D. suzukii* pupae were exposed to *T. anastrephae*, and after 24 h of exposure, they were isolated in microtubes, as described before. Soon after emergence, females were individually placed in plastic cups with pure honey and water (as previously described). The treatments were virgin females (no males were placed in the plastic cups), single-mated females (females were allowed to mate once, and the male was removed soon after mating ended), and presumably multiply-mated females (males were kept with the female during the entire lifespan of the female). The experimental design was completely randomized with 20 replicates per treatment. All replicates were set on a single day.

For parasitism, 15 *D. suzukii* pupae were offered daily during the entire lifespan of the female. After exposure, pupae were removed, placed in acrylic tubes, and after emergence, parasitoids were sexed and counted, and intact pupae were dissected to determine actual parasitism rates. The percentage of parasitized pupae, the viability of parasitized pupae, the number of emerged parasitoids, and the sex ratio were evaluated.

Statistical analysis

Several emerged parasitoids according to a period of the day were compared using generalized linear models (GLM) with a Poisson error distribution ($p \leq 0.05$). Generalized linear models (GLM) with a Poisson distribution were also used to assess the effects of female age and male age (i.e., 1, 2, or 3 days old), mating (i.e., virgin,

single mated or multiply-mated females), and polygyny on the percentage of parasitism, the viability of parasitized pupae, number of offspring, and sex ratio of *T. anastrephae*. The Tukey test ($p \leq 0.05$) was applied for multiple comparisons in case of significance. To verify the occurrence of protandry in the emergence pattern bioassay, a chi-squared test ($p \leq 0.05$) was used to compare the frequency of males and females who emerged in different intervals of the egg-to-adult period. The longevity of virgin, single, and multiply-mated females were subjected to survival analysis using Kaplan–Meier estimator and compared by the log-rank test. All statistical analyses were performed using R software version 4.0.3 (R Core Team 2020).

Results

Emergence pattern

Of the 450 *D. suzukii* exposed to *T. anastrephae* females, 249 parasitoids emerged. Emergence peaked in the first two hours of photophase (07:01–09:00) when 51.41% of parasitoids emerged and decreased along the day ($\chi^2 = 289.43$, $p < 0.0001$, Fig. 1a). The emergence pattern also differed by sex ($\chi^2 = 197.85$, $p < 0.0001$, Fig. 1b). Most males (94.19%) emerged 17 to 21 days after parasitism, while the first female emerged 18 days after, with a steady emergence rate from 19 to 27 days after parasitism.

Influence of parasitoid age on parasitism and sex ratio

The female age affected the number of offspring, while the male age affected the sex ratio of the offspring (Table 1). The higher number of offspring was obtained when females were 1 day old (8.16 ± 0.15), differing from 2 and 3 days old females (7.74 ± 0.14 and 7.70 ± 0.10, respectively), regardless of male age. A more female-biased was obtained when females (regardless of age) were mated with males who were 3 days old (0.64 ± 0.02), differing from 1-day-old males (0.56 ± 0.03). Meanwhile, the sex ratio of the offspring from females mated with 2-day-old males (0.60 ± 0.02) did not differ from the sex ratio of offspring from females mated with either 1- or 3-day-old males.

Occurrence of polygyny and effects on parasitism

All males of *T. anastrephae* tested were able to mate with all 10 females provided. The order in which a female was mated affected the percentage of parasitism ($\chi^2 = 14.14$, df = 179, $p = 0.0169$, Table 2), the viability
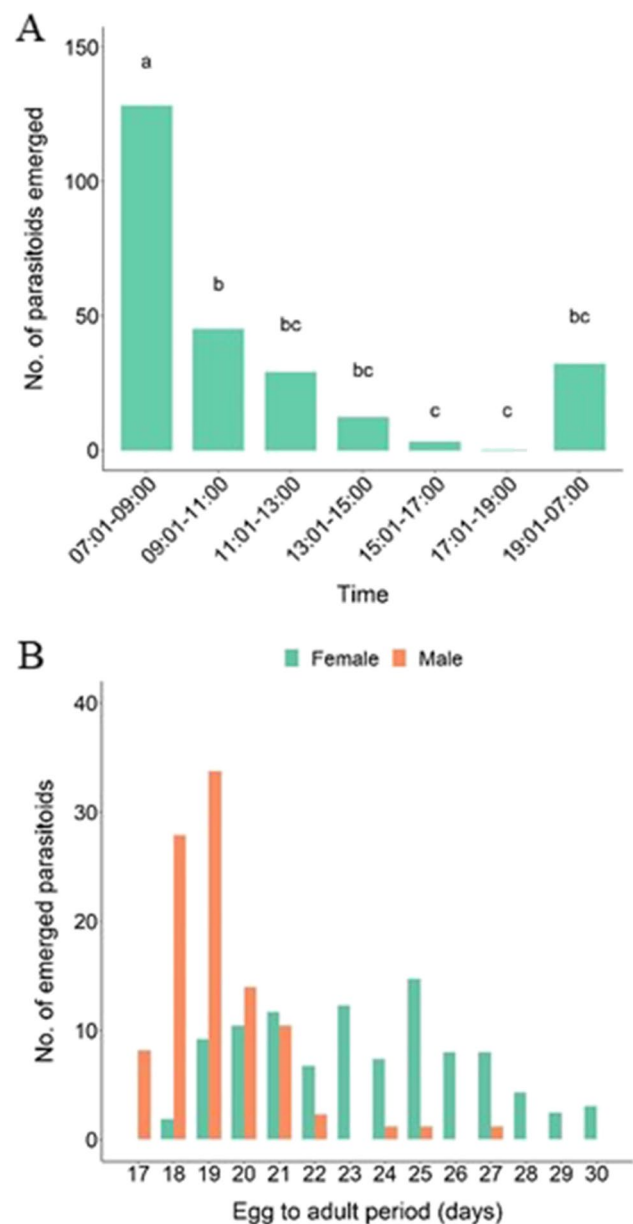

Fig. 1 Emergence rhythm of *Trichopria anastrephae* (considering both, males and females) along the day (**A**) and egg to adult period of males and females of *Trichopria anastrephae* (showed separately by sex) (**B**). Different lowercase letters indicate significant differences among treatments according to the Tukey test ($p \leq 0.05$) in **A**

of parasitized pupae ($\chi^2 = 3.43$, df = 179, $p = 0.0262$, Table 2), and sex ratio of offspring ($\chi^2 = 5.93$, df = 179, $p < 0.0001$, Table 2), but did not affect the number of offspring ($\chi^2 = 1.06$, df = 179, $p = 0.4895$, Table 2). The first female mated showed the highest percentage of parasitism ($63.83 \pm 0.78\%$) and generated the offspring with the highest sex ratio (0.61 ± 0.05), but resulted in the lowest viability of parasitized pupae ($92.92 \pm 0.57\%$).

Table 1 Generalized linear models (GLM) with a Poisson error distribution to evaluate the influence of parasitoid age (female and male) on the percentage of parasitized pupae, number of offspring, the viability of parasitized pupae, and sex ratio of *Trichopria anastrephae*

Dependent variables	Female			Male			Interaction female × male		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
Percentage of parasitism	14.20	153	0.2690	3.37	151	0.7322	11.99	147	0.6958
Number of Offspring	0.84	153	0.0306	0.14	151	0.5582	0.91	147	0.1081
Viability of parasitized pupae	0.05	153	0.9354	0.01	151	0.9851	0.56	147	0.8188
Sex ratio	0.25	153	0.0752	0.29	151	0.0451	0.04	147	0.9200

Table 2 Average (± se) the percentage of parasitism, the viability of parasitoid pupae (%), the total number of offspring, and the sex ratio of offspring produced by females of *Trichopria anastrephae* mated with polygynic males

Order of mating	Percentage of parasitism$^{\alpha}$	Viability of parasitoid pupae (%)$^{\alpha}$	Number of offspring^{ns}	Sex ratio$^{\alpha}$
1°	63.83 ± 0.78a	92.92 ± 0.57b	8.81 ± 0.11	0.61 ± 0.05a
2°	59.02 ± 1.99ab	97.39 ± 0.62a	8.63 ± 0.31	0.54 ± 0.04ab
3°	60.86 ± 1.12ab	94.70 ± 2.11ab	8.70 ± 0.28	0.36 ± 0.05bc
4°	61.53 ± 0.70ab	96.60 ± 0.42ab	8.94 ± 0.10	0.36 ± 0.04bc
5°	59.35 ± 2.66ab	95.21 ± 1.16ab	8.56 ± 0.39	0.29 ± 0.03c
6°	58.90 ± 1.16ab	96.38 ± 0.80ab	8.57 ± 0.19	0.28 ± 0.03c
7°	59.60 ± 1.08ab	96.71 ± 0.42ab	8.67 ± 0.17	0.28 ± 0.04c
8°	58.38 ± 1.06ab	97.08 ± 0.49a	8.51 ± 0.17	0.28 ± 0.04c
9°	58.97 ± 1.15ab	96.68 ± 0.68ab	8.59 ± 0.19	0.33 ± 0.05b
10°	55.41 ± 1.90b	96.59 ± 0.63ab	8.06 ± 0.29	0.30 ± 0.04c

^{ns}Not significant

$^{\alpha}$Different lowercase letters indicate significant differences among treatments according to Tukey test ($p \leq 005$)
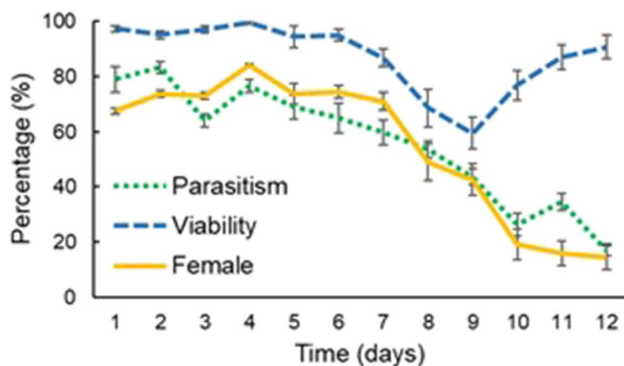

Fig. 2 Percentage of parasitism, the viability of parasitized pupae, and female offspring produced by polyandry *Trichopria anastrephae* females during the observed period. Bars present standard error

Occurrence of polyandry and effects on parasitism

All tested females mated during the 5 opportunities they were given. Even after multiple matings, the percentage of parasitism and female offspring still decreased during the 12 days of observation (Fig. 2). On the other hand, the percentage of viability presented a sharp decrease in the eighth and ninth day after the first mating but showed an increase towards the 12th day (Fig. 2).

Effects of mating on female longevity and parasitism

Mating did not affect the longevity of females ($\chi^2 = 4.50$, df = 57, $p = 0.1000$). Virgin females lived 29.90 ± 1.67 days, while single- and multiply-mated females lived for 35.20 ± 1.46 and 29.00 ± 2.33 days, respectively. Moreover, mating affected the number and sex ratio of offspring ($\chi^2 = 44.12$, df = 57, $p = 0.0107$ and $\chi^2 = 13.96$, df = 57, $p < 0.0001$, respectively, Table 3), but did not affect the percentage of parasitism and viability of parasitoid pupae ($\chi^2 = 15.83$, df = 57, $p = 0.0964$ and $\chi^2 = 0.04$, df = 57, $p = 0.9239$, respectively, Table 3). The highest number of offspring was obtained from virgin females (135.20 ± 2.89), not differing from single-mated females (130.70 ± 6.86), whereas the lowest number of offspring was obtained from multiply-mated females (113.05 ± 5.90). On the other hand, the highest sex ratio of offspring was obtained from multiply-mated females (0.49 ± 0.04), while, not surprisingly, virgin females were unable to produce female descendants.

Table 3 Average (± se) the percentage of parasitism, the viability of parasitized pupae (%), the total number of offspring, and the sex ratio of offspring produced by virgin, single, and multiply-mated females of *Trichopria anastrephae*

Treatments	Percentage of parasitism[ns]	Viability of parasitoid pupae (%)[ns]	Number of offspring [α]	Sex ratio [α]
Virgin	34.73 ± 2.43	92.06 ± 1.09	135.20 ± 2.89a	0.00 ± 0.00c
Single mated	27.76 ± 1.80	91.69 ± 1.04	130.70 ± 6.86ab	0.34 ± 0.04b
Multiply mated	32.05 ± 2.66	92.30 ± 1.13	113.05 ± 5.90b	0.49 ± 0.04a

[ns]Not significant

[α]Different lowercase letters indicate significant differences among treatments according to the Tukey test ($p \leq 0.05$)

Discussion

To ensure their successful reproduction, insects adopt distinct strategies. *Trichopria anastrephae* is a resident pupal parasitoid in South America (Cruz et al. 2011; Garcia et al. 2020), with high potential for *D. suzukii* management (Garcia 2020b), and we elucidate some of their strategies in this paper. First, *T. anastrephae* emerges in the first hours of photophase, and males emerge a few days prior than females. Age impacted reproduction, younger females produced more offspring, and older males produced more female biased. Both sexes are polygamic in laboratory conditions, and the order in which a female is mated by a male affects parasitism, the viability of parasitized pupae, and the sex ratio of the offspring. Lastly, female mating frequency affects reproduction, single mating produces more offspring while multiple matings produce a more female-biased.

Regarding the emergence pattern of *T. anastrephae*, we observed many similarities of this species with other parasitoids, such as a peak following the lighting onset and protandry (Sakai and Ishida 2001; Doyon and Boivin 2005; Bourdais and Hance 2019). The emergence in the first few hours of photophase is probably mediated by photoreceptors and eclosion hormone activation (Bourdais and Hance 2019), and also coincides with more favorable conditions in nature, such as milder temperatures and higher humidity, reducing risks of water loss of newly emerged insects (Lankinen 1986). Also, emergence after the first hours of daylight has been suggested to favor reproductive behavior in parasitoids (Pompanon et al. 1995; Karpova and Reznik 2002; Karpova 2006).

Another point regarding the emergence pattern is that *T. anastrephae* males usually emerge before females but with overlaps, reducing the protandrous status of this species. Other species showing a similar pattern are *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae), *Aphidius ervi* Haliday (Hymenoptera: Braconidae), and *Cotesia glomerata* (Linnaeus) (Hymenoptera: Braconidae) (He et al. 2004; Mazzi et al. 2011; Bourdais and Hance 2019). As a quasi-gregarious species (due to *D. suzukii* pupae distribution in the field being clustered in infested fruits), the overlap in the emergence of males and females may facilitate inbreeding. However, to our knowledge, the costs of inbreeding have not yet been studied for this species, although sib mating is known to have deleterious effects on some hymenopteran species (Heimpel and de Boer 2008). According to our observations, newly emerged males tend to stay closer to the same cluster where they emerge, instead of dispersing, and they mate with newly emerged females as soon as they leave the host pupal case, indicating that sib mating may be a common behavior in this species. This behavior of local mating could reduce genetic diversity but also reduce potential costs of dispersal.

Since males tend to emerge before females, with some overlap, we tested whether the age of the parents would affect the progeny. We found that younger females, regardless of the age of the male they mated, produced larger offspring. This phenomenon may be a consequence of host deprivation since females mated when 2 or 3 days old did not receive any host before mating. It has been shown before that when *T. anastrephae* is host deprived for 3 days, they produce fewer offspring than those that were able to parasitize soon after emergence (Krüger et al. 2019). Meanwhile, females, regardless of their age, mated with older males, and produced offspring with a higher percentage of females. Since all males used in the experiment, regardless of age, were virgins, experience is ruled out as a reason why mating with older males resulted in a more female-biased offspring. The effects of paternal age on the progeny are poorly studied; however, it is possible that older males were able to transfer higher amounts of sperm at the moment of mating, or that sperm viability increases when males age (Garcia-Gonzalez and Simmons 2005).

When males are given the chance to mate with several females, first-mated females produce more female-biased offspring compared to subsequently mated ones. This is consistent with results obtained for other parasitoids (Kant et al. 2012; Makatiani et al. 2013) and suggests a decrease in sperm transferred after each copulation (Boivin et al. 2005). In our experiment, females were replaced right after successful mating by a virgin female, encouraging males to successively mate in a short period. However, males of some species of parasitoids can replenish their sperm when

given an interval between copulations (syn spermatogenic), whereas some males could get permanently sperm depleted (pro spermatogenic) (Boivin et al. 2005).

Although *Trichopria* spp. are expected to be monandrous (Carton et al. 1986), when given the chance, all *T. anastrephae* females tested accepted several matings. However, laboratory strains may present a higher tendency to remate (Burton-Chellew et al. 2007) to avoid male harassment (convenience polyandry) or to replenish their spermathecal (Santolamazza-Carbone and Pestaña 2010). In the field, some parasitoid species usually mate only once upon emergence, before dispersing (Tooke 1955), but the polyandry level of *T. anastrephae* in the field was not yet estimated. It is important to note that even parasitism and sex ratio of offspring produced by polyandry females show a decreasing pattern with time after the first mating and were not recovered by subsequent matings.

A previous study reported that multiple matings may increase the longevity of females of an egg parasitoid (Jacob and Boivin 2005). However, in our experiment, mating status/frequency did not affect female lifespan, suggesting mating presents a low mating cost for this species (Jacob and Evans 2000; Santolamazza-Carbone and Pestaña 2010). Although some species may transfer nutritional resources during mating (Santolamazza-Carbone and Pestaña 2010), in our experiments, mated and unmated females were provided with carbohydrates and hosts during the entire experiment, and the nutritional provision may also have an impact on longevity.

Mating also could influence the parasitism behavior of females. Virgin females produced a higher number of offspring, which was comparable to single-mated females while multiplying mated females produced the lowest. The opposite was found for a species of the same genus, *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae), where virgin females produce less progeny than mated ones (Yi et al. 2020). However, it has been suggested that producing female progeny results in a higher energy cost to the female than producing male progeny (Seyahooei et al. 2018); thus, virgin females, that only produce sons, spend lower energy and can produce more offspring.

As arrhenotoky is the dominant pattern of sex determination in Hymenoptera (Heimpel and de Boer 2008), it was not surprising that virgin females produced male offspring only. When kept with a male counterpart during its lifetime, *T. anastrephae* female produced an overall higher proportion of females than females mated only once. The increase in the sex ratio of the offspring when the female mate more than once was shown previously for other species of parasitoids (Chevrier and Bressac 2002; Sagarra et al. 2002). Multiple mating may help with both the quality and quantity of sperm received from males and positively impact offspring production and sex ratio (Hegazi et al. 2020). For mass-rearing

purposes, an abundance of males should be provided to females, so they could remate as much as needed, resulting in plenty availability of sperm used in sex allocation.

The results presented in these studies give new insights into the relationships of emergence pattern, protandry, polygamy, and mating status on parasitism and progeny viability and sex ratio. Since is known that female-biased offspring are beneficial for biological control since results in increased parasitoid population growth and higher parasitism—consequently higher control of the pest population, studies on mating strategies, and parameters that directly affects parasitism and offspring sex ratio, are crucial. In fact, biological control programs may fail if parasitoid rearing result in male-biased offspring (Makatiani et al. 2013). Information regarding reproduction strategies is important to be considered for ecological purposes and in the production of females in mass rearing of *T. anastrephae* for augmentative release in biological control programs against *D. suzukii*.

Declarations

References

Beers EH, Van Steenwyk RA, Shearer PW, Coates WW, Grant JA (2011) Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag Sci 67:1386–1395. https://doi.org/10.1002/ps.2279

Boivin G, Jacob S, Damiens D (2005) Spermatogeny as a life history index in parasitoid wasps. Oecologia 143:198–202. https://doi.org/10.1007/s00442-004-1800-3

Boughdad A, Haddi K, El Bouazzati A, Nassiri A, Tahiri A, El Anbri C, Eddaya T, Zaid A, Biondi A (2020) First record of the invasive spotted wing Drosophila infesting berry crops in Africa. J Pest Sci 94:261–271. https://doi.org/10.1007/s10340-020-01280-0

Bourdais D, Hance T (2019) Consequence of emergence pattern on inbreeding risk in the aphid parasitoid *Aphidius matricariae* (Hymenoptera: Braconidae). Chronobiol Int 36:838–850. https://doi.org/10.1080/07420528.2019.1593190

Bruck DJ, Bolda M, Tanigoshi L, Klick J, Kleiber J, DeFrancesco J, Gerdeman B, Spitler H (2011) Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manag Sci 67:1375–1385. https://doi.org/10.1002/ps.2242

Burton-Chellew MN, Sykes EM, Patterson S, Shuker DM, West SA (2007) The cost of mating and the relationship between body size and fitness in males of the parasitoid wasp *Nasonia vitripennis*. Evol Ecol Res 9:921–934

Carton Y, Boulétreau M, Van Alphen JJM, Van Lenteren JC (1986) The *Drosophila* parasitic wasps. In: Ashburner M, Carson HL, Thompson JN (eds) The genetics and biology of *Drosophila*, vol 3. Academic Press, pp 348–394

Chevrier C, Bressac C (2002) Sperm storage and use after multiple mating in *Dinarmus basalis* (Hymenoptera: Pteromalidae). J Insect Behav 15:385–398. https://doi.org/10.1023/A:1016269210140

Cruz PP, Neutzling AS, Garcia FRM (2011) Primeiro registro de *Trichopria anastrephae*, parasitoide de moscas-das-frutas, no Rio Grande do Sul. Ciência Rural 41:1297–1299. https://doi.org/10.1590/S0103-84782011000800001

De la Vega GJ, Triñanes F, González A (2021) Effect of *Drosophila suzukii* on blueberry VOCs: chemical cues for a pupal parasitoid, Trichopria anastrephae. J Chem Ecol 47(12):1014–1024. https://doi.org/10.1007/s10886-021-01294-7

Diepenbrock LM, Swoboda-Bhattarai KA, Burrack HJ (2016) Ovipositional preference, fidelity, and fitness of *Drosophila suzukii* in a co-occurring crop and non-crop host system. J Pest Sci 89:761–769. https://doi.org/10.1007/s10340-016-0764-5

Dos Santos LA, Mendes MF, Krüger AP, Blauth ML, Gottschalk MS, Garcia FRM (2017) Global potential distribution of *Drosophila suzukii* (Diptera, Drosophilidae). PLoS ONE 12(3):e0174318. https://doi.org/10.1371/journal.pone.0174318

Doyon J, Boivin G (2005) The effect of development time on the fitness of female *Trichogramma evanescens*. J Insect Sci 5:4. https://doi.org/10.1093/jis/5.1.4

Garcia FRM (2020a) Introduction to *Drosophila suzukii* management. In: Garcia FRM (ed) *Drosophila suzukii* management. Springer, Cham, pp 1–9. https://doi.org/10.1007/978-3-030-62692-1_1

Garcia FRM (2020b) Basis for area-wide management of *Drosophila suzukii* in Latin America. In: Garcia FRM (ed) *Drosophila suzukii* management. Springer, Cham, pp 93–110. https://doi.org/10.1007/978-3-030-62692-1_5

Garcia FRM, Ovruski SM, Suárez L, Cancino J, Liburd OE (2020) Biological control of tephritid fruit flies in the Americas and Hawaii: a review of the use of parasitoids and predators. Insects 11:662. https://doi.org/10.3390/insects11100662

Garcia FRM, Lasa R, Funes CF, Buzzetti K (2022) *Drosophila suzukii* management in Latin America: current status and perspectives. J Econ Entomol 115(4):1008–1023. https://doi.org/10.1093/jee/toac052

Garcia-Gonzalez F, Simmons LW (2005) Sperm viability matters in insect sperm competition. Curr Biol 15:271–275. https://doi.org/10.1016/j.cub.2005.01.032

Geisler FCS, Cunha NS, Martins LN, Oliveira DC, Stupp P, Oliveira IG, Leite FPL, Garcia FRM, Bernardi D (2019) Toxicity of bacterial isolates on adults of *Zaprionus indianus* (Diptera: Drosophilidae) and parasitoids *Trichopria anastrephae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae). J Econ Entomol 112:2817–2823. https://doi.org/10.1093/jee/toz218

Godfray HCJ (1994) Parasitoids, behavioral and evolutionary ecology. Princeton University Press, Princeton

Gress BE, Zalom FG (2018) Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. Pest Manag Sci 75:1270–1276. https://doi.org/10.1002/ps.5240

Hassani IM, Behrman EL, Prigent SR, Gidaszewski N, Ravaomanarivo LHR, Suwalski A, Debat V, David JR, Yassin A (2020) First occurrence of the pest *Drosophila suzukii* (Diptera: Drosophilidae) in the Comoros Archipelago (Western Indian Ocean). Afr Entomol 28:78–83. https://doi.org/10.4001/003.028.0078

He XZ, Wang Q (2008) Reproductive strategies of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). Biol Control 45:281–287. https://doi.org/10.1016/j.biocontrol.2008.03.003

He XZ, Wang Q, Teulon DAJ (2004) Emergence, sexual maturation and oviposition of *Aphidius ervi* (Hymenoptera: Aphidiidae). N Z Plant Prot 57:214–220. https://doi.org/10.30843/nzpp.2004.57.6913

Hegazi EM, Bressac C, Khafagi W (2020) The effects of mating on the reproductive fitness of *Microplitis rufiventris* (Hymenoptera: Braconidae). Eur J Entomol 117:266–272. https://doi.org/10.14411/eje.2020.030

Heimpel GE, de Boer JG (2008) Sex determination in the Hymenoptera. Ann Rev Entomol 53:209–230. https://doi.org/10.1146/annurev.ento.53.103106.093441

Heimpel GE, Lundgren JG (2000) Sex ratios of commercially reared biological control agents. Biol Control 19:77–93. https://doi.org/10.1006/bcon.2000.0849

Jacob S, Boivin G (2005) Costs and benefits of polyandry in the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). Biol Control 32:311–318. https://doi.org/10.1603/0046-225X-29.5.1088

Jacob HS, Evans EW (2000) Influence of carbohydrate foods and mating on the longevity of the parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). Environ Entomol 29:1088–1095

Kant R, Trewick SA, Sandanayaka WRM, Godfrey AJR, Minor MA (2012) Effects of multiple matings on reproductive fitness of male and female *Diaeretiella rapae*. Entomol Exp Appl 145:215–221. https://doi.org/10.1111/eea.12007

Karpova SG (2006) The role of endogenous and exogenous factors in the regulation of synchronous emergence of *Trichogramma embryophagum* Harting and *T. principium* Sug. Et Sor. (Hymenoptera, Trichogrammatidae). Entomol Rev 86:267–282. https://doi.org/10.1134/S001387380603002X

Karpova SG, Reznik SY (2002) Interaction of exogenous factors (light and temperature) in their influence on the daily pattern of adult eclosion in *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae). Eur J Entomol 99:427–436. https://doi.org/10.14411/eje.2002.054

Knapp L, Mazzi D, Finger R (2021) The economic impact of *Drosophila suzukii*: perceived costs and revenue losses of Swiss cherry, plum, and grape growers. Pest Manag Sci 77:3597–3597. https://doi.org/10.1002/ps.6110

Krüger AP, Scheunemann T, Vieira JGA, Morais MC, Bernardi D, Nava DE, Garcia FRM (2019) Effects of extrinsic, intraspecific competition and host deprivation on the biology of *Trichopria anastrephae* (Hymenoptera: Diapriidae) reared on *Drosophila suzukii* (Diptera: Drosophilidae). Neotrop Entomol 48(6):957–965. https://doi.org/10.1007/s13744-019-00705-5

Lankinen P (1986) Geographical variation in circadian eclosion rhythm and photoperiodic adult diapause in *Drosophila littoralis*. J Biol Rhythms 159:123–142. https://doi.org/10.1007/BF00612503

Makatiani JH, Bruce AY, Schulthess F, Takasu K (2013) Reproductive strategies of the larval parasitoid *Microplitis croceipes*. Entomol Exp Appl 148:223–233. https://doi.org/10.1111/eea.12099

Mazzi D, Hatt F, Hein S, Dorn S (2011) Ladies last: diel rhythmicity of adult emergence in a parasitoid with complementary sex determination. Physiol Entomol 36:47–53. https://doi.org/10.1111/j.1365-3032.2010.00761.x

Oliveira DC, Stupp P, Martins LN, Wollmann J, Geisler FCS, Cardoso TDN, Bernardi D, Garcia FRM (2021) Interspecific competition in *Trichopria anastrephae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae) parasitism on pupae of *Drosophila suzukii* (Diptera: Drosophilidae). Phytoparasitica 49:207–215. https://doi.org/10.1007/s12600-020-00843-2

Perez-Lachaud G, Campan M (1995) Influence of previous sexual experience and post-emergence rearing conditions on the mating behavior of *Chryseida bennetti*. Entomol Exp Appl 76:163–170. https://doi.org/10.1111/j.1570-7458.1995.tb01958.x

Pompanon F, Fouillet P, Boulétreau M (1995) Emergence rhythms and protandry in relation to daily patterns of locomotor activity in *Trichogramma* species. Evol Ecol 9:467–477. https://doi.org/10.1007/BF01237829

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.r-project.org/

Ramadan MM, Wang X (2021) Male impact on female reproductive performance of the larval tephritid parasitoid *Diachasmimorpha tryoni* (Hymenoptera: Braconidae). J Econ Entomol 114:1117–1127. https://doi.org/10.1093/jee/toab063

Ridley M (1993) Clutch size and mating frequency in parasitic Hymenoptera. Am Nat 142:893–910

Sagarra LA, Vincent C, Stewart RK (2002) Impact of mating on *Anagyrus kamali* Moursi (Hym., Encyrtidae) lifetime fecundity, reproductive longevity, progeny emergence, and sex ratio. J Appl Entomol 126:400–404. https://doi.org/10.1046/j.1439-0418.2002.00679.x

Sakai T, Ishida N (2001) Circadian rhythm of female mating activities governed by clock genes in *Drosophila*. Proc Nati Acad Sci USA 98:9221–9225. https://doi.org/10.1073/pnas.151443298

Santolamazza-Carbone S, Pestaña M (2010) Polyandry increases male offspring production in the quasi-gregarious egg parasitoid *Anaphes nitens*. Ethol Ecol Evol 22:51–61. https://doi.org/10.1080/03949370903515984

Schlesener DCH, Wollmann J, Krüger AP, Nunes AM, Bernardi D, Garcia FRM (2018) Biology and fertility life table of *Drosophila suzukii* on artificial diets. Entomol Exp Appl 166:932–936. https://doi.org/10.1111/eea.12736

Schlesener DCH, Wollmann J, Pazini JB, Padilha AC, Grützmacher AD, Garcia FRM (2019) Insecticide toxicity to *Drosophila suzukii* (Diptera: Drosophilidae) parasitoids: *Trichopria anastrephae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae). J Econ Entomol 112:1197–1206. https://doi.org/10.1093/jee/toz033

Seyahooei MA, Bagheri A, Bavaghar M, Dousti AF, Parichehreh S (2018) Mating and carbohydrate feeding impact on life-history traits of *Habrobracon hebetor* (Hymenoptera: Braconidae). J Econ Entomol 111:2605–2610. https://doi.org/10.1093/jee/toy253

Shawer R (2020) Chemical control of *Drosophila suzukii*. In: Garcia FRM (ed) *Drosophila suzukii* management. Springer, Cham, pp 133–142. https://doi.org/10.1007/978-3-030-62692-1_7

Steiner S, Henrich N, Ruther J (2008) Mating with sperm-depleted males does not increase female mating frequency in the parasitoid *Lariophagus distinguendus*. Entomol Exp Appl 126:131–137. https://doi.org/10.1111/j.1570-7458.2007.00641.x

Tait G, Mermer S, Stockton D, Lee J, Avosani S, Abrieux A, Anfora G, Beers E, Biondi A, Burrack H, Cha D, Chiu JC, Choi MY, Cloonan K, Crava CM, Daane KM, Dalton DT, Diepenbrook L, Fanning P, Ganjisaffar F, Gómez MI, Gut L, Grassi A, Hamby K, Hoelmer KA, Ioriatti C, Nieri R, Pfab F, Puppato S, Rendon D, Renkema J, Roriguez-Saona C, Rogers M, Sassú F, Schöneberg T, Scott MJ, Seagraves M, Sial A, Van Timmeren S, Wallingford A, Wang X, Yeh DA, Zalom FG, Walton VM (2021) *Drosophila suzukii* (Diptera: Drosophilidae): a decade of research towards a sustainable integrated pest management program. J Econ Entomol 114:1950–1974. https://doi.org/10.1093/jee/toab158

Teder T, Kaasik A, Taits K, Tammaru T (2021) Why do males emerge before females? Sexual size dimorphism drives sexual bimaturism in insects. Biol Rev 96:2461–2475. https://doi.org/10.1111/brv.12762

Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge

Tooke FGC (1955) The eucalyptus snout-beetle, *Gonipterus scutellatus* Gyll. A study of its ecology and control by biological means. Entomological Memoires Department of Agriculture Union of South Africa, Pretoria 3. 1955, 282p

Van Timmeren S, Sial AA, Lanka SK, Spaulding R, Isaacs R (2019) Development of a rapid assessment method for detecting insecticide resistance in spotted wing Drosophila (*Drosophila suzukii* Matsumura). Pest Manag Sci 75:1782–1793. https://doi.org/10.1002/ps.5341

Vieira JGA, Krüger AP, Scheuneumann T, Morais MC, Speriogin HS, Garcia FRM, Nava DE, Bernardi D (2019) Some aspects of the biology of *Trichopria anastrephae* (Hymenoptera: Diapriidae), a resident parasitoid attacking *Drosophila suzukii* (Diptera: Drosophilidae) in Brazil. J Econ Entomol 113:81–87. https://doi.org/10.1093/jee/toz270

Vieira JGA, Krüger AP, Scheunemann T, Garcez AM, Morais MC, Garcia FRM, Nava DE, Bernardi D (2020) Effect of temperature on the development time and life-time fecundity of *Trichopria anastrephae* parasitizing *Drosophila suzukii*. J Appl Entomol 114:857–865. https://doi.org/10.1111/jen.12799

Wollmann J, Schlesener DCH, Ferreira MS, Garcia FRM (2016) Parasitoids of Drosophilidae with potential for parasitism on *Drosophila suzukii* in Brazil. DIS 99:38–42

Yi C, Cai P, Lin J, Liu X, Ao G, Zhang Q, Xia H, Yang J, Ji Q (2020) Life history and host preference of *Trichopria drosophilae* from southern China, one of the effective pupal parasitoids on the Drosophila species. Insects 11(2):103